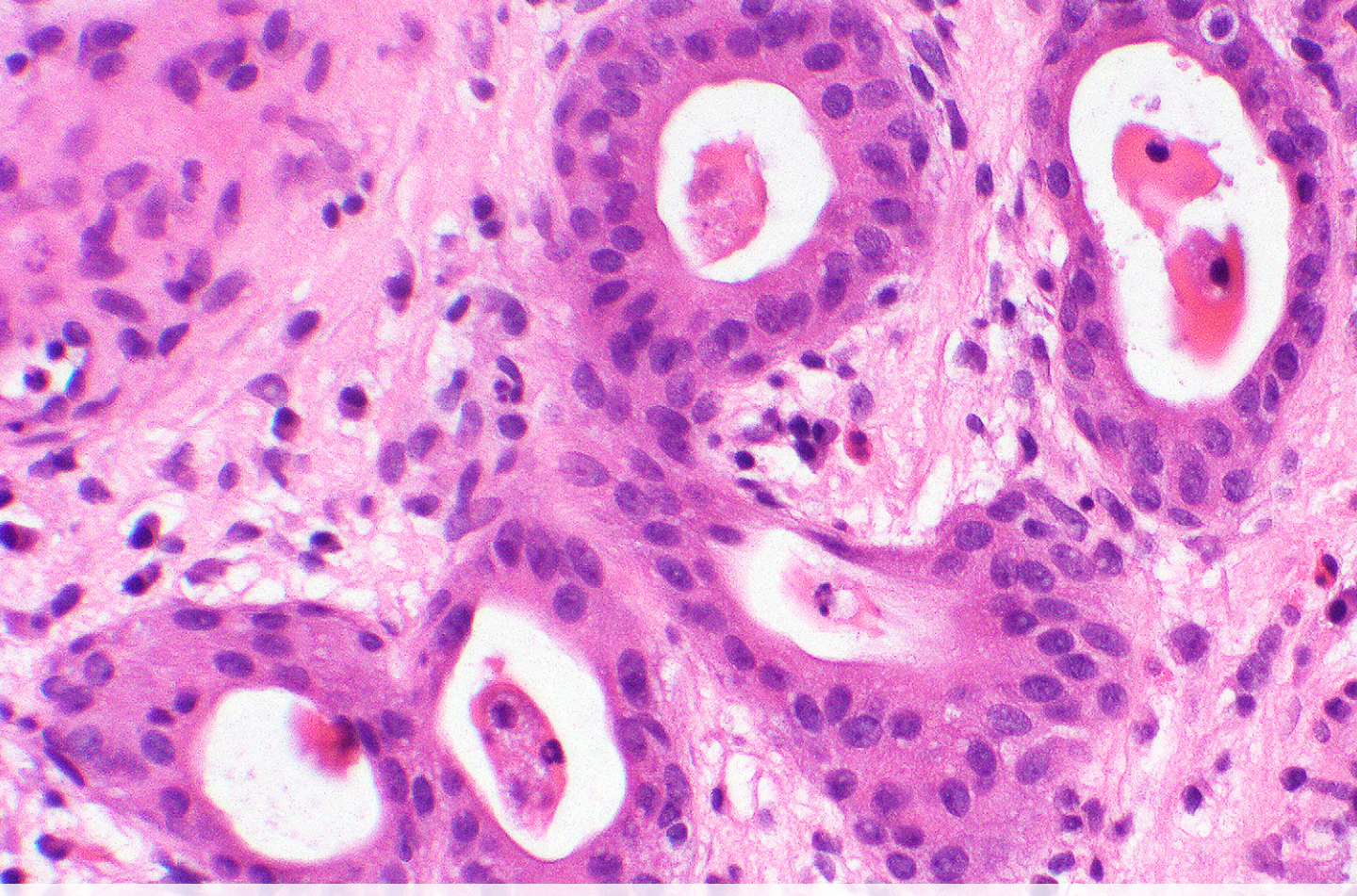

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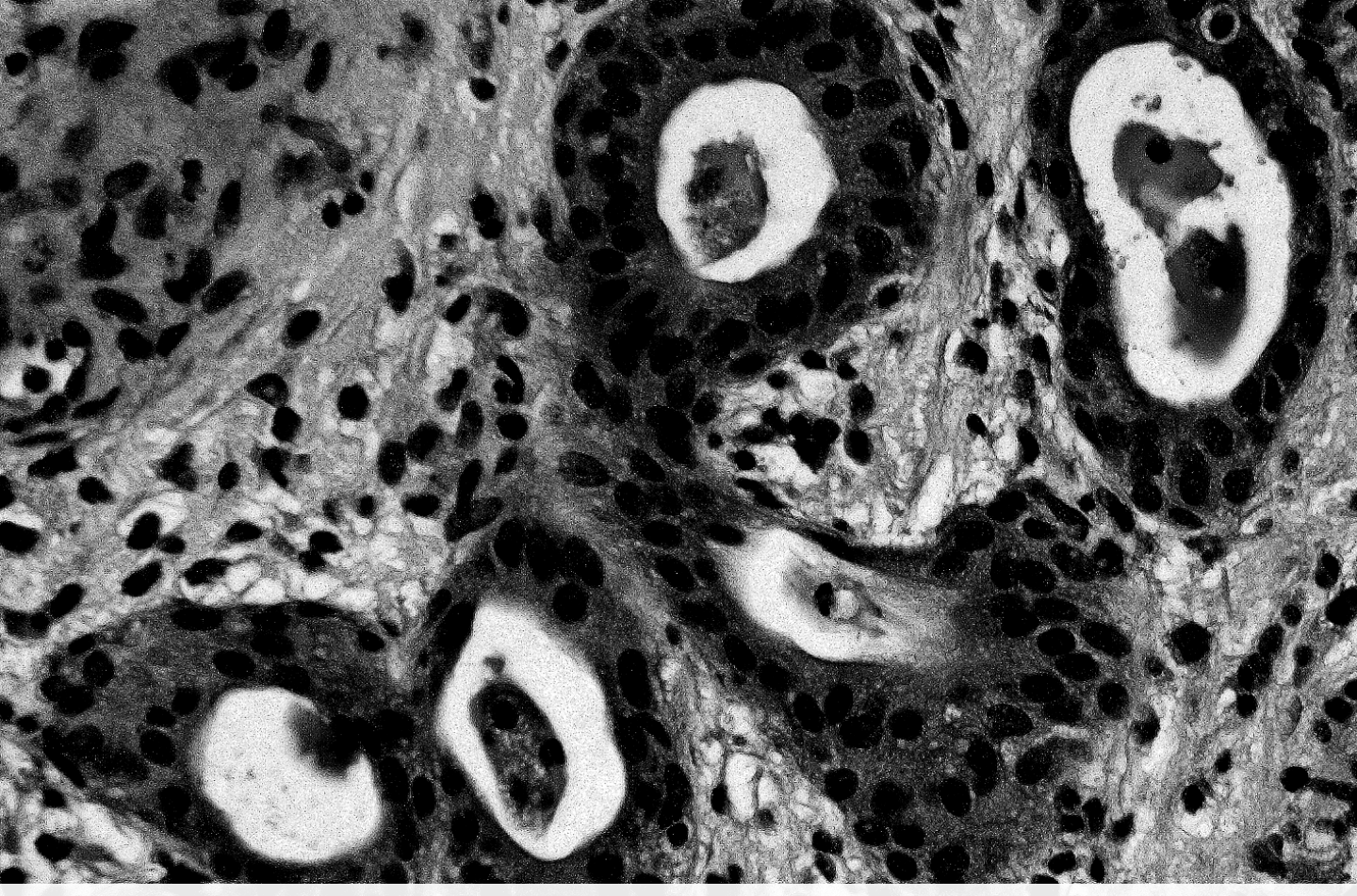

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Abbreviations Used in the Text

ABBREVIATION	FULL TERM	CHAPTER #
18-F FDG	18F-fluorodeoxyglucose	5
^{18}F-F-AraG	2′-deoxy-2′-[^{18}F]fluoro-9-b-Darabinofuranosylguanine	6
^{18}F-FAC	1-(2′-deoxy-2′-[^{18}F]fluoroarabinofuranosyl) cytosine	6
^{18}F-FDG (also 18-F-FDG)	fluorine-18 2-fluoro-2-deoxy-D-glucose	6
34βE12	high molecular weight cytokeratin 34 E12	9
5-ALA	5-aminolevulinic acid	1
5-FU	fluorouracil	6, 8
A/P	anteroposterio (from front to back)	6
ABL1	ABL proto-oncogene 1	4
AC	adjuvant chemotherapy	6
ACS	American Cancer Society	1
ADC	antibody-drug conjugate	8
AE	adverse event	5
AG-ITP	sequential chemotherapy with doxorubicin-gemcitabine (AG) followed by fosfamide, paclitaxel, and cisplatin (ITP)	6
AICR	American Institute for Cancer Research	1
AJCC	American Joint Committee on Cancer	5, 6, 9
AKT	protein kinase B	8
ALA	aminolevulinic acid	5
ALK-1	anaplastic lymphoma kinase	9
AMH	asymptomatic microscopic hematuria	4
Ang	angiopoietin	8
ANXA10	annexin A10	4
APAF1	apoptotic peptidase activating factor 1	4
APC	antigen-presenting cell	8
APOBEC	apolipoprotein B mRNA editing catalytic polypeptide-like	3
AR	androgen receptor	4
ARR	absolute risk reduction	5

ABBREVIATION	FULL TERM	CHAPTER #
AS	active surveillance	5
ASA	American Society of Anesthesiologists	6
ASCI	antigen-specific cancer immunotherapeutic	5
ASCO	American Society of Clinical Oncology	3, 5, 6
ASCO GU	American Society of Clinical Oncology Genitourinary Cancers Symposium	5
ASR	age-standardized rate	1
ASTRO	American Society for Radiation Oncology	6
ATF3	activating transcription factor 3	3
ATP	adenosine triphosphate	3
AUA	American Urological Association	1, 4, 5, 6
AUC	area under the curve	4
AUC	atypical urothelial cell	
AUO	Association of Urologic Oncology	6
AURKA	aurora kinase A	8
B-SCC	bilharzial SCC	9
BATTLE	Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination	8
BBN	n-butyl-n-4-hydroxybutyl nitrosamine	3
BC	bladder cancer	4
BCG	bacillus Calmette-Guérin	1, 3, 4, 5, 6, 8, 9
BCI	Bladder Cancer Index	6
BCRC	Bladder Cancer Research Consortium	6
BFGF	basic fibroblast growth factor	4
BLC	blue-light cystoscopy	5
BLCA-4	bladder cancer 4	4
BMI	body mass index	1, 6
BSC	best supportive care	8
BTA	bladder tumour antigen	4, 5
CA 19-9	carbohydrate antigen 19-9	4
CAE	carcinoembryonic antigen	5
CALGB	Cancer and Leukemia Group B	6
CAP	cyclophosphamide/doxorubicin/cisplatin	6

ABBREVIATION	FULL TERM	CHAPTER #
CAP-ACP	Canadian Association of Pathologists-Association canadienne des pathologistes	2
CAR	chimeric antigen receptor	3
CBI	checkpoint blockade immunotherapy	3
CCD	continent cutaneous diversion	7
CCL2	chemokine ligand 2	3
CDC2	cell division control 2	4
CDK-4/6	cyclin-dependent kinase 4/6	8
CDK2/4	cyclin-dependent kinase 2/4	4
CDKI	cyclin-dependent kinase inhibitor	4
CE	Conformité Européenne	1
CEA	carcinoembryonic antigen	4
ceRNA	competitive endogenous RNA	3
cfDNA	cell-free DNA	3
CGH	comparative genomic hybridisation	3
CHT	chemohyperthermia	5
CI	confidence interval	1, 4, 5, 6
CIN	chromosomal instability	3
circRNA	circular RNA	3
CIS	carcinoma in situ	1, 3, 4, 5, 6
CISCA	cyclophosphamide, doxorubicin, and cisplatin	6, 8
CK18	cytokeratin 18	4
CK19	cytokeratin 19	3
CK20	cytokeratin 20	9
CK7	cytokeratin 7	9
CK8	cytokeratin 8	4
CM	cisplatin and methotrexate	6
CMV	cisplatin, methotrexate, and vinblastine	6
COMPASS	complex of proteins associated with Set1	3
CON	carbogen and nicotinamide	6
COX	cyclooxygenase	5
CPG	Clinical Practice Guideline	6

ABBREVIATION	FULL TERM	CHAPTER #
CPI	checkpoint inhibitor	6
CR	complete response	3, 5, 6, 8
CRH	corticotropin-releasing hormone	4
CROES	Clinical Research Office of the Endourological Society	1
CRP	C-reactive protein	4
CSC	cancer stem cell	3
CSM	cancer-specific mortality	4
CSS	cancer-specific survival	9
CT	computed tomography	1, 3, 5, 6, 9
ct	circulating tumour	8
CTC	Common Terminology Criteria	5
CTC	circulating tumour cell	4
ctDNA	tumour DNA in cell-free DNA	3
CTL	cytotoxic T lymphocyte	5, 8
CTLA-4	cytotoxic T lymphocyte–associated protein 4	3, 4, 8
CTU	computed tomography urography	5
CUA	Canadian Urological Association	5
CUETO	Club Urológico Español de Tratamiento Oncológico (Spanish Urological Club for Oncological Treatment)	4, 5
CVA	cerebrovascular accident	6
CXCR2	C-X-C motif chemokine receptor 2	4
CYFRA 21-1	cytokeratin 19 fragment	4
CYP1A1	cytochrome P450 1A1	3
CYP1B1	cytochrome P450 1B1	3
CYP2E1	cytochrome P450 2E1	3
DCE-MRI	dynamic contrast-enhanced magnetic resonance imaging	6
DD-MVAC (or DDMVAC)	dose-dense methotrexate, vinblastine, doxorubicin, and cisplatin chemotherapy	6, 8
ddPCR	droplet digital polymerase chain reaction	3
DDR	DNA damage response	3
dFdCTP	gemcitabine triphosphate	5
dFdU	2′,2′-difluorodeoxyuridine	5
DFS	disease-free survival	4, 5, 6

ABBREVIATION	FULL TERM	CHAPTER #
DKI	diffusion kurtosis imaging	6
DRE	digital rectal examination	5
DSS	disease-specific survival	3, 5, 9
DVT	deep venous thrombosis	6
DW-MRI	diffusion-weighted magnetic resonance imaging	6
Dy	dysplasia	
E2F1	E2F transcription factor 1	5
EAU	European Association of Urology	1, 4, 5, 6
EBRT	external beam radiation therapy	5, 6
ECOG	Eastern Cooperative Oncology Group	3, 6, 8
EGFR	epidermal growth factor receptor	3, 4, 6
ECUD	extracorporeal urinary diversion	7
eGFR	estimated glomerular filtration rate	7
ELISA	enzyme-linked immunosorbent assay	4
EMA	epithelial membrane antigen	9
EMA	European Medicines Agency	1, 8
EMDA	Electromotive Drug Administration	5
EMT	epithelial-to-mesenchymal transition	3, 8
EORTC	European Organisation for Research and Treatment of Cancer	3, 4, 5, 6, 8
EORTC QLQ BLM30	European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-Bladder Cancer Module	7
EORTC QLQC30	European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire	7
EpCAM	epithelial cell adhesion molecule	5
EPLND (also ePLND)	extended pelvic lymph node dissection	6, 7
ER	estrogen receptor	4
ERAS	enhanced recovery after surgery	6
ERBB2/3	erb-b2 receptor tyrosine kinase 2/3	8
ERP	enhanced recovery program	6
ERUS	European Association of Urology Robotic Urology Section	6
ESRD	end-stage renal disease	8

ABBREVIATION	FULL TERM	CHAPTER #
EUA	examination under anesthesia	5
EZH2	enhancer of zeste-2	3
EZH2i	enhancer of zeste-2 inhibitor	3
FACT-BI	Functional Assessment of Cancer Therapy - Bladder Cancer	6
FAP	alpha-interferon, 5-FU, and cisplatin	8
FDA	US Food and Drug Administration	1, 3, 4, 5, 8
FDG	18F-fluorodeoxyglucose	1
FFPE	formalin-fixed paraffin-embedded	3
FGFR	fibroblast growth factor receptor	3, 5, 9
FGFR1/3	fibroblast growth factor receptor 1/3	8
FGFR3	fibroblast growth factor receptor 3	4, 5
FISH	fluorescence in situ hybridization	1, 3, 4, 5, 8
FOLFOX	oxaliplatin, leucovorin, 5-fluorouracil	9
Fr	French	1
FSFI	Female Sexual Function Index	7
GBP	British Pound Sterling	5
GC	gemcitabine and cisplatin	6, 8
GCa	gemcitabine and carboplatin	8
GEM	genetically-engineered mouse	3
GemRIS	gemcitabine-releasing intravesical system	5
GFR	glomerular filtration rate	6, 8
GHS/QoL	Global Health Status/Quality of Life (GHS/QoL) scale	7
GI	gastrointestinal	6
GM	glandular metaplasia	2
GM-CSF	granulocyte-macrophage colony-stimulating factor	3, 5, 8
GOR	Grade of Recommendation	1, 3, 4, 5, 6, 8, 9
GPD1	glycerol-3-phosphate dehydrogenase 1	3
GSTP1-1	glutathione S-transferase P1-1	5
GTA	gemcitabine, paclitaxel, and doxorubicin	8
GTCa	gemcitabine, paclitaxel, and carboplatin	8

ABBREVIATION	FULL TERM	CHAPTER #
GTP	gemcitabine, paclitaxel, and cisplatin	8
GUONE	Gruppo Uro-Oncologico del Nord Est	6
H&E	hematoxylin and eosin	9
H3	third histone	3
HAL	hexaminolevulinate acid	1, 5
HAT	histone acetyltransferase	3
HDI	Human Development Index	1
HER	human epidermal growth factor receptor	3
HER2	human epidermal growth factor receptor 2	4, 6
HG	high grade	5, 6
HG-RFS	high-grade recurrence-free survival	5
HGUC	high-grade urothelial carcinoma	
HIF1α	hypoxia inducible factor 1 alpha	4
HLA	human leukocyte antigen	3
HNF-1	hepatocyte nuclear factor 1	9
Ho:YAG	holmium:YAG	5
HOXA13	homeobox A13	4
HPV	human papillomavirus	9
HR	hazard ratio	1, 4, 5, 6, 8
HR-QoL (also HRQoL)	health-related quality of life	5, 6
HRAS	HRas proto-oncogene, GTAPase	4
HT	hyperthermia	5
HTh	thermochemotherapy	5
I-SPY-2	Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging and Molecular Analysis-2	8
IAGem	ifosfamide, doxorubicin, and gemcitabine	8
IAP	inhibitor of apoptosis	4
IARC	International Agency for Research on Cancer	1
IBCG	International Bladder Cancer Group	5
IBCNC	International Bladder Cancer Research Consortium	6
IC	immune cell	4
IC	ileal conduit	6, 7

ABBREVIATION	FULL TERM	CHAPTER #
IC_{50}	half maximal inhibitory concentration	6
ICAM-1 (also as ICAM1)	intercellular adhesion molecule 1	4, 5
ICB	immune checkpoint blockade	3
ICCR	International Collaboration on Cancer Reporting	2
ICD-O-3	International Classification of Diseases for Oncology	2
ICH	intravesical chemotherapy	5
ICUD	International Consultation on Urological Diseases	1
IDH	isocitrate dehydrogenase	9
IFN	interferon	3, 4, 5
IGF2	insulin-like growth factor 2	4
IGFBP3	insulin-like growth factor binding protein 3	4
IGFBP5	insulin-like growth factor binding protein 5	4
IHC	immunohistochemistry/immunohistochemical	3, 4, 8
IL	interleukin	3, 5
IL-15R	interleukin-15 receptor	5
IL6	interleukin-6	4
ILCR	International Laparoscopic Cystectomy Registry	6
Ile	isoleucine	3
IM	intramuscular	3
IMP	inosine monophosphate	3
IMPDH	inosine-5′-monophosphate dehydrogenase	3
INB	ileal neobladder	7
INH	isoniazid	5
IONB	ileal orthotopic neobladder	6
IONB-PRO	Ileal Orthotopic Neobladder-Patient Reported Outcome	7
IP	Indiana pouch	6
IPD	individual patient data	5
IPOI	immediate preoperative instillation	5
IPOP	early postoperative instillation	5
IR	intermediate risk	5
IRCC	International Robotic Cystectomy Consortium	6
ISUP	International Society of Urological Pathology	5

ABBREVIATION	FULL TERM	CHAPTER #
ITP	ifosfamide, paclitaxel, and cisplatin	8
ITT	intention-to-treat	8
IV	intravenous	1, 3, 5
IVC	intravesical chemotherapy	5
IVe	intravesical	5
JAK 3	Janus kinase 3	9
K4	fourth lysine	3
KDR	kinase insert domain receptor	9
KMT	lysine methyltransferase	3
KPS	Karnofsky Performance Scale	7
KRAS	Kirsten rat sarcoma viral oncogene homologue	9
KRT	cytokeratin	3
KTP	potassium-titanyl-phosphate	5
LCNEC	large cell neuroendocrine carcinoma	9
LELC	lymphoepithelioma-like carcinoma	2
Leu	leucine	3
LFA-3	leukocyte function-associated antigen-3	5
LG	low grade	5
LGUN	low-grade urothelial neoplasm	
lncRNA	long noncoding RNA	3
LOE	Level of Evidence	1, 3, 4, 5, 6, 8, 9
LPLND	limited pelvic lymph node dissection	6
LRC	laparoscopic radical cystectomy	6
LSI	locus-specific identifier	4
LundTax	Lund University taxonomy	3
LUNG	Lung Cancer Master Protocol	8
LVI	lymphovascular invasion	4, 5
M-CAVI	methotrexate, carboplatin, and vinblastine	8
MA	microsatellite analysis	4
MAb	monoclonal antibody	8
MALT	mucosa-associated lymphoid tissue	9
MAPK	mitogen-activated protein kinase	8

ABBREVIATION	FULL TERM	CHAPTER #
Mb	megabase	3
MCNA	mycobacterial cell wall nucleic acid complex	5
MDA	M.D. Anderson Cancer Center	3
MDK	midkine	4
MEIS1	Meis homeobox 1	4
MESNA	sodium 2-mercaptoethanesulfonate	8
MGH	Massachusetts General Hospital	6
MHOS	Medicare Health Outcomes Survey	6
MIBC	muscle-invasive bladder cancer	1, 3, 4, 5, 6, 8
MIBG	metaiodinebenzylguinidine	9
MIM	missing in metastasis	3
miRNA	microRNA	3
MIUCB	muscle-invasive urothelial carcinoma of the bladder	4
MLL	mixed lineage leukemia	3
MLPA	multiplex ligation–dependent probe amplification	4
MMC	mitomycin C	5, 6
MMP9	matrix metalloproteinase 9	4
MPBC	micropapillary bladder cancer	9
MPC	micropapillary carcinoma	
mpMRI	multiparametric magnetic resonance imaging	6
MR	magnetic resonance	6
MRC	Medical Research Council	5, 6
MRI	magnetic resonance imaging	1, 5, 6, 9
mRNA	messenger RNA	3, 4
MS	mass spectrometry	3
MSig	mutation signature cluster	3
MSKCC	Memorial Sloan Kettering Cancer Center	6
MTD	maximum tolerated dose	5
mTOR	mammalian target of rapamycin	5, 8
mTORC1	mTOR complex 1	8
MU	million units	5
mUC	metastatic urothelial carcinoma	8

ABBREVIATION	FULL TERM	CHAPTER #
MUC-1	mucin-1	5
MVAC	methotrexate/vinblastine/Adriamycin/cisplatin	3, 6, 8
MVD	microvessel density	4
MVEC	methotrexate, vinblastine, epirubicin, and cisplatin	6, 9
NAC	neoadjuvant chemotherapy	3, 6, 9
NB	neobladder	6, 7
NBI	narrow-band imaging	1. 5
NCCN	National Comprehensive Cancer Network	1, 5, 6
NCDB	National Cancer Database	6
NCI	National Cancer Institute	1, 3, 5, 6
NE	neuroendocrine	3
Neo:YAG	neodymium-doped yttrium aluminum garnet	5
NER	nucleotide excision repair	3
NG	nasogastric	5
NGS	next-generation sequencing	3
NGT	nasogastric tube	6
NHGUC	negative for high-grade urothelial carcinoma	
NHS	National Health Service	1
NIBC	noninvasive bladder cancer	4
NICE	National Institute for Health and Care Excellence	5
NK	natural killer	3, 5
NM	nephrogenic metaplasia	2
NMIBC	nonmuscle-invasive bladder cancer	1, 3, 4, 5, 6, 8
NMP22	nuclear matrix protein 22	4, 5
NMR	nuclear magnetic resonance	3
NOS	not otherwise specified	9
NPV	negative predictive value	4
NSAID	nonsteroidal anti-inflammatory drug	5
NSG	NOD scid gamma	3
NVBC	nested variant of bladder cancer	9
OBS	orthotopic bladder substitute/substitution	7
OCT	optical coherence tomography	1

ABBREVIATION	FULL TERM	CHAPTER #
OLA	office laser ablation	5
oncoTICE	BCG, strain TICE	5
ONECUT2	one cut homeobox 2	4
OR	odds ratio	1, 6
ORC	open radical cystectomy	6
ORR	objective response rate	3, 4
ORR	overall response rate	8
OS	overall survival	3, 4, 5, 6
OSR1	odd-skipped related transcription factor 1	4
OTX1	orthodenticle homeobox 1	4
PA	posterioranterior (from back to front)	6
PBO	partial bowel obstruction	6
PC	partial cystectomy	6
PCA	principal component analysis	3
PCaG	paclitaxel, carboplatin, and gemcitabine	6
PCG	paclitaxel, cisplatin, and gemcitabine	6
PCN	percutaneous nephrostomy	7
PCR	polymerase chain reaction	4
pCR	pathologic complete response	6
PD-1	programmed cell death protein 1 (also known as programmed cell death 1)	3, 4, 5, 6, 8, 9
PDE-5	phosphodiesterase-5	7
PD-L1	programmed cell death ligand-1	3, 4, 6, 8, 9
PD-L2	programmed cell death ligand-2	5
PDD	photodynamic diagnosis	1, 5
PDGF	platelet-derived growth factor	4
PDGFR	platelet-derived growth factor receptor	5, 8
PDX	patient-derived xenograft	3
PE	pulmonary embolus	6
PET	positron emission tomography	1, 5, 6
PFS	progression-free survival	3, 4, 5, 6, 8, 9
PI3K	phosphatidylinositol 3 kinase	8,9

ABBREVIATION	FULL TERM	CHAPTER #
PLA2G4A	phospholipase A2, group IVA	3
PLND	pelvic lymph node dissection	6
PO	per os/orally	5
POC	point of care	4
POD	prescription ordering direct	5
POI	postoperative ileus	6
PPARγ	peroxisome proliferator-activated receptor gamma	3
PPD	purified protein derivative	5
PPIX	protoporphyrin IX	1
PPV	positive predictive value	4
PR	partial response	3, 8
PRC2	polycomb-repressor complex-2	3
pri	primary	3
PSA	prostate-specific antigen	5, 9
PSAP	prostate-specific acid phosphatase	9
PSM	positive surgical margin	6
PTEN	phosphatase and tensin homolog	3
PUC	plasmacytoid urothelial carcinoma	9
PUNLMP	papillary urothelial neoplasia of low malignant potential	4, 5
QoL	quality of life	6
qPCR	quantitative polymerase chain reaction	3
qRT-PCR	quantitative reverse-transcription PCR	3
QUADAS-2	Quality Assessment of Diagnostic Accuracy Studies	6
RA	reactive atypia	2
rAd-IFN	recombinant adenoviral interferon	5
RANTES	regulated upon activation normal T cell expressed and secreted	3
RARC	robotic/robot-assisted radical cystectomy	6
Rb	retinoblastoma	4
RB1	retinoblastoma 1	9
RC	radical cystectomy	5, 6, 9
RCT	randomized controlled trial	5,6
RECIST	Response Evaluation Criteria In Solid Tumors	6

ABBREVIATION	FULL TERM	CHAPTER #
recMAGE-A3	MAGE-A3 recombinant protein	5
REMARK	REcommendations for tumour MARKer prognostic studies	4
RF	radiofrequency	5
RFI	recurrence-free interval	5
RFS	recurrence-free survival	3, 4, 5, 6, 9
RISC	Retrospective International Study of Cancers of the Urothelial Tract	6
RNA-Seq	RNA sequencing	3
ROC	receiver operating characteristic	6
RR	response rate	8
RR	relative risk (also called risk ratio)	1, 5, 6
RS	Raman spectroscopy	1
RT	radiotherapy	6
RTK	receptor tyrosine kinase	3
RTOG	Radiation Therapy Oncology Group	5, 6
RXRα	retinoid X receptor alpha	3
s.c.	subcutaneous	3
SAE	serious adverse event	5
SCC	squamous cell carcinoma	3, 5, 6, 9
SCC	squamous-cell carcinoma	3
SCCB	small cell carcinoma of the bladder	9
SCI	spinal cord injury	9
SD	stable disease	3
SE	standard error	6
SEER	Surveillance, Epidemiology, and End Results	1, 6, 9
SES	socio-economic status	1
SHGUC	suspicious for high-grade urothelial carcinoma	2
SHH	sonic hedgehog	3
SI	single-dose intravesical instillation/single instillation	5
SIM2	single-minded family bHLH transcription factor 2	4
SIU	Société Internationale d'Urologie	5
SMG	significantly mutated gene	3

ABBREVIATION	FULL TERM	CHAPTER #
SNP	single nucleotide polymorphism	3, 8
SNV	single nucleotide variant	3
SOGUG	Spanish Oncology Genitourinary Group	6
SPIES	Storz Professional Image Enhancement System	1
SqM	squamous metaplasia	2
SR	systematic review	5
ssDNA	single-stranded DNA	3
STAT	signal transducer and activator of transcription	4
SUI	Société Internationale d'Urologie	1
SUO	Society of Urologic Oncology	1, 4, 5, 6, 9
SV40	simian virus 40	3
SWOG	Southwest Oncology Group	5
TB	tuberculosis	5
TCC	transitional cell carcinoma	6
TCGA	The Cancer Genome Atlas	3, 4, 6, 8
TERT	telomerase reverse transcriptase	3, 4, 9
TEUD	tissue-engineered urinary diversion	7
TGF	transforming growth factor	8
TGFB1	transforming growth factor beta 1	4
Th	T helper	3
TH1	T helper 1	4
Tie2	TEK receptor tyrosine kinase	8
TKI	tyrosine kinase inhibitor	8
Tm:YAG	thulium-doped YAG	5
TMUGS	Tumor Marker Utility Grading System	4
TNF	tumour necrosis factor	3
TNM	Tumour-Node-Metastasis Staging System	5, 9
TPN	total parenteral nutrition	5
TSC1	tuberous sclerosis-1	9
TSC1/2	tuberous sclerosis complex-1/-2	8
TSP1	thrombospondin-1	4
TTF1	thyroid transcription factor 1	9

ABBREVIATION	FULL TERM	CHAPTER #
TUR	transurethral resection	5, 6, 9
TURB	transurethral resection of the bladder	1, 5
TURBT	transurethral resection of the bladder tumour	1, 5, 6, 9
TURP	transurethral resection of the prostate	5
TWIST	twist family bHLH transcription factor 1	4
UA	urinalysis	5
UBC	urinary bladder cancer	4
UC	urothelial carcinoma	3, 8, 9
UCP	urothelial carcinoma of the prostate	5
UD	urinary diversion	7
UICC	Union for International Cancer Control	2
UNC	University of North Carolina	3
uPA	urokinase-type plasminogen activator	4
UPK2	uroplakin 2	3
UPK	uroplakin	3
UPK1B	uroplakin 1B	4
UPUMP	urothelial proliferation of unknown malignant potential	
USPSTF	U.S. Preventive Services Task Force	1
UST	ureterosigmoidostomy	7
UTI	urinary tract infection	5
UTR	untranslated region	3
UTUC	upper tract urothelial carcinoma	4
VA	United States Department of Veterans Affairs	9
VCAN	versican	3
VEGF	vascular endothelial growth factor	3, 4, 6, 8
VEGFR	vascular endothelial growth factor receptor	4, 5, 8
VEGFR2	vascular endothelial growth factor receptor-2	3
VH	variant histology	
VHL	von Hippel-Lindau	9
VLDL	very low-density lipoprotein	3
VST	vesicosigmoidostomy	7
VTE	venous thromboembolism	7

ABBREVIATION	FULL TERM	CHAPTER #
VUC	voided urine cytology	4
WCRF	World Cancer Research Fund	1
WDNET	well-differentiated neuroendocrine carcinoma	9
WHO	World Health Organization	1, 5
WLC	white-light cystoscopy	1, 5
WNT	wingless-type MMTV (mouse mammary tumor virus) integration site	5
β-HCG	β-human chorionic gonadotropin	2

Preface

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The 2017 Société Internationale d'Urologie–International Consultation on Urological Diseases (SIU-ICUD) Joint Consultation on Bladder Cancer represents an update of the 2012 Consultation on the same topic. The ICUD executive committee felt that the field of bladder cancer care and research was evolving so rapidly, that it warranted a new Consultation. To meet these expectations, it was our privilege to invite many of the world's thought leaders in bladder cancer to lead nine committees, with each committee dedicated to one important aspect of clinical care and research. The committee chairs, in turn, invited both established experts and rising stars in the field to compile a comprehensive review on the respective topics. The Consultation itself was held during the 2017 SIU Congress in Lisbon, Portugal. This provided an open forum for debate and discussion of the most recent advances and the most controversial issues in the field. Final drafts of the committee reports incorporated feedback from this Consultation. Importantly, each committee has provided recommendations for clinical practice that have been assigned both levels of evidence and grades of recommendation according to the ICUD Modified Oxford Centre for Evidence-based Medicine Grading System.

This year's Consultation on Bladder Cancer has tackled some potentially contentious issues and addressed all the recent advances in the field. The chapter on localized muscle-invasive disease, for example, under the multidisciplinary leadership of Arnulf Stenzl (Germany), Jason Efstathiou (USA), and Joaquim Bellmunt (USA), reports on new randomized trials comparing robotic and open radical cystectomy. The same chapter also highlights the rise of trimodal therapy as an alternative to radical cystectomy for muscle-invasive bladder cancer. The chapter on urinary diversion, chaired by Joan Palou (Spain), Óscar Rodríguez-Faba (Spain), and Richard Hautmann (Germany), has also critically apprised the issue of open versus robotic surgery, but in the context of intracorporeal urinary diversion after radical cystectomy.

One of the biggest advances in the management of bladder cancer has been the advent of systemic immunotherapy for patients with metastatic bladder cancer. This was an important addition to the chapter on systemic therapy (Axel Merseburger, Germany, and Cora Sternberg, Italy), and was also relevant in the chapters on molecular markers and basic science. The chapter on molecular markers, chaired by Shahrokh Shariat (Austria) and Yair Lotan (USA), provides a comprehensive overview of the current available evidence on serum, urine and tissue markers in bladder cancer.

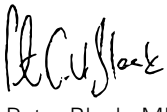
The chapter on pathology, chaired by Eva Compérat (France) and Marek Babjuk (Czech Republic), contains some exciting new features, including especially updated criteria for morphologic classification of variant histologic subtypes and new concepts on immunohistochemistry for disease classification. New elements

of staging and grading have been incorporated, as well as content from the 2017 International Collaboration on Cancer Reporting as it pertains to bladder cancer. This chapter provides an overview of bladder cancer pathology that is unique in this type of document on bladder cancer. A separate chapter on nonurothelial histology (Badrinath Konety, USA, and Antonio López-Beltrán, Spain) delves especially into the clinical ramifications of the different types of bladder cancer.

For the first time, the Consultation includes a chapter on the basic science of bladder cancer. With so much recent progress in understanding the molecular biology of bladder cancer, this chapter has developed into a critical component of the Consultation that does not exist in this form in any similar document. David McConkey (USA) and Seth Lerner (USA) have engaged a remarkable team of highly accomplished scientists from around the globe to assemble a comprehensive, but concise overview of a broad spectrum of topics, from molecular subtypes and metabolomics, to animal models and bladder cancer stem cells. This section should become essential reading for all urologic trainees in the clinic and the lab, as well as those interested in catching up on all recent advances in this field.

Equally important are the chapters on epidemiology, prevention, screening, diagnosis, and evaluation, under the leadership of Ashish Kamat (USA) and Maurizio Brausi (Italy), and on the management of nonmuscle-invasive bladder cancer, under the leadership of Wassim Kassouf (Canada) and Fred Witjes (The Netherlands).

The SIU-ICUD Joint Consultation on Bladder Cancer represents a urologic tour de force that provides a critical resource and an invaluable international reference on bladder cancer for all providers treating and studying this disease. As chairs of this Consultation, it is our extraordinary pleasure to convey, also on behalf of the SIU and the ICUD, our deep appreciation to the committee chairs and all the committee members for their hard work in preparing this SIU-ICUD Joint Consultation on Bladder Cancer. We would also like to acknowledge the tireless efforts of the SIU team, including and especially Christine Albino and Anna Johansen, who did all the hard work behind the scenes to bring all the pieces together and make this Consultation a success.



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Introduction

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Bladder management has a great impact on the quality of life of the spinal cord injured individual. Their days can be dominated by issues of finding an accessible restroom and managing incontinence. There are added matters of medical importance such as recurrent urinary tract infections and renal dysfunction. We all encounter these patients in our practices, yet we lack a central repository for information on this topic. This SIU-ICUD Consultation on the Urologic Management of the Spinal Cord Injured Patient seeks to fill that gap.

This consultation summarizes a state-of-the-art literature review and its recommendations on the urologic management of patients after spinal cord injury (SCI).

After the nine committees were created, the committee chairs presented a summary of their exhaustive review during the 36th Congress of the Société Internationale d'Urologie (SIU) in Buenos Aires, Argentina, in October 2016. The discussions that ensued from those presentations were then incorporated into the final manuscript.

On behalf of the International Consultation on Urological Diseases (ICUD) and its steering committee representing the world's major urological associations (American Urological Association [AUA], Confederación Americana de Urología [CAU], European Association of Urology [EAU], International Continence Society [ICS], SIU, and the Urological Association of Asia [UAA]), it is a great pleasure to thank each of the nine committees' chairmen and members for their hard work in producing this impressive update. The urologic management of patients after SCI can be a poorly understood and oft-neglected topic. It is challenging since it crosses many disciplines, and thus much dedication and effort was required by all involved in this ICUD. As Consultation Chairs, we would like to express our immense gratitude to the SIU leadership, and in particular to Dr Paul Abrams and SIU support staff members, Christine Albino and Anna Johansen, for entrusting us with this important project.

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Evidence-Based Medicine Overview of the Main Steps for Developing and Grading Guideline Recommendations

P. Abrams, S. Khoury, A. Grant

Introduction

The International Consultation on Urological Diseases (ICUD) is a non-governmental organization registered with the World Health Organisation (WHO). In the last ten years, consultations have been organized on BPH, prostate cancer, urinary stone disease, nosocomial infections, erectile dysfunction and urinary incontinence. These consultations have looked at published evidence and produced recommendations at four levels: highly recommended, recommended, optional and not recommended. This method has been useful but the ICUD believes that there should be more explicit statements of the levels of evidence that generate the subsequent grades of recommendations.

The Agency for Health Care Policy and Research (AHCPR) have used specified evidence levels to justify recommendations for the investigation and treatment of a variety of conditions. The Oxford Centre for Evidence-Based Medicine have produced a widely accepted adaptation of the work of AHCPR. (June 5th 2001, www.cebm.net).

The ICUD has examined the Oxford guidelines and discussed with the Oxford group their applicability to the consultations organized by ICUD. It is highly desirable that the recommendations made by the consultations follow an accepted grading system supported by explicit levels of evidence.

The ICUD proposes that future consultations should use a modified version of the Oxford system which can be directly "mapped" onto the Oxford system.

1. First Step

Define the specific questions or statements that the recommendations are supposed to address.

2. Second Step

Analyze and rate (level of evidence) the relevant papers published in the literature.

The analysis of the literature is an important step in preparing recommendations and their guarantee of quality.

2.1 What papers should be included in the analysis?

- Papers published, or accepted for publication in the peer-reviewed issues of journals.
- The committee should do its best to search for papers accepted for publication by the peer-reviewed journals in the relevant field but not yet published.
- Abstracts published in peer-reviewed journals should be identified. If of sufficient interest, the author(s) should be asked for full details of methodology and results. The relevant committee members can then "peer review" the data, and if the data confirms the details in the abstract, then that abstract may be included, with an explanatory footnote. This is a complex issue – it may actually increase publication bias as "uninteresting" abstracts commonly do not progress to full publication.
- Papers published in non-peer-reviewed supplements will not be included. An exhaustive list should be obtained through:
  - I. The major databases covering the last ten years (e.g. Medline, Embase, Cochrane Library, Biosis, Science Citation Index).
  - II. The table of contents of the major journals of urology and other relevant journals, for the last three months, to take into account the possible delay in the indexation of the published papers in the databases.

It is expected that the highly experienced and expert committee members provide additional assurance that no important study would be missed using this review process.

2.2 How are papers analyzed?

Papers published in peer-reviewed journals have differing quality and level of evidence. Each committee will rate the included papers according to levels of evidence (see below).

The level (strength) of evidence provided by an individual study depends on the ability of the study design to minimize the possibility of bias and to maximize attribution.

It is influenced by:

The type of study, whose hierarchy is outlined below:

- Systematic reviews and meta-analysis of randomized controlled trials
- Randomized controlled trials
- Non-randomized cohort studies
- Case-control studies
- Case series
- Expert opinion

How well the study was designed and carried out

Failure to give due attention to key aspects of study methodology increases the risk of bias or confounding factors, and thus reduces the study's reliability.

The use of **standard checklists** is recommended to insure that all relevant aspects are considered and that a consistent approach is used in the methodological assessment of the evidence.

The objective of the checklist is to give a quality rating for individual studies.

How well the study was reported

The ICUD has adopted the CONSORT statement and its widely accepted checklist. The CONSORT statement and the checklist are available at www.consort-statement.org.

2.3 How are papers rated?

Papers are rated following a level of evidence scale.

ICUD has modified the Oxford Centre for Evidence-Based Medicine levels of evidence.

The levels of evidence scales vary between types of studies (i.e. therapy, diagnosis, differential diagnosis/ symptom prevalence study) the Oxford Centre for Evidence-Based Medicine Website: www.cebm.net.

3. Third Step: Synthesis of the Evidence

After the selection of the papers and the rating of the level of evidence of each study, the next step is to compile a summary of the individual studies and the overall direction of the evidence in an **Evidence Table**.

4. Fourth Step: Considered Judgment (Integration of Individual Clinical Expertise)

Having completed a rigorous and objective synthesis of the evidence base, the committee must then make a judgment as to the grade of the recommendation on the basis of this evidence. This requires the exercise of judgment based on clinical experience as well as knowledge of the evidence and the methods used to generate it. Evidence-based medicine requires the integration of individual clinical expertise with the best available external clinical evidence from systematic research. Without the former, practice quickly becomes tyrannized by evidence, for even excellent external evidence may be inapplicable to, or inappropriate for, an individual patient. On the other hand, without current best evidence, practice quickly becomes out of date. Although it is not practical to lay our "rules" for exercising judgment, guideline development groups are asked to consider the evidence in terms of quantity, quality, and consistency, as well as applicability, generalizability and clinical impact.

5. Fifth Step: Final Grading

The grading of the recommendation is intended to strike an appropriate balance between incorporating the complexity of type and quality of the evidence, and maintaining clarity for guideline users.

The recommendations for grading follow the Oxford Centre for Evidence-Based Medicine. The levels of evidence shown below have again been modified in the light of previous consultations. There are now four levels of evidence instead of five.

The grades of recommendation have not been reduced and a "no recommendation possible" grade has been added.

6. Levels of Evidence and Grades of Recommendation for Therapeutic Interventions

All interventions should be judged by the body of evidence for their efficacy, tolerability, safety, clinical effectiveness and cost-effectiveness. It is accepted that, at present, little data exists on cost-effectiveness for most interventions.

6.1 Levels of evidence

Firstly, it should be stated that any level of evidence may be positive (the therapy works) or negative (the therapy doesn't work). A level of evidence is given to each individual study.

Level of Evidence	Criteria
I	▪ Incorporates Oxford 1a, 1b ▪ Usually involves: ▪ meta-analysis of trials (randomized controlled trials [RCTs]) or, ▪ a good-quality RCT or, ▪ "all or none" studies in which treatment is not an option (e.g. in vesicovaginal fistula)
II	▪ Incorporates Oxford 2a, 2b and 2c ▪ Includes: ▪ ***low-quality RCT*** (e.g. <80% follow-up), ▪ ***meta-analysis*** (with homogeneity) of ***good-quality prospective cohort studies*** ▪ May include a single group when individuals who develop the condition are compared with others from within the original cohort group. ▪ There can be parallel cohorts, where those with the condition in the first group are compared with those in the second group
III	▪ Incorporates Oxford 3a, 3b and 4 ▪ Includes: ▪ ***good-quality retrospective case-control studies***, where a group of patients who have a condition are matched appropriately (e.g. for age, sex, etc.) with control individuals who do not have the condition ▪ ***good-quality case series,*** where a complete group of patients, all with the same condition, disease or therapeutic intervention, are described without a comparison control group
IV	▪ Incorporates Oxford 4 ▪ Includes ***expert opinion***, where the opinion is based not on evidence but on "first principles" (e.g. physiological or anatomical) or bench research. ▪ The ***Delphi process*** can be used to give expert opinion greater authority: ▪ involves a series of questions posed to a panel ▪ answers are collected into a series of "options" ▪ these "options" are serially ranked; if a 75% agreement is reached, then a Delphi consensus statement can be made

6.2 Grades of recommendation

The ICUD will use the four grades from the Oxford system. As with levels of evidence, the grades of evidence may apply either positively (procedure is recommended) or negatively (procedure is not recommended). Where there is disparity of evidence, for example if there were three well-conducted RCTs indicating that Drug A was superior to placebo, but one RCT whose results show no difference, then there has to be an individual judgment as to the grade of recommendation given and the rationale explained.

Grade A recommendation usually depends on consistent level I evidence and often means that the recommendation is effectively mandatory and placed within a clinical-care pathway. However, there will be occasions where excellent evidence (level I) does not lead to a Grade A recommendation, for example, if the therapy is prohibitively expensive, dangerous or unethical. Grade A recommendation can follow from Level II evidence. However, a Grade A recommendation needs a greater body of evidence if based on anything except Level I evidence.

Grade B recommendation usually depends on consistent level 2/3 studies, or "majority evidence" from RCTs.

Grade C recommendation usually depends on level 4 studies or "majority evidence" from level 2/3 studies or Delphi processed expert opinion.

Grade D "No recommendation possible" would be used where the evidence is inadequate or conflicting and when expert opinion is delivered without a formal analytical process, such as by Delphi.

7. Levels of Evidence and Grades of Recommendation for Methods of Assessment and Investigation

From initial discussions with the Oxford group, it is clear that application of levels of evidence/grades of recommendation for diagnostic techniques is much more complex than for interventions. The ICUD recommends that, as a minimum, any test should be subjected to three questions:

1. Does the test have good technical performance? For example, do three aliquots of the same urine sample give the same result when subjected to dipstick testing?
2. Does the test have good diagnostic performance, ideally against a "gold standard" measure?
3. Does the test have good therapeutic performance, that is, does the use of the test alter clinical management? Does the use of the test improve outcome?

For the third component (therapeutic performance) the same approach can be used as for section 6.

8. Levels of Evidence and Grades of Recommendation for Basic Science and Epidemiology Studies

The proposed ICUD system does not easily fit into these areas of science. Further research needs to be carried out in order to develop explicit levels of evidence that can lead to recommendations as to the soundness of data in these important aspects of medicine.

Conclusion

The ICUD believes that its consultations should follow the ICUD system of levels of evidence and grades of recommendation, where possible. This system can be mapped to the Oxford system.

There are aspects to the ICUD system that require further research and development, particularly diagnostic performance and cost-effectiveness, and also factors such as patient preference.

Summary of the International Consultation on Urological Disease Modified Oxford Centre for Evidence-Based Medicine Grading System for Guideline Recommendations

Levels of Evidence	Description
I	Meta-analysis of RCTs or high-quality RCT
II	Low-quality RCT or good-quality prospective cohort study
III	Good-quality retrospective case-control study or cohort study
IV	Expert opinion

Abbreviation: RCT=randomized controlled trial

Summary of the International Consultation on Urological Disease Modified Oxford Centre for Evidence-Based Medicine Grading System for Guideline Recommendations

Grades of Recommendation	Description
A	Usually consistent with level I evidence
B	Consistent level II or III evidence or "majority evidence" from RCTs
C	Level IV evidence or "majority evidence" from level II or III studies
D	No recommendation possible because of inadequate or conflicting evidence

RCT=randomized controlled trial

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CONTENTS

Epidemiology, Prevention, Screening, Diagnosis, and Evaluation

Committee 1

1.1 Introduction

This chapter was written by a group of 16 experts from six countries who thoroughly assessed the most recent literature on the epidemiology, prevention, screening, diagnosis, and evaluation of bladder cancer. The experts reviewed the prior recommendations from the International Consultation on Urological Diseases (ICUD) Consultation on Bladder Cancer and updated the recommendations, as appropriate. Some clinical cases are illustrated in the chapter to underline the different pathological features of the tumours and tumour variants. Some new technologies have also been highlighted.

1.2 Epidemiology: General Statistics and Trends

1.2.1 Introduction

Urinary bladder cancer ranks ninth in worldwide cancer incidence (7th among men and 17th among women), with 430,000 new cases in 2012. Approximately 165,000 people die from the disease annually.[1]

About 75% of all bladder cancers occur in men, which, for the most part, is probably a reflection of the differences between men and women historically in smoking prevalence and exposure to occupational carcinogens.[2] Urothelial cell carcinoma is the predominant histological type in bladder cancer. Other histological types, such as squamous cell carcinoma, glandular cell carcinoma, and small cell carcinoma, form only about 5% of all bladder cancers, except in areas with endemic *Schistosoma haematobium* infections, where more squamous cell carcinomas are seen. For example, following the almost complete eradication of *Schistosoma* infections in Egypt, the relative frequency of squamous cell carcinoma dropped from almost 76% to 28%.[3]

Bladder cancer is a very heterogeneous disease; benign tumours may recur within the bladder after surgical removal that will rarely pose a real threat to the patient, or extremely aggressive forms may lead to death shortly after diagnosis. The benign forms of the disease may especially make the interpretation of descriptive statistics on bladder cancer difficult. This can be illustrated by the sudden increase in bladder cancer incidence in the 1970s, after the introduction of the 1973 World Health Organization (WHO) grading system, which reclassified benign papillomas as low-grade papillary carcinomas.[4] Differences in registration practices among cancer registries make it difficult to compare bladder cancer incidence of different world regions, especially when incidence rates are not stratified by disease stage, as some registries include TNM Ta tumours (or papillary urothelial neoplasms of low malignant potential), while others do not. This also complicates the comparison of survival estimates among countries.[5] In addition, differences exist in the registration of metachronous bladder tumours, especially in situations where muscle-invasive bladder cancer (MIBC) is diagnosed in patients who were treated previously for non-muscle-invasive bladder cancer (NMIBC). Some registries report only the first diagnosis of bladder cancer, while others also report a second

diagnosis when MIBC appears during follow-up for NMIBC, or even when a T1 tumour appears after a Ta tumour. It is, therefore, important to realize that differences in bladder cancer burden parameters may be partly artificial.

1.2.2 Bladder cancer incidence and mortality

The absolute number of new bladder cancer diagnoses in 2012 and the age-standardized incidence rates (world standard population), as derived from GLOBOCAN, are listed in **Table 1–1**.[6] Overall, about 9.0 per 100,000 men and 2.2 per 100,000 women develop bladder cancer each year. There is a large geographical variation in the occurrence of bladder cancer, especially among men, with more than 30.0 per 100,000 men diagnosed each year in Spain and Italy, and only 2.0 to 3.0 per 100,000 men diagnosed in some parts of Africa, such as Uganda (**Figure 1–1**). More than half of all bladder cancer cases occur among the 20% of the world population living in countries with a very high Human Development Index (HDI), which is based on health, education, and income, while only 5% of all diagnoses occur in countries with a low HDI.[7] About 3.2 per 100,000 men and 0.9 per 100,000 women die from bladder cancer each year, with somewhat less variation in different parts of the world. Spain and Poland experience the largest mortality rates among men, while countries in Latin America, such as Colombia and Mexico, and Singapore have the lowest rates.

TABLE 1–1 Estimated Number of Bladder Cancer Incident Cases and Deaths by Region of the World in 2012[6]

Area	Incidence					Mortality				
	Men		Women		M:F	Men		Women		M:F
	(n)	(ASR)	*(n)*	(ASR)	(ASR)	*(n)*	(ASR)	*(n)*	(ASR)	(ASR)
World	330,380	9.0	99,413	2.2	4.1	123,051	3.2	42,033	0.9	3.6
By development level										
More developed regions	196,077	16.9	57,766	3.7	4.6	58,914	4.5	21,024	1.1	4.1
Less developed regions	134,303	5.3	41,647	1.5	3.5	64,137	2.6	21,009	0.7	3.7
By human development level										
Very high human development	183,065	16.7	54,713	3.9	4.3	51,927	4.1	19,760	1.1	3.7
High human development	56,697	10.8	15,596	2.2	4.9	24,239	4.5	6,588	0.8	5.6

Abbreviation: ASR, age-standardized rate.
Source: Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol. 2017;71(1):96–108.

continued on **page 7**

TABLE 1–1 Estimated Number of Bladder Cancer Incident Cases and Deaths by Region of the World in 2012[6], *Cont'd*

Area	Incidence					Mortality				
	Men		Women		M:F	Men		Women		M:F
	(n)	(ASR)	*(n)*	(ASR)	(ASR)	*(n)*	(ASR)	*(n)*	(ASR)	(ASR)
Medium human development	79,357	4.7	23,748	1.2	3.9	39,769	2.3	12,226	0.6	3.8
Low human development	11,096	3.1	5,311	1.4	2.2	7,065	2.1	3,443	0.9	2.3
By region										
Africa	17,685	6.3	6,752	2.1	3.0	9,362	3.5	3,906	1.2	2.9
Eastern Africa	2,824	3.3	1,961	2.0	1.7	1,819	2.2	1,290	1.3	1.7
Sub-Saharan Africa	6,460	3.0	4,044	1.6	1.9	3,873	1.9	2,569	1.1	1.7
Middle Africa	610	2.2	441	1.3	1.7	420	1.6	300	0.9	1.8
Northern Africa	11,225	15.1	2,708	3.2	4.7	5,489	7.6	1,337	1.6	4.8
Southern Africa	1,285	7.5	483	1.9	3.9	494	3.0	225	0.9	3.3
Western Africa	1,741	2.1	1,159	1.3	1.6	1,140	1.5	754	0.9	1.7
Caribbean and Central and South America	17,610	6.1	7,234	2.0	3.1	7,078	2.4	3,069	0.8	3.0
Caribbean	1,839	7.6	542	1.8	4.2	773	3.0	284	0.9	3.3
Central America	2,327	3.4	1,430	1.8	1.9	867	1.2	535	0.6	2.0
South America	13,444	6.9	5,262	2.1	3.3	5,438	2.7	2,250	0.9	3.0
North America	58,089	19.5	18,660	5.1	3.8	13,285	4.0	5,307	1.2	3.3
Asia	115,646	5.5	32,922	1.4	3.9	52,816	2.5	16,478	0.6	4.2
Eastern Asia	64,662	5.8	20,789	1.6	3.6	27,271	2.3	10,220	0.7	3.3
Southeastern Asia	10,784	4.3	3,034	1.0	4.3	5,352	2.2	1,517	0.5	4.4
Central and Southern Asia	24,415	3.6	6,159	0.8	4.5	13,413	2.0	3,441	0.5	4.0
Western Asia	15,785	19.0	2,940	3.1	6.1	6,780	8.4	1,300	1.3	6.5
Europe	118,365	17.7	32,932	3.5	5.1	39,522	5.2	12,889	1.1	4.7

Abbreviation: ASR, age-standardized rate.
Source: Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol. 2017;71(1):96–108.

continued on **page 8**

TABLE 1–1 Estimated Number of Bladder Cancer Incident Cases and Deaths by Region of the World in 2012[6], *Cont'd*

Area	Incidence					Mortality				
	Men		Women		M:F	Men		Women		M:F
	(n)	(ASR)	*(n)*	(ASR)	(ASR)	*(n)*	(ASR)	*(n)*	(ASR)	(ASR)
Central and Eastern Europe	30,871	15.1	8,904	2.7	5.6	13,231	6.1	3,543	0.9	6.8
Northern Europe	12,722	12.4	4,645	3.6	3.4	5,174	4.4	2,391	1.5	2.9
Southern Europe	34,786	21.8	8,049	3.8	5.7	11,653	6.0	3,001	1.0	6.0
Western Europe	39,986	19.7	11,334	4.3	4.6	9,464	4.0	3,954	1.1	3.6
Oceania	2,985	10.6	913	2.7	3.9	988	3.2	384	1.0	3.2
Australia/ New Zealand	2,868	11.3	887	2.9	3.9	939	3.3	366	1.0	3.3
Melanesia	84	3.5	21	0.7	5.0	43	2.0	13	0.4	5.0
Micronesia/ Polynesia	33	6.5	5	0.9	7.2	6	1.2	5	0.9	1.3

Abbreviation: ASR, age-standardized rate.
Source: Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol. 2017;71(1):96–108.

FIGURE 1–1
Age-Standardized Incidence Rates of Bladder Cancer in Men in Selected Countries (2003–2007)[6]

Abbreviations: M, male; W, using world-standard population.

Source: Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol. 2017;71(1):96–108.

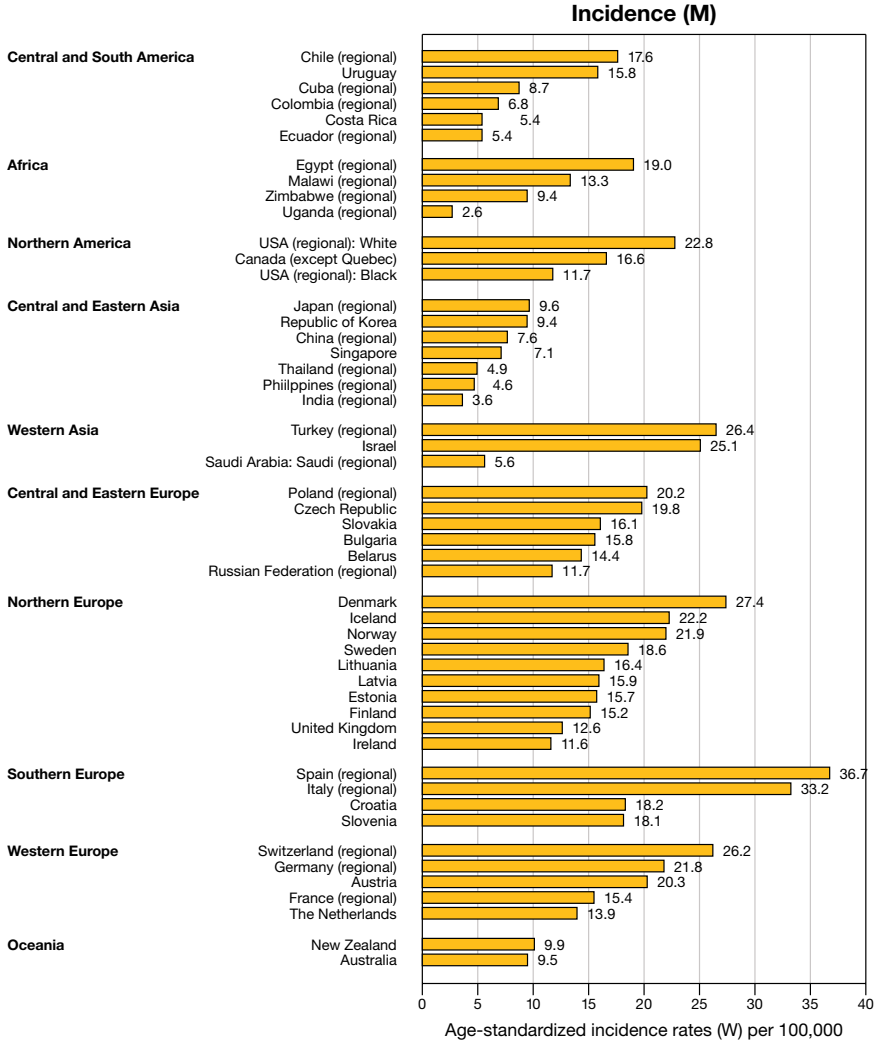


FIGURE 1–1
Age-Standardized and Mortality Rates of Bladder Cancer in Men in Selected Countries (2003–2007)[6]

Abbreviations: M, male; W, using world-standard population.

Source: Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol. 2017;71(1):96–108.

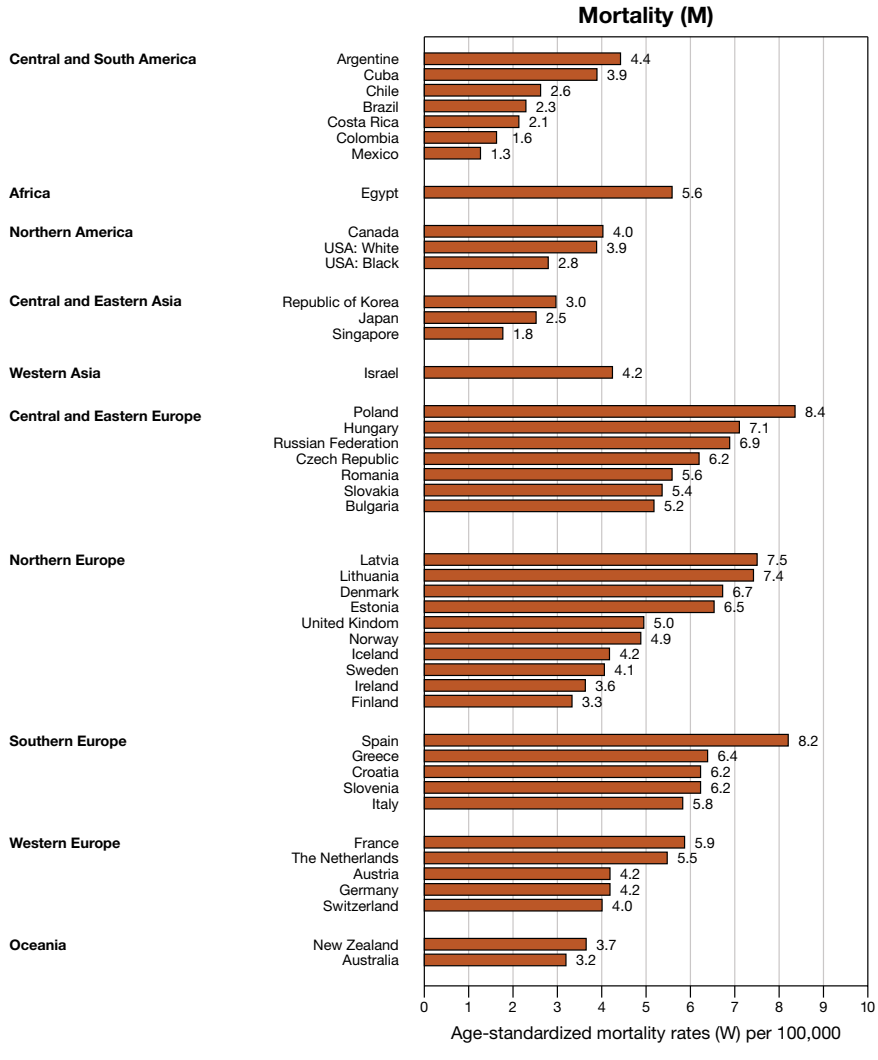


FIGURE 1–1
Age-Standardized Incidence Rates of Bladder Cancer in Women in Selected Countries (2003–2007)[6]

Abbreviations: F, female; W, using world-standard population.

Source: Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol. 2017;71(1):96–108.

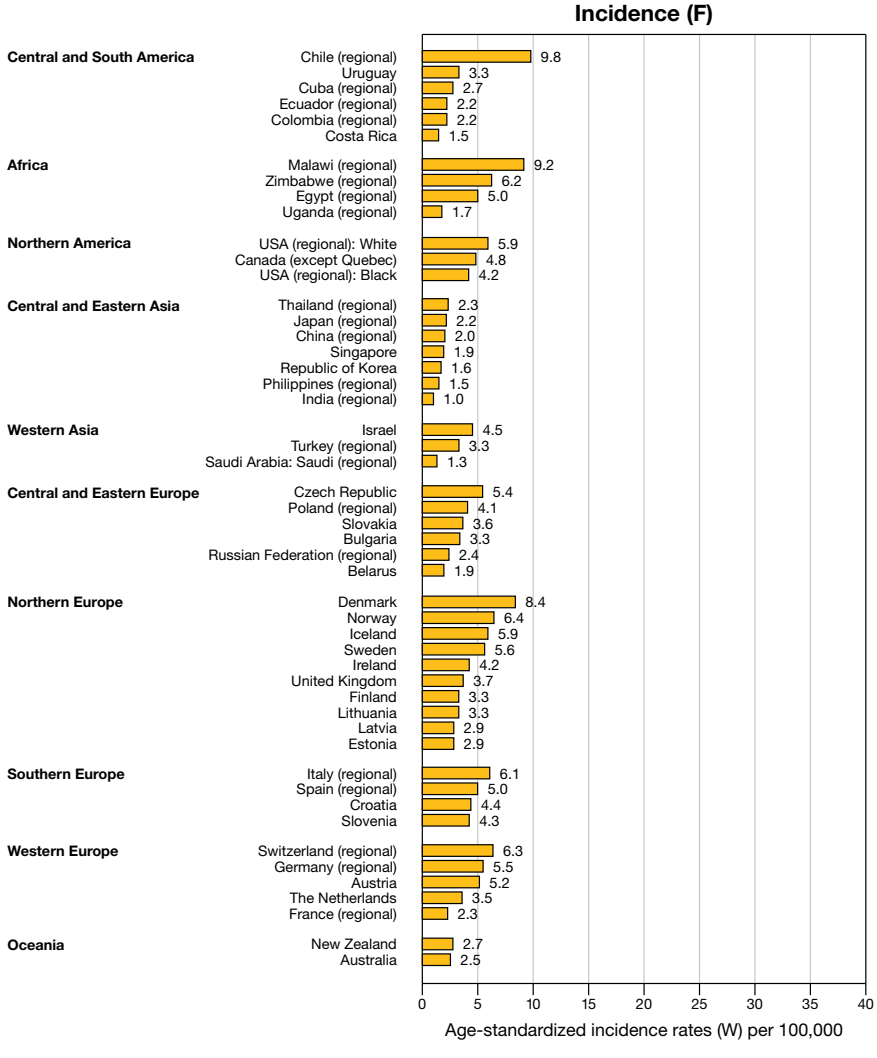
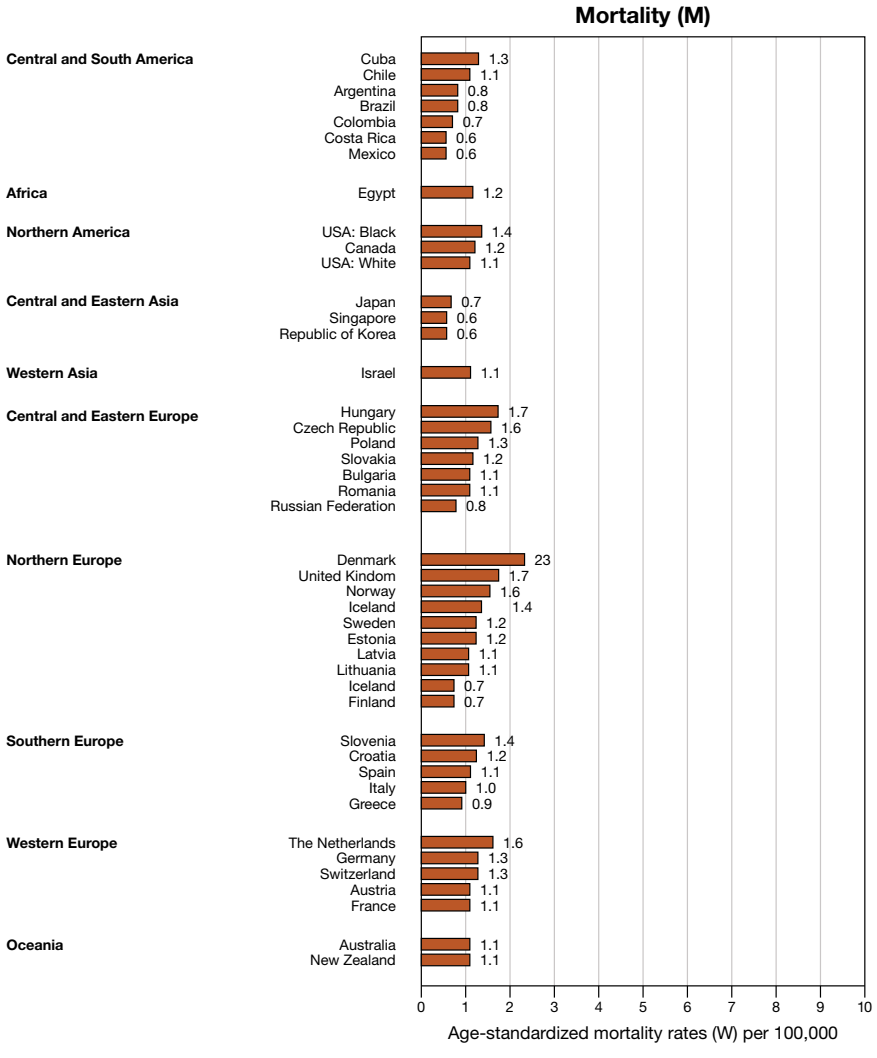


FIGURE 1–1
Age-Standardized Mortality Rates of Bladder Cancer in Women in Selected Countries (2003–2007)[6]

Abbreviations: F, female; W, using world-standard population.

Source: Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol. 2017;71(1):96–108.



1.2.3 Cumulative risks of bladder cancer incidence and death

Standardized incidence rates may be difficult to interpret. An alternative is to look at the cumulative (or lifetime) risks of developing bladder cancer from a certain age (e.g., birth) until a certain age (e.g., 85 years of age). The Netherlands Cancer Registry has reported on such cumulative risks of bladder cancer diagnosis (Ta tumours included) and bladder cancer death. The reported risks were adjusted for competing causes of death and bladder cancer prevalence in the population. The cumulative risk of bladder cancer for men until the age of 85 years was 3.7%, or 1 in 27 men. For women, this risk was almost 1.0%, or 1 in 102 women.[8] In **Table 1–2a** and **Table 1–2b**, the risk for people of different ages of being diagnosed with bladder cancer in the next 10 years is highlighted. For example, a 60-year-old man has a 1.1% (1 in 92 men) risk of being diagnosed with bladder cancer before his 70th birthday. For a 60-year-old woman, this risk is 0.3% (1 in 370 women). About half of these risks can be attributed to the occurrence of Ta tumours. As the Netherlands is a country with an average risk

of bladder cancer, the cumulative risks in other countries may be twice as high or low as those in the Netherlands. As an example, in the United States, the cumulative risks for newborn boys and girls of being diagnosed before the age of 85 years are 3.0% and 0.9%, respectively.[9]

TABLE 1–2A Cumulative Risk for Men of Being Diagnosed With Bladder Cancer From a Certain Age Until a Certain Age (Netherlands Cancer Registry Incidence and Population Data From 2005–2009)[8]

	Until age																				Lifetime risk (until age 85 years)
	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	>95	
From age																					
0	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.03	0.06	0.12	0.26	0.50	0.89	1.47	2.26	3.09	3.74	4.10	4.21	4.23	27
5		0.00	0.00	0.00	0.00	0.01	0.02	0.03	0.06	0.12	0.26	0.50	0.89	1.48	2.27	3.10	3.76	4.12	4.23	4.25	27
10			0.00	0.00	0.00	0.01	0.02	0.03	0.06	0.12	0.26	0.50	0.90	1.48	2.27	3.11	3.76	4.12	4.23	4.26	27
15				0.00	0.00	0.01	0.02	0.03	0.06	0.12	0.26	0.50	0.90	1.48	2.27	3.11	3.77	4.12	4.23	4.26	27
20					0.00	0.01	0.01	0.03	0.06	0.12	0.26	0.50	0.90	1.49	2.27	3.11	3.77	4.13	4.24	4.26	27
25						0.00	0.01	0.03	0.06	0.12	0.26	0.50	0.90	1.49	2.28	3.12	3.78	4.14	4.25	4.27	26
30							0.01	0.02	0.05	0.12	0.25	0.50	0.89	1.49	2.28	3.12	3.78	4.14	4.25	4.28	26
35								0.01	0.04	0.11	0.25	0.49	0.89	1.48	2.28	3.12	3.79	4.15	4.26	4.28	26
40									0.03	0.09	0.23	0.48	0.88	1.47	2.27	3.12	3.79	4.15	4.26	4.29	26
45										0.07	0.20	0.45	0.85	1.46	2.26	3.11	3.79	4.15	4.26	4.29	26
50											0.14	0.39	0.80	1.41	2.22	3.09	3.77	4.14	4.25	4.28	27
55												0.26	0.67	1.29	2.12	3.01	3.70	4.08	4.20	4.22	27
60													0.43	1.08	1.93	2.85	3.57	3.96	4.08	4.11	28
65														0.68	1.59	2.57	3.33	3.74	3.87	3.90	30
70															1.00	2.07	2.92	3.37	3.51	3.55	34
75																1.26	2.26	2.80	2.96	3.00	44
80																	1.33	2.05	2.27	2.32	75
85																		1.18	1.54	1.62	
90																			0.83	1.02	
95																				0.73	

Note that the figures marked in yellow are the 10-year risks of being diagnosed with bladder cancer after the stated starting age.
Source: *Netherlands Comprehensive Cancer Organisation. The Netherlands Cancer Registry. Available: http://www.cijfersoverkanker.nl/home-36.html. Accessed: July 24, 2017.*

TABLE 1–2B Cumulative Risk for Women of Being Diagnosed With Bladder Cancer From a Certain Age Until a Certain Age (Netherlands Cancer Registry Incidence and Population Data From 2005–2009)[8]

From age \ Until age	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	>95	Lifetime risk (until age 85 years)
0	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.03	0.05	0.10	0.18	0.28	0.43	0.62	0.81	0.98	1.10	1.14	1.15	**102**
5		0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.03	0.05	0.10	0.18	0.28	0.43	0.62	0.82	0.99	1.10	1.14	1.15	**102**
10			0.00	0.00	0.00	0.00	0.01	0.01	0.03	0.05	0.10	0.18	0.28	0.43	0.62	0.82	0.99	1.10	1.14	1.15	**101**
15				0.00	0.00	0.00	0.01	0.01	0.03	0.05	0.10	0.18	0.28	0.43	0.62	0.82	0.99	1.10	1.15	1.16	**101**
20					0.00	0.00	0.01	0.01	0.03	0.05	0.10	0.18	0.28	0.43	0.62	0.82	0.99	1.10	1.15	1.16	**101**
25						0.00	0.01	0.01	0.03	0.05	0.10	0.18	0.28	0.43	0.62	0.82	0.99	1.10	1.15	1.16	**101**
30							0.00	0.01	0.02	0.05	0.10	0.17	0.28	0.43	0.62	0.82	0.99	1.10	1.15	1.16	**101**
35								0.01	0.02	0.04	0.09	0.17	0.27	0.42	0.62	0.81	0.98	1.10	1.14	1.15	**102**
40									0.01	0.04	0.09	0.16	0.27	0.42	0.61	0.81	0.98	1.10	1.14	1.15	**102**
45										0.03	0.07	0.15	0.26	0.41	0.60	0.80	0.97	1.09	1.13	1.14	**103**
50											0.05	0.13	0.23	0.38	0.58	0.78	0.96	1.08	1.12	1.13	**105**
55												0.08	0.19	0.34	0.54	0.75	0.92	1.04	1.09	1.10	**108**
60													0.11	0.27	0.47	0.68	0.86	0.99	1.03	1.04	**116**
65														0.16	0.37	0.59	0.78	0.91	0.95	0.97	**128**
70															0.22	0.45	0.65	0.79	0.84	0.85	**154**
75																0.25	0.47	0.61	0.67	0.68	**214**
80																	0.25	0.43	0.49	0.51	**393**
85																		0.24	0.33	0.35	
90																			0.16	0.20	
95																				0.10	

Note that the figures marked in yellow are the 10-year risks of being diagnosed with bladder cancer after the stated starting age.
Source: *Netherlands Comprehensive Cancer Organisation. The Netherlands Cancer Registry. Available: http://www.cijfersoverkanker.nl/home-36.html. Accessed: July 24, 2017.*

Regarding mortality, the cumulative risks of bladder cancer death for men from birth until the age of 85 years are 0.9% (1 in 112) in the Netherlands and 0.6% in the United States. For women, these risks are 0.3% (1 in 323) and 0.2%, respectively (data are not shown).

1.2.4 Time trends in incidence and mortality

The estimated annual percentage change in age-standardized incidence and mortality rates in selected countries around the globe are shown in **Figure 1–2**.[6] Heterogeneous patterns are visible, probably reflecting the different trends in smoking prevalence and registration practices. In most of Western Europe, the incidence has been declining in men but increasing in women. Meanwhile, in Northern Europe, the trend is similar for men and women, decreasing in the Nordic countries and the United Kingdom, but increasing in the Baltic states. Rates are also increasing in Central, Eastern, and Southern Europe. However, they are decreasing in most of the other parts of the world, especially in Oceania. Regarding mortality, most of the selected countries show decreasing rates, except for the Baltic states; some Eastern and Southern European countries, such as Slovenia; and some Central and South American countries, such as Cuba, Mexico, and Brazil. Since trends in mortality are influenced by changes in incidence (which depend on changes in risk factors, registration practices, and diagnostic procedures) and changes in survival (which are, in turn, related to changes in stage distribution and therapeutic management), it is difficult not only to assess the exact cause of any trend, but also to predict whether the observed trends will continue in the future.

FIGURE 1–2
Estimated Annual Percentage Change in Age-Standardized Incidence in Men in Selected Countries (Past 15 Years of Available Data)[6]

Source: Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol. 2017;71(1):96–108.

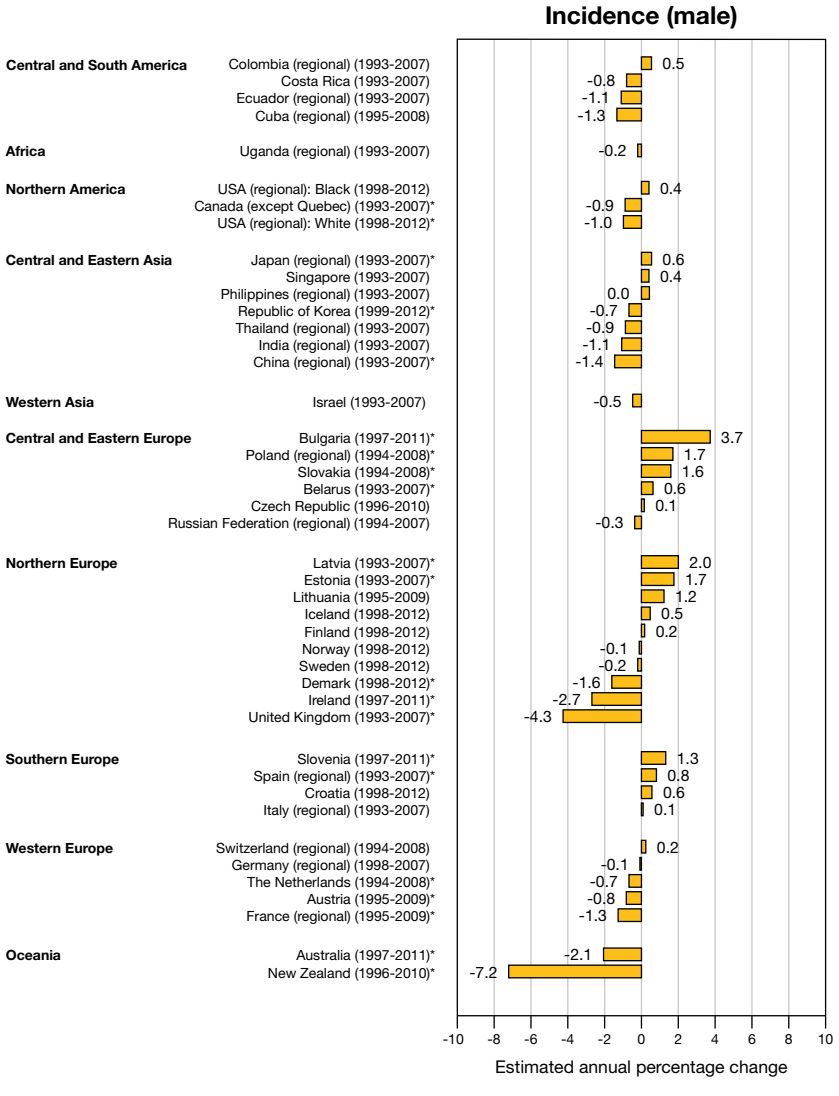


FIGURE 1–2
Estimated Annual Percentage Change in Age-Standardized and Mortality in Men in Selected Countries (Past 15 Years of Available Data)[6]

Source: Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol. 2017;71(1):96–108.

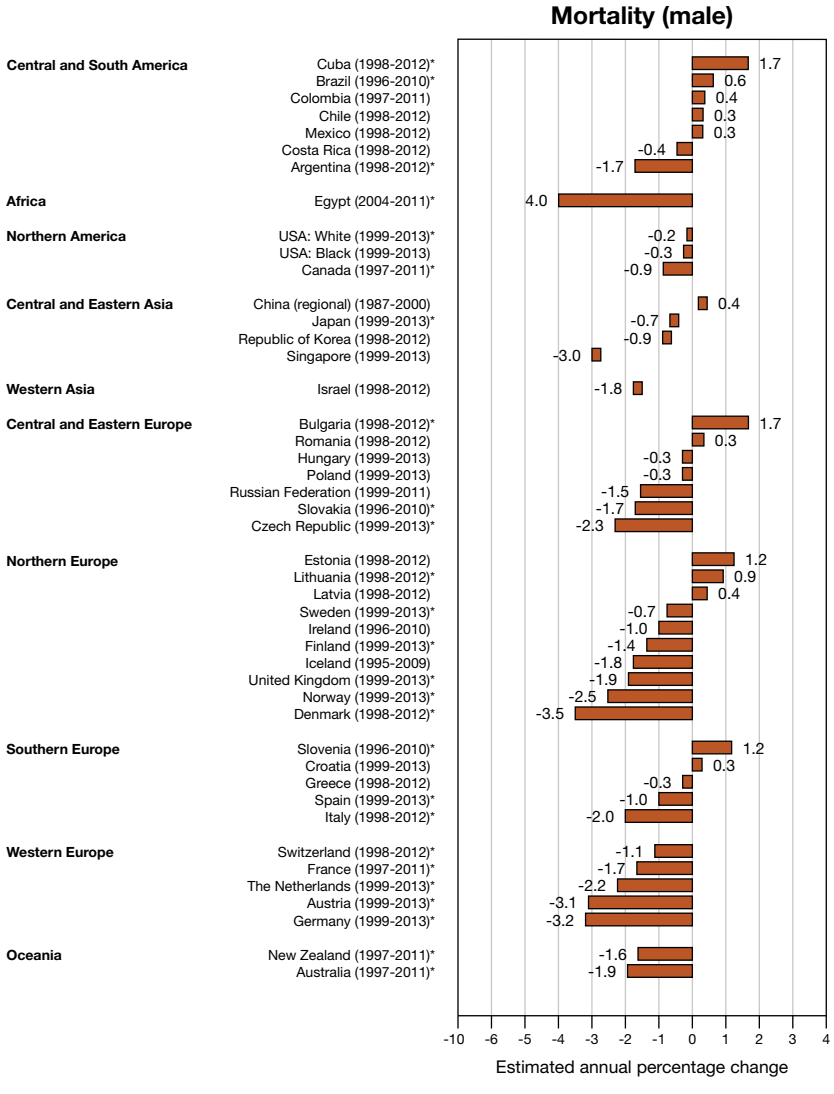


FIGURE 1–2
Estimated Annual Percentage Change in Age-Standardized Incidence in Women in Selected Countries (Past 15 Years of Available Data)[6]

Source: Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol. 2017;71(1):96–108.

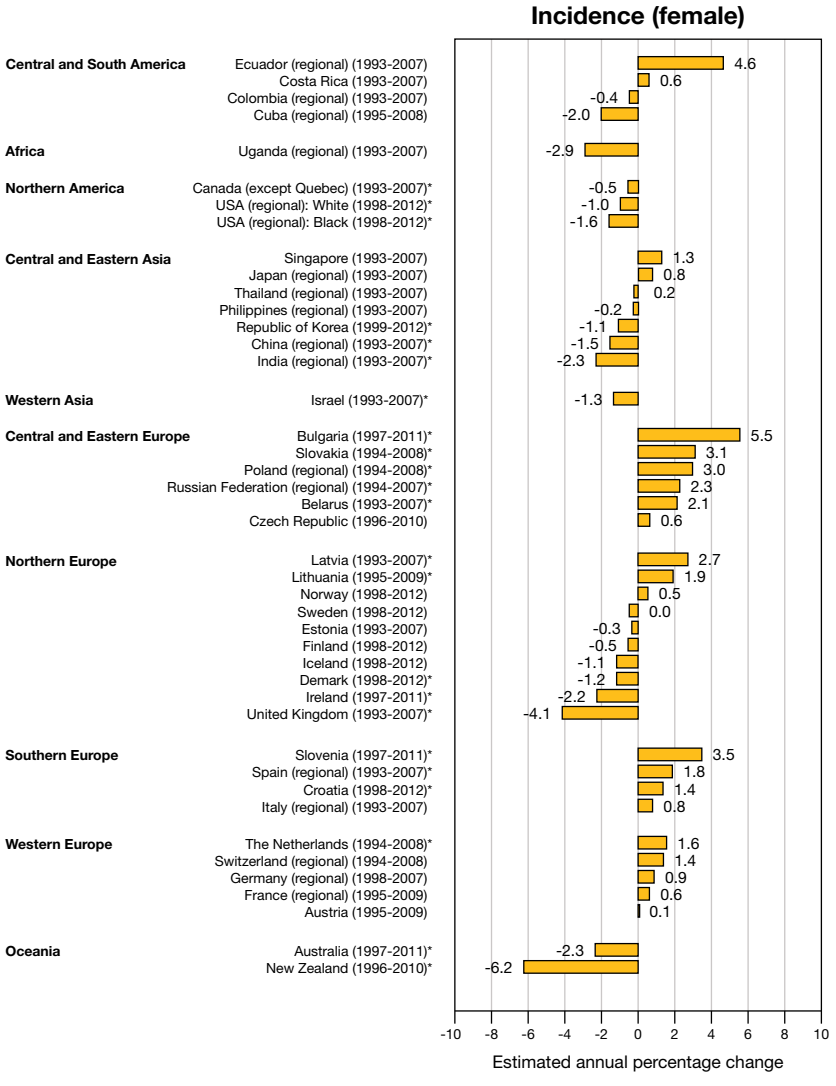
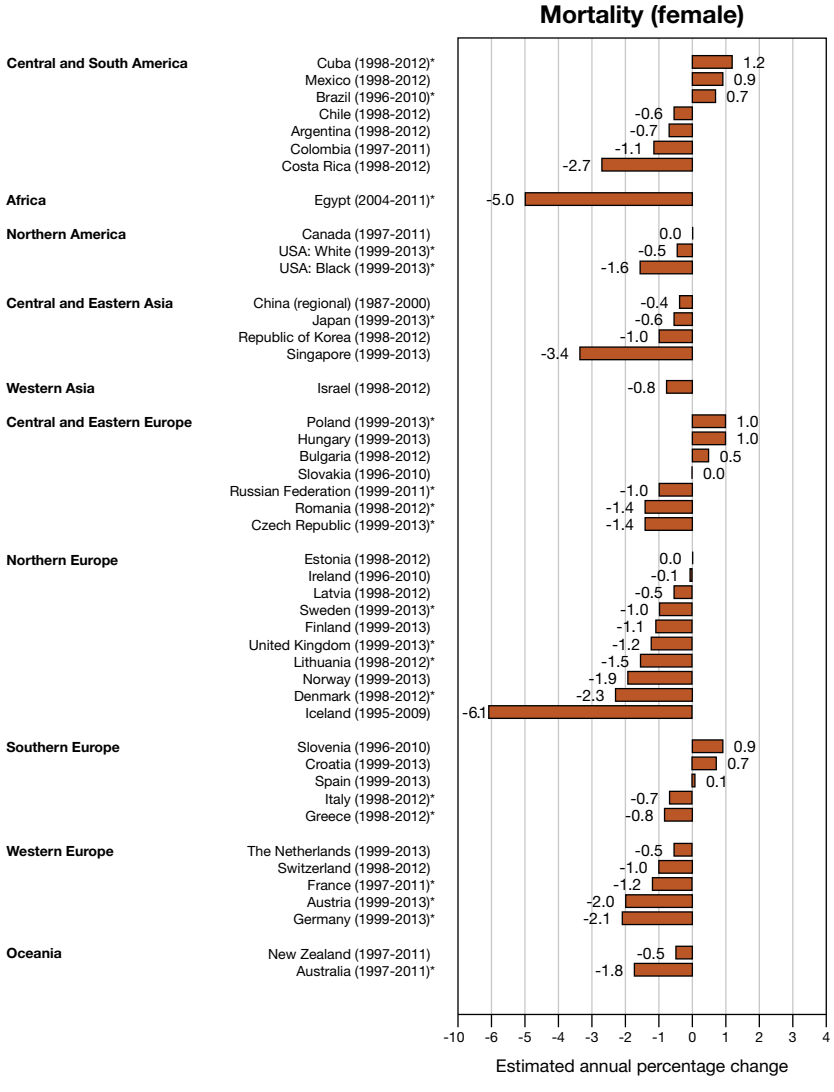


FIGURE 1–2
Estimated Annual Percentage Change in Age-Standardized Mortality in Women in Selected Countries (Past 15 Years of Available Data)[6]

Source: Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol. 2017;71(1):96–108.



1.2.5 Population-based survival estimates

As stated before, population-based estimates on bladder cancer survival are heavily dependent on the proportion of noninvasive tumours in the registries. Survival is also, of course, dependent on the completeness of follow-up. It is safe to say that survival estimates are more valid in countries where a cancer registry can be linked to a valid demographic registry with mortality data. Such a demographic registry is not available, for example, in the United States.[10] In Europe, the 5-year relative survival was reported to be 68.6%, but this estimate varied between 78.8% (95% confidence interval [CI]: 74.4%–83.5%) in Malta and 49.0% (95% CI: 47.1%–50.9%) in Scotland. In Malta, however, 44.8% of all bladder tumours were Ta tumours, while Scotland did not include any Ta tumours.[5] The 5-year relative survival in the United States, based on Surveillance, Epidemiology, and End Results (SEER) data from 1988 to 2013, was 79.1% (invasive plus noninvasive tumours).[9] In the Netherlands, the 5-year relative survival estimates for stage I (noninvasive tumours excluded), II, III, and IV tumours (TNM staging system, sixth edition, 2003–2009) were 80%, 47%, 32%, and 11%, respectively.[8] Survival was higher in men (5-year relative survival: 56%) than in women (5-year relative survival: 45%). This observation was consistent in many studies, and it did not appear to be related to a longer diagnostic delay in women.[11] Strikingly, the 5-year relative survival did not improve at all over the calendar periods of 1989 to 1993, 1994 to 1998, 1999 to 2003, 2004 to 2007, and 2008 to 2012 in the Netherlands. Similarly, stable 5year relative survival rates have also observed in several European countries[5] and in the United States.[9]

1.2.6 Bladder cancer prevalence

Bladder cancer prevalence, like the prevalence of any tumour, is difficult to assess. The number of patients alive with the disease is a function of incidence and duration (prevalence = incidence × duration). For diseases with a poor prognosis, the duration is, in fact, the length of survival. For diseases with a good prognosis, the duration is dependent on how long a patient is considered to be a patient. Some patients consider themselves "cancer survivors" for life, while others consider themselves cured after a couple of years. Therefore, the so-called partial prevalence refers to the number of patients still alive within a defined period after diagnosis (e.g., 5, 10, or 20 years). In the Netherlands in 2016, the 5-year, 10-year, and 20-year prevalence rates were 23,900; 38,200; and 51,300 patients, respectively (noninvasive tumours included).[8] With an absolute incidence of 7,100 cases per year, the 20-year prevalence would be 7.2 times the incidence. When extrapolating this to 430,000 new cases per year globally, it would mean that, currently, 3.1 million people in the world have had a bladder cancer diagnosis during the past 20 years. According to SEER data, the bladder cancer incidence in 2017 in the United States was 79,000 patients, and the prevalence was 696,400 patients.[9] If these figures were to be extrapolated to the global population, it would mean that 3.8 million people of the current world population have had a bladder cancer diagnosis.

1.2.7 A forecast into the quantitative burden of bladder cancer

As bladder cancer survival has hardly changed in the past decades, the future burden of bladder cancer is probably mainly determined by the number of new cases. This number depends heavily on the demographic changes and the prevalence of risk factors. In addition, the world's population is expected to grow to almost 10 billion around 2050, according to the medium fertility scenario of the United Nations Population Fund.[12] Most Western countries will also experience a "double-aging" phenomenon during the next few decades—more elderly with a higher life expectancy. There were approximately 810 million persons aged 60 years or older in the world in 2012, and this number is projected to grow to more than 2 billion by 2050. At that point, older persons will outnumber the population of children (0–14 years) for the first time in human history. Asia has more than half (55%) of the world's older persons, followed by Europe, which accounts for 21% of the total population. Although aging is evolving fast in the more developed regions, the less developed regions will experience faster aging over a much shorter period. Furthermore, the older population is itself aging. Currently, the oldest-old population (aged 80 years or older) accounts for 14% of the population aged 60 years or older. The oldest-old is the fastest growing age segment of the older population. By 2050, 20% of the older population will be aged 80 years or older.[12] At the same time, there will be changes in smoking prevalence, with decreasing rates between 2010 and 2025 in many Western countries, but spectacularly increasing rates in many Asian and African countries.[13] This will mean that the absolute incidence and prevalence of bladder cancer will increase in Western countries, while the age-adjusted risk of the disease will probably decrease. In many lower- and middle-income countries, both the absolute incidence and prevalence, as well as the risk of the disease, will increase enormously. As this phenomenon is not restricted to bladder cancer, but applies to most chronic diseases, the disease burden for these countries will become an even larger problem than it already is.

1.3 Bladder Cancer Prevention

1.3.1 Introduction

Urothelial carcinoma, which comprises about 90% of all bladder cancers, has the highest lifetime cost per patient of all cancers, representing an immense burden on the health care system.[14] Therefore, preventive measures to reduce the risk of developing bladder cancer and to improve bladder cancer outcomes are of importance. This section describes the non-modifiable and modifiable factors that predispose individuals to acquire bladder cancer. Additionally, bladder cancer preventive measures and their efficacy are reviewed. Conclusions on the epidemiological facets of bladder cancer that practitioners might find useful in developing a complete history or plan for bladder cancer patients are provided along with their levels of evidence (LOEs).

The data presented in this section are a summary of the epidemiological literature. Therefore, the data are largely composed of population-based case-control or cohort studies, but rarely randomized controlled clinical trials or meta-analyses of such trials. In many instances, risk factors or preventive interventions that are true for one population in the United States might differ for a population in Eastern Europe, sub-Saharan Africa, and so forth. Therefore, readers should consider these data in the context of the geographical location, patient demographics, and scope of practice investigated to ensure that the recommendations provided are truly relevant to the individuals and populations of any specific locale.

1.3.2 Risk factors

1.3.2.1 Non-modifiable risk factors

1.3.2.1.1 Gender

The incidence of bladder cancer is three times higher in men than in women. Nonetheless, women have a higher mortality rate relative to incidence.[15] While the explanation for why bladder cancer is a more lethal disease in women has not been definitively proven and is likely multifactorial, it has been demonstrated that women present with a higher-grade disease,[16] larger and more multifocal tumours,[17] and a higher rate of variant histology,[18] all of which correspond with poorer outcomes. Other hypotheses for why gender disparities exist in bladder cancer include differences in the metabolism of carcinogens,[19] hormonal dissimilarities,[20] anatomical variations in bladder thickness,[21] rising rates of smoking in women,[22] differential exposure to environmental/occupational hazards,[23] and delays in diagnosis[24] in women.

1.3.2.1.2 Race

In the United States, bladder cancer is most common in Caucasians; however, recent data have emerged suggesting increased trends in African American and Hispanic populations. Although the actual number of new bladder cancer cases is still highest in Caucasians, mortality rates for bladder cancer are higher among non-white patients.[25]

1.3.2.1.3 Genetic susceptibility

Genetic alterations, such as NAT1, NAT2, and GSTM1 null genotypes, have been shown to be associated with an increased susceptibility to bladder cancer.[26] The enzymes for which the genes code play a role in the detoxification pathways of aromatic amines and polycyclic aromatic hydrocarbons. Therefore, these mutations do not intrinsically cause bladder cancer, but do increase susceptibility with exposure to tobacco smoke or other sources of exposure.[27] Females with a history of smoking cigarettes who harbour the GSTM1 null genotype are more prone to developing bladder cancer than their nonsmoking counterparts.[28] Genome-wide association studies have found sequence variants that can increase the risk of bladder cancer; for example, alterations in the urea transporter encoded by SLC14A1[29] are associated with changes in renal urine concentrations and can influence the contact of carcinogens with urothelial surfaces.[30–32] Furthermore, based on an analysis that measured gene-gene interactions in two genome-wide association studies, bladder cancer susceptibility was purportedly associated with decarboxylase protein complexes, which are potential targets for drug therapy.[33]

Germline mutations in FGFR3 have been linked to the development of papillary NMIBC. Furthermore, a variant of the genetic locus rs798766 on chromosome 4p16.3, denoted the T allele, has been associated with low-grade Ta NMIBC in patients also carrying activating mutations in FGFR3.[34]

1.3.2.1.4 Socio-economic status

There is consensus in the literature that socio-economic status (SES) is inversely related to bladder cancer incidence and outcomes; this true with many other types of cancers.[35] The mechanism by which SES impacts bladder epidemiology is through associations with many of the modifiable risk factors discussed in the next section. For example, populations with a low education level, which is an indicator of low SES, were found to have a 20% higher chance of developing bladder cancer, even after adjustment for smoking, which itself is another indicator of low SES.[36] Adults from low-income households are more likely to maintain diets deficient in healthy foods[37] and hold occupations with a higher likelihood of exposure to carcinogens.[38] Low SES, therefore, represents merely the tip of the iceberg, signaling larger societal challenges with disease prevention, access to care, and racial/economic disparities that increase an individual's risk of developing bladder cancer.

1.3.2.1.5 Medical conditions

Certain medical conditions have been linked to bladder cancer incidence. Infamously, infection with the bacterium *S. haematobium* generates a chronic inflammatory state that results in squamous cell carcinoma of the bladder.[39] Conventional urinary tract infections are also a recognized contributor to the risk of developing bladder cancer.[40] Viral infections, specifically human papillomavirus, have been linked to bladder cancer in meta-analyses.[41] The causality of radiation exposure on bladder cancer incidence was established based on long-term studies of World War II atomic bomb survivors.[42] Patients who undergo radiation therapy for prostate[43] and cervical cancers[44] have a documented latent risk of developing urothelial carcinoma, as many as 30 years following treatment. Of the chemotherapeutic agents commonly used to treat neoplasms, only cyclophosphamide has been positively demonstrated to be associated with bladder cancer.[45]

1.3.2.2 Modifiable risk factors

1.3.2.2.1 Smoking

The most common risk factor for the development of bladder cancer is cigarette smoking. While the prevalence rates of smoking have been declining significantly over the past decades, the absolute number of smokers has been increasing because of the growing world population.[46] In fact, estimates indicate that tobacco usage is responsible for at least 50% of all cases. However, the impact of cigarette smoking and tobacco usage is cumulative, with a lag time of more than 20 years between cigarette exposure and diagnosis.[47,48]

Since men smoke more than women, at least in the past, the percentage of cases attributable to smoking is higher in men than in women: 42.8% (males) versus 25.7% (females) in Europe and 34.3% (males) versus 30.1% (females) in the United States. This higher prevalence of smoking among men also explains part of the discrepancy in the incidence of bladder cancer between men and women.[49,50] Consequently, bladder cancer incidence rates are generally highest in regions with high smoking rates. This is best illustrated in the countries of Spain and Italy, where the age-adjusted smoking rates in men in 1980 were 44.4% and 44.3%, respectively.

1.3.2.2.2 Occupational exposure

Occupational risk factors are estimated to account for approximately 8% to 10% of bladder cancer risks, highlighting the need for awareness of these exposures in at-risk patient populations.[51] Several meta-analyses have been performed looking at occupational exposure and risk of bladder cancer. The most comprehensive of all to date is that by Cumberbatch *et al.*[52] These authors identified occupations based on the studies included. They found the following occupations had a statistically significant, 20% increased risk of bladder cancer development: tobacco workers, dye workers, chimney sweeps, nurses, rubber workers, waiters, aluminum workers, hairdressers, printers, seamen, oil and petroleum workers, shoe and leather workers, and plumbers. Protective effects were found in farmers, gardeners, teachers, forestry workers, religious and legal workers, and economically inactive workers.[53]

1.3.2.2.3 Environmental pollution

Recently, reports have demonstrated the effects of environmental pollution on bladder cancer risk, particularly in water and food supplies. A hospital-based case-control study from Spain reported that consumption of drinking water with high nitrate levels for more than 20 years was associated with a 1.4 times increased risk of bladder cancer.[54] Furthermore, arsenic pollution was correlated with bladder cancer risk in Argentina, Chile, and Bangladesh.[55,56] Ambient air pollution is thought to contribute to bladder cancer risk in underdeveloped countries. The direct combustion of chemicals from diesel and gasoline engine exhausts, stationary power plants, and indoor air pollution are all major sources of air pollution.[57]

1.3.3 Primary prevention of bladder cancer

1.3.3.1 Smoking cessation

Smoking cessation prior to the development of bladder cancer reduces the overall lifetime risk of bladder cancer by almost 40% within 5 years after quitting,[58] although this decrease in risk does not reach the level of risk of never smokers.[59] Although the unexplained and irreversible effects of

smoking make the complete elimination of risk impossible,[60] efforts to emphasize that smoking cessation and prevention of smoking initiation prevent bladder cancer should remain high on public health policy agendas.

1.3.3.2 Occupational risk avoidance

There is no question that certain occupational exposures increase the risk of bladder cancer.[61] Thus, if these exposures could be modified, at least in theory, these bladder cancer cases could be prevented. Specifically, working conditions involving exposure to aromatic amines, polycyclic aromatic hydrocarbons, tobacco smoke, heavy metals, and combustion products should be given priority to reduce exposure to these compounds.[62,63]

1.3.3.3 Environmental pollution

In 2010, the International Agency for Research on Cancer (IARC) listed outdoor air pollution as a modifiable risk factor for the development of bladder cancer, citing limited, but consistent, epidemiological data.[64] Indoor air quality and, most significantly, the presence of secondhand smoke are also important risk factors for bladder cancer.[65] Reducing the contribution of environmental pollution to bladder cancer depends on public awareness and the election of local, state, and national policy makers who include clean air initiatives, green energy sources, and smoking cessation in their agenda.

1.3.3.4 Diet

In a systematic review of all meta-analyses, based on both case-control and cohort studies, on dietary factors in relation to primary bladder cancer risk, statistically significant associations were found with higher body levels of selenium (relative risk [RR]: 0.61) and vitamin D (RR: 0.75), and higher intakes of vitamin A (RR: 0.82), vitamin E (RR: 0.82), folate (RR: 0.84), fruit (RR: 0.77), vegetables (RR: 0.83), citrus fruit (RR: 0.85), cruciferous vegetables (RR: 0.84), and processed meat (RR: 1.22).[66] However, in a comprehensive report on bladder cancer, the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) judged the evidence on most of these dietary factors as limited and inconclusive.[67] The report is the world's largest and most authoritative source of scientific research on cancer prevention through diet, nutrition, and physical activity. It is only based on cohort studies, nested case-control studies, and randomized controlled studies, and does not include case-control studies. The WCRF/AICR established that there was probable evidence that drinking water containing arsenic increases the risk of bladder cancer. Further, there was limited evidence that greater consumption of vegetables and fruit, and greater consumption of tea decrease the risk of bladder cancer. This may be due to their constituents, which have antioxidative and possibly antiproliferative properties.

1.3.3.5 Body mass index

Obesity is associated with a small increase in bladder cancer risk, as reported by three meta-analyses.[68–70] The most comprehensive meta-analysis, which included 15 prospective cohort studies, 14.2 million subjects, and 38,072 bladder cancer patients, found that, compared with being normal weight, being overweight (pooled RR: 1.07; 95% CI: 1.01–1.14; I^2=37.6%; $p_{heterogeneity}$=0.029) and being obese (pooled RR: 1.10; 95% CI: 1.06–1.14; I^2=15.5%; $p_{heterogeneity}$=0.241) increased the risk of bladder cancer.[71] For each 5 kg/m^2 increase in body mass index (BMI), the risk of bladder cancer increased in a linear fashion by 4.2% (pooled RR: 1.04; 95% CI: 1.01–1.07; I^2=32.1%; $p_{heterogeneity}$=0.172). In

their report on bladder cancer, based on 16 prospective cohort studies, the WCRF/AICR judged the evidence on an association between obesity and bladder cancer risk as limited and inconclusive.[72] Although results were in the same direction, they were not statistically significant (pooled RR per 5 kg/m^2: 1.03; 95% CI: 0.97–1.09; I^2=55.1%; $p_{heterogeneity}$<0.01).

1.3.3.6 Physical activity

A meta-analysis of five case-control and 10 cohort studies in 5,402,369 subjects and 27,784 bladder cancer patients showed that high versus low levels of physical activity were associated with a decreased risk of bladder cancer (summary RR: 0.85; 95% CI: 0.74–0.98; I^2=83%; $p_{heterogeneity}$<0.001), with similar results for cohort studies (summary RR: 0.89; 95% CI: 0.80–1.00; I^2=64%) and case-control studies (summary RR: 0.71; 95% CI: 0.43–1.16; I^2=87%).[73] In a pooled analysis of 12 prospective US and European cohort studies including 1.44 million subjects and 9,073 bladder cancer patients, the 90th versus the 10th percentile of self-reported leisure-time physical activity was associated with a decreased risk of bladder cancer (pooled hazard ratio [HR]: 0.87; 95% CI: 0.82–0.92; $p_{heterogeneity}$=0.84).[74] The WCRF/AICR's report on bladder cancer, which partly included different cohort studies, reported similar but nonstatistically significant results (summary RR: 0.94; 95% CI: 0.83–1.06; I^2=83%; $p_{heterogeneity}$<0.001), and weighed the evidence on an association between physical activity and bladder cancer risk as limited and inconclusive.[75]

1.3.4 Tertiary prevention of bladder cancer

1.3.4.1 Smoking cessation

A systematic review[76] reported that the majority of studies found that smoking was associated with disease recurrence in urothelial bladder cancer patients treated with transurethral resection of the bladder (TURB), but the evidence on associations with progression, cancer-specific mortality, and all-cause mortality was limited. The evidence on an association between smoking and response to intravesical therapy was also limited. No association between smoking and outcomes was found in patients treated with radical cystectomy. Three of six studies found an association between smoking cessation and a reduced risk of recurrence and progression in urothelial bladder cancer patients treated with TURB. In addition, one of two studies in urothelial bladder cancer patients treated with radical cystectomy linked smoking cessation to a reduced risk of recurrence, cancer-specific mortality, and all-cause mortality.

A meta-analysis of 15 studies including a total of 10,192 patients[77] found an association between current smoking versus never smoking and an increased risk of recurrence (pooled RR: 1.23; 95% CI: 1.05–1.45; I^2=56.3%; $p_{heterogeneity}$=0.004) and cancer-specific mortality (pooled RR: 1.28; 95% CI: 1.07–1.52; I^2=0%; $p_{heterogeneity}$=0.462), but not with progression (pooled RR: 1.11; 95% CI: 0.71–1.75; I^2=55.4%; $p_{heterogeneity}$=0.067). Former smoking versus never smoking was also associated with an increased risk of recurrence (pooled RR: 1.22; 95% CI: 1.09–1.37; I^2=35.2%; $p_{heterogeneity}$=0.087) and cancer-specific mortality (pooled RR: 1.20; 95% CI: 1.03–1.41; I^2=0%; $p_{heterogeneity}$=0.964), but not with progression (pooled RR: 1.16; 95% CI: 0.92–1.46; I^2=0%; $p_{heterogeneity}$=0.853). However, estimates were based on historical cohort studies only, and prospective cohort studies with a longer follow-up are required to confirm these findings.

1.3.4.2 Diet

Four small cohort studies (102–267 patients) investigated the association between dietary factors and bladder cancer outcomes, but only single foods in solitary studies were evaluated. Low versus high prediagnosis dietary intake of vitamin A was associated with a higher recurrence rate per 1,000 person-months (p=0.02), but not with risk of recurrence (RR: 1.34; 95% CI: 0.5–3.34).[78] Ever versus never prediagnosis consumption of beverages and artificial sweeteners was investigated in relation to 5-year overall mortality, which may not have been a very relevant endpoint for bladder cancer. An inverse association with alcoholic beverages was observed (HR: 0.46; 95% CI: 0.26–0.79), whereas no associations with nonalcoholic beverages and artificial sweeteners were found.[79] No associations between postdiagnosis total fluid intake and risk of recurrence were reported.[80] Consumption of ≥1 versus <1 serving/month of raw broccoli was associated with reduced cancer-specific mortality (HR: 0.43; 95% CI: 0.25–0.74), while no associations with total fruits, total vegetables, or individually cooked or raw cruciferous vegetables were found.[81]

1.3.4.3 Body mass index

Twelve historical cohort studies and one prospective cohort study investigated the association between BMI and clinical outcomes of bladder cancer, and yielded inconsistent results. Three studies investigated the association between BMI and risk of recurrence and progression exclusively in NMIBC patients. Kluth *et al.*[82] showed in 892 high-grade T1 patients that a BMI ≥30 versus <30 kg/m^2 was associated with an increased risk of recurrence (HR: 2.66; 95% CI: 2.12–3.32) and progression (HR: 1.49; 95% CI: 1.00–2.21). This was confirmed by Xu *et al.*[83] in 469 NMIBC patients with Ta and T1 tumours. In 338 NMIBC patients with Ta, T1, and Tis tumours, Wyszynski *et al.*[84] found no statistically significantly associations between being overweight or obese compared with being normal weight and risk of recurrence (HR: 1.33; 95% CI: 0.94–1.89), except when analyses were restricted to current smokers (HR: 2.24; 95% CI: 1.15–4.34).

Six studies were performed in NMIBC and MIBC patients combined, and had inconsistent results. Chromecki *et al.*,[85] in a multicentre study of 4,118 patients, found an increased risk of recurrence (HR: 1.67; 95% CI: 1.46–1.91), cancer-specific mortality (HR: 1.43; 95% CI: 1.24–1.66), and overall mortality (HR: 1.81; 95% CI: 1.60–2.05) for obesity versus normal weight. These findings were confirmed by Dabi *et al.*[86] in an institutional cohort of 701 patients. Kwon *et al.*[87] showed a decreased risk of recurrence (HR: 0.52; 95% CI: 0.37–0.73) and cancer-specific mortality (HR: 0.52; 95% CI: 0.37–0.73) for obesity versus normal weight. However, Bachir *et al.*[88] showed no statistically significant associations. Progression was not investigated in any of these studies.

Four studies were conducted exclusively in MIBC patients. Two studies reported a reduced risk of overall mortality with a higher versus lower BMI,[89,90] while the other two studies did not show any statistically significant associations.[91,92]

1.3.4.4 Physical activity

To date, no studies have reported on physical activity in relation to bladder cancer prognosis.

1.3.4.5 Dietary supplements

Fourteen randomized controlled trials investigated the effects of dietary supplements on risk of recurrence: etretinate,[93–95] fenretinide,[96,97] vitamin B6 (pyridoxine),[98,99] vitamin E (tocopherol),[100] multivitamins,[101,102] *Lactobacillus casei*,[103–105] and selenium.[106] The results were inconsistent. One trial was conducted in NMIBC and MIBC patients combined,[107] and all others were conducted in NMIBC patients only.

Supplementation with fenretinide,[108,109] vitamin B6,[110,111] and selenium[112] was found not to affect recurrence. Three studies that evaluated the effects of etretinate had conflicting results, with one small study[113] reporting a lower recurrence rate in the etretinate group versus the placebo group (60% vs. 87%, $p<0.01$) and two larger studies[114,115] not reporting any differences. A small trial in 46 NMIBC patients reported that a daily intake of 400 IU of vitamin E after diagnosis resulted in a lower risk of recurrence (RR: 0.53; 95% CI: 0.11–0.94) compared to the control.[116] Two trials that evaluated the effects of supplementation with a megadose of multivitamins had conflicting results: one trial of 65 NMIBC and MIBC patients found a reduced risk of recurrence in the group that received the recommended daily allowance of a supplement plus a megadose of vitamin A, B6, C, E, and zinc compared to the group that received the supplement alone.[117] In contrast, a large multicentre trial in 670 bacillus Calmette-Guérin (BCG)–naïve patients showed no differences in recurrence-free survival.[118] Two studies reported that oral administration of 3 gram/day of an *L. casei* preparation resulted in a lower recurrence rate (HR: 0.57; 95% CI: 0.35–0.93)[119] or a 1.8 times prolonged recurrence-free survival interval compared to the control group (350 days vs. 195 days, respectively; HR: 2.41; $p=0.028$).[120] The other study found no overall differences in recurrence-free intervals, except when the analysis was restricted to patients with primary multiple tumours or recurrent single tumours (688 days vs. 543 days; HR: 2.58; $p=0.013$).[121]

1.3.4.6 Interventions

Urologists are in a unique position to encourage patients to stop smoking. Patients who receive smoking cessation advice from their urologists have been shown to be 2.3 times more likely to attempt quitting.[122] A study showed that smokers with a new diagnosis of bladder cancer were almost 5.0 times more likely to quit smoking than those in the general population (48% vs. 10%, respectively; $p<0.001$). A diagnosis of bladder cancer and advice from a urologist were the most often cited reasons for quitting.[123]

Urologists should give advice that clearly connects the patient's illness or potential illness with smoking. A 5-minute, brief smoking cessation intervention can be easily incorporated into the daily clinical practice.[124] Brief interventions, along with straightforward smoking cessation advice—for example, "As your urologist, I must advise you that smoking is risky for your health, and it is important that you stop"—have been shown to increase smoking cessation.[125] Failure to address smoking is interpreted as a sign of acceptance by the patient. Optimal smoking cessation counseling can be further individualized and, in some cases, rely on strict collaboration with specialized institutions.[125] Continuing to assist the patient in abstaining from smoking by referral to a smoking cessation clinic, telephone quitting line, psychologist for support, and/or patient support group is often a necessary element to achieve permanent smoking cessation. Furthermore, providing pharmacological smoking cessation therapy, such as nicotine replacement therapy, varenicline, or bupropion, has been proven effective.[125]

1.3.5 Recommendations

Smoking cessation is recommended as a means to reduce the risk of bladder cancer. **[LOE 3; Grade of Recommendation [GOR] C]**

No recommendations can be made for diet, body weight, and physical activity with respect to reducing bladder cancer risk. **[LOE 3; GOR D]**

1.4 Screening and Early Detection

1.4.1 Screening and detection: methodology

Cystoscopy and biopsy/resection remain the primary methods of detecting and documenting the presence of bladder cancer, as per the American Urological Association (AUA)/Society of Urologic Oncology (SUO) 2016 guideline on NMIBC.[126] The use of enhanced cystoscopy (fluorescence or narrow-band imaging [NBI]) in the initial detection of bladder cancer has not been adequately evaluated. While most bladder cancers are initially detected by cystoscopy, imaging (computed tomography [CT], magnetic resonance imaging [MRI], and ultrasound) may initially indicate that bladder cancer is present. The use of cytology and other biomarkers in the setting of initial detection (e.g., evaluating microscopic hematuria) is controversial.[127]

1.4.2 Screening general populations

Bladder cancer is a worldwide problem, ranking number nine on the list of most common cancers.[128] It is also among the most expensive cancers to treat,[129] and a cause of considerable morbidity and mortality. Prompt detection and treatment of non-muscle-invasive disease offer the potential to prevent invasion and metastasis. Interventions with well-tolerated treatments, such as BCG, have been shown to improve survival.[130] These factors, seemingly, make bladder cancer an ideal candidate for screening. However, while a study from the early 1990s suggested the possibility that screening could detect early bladder cancer and possibly improve survival,[131] a more contemporary effort produced a low diagnostic yield, raising doubts about the feasibility of this approach.[132] A report by the US Preventive Services Task Force (USPSTF) in 2011 concluded that there was insufficient evidence to balance the benefits and risks of screening for bladder cancer in asymptomatic adults.[133]

1.4.3 Screening high-risk populations

Screening people who are at increased risk of developing cancer appears to be the solution to the challenges posed in evaluating unselected populations. High-risk populations have been identified, and a risk prediction model for bladder cancer exists.[134] A variety of detection methods exists, as discussed previously, and the risk of overtreatment is relatively low. Screening well-defined groups with occupational exposure to putative carcinogens has resulted in the detection of bladder cancer, with incidences of 0.3% to 1.6%.[135–138] However, contemporary attempts at screening high-risk groups have not focused exclusively on occupational exposure and have yielded a much lower rate of detection.[139] Despite these efforts, there is still no high LOE **[LOE 1, LOE 2]** to recommend screening and early detection of bladder cancer.

While there may be a limited role for screening in well-defined groups with high-risk occupational exposure, there are several factors that may limit the usefulness of bladder cancer screening in other high-risk populations. First, there is high contamination in the generally targeted population (people aged 50 years or older). Many of the individuals in this population will have had urinalyses for other reasons, with the potential for detecting asymptomatic hematuria, which may lead to a diagnosis of bladder cancer.[139] Second, there is no consensus on the best methodology to perform screening. A

variety of tests and strategies have been employed, and current noninvasive testing results in many false-positives. Third, bladder cancer appears to develop along two distinct pathways.[140] Identifying the more common low-risk bladder cancers earlier is not likely to result in improvement in survival, and identifying the high-risk bladder cancers before they become muscle-invasive may not always be possible. This combination of factors is reflected in the recommendations for the screening of bladder cancer (**Table 1–3**).

TABLE 1–3 Recommendations for Bladder Cancer Screening by Major Organizations

Organization	Recommendations/comments
ICUD-EAU International Consultation on Bladder Cancer 2012[141]	▪ There is insufficient evidence on the impact of screening on bladder cancer survival. ▪ Bladder cancer screening may be confined to high-risk patients. ▪ Prevention of bladder cancer includes eliminating active and passive smoking.
NCI (United States)	▪ There is inadequate evidence to determine whether screening for bladder cancer would impact mortality. ▪ There is fair evidence that screening would result in unnecessary procedures with associated morbidity.
ACS	▪ Screening of bladder cancer is not included in their list of recommended cancer screenings.
USPSTF (2011 recommendations)[133,142]	▪ There is no high-quality evidence that screening adults for bladder cancer improves the outcomes compared to no screening. ▪ Current evidence is insufficient to assess the benefits or harms of screening. ▪ If screening is offered, the patient should understand that there is uncertainty about the benefits and harms.

Abbreviations: ACS, American Cancer Society; EAU, European Association of Urology; ICUD, International Consultation on Urological Diseases; NCI, National Cancer Institute; USPSTF, U.S. Preventive Services Task Force.

This *Société Internationale d'Urologie* and International Consultation on Urological Diseases (SIU-ICUD) Joint Consultation does not recommend screening and early detection of bladder cancer in the absence of high-level evidence **[LOE 1, LOE 2]**. This finding underlines the need for more work on this issue. Efforts are under way to "screen smarter."[143,144]

1.4.4 Detection: hematuria

Hematuria is the classic symptom/sign of bladder cancer, but its presence is not rare. The prevalence of microscopic hematuria detected in the general population ranges from 2.4% to 31.1%,[145] and far more benign urological conditions contribute to the finding of hematuria than bladder cancer. The importance of hematuria is very much context-dependent. The incidence of bladder cancer in women under the age of 50 years with microscopic hematuria is low.[146,147] Although there is no consensus on the definition of microscopic hematuria and its evaluation,[127] there are several factors associated with an increased risk of bladder cancer when hematuria is present. These include sex (men over 35 years and women over 50 years), smoking, and gross hematuria.[145–147] Cystoscopy and upper tract imaging are the recommended methods for evaluation.[127] Evaluating microscopic hematuria in high-risk groups with cystoscopy and imaging is recommended. **[LOE 3; GOR B]**

1.4.5 Detection: other

While hematuria is the most common presentation of bladder cancer, incidental detection by imaging and endoscopy does occur.[148–150] In particular, Carcinoma *in situ* (CIS) may present with dysuria and outflow obstruction.[151] Furthermore, patients with bladder cancer who initially present with a urinary tract infection may have a higher-stage disease and increased mortality.[152] The low frequency of bladder cancer in patients exhibiting these alternative presentations makes detection in these circumstances challenging.

1.4.6 Recommendations

The investigation of hematuria should include imaging of the upper tracts. **[LOE 3; GOR B]**

- Bladder cancer screening, if undertaken, should be confined to high-risk patients. **[LOE 3; GOR C]**
- Bladder cancer screening is not recommended for the general population. **[LOE 3; GOR C]**
- Screening can consist of an annual cytology and dipstick. **[LOE 4; GOR C]**

- Urine cytology and cystoscopy should be used for symptomatic or gross hematuria, or in patients with risk factors for urothelial carcinoma. **[LOE 3; GOR B]**

- For patients with asymptomatic microscopic hematuria without risk factors for urothelial carcinoma, urine cytology or cystoscopy can be used. **[LOE 4; GOR D]**

1.5 Endoscopic Examination of the Lower Urinary Tract: Methods, Techniques, Fluorescence, and Optical Advances

1.5.1 Introduction

One of the cornerstones of the diagnosis and management of urothelial carcinoma is cystoscopic examination of the lower urinary tract. This section provides an overview of white-light cystoscopy (WLC), techniques of lower urinary tract endoscopy, and newer optical technologies that can enhance endoscopy, including fluorescence cystoscopy, Raman spectroscopy (RS), and optical coherence tomography (OCT). The evidence presented in this section is level 3 unless stated otherwise, and all recommendations presented in this section are grade C unless stated otherwise.

1.5.2 White-light cystoscopy

1.5.2.1 Introduction

White-light endoscopic examination of both the urethra and the bladder remains the gold standard for screening and diagnosis of multiple diseases of the lower urinary tract, including urothelial carcinoma. Cystoscopy not only permits visualization of the bladder urothelium but also affords access to the ureteral orifices to facilitate assessment and treatment of the upper urinary tract.

Cystoscopy can be performed utilizing either rigid or flexible endoscopes, depending on the clinical circumstances. The standard calibre measurement for all endoscopes is based on the French (Fr) scale, in which 1 Fr equals 0.33 mm (e.g., a 12-Fr endoscope has a diameter of 4 mm). Endoscopes from 8 to 12 Fr are typically used for pediatric patients, and endoscopes from 16 to 28 Fr are typically used for adult patients.

1.5.2.2 Rigid cystoscopy

For screening and diagnostic work, most often, sheaths of 20 to 22 Fr are used for adult patients, and smaller sizes are used for pediatric patients. For suspected urethral neoplasms, a 0-degree lens is useful. For examination of the prostatic urethra, the bladder trigone, and the bladder wall, except the part immediately adjacent to the bladder neck, a 30-degree lens is generally used. For more acute or difficult angles, particularly at the anterior bladder neck, a 70-degree or 90-degree lens may be employed; however, in such cases, use of adjunctive implements, such as catheters or biopsy forceps, is typically not possible without specialized deflecting bridge adaptors.

The large bore of rigid cystoscopes typically allows for excellent irrigation flow and visualization, even when there is mild to moderate bloody urine or debris. The bore also provides a port that can accommodate a variety of instruments. However, because of their large size and rigid nature, rigid cystoscopes typically cannot be used effectively in the office. Most often, particularly in men, rigid

cystoscopes are used in an operating room with the patient under general or some form of regional anesthesia. In addition, for rigid cystoscopes to be used effectively, the patient must be placed in a dorsal lithotomy position, and in the occasional patient, this may pose difficulties.

1.5.2.3 Flexible cystoscopy

Rigid cystoscopy was the standard of care in urology for many years, but starting in the early 1970s, the advent of better fibre-optic technologies permitted the development of flexible instruments that could be used more easily than rigid cystoscopes in the office. The first published use of a flexible fibre cystoscope for examination of the bladder neck was by Tsuchida and Sugawara in 1973.[153] Their report was followed over a decade later, in 1984, by the development of the first commercial flexible instrument built specifically for cystoscopy. Since that time, the use of flexible fibre-optic cystoscopy has increased rapidly, and flexible cystoscopy is now the standard method for diagnosis and surveillance of a variety of lower urinary tract disorders, including urothelial carcinoma. The fact that flexible cystoscopy can generally be performed without anesthesia has led to widespread acceptance of this procedure—it is now the most common office-based procedure performed by urologists in developed countries such as the United States. Over time, manufacturers have developed improved optics, permitting smaller-calibre scopes while maintaining high image quality. Additionally, the development of a working channel allowed for the use of flexible instruments. This, coupled with the advent of active deflection, has permitted a range of office-based procedures to, potentially, be done without the need for general anesthesia. In addition to decreased discomfort, the other advantage of flexible cystoscopy is the use of active deflection to improve visualization of the anterior bladder neck.

Despite the advantages and widespread use, flexible cystoscopes have some disadvantages compared to rigid endoscopes, such as the small irrigation port and lack of a separate working sheath, which limit the ability to irrigate and use instruments simultaneously. Typically, only the smallest of tumours can be ablated or biopsied using only flexible endoscopy. Finally, flexible endoscopes are, typically, more costly and more prone to damage than rigid endoscopes if not handled properly, such as during cleaning and sterilization between cases.

The flexible fibre-optic cystoscopes commonly in use today also have some optical disadvantages compared to rigid endoscopes. These disadvantages are due to the inherent imaging limitations of fibre-optic technology. The diameter of the glass fibres carrying the image is finite. This results in the appearance of a pixelated or "screen door" image.[154] The shaft of a flexible cystoscope typically has three fibre-optic bundles: two carry the light from the generating source, and the third carries the image back to the eyepiece. As a consequence, the image obtained is actually a composite matrix of each of the individual fibres in the bundle. This creates an image in which the individual dots in the matrix have merged into one image, analogous to what is seen in newspaper images. This is an inherent limitation of fibre-optic technology, but digital endoscopy, as discussed in the next section, overcomes these limitations.

1.5.2.4 Advances in white-light cystoscopy

Video cameras as an adjunct to endoscopy were first introduced by French researchers in 1956.[154,155] This concept has led to improved ergonomics, improved safety through avoidance of contact with body fluids, enhanced patient and resident education, and improved documentation of findings and sharing of information among physicians through the use of digital cameras and recording devices.[155]

One study showed that male patients who were able to monitor their cystoscopy by watching the procedure on a video monitor experienced up to 40% less pain and discomfort during endoscopy compared to patients who did not watch their procedure on a video monitor.[156]

Perhaps the biggest recent advance in WLC has been the development of digital endoscopy. With this method, light from the generating source still travels through the traditional fibre-optic imaging bundle. However, the image is no longer carried back along the fibre-optic bundle to the eyepiece. Instead, there is a digital sensor at the very tip of the endoscope that is based on a charge-coupled device and complementary metal oxide semiconductor chips. The image is acquired through the digital sensor through millions of photodiodes. The photodiodes convert the photons of light into an electric current. Subsequently, the current is transformed into voltage, and then amplified and converted to a digital format.[154] The semiconductor chips transfer the information to a receiver, which then presents the image on a monitor and/or stores it digitally.

Digital endoscopy offers the promise of improved optical resolution, contrast, and colour differentiation. It may be more durable than traditional flexible endoscopy. *Ex vivo* studies have suggested that distal endoscopes are superior to more traditional fibre-optic cystoscopes in terms of resolution, contrast discrimination, and red-colour differentiation.[157]

First introduced commercially in 2005, digital endoscopes have been studied, to a limited degree, in the clinical setting. Quayle *et al.*[158] found that digital sensor–based flexible scopes had better optics than fibre-optic cystoscopes. Okhunov *et al.* conducted a prospective clinical trial comparing digital cystoscopes with fibre-optic cystoscopes for office-based flexible cystoscopy in over 1,000 patients.[159] These authors reported that operating surgeons found that the digital cystoscopes were lighter and easier to manoeuvre. There was better deflection with the instruments placed in the working channels of the digital endoscopes, without apparent loss of instrument functionality. Both types of endoscopes were found to be relatively durable; both types had a 0.2% repair rate over the course of the study. It should be emphasized that digital endoscopes are often more expensive, so whether they will ultimately prove to be cost-effective remains to be seen. The development of digital endoscopes has, however, permitted the development of other promising technologies, which will be discussed later in this chapter, such as NBI.

1.5.2.5 Conclusions

WLC, whether performed in the office utilizing flexible instruments or performed in the operating room using rigid instruments, is the standard approach for the diagnosis and management of lower urinary tract diseases, including urothelial carcinoma, and is the gold standard against which other approaches must be compared.

1.5.3 Photodynamic diagnosis of bladder tumours

1.5.3.1 Principles of photodynamic diagnosis

5-Aminolevulinic acid (5-ALA) is the building block of heme, a key molecule in the mitochondrial functions of normal cells that is also crucial in cancer bioenergetics.[160] Upon administration of 5-ALA, cancer cells, which exhibit distinct alterations in 5-ALA transport and heme synthesis,[161]

accumulate the direct precursor of heme, protoporphyrin IX (PPIX). PPIX shows red fluorescence under blue-light excitation,[162] which, due to its accumulation in solid tumours, led to laser-based tissue characterization[163] and photodynamic diagnosis (PDD).

However, while it is observed in most solid tumours,[164] PPIX fluorescence is not cancer-specific. It can be the consequence of the summation of ancillary PPIX synthesis in normal cells or the accumulation of PPIX in normal cells lacking one or the other of the transporters or enzymes involved in heme synthesis (e.g., neutrophils). Therefore, it must be understood that PPIX is evocative but not specific to cancer, and it must be analyzed comparatively to the adjacent tissue.

1.5.3.2 Practical considerations

1.5.3.2.1 Source of 5-aminolevulinic acid

Early experiences validated the clinical applicability of 5-ALA intravesical instillations in the PDD of bladder cancer.[165] However, individual variability in the intensity of the signals and photobleaching, as well as the desire to ensure high levels of accumulation of PPIX, which is also a potent photosensitizer in photodynamic treatment, spurred research on derivatives with better lipid solubility and bioavailability.[166] Ester derivatives were synthesized and shown to accelerate and regularize PPIX accumulation, and to optimize urothelial cell necrosis upon illumination.[166] One of these derivatives, was selected by Photocure ASA (Norway) to undergo the full process of approval by the UK National Health Service (NHS; first approved on September 9, 2004), the European Medicines Agency (EMA; Hexvix®), and the US Food and Drug Administration (FDA; Cysview® in combination with the Karl Storz D-Light C Photodynamic Diagnosis system; first approved on May 28, 2010). A user-friendly, prefilled syringe was recently approved and released in Europe and the United States.

1.5.3.2.2 Hexyl aminolevulinic acid and photodynamic diagnosis workflow

The bladder is transiently catheterized before instillation of a 50-mL bolus containing either 85 mg of hexyl aminolevulinic acid (HAL) in the European formulation or 100 mg in the US formulation. The drug should be retained for 1 hour to allow uptake by the mucosa and cellular processing to PPIX. Given the kinetics of uptake and accumulation, there is no clear advantage in keeping the drug longer than 1 hour, so patients are allowed to empty their bladder thereafter. On the other hand, while the instillation is painless, patients with storage phase symptoms may find it hard to keep the drug for a full hour. Although the efficacy of HAL has not been established when the solution is retained for less than 1 hour, a contact of 15 to 30 minutes is usually sufficient to elicit a detectable signal. In clinical trials, HAL instillations were well tolerated, with most side effects related to the procedure rather than the drug.[167] A single case report described the occurrence of nonimmunoglobulin E–mediated anaphylactic shock at the end of fluorescence cystoscopy and transurethral resection of the prostate, although the relationship with HAL was not clearly established.[168] No similar reports have described this ever since. Due to its mechanism of action, HAL should not be used in patients with porphyria or a family history of porphyria, and when gross bleeding may result in exposure of the greater circulation to the drug.

Of note, while HAL is the only drug validated for bladder PDD, a large wealth of experience is also available on 5-ALA, which preceded HAL.[169,170] 5-ALA has the distinct advantage of being amenable not only to bladder instillations,[169] but also, as shown by central nervous system tumour research,[164,171] to oral or intravenous (IV) treatments. The latter might reduce the hassle of preoperative bladder catheterization and open the way to upper tract PDD.[172]

1.5.3.2.3 Imaging equipment required for hexyl aminolevulinic acid and photodynamic diagnosis

PDD equipment addresses four separate technical challenges: (1) the ability to conduct an optimal white-light examination, (2) the excitation of light of adequate intensity and wavelength (405 nm), (3) the capture and optimization of the output image, and (4) the transmission to the monitor's screen.

Research on the source lights and cables (e.g., Argon lamps, light-emitting diodes, and gel cables), the telescopes, the camera heads, and the monitors (high-definition monitors and 4K resolution) is thriving. Proprietary improvements in the technology setup are continuously being introduced by the three main manufacturers for operative endoscopy equipment in order to optimize the vision and increase the contrast between the weak red signal elicited by PPIX accumulation (main emission at 635 nm, secondary emission at 690 nm[166]) and the blue background of normal mucosa.[173] Of note, PDD is now available for both rigid and flexible endoscopy. In addition, continuous efforts are being made by all three manufacturers to develop trimodality systems that would combine high-definition white-light imaging, PDD, and either NBI (Olympus) or augmented reality (e.g., Storz Professional Image Enhancement System [SPIES]).

1.5.3.3 Evidence in support of photodynamic diagnosis

Meta-analyses are available on 5-ALA[174] and on HAL PDD[170,175] (**Table 1–4**). The results can be analyzed according to two perspectives: the research of additional tumours in patients known to have cancer using white-light examination (detection rate) or the analysis of the consequences for patients of PDD (residual tumour rate, recurrence at check cystoscopy, recurrence-free survival, and progression-free survival).

TABLE 1–4 Summary of Five Meta-Analyses Comparing Photodynamic Diagnosis With White-light Cystoscopy

Reference	Comments	Reports (participants)	Comparison vs. WLC
Kausch *et al.*,[170] 2010	5-ALA and HAL	*n*=18 (1,616)	▪ Additional detection rate: 20% (95% CI: 8–35) ▪ Additional detection rate of CIS: 39% (95% CI: 23–57) ▪ Residual tumour rate: 28% (95% CI: 15%–52%)
Mowatt *et al.*,[174] 2011	5-ALA and HAL	*n*=27 (2,949)	▪ Improved sensitivity: 92% (95% CI: 80–100) vs. 71% (95% CI: 49–93) ▪ Lower specificity: 57% (95% CI: 36–79) vs. 72% (95% CI: 47–96) ▪ Reduced risk of residual tumours at check cystoscopy: RR: 37% (95% CI: 20%–69%) ▪ Longer recurrence-free survival: RR: 137% (95% CI: 118–159)
Shen *et al.*,[176] 2012	5-ALA and HAL	*n*=14 (4,078)	▪ No significant differences in tumour detection rate for patient perspective: RR: 99% (95% CI: 96–103) ▪ No significant differences in CIS detection rate for patient perspective: RR: 82% (95% CI: 67–102) ▪ Higher rate of false-positives: RR: 69% (95% CI: 49–97) ▪ Reduced risk of residual tumours at protocol re-resection: RR: 36% (95% CI: 19%–68%)
Rink *et al.*,[167] 2013	5-ALA and HAL	*n*= 44 (8,740)	▪ Additional detection rate of papillary tumours (range): pTa: 8.6%–29%; pT1: 7%-25% ▪ Additional detection rate of CIS (range): 16%–76% ▪ Reduced risk of residual tumours: 20%
Burger *et al.*,[175] 2013	HAL, based on raw data	*n*=9 (1,345)	▪ Additional detection rate of Ta lesions: >14.7% ▪ Additional detection of CIS: >40.8% ▪ Reduced 12-month recurrence rate: 34.5% vs. 45.4%

Abbreviations: 5-ALA, 5-aminolevulinic acid; CI, confidence interval; CIS, carcinoma in situ; HAL, hexyl aminolevulinic acid; RR, relative risk; WLC, white-light cystoscopy.

PubMed® search keywords: photodynamic diagnosis, bladder cancer, meta-analysis, English language.

Note that similar series might be included in different reports.

Regarding the individual tumour perspective, all meta-analyses consistently pointed out a strong improvement in detection, notably for CIS,[167,170,175] compared to white-light imaging, albeit at the cost of reduced specificity.

Indeed, red fluorescence is not tantamount to cancer, and false-positive results may impair the surgeon's confidence in PDD. This is mostly true at the beginning of one's experience and can be addressed with information on the classic pitfalls of the technique, such as tangential view of the bladder neck and trigone, mucosal folds, ureteral orifices, and diverticula. Introductory courses in reference centres and reports on the typical situations with false-positive lesions should also be promoted to address the enthusiasm of neophytes (**Figure 1–3**). Most false-positive results relate to flat lesions, which are among the most demanding in uropathology.[177] Carcinoma *in situ* is typically revealed by a flat, well-demarcated, PDD-positive area, whose mucosa is easily detached as a pink veil by gently stroking the mucosa with the resection loop.[162] The proposed classification[178] for CIS and immunohistochemistry by McKenny and colleagues is of help when facing rare forms with denuded mucosa or undermining the growth of cancer cells under normal umbrella cells (**Figure 1–4**). Other PDD-positive, flat entities of weak intensity, such as urothelial dysplasia or flat urothelial hyperplasia,[179] that may share genetic alterations with papillary tumours in the vicinity[180] should also be described, and not simply reported as negative.

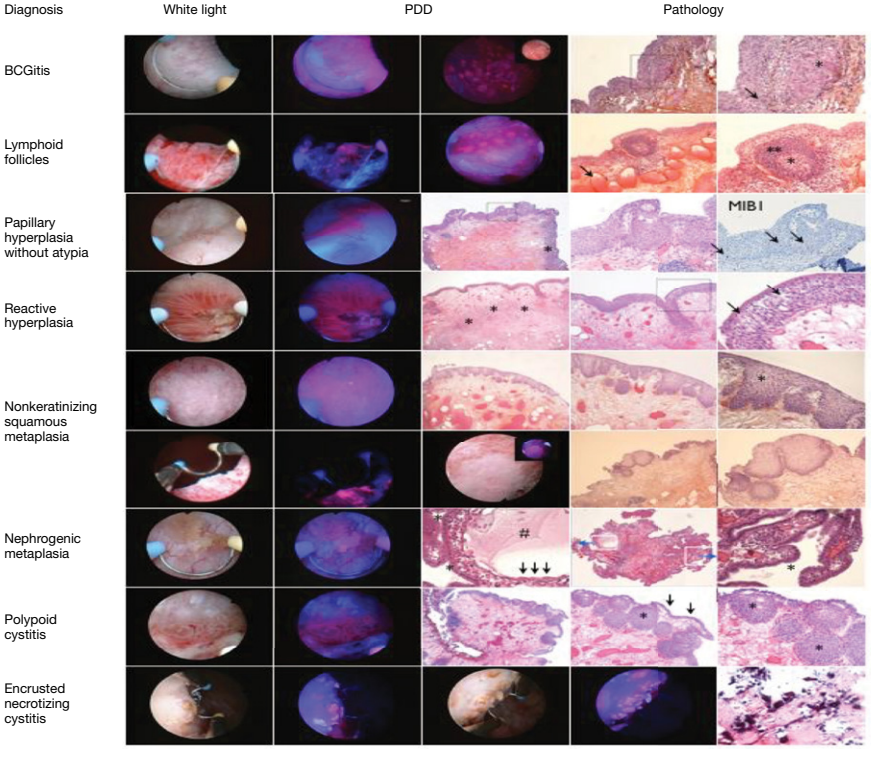


FIGURE 1–3
Representative Cases of Photodynamic Diagnosis–Positive Noncancerous Lesions

Abbreviations:
BCG, bacillus Calmette-Guérin; PDD, photodynamic diagnosis.

Source: Sections and pathology descriptions are courtesy of Dr. Catherine Mazerolles.

BCGitis

Case is a 45-year-old female with a history of pT1aG3 (WHO, 1973) high-grade (WHO, 2004) tumours. Control after six BCG courses shows on WLC the typical cobblestone-like aspect with distinct flat areas separated by discrete folds. PDD shows intense and well-delineated fluorescent areas.

Pathology: Similar organization with slightly undulated mucosa and discrete exocytosis characterized by few lymphocytes (arrow) in the basal layers of the urothelium is shown. Typical, noncaseating epithelioid granuloma (box) with numerous giant cells (*) is evidenced in the superficial lamina propria and surrounded by densified connective tissue.

Lymphoid follicles

Case is a 38-year-old male with neurogenic bladder (spina bifida) who underwent clean intermittent self-catheterization of the bladder. Follow-up with WLC shows a typical "Christmas tree" neurogenic bladder with raised white lesions (1 mm) on the bladder neck, and anterior and lateral walls. The lesions are intensely fluorescent on PDD, giving a "panther fur" appearance.

Pathology: Note the numerous congestive veins (arrow) at low-power magnification, which is consistent with intense inflammation. Lymphoid follicles located underneath a thin epithelial layer are composed of a germinal centre characterized by clear immature cells (*), surrounded by the mantle zone with densely packed small lymphocytes (**).

Papillary hyperplasia without atypia

Case is a 65-year-old male with a history of pTaG1 NMIBC. WLC shows a rough, irregular area above the left ureteral orifice, with intense fluorescence on PDD.

Pathology: Undulating folds of urothelium (arrow), with no suggestion of papillary cancer, such as significant cytological atypia or well-developed branching fibrovascular cores, are shown. As evidenced by MIB1, cycling cells are few and restricted to the basal layers (arrows), confirming the diagnosis of papillary hyperplasia without atypia. Note that there is minimal coagulation necrosis (*), which is in line with the use of bipolar resection.

Reactive hyperplasia

Case is a 72-year-old female. Second-look resection 6 weeks after TURB for pT1G3 shows converging folds of edematous mucosa that are fluorescent on PDD.

Pathology: Low-power magnification shows intense edema in the superficial lamina propria, with numerous congestive capillaries and resorptive granuloma subsequent to prior TURB (*). High power shows mucosal hyperplasia with more than seven layers of urothelial cells that are dissociated by extracellular edema (spongiosis; arrow).

Nonkeratinizing squamous metaplasia (top)

Case is a 42-year-old woman with a whitish and well-delineated area of the trigone on WLC. Note the congestive aspect of the adjacent mucosa. PDD shows a faint and inhomogeneous fluorescence.

Pathology: Low-power magnification evidences foci of nonkeratinizing squamous metaplasia set on a congestive and edematous lamina propria. High power highlights clear cells with abundant intracytoplasmic glycogen (*) and lack of keratinization, similar to the vaginal and cervical squamous epithelium. This condition is related to estrogen exposure and often observed in women, most typically in the trigone and bladder neck areas.

Nonkeratinizing squamous metaplasia (bottom)

Case is a 53-year-old male with a history of intermediate-risk NMIBC treated by TURB and BCG. Control cystoscopy 3 years after the last recurrence evidences slightly raised islets (1–2 mm) that show highly fluorescent spots on PDD.

Pathology: Typical nonkeratinizing squamous metaplasia is also observed in chronic irritation, such as stones, nonfunctioning bladder, or, as shown here, after BCG.

Nephrogenic metaplasia

Case is an 86-year-old male with a history of recurrent, multifocal pTaG3 (WHO, 1973) high-grade (WHO, 2004) tumours and control cystoscopy during BCG maintenance.

Pathology: Low power shows small finger-like papillary fronds and tubular structures that extend within the lamina propria, where they can be dilated with protein material (#). High power shows the hallmark nephrogenic metaplasia—the presence of cuboidal (*) or hobnail (arrow) cells with clear or eosinophilic cytoplasm.

Polypoid cystitis

Case is a follow-up cystoscopy of a 73-year-old male with a history of pT1G3 (WHO, 1973) high-grade (WHO, 2004) NMIBC treated conservatively. It shows, on the right lateral wall, a sessile lesion composed of distinct round-shaped units that are slightly fluorescent.

Pathology: Low power shows that the mucosal surface assumes a polypoid contour, although without well-defined papillary fronds. Note the extensive edema of the lamina propria, with congestive, branched capillaries. High power shows the urothelial layer is thin (three cell layers; arrows) with numerous Von Brunn nests (*), which contribute to the polypoid appearance observed at endoscopy.

Encrusted necrotizing cystitis

Case is a 67-year-old with a history of external beam radiation therapy of the prostate (72 Gy). NMIBC was detected 4 years after, and treated by TURB and adjuvant mitomycin C. Patient complains of recurrent urinary tract infections and presents with suspicious urine cytology. Cystoscopy shows dense and slightly exophytic fluorescent foci set on an atrophic mucosa.

Pathology: Due to extensive calcification, sectioning is not optimal. Calcifications are set on necrotic and fibrotic tissues, and the urothelium is not observed.

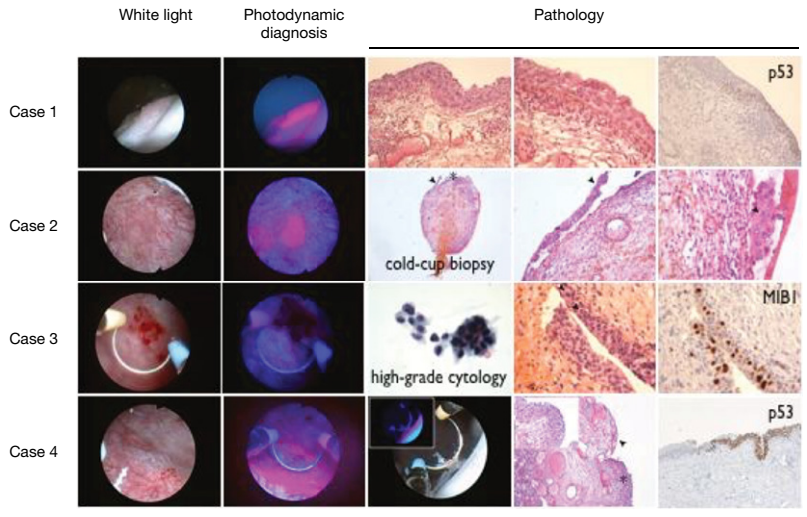


FIGURE 1–4
Representative Illustrations of Different Forms of Carcinoma *In Situ*

Abbreviations:
BCG, bacillus Calmette-Guérin; PDD, photodynamic diagnosis.

Source: Sections and pathology descriptions are courtesy of Dr. Catherine Mazerolles.

Case 1

Case shows a discrete area that is slightly raised and intensely fluorescent in the vicinity of a single exophytic high-grade lesion (pT1HG, not shown).

Pathology: Pleiomorphic cells with hyperchromatic nuclei are found underneath the most superficial layer of the mucosa that retains terminal differentiation (arrowhead), as reported in the classification by McKenny and colleagues[178] as an "undermining" variant of CIS. Note the intense p53 staining in this contingent.

Case 2

Case shows a slightly uneven surface on WLC and is intensely fluorescent on PDD.

Pathology: Low power is evocative of the erosive form of CIS, as the urothelial layer is detached (arrowhead) or denuded (*). At high power, large pleomorphic cells with abundant cytoplasm are grouped in clusters and remain attached to the basal layer (arrowhead).

Case 3

Case is a 71-year-old male who received external beam radiation therapy for prostate cancer 7 years ago and has positive cytology with no evidence of disease on WLC. PDD shows a faint irregular signal on the right bladder wall.

Pathology: The urothelial layer is thin and disorganized. Note the presence of large proliferative cells with prominent nuclei (arrowhead). Proliferative cells (MIB1) are interspersed between normal cells in a pagetoid variant of CIS.

Case 4

Case is a 70-year-old male with a history of pT1G3 treated by BCG and maintenance therapy. Positive cytology but unevocative WLC spurred two successive random biopsies that failed to show any significant abnormalities. PDD shows three large flat and fluorescent areas. The detached urothelium was intensely fluorescent (inset).

Pathology: Low power shows denuded areas (arrowhead), when present (*). The urothelium is normal-looking. High power (box) shows faint abnormalities in the guise of slight architectural disorganization and the presence of large nuclei that were not sufficient to characterize CIS. However, intense and discrete p53 staining confirms malignancy.

A meta-analysis by Shen *et al.*[176] failed to demonstrate any differences in detection, although PDD improved the quality of resection, as shown by reduction of the residual tumour rate. Improvements in the 12-month recurrence rate[175] and longer recurrence-free survival[174] attest to the improvement in the quality of resection and better detection.

A distinct meta-analysis of 11 randomized controlled trials addressed specifically the question of the therapeutic outcome after PDD-assisted transurethral resection of bladder tumour (TURBT), confirming the value of 5-ALA and HAL PDD in the prevention of recurrence. Intriguingly, the meta-analysis also suggested better prevention of recurrence after resection with 5-ALA compared to HAL.[181] No improvements were observed in the progression rate.

With respect to cost, several simulations were modeled from the results of the literature. Even when the strong national variations that exist in health economics were taken into account, PDD at the time of TURBT was the dominant option, leading to significant savings over time,[182–184] despite the need for investment in a specialized telescope, light source, and camera head.

1.5.3.4 Conclusions

Although not cancer-specific, PDD is a robust adjunct to WLC in the detection of papillary and nonpapillary forms of cancer, improving the quality of resection and recurrence-free survival. Better detection comes at the cost of lower specificity, which can be controlled by training and experience.

The limitations pertain to the logistics of HAL instillation into the bladder and the use of a dedicated endoscopy system (light source, telescope, and camera head).

1.5.4 Narrow-band imaging of bladder tumours

1.5.4.1 Principles of narrow-band imaging

For NBI, modified optical filters are used in the light source of a video endoscope system to narrow the bandwidth of spectral transmittance. NBI enhances the differences in penetration depth between wavelengths. Light penetration depth within tissue is highly dependent on the wavelength: shorter wavelengths produce only superficial penetration, and longer wavelengths produce deeper penetration. Blue light, therefore, penetrates superficially, while red light penetrates deeply.[185] NBI narrows the bandwidth of light output from the endoscope system to between 415 and 540 nm. The relative intensities of blue and green light are increased, while the intensity of red light is decreased to a minimum. The narrow bandwidth of green and blue light is strongly absorbed by hemoglobin, so NBI enhances the visibility of surface capillaries and blood vessels in the submucosa, without the use of dyes **(Figure 1–5)**.

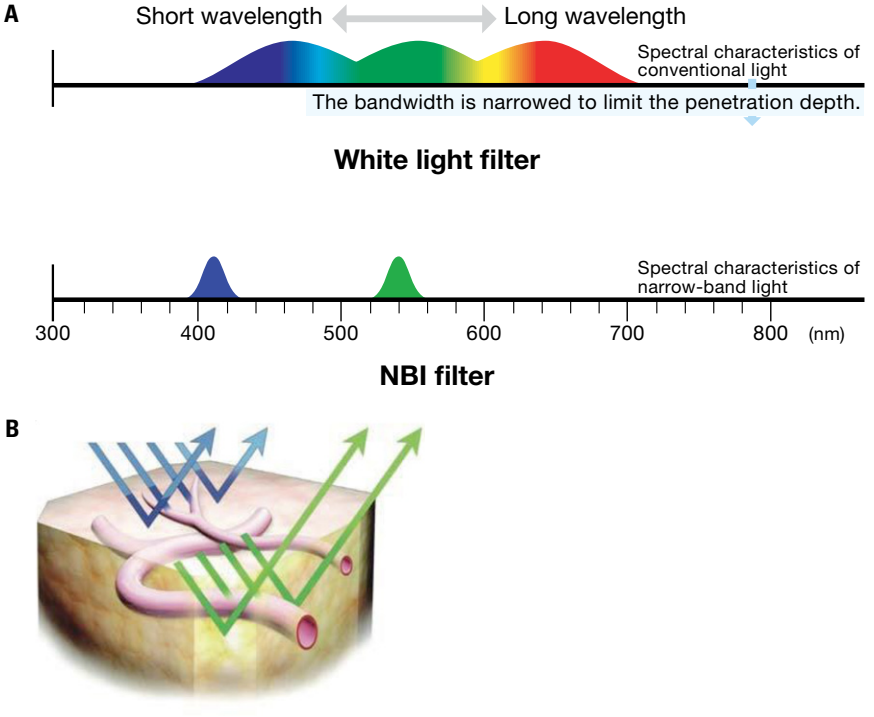


FIGURE 1–5
Representation of (a) the Narrowed Bandwidth of Light and (b) Its Penetration of Tissue[270]

Source: Watanabe A, Fujita M. Case Study of NBI (Specific Wavelength Light) Endoscopy. Vol 1. Japan; Olympus Medical Systems Corporation:1–16.

NBI cystoscopy is easy for surgeons to adopt, and it has a high sensitivity in detecting small papillary and otherwise undetectable cancers, especially CIS. NBI was initially shown to be useful in gastrointestinal diseases, particularly in the detection of adenomas during colonoscopy and in the follow-up of Barrett esophagus.[186] The first report of NBI in bladder cancer was published by Bryan *et al.* in 2008.[187] These authors performed WLC and subsequent NBI flexible cystoscopy to detect bladder cancer in 29 patients with recurrent NMIBC. NBI flexible cystoscopy provided much better visualization of bladder cancer than conventional WLC flexible cystoscopy. NBI cystoscopy revealed 15 additional tumours (in 12 patients) not detected by WLC. However, these additional tumours were not confirmed histopathologically because all the tumours were treated by diathermy ablation following cystoscopic examination. With NBI cystoscopy, the vasculature appears dark green or black against the almost white, normal urothelium, whereas with WLC, tumours appear red in a background of pink, normal urothelium **(Figure 1–5)**.

1.5.4.2 Practical considerations

Systems that have integrated NBI and WLC are now commercially available for use with both rigid and flexible endoscopes. One of the practical advantages of NBI is that, with the push of a button, the NBI mode is activated by mechanical insertion of a narrow-band filter in front of the white-light source. This obviates the need for instillation of any fluorophores, which is required for PDD. Several small single-centre series have suggested that there is a relatively short learning curve with the use of NBI. Herr *et al.* demonstrated that both experienced and relatively inexperienced urologists quickly learned NBI cystoscopy as a supplement to WLC.[188] That is, the use of NBI cystoscopy to detect and characterize bladder tumours was easy to learn, and it was fairly quick to apply to the clinical routine of urologists. An observation that was also demonstrated in a study by Bryan *et al.*[189]

1.5.4.3 Evidence in support of narrow-band imaging

A large number of studies have now been published comparing NBI with WLC in the detection of bladder cancer. Sensitivity and specificity rates for NBI cystoscopy and WLC in patients with NMIBC and CIS are presented in **Table 1–5**. Herr *et al.* described a series of 427 patients who underwent follow-up with WLC and NBI cystoscopy, 103 of whom had tumour recurrence.[190] Ninety patients (sensitivity: 87%) had their disease diagnosed by WLC, and the other 13 patients (sensitivity: 100%) had their disease detected only by NBI cystoscopy. This included eight patients with CIS, four patients with Ta disease, and one patient with T1 disease. NBI cystoscopy performed better than WLC in demarcating the margins of CIS lesions from the surrounding normal-appearing mucosa. In another series, Tatsugami *et al.* performed WLC and then NBI cystoscopy in 104 consecutive patients.[185] In 39 (26.9%) of 161 suspicious tumour sites, bladder tumours were identified only by NBI. The tumours were CIS in 25 patients, Ta tumours in 12 patients, and T1 tumours in two patients. In this entire series, 14 of 30 patients with CIS had their disease detected only by NBI. However, the aforementioned studies were subject to potential observer bias since WLC and NBI cystoscopy were performed by the same urologist. To mitigate this, Cauberg *et al.* conducted a trial in which WLC and NBI cystoscopy were performed by different surgeons in 95 patients.[191] Seventy-eight patients had histopathologically confirmed NMIBC. NBI identified additional tumours in 28 of those 78 patients. In contrast, WLC identified additional tumours in only three of those 78 patients.

TABLE 1–5 Comparison of Sensitivity and Specificity Between Narrow-band Imaging and White-light Cystoscopy for Non-Muscle-Invasive Bladder Cancer

Reference	Number of patients	NBI		WLC	
		Sensitivity	Specificity	Sensitivity	Specificity
Tatsugami *et al.*[185]	104	92.7	70.9	57.3	86.2
Herr *et al.*[190]	427	100.0	82.0	87.0	85.0
Cauberg *et al.*[191]	95	94.7	68.4	79.2	75.5
Ye *et al.*[192]	384	98.8	60.9	75.4	58.6
Drejer *et al.*[193]	955	100.0	86.5	83.1	92.1

Abbreviations: NBI, narrow-band imaging; WLC, white-light cystoscopy.

Possible limitations of these published studies are their monocentric nature and possible observer bias since WLC and NBI were performed sequentially and observed by the same urologist. In addition, they were not randomized. A study by Geavlete *et al.* randomized 220 patients at a single institution to undergo WLC and standard monopolar TURBT versus a combination of WLC and NBI endoscopy plus resection utilizing bipolar plasma vaporization.[194] While this was only a single-centre study, it did demonstrate that NBI had a higher detection rate of cancer (95% vs. 84%), with a nonsignificant increase in the false-positive rate (14% vs. 12%). While the recurrence rates for the combined NBI and bipolar plasma vaporization were lower, it was not possible to determine whether this was driven by the use of NBI or the bipolar plasma vaporization resection technique. More recently, Ye *et al.* conducted a study designed to compare the rate of detection of NMIBC by NBI cystoscopy versus WLC in a multicentre setting, using a randomized sequence of the two procedures. Three hundred and eighty-four consecutive patients from eight academic centres in China were included in this prospective, multicentre, phase 3 trial.[192] One hundred and three patients had confirmed bladder tumours. NBI had a higher sensitivity compared to WLC (98.8 vs. 75.4%, respectively), but a comparable specificity (60.9% vs. 58.6%, respectively) and positive predictive value (76.0% vs. 69.6%, respectively). These studies, as well as others discussed later, focused on the potential of NBI to reduce the risk of recurrence and consistently showed that NBI has improved sensitivity compared to WLC. Three recent meta-analyses have also been conducted and reached the same fundamental conclusion.[195–197]

NBI technology can be used for rigid cystoscopy and bladder tumour resection. One feasibility report of TURBT performed with NBI cystoscopy indicated shorter operative times, shorter time to catheter removal, and shorter time to hospital discharge with NBI cystoscopy versus WLC, although the differences were not statistically significant.[198] The authors speculated that the use of NBI cystoscopy for TURBT may substantially reduce the recurrence rate of bladder tumours. It is important to note, however, that the observer in this trial was allowed to take a "second look" with the alternative form of imaging, which may have introduced bias.

Two trials have evaluated the ability of NBI to detect residual cancer in patients with high-risk NMIBC after initial endoscopic resection. In a series of 47 patients, all patients were examined with WLC and NBI cystoscopy, and underwent a second TURBT procedure approximately 1 month after

the initial TURBT.[199] Overall, 16 of 47 patients were discovered to have residual or recurrent cancer, including six patients with high-grade cancers detected only by NBI. In another series, 61 patients were evaluated using both WLC and NBI cystoscopy 3 months after beginning induction therapy with BCG. NBI correctly identified 21 of 22 cases of residual cancer.[200]

Another trial compared the usefulness of WLC and NBI cystoscopy in monitoring disease recurrence during follow-up.[201] Patients were followed up with WLC for 3 years and with NBI cystoscopy for the next 3 years. The trial revealed fewer tumour recurrences, a smaller number of recurrent tumours, and longer recurrence-free survival times with NBI cystoscopy. Small solitary papillary tumours and clusters of papillary tumours were more efficiently treated with NBI cystoscopy than with WLC. However, only patients with frequently recurring tumours were included in this study. Hence, the authors were unable to discriminate between the natural history of the disease and the influence of NBI cystoscopy on outcomes. With longer follow-up periods, tumours generally recur less frequently,[202] and so more trials with longer follow-ups are needed to confirm this trial' s conclusions.

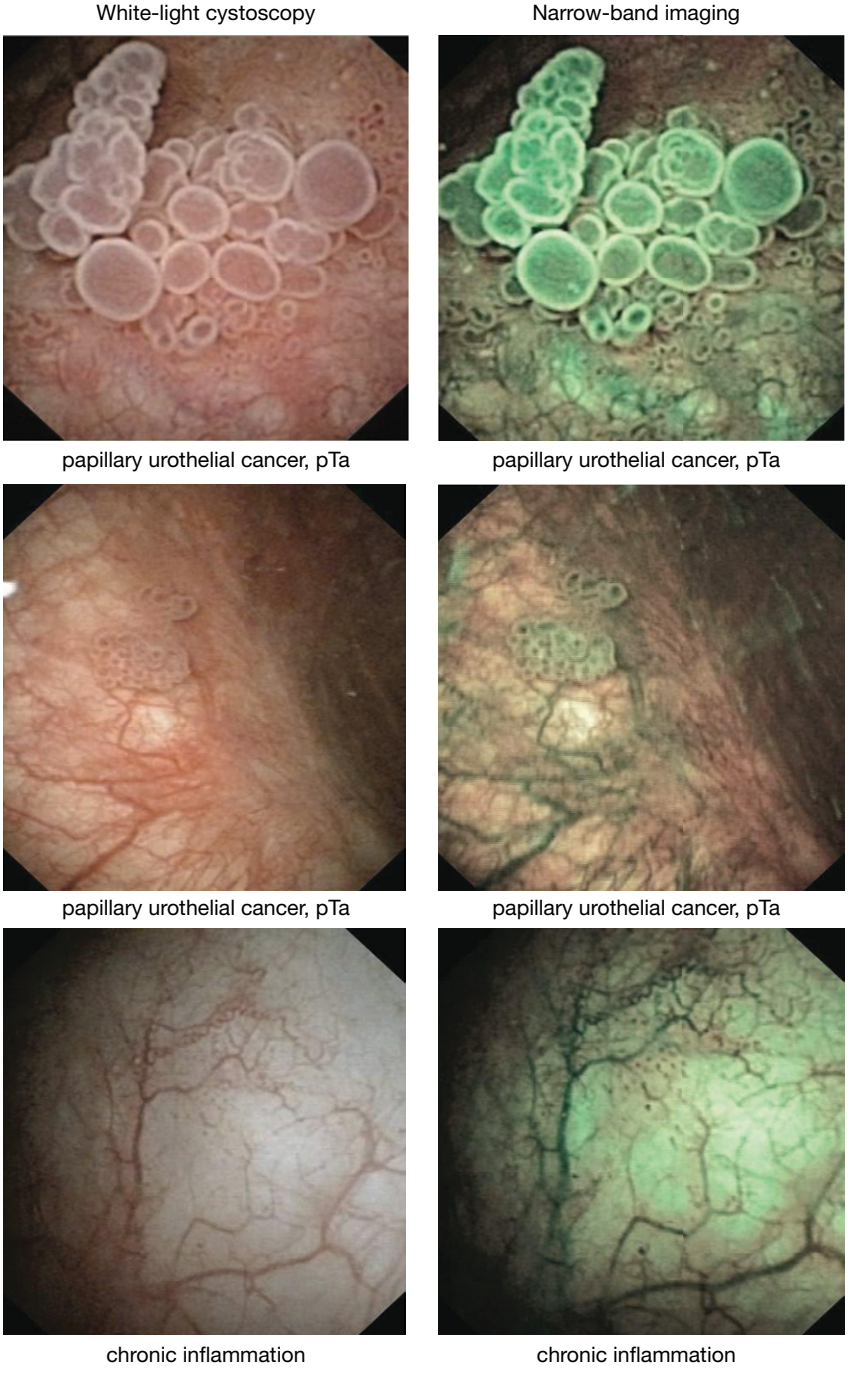
A critical question is whether the use of NBI can reduce the risk of recurrence in patients undergoing TURBT for NMIBC. By permitting better visualization of the margins of non-muscle-invasive papillary and flat bladder lesions, NBI cystoscopy facilitates a more thorough excision of the tumour. This, in theory, could result in a lower rate of tumour recurrence with the use of NBI. Fluorescence cystoscopy (PDD) using 5-ALA or its hexyl ester has been recognized to improve the detection of non-muscle-invasive papillary and flat bladder lesions compared to WLC. It has also been shown to reduce the recurrence rate of NMIBC.[203] Several prospective, randomized trials have now been published that have examined this important question regarding NBI. Naselli and colleagues performed a small, two-centre trial that randomized 148 patients with NMIBC to receive WLC- versus NBI-directed TURBT. They found that the use of NBI reduced the 1-year recurrence risk by 40% (odds ratio [OR]: 0.62; 95% CI: 0.4–0.92), without significantly increasing the false-positive tumour detection rate.[204] More recently, the Clinical Research Office of the Endourological Society (CROES) published the preliminary results of a prospective randomized trial of NBI plus WLC-directed (NBI-assisted) versus WLC-directed (WLC alone) TURBT in patients with NMIBC.[205] This large, multicentre, well-designed trial randomized 965 patients to NBI-assisted versus WLC alone TURBT. In a preliminary analysis of 597 patients who had completed 1 year of follow-up, the overall recurrence rates were not significantly different for NBI TURBT and WLC TURBT (27% and 25%, respectively). A subgroup analysis found that patients with a low risk of disease recurrence had a significantly lower recurrence rate with NBI-assisted versus WLC alone TURBT at 1 year (6% vs. 27%, respectively). The mean endoscopic resection time for NBI-assisted was 3 minutes longer, but had significantly better sensitivity in tumour detection versus WLC alone. It is important to recognize that these are the preliminary results of a large trial, so any firm conclusions should await the final results of the study, which will, hopefully, become available within the next year or two. Two meta-analyses have also now been done that have examined the effect of NBI on reducing the risk of recurrence, and both concluded that NBI improves recurrence-free survival at 3 and 12 months, although the quality of the trials contributing to the data itself was quite variable.[197,206]

One notable deficiency of NBI cystoscopy in bladder cancer detection is its low specificity (high false-positive rate), which can lead to unnecessary resection of noncancerous tissue. For example, NBI cystoscopy may pick up areas of neoangiogenesis, which is seen in interstitial cystitis/painful

bladder syndrome and is not related to urothelial carcinoma.[207] Intravesical agents (immunotherapy or chemotherapy), cystitis, and hematuria may be associated with inflammatory mucosal appearances mimicking urothelial tumours (see **Figure 1–6**).

FIGURE 1–6
Examples of the Differences Between Tumours Visualized With White-light Cystoscopy (Left) and Narrow-band Imaging Cystoscopy (Right)

Source: Photos were cited from Watanabe A, Fujita M. Case Study of NBI (Specific Wavelength Light) Endoscopy. Vol 1. Japan; Olympus Medical Systems Corporation:1–16.



1.5.4.4 Conclusions

NBI has been shown to be a potentially useful adjunct to WLC in the detection of bladder cancer. It is practical to use in combination with modern digital endoscopes and appears to have a reasonably short learning curve. It consistently demonstrates improved sensitivity over WLC in the detection of bladder cancer, although this likely comes at the cost of lower specificity and a slightly lower positive predictive value. As an adjunct to TURBT, it may improve recurrence-free survival in the short term (1 year), particularly in those with the lowest risk of recurrence, but the final long-term results of several ongoing clinical trials will be needed to truly confirm this.

1.5.5 New optical imaging techniques for evaluating bladder tumours

1.5.5.1 Raman spectroscopy

1.5.5.1.1 Principles of Raman spectroscopy

In 1930, Raman was awarded the Nobel Prize in Physics for his demonstration that light is scattered by atoms or molecules into two main components, one termed *ordinary*, with the same wavelength as the incident beam, and the other termed *modified*, whose spectrum—the "Raman signature"—is specific to the compound that is illuminated.[208] The concept of Raman spectroscopy (RS) opened the door to molecular imaging, which recently gained momentum due to advances in lasers (1964 Nobel Prize in Physics) and charge-coupled device camera heads (2009 Nobel Prize in Physics).[209] Technology developments, such as Raman difference spectroscopy,[210] which addresses the issue of ambient light and autofluorescence of tissues, are anticipated, in the near future, to offer rapid, label-free, noninvasive information on the structure and biochemistry of tissues and cells. Software-assisted diagnostic systems and wide-field technology are being introduced in operative endoscopy, notably in endoscopic gastric cancer detection.[211]

1.5.5.1.2 Raman spectroscopy in the detection of bladder cancer

In 2004, Crow *et al.* reported on the first application of RS in bladder tissue samples.[212] This landmark *in vitro* study confirmed that Raman spectra could be acquired in a nondestructive manner for the sample and within a time frame (10 seconds) compatible with clinical use. In addition, specific signatures of high sensitivity and specificity were observed in clinical situations ranging from inflammation to muscle-invasive disease, suggesting that RS may be of help in demanding situations, such as distinguishing between cystitis and CIS.

The first clinical report on bladder endoscopy used a miniaturized probe (2.1 mm) and short integration times (<5 seconds) to confirm the safety of the procedure, validate the diagnostic value of spectral analysis, and detail cancer-specific biochemical signatures (loss of hydroxyproline peak, and increased concentrations of nucleotides and amino acids) of high sensitivity (85%) and specificity (79%). Biochemical signatures were measured up to a depth of 2 mm, extending far beyond the epithelial layer to the submucosa and muscle layers. Cancer invasion was characterized by lower intensities of lipid and protein peaks, as it reduced on the spectra the relative contribution of submucosal fatty tissues.[213] Finally, the authors showed that RS was amenable to bladder walls exposed to 5-ALA or HAL, suggesting that it could offer real-time information on suspicious PDD areas, which would be a strong advantage and optimize the sensitivity of PDD.

1.5.5.1.3 Future direction of Raman spectroscopy

While RS is close to being used in routine clinical applications in other domains, such as gastroscopy,[211] the exciting first steps of its use in the urology literature remain preliminary and have not been extensively studied to date. Interaction with the optical industry is certainly needed to develop interfaces that would translate the complexity of the spectra and signatures into applicable information. Given the small size of the Raman probes designed so far, it would make sense to complement RS with advanced technology, such as PDD or NBI. The latter could be used to screen the entirety of the bladder lining in a timely manner, while RS could qualify regions of interest in biopsy, resection, or simple surveillance.

1.5.5.2 Optical coherence tomography

1.5.5.2.1 Principles of optical coherence tomography

OCT is the optical analogue to ultrasound imaging and involves using infrared light waves rather than acoustic waves. The light reflected from internal microstructures is measured by interferometry and is used to produce two-dimensional maps of high resolution from the backscattering of cellular structures.[214] OCT allows for nondestructive cross-sectional imaging of live tissues. It is routinely applied in ophthalmology, and is amenable to diagnostic and operative endoscopy.

1.5.5.2.2 Optical coherence tomography in the detection of bladder cancer

OCT was first applied to tissue samples, where it was used to analyze the integrity of the different layers of the bladder and to detect invasion of the lamina propria,[215] although it failed to detect cellular alterations within the mucosal layer.[216]

After several preliminary experiments, some *Conformité Européenne* (CE)-marked and FDA-approved miniaturized probes (outer diameter: 2–3 mm) were developed and used to compare OCT video-recorded endoscopic images with microscopic analysis of cold-cup biopsies. Although high sensitivity values were reported for the diagnosis of cancer and invasion beyond the lamina propria, specificity was suboptimal.[217] The maturity of the technology remains in question, due to the small size of the sample analyzed at each scan and the proficiency in bladder histology needed to make the most of the two-dimensional monochrome images that are generated.

1.5.5.2.3 Future direction of optical coherence tomography

OCT images provide a valuable insight into the bladder wall structure, up to a depth of a few millimetres, making it of high interest in the analysis, in a noninvasive manner, of the structure of the submucosal layers. However, cross-sectional scans cover just a few millimetres of width and depth, and require expertise to analyze. Postprocedure analysis of the scans also limits the clinical value of the technology. Real-time, user-friendly algorithms of detection[218] and integration with PDD or NBI for cross-reference[219] may facilitate the transition of OCT from a promising concept to a valuable technology.

1.5.6 Conclusions/summary

WLC is the standard approach for the diagnosis and management of lower urinary tract disease, including urothelial carcinoma, and is the gold standard against which other approaches must be compared. Although not cancer-specific, PDD is a robust adjunct to WLC in the detection of

papillary and nonpapillary forms of cancer, improving the quality of resection and recurrence-free survival. Better detection comes at the cost of lower specificity, which can be controlled with training and experience. NBI has been shown to be a potentially useful adjunct to WLC in the detection of bladder cancer. It is practical to use in combination with modern digital endoscopes and appears to have a reasonably short learning curve. It consistently demonstrates improved sensitivity over WLC in the detection of bladder cancer, although this likely comes at the cost of lower specificity and a slightly lower positive predictive value. RS and OCT are promising new technologies that require further study before being entered into routine clinical practice.

1.5.7 Recommendations

- WLC is the gold standard for the evaluation of the lower urinary tract and is the standard against which other approaches must be compared. **[LOE 3; GOR B]**

- A bladder diagram should be utilized at the time of first cystoscopy to localize precisely the tumour area and to facilitate a future transurethral resection. **[LOE 4; GOR C]**

- PDD may be used:
  - As an adjunct to WLC for the detection of bladder cancer during endoscopic evaluation. **[LOE 1; GOR B]**
  - As an adjunct to WLC during the transurethral resection of a suspected bladder cancer. **[LOE 1; GOR B]**

- NBI may be used:
  - As an adjunct to WLC for the detection of bladder cancer during endoscopic evaluation. **[LOE 2; GOR C]**
  - As an adjunct to WLC during the transurethral resection of a suspected bladder cancer. **[LOE 2; GOR C]**

- Voided urine cytology should be used during monitoring of high-grade tumour recurrence. **[LOE 3; GOR B]**
  - Cytology may be used to differentiate high-grade from low-grade urothelial carcinomas prior to TURBT, so as to guide the procedure. **[LOE 4; GOR C]**

- Bladder wash cytology may be considered for high-risk situations due to the higher diagnostic yield than voided cytology. Minimal manipulation should be performed prior to bladder wash. Residual urine mixed with the bladder wash specimen should be sent for cytology. **[LOE 4]**

- In general, no recommendations **[GOR D]** can be made for urinary markers in the diagnosis or follow-up of bladder cancer. **[LOE 4]**
  - Some urinary markers (e.g., fluorescence *in situ* hybridization [FISH]) may be used in the setting of atypical cytology with negative cystoscopy. **[LOE 3; GOR C]**
  - Some urinary markers (e.g., FISH) may be used for predicting the risk of recurrence in patients on BCG therapy. **[LOE 3; GOR C]**

1.6 Transurethral Resection of Bladder Tumours

1.6.1 Surgical technique

1.6.1.1 Introduction

The foundation of decision-making for a patient with bladder cancer is the endoscopic examination of the bladder and the subsequent removal of all obvious papillary and sessile tumours, when technically possible, as well as the biopsy of suspicious areas, which may or may not be malignant. The challenge of removal of the entire tumour by endoscopic resection (TURBT) is evidenced by the relatively high residual tumour rate in tumour resection restaging for tumours that have invaded the lamina propria (T1). The quality of the surgical resection impacts the risk of tumour recurrence. To improve surgical quality when performing TURBT, a standardized procedural checklist was developed[220] and subsequently employed.[221] The checklist is expected to improve the quality of resection and reporting of procedures. In addition to adherence to some basic technical aspects of endoscopic bladder tumour excision, the urologist must be flexible, according to the individual patient, and consider age, frailty, comorbidities, prior surgeries, etc.

Preparation for a cystoscopy and TURBT begins with a careful preoperative history and physical examination. It is critical that the patient's history is reviewed, with particular attention paid to any prior genitourinary tract surgeries and any prior bladder cancer history, including pathology, cystoscopy, and operative reports when applicable. The status of the upper urinary tract should be reviewed and documented to prepare for the TURBT, by ascertaining whether there are any abnormalities in the upper tract, such as an obstruction or neoplasm, that may impact subsequent management decisions.

1.6.1.2 Anesthesia options

The urologist's preoperative planning should include a discussion with the patient and anesthesiologist regarding the type of anesthesia that will be used. The options may include endotracheal intubation, spinal anesthesia, and laryngeal mass airway. Complete paralysis is recommended to decrease patient movement during the procedure and to lessen the possibility of the obturator reflex, which could result in bladder perforation and premature termination of the procedure. It is most frustrating to try to resect a tumour located on the lateral, posterior, or anterior walls if the patient is moving as a result of abdominal breathing or insufficient sedation. Thus, unless there is some contraindication, complete paralysis is preferred. Many patients with bladder cancer are elderly and/or have chronic obstructive pulmonary disease, and a spinal anesthetic is an alternative.

1.6.1.3 Technique

1.6.1.3.1 Introduction

The initial part of TURBT begins with urethroscopy to survey the entire urethra, including the prostatic urethra in men. The urologist should avoid trauma to the urothelium during the initial instrumentation, as this may lead to a urethral stricture or bleeding, which may hinder a close examination of all areas of the bladder. The initial endoscopy can be performed with a cystoscope sheath, an

optical dilator, or the resectoscope sheath and a visual obturator. A 12-degree lens is suggested for the urethroscopy. Blind placement of the resectoscope sheath should be avoided. Once the sheath has entered the bladder, the urologist should consider collecting the urine and placing it in a container. Depending on the clinical scenario, this can be sent for cytological assessment. If the cytology findings are particularly important (e.g., following BCG therapy, the clinician can also perform bladder irrigation or barbotage with saline to maximize the cellular yield), it is recommended that the initial endoscopy of the bladder be performed with a 70-degree lens since this provides the most comprehensive view of the bladder. The location and shape of the ureteral orifices should be noted. The location and configuration of all the tumours should be documented, and a plan should be established for the sequence of the resection; tumours most likely to cause the obturator reflex or bladder perforation should be removed last, and one can begin with the easy resections. Increasingly, urologists have the capability to capture images/videos of the tumours, and these provide a useful resource for documenting the neoplastic diathesis of the patient, as well as for educating the patient and their family.

Urologists are reasonably accurate at guessing the grade and stage of bladder tumours. This is important, as the extent of tumour resection can be tailored to the type of tumour. Thus, if a patient has one or more tumours that are papillary, and appear to be low grade and confined to the urothelium (i.e., Ta), and particularly if the patient has a history of similar tumours, the urologist can minimize trauma to the bladder by removing the tumours by cold-cup resection and subsequent fulguration of the biopsy site, fulguration only, or limited TURBT with the resectoscope. Since patients with these low-risk tumours may have frequent subsequent tumour events, one should minimize trauma to the bladder. Not every TURBT requires muscle to be present in the specimen. Thus, if the tumour is almost certainly a Ta tumour, there is no need to have muscle present.

If the urologist feels the tumour might invade the lamina propria (i.e., T1 or higher stage), they should attempt to resect the entire tumour and include muscularis propria in the resected tissue. This is important, as it allows the pathologist to properly stage the tumour by determining whether the cancer has invaded the lamina propria or muscularis propria. If tissue from these two layers of the bladder is missing, then, obviously, the pathologist cannot comment on whether the cancer has invaded these layers. If only tissue from the urothelium and lamina propria are present in the histological material, then the pathologist can report whether or not the cancer has invaded the lamina propria, but they must state that there is no muscularis propria in the removed tissue. Thus, they cannot comment on muscle invasion. The urologist should minimize the cautery effect on the tissue for the same reason (i.e., aid the pathologist in being able to comment on the presence and extent of any invasion). When tumour tissue is scarce, cold-cup biopsies are preferred over cautery to limit the possibility of charring, which can disrupt pathological assessment.

The approach for a tumour that appears to have invaded the muscularis propria depends on the clinical situation. If the size of the tumour and the clinical scenario (e.g., age, medical status, and comorbidities of the patient) suggest that bladder preservation may be a treatment approach, then the urologist should attempt to perform as complete a resection as possible. This is an important criterion for the success of a bladder preservation strategy for MIBC. One of the criteria for a successful attempt at bladder preservation with chemotherapy and radiation is a clinically complete resection. Even if a radical cystectomy would ordinarily be the choice of the urologist as they begin the endoscopic resection of a patient with *c*T2–*c*T3 bladder cancer, they cannot be certain what the patient

may elect for treatment or what the complete cardiovascular investigation may allow. On the other hand, if the tumour is very extensive, the urologist has to be judicious concerning the extent of the resection, as hemostasis is critical during the resection.

The urologist should be aware of the height of the bladder irrigation fluid. If the irrigation solution is too high (a common problem), the bladder will be too distended, thus thinning the wall and increasing the risk of perforation. The urologist should examine the bladder, with various degrees of filling, to ensure maximal visualization of any tumour. During the resection, the optimal situation is to keep the bladder capacity at about 50%. The goal is to resect the tumour and obtain tissue at the proper depth, which depends on the type of tumour, without risking perforation of the bladder.

There are two basic approaches to performing TURBT: staged resection and en bloc resection.

1.6.1.3.2 Staged resection

A staged TURBT is performed in several phases. First, the exophytic portion of the tumour is resected. The surgeon begins at one end and resects toward the other end if possible. The next layer of tissue is resected in a similar fashion. Layers of tissue are resected in this manner until the base of the tumour is reached. Finally, the base of the tumour is resected. The resected tissue may be sent together for collective analysis, or the tissue from each stage may be sent separately for differential analysis.[222] Once again, the goal is to remove all obvious tumours in a safe and efficient manner, with hemostasis and depth determined by the urologist's clinical impression of the stage and grade of the tumour. The degree to which this can be accomplished varies widely. Thus, in a thin patient with a few papillary, low-grade–appearing Ta tumours, the procedure is relatively easy. In contrast, TURBT in an obese man with a large prostate and with tumours located at the anterior wall can be very difficult.

Since the collection of the resected tissue is valuable, it is paramount not to lose any of the tissue. One way of aiding this process is to use a strainer to collect the fluid. This leaves the resectoscope sheath during the multiple times the urologist removes the actual working element to allow the irrigant to leave the bladder. Although the drapes also have a filter, a strainer seems to facilitate the collection of the specimen and lessen the chance of losing some of the resected tissue.

1.6.1.3.3 En bloc resection

En bloc resection may be used for small tumours, generally those less than 3 cm in greatest dimension. The reported advantages of an en bloc resection include more accurate pathological assessment because of decreased cautery artifact, avoidance of tumour fragmentation, and preservation of the spatial orientation of the tumour relative to the bladder wall. There have been no comparative studies of en bloc TURBT versus staged TURBT.

1.6.1.3.4 Bipolar electrocautery

During bipolar electrocautery, the electric current is restricted to the area between two polar elements. Thus, the patient's body is taken out of the electric current loop, and the risk of inadvertent burning at a distant site is significantly reduced. Bipolar electrocautery allows for resection to occur with isotonic fluid (i.e., saline), which decreases the risk of complications, such as transurethral resection of the prostate syndrome, which, theoretically, can occur during resection with water or glycine; however, this is a very rare event.

Bipolar and holmium laser treatment of bladder tumours has been shown to decrease the obturator nerve reflex, bladder perforation, and bleeding compared with conventional monopolar electrocautery.[223] Comparisons between monopolar and bipolar TURBT have been conducted.[224–226] A meta-analysis including six randomized controlled trials reported shorter operative times, less blood loss, and shorter hospital stays, along with fewer complications from the obturator nerve reflex and bladder perforation.[227] It should be added that the overall complication rate for TURBT is quite low, and the difference between monopolar and bipolar TURBT is relatively small.

Cautery can damage tissue and interfere with pathological interpretation.[228] Therefore, consideration of the surgical technique should include consideration of the effects on pathological assessment.

In a blinded comparison of TURBT specimens from monopolar versus bipolar resected bladder tumours, tissues from bipolar TURBT were smaller compared to specimens collected from monopolar cautery. However, there were no differences in the cautery artifact or ability to interpret the pathological stage/grade.[229]

1.6.1.3.5 Tumour in a diverticulum of the bladder

A special scenario is the presence of a bladder tumour in a diverticulum of the bladder. A diverticulum lacks muscularis propria. Thus, the urologist must be careful in their approach to removing a tumour in this location. In most cases, an initial specimen can be obtained with cold-cup forceps, and depending on the size of the tumour, the entire base can then be cauterized. If the patient has a history of only low-grade Ta tumours, and the tumour in the diverticulum appears to be consistent with this grade and stage, all of the tumour can be cauterized. If the tumour is likely to be an invasive tumour, then a specimen is needed to make this determination. This can be done by cold-cup or limited, careful electrocautery, and a partial or total cystectomy should be considered, depending on the grade and stage of other tumours and other factors related to the patient's profile.

1.6.1.3.6 Tumour at the ureteral orifice

Another special circumstance is a tumour at the ureteral orifice. In general, one can resect the tumour as completely as needed to ensure as complete a resection as possible and then decide on the need for a ureteral stent. In most cases, if one is judicious in performing TURBT, with limited coagulation current of such a tumour, a stent can be avoided.

Another consideration is the choice of loop required to remove the tissue. Most urologists use a right-angle loop in TURBT, regardless of the tumour's location. The so-called bladder wall loop, however, is designed for the contour of the bladder, and is useful for resecting tumours located in the posterior or lateral walls. Alternatively, one can bend the right-angle loop and proceed with the resection of tumours located in areas other than the trigone.

1.6.1.3.7 Tumour in the prostatic urethra

The urologist may be confronted with papillary or sessile tumours in the prostatic urethra. In general, they should proceed in a similar manner as one would to resect a tumour in the bladder. If the tumour appears to be papillary and Ta, then a limited resection is appropriate. If there is any concern that the tumour might be high grade, and thus invade the prostatic ducts or stroma, then a more

extensive resection of the prostate is required. It is important to convey as much information to the pathologist as possible in these situations. This will allow them to provide the optimal information needed to proceed with the management of the patient.

1.6.1.3.8 Repeat transurethral resection of bladder tumours

Another special circumstance is the repeat TURBT. A patient with a diagnosis of a high-grade T1 tumour should be taken to the operating room for a repeat resection to ensure accurate staging and removal of all the tumour if possible. In these circumstances, urine for cytology is the most helpful, as positive cytology for a high-grade tumour and a negative re-TURBT specimen suggest a residual tumour, either CIS in the bladder, prostatic urethra, or upper tract, or a missed tumour. The bladder lining will likely be thinner. Thus, this requires more diligence regarding the actual resection. Nonetheless, muscularis propria is required in re-TURBT tissue.

1.6.2 Pathological processing

The pathological evaluation of bladder tumour specimens acquired by TURBT differs markedly from the usual pathological evaluation of tumour specimens. This is due, in large part, to the nature of the procedure, which cuts the bladder tumour into multiple fragments with electrocautery. The pathologist receives a specimen without any anatomical orientation, has to process multiple fragments of the specimen, and has to deal with potentially extensive cautery artifact. The most critical information gained from pathological interpretation of TURBT specimens is information about whether there is invasion of the lamina propria or muscularis propria. It is imperative that TURBT specimens be handled and processed optimally to help the pathologist make the most accurate assessment.

1.6.3 Tissue fixation

In the operating room, all tissues must be placed immediately in 10% neutral buffered formalin in a 10:1 ratio by volume of fixative to tissue to ensure adequate fixation. Filling a specimen cup with tumour chips without adequate fixative will lead to poor fixation and increased tissue degradation. Alternatively, 4% paraformaldehyde may be used instead of neutral buffered formalin. In fact, 4% paraformaldehyde may even be a superior fixative for immunohistochemistry, as it tends to yield reduced background staining. However, it must be freshly made just before use. The duration of fixation can be 12 to 24 hours for TURBT specimens since each tissue fragment tends to be small.

1.6.4 Conclusions and levels of evidence

Section	Topic	Conclusions and levels of evidence
1.6.1	Surgical technique:	▪ A standardized checklist should be used during transurethral bladder tumour resection. **[LOE 3]** ▪ Following complete endoscopic examination of the bladder and urethra using 12- to 70-degree lenses, a strategy for the safe removal of all intravesical components of papillary bladder tumours should be devised before initiating resection of tumours. **[LOE 4]**
1.6.1.2	Anesthesia options:	▪ Complete paralysis is recommended to avoid the obturator reflex. **[LOE 4]**
1.6.1.3	Technique:	▪ If the urologist feels the tumour might invade the lamina propria (i.e., T1), they should attempt to resect the entire tumour and include muscularis propria in the resected tissue. **[LOE 4]** ▪ The urologist should minimize the cautery effect on the tissue for the same reason (i.e., aid the pathologist in being able to comment on the presence and extent of any invasion). **[LOE 4]** ▪ When tumour tissue is scarce, cold-cup biopsy is preferred over cautery to limit the possibility of charring, which can disrupt pathological assessment. **[LOE 4]** ▪ Surgical en bloc resection may be used for small tumours, generally those less than 3 cm in greatest dimension. **[LOE 4]** ▪ Bipolar transurethral resection may result in fewer complications from the obturator nerve reflex and bladder perforation. **[LOE 3]** ▪ Repeat resection is recommended for patients with T1 tumours. **[LOE 2]**
1.6.3	Tissue fixation:	▪ Tissue should be placed immediately in 10% neutral buffered formalin in a 10:1 ratio of volume of fixative to tissue to ensure adequate fixation. **[LOE 4]**

1.6.5 Recommendations

- Imaging of the upper tracts is necessary in the investigation of hematuria. **[GOR B]**
  - CT urography should be performed in patients suspected of having urothelial carcinoma. **[LOE 3; GOR B]**
  - IV urography, regular CT, ultrasound, and MRI are options. **[LOE 3; GOR C]**

- CT scan of the abdomen and pelvis with IV contrast, including an excretory phase study, is recommended as the imaging modality for the investigation of upper tract lesions, and nodal and distant metastases within the abdomen and pelvis in patients with bladder cancer. This modality is more favoured than MRI. MRI of the abdomen and pelvis with IV contrast should be considered in patients who cannot tolerate CT contrast. **[LOE 3; GOR C]**

- Imaging for staging should be obtained prior to TURBT or 7 days after TURBT to avoid artifacts. **[LOE 4; GOR C]**

- Metastatic work-up of patients with a diagnosis of urothelial cancer should include:
  - Chest radiography **[LOE 4; GOR B]**
  - Bone scan for patients with bone pain or elevated alkaline phosphatase concentrations **[LOE 4; GOR B]**

- Diffusion-weighted MRI has poor sensitivity in differentiating between Ta, T1, and T2 bladder tumours. Diffusion-weighted MRI may be used to identify T3 and T4 disease. **[LOE 3; GOR B]**

- Positron emission tomography (PET) scanning appears to have the greatest accuracy in the detection of nodal metastases. **[LOE 4; GOR C]**

1.7 Imaging and Bladder Cancer

1.7.1 Role of imaging in the diagnosis and staging of bladder cancer

1.7.1.1 Imaging in the diagnosis of bladder carcinoma

Most patients with bladder cancer present with hematuria. Hence, they undergo an imaging work-up to exclude other common causes of hematuria prior to a definitive diagnosis of bladder cancer. The work-up of hematuria includes clinical examination, imaging, and cystoscopy. Age, sex, gross versus microscopic hematuria, and presentation of clinical symptoms dictate the need for and the modality of imaging used for the work-up of hematuria. Usually, the focus of imaging is to detect structural abnormalities of the kidneys and the upper tracts.[230–232] Imaging may include ultrasound of the kidneys, excretory urography, plain radiographs of the abdomen, CT, and MRI.

Use of imaging to detect bladder carcinoma is not common due to certain limitations of the imaging modalities and the costs involved. Excretory urography has traditionally been used for excluding synchronous tumours in the upper urinary tract in a patient with bladder carcinoma. Its sensitivity in detecting bladder carcinoma is low and variable.[233] Ultrasound of the pelvis using a high-frequency probe may have a role in noninvasive screening for bladder carcinoma in high-risk individuals. However, there is no good evidence to support its use in routine clinical practice.[234,235] Cystoscopy and biopsy remain the gold standard for diagnosing bladder carcinoma in high-risk patients.

1.7.1.2 Imaging in staging bladder carcinoma

Imaging has a role in staging muscle-invasive bladder carcinoma. The primary purpose of imaging is to assess the degree of local invasion, and to detect nodal and distant visceral metastases. Patients with bladder carcinoma are at an increased risk of developing synchronous and metachronous tumours in the upper tracts. Hence, a strategy to image the upper tracts is commonly utilized in patients who undergo imaging work-up for bladder carcinoma. However, the incidence of upper tract malignancies is rare. In the largest series reported that studied patients with Ta bladder tumours, 0.3% were diagnosed with synchronous or metachronous upper tract malignancies.[236] Even in patients with muscle-invasive disease, the incidence of upper tract carcinoma is rare. There are no good data to suggest that routine surveillance of the upper tract in these patients will lead to a better prognosis than when these patients present with symptoms.[237] Hence, a risk-stratified approach for upper tract surveillance should be considered, weighing the pros and cons, and whether the additional burden of upper tract surveillance in these patients is warranted.

1.7.1.3 Depth of invasion (T staging)

1.7.1.3.1 Ultrasound

A distended bladder offers a good acoustic window that can help in the detection and staging of bladder carcinoma. With a high-frequency probe, the bladder wall can be seen as three layers—a middle hypoechoic layer of muscularis sandwiched between two relatively hypoechoic layers of serosa and submucosa. Disruption of the middle hypoechoic layer is considered to represent muscle-invasive disease.[238] However, there are significant limitations in the use of ultrasound. First, there are challenges in the detection of bladder tumours. Small lesions less than 0.5 cm in size and plaque-like

lesions are difficult to detect. Second, the detectability of the lesions depends on the location of the tumours. Lesions in the anterior and inferior walls of the bladder, and in the bladder neck are difficult to detect and, hence, stage.[239] Last, large lesions and lesions with calcification pose significant technical challenges, making consistent identification of the three layers of the bladder difficult. In addition, ultrasound is limited in its ability to detect enlarged pelvic and retroperitoneal nodes. Hence, it cannot be relied on by itself for comprehensive staging.[240]

1.7.1.3.2 Computed tomography

CT is the most widely used imaging study to stage bladder carcinoma. In patients who need assessment of the upper tracts for synchronous malignancies, CT scans play an important role and can be optimized to assess both the upper tract and the urinary bladder.

CT may be helpful in identifying gross or macroscopic extravesical invasion of bladder carcinoma. Several studies show significant variability in the accuracy of CT in local staging, ranging from 40% to 85%.[240,241] Several of these studies predate the widespread use of modern multidetector CT and postprocessing capabilities. Small studies have shown improved accuracy with these technological advances in local staging.[242] However, difficulty in finding the fat planes reliably in all patients, poor contrast resolution in the pelvis, and inability to distinguish between inflammatory perivesical stranding and tumour infiltration remain major limitations in the accurate staging using CT.[243] Both overstaging and understaging remain a problem.[241] Indirect signs of muscle invasion, such as retraction of the bladder wall, are also unreliable, as these may vary with the degree of distention of the bladder.

1.7.1.3.3 Magnetic resonance imaging

MRI offers significantly improved soft-tissue resolution compared to CT, with striking contrast between the low signal of the bladder wall and the high signal of the surrounding fat in both T1- and T2-weighted sequences, thereby giving MRI a distinct advantage over CT in detecting adjacent organ involvement. Bladder carcinoma is enhanced after injection with a contrast medium on T1-weighted images. This characteristic can be exploited to detect extension into perivesical fat when fat suppression techniques are used. In addition, disruption of the detrusor muscle can be detected in T2-weighted sequences, indicating deep muscle invasion.[244] Contrast-enhanced T1-weighted sequences and T2-weighted sequences yield an accuracy of 73% to 96% in distinguishing organ-confined disease from non–organ-confined disease.[240,243,245] The addition of diffusion-weighted sequences has been found to further improve the staging accuracy of MRI.[244,246] Tumours have restricted diffusion relative to non-neoplastic tissues, including inflammatory and fibrotic changes related to treatment. In addition to improved local staging, diffusion-weighted imaging studies can also be helpful in the assessment of therapeutic response by differentiating inflammatory from fibrotic changes in patients receiving chemoradiation.[247] However, diffusion-weighted imaging suffers from a low signal-to-noise ratio and is susceptible to several artifacts. Its role in the staging of bladder carcinoma has yet to be determined.

MRI has limitations in the assessment of the upper tracts compared to CT. This is due to several factors, including poorer spatial resolution and long acquisition times leading to image degradation from motion. Hence, although MRI offers superior local staging, CT urography offers the potential for comprehensive evaluation, including assessment of the upper tracts. The choice of modality should be made after factoring in the individual patient risks, availability of resources, and local expertise.

1.7.1.4 Nodal staging (N)

MRI is slightly superior to CT in detecting pelvic lymph nodes.[248] However, there are no well-established criteria to distinguish between malignant and benign lymph nodes on CT and MRI, apart from size. This limits the detection of metastatic nodes in normal-sized nodes. Diffusion-weighted imaging does not have a sufficient signal-to-noise ratio to make a significant impact on small pelvic nodes. Use of other morphological criteria, such as shape and margins, may be more helpful. Use of lymphotropic nanoparticle–enhanced MRI has shown promise in detecting micrometastasis in normal-sized lymph nodes in patients with bladder carcinoma, with a detection of up to 92% and a sensitivity of up to 96%.[249,250] However, studies investigating this have been small, and its impact on patient management has not been well studied. Use of ^{18}F-fluorodeoxyglucose (FDG) PET/CT does not appear to improve characterization of lymph node metastasis compared to contrast-enhanced CT alone.[251,252]

The incidence of lymph node metastasis depends on the depth of invasion. In patients with T2b disease and in patients with extravesical invasion, the incidence of lymph node metastasis is 20% to 30% and 50% to 60%, respectively. For a suspicious lymph node in such patients, a fine-needle aspiration biopsy should be considered.

1.7.1.5 Distant metastasis

Bladder carcinoma spreads hematogenously. The incidence of metastasis increases in a linear fashion as the grade of the tumour and the stage of the tumour increase. In patients with muscle-invasive and high-risk disease, preoperative imaging has been shown to improve overall survival, indicating a better selection of patients for cystectomy.[253] The liver, bones, and lungs are common sites of metastasis, in decreasing order of frequency.[254] CT scan of the chest, abdomen, and pelvis and bone scans are indicated in these patients.

Use of FDG PET/CT has been shown to have an impact on patient management. In a prospective study that used the National Oncologic PET Registry, the physicians surveyed reported a change in management in 47% of patients based on PET/CT findings, even after adjusting for the impact on patients, in whom a different imaging test, such as CT or MRI, may have led to the same management strategy. This management included avoidance of additional testing, need for biopsies, and addition of systemic chemotherapy.[255] PET/CT has also been shown to detect occult metastasis in patients with negative conventional imaging scans (CT and bone scans), thereby significantly impacting management and suggesting a role for PET/CET in presurgical screening in patients selected for radical cystectomy.[256]

1.7.2 Role of imaging in follow-up of bladder cancer

In patients with NMIBC, the natural history after treatment is often characterized by recurrence, even for solitary, small, low-grade papillary tumours.[257] Cystoscopic evaluation remains the gold standard for surveillance and should occur 3 months after initial treatment. The modality and frequency of imaging in these patients are based on the risk profile. Patients with MIBC benefit most from imaging follow-up after treatment.[258]

1.7.2.1 Local recurrence and surveillance of upper tract disease

Imaging has a limited role in the surveillance of NMIBC after primary treatment. Patients with low-risk bladder cancer have a low incidence (0.6%–0.9%) of subsequent upper tract malignancies. Hence, routine surveillance of the upper tract is not recommended.[257] Patients with muscle-invasive carcinoma, high-grade carcinoma, T1 disease, and multifocal disease are at a high risk of upper tract recurrence, which can be seen in up to 10% of patients.[257–259] Follow-up with cross-sectional imaging designed to study the upper tract is recommended in these patients for 2 to 3 years. The major urological guidelines, such those by the AUA, EAU, and National Comprehensive Cancer Network® (NCCN®), concord on the fact that patients with NMIBC need imaging surveillance due to the risk of recurrence and progression; however, the frequency of imaging should reflect the degree of risk.[257,259,260]

In patients with MIBC, local recurrence occurs in soft tissues at the original surgical site or in the lymph nodes in the area of the previous lymph node dissection. Node involvement above the aortic bifurcation is considered distant metastasis. Locoregional recurrence of bladder cancer following cystectomy develops in 5% to 15% of patients. In MIBC, upper urinary tract carcinoma occurs in 1.8% to 6% of patients.[261]

1.7.2.1.1 Ultrasound

For the follow-up of patients diagnosed initially with NMIBC (Ta, low grade [G1–G2]), ultrasound can be used in cases where cystoscopy is not possible or is refused by the patient. However, there are limitations in the detection of small lesions.

1.7.2.1.2 Computed tomography

CT is the most appropriate test for follow-up. CT can be used to assess pelvic recurrence as well as upper urinary tract disease.[261,262] The detection of upper urinary tract urothelial carcinoma is limited with conventional CT, compared with CT urography, and it is currently the standard imaging technique.[261] CT urography has an important role in surveillance due to its superior diagnostic accuracy in upper urinary tract disease, which is above that of other imaging techniques. It has a sensitivity of 67% to 100% and a specificity of 93% to 99%.[262] In NMIBC, CT urography is recommended yearly, while in patients with MIBC, it should be performed every 3 to 6 months for 2 to 3 years and thereafter annually.[259,260]

1.7.2.1.3 Excretory urography

Excretory urography is of limited value in the assessment of the upper urinary tract due to its poorer contrast resolution. In addition, the area of interest is often obscured by overlying stool and bowel gas, and urinary segments are often unclear. Thus, it should only be reserved for when CT urography

is not available.[261] The sensitivity of excretory urography in the detection of upper urinary tract lesions is 80.4%, and its specificity is 81%. Its overall accuracy in the localization of upper urinary tract carcinoma is inferior to CT urography.[261,262]

1.7.2.1.4 Magnetic resonance imaging

MRI is comparable to CT in its effectiveness to demonstrate local recurrence, although MRI offers superior soft-tissue contrast. MRI without contrast is generally superior to CT without contrast due to this reason. MRI urography is a viable alternative for patients who cannot undergo CT urography, such as when radiation or iodinated contrast media are contraindicated. The sensitivity of contrast-enhanced MRI urography is 75% for tumours smaller than 2 cm.[263]

Diffusion-weighted MRI is a functional imaging technique for monitoring therapeutic response, especially for bladder-sparing strategies in MIBC. Diffusion-weighted MRI also has improved accuracy in detecting residual disease after induction low-dose chemoradiation, with an accuracy of 80%. However, it is of limited value in small lesions.[264]

1.7.2.2 Nodal and distant metastasis

CT and MRI both are equivalent in their ability to detect metastatic lymph nodes, but this ability is low, as both modalities depend on the size and shape of the lymph nodes. Pelvic nodes more than 8 mm in short axis diameter and para-aortic nodes larger than 10 mm in short axis diameter are considered metastatic.[265]

Diffusion-weighted MRI is more useful in detecting pelvic lymph node metastasis. The use of lymphotropic ultra-small superparamagnetic particles as an MRI contrast agent may improve the detection of metastasis in normal-sized pelvic lymph nodes in bladder cancer, but has not attained widespread use.[266]

1.7.2.2.1 Positron emission tomography/computed tomography

PET/CT has a role in the surveillance of MIBC, and it may be performed, if not previously done, at staging. It is useful to resolve equivocal abnormal findings in CT, and it can potentially identify other sites of metastasis after primary treatment.[267]

PET/CT efficiently detects local recurrence and is useful for evaluating nodal metastasis, with a sensitivity of up to 92% and an accuracy of 90%.[267]

Distant recurrences are seen in up to 50% of patients, depending on the stage and nodal involvement. Most of the recurrences occur in the lymph nodes, lung, liver, and bone, often within 24 months.[233]

Judicious use of PET/CT in patients with suspected recurrent bladder cancer has a sensitivity of 87% and a positive predictive value of 95%, allowing for a change in treatment decisions in about 40% of cases.[267] However, it should not be used routinely for surveillance imaging because there are no data to support its superiority over conventional imaging.

The major urological guidelines, such those by the AUA, EAU, and NCCN, concord on the fact that, in clinical practice, CT and MRI are the imaging techniques most used. Although FDG PET/CT has a potential clinical use, there are insufficient data to recommend its use routinely.[258,259,266]

Chest radiography with posteroanterior and lateral views is recommended every 3 to 6 months in the first 2 years and then yearly.[233] Chest CT with IV contrast is reserved for follow-up when chest x-ray is abnormal or equivocal.

PET/CT may be performed in high-risk patients or when metastatic disease is suspected. In symptomatic or high-risk patients, or patients with laboratory indicators of bone metastasis, bone scans or PET/CT should be performed. ^{18}F-fluoride PET/CT has a higher sensitivity, specificity, and positive predictive value than conventional bone scans. However, its high cost and availability are limitations.[268] Whole-body MRI may be used to investigate bone metastasis, and it has a higher sensitivity and specificity than bone scans. Imaging for brain metastasis is recommended only in symptomatic patients.

The major urological guidelines, such as those by the AUA, EAU, and NCCN, concord on the fact that it is essential to evaluate for the presence of distant metastasis; CT and MRI are the diagnostic techniques of choice; and PET/CT has the potential for clinical use in the follow-up of metastatic bladder cancer.[258,259,266]

1.7.2.3 Monitoring response to therapy

CT and MRI are currently the best available imaging studies for measuring target lesions to monitor response to medical treatment of nodal and visceral metastatic disease. PET/CT may also allow for the identification of early responders by demonstrating decreased radiotracer activity earlier than morphological response.[269]

1.7.3 Recommendations

- Surveillance with CT or MRI is recommended for monitoring of recurrent or metastatic disease. **[LOE 4; GOR C]**
- At the beginning of TURBT, the bladder should be thoroughly viewed using a 12-degree lens, or both 30- and 70-degree lenses to ensure as complete an endoscopy as possible. **[LOE 4; GOR C]**
- A strategy for the safe removal of all intravesical components of papillary bladder tumours should be devised before initiating resection. **[LOE 4; GOR C]**
- Patients undergoing TURBT should be given appropriate prophylactic antibiotics. **[LOE 3; GOR B]**
- During resection, three key principles must be ensured to improve pathological interpretation of the resected bladder tumour: **[LOE 4; GOR C]**
  - Limiting cautery artifact
  - Ensuring adequate depth of biopsy according to the type of tumour
  - Proper handling of tissue for pathological processing following removal
- Complete tumour resection should be attempted in all patients, except in those with diffuse CIS. **[LOE 3; GOR C]**
- The following should be documented for each tumour noted or resected: **[LOE 3; GOR C]**
  - Shape (papillary or sessile), size, and location of the tumour

- Suspected CIS
- Appearance of the base of the tumour
- Visible detrusor muscle, whether present or not

- A separate specimen from the tumour base should be considered when the tumour appears to have invaded the lamina propria or deeper. **[LOE 3; GOR C]**
- Use of cold-cup biopsy is recommended when possible to minimize cautery artifact, especially for small papillary tumours. **[LOE 4; GOR C]**
- For tumours in a diverticulum, aggressive resection should be avoided to reduce the risk of perforation. **[LOE 4; GOR C]**
- If the ureteral orifice is resected, cutting current should be used, and a functional study should be performed 3 to 6 weeks later. **[LOE 4; GOR C]**
- There is insufficient information **[LOE 4; GOR D]** to support any specific energy modality for TURBT.
- Random biopsies of the bladder are not routinely recommended **[LOE 4; GOR C]**, but random biopsies of the bladder may be indicated in patients:
  - With positive findings on urine cytology and normal cystoscopy **[LOE 3; GOR B]**
  - Who are considered candidates for partial cystectomy **[LOE 3; GOR C]**
- Prostatic urethral biopsy/resection should be considered in cases of CIS or visible abnormalities of the prostatic urothelium. **[LOE 3; GOR B]**
- Prostate urethral biopsy is not useful in counseling patients for neobladder. **[LOE 3; GOR C]**
- Transurethral prostatic resection biopsy can be useful in counseling patients for neoadjuvant chemotherapy by identifying cT4 disease. **[LOE 3; GOR C]**
- A second TURBT should be performed in all patients with a high-grade T1 lesion, regardless of the presence or absence of muscularis propria. **[LOE 3; GOR B]**
- A second TURBT may be considered for select high-grade Ta lesions. **[LOE 3; GOR C]**
- The optimal timing of repeat TURBT is 4 to 6 weeks after the first resection. **[LOE 4; GOR C]**

1.8 Summary of Recommendations

- Smoking cessation is recommended as a means to reduce the risk of bladder cancer. **[LOE 3; GOR C]**
- No recommendations can be made for diet, body weight, and physical activity with respect to reducing bladder cancer risk. **[LOE 3; GOR D]**
- Bladder cancer screening, if undertaken, should be confined to high-risk patients. **[LOE 3; GOR C]**
  - Bladder cancer screening is not recommended for the general population. **[LOE 3; GOR C]**
  - Screening can consist of an annual cytology and dipstick. **[LOE 4; GOR C]**
- The investigation of hematuria should include imaging of the upper tracts. **[LOE 3; GOR B]**
- Urine cytology and cystoscopy should be used for symptomatic or gross hematuria, or in patients with risk factors for urothelial carcinoma. **[LOE 3; GOR B]**
- For patients with asymptomatic microscopic hematuria without risk factors for urothelial carcinoma, urine cytology or cystoscopy can be used. **[LOE 4; GOR D]**
- White-light cystoscopy (WLC) is the gold standard for the evaluation of the lower urinary tract and is the standard against which other approaches must be compared. **[LOE 3; GOR B]**
- A bladder diagram should be utilized at the time of first cystoscopy to localize precisely the tumour area and to facilitate a future transurethral resection. **[LOE 4; GOR C]**
- Photodynamic diagnosis (PDD) may be used:
  - As an adjunct to WLC for the detection of bladder cancer during endoscopic evaluation **[LOE 1; GOR B]**
  - As an adjunct to WLC during the transurethral resection of a suspected bladder cancer **[LOE 1; GOR B]**
- Narrow-band imaging (NBI) may be used:
  - As an adjunct to WLC for the detection of bladder cancer during endoscopic evaluation **[LOE 2; GOR C]**
  - As an adjunct to WLC during the transurethral resection of a suspected bladder cancer **[LOE 2; GOR C]**
- Voided urine cytology should be used during monitoring of high-grade tumour recurrence. **[LOE 3; GOR B]**
  - Cytology may be used to differentiate high-grade from low-grade urothelial carcinomas prior to transurethral resection of bladder tumour (TURBT), so as to guide the procedure. **[LOE 4; GOR C]**
- Bladder wash cytology may be considered for high-risk situations due to the higher diagnostic yield than voided cytology. Minimal manipulation should be performed prior to bladder wash. Residual urine mixed with the bladder wash specimen should be sent for cytology. **[LOE 4]**
- In general, no recommendations **[GOR D]** can be made for urinary markers in the diagnosis or follow-up of bladder cancer. **[LOE 4]**
  - Some urinary markers (e.g., fluorescence in situ hybridization [FISH]) may be used in the setting of atypical cytology with negative cystoscopy. **[LOE 3; GOR C]**
  - Some urinary markers (e.g., FISH) may be used for predicting the risk of recurrence in patients on bacillus Calmette-Guérin (BCG) therapy. **[LOE 3; GOR C]**
- Imaging of the upper tracts is necessary in the investigation of hematuria. **[GOR B]**
  - Computed tomography (CT) urography should be performed in patients suspected of having urothelial carcinoma. **[LOE 3; GOR B]**
  - Intravenous (IV) urography, regular CT, ultrasound, and magnetic resonance imaging (MRI) are options. **[LOE 3; GOR C]**

- CT scan of the abdomen and pelvis with IV contrast, including an excretory phase study, is recommended as the imaging modality for the investigation of upper tract lesions, and nodal and distant metastases within the abdomen and pelvis in patients with bladder cancer. This modality is more favoured than MRI. MRI of the abdomen and pelvis with IV contrast should be considered in patients who cannot tolerate CT contrast. **[LOE 3; GOR C]**
- Imaging for staging should be obtained prior to TURBT or 7 days after TURBT to avoid artifacts. **[LOE 4; GOR C]**
- Metastatic work-up of patients with a diagnosis of urothelial cancer should include:
  - Chest radiography **[LOE 4; GOR B]**
  - Bone scan for patients with bone pain or elevated alkaline phosphatase concentrations **[LOE 4; GOR B]**
- Diffusion-weighted MRI has poor sensitivity in differentiating between Ta, T1, and T2 bladder tumours. Diffusion-weighted MRI may be used to identify T3 and T4 disease. **[LOE 3; GOR B]**
- Positron emission tomography (PET) scanning appears to have the greatest accuracy in the detection of nodal metastases. **[LOE 4; GOR C]**
- Surveillance with CT or MRI is recommended for monitoring of recurrent or metastatic disease. **[LOE 4; GOR C]**
- At the beginning of TURBT, the bladder should be thoroughly viewed using a 12-degree lens, or both 30- and 70-degree lenses to ensure as complete an endoscopy as possible. **[LOE 4; GOR C]**
- A strategy for the safe removal of all intravesical components of papillary bladder tumours should be devised before initiating resection. **[LOE 4; GOR C]**
- Patients undergoing TURBT should be given appropriate prophylactic antibiotics. **[LOE 3; GOR B]**
- During resection, three key principles must be ensured to improve pathological interpretation of the resected bladder tumour: **[LOE 4; GOR C]**
  - Limiting cautery artifact
  - Ensuring adequate depth of biopsy according to the type of tumour
  - Proper handling of tissue for pathological processing following removal
- Complete tumour resection should be attempted in all patients, except in those with diffuse carcinoma *in situ* (CIS). **[LOE 3; GOR C]**
- The following should be documented for each tumour noted or resected: **[LOE 3; GOR C]**
  - Shape (papillary or sessile), size, and location of the tumour
  - Suspected CIS
  - Appearance of the base of the tumour
  - Visible detrusor muscle, whether present or not
- A separate specimen from the tumour base should be considered when the tumour appears to have invaded the lamina propria or deeper. **[LOE 3; GOR C]**
- Use of cold-cup biopsy is recommended when possible to minimize cautery artifact, especially for small papillary tumours. **[LOE 4; GOR C]**
- For tumours in a diverticulum, aggressive resection should be avoided to reduce the risk of perforation. **[LOE 4; GOR C]**
- If the ureteral orifice is resected, cutting current should be used, and a functional study should be performed 3 to 6 weeks later. **[LOE 4; GOR C]**
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- Random biopsies of the bladder are not routinely recommended **[LOE 4; GOR C]**, but random biopsies of the bladder may be indicated in patients:
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- Prostate urethral biopsy is not useful in counseling patients for neobladder. **[LOE 3; GOR C]**
- Transurethral prostatic resection biopsy can be useful in counseling patients for neoadjuvant chemotherapy by identifying cT4 disease. **[LOE 3; GOR C]**
- A second TURBT should be performed in all patients with a high-grade T1 lesion, regardless of the presence or absence of muscularis propria. **[LOE 3; GOR B]**
- A second TURBT may be considered for select high-grade Ta lesions. **[LOE 3; GOR C]**
- The optimal timing of repeat TURBT is 4 to 6 weeks after the first resection. **[LOE 4; GOR C]**

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C2

Pathology

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CONTENTS

Pathology

2.1 Introduction

The International Collaboration on Cancer Reporting (ICCR) was founded by the Royal Colleges of Pathologists of Australasia and of the United Kingdom, the College of American Pathologists, and the Canadian Association of Pathologists-Association canadienne des pathologistes (CAP-ACP), in association with the Canadian Partnership Against Cancer (CPAC). The ICCR's intent is to produce internationally standardized and evidence-based data sets for the pathology reporting of cancer. New insights in bladder cancer pathology and in the molecular-genetic basis of the disease continuously drive the need to adapt pathology reporting, with the aim of advancing international benchmarking in cancer management worldwide. In this chapter, we provide an update on the latest ideas and concepts of the World Health Organisation (WHO) 2016. Of particular interest are the ongoing controversy in grading, pathological staging, and substaging, as well as the reporting and nomenclature of variant-histology bladder cancers.

In this document, several authors have provided new consensus guidelines and insights with an extensive literature overview. The group of authors was deliberately kept small and included 12 experts, known worldwide in the field of urology and uropathology.

The chapter is kept short to provide concise consensus guidelines to guarantee the best patient care. As such, they should be easy to understand for non-pathologists. It was our intention to orient this chapter toward clinicians and address their needs in daily practice.

We focused on the following issues:

1. Bladder wall anatomy and histology with their regional variations
2. Grading of bladder cancer, comparing the 2004 and 1973 WHO grading systems
3. Staging of bladder cancer regarding the issue of pT1 substaging
4. Histological variants of bladder cancer in light of molecular classifications
5. Prognostic insights into different subtypes
6. Rare issues, such as bladder diverticulum and urachus carcinoma
7. Immunohistochemistry and its role in the latest molecular findings
8. ICCR worldwide consensus reporting
9. Urinary cytology after the adoption of the Paris system
10. Handling of different types of samples
11. Indications for frozen section specimens
12. The need for clinical information and collaboration with clinicians

2.2 Normal Urothelium and Bladder Wall Histoanatomy

2.2.1 Histoanatomy of the bladder

The urinary bladder wall is composed of three layers: the mucosa, the muscularis propria, and the adventitia or serosa. The mucosa comprises the urothelium, the basal lamina, and the lamina propria. Normal urothelium is composed of three different layers. The most superficial layer is covered by umbrella cells. Due to DNA multiploidization and endoreplication, their nuclei might display an increased and variable size of nuclei, but these features should not be mistaken as carcinoma *in situ* (CIS). Mild degrees of variation in architecture without cytological atypia can be allowed in the urothelium; usually no mitosis is seen. The middle layer is composed of intermediate cells and the the deepest layer is made of basal cells. Basal cells are small, have a high nuclear:cytoplasm ratio, and some mitoses may be present. Urothelial stem cells are in the basal cell compartment, but their identification remains controversial because of the lack of specific markers.[1] [**Level of Evidence 3, Grade of Recommendation C**]

2.2.2 Topographic variations (regions of the bladder) and their potential impact on bladder cancer outcomes

The urinary bladder is mapped into the bladder neck, trigone, anterior bladder wall, posterior bladder wall, dome, lateral bladder wall, and ureteric insertion sites, each coded separately according to the International Classification of Diseases for Oncology (ICD-O-3) to be used for data collection in cancer registries.[2–4] These are topographic regions with considerable histoanatomic differences. The mucosa of the bladder, histologically lined by urothelium, is undulated, more pronounced, and wavering toward the dome, and relatively more flat toward the trigone and bladder neck.[4]

At the trigone, the lamina propria is thinnest (0.46 mm–1.58 mm), and about half the dimension of and narrower than in the dome (0.98–3.07 mm).[4] The thickness of the lamina propria in the anterior, posterior, and lateral walls is approximately similar (0.72 mm–2.55 mm). To put these dimensions in perspective, the mean depth of pT1 invasive urothelial carcinoma (UC) is 1.1 mm to 1.5 mm (range: 0.1 mm–5 mm).[5,6] Lamina propria is composed of an extracellular matrix containing several types of cells, including fibroblasts, myofibroblasts/interstitial cells, immune cells, and afferent and efferent neurons. In addition, lamina propria contains blood and lymphatic vessels, elastic fibres, and smooth muscle fascicles (muscularis mucosae). [**Grade D**]

Another structure in the lamina propria that varies significantly in size, location and distribution is the muscularis mucosae layer, considered a landmark proposed for one of the pT1 substaging systems; it is often not discernible at the trigone.[4,7]

These variations in the lamina propria must be factored in when considering proposals for pT1 substaging (see **Chapter 4, Section 2**). The trigonal region of the urinary bladder is most distinctive because it contains two additional unique muscularis propria muscle bundles. Superficially, thinner

muscle bundles from the intramural ureters meet centrally and merge with bundles from the contralateral ureter, thus forming the superior border of the trigone (musculus interuretericus).[8] The second layer is the trigonal muscle itself, which is a continuation of the bladder sphincter musculature (musculus sphincter vesicae).[8] Thus, the muscularis propria muscle bundles of the trigone are more superficial and frequently show a gradual diminution in size, superficially attaining an almost suburothelial disposition.[4] Similarly, where the ureters are inserted, superficial smaller muscle bundles of the ureter are layered on the bladder detrusor muscle.[4] These variances in size and location of muscularis propria muscle may compound the pT stage assignment at these sites. [**Grade D**] At the trigone, our approach is to designate a tumour as muscularis propria–invasive if any of the thicker bundles, even within the superficial muscle layer, are involved.

These regional variations have a potential impact on bladder cancer prognosis. Approximately one-third of bladder tumours arise from the trigone, bladder neck, and ureteral orifice regions, and a slightly greater number from the lateral walls.[9] Early data suggest that tumours in the trigone and bladder neck have greater risk for progression.[9–16] In a prospective study of almost 400 transurethral resections (TURs) with nonmuscle-invasive cancers treated with intravesical bacillus Calmette-Guérin (BCG) or chemotherapy, involvement of prostatic urethra, bladder neck, trigone, and posterior wall (designated as high-risk regions) were significantly related to a shorter recurrence-free interval.[16] Similarly, trigonal cancers showed a higher risk for lymph-node metastasis and decreased cancer-specific survival in multivariate analysis in a large cystectomy series.[12] Bladder neck and trigone tumours have also been associated with an increased risk for prostatic urethral involvement.[17] [**Level 3, Grade C**] Data are more limited and conflicting regarding the regional influence on muscularis propria sampling and adequacy in TUR specimens.[18,19] Two centres demonstrated that the lateral, dome, and anterior locations are independently associated with absent muscularis propria in TURs; another study showed that tumour location had no impact on sampling muscularis propria, negating any influence of regional variations in histology.[18,19]

Adipose tissue in the bladder is not restricted to perivesical adipose tissue. It is seen in 53% and 100% of cystectomies within the lamina propria and muscularis propria,respectively; it is located predominantly in the deep lamina propria (at or below the muscularis mucosae).[20] [**Level 3**] Thus in TUR specimens, adipose tissue in lamina propria or muscularis propria should not be misinterpreted as perivesical soft tissue in location and cancer involvement overstaged as pT3; this has serious management and prognostic implications.[2–4,20] The perivesical soft tissue is almost exclusively composed of adipose tissue with variable vascularity. Delineation of the perivesical adipose tissue from the deep (outer) muscularis propria is typically indistinct because muscle bundles of the latter haphazardly merge with the perivesical adipose tissue. Thus, substaging pT2b/pT3a of muscle-invasive tumours can only be performed in cystectomy specimens and by using low-power magnification, because the junction of muscularis propria and the perivesical adipose tissue is typically ill-defined.

2.3 Epithelial Changes and Flat Lesions

2.3.1 Urothelial denudation

Instrumentation and intravesical therapy are known contributors to denudation at cystoscopy or in bladder specimens. Another reason for denudation is UC *in situ* (CIS). The presence of extensive denudation in bladder biopsy samples with prominent vascularization should at least evoke the presence of CIS.[21] In cold-cup biopsies with "denudation" cystitis, more than half of patients had concurrent positive urine cytology, stressing the importance of performing concurrent cytology, which has high sensitivity for detecting UC.[22] Denudation may also have some implications in papillary urothelial lesions. Papillary urothelial lesions with extensive urothelial denudation are more often high-grade carcinomas.[23] In denuded papillary lesions, pathologists should carefully search for residual high-grade cells to avoid underdiagnosis. Denuded flat urothelial neoplasms, papillary urothelial neoplasms, or both may occur with prominent cautery artifact or in an anatomically confined area implicating iatrogenic or mechanical contributing factors for the epithelial exfoliation. Extensive or complete urothelial denudation in bladder biopsy samples must be reported. Correlation of denuded biopsy samples with concurrent cytology results may yield a positive diagnosis of malignancy in these patients. Urothelial denudation in cold-cup biopsies of cystoscopically abnormal areas in patients with prior CIS and without any recent intravesical interventions should be dealt with caution by urologists. [**Grade C**]

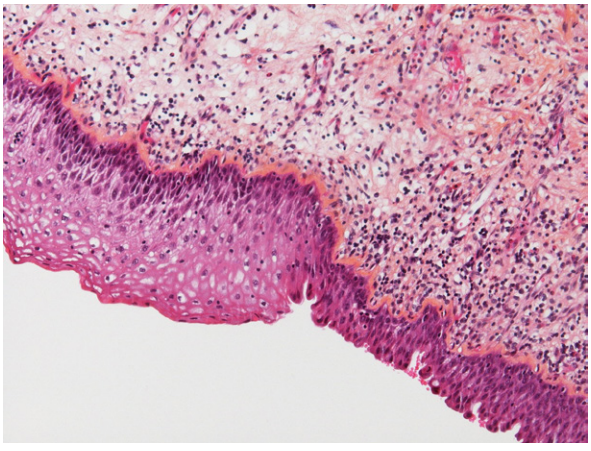
2.3.2 Metaplasia

Several types of metaplasia exist and must not be misdiagnosed.

2.3.2.1 Squamous metaplasia

The most frequent is squamous metaplasia (SqM) without keratinization, which is common in females (**Figure 2–1**). [**Level 2**] SqM with keratinization is characterized by the presence of parakeratosis, hyperkeratosis, and even granular layers. SqM has been considered a precancerous lesion by some authors, especially the keratinizing type. Guo *et al.* showed increasing numbers of squamous cell carcinomas after detection of SqM, but the number of cases was limited.[24] Keratinizing SqM should be considered a precancerous lesion and should be reported.[24] [**Level 3**] Human papillomavirus (HPV) is commonly absent.

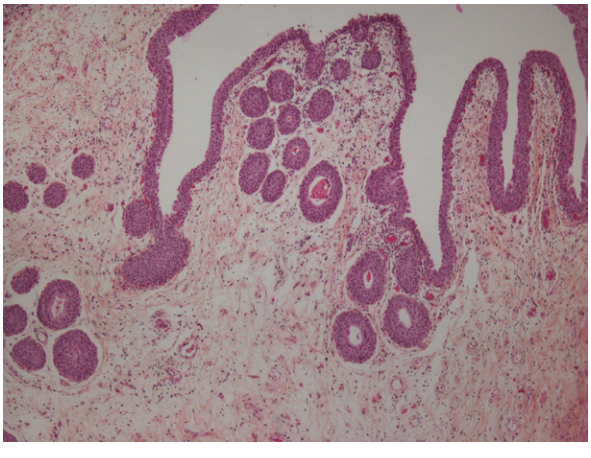
FIGURE 2–1
Squamous Metaplasia—Difference From Normal Urothelium



2.3.2.2 Glandular metaplasia

Glandular metaplasia (GM) might be seen on the surface of the urothelium as a response to chronic irritation or inflammation, such as neurogenic overactive bladder, bladder extrophy, long-term catheterization, or a history of calculi (**Figure 2–2**). Data on the value of GM as a precancerous lesion are discordant. Recent data confirm that intestinal metaplasia and cystitis glandularis involve divergent pathways.[25] Intestinal metaplasia is rarely a precursor lesion of adenocarcinoma of the urinary bladder.[26] [**Grade C**]

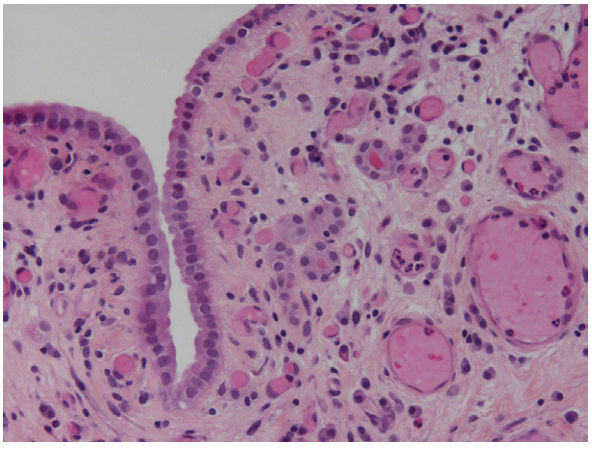
FIGURE 2–2
Glandular Metaplasia



2.3.2.3 Nephrogenic metaplasia

Nephrogenic metaplasia (NM) is seen in bladder walls with a history of injuries, such as previous urological instrumentation, predominantly in males. NM can mimic polypoid hyperplastic lesions, and at cystoscopy, it sometimes has a velvet-like appearance (**Figure 2–3**). Its recognition might be challenging in cases of inflammatory submucosa with swollen endothelial cells lining florid capillary proliferation, which may mimic or distort aspects. [**Grade C**]

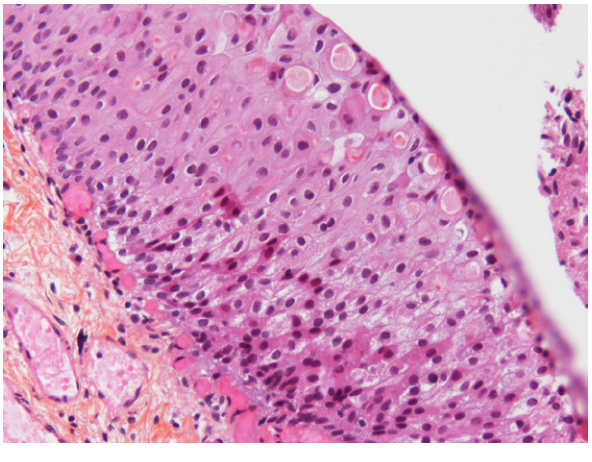
FIGURE 2–3
Nephrogenic Metaplasia



2.3.3 Urothelial proliferation of unknown malignant potential

When the urothelium is thickened (>7 layers), but lacks cytologic atypia, a diagnosis of urothelial proliferation of unknown malignant potential (UPUMP) (previously known as flat urothelial hyperplasia) should be considered. It may have some degree of nuclear enlargement and may surround low-grade pTa tumours (**Figure 2–4**). UPUMP is mostly seen in cases of follow-up of pTa tumours and is generally considered a minor finding. [**Level 3**] Several studies demonstrate genetic abnormalities already present in hyperplasia, such as a loss of heterozygosity of the FGFR-3 gene and deletion of chromosome 9 in 70% of UPUMP cases.[22,27,28] The inability to distinguish reactive hyperplasia from neoplastic hyperplasia by morphology criteria is the reason why the WHO 2016 changed its terminology.[29]

FIGURE 2–4
Urothelial Proliferation of Unknown Malignant Potential

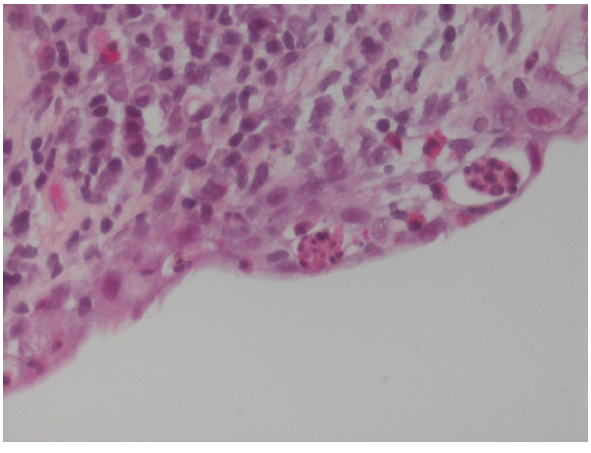


2.3.4 Flat lesions with atypia

2.3.4.1 Reactive atypia

Reactive atypia (RA) is sometimes difficult to classify and is not standardized in classifications. The inflammatory context usually helps to correctly diagnose these lesions and not to misdiagnose them as CIS (**Figure 2–5**). Increasing size of nuclei, with a globally preserved architecture and increasing cellular density, are common, as well as increasing vascularization.[30,31] Several instances of RA with mitosis have been described, but they should be confined to the lower layers of urothelium. Atypical mitoses would rule out reactive atypia.[31] [**Level 2**] A pitfall may be denuded areas or atrophy of the urothelium. Contrary to CIS, no increasing ratio between nucleus and cytoplasm, hyperchromatic nuclei, or membrane irregularity are observed.[32] If RA is suspected, clinical history, such as a history of previous biopsies, is very important to avoid their overdiagnosis.[33]

FIGURE 2–5
Reactive Atypia With Intraurothelial Abcedation



2.3.4.2 Urothelial atypia of unknown significance

This entity is not very well defined and one should reserve this terminology only for very challenging cases with considerable inflammation, where one cannot entirely exclude CIS or dysplasia. [**Grade D**] This diagnosis should only be rarely made. Urologists must be aware that this entity needs anti-inflammatory treatment and new biopsies to allow a final diagnosis.

2.3.4.3 Therapy-associated atypia

2.3.4.3.1 Truncated papillae

These kinds of lesions are not well known by many pathologists and they have been described after mitomycin-C therapy. The top of the papillae is destroyed by chemotherapy. Together with inflammation and denudation, truncated papillae should not be mistaken as CIS.[32,34] [**Level D**]

2.3.4.3.2 Treated papillary carcinoma/granulomas

After BCG therapy, granulomas containing epithelioid histocytes and multinucleated giant cells are common. When sending biopsies or resection material after treatment, it is important to include the clinical history of the patient, otherwise, differential diagnosis with urogenital tuberculosis might be difficult in extensive necrotic cases. [**Level D**]

2.3.4.3.3 Radiation/chemotherapy cystitis

The microscopic changes (pseudocarcinomatous hyperplasia) are very impressive, but theycan be misleading. These lesions may be indistinguishable from micro-invasive carcinoma in the absence ofclinical history. Radiation cystitis may persist for years after therapy.[35,36] [**Level C**]

2.3.5 Dysplastic lesions

Dysplasia (Dy) is one of the major problems if isolated. Dy is characterized by nuclear and architectural abnormalities with normal thickness of the urothelium.[30,37] Its cytonuclear atypia falls short of a diagnosis of CIS. Dy is commonly seen in patients with bladder neoplasia. In Dy patients without associated bladder carcinoma, the risk of developing cancer has been estimated at only 19% in an 8-year mean follow-up when Dy are present, while it has been observed in 32% to 83% of patients when associated with CIS.[38,39] [**Level 2**] One possible explanation is the coexistence of two main molecular pathways of bladder tumour progression involving p53 (from flat dysplasia to CIS) or loss of heterozygosity on chromosome 9 (from Dy to low-grade papillary carcinoma, but no CIS), with common interactions and overlapping.[38] Two main differential diagnoses remain: RA and CIS. Immunohistochemistry may be helpful to distinguish inflammation and CIS to a certain point, but probably will not resolve the problem completely.[40,41] Several studies have demonstrated increasing incidence of Dy if associated with CIS or papillary carcinoma, but when it is isolated, there is a dearth of recent studies concerning its prognosis.[42,43] [**Level 3**] One reason for the lack of data may be the problem of reproducibility in diagnosing Dy.[22,29,44,45] Urologists generally do not treat patients with isolated Dy, but may monitor the patient by cytology.

2.3.6 Carcinoma *in situ*

CIS is defined as a flat noninvasive lesion characterized by the presence of cells with large, irregular, hyperchromatic nuclei that may be either present in the entire thickness of the epithelium or only a part of it, or even only in von Brunn nests.[42,46] CIS is always a high-grade lesion. The strictest criteria include: loss of polarity, loss of cytologic glycogen, nuclear enlargement, increased nuclear:cytoplasmic ratio, eccentric positioning of nuclei with nuclear clustering, nuclear pleomorphism, coarse granular and irregularly distributed chromatin, and a few large nucleoli. Mitosis may be present within the whole thickness of the urothelium (**Figures 2-6 and 2-7**). These criteria can be complemented with a panel of immunohistochemical antibodies (CD44, CK20, AMACR, and others; see Chapter 2, Section 7), but only help to distinguish between reactive–regenerative atypia and CIS.[24] Several different histological patterns of CIS exist, but it is not essential to subclassify CIS in the pathology report, because different patterns do not make any difference in clinical follow-up.[40,47] Different patterns can coincide in the same patient.[48] In rare cases, the phenotype of the CIS looks like an *in situ* adenocarcinoma or an *in situ* squamous cell carcinoma.[49]

Diagnosis of isolated CIS is not uncommon; its features are well defined and pathologists are familair with CIS. It is generally associated with a positive urine cytology. Frequently, denuded urothelium exists, underlining the discohesive nature of tumour cells. Owens showed in a study that the majority of denuded lesions were of high grade. In cases of significant denudation, urinary cytology may be helpful to determine diagnosis.[34] Nevertheless, pathologists should be careful, especially in cases of cautery artefacts or in anatomically confined areas.[50]

The finding of lesions composed of very short microscopic papillae coated with urothelium with the referred atypia can be considered CIS with incipient papillae. CIS of the urethra may extend into underlying prostate ducts, and from renal upper urinary tract into renal collecting ducts; both extensions must be considered as CIS. Development of true invasion is seen in 20% to 30% of the cases with worse prognosis among patients with primary CIS.[25,26] [**Level 2**]

Differential diagnosis may be difficult with RA if cells display few atypia. Clinical history of radiotherapy should be mentioned by urologists; otherwise, reactive changing linked to the treatment may be misinterpreted. One should also think of CIS extending to von Brunn nests, or exclusively being present in the latter. This aspect must not be mistaken for microinvasion.

FIGURE 2–6
Carcinoma *in situ*

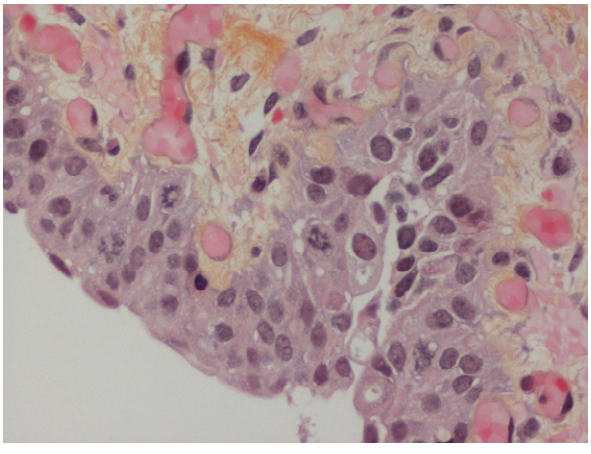
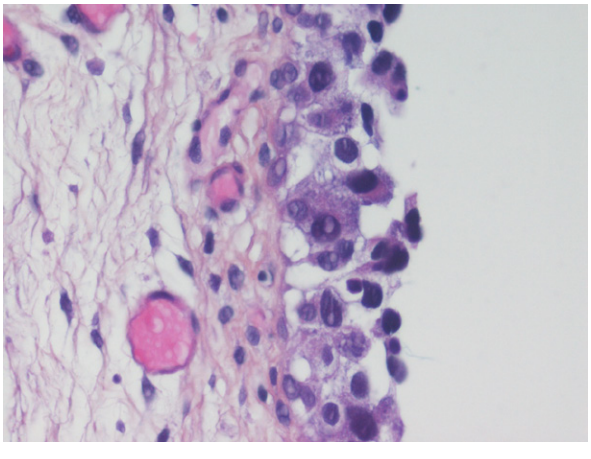


FIGURE 2–7
Carcinoma *in situ*



2.3.7 Flat lesions and immunohistochemistry

The distinction of UC *in situ* from urothelial dysplasia and reactive urothelial atypia can be challenging in biopsy and TUR specimens. A role for immunohistochemistry as an adjunct in this differential diagnosis has been explored using a variety of immunohistochemical markers with variable success. The most frequently used markers have been cytokeratin 20, p53 protein, and CD44s.[40,41,51,52] Diffuse expression of CK20 is present in 72% to 100% of cases, a pattern not seen in RA. [**Level 2**] Strong and diffuse expression of p53 can be found in up to 80% of cases of CIS; however, interpretation can be difficult, as Dy and RA can show positive cells. There is loss of normal expression of CD44s in up to 70% of cases, a finding not seen in reactive lesions. These markers have also been applied in cases of post-radiation atypia, post-chemotherapy, and following BCG therapy, with similar results.[53,54] Proliferation, as determined by Ki67, has also been studied and is typically very high in CIS; however, there is too much overlap with Dy and RA to make this a reliable marker in individual cases.[41,55,56] If immunohistochemistry is going to be used in this differential diagnosis, the International Society of Urological Pathology (ISUP) recommends using a panel of markers, as no single marker is sensitive or specific enough to be relied on.[57]

2.4 Grading of Bladder Cancer

2.4.1 Updates on grading of noninvasive and invasive urothelial carcinoma of the urinary bladder

Currently, two grading systems for bladder cancer co-exist: the 1973 WHO and the 2004 WHO/ISUP grading systems. Grading of bladder cancer has most clinical relevance for nonmuscle-invasive bladder cancers. Current guidelines of the most influential urological societies (European Association of Urology [EAU], American Urological Association [AUA]) differ in their recommendation on grading of nonmuscle-invasive bladder cancers.[58,59] The EAU continues to recommend the pathology reporting of both the 1973 and 2004 WHO grading systems for nonmuscle-invasive bladder cancer whereas the AUA merely mentions that the WHO/ISUP 2004 classification, which designates tumours as "low-grade" or "high-grade," is currently the most widely used system in the US. The EAU recommends the use of 1973 and 2004 grading in parallel by pointing to the limited evidence that one of the systems would be superior to the other. Further, their European Organisation for Research and Treatment of Cancer (EORTC) risk tables of bladder cancer makes use of the WHO 1973 grading system.[60] [**Level 2**] Although the AUA refers in their guideline to the EORTC risk calculator, they also present their own risk stratification system for nonmuscle-invasive bladder cancer based on WHO 2004 grading.[59] The AUA panel states that the AUA risk stratification lacks evidence regarding its actual impact on disease outcome.

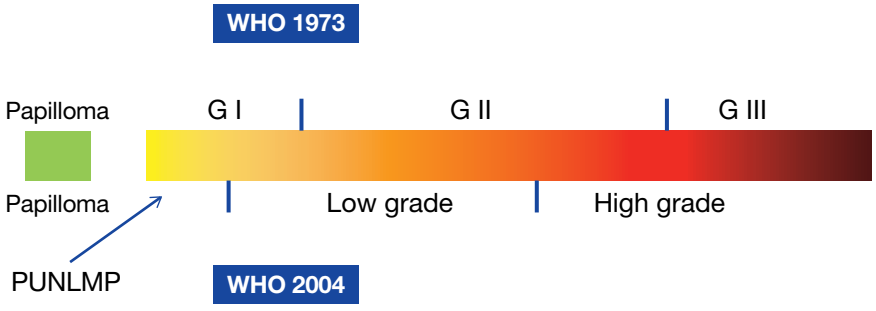
Since its introduction in 1998, the different components of the 2004 WHO (1998 ISUP/WHO) grading system for urothelial neoplasms have been the subject of several clinicopathological validation studies, observer variability studies, and comparative analyses with the earlier 1973 WHO grading system. The main criticism of the 1973 grading system was 1) lack of detailed grading criteria resulting in low inter-observer reproducibility; 2) predominance of absolute number of patients showing progression to muscle-invasive bladder cancer in the grade 2 category, rather than the grade 3 category; and 3) the attribution of the carcinoma label to the subset of bladder tumours considered at very low risk of progression.[61] Further, the use by some pathologists of intermediate categories, such as grade 1 to 2 or grade 2 to 3, when they encountered grade heterogeneity within a tumour was thought to further diminish the value of the 1973 WHO grading.

The 2004 WHO grading system tried to address the lack of reproducibility among pathologists by 1) essentially reducing the number of carcinoma categories to two grades, a low-grade and a high-grade category and 2) by specifying in greater detail the various categories. The first was achieved by labelling the category of noninvasive papillary urothelial neoplasms lacking cytonuclear atypia as papillary urothelial neoplasm of low malignant potential (PUNLMP) instead of carcinoma. It was expected that a lower recurrence rate and negligible progression rate in this category of patients might lead to a de-intensification of their monitoring. Finally, the anomaly of the 1973 WHO grading that most patients (in absolute numbers) with disease progression were in the grade 2 category was solved by the two-tier 2004 WHO carcinoma grading, with the vast majority of "progressors" placed in the high-grade category. It was further thought that the 2004 WHO grading would result in a

better correlation of histopathology and cytology, assuming that the entire category of high-grade carcinomas would be detectable by urine cytology, whereas low-grade carcinomas would be mostly undetectable.

Importantly, due to differing histopathological criteria of the 1973 and 2004 WHO classifications, the grades of one system cannot be directly translated into the other and their correlation would require a pathology review (**Figure 2–8**). Therefore, the exclusive use of the 2004 grading would prevent clinicians from using the EORTC risk tables, which became available around the same time, as an important clinical decision-taking tool for nonmuscle-invasive bladder cancers.[60]

FIGURE 2–8
Comparison of the World Health Organisation 1973 and World Health Organisation/ International Society of Urological Pathology 2004 Grading System



Analysis of some studies directly comparing the prognostic impact of the two grading systems follow. Some recent reviews on bladder cancer grading on nonmuscle-invasive bladder cancers combined noninvasive (pTa) and invasive (pT1) bladder cancers. Based on their systematic literature review, Soukup *et al.* concluded that both grading systems predict progression and recurrence, although pathologists vary in their reporting.[62] [**Level 2**] Since clinical outcome is influenced strongly by pathological stage, for this review, studies allowing a separate analysis of stage-specific grading were selected.

2.4.2 Grading noninvasive (pTa) papillary urothelial neoplasms: tumour distribution and impact on outcomes of World Health Organisation 2004 grading versus 1973 grading

Table 2–1 gives the distribution of noninvasive papillary urothelial neoplasms according to the 2004 WHO/ISUP and 1973 WHO grading systems.

TABLE 2–1 Distribution of Noninvasive Papillary Urothelial Neoplasms According to 2004 World Health Organisation/International Society of Urological Pathology and 1973 World Health Organisation Grading Systems

Study	Total	2004 WHO / ISUP				1973 WHO			
		Papilloma (%)	PUNLMP (%)	LG (%)	HG (%)	Papilloma (%)	G1 (%)	G2 (%)	G3 (%)
Cao 2010	172	-	0	65 (36)	117 (64)	-	44 (26)	121 (70)	7 (4)
May 2010	200	-	1 (0.5)	149 (74)	50 (25)	-	82 (41)	109 (54)	9 (5)
Burger 2007	114	-	6 (5)	77 (71)	26 (24)	-	58 (53)	46 (42)	5 (5)
Schned 2007	504	-	179 (38)	214 (46)	73 (16)	-	295 (58)	154 (31)	55 (11)
Yin and Leong 2004	84	3 (4%)	32 (38)	46 (55)	3 (4)	0	12 (14)	53 (63)	19 (23)
Samaratunga 2002	134	3 (2%)	29 (22)	73 (54)	29 (22)	7 (5)	42 (31)	79 (59)	6 (4)
Oosterhuis 2002	322	18 (5)	116 (36)	141 (44)	45 (14)	2 (1)	31 (9)	286 (84)	1 (1)
Pan 2010*	1,006	-	212 (21)	603 (60)	191 (19)				
Range		2-5%	0-38%	36-74%	4-64%	0-5%	9-58%	31-84%	1-23%

**This study did not compare 2004 and 1973 WHO grading, but was included in the table because it represents the largest reviewed series of nonmuscle-invasive bladder cancers.*

Abbreviations: G1, grade 1; G2, grade 2; G3, grade 3; HG, high grade; ISUP, International Society of Urological Pathology; LG, low grade; PUNLMP, papillary urothelial neoplasm of low malignant potential; WHO, World Health Organisation.

In most studies, low-grade papillary carcinoma represented the largest grade category by the 2004 WHO system which comprised 36% to 74% of the papillary tumours, and G2 (31%–84%) was the largest when 1973 WHO system was applied (**Table 2–1**). High-grade papillary carcinomas (2004 WHO) are generally more frequent than G3 (1973 WHO): in 6 of 7 series, high-grade papillary carcinoma constituted >10% of tumours, whereas G3 was comparatively less common and constituted >5% of tumours in only 2 out of the 7 studies. [**Level 2**] This is not surprising, since the 1973 WHO grade 3 constitutes the subset of high-grade carcinomas with the most aggressive (CIS-like) pathological features.

Maclennan *et al.* reported an incidence of PUNLMP of 12% to 39% in their literature review, with a corresponding recurrence rate of 25% to 60%.[61] Most studies show, as expected, a lower number of PUNLMP than G1 tumours. [**Level 2**] In summary, **Table 2–1** shows that the distribution of low-grade UC (36%–74%) and high-grade UC (4%–64%) seems to be more balanced than the three-tier 1973 grading system, as it confirms the strong preponderance of grade 2 carcinomas (31%–84%) as compared to grade 3 carcinomas (1%–23%). Differences in patient cohort geography may partly explain the broad differences in grade distribution of the cited studies, but the influence of the well-established variability in bladder cancer grading among pathologists is likely an important factor, too.

Since its introduction, several studies compared the impact on disease outcome of the 1998 ISUP/WHO (2004 WHO) system and the 1973 WHO system in the same patient cohort of noninvasive (pTa) papillary urothelial neoplasms.[63] Some studies have shown a limited advantage for the 2004 WHO grading system over the 1973 WHO grading system. Cao *et al.* reviewed 172 pTa (out of 269 nonmuscle-invasive tumours) with long follow-up (up to 10 years) and demonstrated near-significant differences between low-grade and high-grade papillary carcinoma in recurrence-free survival (p=0.05, log-rank test) and significant differences in progression-free survival (p=0.01, log-rank test).[64] Strikingly, no PUNLMP was identified in their study cohort. The 1973 WHO system, in contrast, showed significant difference only in progression-free survival (p=0.03, log-rank test). [**Level 2**] It should be noted that their series comprised only 7 pTa grade 3 carcinomas, limiting the value of this comparison. Yin and Leong reviewed 84 pTa tumours and showed significant differences in recurrence within 36 months between PUNLMP (17% recurrence) and low-grade papillary carcinoma (45% recurrence), and between low-grade papillary carcinoma and high-grade papillary carcinoma (74% recurrence) (p<0.5).[65] In contrast, no significant difference in recurrence was observed among the 1973 WHO grades (urothelial papilloma, grade 1, grade 2, and grade 3, with recurrence rates of 0%, 41%, 54%, and 67%, respectively). There were only 3 pTa cases with grade 3 carcinomas and no data on carcinoma progression were provided. Other studies did not show a clear-cut advantage in terms of impact to outcome for the 2004 WHO system over the 1973 WHO grading system. May *et al.* compared the prognostic implications of both WHO grading systems in 200 noninvasive tumours and with mean follow-up of 72 months using consensus diagnosis of 4 genitourinary pathologists.[66] Interestingly, no PUNLMP was identified in their series. They demonstrated a significant difference in 5-year recurrence rate between low-grade and high-grade papillary UC (2004 WHO), but 5-year progression-free survival was not significantly different. Similarly, grades 1, 2, and 3 (1973 WHO) pTa carcinomas had significantly different 5-year recurrence-free survival rates, as well as 5-year progression-free survival rates. Schned *et al.* reviewed 504 noninvasive tumours (mean follow-up 7.2 years) and similarly showed a gradient of progressively lower overall survival times from the lowest to the highest grade of tumours in both 2004 and 1973 WHO grading systems. The study, however, did not provide information on recurrence, progression, or disease-specific survival of these cases.[67] Samaratunga *et al.* investigated 134 patients with pTa tumours and showed no statistically significant difference in recurrence rates among the 1998 ISUP/WHO grades.[68] The only statistically significant difference in the 1973 WHO system was that grade 3 had increased recurrences per year compared with papilloma (p=0.02), grade 1 (p=0.0001), and grade 2 (p=0.0001). Separate analyses showed both 2004 WHO system and 1973 WHO system independently predicted progression (p=0.003 and p=0.002, respectively) together with tumour size.

The study by Hölmang *et al.* confirmed the usefulness of the 2004 WHO grading system, but also highlighted a potential advantage for the 1973 WHO grading system.[69] Some 363 primary pTa tumours with follow-up of at least 5 years were classified according to the 1998 ISUP/WHO grading system.[63] Recurrence rates were significantly less frequent in PUNLMP compared to low-grade papillary carcinoma and most frequent in high-grade papillary carcinoma. Progression was 0%, 4%, and 23% for PUNLMP, low-grade papillary carcinoma, and high-grade papillary carcinoma, respectively (p<0.0001). However, when the 108 high-grade papillary carcinomas where further subdivided by 1973 WHO system into grade 2 (95) and grade 3 (13), there was a significant difference in progression of 20% and 45%, respectively (p<0.0022). Lokeshwar *et al.* reported a significant grade shift in

their population of Ta bladder cancer patients upon implementation of the 2004 WHO grading in their institution.[70] This shift was not parallelled by a change in disease progression, implying that the change in grading system would negatively impact patient management, morbidity, and costs.

To date, only 3 studies have compared the prognostic value of the two grading systems for pT1 bladder cancers.[64,71,72] In the study by Cao *et al.*, 41 of 42 (98%) pT1 tumours were classified as high grade based on the 2004 WHO system.[64] By the 1973 WHO system, these high-grade tumours were classified into 27 (64%) grade 2 and 15 (36%) grade 3. No significant differences were noted in recurrence-free survival, progression-free survival, or overall survival between grade 2 and grade 3 pT1 tumours. The very low frequency of low-grade pT1 carcinomas precluded an assessment of the impact of the 2004 WHO grading in this small series of pT1 cancers. The next two much larger studies on 134 and 310 pT1 bladder cancer patients confirmed that low-grade pT1 carcinomas were very uncommon, reducing the 2004 WHO grading essentially to a single grade system. One study demonstrated that 1973 WHO approached significance as a prognosticator for progression to muscle-invasive carcinoma, whereas the other larger study demonstrated that the 1973 WHO grading was a prognosticator for disease-specific survival, with 10-year disease-specific survival of 96% for grade 2 and 78% for grade 3 pT1 bladder cancers ($p<0.001$).[71,72]

For muscle-invasive bladder cancers, the prognostic impact of grading is even more limited than in pT1 bladder cancers. The WHO 2016 classification recognizes that the overwhelming majority of invasive bladder cancers are high grade (2004 WHO), but some may be labelled as low grade, such as the nested variant.[73] Since the nested variant is considered an aggressive bladder cancer, this may be considered as an anomaly; to that end, the latest International Consultation On Urologic Disease (ICUD) recommends categorizing all invasive bladder cancers, irrespective of extent of invasion, as high grade.[74]

In terms of impact on disease outcome, both grading systems provide important prognostic information in most of the published studies of noninvasive papillary urothelial neoplasms, with the 2004 WHO generally demonstrating essentially similar results as the 1973 WHO grading. Most importantly, far more patients with noninvasive bladder cancer are now in the high-grade category as compared to grade 3 (1973 WHO), resulting in more patients undergoing BCG treatment.[70] There is evidence suggesting that high-grade papillary carcinoma is heterogeneous in terms of outcome, and subdivision into grade 2 and grade 3 by the 1973 WHO system may provide additional prognostic information for the pT1 bladder cancers.[72] In addition to clinicopathological arguments to distinguish a subset of high-grade UCs at the aggressive (CIS-like) spectrum, molecular genetic data seem to support this notion.[75,76] This data may be helpful in future refinements of the WHO grading system to increase the predictive power of high-grade tumours focusing on morphologic or molecular subcategorization, or both, while eliminating the need for two distinct grading systems. [**Level 3**]

2.4.3. Interobserver and intraobserver variation studies

Most published observer variability studies show that both 2004 and 1973 WHO grading systems for bladder urothelial neoplasms suffer from substantial interobserver agreement among pathologists, with only moderate agreement.[77] Among the WHO 2004 grades, the distinction of PUNLMP from low-grade papillary carcinoma appeared to be the most difficult. In an interobserver variation study of four pathologists, the frequency of PUNLMP ranged between 10% and 47% with a recurrence risk varying from 47% to 69%.[78] Condensing PUNLMP and low-grade papillary carcinoma would obviously improve the grading reproducibility of the 2004 WHO system, but agreement still remained low (with kappa values between 0.33 and 0.73), according to one paper.[78]

Reasons for lack of reproducibility among pathologists for bladder cancer grading is the lack of clearly definable hallmarks in the wide spectrum of gradual cytonuclear changes, varying from entirely bland to highly anaplastic, and the equally gradual transition from no disorder to complete disarray in urothelial neoplasms. Another reason for interobserver variability may be tumour heterogeneity: for instance, it is not clear whether a very small focus of high-grade carcinoma should be accounted for amid predominantly low-grade papillary tumours (see Chapter 3, Section 3). It is likely that pathologists working in the same pathology department display less variability in grading as compared to pathologists of separate laboratories, because in the former situation, internal consultations may help reduce this variability. Web-based learning using image depositions of various bladder cancer grades may help resolve this issue. The few studies on intraobserver reproducibility showed no differences between the 2004 and 1973 grading systems, since both showed moderate to substantial agreement.[79] Summarizing, interobserver variation remains an important challenge for the grading of bladder cancer. Particularly, the distinction of PUNLMP from low-grade UC remains difficult.[78] [**Level 3**]

2.4.4. Impact of grade heterogeneity

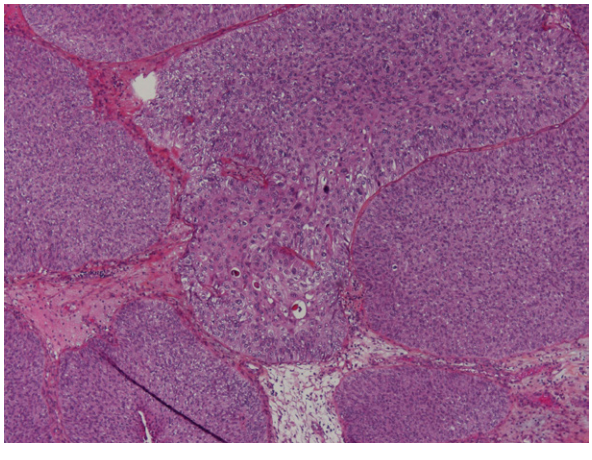
Admixture of at least two different grades in a papillary urothelial neoplasm is not uncommon,, and is reported in 3% to 43% of tumours (**Figure 2–9**).[80–82] The 1998 ISUP consensus, cognizant of urothelial tumours with variable histology, suggests that grade should be reported according to the highest grade present in heterogeneous tumours.[83] The WHO 2016 classification also mentions that the conventional approach is to grade a bladder cancer based on the highest grade component, but cites a lack of consensus on whether to use a threshold of the percentage of high-grade cancer.[21] Some studies used a 5% minimum cut-off, while another study found no difference in 5-year progression-free and disease-free survival at a threshold of 10%.[66,84] An argument to report the presence of a minor (<5%) high-grade component in an otherwise low-grade bladder cancer would be the presence of distinct genetic abnormalities associated with aggressive cancer even in the low-grade component.[85] [**Grade 3**]

A few authors investigated the prognostic impact of the presence of two grades when at least 5% of carcinomas had a grade different than the predominant grade, by examining whether prognosis was determined by worst grade or by combining the grades and assigning a separate mixed grade. Cheng *et al.* noted a significantly more favourable outcome of mixed low-grade and high-grade pTa carcinomas as compared to pure high-grade carcinomas ($p<0.02$), but a drawback of their study is the wide range in therapies given to their patients.[82,86,87] In a series of 153 nonmuscle-invasive bladder cancers, Schubert *et al.* investigated the impact of grade heterogeneity on response to BCG treatment, comparing those with less than 50% high-grade component to those with pure high-grade carcinomas.[87] The 50% cutoff was chosen because the paper by Cheng *et al.* suggested that those with more than 50% high-grade carcinomas would behave worse.[82] About 88% of patients with a mixed grade (<50% high-grade component) responded to BCG versus only 54% in the pure high-grade category ($p=0.03$). [**Grade B**]

For grade heterogeneity in muscle-invasive carcinomas, Kruger *et al.* showed a very limited prognostic impact when they examined 151 muscle-invasive bladder carcinomas.[86]

Summarizing, there is a lack of good outcome data, both on the use of a cutoff level for a high-grade component to be included in the final grade and on the prognosis of mixed-grade carcinomas. Therefore, it is not possible to make a strong recommendation regarding the reporting of mixed-grade, nonmuscle-invasive bladder cancers. The 2004 and 2016 WHO systems recommend grading of heterogeneous tumours based on the highest grade present in a tumour, but do not provide a cutoff value required to upgrade a predominantly low-grade nonmuscle-invasive bladder carcinoma. [**Level 3**] A reasonable approach would be to perform grading based on the highest grade present and in those cases where the high-grade component is less than 10%, this observation should be communicated to the clinician in the pathology report.

FIGURE 2–9
Heterogeneous Lesion With 10% High-grade Lesion in the Middle Part



2.5 Histological Description of pTa Tumours

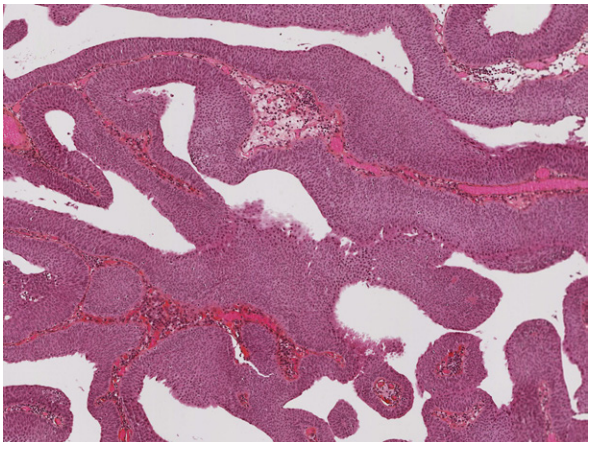
2.5.1 Noninvasive papillary urothelial carcinoma

Three different categories can be reported in the pTa group of bladder neoplasiaL PUNLMP, pTa low-grade tumours, and pTa-high grade tumours.

2.5.1.1 Papillary urothelial neoplasm of low malignant potential

The PUNLMP category was adopted by the 2004 WHO; it does not carry the label "cancer," which is important, considering that PUNLMPs are frequently present in younger patients, who would have to carry the diagnosis for their whole life, with all the psychological and—in some countries—financial consequences implied. PUNLMP should only be applied to a very restricted group of tumours and criteria should be applied in the strictest way. The papillae of this exophytic lesion are discreet, slender, and not fused, covered by mostly normal urothelial cells, with absent or minimal cytological atypia (**Figure 2–10**). The cell density can be slightly increased; polarity is preserved; nuclei can be increased, when compared to normal tissue. Umbrella cells are mostly present. Mitosis, if present, is the exception and only located in the basal layers. Necrosis is absent. The prognosis of these lesions is very good, the risk of recurrence is undeniably present, but the risk of progression is low. [**Level 2**]

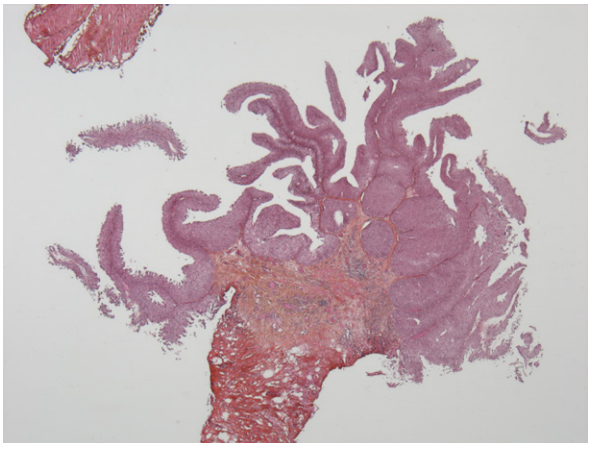
FIGURE 2–10
Papillary Urothelial Neoplasm of Low Malignant Potential



2.5.1.2 Noninvasive papillary low-grade urothelial carcinoma

The low-grade pTa carcinoma is characterized by orderly arranged papillae. Variations of polarity and nuclear size, shape, and chromatin distribution are not very important, but there exists a definitive cytological disorder (**Figure 2–11**). Mitosis is rare. Sometimes, with the problems of inclusion and tangential cutting, it can be difficult to analyze architectural aspects. They can be mistaken for irregular urothelium; fused glands can also be overgraded. Nevertheless, in case of different aspects in the same lesion, the highest grade should be considered. The histological differences between PUNLMP and low-grade pTa can sometimes be subtle. [**Level 2**]

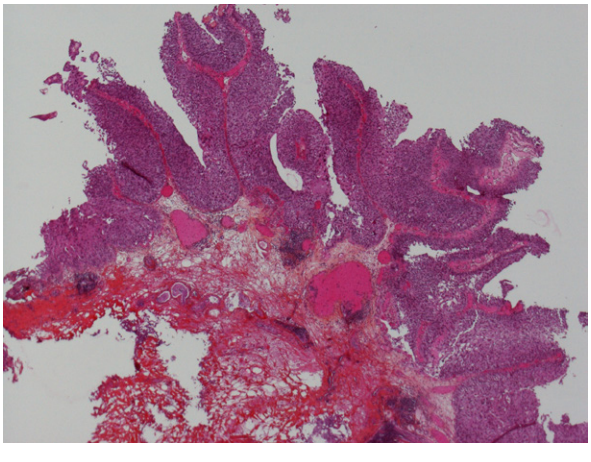
FIGURE 2–11
Transurethral Resection of the Bladder, Low-grade pTa



2.5.1.3 Noninvasive papillary high-grade urothelial carcinoma

High-grade lesions are characterized by sometimes totally disorderly appearance at low magnification. This disorder is linked to cytonuclear as well as architectural disorganization (**Figure 2–12**). The spectrum of pleomorphism can range from moderate to marked. The WHO/ISUP Bladder Consensus Conference Committee recommended a comment on the degree of nuclear anaplasia.[83] Nuclei have prominent nucleoli, nuclei are pleomorphic, and mitosis is frequent. Intraepithelial necrosis may be present. The thickness of the urothelium can vary considerably; the papillae are fused and display an anarchistic way of growth. High-grade tumours can be pTa, but also pT1 to pT4. Usually, pT2 to pT4 UCs are automatically considered high grade, according to the WHO 2004/2016 classification. All tumours classified in the 1973 classification as grade 3 as well as some assigned grade 2 in this classification belong to this entity. [**Level 2**]

FIGURE 2–12
Transurethral Resection of the Bladder, High-grade pTa

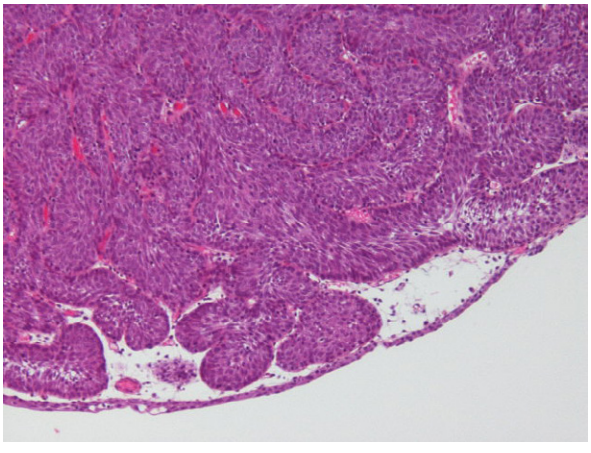


2.5.2 Inverted (endophytic) urothelial neoplasms

2.5.2.1 Inverted papilloma

Inverted papilloma is a distinct tumour composed of thin anastomosing cords and trabeculae of bland urothelial cells within the lamina propria, and typically covered by a normal or attenuated urothelial lining without cytologic atypia, but peripheral nuclear palisading. Surrounding stromal reaction is lacking as well as extension into the muscularis propria. Cells are bland; in rare tumours, areas typical of inverted papilloma may be admixed with an exophytic component identical to urothelial papilloma.[88,89] Inverted papilloma is considered a benign neoplasm with a low recurrence rate of less than 2% (**Figure 2–13**).[88–91] [**Level 2**] A similarly benign course has also been reported in tumours showing mixed inverted and endophytic components.[88,89]

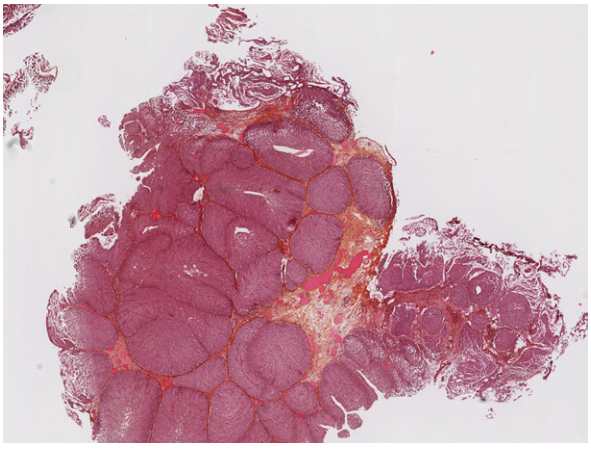
FIGURE 2–13
Inverted Growth, Inverted Papilloma



2.5.2.2 Inverted noninvasive urothelial neoplasms

PUNLMP, noninvasive low-grade papillary UC, and noninvasive high-grade papillary UC can have variable degrees of inverted or endophytic growth component, which refers to the presence of noninvasive urothelial nests within the lamina propria. By definition, those nests should not involve the muscularis propria and do not display the typical features of invasion. Tumours harbouring an inverted component are graded by applying the same cytological criteria used in papillary tumours, regardless of the extent of the inverted component. In tumours showing an inverted component, the descriptive term "with inverted growth pattern" can be added to the diagnosis in order to account for its presence (for example, noninvasive low-grade papillary UC with inverted growth pattern). In tumours predominantly or exclusively composed of inverted growth pattern, terminologies such as inverted PUNLMP, inverted noninvasive low-grade papillary UC, and inverted noninvasive high-grade papillary UC have been proposed (**Figure 2–14**).[92] The clinical significance of the inverted growth pattern in noninvasive urothelial tumours has not been fully studied. In one large study conducted on PUNLMP, 6% were inverted PUNLMP (12/189), and none of them had a documented recurrence or progression on follow-up.[92] In comparison, the same study reported 20.1% recurrence rates and 11.1% grade progression rates in usual PUNLMP (noninverted). [**Level 2**]

FIGURE 2–14
Inverted pTa Tumour, Endophytic and Exophytic Growth



2.5.2.3 Inverted invasive urothelial neoplasms

Although most invasive UCs show the classic features of invasion, a minority invades in the form of endophytic nests overlapping with noninvasive inverted tumours (**Figures 2-15 and 2-16**). Two patterns of invasion have been described: the first, which takes the form of cords and nests with slender trabecular formation, and occasional anastomoses, mimics inverted papilloma and has been referred to in the literature as invasive UC with inverted papilloma-like pattern;[93] The second is that of invasive nests of medium to large size with overall smooth contour, and absent or minimal stromal reaction, mimicking noninvasive papillary urothelial neoplasms with inverted growth component. Many terms have been proposed to describe such cases, including invasive UC, "the inverted variant," "with broad front," or "large nested variant."[93–95] The degree and extent of nuclear atypia in those lesions varies and as such, the differential diagnosis is with noninvasive low-grade as well as high-grade UCs. In that regard, the term "large nested variant" has been recently retained in the latest WHO bluebook under the "nested UC variant" section to describe similar tumours at the architectural level and displaying only minimal to mild nuclear atypia.[21,95]

Overall, inverted invasive UC are distinguished from noninvasive lesions by the presence of one or more of the following features, the extent of which varies in individual cases: muscularis propria invasion; component of usual invasive UC; irregular ragged nests contour; retraction artifact; stromal reaction; or lymphovascular invasion. Although data about the clinical behaviour of those tumours is still scarce, recent studies seem to indicate that they do not behave differently than usual invasive UC.[94–96] [**Level 3**]

FIGURE 2–15
Large Nested Urothelial Carcinoma With Bland Features, but Invading the Detrusor Muscle

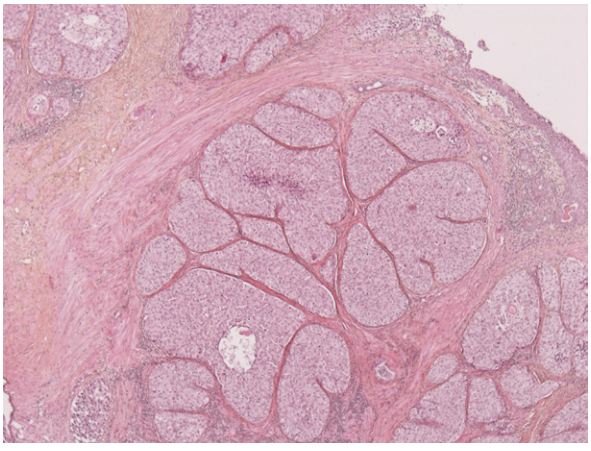
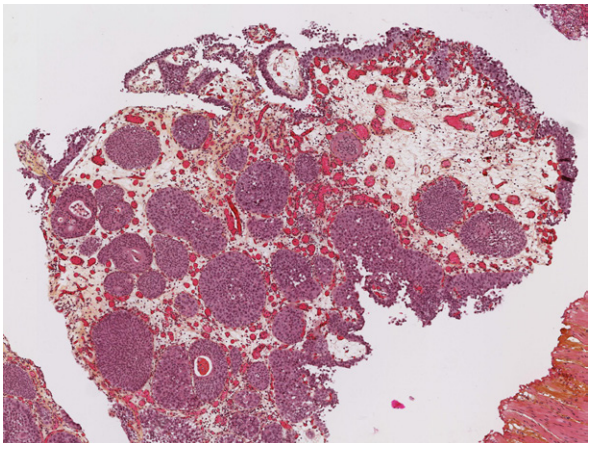


FIGURE 2–16
High-grade Lesion Associated With Endophytic Growth (difficult to judge invasiveness)



2.6 T1 Bladder Cancer

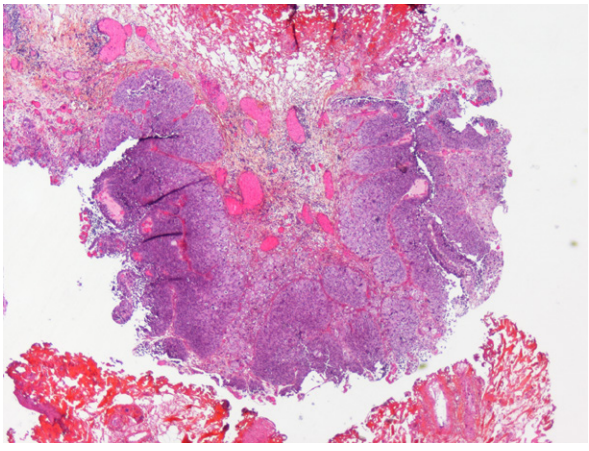
2.6.1 Definition of invasiveness

For a pathologist, the term "invasive UC" indicates the presence of invasive tumour nests beneath the urothelial basement membrane including invasion limited to the lamina propria (pT1). Urologists, however, may use this term in their daily practice to refer to muscle-invasive disease (pT2). Nonmuscle-invasive bladder cancer (NMIBC) is clinically regarded as one disease with different risk categories and treatment modalities. Within NMIBC, the 2016 WHO classification of bladder cancers distinguishes pTa and pT1 tumours. As pT1 tumours are known to recur and progress more frequently than their pTa counterparts, urologists may tend to treat pT1 bladder cancer more aggressively, and early cystectomy may even be considered for pT1 tumours with high-risk features, such as those associated with CIS or aggressive UC variants.[97] [**Level 3**] Therefore, accurate staging of NMIBC and identifying potential progressors among patients with pT1 tumours is of major clinical importance.

Criteria of infiltration of the lamina propria are: a) small nests with irregular contours and cords of neoplastic cells; b) absence of basement membrane with loss of the capillaries that are aligned in it; c) eosinophilia of the cytoplasm of the cells; and d) the reaction of the surrounding stroma, including the retraction of the stroma (**Figure 2–17**). For the correct evaluation of the extension, it is advisable to: a) evaluate only the areas with perpendicular section to the basement membrane; b) avoid the evaluation of artifact areas by crushing; and c) avoid the evaluation in previously resected areas, since the cicatrization rendersthe anatomical recognition of the anatomic layers impossible.

The recognition of early lamina propria invasion (pTa vs. pT1) can occasionally represent major challenges.[98,99] [**Level 2**] Assessment of invasion of the lamina propria may be very difficult or even impossible in cases of superficial sampling of the tumour.[100] The presence of one or two scattered single tumour cells, lack of spatial orientation, and tangential sectioning all raise the question of whether invasion is truly present. Furthermore, tumours with endophytic or inverted papilloma-like growth contours also represent a diagnostic challenge.[14] Von Brunn nests or benign proliferative urothelial cells may also show pseudo-invasive features, and it may be difficult to distinguish from an invasive carcinoma such as the nested variant.[16] Fibrohyaline stroma surrounding capillary networks may also hinder the interpretation of the sample. In such a case, the recognition of subtle features of invasion may be compounded when von Brunn nests have been distorted by inflammation or cautery artefacts.[8] When small clusters are present, distinction between pTa and focally invasive carcinoma can be difficult. Nevertheless, it is worth emphasizing that the terminology of microinvasive cancer is obsolete. Diagnosing early or superficial invasion can be very challenging, but as soon as the tumour grows into the lamina propria, it must be considered a pT1 tumour. [**Level 2**]

FIGURE 2–17
pT1 Tumour Invading the Lamina Propria



2.6.2 Substaging of pT1 bladder cancer

In 1990 Younes *et al.* observed that high-grade carcinomas with invasion of the subepithelial tissue had different outcomes, according to the depth of invasion.[101,102] Therefore, a subdivision of category T1 was advocated. This observation has been repeatedly confirmed by other authors.[97,103–106] The largest study to date using muscularis mucosae as a landmark is the paper of Rouprêt *et al.* which included 587 pT1 cases with a single centralized pathologist's analysis.[97,104,107] They divided pT1 bladder cancers into pT1a and pT1b (above, or into or beyond the muscularis mucosae) (**Figures 2-18 and 2-19**). On multivariable analysis, pT1b tumours had a significantly worse recurrence-free survival, progression-free survival, and cancer-specific survival. Based on this study and a literature review, three findings can be underlined. First, the ability to identify muscularis mucosae in a TUR ranges from 58% to 100%. Second, on univariate analysis, muscularis mucosae invasion was a significant predictor of recurrence-free survival, progression-free survival, and cancer-specific survival in 30%, 88%, and 81% of the studies, respectively. Third, on multivariate analysis, the numbers were 25% for recurrence-free survival, 82% for progression-free survival, and 50% for cancer-specific survival.[107] However, there is a limitation in identifying the level of muscularis mucosae in the transurethral resection of the bladder (TURB) material, since it is not always present; instead, the thick venous vessels (plexus venosus) should be used as a surrogate.

A few other metrics have been proposed to quantify the depth and extent of subepithelial tissue.[108] These include the measurement of 1) the depth in mm (perpendicular to the surface) or 2) diameter (in any direction) of the invasive focus. The latter method defines microinvasion- or focal invasion (T1m) as a single invasive focus <0.5 mm invasion (within one high-power field, 400x) and T1 extensive-invasive (T1e) as ≥0.5 mm extensive invasion of lamina propria.[109] A review of several studies demonstrates that the latter approach performs well, as it is simple and applicable in nearly 100% of cases. In some studies, the maximum depth of invasion perpendicular to the mucosal surface correlated with recurrence and progression.[99] The limitation of this method is that it requires both a well-oriented specimen and a urothelial surface to measure from. The 2004 and 2016 WHO recommend substaging pT1 tumours, but does not indicate which method to use. The ICCR group considers that

two methods of reporting should be suggested to pathologists. Therefore, diameter and extension, or pT1a and pT1b (staging according to the muscularis mucosae), have been included as a "recommended" element to be enclosed in a TURB pathology report. [**Level 2**]

FIGURE 2–18
Invasive pT1a Tumour (the invasion is only in the upper part of the lamina propria)

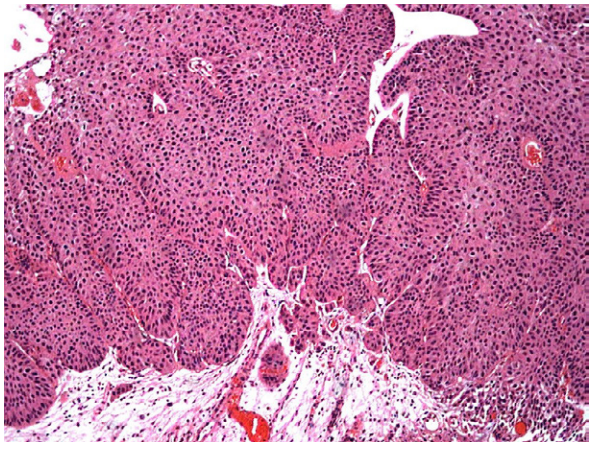
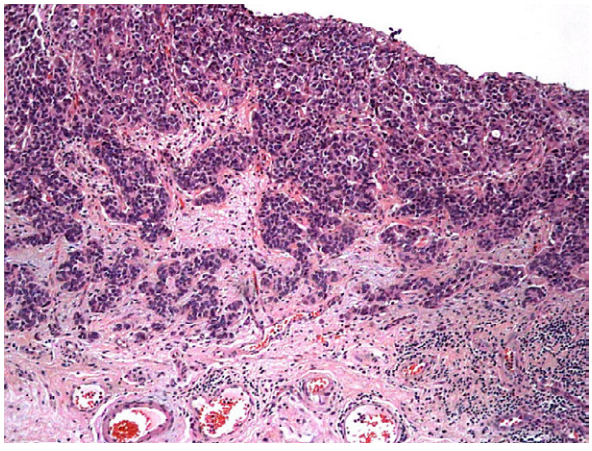


FIGURE 2–19
Invasive pT1b Urothelial Carcinoma (tumour growing into the muscularis mucosae)



2.6.3 Upstaging and downstaging

Several staging-related inter- and intraobserver reproducibility studies have been conducted.[99] Even in cases with good material without artefacts, interobserver discrepancy was still shown to exist among pathologists.[99,100] The reports of upstaging and downstaging of pT1 tumours in up to 55% of cases after central histopathological review are numerous. An average of 5% of pT1 cases were upstaged to pT2 and 14% of pT2 cases were downstaged to pT1 after central pathology review; up to 10% of pTa tumours were reclassified as pT1 and up to 50% of pT1 tumours were reclassified as pTa.[97,99] [**Level 2**] A recent study with internationally known uropathologists annotated lamina propria invasion on virtual slides in the selected difficult TURBs. A majority consensus in 72% of the 25 cases and a multi-rater kappa score of 0.47 was found.[99] [**Level 2**] Therefore, it is wise to recommend internal consultations for pT1 bladder cancers as a quality assurance method.[97]

2.7 Muscle-invasive Carcinomas and Cystectomy

Issues in staging can also be rarely encountered in radical cystectomies. As an example, the tumour may come in contact with the muscularis propria, without penetrating into it (pT1 vs. pT2). In this setting, it is recommended to keep the stage as pT1 (**Figure 2–20**). Another challenging scenario is distinguishing pT2b and pT3a in deeply invasive tumours that are surrounded by a fibrotic or desmoplastic stroma without infiltration and within adipocytes. Since the border separating the outer muscularis propria and the perivesical fat is not well delineated, evaluating tumours in this area can be subjective (**Figure 2–21**).[102] [**Level 3**] A recent paper showed that interpretation of cases with microscopic invasion of the perivesical fat is extremely challenging, as dense fibrosis, desmoplasia, obscuring inflammation, and lymphovascular invasion at the tumour border can influence the interpretation. This study highlighted the need to adopt common criteria for a definition of pT3a disease.[110]

In terms of gross handling and in contrast to the prostate, no official consensual guidelines have been published to date, with the exception of some rare available protocols.[111] Therefore, it is at the pathologists' discretion, how many and which samples to provide.

Furthermore and as already mentioned, some variant histologies are more difficult to evaluate than others. This is not solely related to the pathologist's ability, but to the subtle histological features that can be very difficult to recognize prospectively. As an example, the nested variant and the large nested variant may be difficult to differentiate from inverted growth pattern of noninvasive tumours, especially if the tumour does not involve the muscularis propria and if it lacks the definitive features of invasion.[94,95] These two variants display mild atypia and grow as well-delineated nests with mild or even absent surrounding stromal reaction. In such settings in which definitive invasion cannot be established, it is important to communicate the case's level of difficulty in the pathology report and to recommend a second resection in case the first resection did not show any muscle (**Figure 2–22**).[111,112]

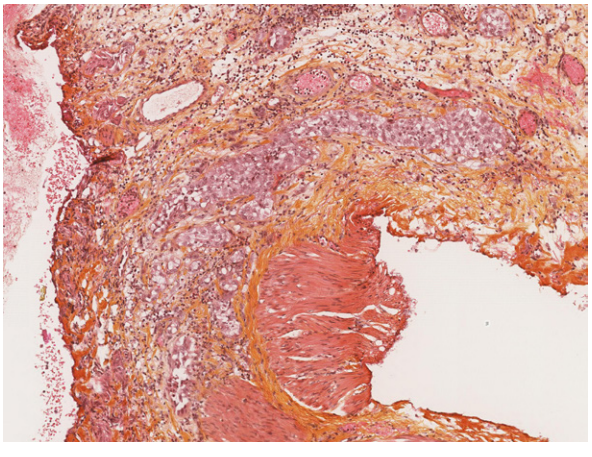


FIGURE 2–20
The Tumour in Contact With the Detrusor Muscle (no invasion, still considered as pT1)

FIGURE 2–21
Urothelial Carcinoma Beyond the Detrusor Muscle (but no real contact with the adipose tissue surrounding the bladder)

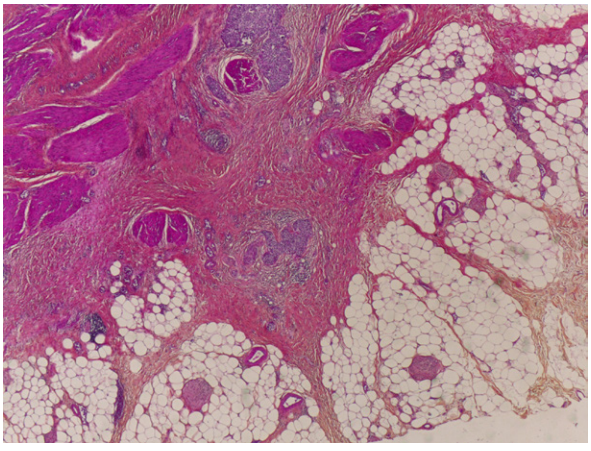
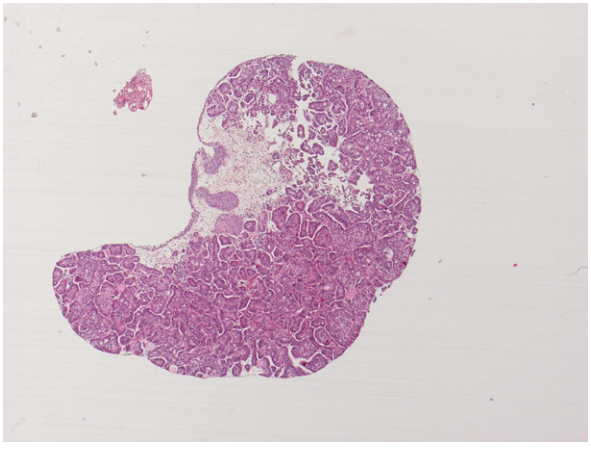


FIGURE 2–22
Transurethral Resection of the Bladder Without Muscle Layer



2.8. Diverticula

A well-known problem is staging in diverticula, a common finding in urology occurring more frequently in men. Two types of diverticula exist, the acquired and the congenital forms, the latter being rare. Development of UC in diverticula has been described in 1% to 14%, according to different studies.[113,114] While in congenital diverticula, muscularis propria is absent, in acquired ones, the muscularis propria is present, but extremely thinned and sometimes not easy to see. Therefore, only pTa, pT1, and pT3 tumours can be observed.[114] [**Level 2**] In If the pT2 stage cannot be given due to lack of detrusor muscle, the pathologist should be descriptive, and explain how far the tumour grows into the fibrotic area (for example, inner or outer half); no consensus exists about this issue. Interestingly, some data show that pT3a tumours seem to have the same outcome as in the bladder.[114] [**Level 3**] On the other hand, grade, multifocality, and hydronephrosis have been shown to be significant in patient outcome.[115]

2.9. Urachus

Urachal carcinomas can carry diagnostic challenges. These tumours are mostly present in the anterior bladder wall or in the dome and can extend to the umbilicus. Although different histological types can be seen, adenocarcinoma is the most frequent subtype. The WHO 2016 added for the first time a chapter on urachus and included the criteria suggested by Gopalanas as necessary to diagnose a urachal adenocarcinoma (**Table 2–2**). The currently recommended staging system for urachal carcinomas is the Sheldon system (**Table 2–3**).[116,117]

TABLE 2–2 Criteria for Diagnosis of Urachal Adenocarcinoma (adapted from Gopalan *et al.*[116])

1. Location of the tumour in the bladder dome and/or anterior wall
2. Epicentre of carcinoma in bladder wall
3. Absence of widespread cystitis cystica, cystica glandularis, or both beyond the dome or anterior wall
4. Absence of known primary elsewhere

The prognosis of the different histological subtypes is not well established. The 5-year survival ranges between 40% and 64%, independent of the staging system.[116,117] Tumours confined to the bladder have a better prognosis. [**Level 3**]

TABLE 2–3 The Sheldon Staging System for Urachal Carcinoma

Stage I		Confined to urachal mucosa
Stage II		Confined to urachus
Stage III	IIIA	Extension to bladder
	IIIB	Periurachal and vesical fat invasion
	IIIC	Extension to peritoneum
	IIID	Extension to viscera other than bladder
Stage IV	IVA	Metastases to regional lymph nodes
	IV	Metastases to other organs

2.10 Complementary Prognostic Factors

2.10.1 Lymphovascular invasion and pT1

Approximately 10% of pT1 urothelial cell carcinomas have lymphovascular invasion, but it seems that this invasion has statistically significant prognostic value only in lymph node–negative cases.[100,105]

The criteria for the recognition of microvascular invasion must be as strict as those already described to evaluate the subepithelial invasion; thus, there are a number of characteristics which help identify a true capillary vessel (**Figure 2–23**). Proximity of arterioles is favourable, while an ectatic capillary network around a supposed vessel may be less favourable, because it may indicate a focus of invasion with capillary neoproliferation around it, and certain presence of endothelial cells. The invaded vessels are usually occasional and isolated, whereas the existence of multiple thrombus-like figures must make us doubt that it is an invaded vessel. Also, the thrombi are usually floating. The tumoural thrombus is composed of tightly cohesive cells with a smooth border and a shrunken cytoplasm, and the cells in the periphery have a shell-like aspect. In pseudoinvasion by tissular retraction, the pseudothrombus has a rough surface with a blurred outline, and shreds of cytoplasm may be present between the supposed pseudothrombus and the vessel wall.[97]

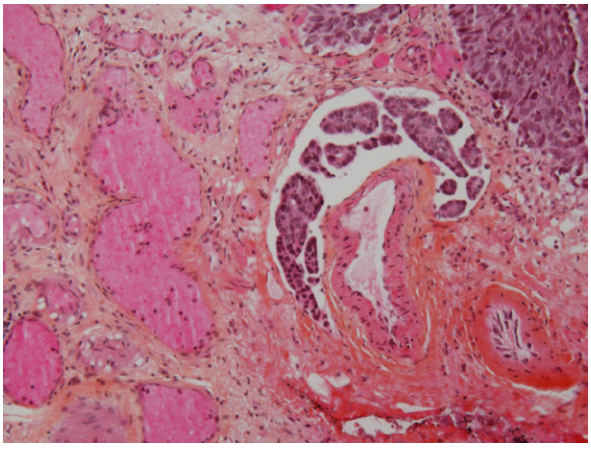


FIGURE 2–23
Lymphovascular Invasion of a Urothelial Carcinoma (micropapillary type)

2.10.2 Variant histologies

Urachal carcinoma is known to be a heterogeneous disease and reporting variant histology (VH) is recommended because of prognostic and therapeutic implications. In light of the evolution of the molecular classification, morphological variants become increasingly important. Therefore, reporting and recognizing VH is a major challenge. A recent paper including 779 patients demonstrates that VH occurs in approximately 27.3%.[118] [**Level 3**] Micropapillary carcinoma (MPC) and squamous differentiation were the most frequent variants. Poor agreement was reported for MPC. These findings join the results of the paper written by Sangoi *et al*., who demonstrated that MPCs were well reported in their classical form with an agreement of 93%, whereas in case of atypical aspects, the agreement was poor, with a median kappa of 0.54.[119] [**Level 2**] Shah *et al.* showed that several VHs are often underreported, especially the lymphoepithelial, plasmacytoid, and nested ones.[120]

Several authors show the survival of VH. Interestingly, patients with squamous cell differentiation seem to have a better outcome than those with MPC and nested carcinomas.[121–123] MPC and nested carcinomas both have bad outcomes.[124] Williams and Kamat suggested that in cases of cT1-N0-M0 MPC, cystectomy should be carried out immediately without delay. There was a difference of disease-specific survival of 100% in patients with immediate cystectomy. On the other hand, patients with delayed cystectomies reported 45% of progression and 35% will be node-positive.[125] Some data suggest that patients with basal-type UCs which morphologically resemble squamous cell carcinomas benefit from neoadjuvant chemotherapy.[126] It is very important that VHs are reported. [**Level 2**] The ICCR underlines the importance of reporting the different types of urachal carcinoma.

2.11 Histologic Types of Bladder Cancer and Variants of Urothelial Carcinoma

2.11.1 Introduction

Bladder cancer can show an incredible diversity in morphology. UC accounts for more than 90% of carcinomas involving the bladder, with a subset of these cases displaying variant morphology. When present, the percentage of each UC variant should be reported. Other major subtypes of bladder cancer include squamous cell carcinoma, adenocarcinoma, and neuroendocrine carcinoma when they occur in pure form.

Emerging molecular evidence has identified relevant alterations in some variants and subtypes, although many of these findings have not yet modified routine clinical practice. In addition, the rarity of many bladder cancer variants and subtypes limits the ability to validate many of these findings. As precision medicine continues to evolve, deeper understanding of pathogenesis and disease outcomes in the setting of variant or subtype morphology is needed to better define commonalities and differences to conventional UC.

Several features are also shared between bladder cancer variants and subtypes. Clinical features frequently overlap and include gross or microscopic hematuria, voiding dysfunction, or abdominal pain, among other findings that are nonspecific. Cystoscopy and gross pathology findings often do not identify variants, with the exception of a subset of cases of mucin-rich adenocarcinoma, or squamous cell carcinoma. The remainder of cases range in gross appearance from exophytic masses to infiltrative lesions that appear ulcerated, nodular, or friable.

UC variants occur in the setting of a concurrent or precedent UC identified on pathology. The percentage of the variant morphology can vary and several variants can co-exist in one patient. In addition, variants identified at one time point in sampling may differ from those identified at another time point, which is a very important finding. This has also been shown by molecular data recently.[127] Whenever variant morphology is identified, it should be reported and the percentage of each variant should also be specified.

2.11.2 Urothelial carcinoma with divergent differentiation

Reporting of UC that contains foci of squamous, glandular, or trophoblastic differentiation, with or without other forms of differentiation, are not included in subsequent variant categories.[128–137] These carcinomas are diagnosed as "UC with _____ differentiation" and the percentage of the variant morphology reported.

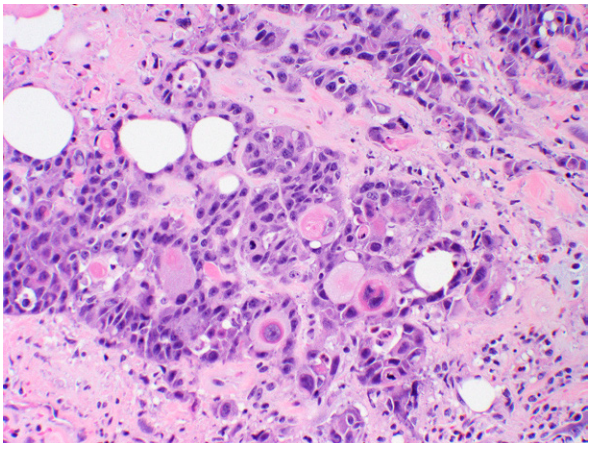
Behaviour is similar to conventional UC. In rare cases with extensive production of ß-human chorionic gonadotropin (ß-HCG), gynecomastia may be present. A choriocarcinomatous component may be fungating and hemorrhagic.

Divergent differentiation is the most common variant morphology and is present in up to 40% of bladder cancer specimens, depending on the study, the specimen type, and the variant reported.[128–137] In sequential specimens from the same patient, variant morphology and extent of variant present may differ.[138] Squamous carcinoma is the most common form of divergent differentiation, followed in incidence by glandular, trophoblastic, and other forms of divergent differentiation. Squamous differentiation has not been considered to be causal in squamous differentiation, although HPV may be periodically identified in UC with or without squamous differentiation.[139,140]

2.11.3 Urothelial carcinoma with squamous differentiation

Squamous differentiation is defined by the presence of either keratin production or desmosome formation, and is found in up to 40% of UC and is most frequent in invasive high-grade tumours. The urothelial component of these tumours expresses markers of urothelial differentiation. In areas of squamous differentiation, expression of markers such as GATA-3, and uroplakin II or III are often reduced or lost, while markers associated with squamous cell carcinoma, such as cytokeratin 14, MAC387, caveolin-1, caveolin-2, desmocolin-2, and desmoglein-3, are expressed.[141–144] In specimens from the bladder with pure squamous histology, retained expression of markers such as GATA-3 or uroplakin would suggest a diagnosis of UC with squamous differentiation, rather than primary (or secondary) squamous cell carcinoma (**Figure 2–24**).[144]

FIGURE 2–24
Urothelial Carcinoma With Squamous Differentiation

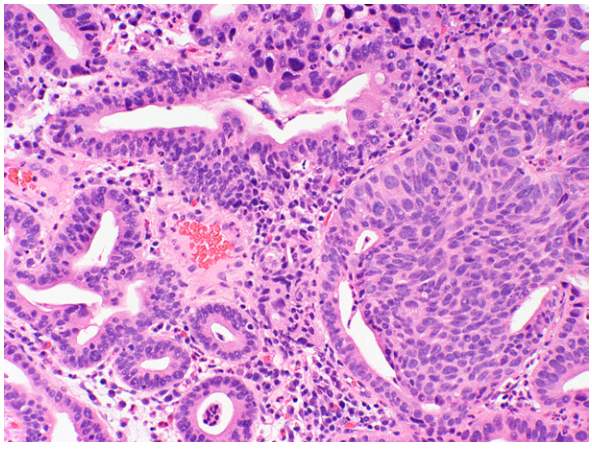


2.11.4 Urothelial carcinoma with glandular differentiation

Glandular differentiation may resemble the spectrum of mucin production and signet ring cells. It is less common than squamous differentiation, but has still been reported in as high as 18% of high-grade UC (**Figure 2–25**). In most instances, the glandular differentiation takes on an enteric morphology including mucinous and signet ring types. Because of this, the glandular component will often show an immunohistochemical pattern similar to primary bladder and colonic adenocarcinoma,

including expression of CDX2 and MUC5AC-apomucin.[145,146] The glandular component can either retain or lose expression of urothelial markers such as GATA-3, p63, and cytokeratin 34ßE12.[146,147] There has been membranous, but not nuclear ß-catenin expression in the glandular component of cases studied.[146] The finding of GATA-3 expression, with or without p63 expression, in a biopsy or TUR that is pure adenocarcinoma morphologically would be highly suggestive that the diagnosis is UC with glandular differentiation.[146]

FIGURE 2–25
Urothelial Carcinoma With Glandular Differentiation



2.11.5 Urothelial carcinoma with trophoblastic differentiation

Syncytiotrophoblastic giant cells may be present and are associated in some cases with an elevation in serum ß-HCG (**Figure 2–26**).[148–156] High-grade UC frequently will express ß-HCG if evaluated by immunohistochemistry (up to 35%) and this is reflected by elevated serum HCG levels in up to 76% of patients with metastatic UC.[150,153] In some cases, the tumour contains multinucleated giant cells that resemble syncytiotrophoblastic giant cells. The diagnosis can be confirmed by immunohistochemistry with expression of ß-HCG or inhibin.[153,154] Osteoclastic giant cells, that should also be considered, will be cytokeratin-negative and will express histiocytic markers such as CD68.[157]

Differential diagnosis includes: 1) squamous cell carcinoma of the bladder; 2) primary adenocarcinoma of the bladder; and 3) other forms of metastatic cancer, such as metastatic choriocarcinoma.

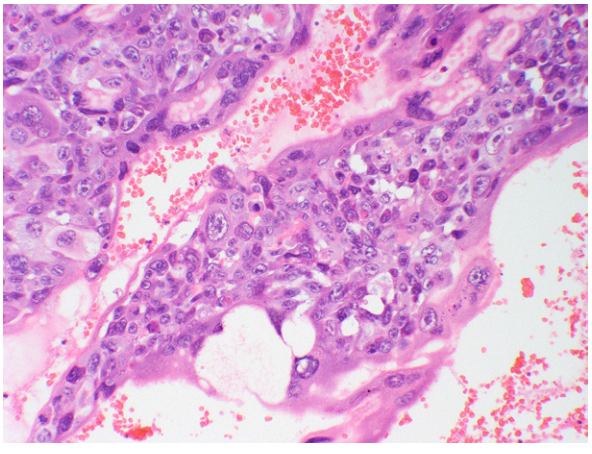
Distinction between UC with extensive squamous differentiation or extensive glandular differentiation may be difficult to impossible to distinguish from pure squamous cell carcinoma or pure adenocarcinoma, respectively. Convention is to evaluate the invasive carcinoma component and when there is pure single-lineage morphology and also absence of a prior diagnosis of UC, to use the diagnosis "squamous cell carcinoma" or "adenocarcinoma." Distinction from metastatic choriocarcinoma can only be performed clinically through evaluation of the testis or gynecologic tract for alternative primary sites.

No ancillary tests are required for the diagnosis of divergent differentiation in the bladder and routine stains for ß-HCG are currently not recommended. However, metastatic UC with extensive squamous or glandular differentiation may be challenging to distinguish from primary lesions at sites such as

the lung. Studies show that many of these cases may show markers of UC differentiation in portions of the tumour, although a large proportion of these markers overlap with the squamous phenotype.[144,158] Foci of glandular differentiation may develop markers in line with an adenocarcinoma lineage, such as CDX2. Thus, careful assessment of current and precedent specimens, and detailed knowledge of the clinical history is essential to complement any immunohistochemical testing performed.

The prognosis of squamous and glandular differentiation was considered historically to indicate a worse prognosis, although recent studies have challenged this finding.[128–137] Whereas UC with squamous differentiation may present at a higher stage than conventional UC, there does not appear to be a significant difference in stage-matched survival outcomes between these two entities.[134,136,137]

FIGURE 2–26
Urothelial Carcinoma With Trophoblastic Differentiation



2.11.6 Nested urothelial carcinoma

Diagnosis may be delayed due to lack of overt malignant features, especially in small biopsy samples. Frequent recurrence and progression to muscularis propria invasion is common.[159]

This bland variant of UC is composed of small nests and large nests, with or without small-to-medium tubules, that can mimic benign urothelial processes on limited sample.[95,137,160–165] Nested UC occurs most commonly in the bladder, in contrast to other sites of the urinary tract with an incidence of less than 1% of UCs.

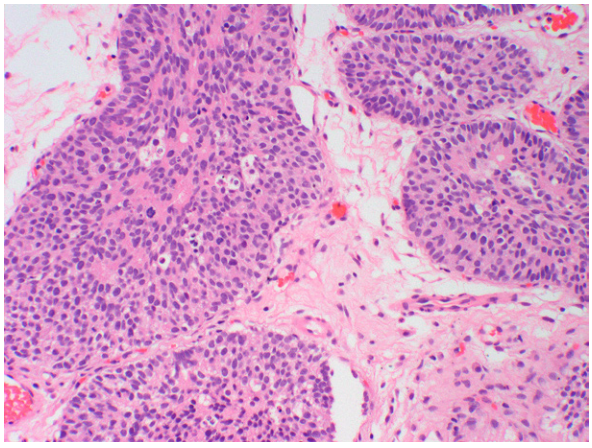
The nested UC may show a variety of architectural features that includes the presence of small nests or large nests (**Figure 2-27**), with or without small tubules, often haphazardly distributed away from the surface urothelium, and may infiltrate into deeper layers of the bladder wall. Diagnostic challenges exist, especially in small specimens. Cytologic atypia is most prominent in the deeper component of the invasive carcinoma.[166,167]

Differential diagnosis includes: 1) florid proliferation of von Brunn nests; 2) cystitis cystica et glandularis; 3) nephrogenic adenoma; 4) inverted growth pattern of a bland exophytic lesion; and 5) adenocarcinoma. Benign processes of the urothelium, including von Brunn nest proliferation and cystitis cystica et glandularis, typically closely approximate the surface urothelium and are not distributed into deeper layers of the lamina propria.[166]

Nested UC shows a similar immunohistochemical profile to conventional UC.[137,168,169] TERT promoter mutations have been reported in a subset of cases and may be of assistance in the diagnosis of some challenging cases.[170]

Nested UC is frequently invasive into the muscularis propria. Recent large studies suggest that outcomes may be similar to conventional UC.[69,75,159,168,171]

FIGURE 2–27
Nested Urothelial Carcinoma Containing Small Nests



2.11.7 Micropapillary urothelial carcinoma

This variant, relatively well known by the urological community, occurs in up to 8% of UCs. MPC has been reported to occur in a significantly higher proportion of men than conventional UC, with a male:female ratio of 5:1 to 10:1.[172] A reduced response to BCG therapy has been reported and use of early cystectomy prior to progression to muscularis propria–invasive disease has been proposed as a treatment strategy for cT1 MPC.[173]

MPC is characterized by filiform, avascular papillary cores reminiscent of ovarian papillary serous carcinoma.[172] MPC terminology should only be applied in the context of invasive disease. MPC is frequently admixed with conventional UC and there is no established cutoff of MPC percentage within a carcinoma required to either include or exclude cases from this diagnostic category.[174,175] Features associated with MPC include the presence of thin, highly branched papillary structures that lack a true fibrovascular core.[172,176] This variant is associated with prominent retraction artifact, resulting in the presence of multiple tumour clusters present within a single retraction space when papillae are cut in cross-section (**Figure 2-28**), which can mimic angiolymphatic invasion. Published series have included cases with <10% of MPC to pure forms.[119,172,173,176,177]

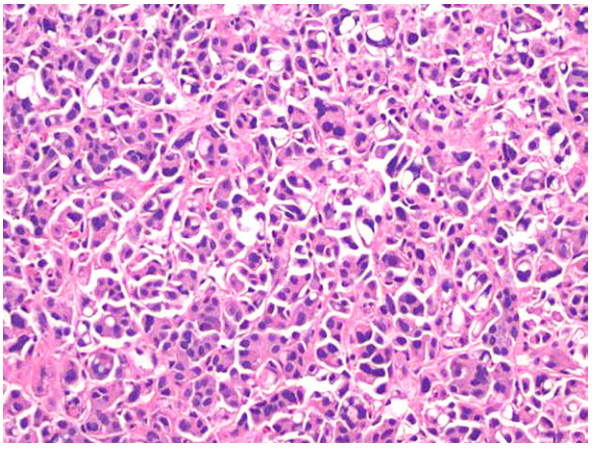
Differential diagnosis includes: 1) distinction of urothelial MPC from secondary carcinomas with micropapillary features and 2) the distinction between urothelial MPC from conventional UC with extensive retraction artifact. High interobserver variability is common in this variant, especially in cases that show limited regions of micropapillary morphology or in "non-classic" forms (kappa 0.54).[119]

The immunophenotype of micropapillary UC is similar to usual UC as regards expression of the commonly applied markers of urothelial differentiation. By immunohistochemistry, MPC expresses markers in common with conventional UC that can include CK7, CK20, high-molecular weight cytokeratin, GATA-3, uroplakin II, uroplakin III, p63 and thrombomodulin.[169,177–181] These usually express MUC1 and MUC2, but these markers are not useful in separating usual UC with clefts from micropapillary carcinoma.[180,182] In pure micropapillary carcinoma lacking a typical UC component, the possibility of metastatic micropapillary carcinoma from another site (such as the lung or breast) or serous papillary carcinoma of the female genital tract are considerations. Immunohistochemical expression of TTF-1 or mammaglobin would favour lung or breast origin; however, TTF-1 has been found in a small percentage (5%) of UCs studied.[181,183] Expression of WT-1 and PAX8 would favour a female genital tract origin.[181,184]

No ancillary test is currently recommended for the diagnosis of MPC. Amplification with or without overexpression of ERBB2 (HER2) occurs at a much higher rate in MPC than in conventional UC.[185] Activating mutations in the extracellular domain of ERBB2 also occur more frequently in MPC.[186] MUC1 and CA-125 overexpression using immunohistochemistry has also been reported.[180]

MPC often presents at a higher stage than conventional UC and may be associated with an increased risk of lymph node and distant metastasis. The percentage of the micropapillary component within a carcinoma appears to correlate with an increasing risk of aggressive biological behaviour.[187]

FIGURE 2–28
Micropapillary Urothelial Carcinoma With Prominent Ring Forms



2.11.8 Rare entities with <3% incidence

2.11.8.1 Microcystic urothelial carcinoma

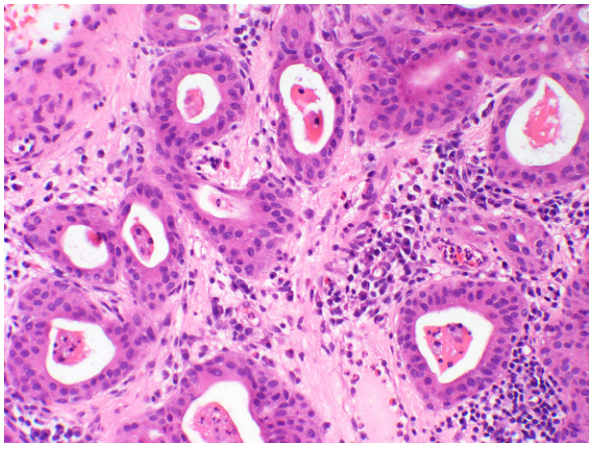
Diagnosis of this variant may be delayed due to lack of overt malignant features, especially in small biopsy samples. This carcinoma often presents at an advanced pathological stage.[188]

Bland variant of UC consists of rounded nests of UC that are approximately 1 mm to 2 mm in diameter.[167,188–190] Microcystic UC consists of dilated microcysts (**Figure 2–29**). Denudation may be seen. Occasionally, macrocysts may be present. Luminal secretions and necrotic debris, with or without calcifications, may be present. Stromal reaction varies, but may be absent in some cases. The infiltrative nature of the process is best appreciated on specimens in which the deeper aspects of the tumour can be visualized.

Differential diagnosis includes: 1) cystitis cystica et glandularis or nephrogenic adenoma and 2) other variant malignant processes, such as invasive nested UC with small tubules and invasive primary or secondary adenocarcinoma.

The immunoprofile of microcystic UC is similar to that of conventional UC, with the exception that immunoreactivity to uroplakin III and thrombomodulin may be less robust.[169] Limited studies show that outcomes are similar to conventional UC.[191]

FIGURE 2–29
Lymphoepithelioma-like Carcinoma



2.11.8.2 Plasmacytoid urothelial carcinoma

This relatively uncommon variant of UC has a reported incidence of less than 3% of UCs and shares demographic features with conventional UC. However, the clinical presentation is often associated with diffuse growth and peritoneal carcinomatosis.[192–196] The bladder is often edematous and the bladder wall diffusely involved. Also newly termed "signet ring–like/diffuse type," this carcinoma consists of dyscohesive cells that resemble plasma cells or signet ring cells.[192,197–209] The signet ring component has newly been added in the WHO 2016 classification.[21] These tumours express markers such as GATA-3, thrombomodulin, p63, uroplakin II and III, cytokeratin 7, and cytokeratin 20, similar to usual UC.[204,205,210] In some cases, the tumour cells are quite small and can closely mimic plasma cells. Because of this, expression of CD138 has been applied and has shown consistent expression in

plasmacytoid UC and other non-plasmacytoid UCs. The tumour demonstrates loss of E-cadherin expression by immunohistochemistry.[169,178,192,199,201,204,205,211–215] In a high percentage of these tumours, there is loss of E-cadherin expression (70%–100%).[201,210,216,217] Mucin stains will highlight intracytoplasmic mucin, although this finding remains consistent with a diagnosis of plasmacytoid UC. Extracellular mucin is not permitted for this diagnostic category.

If metastatic lobular carcinoma of the breast is considered in the diagnosis, it is important to be aware that a small percentage of UCs, including the plasmacytoid variant, can express estrogen receptor (α), but progesterone receptor expression has only very rarely been shown.[216,218,219]

Differential diagnosis includes: 1) metastatic or primary signet ring cell carcinoma; 2) lymphoma/plasmacytoma; 3) melanoma; 4) rhabdomyosarcoma; and 5) metastatic lobular breast carcinoma. This carcinoma presents at an advanced stage and is commonly associated with diffuse bladder wall involvement, carcinomatosis, and positive surgical margins.[194–196] Outcomes are extremely poor.[194,211,214]

2.11.8.3 Lymphoepithelioma-like urothelial carcinoma

The tumour displays a male predominance. The lymphoepithelioma-like carcinoma (LELC) resembles lymphoepithelioma of the head and neck region in which carcinoma cells are admixed with a dense, and often obscuring, inflammatory infiltrate.[197,220–225] Nuclear pleomorphism and brisk mitotic activity may be seen.[226] Like other UC, LELC may occur in pure form or admixed with UC.[220,222–224] LELC shows an identical profile to conventional UC, although one series suggests that CK20 may be less frequently positive in this variant, but it does not show an association with Epstein-Barr virus infection.[222,224,227,228]

Differential diagnosis includes: 1) a lymphoproliferative disorder and 2) chronic cystitis. In any bladder specimen, the finding of a dense inflammatory infiltrate should prompt microscopic assessment for carcinoma cells and judicious use of immunohistochemical stains for cytokeratin in challenging cases.

One of the larger series suggests that LELC in pure form may portend good outcomes and improved response to chemotherapy,[224] although a separate study suggests similar outcomes to conventional UC.[223,224]

2.11.8.4 Lipid-rich urothelial carcinoma

These are UCs containing large, lipid-filled cells resembling lipoblasts,[229,230] an uncommon variant of UC with male predominance. Lipid-rich UCs are composed of large neoplastic cells that contain one or more clear lipid vacuoles that indent the nucleus.[198,229–231] No predefined cutoff of lipid-rich cells is required for this diagnosis. Immunohistochemistry is similar to conventional UC. S100 immunostain is negative.

Differential diagnosis includes: 1) sarcomatoid UC with lipomatous differentiation and 2) signet ring cell carcinoma.

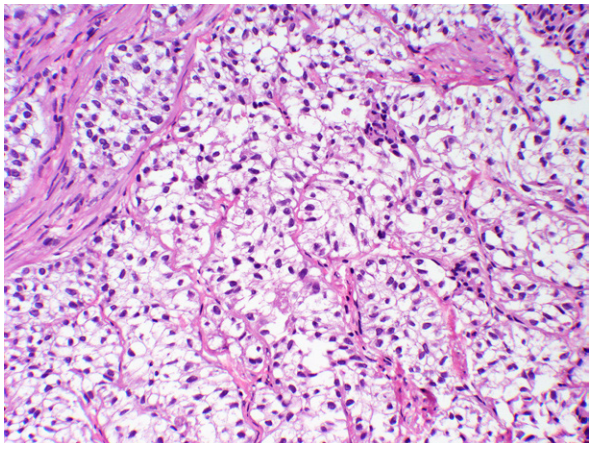
The majority of lipid-rich UCs present at an advanced pathological stage and the vast majority of patients show progressive disease. Up to two-thirds of patients may die from disease within 3 years.[229,230]

2.11.8.5 Clear cell urothelial carcinoma

The clinical appearance is similar to conventional UC. This invasive UC contains a predominance of large carcinoma cells with optically clear cytoplasm containing glycogen[232–235] (**Figure 2–30**). Clear cell UC often occurs in association with conventional UC or other UC variants. Immunohistochemical staining patterns are identical to conventional UC.[169] The cytoplasmic glycogen content is positive for periodic acid–Schiff stain and sensitive to diastase digestion.[199]

Differential diagnosis includes: 1) clear cell adenocarcinoma; 2) metastatic clear cell renal cell carcinoma; 3) squamous cell carcinoma with cytoplasmic clearing; 4) artefactual clearing caused by cautery; and 5) paraganglioma.[236–238] The number of reported cases is low and it is unclear if outcomes differ from conventional UC.

FIGURE 2–30
Clear Cell Urothelial Carcinoma



2.11.8.6 Sarcomatoid urothelial carcinoma

For sarcomatoid carcinoma in the urinary bladder where there is a recognized carcinoma component (urothelial or other), immunohistochemistry generally does not have a role in diagnosis. There are cases with a particularly exuberant stromal response (UC with pseudosarcomatous stroma) or prominent myxoid stroma background where it can be difficult to determine the nature of the stromal component. Specific risk factors for the development of sarcomatoid UC include radiation exposure and cyclophosphamide administration.[239,240] It is often a solitary, firm carcinoma that demonstrates a white, fleshy cut surface that may contain hemorrhage and necrosis.

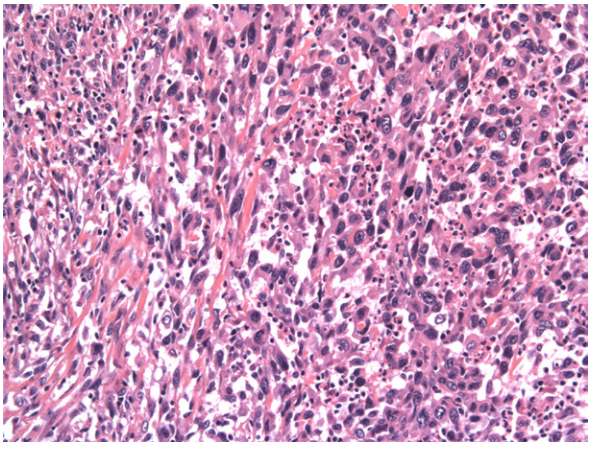
Sarcomatoid UC may be present in pure form or mixed with other forms of bladder cancer, including UC, squamous cell carcinoma, adenocarcinoma, or small cell carcinoma.[241–254] Although the majority of sarcomatoid carcinomas demonstrate highly atypical cells with nondescript spindle cell morphology, a variety of mesenchymal-like patterns may be evident [242,244,255] **(Figure 2-31)**. Heterologous elements including osteoid and chondroid material may be present. For cases with a pure sarcomatoid morphology, distinction from true sarcoma is necessary. Cytokeratin expression can be demonstrated in most sarcomatoid carcinomas.[244,254,256,257] It is important to remember, however, that some sarcomas (such as leiomyosarcoma) can also express cytokeratin (typically low molecular weight),

as can myofibroblastic proliferations.[256,258] High molecular-weight cytokeratin expression (34ßE12) would indicate sarcomatoid carcinoma.[256] Positivity for both p63 and GATA-3 can be present in many sarcomatoid UCs.[256,259,260]

Differential diagnosis includes: 1) benign spindle cell proliferations in the bladder, including inflammatory myofibroblastic tumour or reactive fibroblastic proliferations, and 2) malignant sarcomatous lesions. Differentiation from benign processes requires careful assessment of nuclear pleomorphism and morphology. ALK-1 immunoreactivity, if present, is helpful in establishing a diagnosis of inflammatory myofibroblastic tumour.[256] One recent paper has shown that use of a panel of UC markers, including but not limited to uroplakins, GATA-3, p63, CK7, and pancytokeratin, will often show at least one immunoreactive marker.[257] Sarcomatoid UC shows markers of epithelial-to-mesenchymal transition including ZEB1, SNAIL, and others, highlighting a potential pathogenic mechanism of sarcomatoid carcinoma development.[257] TERT promoter mutations have been recently reported in sarcomatoid UC of the upper tract.[260]

Outcomes are very poor for patients with a diagnosis of sarcomatoid UC, with a 5-year cancer-specific survival after cystectomy of 20% and median overall survival of 14 months.[241–252] Metastatic spread is common.

FIGURE 2–31
Sarcomatoid Urothelial Carcinoma



2.11.8.7 Giant cell urothelial carcinoma

Clinical behaviour is similar to conventional UC, although reported cases are extremely limited.[261] This uncommon variant consists of at least 20% giant cell component, which is defined as highly bizarre, pleomorphic, multinucleated tumour giant cells with abundant eosinophilic or amphophilic cytoplasm that resemble giant cell carcinoma of the lung.[128,130,131,135,261] Brisk mitotic activity, atypical mitotic figures, angiolymphatic invasion, and necrosis are common. Giant cell UC often expresses immunohistochemical markers consistent with epithelial derivation, urothelial derivation, or both, including cytokeratin, CK7, Cam5.2, p63, thrombomodulin, and uroplakins.

Differential diagnosis includes: 1) UC with syncytiotrophoblastic giant cells consistent with divergent differentiation and 2) UC with osteoclast-like giant cells.

Metastatic disease is common. The prognosis is uniformly poor, with death from disease within 2 years.[157,261]

2.11.8.8 Poorly-differentiated urothelial carcinoma (including those with osteoclast-like giant cells)

This overarching category with extremely limited reported cases encompasses poorly-differentiated, high-grade carcinomas and includes carcinomas that contain osteoclast-like giant cells and resemble osteoclastic giant cell tumours of bone or soft parts.[262–266] A subset of these tumours contains osteoclast-like giant cells that resemble giant cell tumour of the bone; the osteoclast-like giant cells will show immunoreactivity for CD68, LCA, CD51, and CD54.[157,262–266] A subset of mononuclear cells may show immunoreactivity for α-smooth muscle actin, desmin, and S-100.

Differential diagnosis includes: 1) UC with trophoblastic giant cells; 2) giant cell UC, 3) sarcomatoid UC; and 4) metastatic carcinoma. These carcinomas have a very poor prognosis. Lung metastasis and death from disease within one year of surgery have been reported.

2.11.9 Squamous cell carcinoma

2.11.9.1 Squamous cell carcinoma not associated with schistosomiasis

Squamous cell carcinoma is the most common pure variant of UC comprising approximately 5% of bladder cancer in the Western world, although squamous differentiation is much more common as a component of UC with mixed histologic features.[267,268] At cystoscopy, they appear as solitary polypoid, sessile lesions associated with white keratin debris. The presence of necrosis and ulceration is common.[238] Proper identification of squamous cell carcinoma may be difficult in biopsy samples due to overlapping morphologic features with high-grade UC. Risk factors include longstanding inflammation/irritation due to many factors, such as indwelling catheters, infection, calculi, bladder-outlet obstruction, and fistula.[24,269–271] Tobacco smoking has been established as a significant risk factor.[272,273] HPV is not usually associated.[274–276] In women with HPV-associated carcinoma involving the urothelial tract, it is imperative to rule out extension from a gynecological primary disease.[277] The peak incidence is in the seventh decade of life.[42,268] It is more common in men and African-Americans are twice as likely to be affected.[278] Clinical presentation includes hematuria and irritative voiding symptoms, such as dysuria, urgency, suprapubic pain.

In this neoplasm, composed entirely of neoplastic squamous cells, it is common for the surface urothelium to exhibit squamous metaplasia, with or without dysplasia, as well as squamous CIS. By convention, the presence of any "usual" UC, either *in situ* or invasive, warrants a diagnosis of UC with squamous differentiation.[279] Whether this very rigid approach to classification is appropriate, particularly in cases where all or the overwhelming majority of the invasive tumour is composed of squamous cell carcinoma, remains to be proven. Evaluation for p16 expression is of no value, as up to 60% of UC are positive for this marker.[280,281]

Differential diagnosis includes: 1) UC with extensive squamous differentiation as well as any squamous cell carcinoma; 2) in women, direct extension from a tumour arising from the gynecological tract must be considered.

Reliable outcome data are hindered by the paucity of studies with well-characterized tumours, similar treatment strategies, and adequate clinical follow-up. Some investigators have reported disease-free survival rates following cystectomy ranging from 43% to 57% with poor outcome associated to advanced stage at presentation and regional lymph-node metastasis in up 24% of cases at cystectomy.[122,134,238,282] When controlling for clinicopathological factors such as stage, outcomes appear to be similar between squamous cell carcinomas and usual UC, with a 5-year disease-specific survival of 57%.

A key challenge is in the management of patients who present with squamous cell carcinoma on initial biopsy or TUR, where sampling may miss areas of the tumour with usual UC morphology (UC with squamous differentiation). This is particularly true in the modern era of neoadjuvant therapy prior to cystectomy, which ultimately may be the only type of sample that is almost certainly a pure squamous carcinoma. Another challenge is the management of *in situ* squamous lesions found on biopsy, particularly squamous CIS, where at least one clinical study demonstrated progression to invasive disease in over 79% of cases.[24]

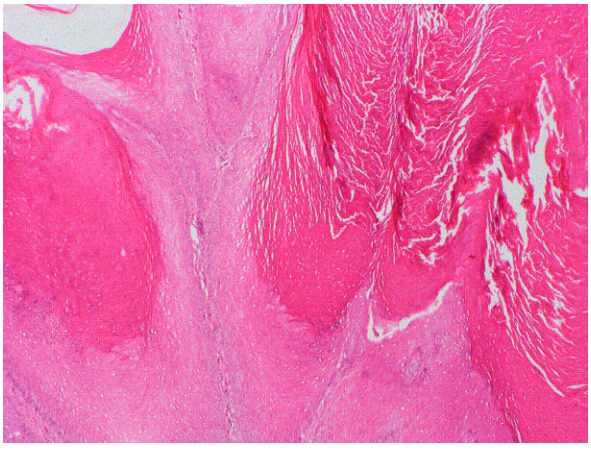
2.11.9.2 Verrucous carcinoma (with and without association to urinary schistosomiasis)

This tumour occurs more often in men than women and in regions where urinary schistosomiasis is endemic. Peak incidence is in the fifth decade of life in tumours associated with schistosomiasis and a decade later in cases not associated with the disease.[283–285] Very rarely have these tumours been associated with condyloma acuminatum, bladder diverticula, or chronic urinary tract infection.[286–290] Irritative urinary symptoms at presentation are the norm, but not hematuria. These tumours are usually solitary, exophytic and "wartlike." The surface is white and flaky due to the presence of keratin. Verrucous carcinoma is a rare subtype of squamous cell carcinoma, with broad "pushing" infiltrative borders. In areas where urinary schistosomiasis is endemic, verrucous carcinoma accounts for up to 3.4% of all bladder carcinomas and 4.6% to 6.5% of squamous cell carcinomas.[283–285]

The exophytic component is composed of elongated papillae lined by squamous epithelium with parakeratosis (**Figure 2–32**). By definition, irregular, frankly invasive nests are not present. Parasite ova may be present within the bladder wall in cases associated with schistosomiasis.

Differential diagnosis includes: 1) well-differentiated component of squamous cell carcinoma; 2) other entities in the differential diagnosis include verrucous squamous hyperplasia, squamous papilloma, and condyloma acuminatum, the last of which may be associated with HPV infection; 3) secondary involvement of the bladder by well-differentiated squamous cell carcinoma arising in the gynecological tract or anus is another consideration. The 5-year overall survival for schistosomiasis-associated squamous cell carcinoma is approximately 50%, although this likely includes cases other than verrucous carcinoma. As expected, survival is associated with tumour grade, stage, and lymph node status. Cases not associated with schistosomiasis have reported very favourable clinical outcomes, although patient follow-up is short and the number of cases modest. Tumour recurrence may follow incomplete resection, although cystectomy in this setting is curative.[288,290,291] Given the virtual impossibility of making this diagnosis on biopsy of TUR, it may well be that the diagnosis can only be rendered at the time of radical cystectomy that remains the treatment of choice. Radiation therapy is not advocated due to the possibility of development of higher-grade, more aggressive disease, as has been documented at other sites.[284,287,292–294]

FIGURE 2–32
Verrucous Carcinoma



2.11.10 Adenocarcinoma

2.11.10.1 Primary bladder adenocarcinoma

Pure adenocarcinoma of urothelial origin is rare limited data are available. In large series, adenocarcinomas constituted 0.55% to 2.6% of all urinary bladder malignancies.[295–299] There is an association between schistosomiasis and bladder adenocarcinoma, and in a series from Egypt, 5.2% to 11.4% of bladder tumours were found to be adenocarcinomas.[300–302] An increased incidence of these tumours is also seen in individuals with bladder extrophy where they occurred in approximately 10% of cases.[302,303] Adenocarcinoma of the bladder occurs most frequently in the fifth to seventh decades, although rare pediatric cases have been reported.[304] Hematuria is the most common presenting symptom for bladder adenocarcinoma; other presenting features are irritative bladder symptoms, flank pain secondary to outflow obstruction, suprapubic pain, urinary frequency, dysuria, and mucosuria.[295,297,298,305–308]

At cystoscopy, primary bladder adenocarcinoma is more frequently solid or sessile than papillary, and there is usually extensive ulceration.[304,306,309] Tumours may be mucinous and hemorrhage is frequently seen. Adenocarcinomas are solitary in >50% of cases. While the trigone is the most common primary site, all parts of the bladder may be involved.[295,298,304–306,309,310]

Signet ring cell carcinoma is now considered to belong in the plasmacytoid UC category.[311] Adenocarcinomas with extravasated mucin-containing signet ring cells fall into the category of mucinous adenocarcinomas, rather than signet ring cell carcinomas, which, by definition, lack extracellular mucin.[304,307,311,312]

Various grading systems, including that of the WHO, have been used for these tumours.[309,313] Since these tumours commonly present with locally advanced disease, the clinical value of grading the these tumours is questionable, as is the case in invasive UC. Bladder adenocarcinoma is often found in association with cystitis glandularis and urothelial intestinal metaplasia.[212,314]

There are no reliable immunohistochemical markers to distinguish between primary adenocarcinoma or metastasis from an adenocarcinoma.[212] There have been many studies evaluating a broad range of markers in an effort to identify a reliable method to distinguish primary from secondary adenocarcinoma.[146,147,315–320] The conclusion of these studies has been that, for the individual case, immunohistochemistry cannot reliably distinguish primary from secondary adenocarcinoma. The most reliable marker that has emerged is ß-catenin, where strong nuclear expression is much less frequent in primary (<10%) versus secondary enteric adenocarcinoma (>90%).[146,147,316,320] In contrast, a membranous reactivity pattern is more typical of primary than secondary adenocarcinoma.[146]

Interestingly, a recent study demonstrated striking molecular similarities between colorectal adenocarcinomas and adenocarcinomas arising within the urinary bladder.[321] Despite the significant overlap, FGFR3 and HRAS mutations, and APC, CTNNB1 and IDH2 alterations were found to be mutually exclusive between primary bladder adenocarcinoma and high-grade UC.

Differential diagnosis includes: 1) other forms of bladder neoplasia and secondary adenocarcinoma of the stomach, appendix, large bowel, pancreatobiliary system, breast, endometrium, and prostate gland; 2) cystitis cystica with intestinal metaplasia and extravasated mucin; 3) infiltration of the bladder by prostatic adenocarcinoma; and 4) colonic adenocarcinoma infiltrating or metastatic to the bladder that may mimic bladder adenocarcinoma (although careful examination will confirm that colonic metastases do not have any *in situ* component). Most studies relating to the outcome of primary adenocarcinomas of the urinary bladder are hampered by small sample size. Further confounding factors are that in many series, urachal and nonurachal carcinomas are admixed.[297–299] In further series, adenocarcinomas containing areas of typical UC were included.[305] Bladder adenocarcinomas are often of advanced stage at presentation. Lymphadenopathy was visible in 25% of cases and in 25%, there was direct invasion of the rectus muscle.[322] Metastases are present in approximately 25% of cases, with secondary sites, in descending order of frequency, being liver, bone, regional lymph nodes, adrenals, peritoneum, and skin.[295,304,305,309,323] Bladder adenocarcinoma has a poor prognosis with 5-year survivals of 11% to 55% being reported.[242]

2.11.10.2 Urachal carcinoma

Although urachal remnants are seen in up to one-third of postmortems, urachal malignancies are rare tumours with many reports consisting of single cases and small series.[116,324,325] Urachal carcinomas have been shown to constitute 0.07% to 0.7% of bladder carcinomas in North America and Europe, and 0.55% to 1.2% of bladder carcinomas in Japan.[326]

There is a male predominance for all types of urachal cancers with male:female ratios of 1.8:1 to 3:1 being reported.[325,327,328] In various series, patients ranged from 50 to 60.7 years of age.[116,117,325,327–331] Hematuria is the most common presenting feature in 55% to 80% of cases.[116,117,326,328] Other presenting signs and symptoms are abdominal mass or pain, suprapubic mass, umbilical mass or discharge, recurrent urinary tract infections, irritative voiding symptoms, or urinary outflow obstruction, while mucinuria is seen in 25% of urachal adenocarcinomas.[116,327,328]

The great majority of these tumours are adenocarcinomas originating from vestigial urachal epithelium that closely resemble colonic adenocarcinoma.[327] Less frequently, the tumour has a signet ring cell morphology, although it is usually associated with extravasated mucin.[212,325,328]

There is usually infiltration into the bladder wall, although occasionally, this may be absent, with the tumour having a pushing margin and being clearly demarcated from adjacent bladder tissue. Tumours are usually bulky. Less frequently, urachal carcinomas infiltrate the bladder mucosa[116] and may grossly appear as solitary ulcerated, mucinous, or papillary masses.[116,327]

Because of the morphologic overlap of vesical and urachal adenocarcinoma, several diagnostic criteria for urachal tumours have been proposed. For this reason, the criteria of Gopalan *et al.* are recommended.[116]

Typical urachal adenocarcinomas may contain focal areas of rare forms of UC. Several cases of urachal sarcoma with features resembling leiomyosarcoma, rhabdomyosarcoma, and hemangiopericytoma have also been reported.[327,332–334]

In larger series, tumour grading is reported, although no formal grading system for these tumours has been established.[329] Studies on the immunohistochemical expression of urachal carcinomas are limited.[335] Similar to vesical and colonic adenocarcinomas, stains are of no particular diagnostic value and the routine use of these assays is discouraged.

Differential diagnosis includes primary adenocarcinoma of the bladder, and metastatic colonic, ovarian, and prostatic carcinomas.

In earlier series, the prognosis of urachal carcinoma was found to be poor, with an overall survivals of 22.7% and 31% and a 5-year survival of 61% being reported.[326,336] In more recent studies, mean overall survival was 122 months and a meta-analysis of 312 patients showed 5-year postoperative cancer-free survivals ranging from 43% to 70%.[117,325,328–330,336–342]

A staging system for urachal carcinoma was established by Sheldon *et al.*[117] Advanced tumour stage is associated with a less favourable outcome, with reported 5-year survivals of 93% for patients with tumour confined to the urachus and bladder, 69% for extra-vesical and periurachal tumours, and 0% for tumours within the peritoneal cavity.[325,328]

Successful management of urachal carcinoma depends on complete surgical excision of tumour.[116,325] Salvage surgery has shown to result in a long-term cure for 50% of patients with local recurrence.[328] Adjuvant chemotherapy currently has a limited efficacy for urachal adenocarcinoma.[116,324,329,343,344]

2.11.11 Tumours of mullerian type

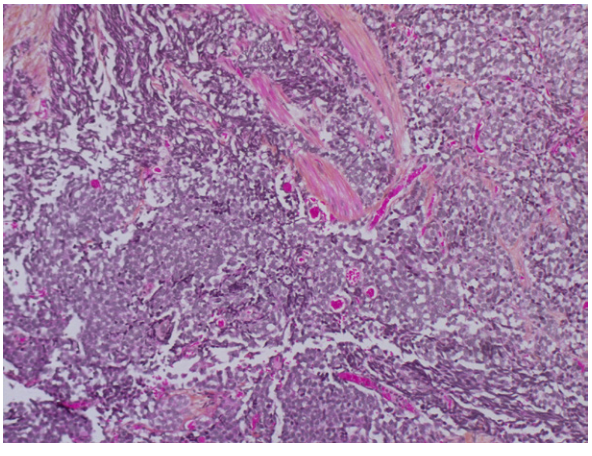
These are rare neoplasms of two fundamental types. The first group arises in females from endometriosis, endocervicosis, or endosalpingiosis (so-called mullerianosis) and are histogenetically as well as morphologically identical to mullerian clear cell carcinoma. The second group includes neoplasms without a proven association with endometriosis, but with morphologic similarity to tumours having such an association. Even when both subsets are combined, this is among the rarest of all bladder cancers, accounting for no more than 0.01% of cases. This designation should be reserved for tumours resembling to a significant degree clear cell adenocarcinoma of mullerian type as encountered in the female genital tract.[236,345]

The great majority (approximately 80%) of these tumours occurs in females. There is a wide age distribution in adult life from the early 20s to later years, but the majority occurs in a somewhat older population (mean 57 years of age). It is conceivable that in a female, the combination of symptoms with the menstrual cycle could indicate an endometriosis-associated carcinoma. Most of the reported tumours have been polypoid or papillary. The typical triad is that of tubulocystic, papillary, and diffuse (solid) arrangements. The most common is tubulocystic, in which the lumens of these formations often contain basophilic or eosinophilic secretions that may be mucin-positive (**Figure 2-33**). Tumours with endometrioid-type morphology are exclusively seen in women.[345]

Differential diagnosis should focus on: 1) nephrogenic adenoma; 2) UC with glandular differentiation; and 3) metastatic renal cell carcinoma.

True adenocarcinomas of mullerian type would be expected to have an immunohistochemical profile appropriate for such tumours, including expression of PAX8 and HNF-1ß.[162,346,347] Most of the reported tumours stain immunohistochemically for CA-125 and CK7. There is no evidence that the behaviour, stage for stage, is different from usual bladder cancer.

FIGURE 2–33
Neuroendocrine Carcinoma



2.11.12 Neuroendocrine neoplasms

2.11.12.1 Tumours of the bladder

Neuroendocrine carcinoma occurs in pure form or mixed with other carcinoma types, most often UC.[348] Large cell neuroendocrine carcinoma of the urinary bladder is extremely rare with fewer than 10 cases reported in the literature.[349] Small cell carcinoma of the urinary bladder, although relatively much more common than both large cell neuroendocrine carcinoma and well differentiated neuroendocrine tumour, is a rare primary bladder malignancy, accounting for fewer than 10% of urinary bladder cancers (**Figure 2–33**).[350–353] Age at diagnosis ranges from 32 to 82 years. Patients usually present with hematuria, which may be accompanied by dysuria, frequency, nocturia, urinary obstructive symptoms, or localized abdominal or pelvic pain.[351,354] Small cell carcinoma may manifest as a single large, solid, polypoid, sessile, or ulcerated mass in the lateral walls and dome of the bladder, and rarely in bladder diverticula, which may be infiltrative at presentation.[267,350] Well-differentiated neuroendocrine tumours, also called carcinoid tumours, are fleetingly rare. Large cell neuroendocrine

carcinoma is poorly differentiated and high-grade. At low magnification, it demonstrates a noticeable histologic pattern of growth in the form of nests and trabeculae. In tumours with mixed histologies, what is certain is that the presence of any small cell carcinoma component must be mentioned in the report and a percentage given.[279] In most cases, neuroendocrine markers including synaptophysin, chromogranin, and CD56 can be demonstrated, with CD56 and synaptophysin being most sensitive.[348,349,355–357] Although cases can be occasionally cytokeratin-negative, in most of them, there is positive expression, including a "dot-like" perinuclear distribution.[348,349] These tumours will frequently express TTF-1.[358,359] For the most part, markers such as uroplakin, GATA-3, p63, and high molecular-weight cytokeratin are not expressed or expressed in only a small percentage of cases.[357,359] For pure tumours, the possibility of origin elsewhere must be considered. Most often, prostatic small cell carcinoma is the case. If the tumour expresses ERG, present in approximately 50% of prostatic small cell carcinomas, that would be helpful in confirming a prostatic origin.[360]

In the differential diagnosis, large cell neuroendocrine carcinoma should not to be confused with a metastatic deposit of its pulmonary counterpart, while small cell carcinoma should not be mistaken for malignant lymphoma or poorly differentiated UC. Pulmonary metastasis or extension from adjacent viscera must be ruled out by clinicoradiologic correlation as well as alveolar rhabdomyosarcoma.[350]

Large and small cell neuroendocrine carcinoma appear to behave aggressively with high metastatic potential and mostly fatal outcome.[361,362] Neuroendocrine carcinoma often presents at an advanced stage, with up to 94% of cases having muscularis propria invasion or extravesical extension. Metastasis at time of presentation is not uncommon, to sites that include regional lymph nodes, liver, bone, and lung. Mean survival ranges from 6 to 34.9 months, and the reported 5-year survival rate ranges from 8% to 40%. Organ-confined disease is more amenable to therapy and is associated with more favourable patient survival. In sum, prognosis is influenced by disease extent at diagnosis, employment of chemotherapy, and the patient's performance status.[354,356,363]

Carcinoid tumours of the urinary bladder have been reported to occur in adults ranging from 29 to 75 years of age. Carcinoid tumours usually present as small polypoid masses at the bladder neck or trigone.[364]

Microscopically, carcinoid tumours are submucosal and confined within the lamina propria, often associated with adjacent cystitis cystica et glandularis. Carcinoid tumour cells are immunopositive for neuroendocrine markers chromogranin, synaptophysin, serotonin, and neuron-specific enolase, and for cytokeratin AE1/AE3.

In the differential diagnosis, carcinoid tumours can be histologically confused with nested variant of UC, inverted urothelial papilloma, and metastatic tumours arising in the prostate, gastrointestinal tract, and lung.

In the largest series to date, all 6 pure carcinoid tumours with similar morphology had excellent prognosis.[364]

2.12 Role of Immunohistochemistry

2.12.1 Markers of urothelial differentiation

In most cases of UC arising in the urinary tract, the diagnosis is readily accomplished based on the morphologic features. In certain situations, however, immunohistochemistry may be helpful in determining the urothelial nature of a tumour within the urinary tract. This most often arises when the morphology is not specific and the possibility of direct spread or metastasis from another location is considered in the differential diagnosis. These occur in the setting of poorly differentiated tumours and also with certain variant histologies that raise concerns about another primary tumour. In this section, the antibodies most often used to indicate urothelial differentiation are reviewed. In general, other than uroplakin III, the markers discussed below are not specific and their applicability depends on the specifics of the differential diagnosis being considered.[52,57] In 2014, the ISUP published recommendations on the use of immunohistochemistry in urinary bladder lesions; this brief review generally follows and updates the information presented in that document.[57]

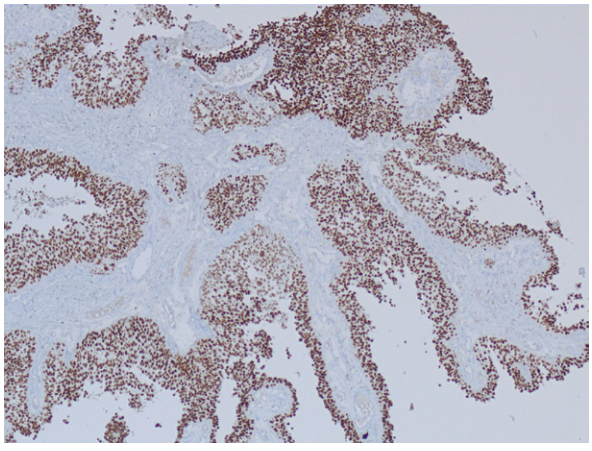
2.12.1.1 Uroplakin II and III

The uroplakin III transmembrane protein is expressed by normal urothelial cells and is generally considered to be the most specific marker of urothelial differentiation in use today.[365,366] The limitation of this marker is its lack of sensitivity. Further, it tends to lose expression with increasing grade and so it is often not expressed in tumours where it would most often be helpful, especially for muscle-invasive tumours.[367] More recently, antibodies to uroplakin II have become commercially available. This marker is also highly specific for urothelial differentiation and in several reports, has been demonstrated to have greater sensitivity than uroplakin III.[368–370] For example, in a study of 174 cases of UC, the sensitivity of uroplakin II was 77% compared to 54% for uroplakin III.[368] In another report on 89 cases of invasive high-grade UC, 80% expressed uroplakin II.[371] This report also included evaluation of lymph node metastases in 35 patients, and uroplakin II expression was present in 90% of cases where it was also expressed in the paired primary tumour.[371]

2.12.1.2 GATA-3

This nuclear transcription factor has been recognized as being expressed in urothelial tumours for over a decade and enjoys widespread use as a marker of urothelial differentiation.[372] GATA-3 has proven to be a highly sensitive marker being expressed in 80% to 100% of high-grade UCs (**Figure 2–34**).[370,371,373] The major limiting factor of GATA-3 is its lack of specificity. GATA-3 is expressed by a high percentage of breast ductal and lobular carcinomas.[373,374] Many other tumours can be GATA-3–positive in a smaller percentage of cases, including squamous cell carcinoma, lung adenocarcinoma, renal cell carcinoma, trophoblastic tumours, yolk sac tumour, pancreatic adenocarcinoma, colonic adenocarcinoma, and endometrial adenocarcinoma.[373,375–377] It is also expressed in paraganglioma of the bladder and occasionally in primary bladder adenocarcinoma.[378,379] Only rarely has it been expressed by prostatic adenocarcinoma.[370]

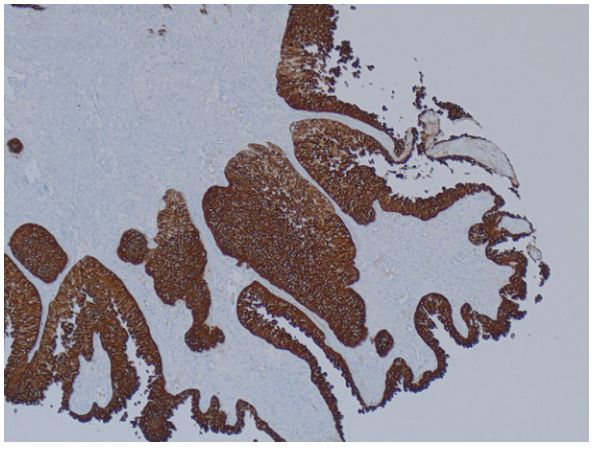
FIGURE 2–34
GATA-3 Staining; Nuclear Expression of the Antibody



2.12.1.3 Cytokeratins

One of the most characteristic features of UC is the co-expression of cytokeratins 7 and 20.[380–382] When present, this would strongly support a diagnosis of UC. Expression of these markers is grade-dependent and for high-grade UC, cytokeratin 7 is expressed more often than cytokeratin 20 (**Figure 2–35**).[57] Co-expression is reported in 50% to 62% of cases.[382,383] Of note, up to 14% of UCs have been reported to have no expression of either cytokeratin 7 or 20.[380,382,383] High molecular-weight cytokeratin, in particular the 34ßE12 clone, is expressed in 65% to 97% of cases, again related to tumour grade.[366,382,384] It has recently been reported that lack of cytokeratin 20 expression is a feature of the so-called basal phenotype of UC.[385] The basal cell phenotype has also been reported to have reduced GATA-3 and uroplakin II expression.[386]

FIGURE 2–35
Cytokeratin 7 Expression in the Urothelium



2.12.1.4 S100A1

In 2007, a couple of publications highlighted the potential for use of S100A as a marker of urothelial differentiation.[372,387] Subsequent studies have confirmed the high frequency of expression of S100P in UC.[144,388] It is not specific for UC, however, and is also expressed in colonic adenocarcinoma, gastric adenocarcinoma, breast carcinoma, pancreaticobiliary adenocarcinoma, among others.[389,390]

2.12.1.5 p63

The p63 protein has also been widely used in the diagnosis of UC because of its high sensitivity, being expressed in 81% to 92% of UCs.[357,382] It has been most useful in the differential diagnosis with poorly differentiated prostatic adenocarcinoma where p63 is only rarely expressed.[382,391–393] It is also nonspecific, being expressed in squamous cell carcinoma and a variety of other tumours.

2.12.1.6 p40 (ΔNp63)

The ΔNp63 isoform (p40) has been shown to be expressed in a significant proportion of UCs.[394,395] Interest in this marker is related to its potential use as a prognostic marker, with loss of expression associated with an increased risk of progression for UC.[396–398] These studies have also shown that p40 expression is present in almost 90% of high-grade invasive UCs.[371] It is also expressed in squamous cell carcinoma from multiple sites, including the head and neck, lung, and skin.[394] Similar to p63, p40 highlights prostatic basal cells and is not expressed by prostatic adenocarcinoma, though there are limited studies on this.[394]

2.12.1.7 Thrombomodulin

Thrombomodulin is expressed by up to 90% of UCs, but the expression is grade-dependent and is significantly lower in high-grade tumours.[366,383,399] Immunohistochemistry is commonly employed, and sometimes essential, in routine diagnostic practice to confirm urothelial differentiation. More data are needed before recommending this marker for routine application. [**Level 4**]

2.12.2 Urothelial carcinoma variants

Studies on the expression of the above markers and others in variants of UC are quite variable in the literature. For the most part, the expression has been similar in most variants as in usual UC.[213,259,400] [**Level 4**] It is beyond the scope of this section to comprehensively catalogue all the case reports or small case series of uncommon variants. The following sections will focus on larger series, and markers that are particularly relevant to the specific variant and its differential diagnosis. Variants without specific issues regarding the immunohistochemical profile or differential diagnosis are not discussed (such as nested variant, microcystic variant, inverted papilloma-like variant, and others).

2.12.3 Other carcinoma types

2.12.3.1 Introduction

The urinary bladder can be involved with tumours originating at other sites, either by direct invasion or by metastatic spread, with the former accounting for approximately 70% of cases.[401] Most tumours that directly invade the bladder originate in the gastrointestinal tract, the prostate gland, the uterus, or cervix. Colorectal adenocarcinoma and squamous cell carcinoma of the cervix have already been discussed and will not be covered here. Metastases from some sites have also been covered in the relevant sections above. Following are other tumours that can be particular problematic.

2.12.3.2 Prostatic adenocarcinoma

In most cases, the morphology of the tumour makes it readily apparent that it is of prostatic origin or at least strongly suggests that possibility. The ductal variant, that can have a prominent papillary architecture, is particularly prone to misdiagnosis when presenting in the urinary bladder. In some cases of poorly differentiated prostatic adenocarcinoma, immunohistochemistry becomes a critical tool in distinguishing it from high-grade UC. Immunoreactivity for prostate-specific antigen, prostate-specific acid phosphatase, and p501s (prostein), with or without NKX3.1, would confirm the prostatic origin.[57,387] Alpha-methylacyl-CoA racemase (p504s) is not helpful, as it is expressed by both UC and prostatic adenocarcinoma. GATA-3 and p63 have been reported to be expressed only rarely by prostatic adenocarcinoma.[370,391]

2.12.3.3 Endocervical and endometrial adenocarcinoma

Morphologically, most endocervical adenocarcinomas are distinctive and do not resemble primary bladder adenocarcinoma. In challenging cases, demonstration of HPV (by immunohistochemistry or *in situ* hybridization) and PAX8 would confirm a cervical origin.[402–404]

2.12.3.4 Renal cell carcinoma

Metastatic renal cell carcinoma to the urinary bladder is well described in the literature.[405] Most cases of the clear cell variant of UC do not resemble clear cell renal cell carcinoma; however, occasionally, some resemblance can be present. In those cases where there is diagnostic difficulty, immunohistochemistry can readily distinguish the two, with renal cell carcinoma expressing PAX8.

2.12.3.5 Malignant melanoma

Primary malignant melanoma occurs rarely in the urinary bladder and when found, it is most often metastatic. In cases where this possibility is considered, based on morphology or clinical history, demonstration of melanotic markers (HMB-45, melan-A, S100 protein) and the absence of epithelial markers can confirm the diagnosis. Immunohistochemistry is commonly employed, and sometimes essential, in routine diagnostic practice for the distinction between a primary bladder carcinoma and secondary involvement from another anatomic site. The exact immunohistochemical panel required, if any, depends on the histologic features of a given case. [**Level 4**]

2.13 Recommended Nomenclature for Urine Cytology

Pathologists and cytologists are an active part in decision-making via their diagnostic abilities. They help clinicians choose the optimal management options. There exists a real need for a more standardized terminology in urinary cytology. The Bethesda system for cervical cytology terminology initiated the standardization in cytopathology. The College of American Pathologists encouraged the use of standardization of nongynecologic terminology.

2.13.1 Nomenclature and reporting

Urine cytology is a simple, noninvasive, and inexpensive tool in the diagnosis and follow-up of patients with UC that is generally used in conjunction with cystoscopy and imaging to guide an individual patient's work-up, management, and follow-up planning. A negative cytology result associated with a normal cystoscopy is quite specific and reassuring as to the absence of a high-grade lesion. Conversely, a positive result accompanied by a negative cystoscopy or biopsy is usually an indication for a more aggressive follow-up and repeat biopsy with the potential evaluation of the upper urinary tract.

Despite the fact that urine cytology was reported to have high specificity for high-grade UC, the clinical relevance of nondefinitive cytological diagnoses such as "atypical urothelial cells" remains ambiguous, due to the inter-institutional differences in the diagnostic categories used as well as in the criteria applied, which has resulted in a significant variation in both the percentages of the cytological diagnostic categories and their association with concurrent or subsequent histologically-confirmed malignant tumours.[406–409] This, coupled with the fact that urine cytology has a low yield for low-grade urothelial lesions (noninvasive low-grade papillary UC, papillary urothelial neoplasm of low malignant potential, papilloma), which are molecularly distinct tumours that are easily detected clinically and that have low rates of progression, are the two conceptual pillars based on which the Paris system (**Table 2–4**) of reporting urine cytology was built.[410–414] The Paris system provides a long needed uniformity in the terminology and the criteria used, which are geared toward the detection of high-grade urothelial carcinoma (HGUC).

The diagnostic categories are: 1) nondiagnostic/unsatisfactory; 2) negative for high-grade urothelial carcinoma (NHGUC); 3) atypical urothelial cells (AUC); 4) suspicious for high-grade urothelial carcinoma (SHGUC); 5) HGUC; 6)-low-grade urothelial neoplasm (LGUN); and 7) secondary malignancies.[415] In the Paris system, the NHGUC category was designed to include the majority of cases, including those showing cellular fragments or cells with minimal atypia which can be accounted for by a nonneoplastic etiology, such as urolithiasis, infection, chemotherapy, radiation, BCG treatment, or polyomavirus (BK virus) infection.[415–418] The AUC category includes cells showing an increased nuclear:cytoplasmic ratio (>0.5), and one or more atypical cytological features, depending on whether the cells are degenerated or nondegenerated. The SHGUC and the HGUC categories share the same cytological features, including nondegenerated urothelial cells with a nuclear:cytoplasmic ratio of >0.7 and severe hyperchromasia, with either irregular nuclear membranes or clumpy chromatin pattern, or both. The main difference between the two categories is the number of severely abnormal

urothelial cells, with the HGUC diagnosis reserved for cases with a minimum of 5 to 10 cells.[419,420] The LGUN category is reserved for the very rare cases showing fibrovascular cores associated with cells that display cytological atypia falling short of a SHGUC/HGUC diagnosis.

In addition to its quest for uniformity, the aim of the Paris system is to minimize the rate of AUC diagnoses and to transform this category into a standardized, reproducible, and clinically meaningful category. In one recent study, implementing the Paris system resulted in fewer cases being assigned an AUC diagnosis (39% vs. 26%), while the association of AUCs with subsequent biopsy-proven HGUC increased from 33% to 53%.[17] [**Level 2**] No single ancillary test is clearly recommended in urine cytology; the optimal role remains to be defined. Fluorescence *in situ* hybridization assay examination can still be used in difficult cases; nevertheless, the robust Paris system provides good definitions of narrow diagnostic groups and decreases the necessity of ancillary testing. [**Level 3**]

TABLE 2–4 Terminology of the Paris System

Adequacy of urine specimens (Adequacy) (Nondiagnostic/unsatisfactory)
Negative for high-grade urothelial carcinoma (Negative)
Atypical urothelial cells
Suspicious for high-grade urothelial carcinoma (Suspicious)
High-grade urothelial carcinoma
Low-grade urothelial neoplasm
Secondary malignancies

2.14. Optimal Management: The Urologist's Point of View

Optimal management of NMIBC is based on a continuum of expertise. It is of crucial importance for the urologist to share relevant facts with the pathologist when referring a resection specimen for analysis, in order to receive, in return, the information required for understanding the severity of the disease and for personalizingtreatment.[58,421] This information includes personal history of the patient; prior intravesical treatments; the gross presentation of the tumour in terms of size, appearance, and position within the bladder; and finally, the technique used for procuring the specimens.

It is important to inform the pathologist after adjuvant treatment with intravesical BCG or mitomycin C, where it may be hard to distinguish at cystoscopy between the anticipated therapeutic effect and cancer recurrence. BCG therapy induces acute and chronic inflammation with small granuloma within the superficial layer of the lamina propria. The epithelium shows slightly raised photodynamic excitation at photodynamic diagnosis, and can be mistaken for recurrent CIS and subjected to biopsy during endoscopy. Mitomycin C antitumour action is mediated by crosslinking to the DNA of the epithelial cells, resulting in superficial cell death and exfoliation. Abundant exfoliation of large multinucleated cells can be observed for a long time after treatment and may be evocative of high-grade cancer on washout cytology, while areas of denudation at endoscopy may impose for a denuding form of CIS.[422]

Systemic treatments may also affect the bladder epithelium through the urinary excretion of toxic by-products, and may induce acute hemorrhagic cystitis[6] or increase in the long term the risks of bladder cancer.[423,424] Significant changes were also reported for other systemic chemotherapies, including pre-emptive cisplatin-based regimens for MIBC where cancer regression can be graded and may have prognostic value.[425]

More recently, urine metabolites of ketamine, an anesthetic drug, were shown to have an impact on symptoms, such as induction of irritative voiding. These symptoms are also evocative of CIS and may lead to diagnostic cystoscopy and biopsy.[426]

The bladder mucosa is also exposed to a wide variety of infectious agents. History of chronic infection, indwelling catheters, bladder stones, or history of spinal cord injury must be highlighted, as they strongly relate to squamous cell carcinoma.[427] Viral infections are not uncommon in immunocompromised patients, such as the transplant recipient. The BK polyomavirus is quite prevalent and suspected to bear oncogenic potential.[428,429] Viral cytopathic effect includes sloughing of altered epithelial cells with nuclear inclusions—the "decoy cells," which can be mistaken in urine cytology for atypias of uncertain significance.[430]

The bladder may be affected by radiation therapy of neighbouring organs, such as the prostate, the rectum, or the female genital tract.[431] While typical signs of mild radiation cystitis such as tortuous microvessels, telangiectasia, and atrophic mucosa are readily understood, biopsy or resection may be

needed to research early signs of radiation-induced cancer. In conclusion, informing the pathologist of the patients' history is important to optimize the comprehension of the observed tissue alterations (**Table 2–5**).

TABLE 2–5 Summary of Conditions Warranting Information

Information	In relation with
Intravesical treatments	
BCG	Inflammation, granulomas
chemotherapy	Atypical washout cytology, denudation
Systemic treatments	
Immunosuppressive drugs	Viral infections, BK polyomavirus
Cyclophosphamide	Acrolein cystitis
Platinum salt preemptive chemotherapy	Tumour regression
Recreational drugs	Alteration of E-cadherin
Geography	Schistosomiasis
Chronic infection, irritation	Squamous cell carcinoma

2.14.1 The value of endoscopy

Visual understanding should improve our ability to comprehend the subtle changes associated with bladder cancer. Indeed, of the 6 independent factors predictive of recurrence and progression highlighted in the EORTC risk calculator, 2 pertain to the technology used for detection (number of lesions and presence of CIS). Conventional endoscopy images the bladder wall through the reflection of white light by the different structures of the submucosa. Although this method is still considered the gold standard in endoscopy, it was repeatedly shown to overlook cancer lesions, both flat and exophytic, compared to more advanced forms of imaging such as hexyl aminolevulinate photodynamic diagnosis and narrow-band imaging.[432–435] This limitation was instrumental in the decision of the National Institute for Health and Care Excellence guideline (nice.org.uk/guidance/ng2, 25/2/2015) to insist on complementing white light–guided TUR of the bladder tumour with one of photodynamic diagnosis, narrow-band imaging, cytology, or a urinary biomarker test to people with suspected bladder cancer," as abnormalities revealed only through advanced imaging techniques may be of subtle endoscopic appearance requiring the full attention of the pathologist.[436] The urologist's skills in endoscopy and resection must be complemented by the pathologist's expertise. This cooperation encompasses information on patient history, endoscopic appearance of the lesions, and their position within the bladder.

2.15 Updated Protocols for Examination and Handling of Specimens From Patients With Urinary Bladder Carcinoma

2.15.1 Biopsy management

Identification data should be verified and confirmed. All tissues must be submitted and processed. If specimens are submitted separately by the urologist, they should be processed separately. An attempt should be made to properly embed the tissue on edge to allow visualization of mucosa and tissues beneath the mucosa. It is necessary to excercise extra care in handling fixed specimens because of the fragility of neoplastic tissue (that is, papillary lesions). For histologic evaluation, different levels of tissue sections for each biopsy can be prepared. However, pathologists can request deeper level sections at their discretion in any given case, where the urothelium is denuded or absent (that is, artifact, denuding cystitis, CIS).

2.15.2 Transurethral resection

Identification data should be verified and confirmed. If samples are submitted separately by the urologist, they must be processed separately, as submitted by clinicians. TUR specimens are usually submitted completely. Some protocols allow the submission of 1 bloc per 1 cm of tumour tissue. However, the vast majority of TUR specimens are small samples and those exceeding 10 cm are rather rare. One can submit the entire tissue, even in large quantity, for complete histologic assessment, but no official recommendations exist. Due to fragmentation and cauterization of TUR samples, it is exceedingly difficult to orient such samples and to embed on edge. Nevertheless, any effort to identify the mucosal aspect should be made. As in biopsy material, at least 3 levels of tissue sections for each biopsy should be prepared for histologic evaluation.

2.15.3 Monobloc resections

Monobloc resections are a relatively new technique, where the tumour is first elevated with saline installations and then cut with a hydroknife. This technique allows a better overview of the whole specimen. In huge samples, the resection limits should be inked. This technique allows the pathologist a very accurate reporting of staging and also of evaluation of the resection margins.[437]

2.15.4 Cystectomy

Identification data should be verified and confirmed. If specimens are submitted separately by the urologist, they should be processed separately, as submitted by clinicians.

The outer dimension of the bladder, as well as the length and diameter of attached ureters, should be recorded. In males, the prostate, seminal vesicles, and vas deferens should be measured. In females, the measurement of the anterior vaginal wall should be documented. Bladders that arrive intact are inflated with formalin through the urethra, or, if previously opened, must be pinned out for proper fixation overnight. Inking of the whole surface of the specimen is suggested by some protocols.[111,438] Resection margins, including urethra (prostatic urethra) and ureters, should be identified and submitted for histologic evaluation (ideally, crosssections).

Tumour or mucosal ulcerations (in cases when no residual tumour is present or CIS is suspected) should be measured, documented, and submitted. Representative sections from the tumour and deepest invasion must be submitted for histologic evaluation. When the tumour is present near a surgical margin, inking of such an area is strongly recommended. The tumour should be sampled in full thickness, including surgical margin (mostly 2 to 3 blocs is sufficient to cover the whole bladder wall including the tumour). Representative sections of the dome, trigone, anterior, posterior, and lateral walls should be submitted.

In cystoprostatectomy specimens, prostate should be adequately sampled. One option is to use whole-mount sections containing the prostate en toto. Alternatively, representative sections from both sides should be taken, including perpendicular sections of the apical margins.[439,440]

Another option is to take parts of the prostatectomy apex, middle, and base seminal vesicles. This protocol allows detection of prostate cancer in 25% of cases.[111]

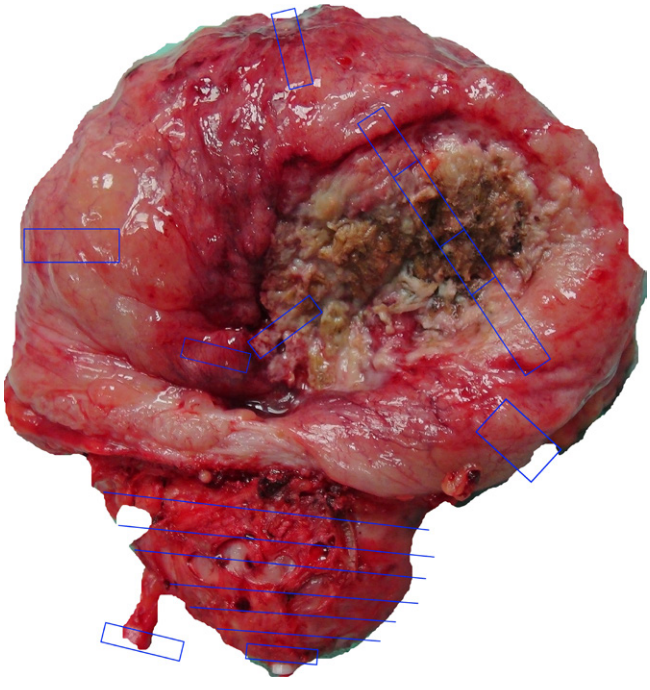
Lymph nodes (if present) must be dissected away from the lymphadenectomy specimens, and all grossly and tentatively identified lymph nodes to be submitted.[441]

In case of segmental cystectomy, bladder-wall resection margins should be identified for possible tumour involvement. Complete embedding of surgical margins is preferable.

Submitted blocs (checklist) (**Figure 2-36**): tumour (1 bloc per 1 cm, minimum-whole thickness of the tumour and bladder wall); uninvolved bladder mucosa, including bladder neck, trigone, anterior, posterior, lateral wall, and dome; resection margins, including urethra and ureters; prostate (see above for details); other organs, if present; all lymph nodes, if present.[438]

Nevertheless, it must be emphasized that no standardized protocol exists for any type of cystectomy.

FIGURE 2–36
Submitted Blocs Checklist



2.15.5 Fresh frozen sections

Intraoperative examination of histologic specimens for urinary bladder is relatively rare and only exceptionally indicated.[442] There are some rare situations when frozen sections can be useful, although they are not recommended.[443] Surgical margins of ureters and urethra can be submitted for frozen section. The important point is to evaluate mucosal margin for malignancy (CIS or invasive carcinoma). Preferably, the true margin should be designated by the surgeon (ink, or suture or ligature). It is crucial to identify the lumen and properly orientate the specimen.

Soft tissue surgical margins are more problematic, as lipomatous tissue is not suitable for frozen section. Hence, careful gross evaluation should be performed.

Gross examination is important to identify tumour extent when assessing surgical margins in partial cystectomy. Depending on the distance of the tumour from margin, representative sections of the margin, either en face or perpendicularly, can be submitted for frozen section examination, but this setting should remain an exception.

Urinary bladder neck in radical prostatectomy for prostatic adenocarcinoma is usually a small tissue sample which should be completely submitted for frozen section. Orientation of the sample is mostly impossible; therefore, this setting should be avoided.

Normally, frozen sections are not recommended, they should only be used in particular settings, and should remain the exception.

2.15.6 Lymph node dissection and how to report

The same problem with gross handling is also found in lymph node dissections. Lymph node dissection is the most reliable method for status staging and is of major importance for guiding adjuvant treatment.[443,444] Currently, no standardization in the sampling of lymph node dissection exists. It falls to the discretion of the pathologist on how he proceeds, since no universally accepted quality parameter exists.[445] Counting inguinal lymph nodes is controversial and a difficult issue in uropathology. The number of resected lymph nodes is important and some papers claim the importance of the socalled "lymph node density." Stein *et al.* published this prognostic factor first in 2003.[446] They characterized lymph node density as lymph nodes involved with tumour divided by the total number of lymph node s removed. This concept is clearly not up to date any more.

A recent paper compared the prognostic value of the American Joint Committee on Cancer (AJCC) TNM Staging System with that of lymph node density in patients with lymph node–positive bladder cancer who received extended or superextended pelvic lymphadenectomy. The authors concluded that lymph node density shows a better prognostic value than the AJCC TNM Staging System in patients with lymph node–positive bladder cancer undergoing extended or superextended pelvic lymphadenectomy.[447]

Therefore, one must be aware that the pathological examination plays a major role. No optimum method has been recommended; normally, lymph node dissections are manually palpated and dissected. The major problem is that very often, these lymph nodes are extensively infiltrated by fat, have elongated tortuous features, and even when totally embedded, the count on the slide is far from evident. There is also no consensus on how to treat the remaining fat tissue around the manually palpable lymph nodes. Total submission of the lymph node dissection has been claimed by several authors and increases the number of lymph nodes.[448,449] But cost and time should also be evaluated, and the clear benefit has not been established.

The enumeration is difficult, and depends on the subjective interpretation in several scenarios. Even when following the histological description of a lymph node (capsule, subcapsular sinus, and lymphoid tissue), it is still unclear how to count lymphoid aggregates without capsule and how to report them, in case of invasion by a UC.[450] Furthermore, when cutting a lymph node, a single incurvated lymph node can give the impression of being 2 lymph nodes, but also, small lymph nodes can exit in the fat around a huge lymph node. Counting on the slides is probably the most precise way.

Most of the time, metastatic lymph node invasion is already obvious in gross findings. The eighth edition of the Union for International Cancer Control (UICC) pathologic TNM classification divides lymph nodes into 4 groups according to the number and location of positive lymph nodes (**Table 2–6**).[451] This edition differs slightly from the eighth AJCC edition.[441]

TABLE 2–6 Regional Lymph Nodes According to the Union for International Cancer Control Pathologic TNM Classification, Eighth Edition

N0	No regional lymph node metastasis
N1	Metastasis to a single lymph node in the true pelvis (hypogastric, obturator, external iliac, presacral)
N2	Metastasis to multiple lymph nodes in the rue pelvis (hypogastric, obturator, external iliac, presacral)
N3	Metastasis to common iliac lymph nodes

Nodal cancer volume, the size of the largest metastatic tumour deposit, and extranodal extension play a role for the prognosis.[452] The problem of micrometastases, is on the other hand, not completely resolved, no recommendations for section cutting exist, and step-sectioning, together with immunohistochemical stains, are not recommended. A recent paper by Engvad *et al.* does not recommend the use of routine cytokeratin staining, although they do recommend extensive pathological examination of lymph node dissections.[453]

A further problem is how to consider lymph node dissections after neoadjuvant chemotherapy. We do not have real robust data on whether the number of lymph nodes decreases under neoadjuvant chemotherapy, and again, no recommendations exist on how to report involution into fibrosis in lymph node metastases after chemotherapy.[445]

There is an urgent need to establish a more consistent and standardized approach to both gross and histologic evaluation, which requires close collaboration between pathologists and urologists. Routine reporting on the lymph node metastatic size and extranodal extension are recommended by the ICCR group, which recommends reporting the following items: lymph nodes submitted, number of lymph nodes examined, number of positive lymph nodes, presence or absence of extracapsular spread (recommended), and size of the largest metastases (in mm). It is also important to report the size of the positive lymph nodes, and to establish a relationship between lymph nodes and tumour invasion. No recommendations exist about how to report extranodal tumour deposits.

2.15.7. Standardized reporting

The ICCR produces common, internationally validated and evidence-based pathology data sets for cancer reporting, through broad collaboration with major cancer and pathology organizations and colleges. It aims to encourage the uniform uptake of a single pathology reporting standard across the world. The ultimate goal of the ICCR is to improve cancer patient outcomes worldwide and to advance international benchmarking in cancer management.

Standardization of pathology reports has become an essential step in the process of improving patient care. Not only does it ensure that the same histological elements are reported, but it also allows more accurate comparison of different studies conducted in different institutions or countries. This being said, standardizing reports can be meaningless, if clear and reproducible histological criteria that define different elements are not established. In the last 20 years, the WHO classification made several steps toward standardization by providing detailed descriptions of different entities and histological elements.[21] The second major step forward was the ICCR group, which was founded by major pathology organizations. The main goal of this initiative was to produce internationally standardized and evidence-based data sets for the pathology reporting of cancer in order to improve cancer patient outcomes worldwide and to advance international benchmarking in cancer management. The ICCR constructed website has important advantages, such as a bookmarked guide and a hyperlinked guide, which was designed to be viewed when connected to the Internet, where explanatory texts on requested elements can be found. Furthermore, MS Word and MS Excel documents are available, which include the data set content to assist with implementation. The bladder data set has been developed for the reporting of many specimen types, including cystectomy, cystoprostatectomy, or diverticulectomy. The protocols apply to primary carcinomas (noninvasive and invasive), with or without associated epithelial lesions. Urothelial tumours diagnosed as papilloma or PUNLMP are not carcinomas and this data set does not apply to those diagnoses. TUR and biopsy specimens are dealt with in a separate data set.

In the bladder data sets, two types of elements were included: required (req) or recommended (rec). Required elements are those that are prognostically important and act as a basis in clinical management. Evidence-based support at Level III-2 or above (based on prognostic factors in the National Health and Medical Research Council levels of evidence document and defined as "Analysis of prognostic factors amongst persons in a single arm of a randomized controlled trial") is needed for the element to be considered required. Required elements are mandatory reporting items that should be included in every pathology report. In comparison, recommended elements are those for which reporting may be clinically important and considered to be good practice, but which are not yet validated or regularly used in patient management.[454]

In the ICCR bladder data sets, the following elements are included: clinical information (rec and req in TURB); specimen site (req); additional specimens submitted (req); operative procedure (req); bloc identification key (req and rec in TURB); histological tumour type (req); presence of noninvasive carcinoma (req); associated epithelial lesions (req and rec according to the operative procedure); histological grade (req); extent of invasion (req); macroscopic extent of invasion (req); microscopic extent of invasion (req); tumour focality (rec); substaging T1 disease (rec); and lymphovascular invasion (req). Some items can only be applied in cystectomy specimens, such as response to neoadjuvant therapy (rec), margin status (req), lymph node status (req), histologically confirmed distant metastases (req), coexistent pathology (rec), and pathologic staging (req if applicable) (**Table 2–7**).

The objective of providing uniform reporting and treatment for bladder cancer is expected to be facilitated by using these data sets, along with the WHO 2016 and the EAU guidelines.

TABLE 2–7 Required and Recommended Items in a Pathology Report According to the International Collaboration on Cancer Reporting

Item	Recommended	Required
Clinical information	✔	✔
Specimen site	-	✔
Additional specimens submitted	-	✔
Operative procedure	-	+
Bloc identification key	✔ (TURB)	✔
Histological tumour type	-	✔
Presence of invasive carcinoma	-	✔
Associated epithelial lesions (depends on operative procedure)	✔	✔
Histological grade	-	✔
Extent of invasion	-	✔
Macroscopic extent of invasion	-	✔
Microscopic extent of invasion	-	✔
Tumour focality	✔	-
Substaging T1 disease	✔	-
Lymphovascular invasion	-	✔
Only for cystectomies		
Response to neoadjuvant therapy	-	✔
Margin status	-	✔
Lymph node status	-	✔
Histologically confirmed metastasis	-	✔
Coexistent pathology	✔	-
Histological staging (if applicable)	✔	-

2.16 Levels of Evidence and Grades of Recommendation

2.16.1 Levels of evidence

Level 1: Meta-analysis of randomized trials of a good-quality randomized trial
Level 2: Low-quality randomized trial or meta-analysis of good-quality, prospective cohort studies
Llevel 3: Good-quality retrospective case-control studies or case series
Level 4: Expert opinion based on "first principles" or bench research, not on evidence

2.16.2 Grades of recommendation

Grade A: Usually consistent level 1 evidence
Grade B: Consistent level 2 or 3 evidence, or "majority evidence" from randomized trials
Grade C: Level 4 evidence, "majority evidence" from level 2 to 3 studies, expert opinion
Grade D: No recommendation possible because of inadequate or conflicting evidence

Adapted from: Abrams P, Grant A, Khoury S. Evidence-based medicine: Overview of the main steps for developing and grading guideline recommendations. In: Abrams P, Cardozo L, Khoury S, Wein A, eds. *Incontinence: Basics and Evaluation.* Vol 1. Paris: Health Publication Ltd; 2005:10-11.

2.16.3 Summary of recommendations

- Make sure to know from which part of the bladder the sample has been taken (impact on staging).
- Flat lesions must be reported.
- Clinical information is mandatory, especially when characterizing flat lesions.
- Both 1973 and 2004 WHO grading systems provide important prognostic information about noninvasive papillary urothelial neoplasms, as both versions demonstrate generally similar results.
- Grading of heterogeneous lesions should be based on the highest grade; if present and if the high-grade component is lower than 10%, this observation should be communicated to the clinician in the pathology report.
- pT1 substaging is recommended, but not the methodology.
- In diverticula, no stage pT2 should be given. [**Level 2**]
- Urachal carcinoma should be staged according to the Sheldon system.

- Complementary prognostic factors are lymphovascular invasion and variant histologies.
- Numerous different histologic variants exist and should be mentioned in a report.
- No recommendation exists on how to handle resections, grossing of cystectomies, and lymph node dissections.
- Frozen sections during surgery are not recommended, but can be done in special settings.
- Standardized reporting has been proposed by the ICCR.

2.17 List of Figures

2.18 References

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C3

Basic Science

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3.1 Introduction

Recent developments have generated significant interest in bladder cancer as a model system for basic and translational research. Whole genome characterizations of both nonmuscle-invasive and muscle-invasive bladder cancers have provided the first high-resolution descriptions of cancer heterogeneity, and the appreciation that immune checkpoint blockade (ICB) is clinically active in a subset of advanced cancers has attracted significant industrial interest and investment in the disease. Researchers also benefit from ready access to longitudinal tissue samples and a relatively large (and rapidly growing) array of preclinical human and mouse models for mechanistic interrogation. Here we provide a fairly comprehensive overview of some of the most impactful basic research topics currently being explored in the disease. Each section of this chapter was prepared by internationally recognized experts in the field, and the bibliography should serve as an excellent resource for investigators in the field.

3.2 Developmental Aspects of Human Urothelial Differentiation *In Vitro* and *In Vivo*

3.2.1 Introduction

The human urinary bladder plays an essential role in providing a reservoir for the secure temporary storage of urine from the kidneys. Inherent to this role is the ability of the bladder to accommodate changing volumes of urine at low pressures and to prevent toxic urinary contents from reabsorption by the body. These properties are critically dependent on the unique properties of the urothelium: the transitional epithelium that lines the major portion of the urinary tract, including the bladder. The urothelium is highly specialized as a mitotically quiescent tissue barrier to urine that nevertheless retains the capacity for rapid cellular regeneration and repair. The mechanisms that have evolved to regulate urothelial homeostasis at these extremes of mitotic activity are not only central to understanding the normal physiology of the urinary system, but to interpreting the drivers and pathways of chronic benign and malignant urothelial diseases. In addition, the genes and proteins that define the specialized features of the urothelium have use as markers in basic research and for diagnostic/prognostic pathology. This chapter describes the morphology, molecular cell biology, and function of the urothelium, with emphasis on human tissues. In particular, consideration is given to developmental processes and differentiation, and how these inform and are informed by malignant transformation.

3.2.2 What is the urothelium?

As a transitional epithelium, the urothelium is stratified into morphologically distinct basal, intermediate, and superficial cell zones **(Figure 3–1).** The basal cells attach to a capillary-rich basement membrane, whilst the superficial cells are uniquely specialized as the main urine-facing barrier. During the micturition cycle, the urothelium accommodates the large change in intraluminal surface area through the intermediate cell layer, which varies from three to five or more cells thick, depending on bladder distention, and the surface area of the superficial cell layer, through invagination of the apical membrane. These features contribute to maintaining the urinary tract as a low-pressure nonrefluxing system that protects the kidneys from damaging pressures and ascending bacterial infections. Although beyond the scope of this review, the urothelium also plays a critical role in the innate sensing and defence against uropathogenic *Escherichia coli* (*E.coli*) via flagellin-activation of toll-like receptor 5.[1,2]

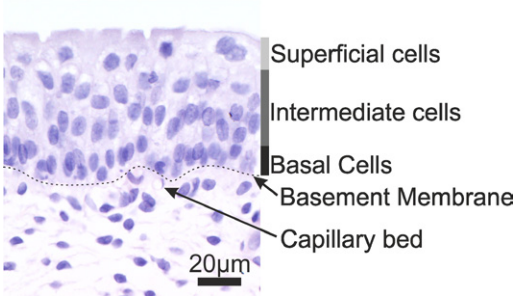


FIGURE 3–1
Hematoxylin- and Eosin-Stained Section of Normal Human Bladder Tissue Illustrating the Basal, Intermediate, and Superficial Compartments of the Urothelium

Note the frequently observed binucleated cell in the superficial cell layer.

Within the urothelium, the basal, intermediate, and superficial cells display differential patterns of protein expression. Of particular note are the intermediate filament-forming cytokeratin (KRT) proteins that show epithelial tissue-associated and differentiation stage-related expression patterns; these provide useful biomarkers in both normal urothelium and in bladder cancer. In normal human urothelium, KRT7, KRT8, KRT18, and KRT19 are detectable throughout; KRT5 and KRT17 are found basally and into the intermediate cell zone; KRT13 is present in all but the superficial cell layer, whilst KRT20 is restricted to superficial cells.[3] Changes in the normal KRT expression and/or distribution pattern can be informative histopathologically. Thus, although KRT14 is absent from normal human urothelium, its occurrence is associated with squamous metaplasia.[4] Likewise, a change from superficial to full thickness KRT20 expression is associated with dysplasia[5] and recurrent noninvasive papillary tumours.[6,7] Taking this to a transcriptomic level, **Figure 3–2** shows a principal component analysis (PCA) of the most expressed KRT transcripts in The Cancer Genome Atlas (TCGA) cohort of muscle invasive bladder cancers (MIBCs).

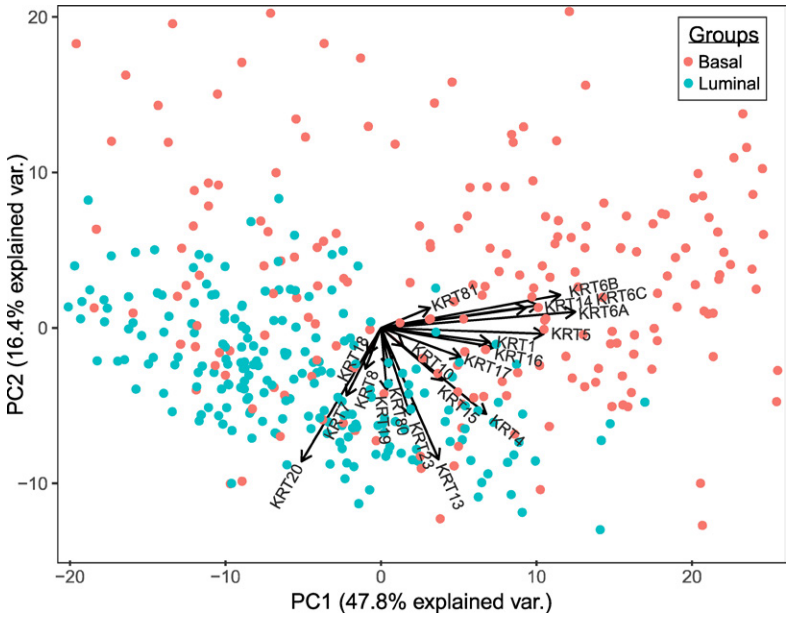


FIGURE 3–2
PCA of The Cancer Genome Atlas Cohort of MIBCs

Expression values were downloaded as normalized counts from the Genome Data Commons, and values for the KRT family of genes was extracted. KRT genes with a mean expression of >1,000 counts/tumour were log2 transformed and included in the unscaled PCA (performed in R3.4.1). TCGA tumours were classified as basal/luminal using a prediction analysis for microarray classifier. Five outlying values for luminal tumours were excluded from this figure to allow a focus on the central area of the PCA plot.

Various urothelial differentiation-restricted proteins are associated with the specialized barrier-forming superficial cell. The best characterized are the species-conserved, integral membrane proteins, known collectively as uroplakins (UPKs), of which there are four major species. The 27,000 Mr UPK1A and the 28,000 Mr UPK1B proteins are members of the tetraspanin family and interact respectively with the unrelated single transmembrane 15,000 Mr UPK2 and 47,000 Mr UPK3 proteins.[8] Combined, the UPKs constitute the unique asymmetric unit membrane plaques of superficial bladder urothelial cells that assemble in the Golgi and translocate as fusiform vesicles to extend the superficial apical membrane during bladder filling.[9]

The urothelium is one of the "tightest" (ie least permeable) epithelial tissues of the body, with well-developed terminal tight junctions between the superficial cells restricting the free movement of solutes between cells. Epithelial tightness is typically assessed experimentally by measuring transepithelial electrical resistance. With 500 $\Omega.cm^2$ being the threshold for classifying epithelia as tight, the transepithelial electrical resistance of human urothelium *in vitro* is in the order of 3000 $\Omega.cm^2$.[10] The claudin composition of the tight junction is considered the main barrier-defining component. Of the 24 members of the claudin superfamily, human urothelium expresses at least claudins 3, 4, 5, and 7,[11] with claudin 3 associated spatially and functionally with tight barrier function.[12]

It is axiomatic that carcinogenesis is associated with loss of tight junctions and this is well-supported immunohistologically in bladder cancer (reviewed in references 13 and 14). Furthermore, the invasiveness of bladder tumour cells can be inhibited by claudin overexpression in experimental models.[15] At the transcriptomic level, a subgroup of undifferentiated (basal) MIBCs has emerged that shows extreme loss of claudin transcript (particularly CLDN7).[16] These "claudin-low" tumours lack E-cadherin expression with signs of epithelial-to-mesenchymal transition[16] and are highly immune-infiltrated and associated with poor patient survival,[17] irrespective of treatment.

3.2.3 Urothelial turnover and repair: implications for bladder cancer

Considering the lifelong exposure to concentrated toxicants, the urothelium evades carcinogenesis through its stable tight barrier, its state of replicative quiescence, and its capacity for repair. This leads to the idea that stressed or damaged urothelium defaults to a repair programme, unlike other epithelia with nonessential barrier functions, where the predominant response to stress is cell death.

Urothelium is a tissue of mitotic extremes and, in response to injury, is capable of very rapid proliferation in pursuit of urinary barrier restitution. Although various metrics are reported, such as 1% of cells being in cell cycle at any one time and an estimated turnover time of 1 year,[18,19] human urothelium does not have a proliferative cell compartment. It is perhaps more appropriate to consider the urothelium as having a facultative regeneration cycle, insofar as it is mitotically quiescent but responds to damage by rapid entry into cell cycle and proliferation. Indeed, when present, mitotic figures are observed in all three layers.[20] In this way, urothelium more closely resembles tissues such as lymphocytes and liver, which are characterized by proliferation on demand. One implication of this responsiveness relates to the relationship between quiescence, regeneration, and differentiation in normal urothelium and how the implicit feedback mechanisms are circumvented in cancer. Cancer is often described as a wound-healing event that fails to resolve but, at this point, surprisingly little is known about the repair and regenerative mechanisms of normal human urothelium. Another implication is in understanding the potential role of telomerase in rapidly proliferating normal cells in light of the recent association between telomerase activation and neoplastic transformation in urothelium.[21]

Compared to human urothelium, where cells rest in a G0 or non-cell cycle state,[22] rodent urothelium remains in the cell cycle at all times, as evidenced from immunolabelling with cell cycle markers such as Ki67.[23] Cell cycle analysis of rat urothelial cells has revealed a high percentage of cells in G2/M.[24,25] The functional consequence of such a fundamental difference in cell cycle regulation between species is unknown, although telomerase may be the key. Whereas telomerase activation is heavily implicated in neoplastic transformation of human urothelium, it remains constitutively active in rodent somatic cells and, by not providing a tumour block, may underpin the greater rate of tumour initiation in rodents.

Also important in considering the urothelial regeneration question is whether urothelium has a defined stem cell population, as this could influence whether therapeutic strategies in cancer should target a particular population. Extrapolating from other epithelia, it is generally assumed that urothelium has a "programmed" basal stem cell population and undergoes hierarchical differentiation from basal through intermediate to superficial urothelial cell layers. A variety of evidence has been used to support this view.[26-29] Although these observations are supportive of a basal progenitor cell type sustaining a hierarchical differentiation program, they are not definitive and there has been no unequivocal identification or isolation of a definitive stem cell from urothelium.

Some evidence is beginning to emerge to support a nonhierarchical model. As all cells (including superficial cells) retain the potential to proliferate in response to tissue damage,[20] this suggests that urothelial tissue homeostasis may involve cellular plasticity, where individual cells are locally

responsive to external cues. Following separation of freshly isolated urothelial cells into basal and suprabasal populations, both subpopulations assume a similar proliferative phenotype *in vitro*, with both producing progeny capable of differentiation to a functional barrier urothelium with distinct basal, intermediate, and superficial compartments; this includes the "backwards" production of basal cells from the suprabasal-derived subpopulation.[30] This could be relevant to urothelial cancer if cells function outside of a unidirectional hierarchical model and are able to interconvert to a stem-like state. Controversy in the field regarding the existence of a repopulating stem cell in normal adult urothelium translates into ambiguity in our understanding of urothelial carcinogenesis. In breast cancer, there is some support for a "cell-of-origin" hypothesis wherein the varying subtypes of tumour derive from different originating cells;[31] however, to date there is little evidence supporting this model in human bladder carcinogenesis.

3.2.4 The embryological roots of urothelial development

The embryological development of the bladder and associated structures is complex and outside the scope of this review. However, parts of this process are worth reflecting upon when considering the potential of the urothelium to undergo alterations of the differentiation state during metaplastic or neoplastic change. A critical part of the developmental process are the spatial/temporal interactions between epithelial and mesenchymal precursors that orchestrate the formation of differentiated tissues and organ structures.

The urothelium is unusual in that, developmentally, it arises convergently from two embryological origins: the endodermally derived urinogenital sinus, which gives rise to the urothelium of the urinary bladder (including the trigone region),[32] and the mesodermal Wolffian duct, which gives rise to the urothelium of the ureter. This provides a useful basis for examining regulatory factors, with bladder and ureteric human showing comparable phenotype and transcriptomes.[33,34]

Urinogenital sinus epithelium not only gives rise to the urothelium, but also to the stratified squamous epithelium of the vagina and the glandular-type epithelium of the prostate, indicating the diverse differentiation potential of cells of urogenital sinus derivation and reflecting the common metaplastic states of squamous or, more rarely, glandular metaplasia. A critical part of the developmental process is the spatial/temporal interactions between epithelial and mesenchymal precursors that regulate the formation of differentiated tissues and organ structures. Tissue recombination experiments in rodents have shown that the fetal, but not adult, mesenchyme produces signals that can direct epithelial cells to these different endpoints, indicating that signalling is instructive. There is work needed to understand the molecular basis of these inductive signals with, for example, evidence emerging to suggest that isoform switching in peroxisome proliferator-activated receptor gamma (PPARγ) may regulate the specialization of urothelium versus prostatic epithelium.[35]

3.2.5 Developmental aspects of human urothelial differentiation *in vitro* and *in vivo*

In serum-free culture and in the absence of nuclear receptor ligands, normal human urothelial cells assume a nondifferentiated, KRT14$^+$ squamous phenotype with autocrine activated estimated glomerular filtration rate growth regulation, and retain the capacity to respond to differentiation-inducing

signals.[36] Whereas squamous differentiation is reversible with retinoids, the cells nevertheless fail to express differentiation-associated genes.[37] By contrast, PPARγ has been identified as a master regulator, activation of which induces expression of genes associated with urothelial differentiation, including UPKs, cytokeratins, and claudins.[38,39] PPARγ-mediated differentiation is mediated indirectly via a network of intermediary transcription factors that appear to function as a heterarchical network, including FOXA1,[40] ELF3,[33] and GATA3.[41] Opposing this, p63 has emerged as an important driver of the more basal squamous phenotype that cells adopt in cultures without nuclear receptor activation. In absence of PPARγ-stimulation, p63 represses genes normally activated as part of the urothelial specialization program.[41]

In vitro systems are open to questions of *in vivo* relevance. In seeking to relate the role of PPARγ activation to human urothelial development *in vivo*, we have examined the timing of PPARγ expression against the emergence of a differentiated transitional phenotype during late embryonal and fetal development. Up to 6 weeks' gestation, the presumptive urothelium is a single-cell layer that becomes a two-cell layer at around 8 weeks, followed by a two-to-three–cell layer at 10 weeks. At 10 weeks' gestation, the first expression of a transitional differentiated phenotype emerges, accompanied by a normal transitional KRT expression profile and apical membrane expression of UPKs. Supporting the role of PPARγ in urothelial differentiation is the demonstration that PPARγ and its heterodimerization partner, retinoid X receptor alpha (RXRα), are both first detected with nuclear localization at the 10-week stage, indicating that their presence is associated with transitional differentiation from the very earliest emergence of this phenotype **(Figure 3–3).**

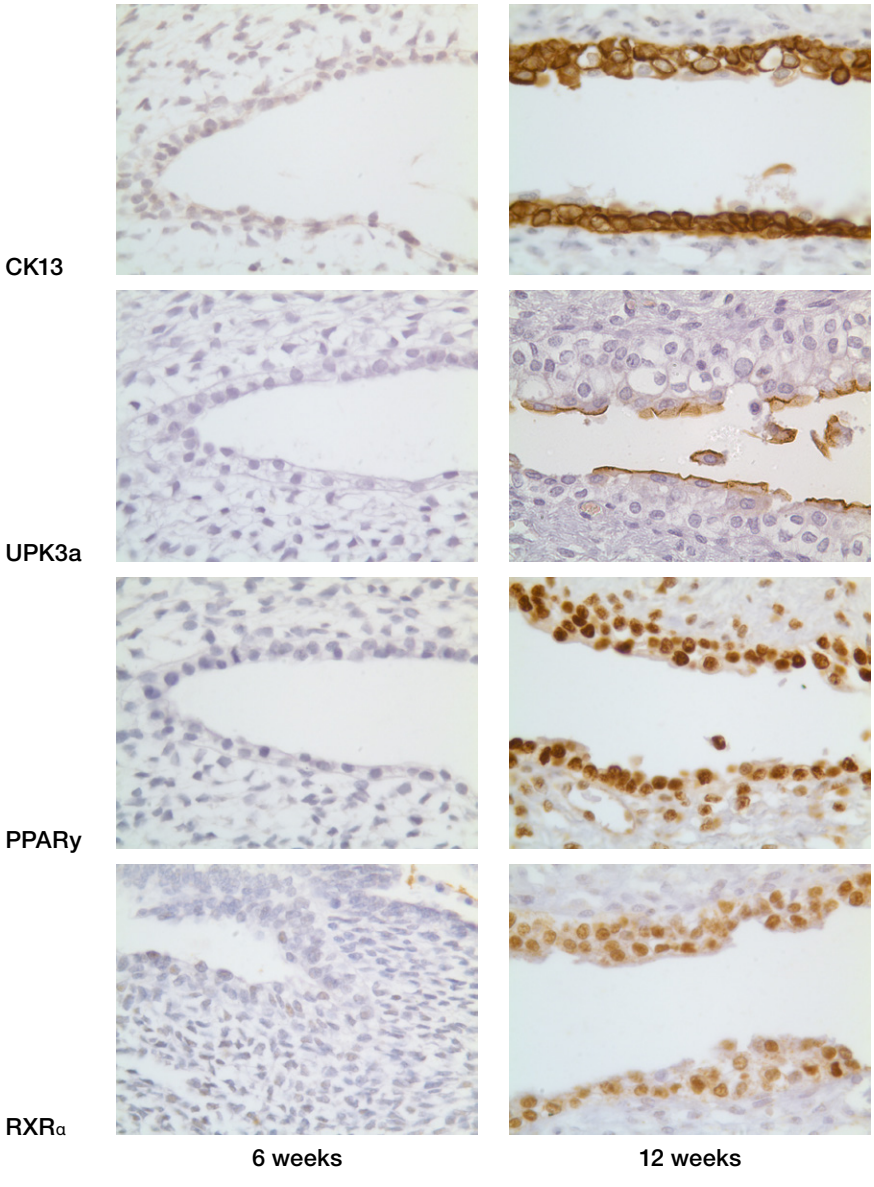


FIGURE 3–3
Immunohistochemistry of Human Fetal Bladder at 6 (Left) and 12 Weeks' (Right) Gestation (All Taken Using x63 Objective)

At 6 weeks, a single-layer epithelium was evident with no or equivocal expression of CK13, UPK3a, PPARγ, or its heterodimerization partner, RXRα. At 12 weeks' gestation, a three-layered urothelium is present, within which CK13 is intensely expressed in basal and intermediate cells, UPK3a is expressed along the apical edge of superficial urothelial cells where preserved, and there is diffuse nuclear labelling of PPARγ and RXRα.

In addition to their well-characterized role in transcellular urinary barrier function, the UPKs are critical to the normal development of the urinary tract. In mice deficient for single UPK genes, the urothelium fails to polarize or develop fully differentiated superficial cells, forming a hyperplastic permeable barrier that sometimes occludes the ureter, causing obstruction, vesicoureteral reflux,

and hydronephrosis.[42,43] Whilst there is no genetic link between UPK3A and vesicoureteral reflux in patients,[44] subtle point mutations have been associated with severe congenital anomalies such as renal adysplasia[45,46] indicating a more severe spectrum, presumably as a result of the spatial and temporal misalignment of tissue-inductive processes during renal and urinary tract development. The link between UPK loss and hyperplasia is compelling, given the propensity for urothelium to switch between differentiated quiescent and regenerative states, but the observation has yet to extend to our understanding of urothelial tumourigenesis.

There appear to be strong parallels between the regulation of urothelial differentiation observed *in vitro* and the groupings established by transcriptomic analysis of MIBC. Undifferentiated basal tumours depend on autocrine activation of the epidermal growth factor receptor for growth[47] and are enriched for p63 gene expression signatures, whereas the hallmark differentiated luminal tumours have a PPARγ-activated profile.[48] Of the intermediary transcription factors identified in differentiating urothelial cells,[33] ELF3 has emerged as frequently mutated in its coding sequence (in ~9% of tumours) and these, presumed activating, mutations are associated with a luminal phenotype.[49] Whilst many MIBC tumours exhibit a fixed homogeneous differentiation state, it is important also to note that others display the plasticity required to create grossly normal structures exhibiting polarity and stratification in KRT expression.[50] It is appealing to consider how far the *in vitro* models can provide insight, for example, whether the identification of an autocrine TGFβR-Smad3 signalling pathway in differentiated urothelial regeneration[34] *in vitro* has relevance for luminal MIBC.

3.2.6 Concluding points

The evolutionary need for a urinary barrier has led to development of an epithelium, the urothelium that is structurally and functionally specialized for its role. The mitotically quiescent barrier protects the underlying cells from potentially carcinogenic or toxic exposure, further minimized by the capacity to respond to damage through regeneration and barrier restitution. Whereas a subset of urothelial cancers appear to "escape" the constraints of differentiation and assume the primitive "basal" phenotype of a squamous autocrine epidermal growth factor receptor (EGFR)-regulated cell, it appears that other "luminal" cancers hijack the differentiated urothelial transcriptomic program, possibly showing a selection dependency on the pathways that support regeneration and repair in the normal tissue. This raises interesting questions about what initiates and selects for MIBCs that follow the luminal versus basal routes and how different therapeutic agents might target each. Further research into this remarkable epithelium may reveal other secrets, such as the origin and identity of the natural ligands that drive the fetal development and differentiation of urothelium and the nature of the reversible switch that regulates the balance between regenerative and quiescent phenotypes.

3.3 Overview of Mutational Events

Patient age, gender distribution, and risk factors are broadly similar in populations of patients with the two major bladder cancer disease types (nonmuscle invasive bladder cancer [NMIBC] and MIBC). However, the tumours themselves show distinct mutational features, ranging from overall mutational burden at single nucleotide level through alterations that generate aneuploidy, chromosomal rearrangements, and DNA copy number alterations. There are also major differences in evolution of the mutational landscape over time and space in these two groups.

The somatic mutation patterns found reflect damage caused by carcinogens and the DNA damage repair mechanisms that have operated during disease pathogenesis. Bladder cancer risk has proven links to both smoking and occupational exposures and, of these, cigarette smoking is linked to more cases than any other known agent. It has been estimated that current smokers have a three- to four-fold higher risk than nonsmokers, and this is linked to approximately half of all cases in males and a third to a half of cases in females.[51,52] Other lifestyle factors, including alcohol consumption, coffee drinking, fluid intake, and dietary factors, have been suggested, but some data are contradictory and/or inconclusive.[53] Several occupations are implicated[54] with known causative carcinogens including beta naphthylamine, benzidine, and polycyclic aromatic hydrocarbons.

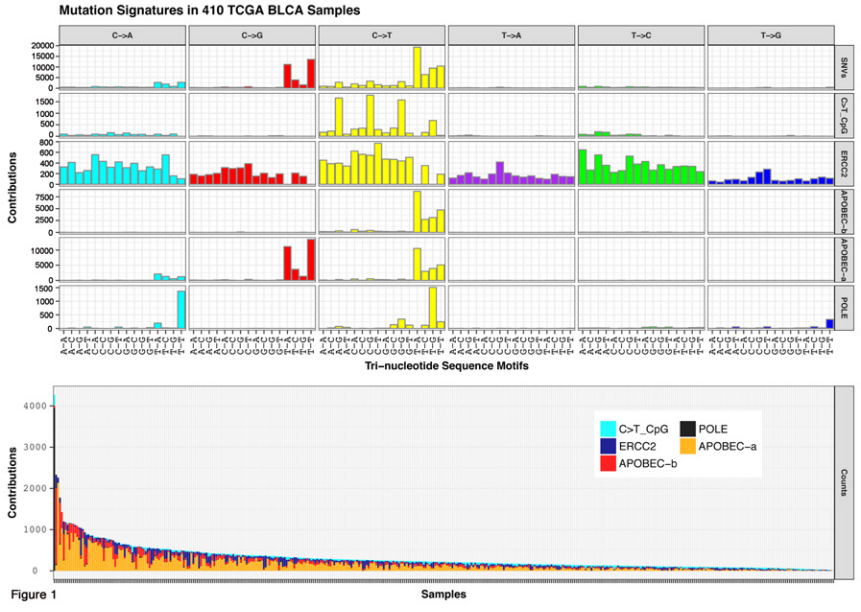
The urine of smokers contains a range of carcinogens and related metabolites, including polycyclic aromatic hydrocarbons and aromatic amine metabolites.[55] Such urine is mutagenic, and this has been attributed in particular to the content of aromatic and/or heterocyclic amines.[56] The mutations induced include single base changes and DNA double-strand breaks. Compatible with this, carcinogen adducts and elevated levels of the DNA double-strand break marker phosphorylated histone 2A have been identified in bladder tumours from smokers.[57]

Whole genome and whole exome sequencing of bladder tumours has recently provided a detailed view of their mutational landscapes. This has not only identified genes that are critical bladder cancer "drivers," but also allows potential links of mutational signatures to carcinogen exposure and lifestyle exposures to be examined. MIBC contain more DNA alterations than most other adult malignancies apart from melanoma and lung.[58] The TCGA study of MIBC has reported mean and median nonsynonymous somatic mutation rates of 8.2 and 5.8 per megabase (Mb), respectively.[59] In NMIBC, mean overall mutation rates (synonymous and nonsynonymous) per Mb are much lower (1.8–2.41 per Mb).[60,61]

C > T transitions are the most common single nucleotide variant (SNV) seen in MIBC (51%), followed by C > G (27%),[59] with similar frequencies in NMIBC (40%–50% C > T; 20%–30% C > G).[60-62] In the National Cancer Institute (NCI) TCGA whole exome sequencing data set of 412 MIBC, Bayesian non-negative matrix factorization identified five different mutation processes. Two of these were variants of the hallmark apolipoprotein B messenger RNA (mRNA) editing catalytic polypeptide-like (APOBEC) mutagenesis signature, consisting of TC(A or T) -> T(G or T)(A or T), that accounted for 67% of the mutations seen. C > T at CpG sites and *ERCC2* mutations accounted for the remainder of mutations, with the exception of one sample with a very high mutation rate due to P286R mutation in *POLE* **(Figure 3–4)**.

FIGURE 3–4
(Top) The Spectrum of Total SNVs and Five Mutational Signatures in 96 Base Substitution Contexts (Mutated Pyrimidines and Adjacent 5′ and 3′ Bases) in MIBC (9)

(Bottom) The Number of Mutations Assigned to Each Mutational Process Across 410 Samples From TCGA (9)



(Top) Note that, because the dynamic range for the signatures is large, y axis upper limits are different for each signature. The POLE signature was present exclusively in a single ultra-mutated sample, with >4,000 SNVs and a *POLE* mutation (P286R), and the activity of ERCC2 signature was significantly associated with *ERCC2* mutations.

(Bottom) The activity of APOBEC-a and -b signatures accounted for 67% of all SNVs and played a major mutagenic source with the *ERCC2* signature (20%), except the *POLE* signature.

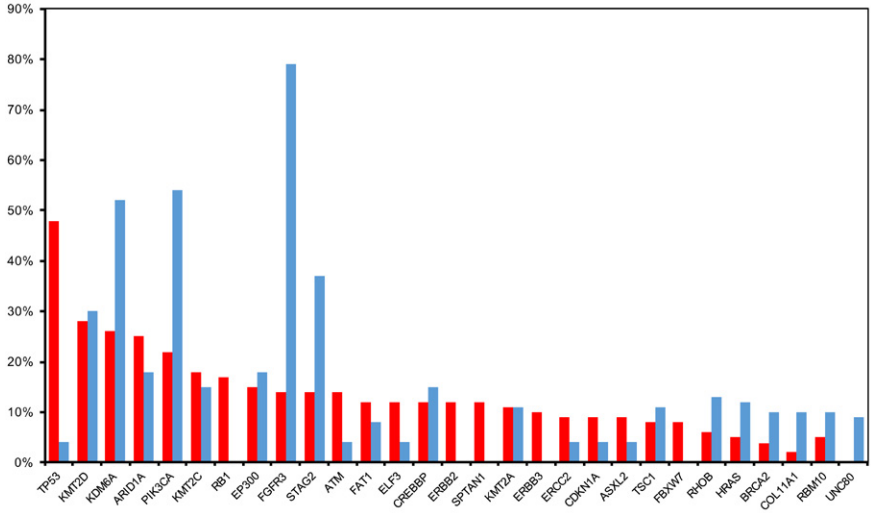
Unsupervised clustering analysis of samples by signature led to identification of four mutational signature clusters in the TCGA MIBC set. Patients with mutation signature cluster (MSig) 1 (~10%) cancers had very high APOBEC-signature mutagenesis and high mutation burden (>20 mutations/Mb), and showed an exceptional 75% 5-year survival rate. On the other hand, patients with MSig2 (nearly 50%) had the lowest mutation rate (<5 mutations/Mb) and poorest 5-year survival (22%). MSig4 cluster samples were enriched in both ERCC2 signature mutations (average contribution 49% vs 17% in all others), and *ERCC2* mutations (24 out of 39, $p=10^{-13}$). ERCC2 signature mutations were highest in smokers with *ERCC2* mutations ($p=6.9 \times 10^{-13}$); for cases with wild-type *ERCC2,* ERCC2 signature mutations were enriched in smokers compared to nonsmokers.

NMIBC also show a strong APOBEC mutagenesis signature.[60,61,63] In a small series of stage Ta tumours, APOBEC signature was enriched in 18 of 24 samples, and 35% of all mutations bore this signature. The number of mutations with APOBEC signature strongly correlated with the total number of mutations except in two outliers, one of which contained a *POLE* mutation and the other a mutation in *POLE2,* and the signature was significantly enriched in the more genomically unstable tumours.[60]

Within the large numbers of somatic mutations identified by genome sequencing, it is important to identify those genes that make an essential contribution to tumour development and phenotype. Thus, efforts have been made to separate so-called "driver" from "passenger" mutations.[64] Algorithms have been developed to identify significantly mutated genes (SMGs) that have higher mutation rate than the background mutation rate. SMGs in MIBC and NMIBC show differences both in diversity and spectrum.[59-62,65,66] Whole exome sequence data have identified 53 SMGs in MIBC, with *TP53, KMT2D, KDM6A, ARID1A, PIK3CA, KMT2C, RB1,* and *EP300* being the most commonly mutated in order of decreasing frequency from 48% to 15%. The remaining 48 genes are mutated in fewer patients, as infrequently as 2%. In addition, multiple other large genes, especially those involved in chromatin remodelling/regulation, were also mutated at significant frequency (up to 15%), but did not achieve statistical significance due to their size.[59]

In contrast, NMIBC form a much more homogeneous group with a shorter "tail" of infrequently mutated genes.[60-62] These have a high frequency of oncogenes activated by point mutation rather than amplification (*fibroblast growth factor receptor 3 [FGFR3], PIK3CA*), several commonly inactivated tumour suppressor genes, including *STAG2, KDM6A,* and *KMT2D* that are inactivated in 30% to 55% of cases, and almost no mutations in *TP53* and *RB1.* Single nucleotide mutations in the promoter of *TERT* are the most common alteration reported, found in >70% of tumours of all grades and stages.[67-69] Chromatin modifier gene mutations feature strongly in both groups but are more frequent in NMIBC **(Figure 3–5).**[60]

FIGURE 3–5
Nonsynonymous Mutation Frequencies in Muscle-Invasive Bladder Cancer and in Noninvasive Bladder Cancer (Stage Ta), Where One or Other Cohort Had a Reported Frequency of >9%



Zero frequencies for noninvasive bladder cancer indicate absence of mutation in the relatively small discovery series analyzed and lack of inclusion in subsequent targeted mutation screening.

Many alterations in bladder tumours involve structural changes in the genome, including allelic losses, alterations in DNA copy number, and genomic rearrangements, some of which generate fusion genes. Numerical chromosomal changes have long been described in bladder cancer. In general, aneuploidy (deviation from the normal 46 chromosome content of a somatic cell) is associated with MIBC and near-diploid karyotype with NMIBC.[70] Early studies reported loss of chromosome 9 as

a common alteration in all bladder tumours, and identified a range of other changes that have since been confirmed using other techniques such as comparative genomic hybridization (CGH) and fluorescence *in situ* hybridization (FISH). Array-based CGH and, recently, next generation sequence analysis have allowed high-resolution mapping of copy number alterations, and these analyses confirm that the fraction of the genome altered is much higher in MIBC.[59,71,72]

NMIBCs, particularly stage Ta tumours, have stable genomes with few common alterations except those involving chromosome 9. Other alterations in NMIBC include loss of Y, loss of 11p, and gains of material from 1q and 20q. In contrast, MIBCs often show chromosomal instability (CIN), and have many copy number alterations including high level amplifications, subchromosomal deletions, and rearrangements.[59,60,66,73,74] CIN may arise through a range of mechanisms including weak or absent attachment to the mitotic spindle or by chromosome nondisjunction, resulting in whole chromosome losses or gains.[75] Absence of normal cell cycle and/or mitotic checkpoints, in particular *TP53* mutation with consequent loss of p21 response, is implicated in the generation of CIN.[75] Both *TP53* and *RB1* inactivation are found in carcinoma *in situ* (CIS),[76,77] a predicted precursor of MIBC, suggesting a loss of checkpoint control and an early predisposition for aneuploidy in these cells. The imbalanced genome and transcriptome induced by whole chromosome losses or gains in a diploid cell induces cellular stresses that can be buffered by whole genome doubling. This appears common in MIBC, with a finding of subtetraploid karyotype and molecular evidence for whole genome doubling in many cases.[59,74,78] An alternative route to CIN is via telomere shortening leading to so-called Breakage-Fusion-Bridge cycles that generate chromosomal rearrangements via chromosomal breakage and rejoining.[79] Very short telomeres have been reported in CIS, suggesting that this process may occur in the precursors of MIBC.[80] Compatible with these mutational mechanisms, many of the driver mutational events identified in these CIN-driven tumours result from DNA losses, gains, high-level amplifications, or rearrangements. In MIBC, *CDKN2A* is very commonly inactivated (>40%), almost always via deletion. It is also notable that genes with predicted dominant oncogenic potential, e.g. *E2F3, CCND1, EGFR, ERBB2*, are commonly activated by amplification in MIBC.

Although only a few whole bladder cancer genomes have been sequenced to date, this has revealed the complexity of structural alterations in MIBC in more detail. For example, chromothripsis, a catastrophic chromosomal shattering and repair event,[81] appears to be linked to *TP53* mutation and is implicated in the generation of clustered breakpoints and segmental copy number alterations in MIBC, some of which may generate driver events.[82,83] In the genomes analyzed to date, chromothripsis has not resulted in generation of frequent gene fusions, which rather appear to be generated by Breakage-Fusion-Bridge cycles. Overall, few fusion genes have been identified, with FGFR3-TACC3 and fusions with PPARG the only recurrent fusions identified to date.[59,65,66,84]

In the absence of CIN as an early driver of tumour development in NMIBC, oncogenic point mutations in *FGFR3, HRAS* and *PIK3CA* dominate, compatible with the action of mutagenic processes that induce single nucleotide alterations. Overall, NMIBCs appear to be driven largely by increased cell proliferation in the presence of intact cell cycle and mitotic checkpoints. It is notable that some MIBCs bear features similar to those found in NMIBC, including *FGFR3* alterations. In such cases, a common finding is homozygous deletion of *CDKN2A*.[85] As this locus encodes two proteins (p14ARF

and p16) that regulate the p53 and Rb pathways, respectively, this may represent a mutational mechanism through which low-grade papillary NMIBC can progress to MIBC with concomitant loss of genomic stability.

In the presence of a high mutation rate, intratumour mutational heterogeneity and subclonal evolution during the disease course are likely. Whole genome and whole exome sequencing have revealed considerable intratumour heterogeneity, particularly in MIBC, and have provided phylogenetic evidence for tumour evolution in local recurrences over time, following chemotherapy and during the development of metastases. Overall, clonal diversity increases with tumour stage.[82] In NMIBC, in addition to sequencing of single tumours, sequencing of synchronous multifocal lesions, metachronous tumours from the same patient, and paired samples before and following disease progression has been reported.[61,63,86,87] In tumours that later progressed, higher levels of intratumour heterogeneity were present, and these had more APOBEC-related mutations than those that did not, implying that this process was activated at a late stage. Phylogenetic analysis of these samples confirmed overall monclonality.[63] Analysis of paired pre- and post-progression samples also confirmed a common origin, with increased numbers of mutations and divergence in SNVs, indels, and breakpoint content in progressed tumours, and few rearrangements in common with the related preprogression samples. As might be expected, the ancestral clones contained mutations in several genes known to be commonly mutated in NMIBC (*FGFR3, KDM6A, PIK3CA*). In all cases, expansion of a minor or undetectable subclone from the primary tumour was detected in the progressed tumour.[61]

In MIBC, localized tumour and related metastases, samples before and following chemotherapy, and multiple samples from the same tumours have been sequenced.[73,88,89] Multiregion analysis of samples taken at cystectomy from unifocal and multifocal tumours and related "normal" urothelium from four patients showed overall monoclonality of tumours. In the multifocal lesions, there was much spatial intratumour heterogeneity, and low levels of mutations found in the tumours were detected in "normal" urothelium elsewhere in the bladder, indicating the presence of abnormal cells in broad regions of the bladder. In the unifocal lesions, spatial heterogeneity was less and there was little evidence for mutations in the normal urothelium.[89] Multiregion analysis of three primary and metastatic tumour pairs indicated much greater heterogeneity in the metastases and relatively low spatial heterogeneity in the primary tumours.

Change in the mutational landscape of MIBC over time is particularly pronounced following chemotherapy. Whole exome sequencing and clonality analysis of 16 paired pre- and post- chemotherapy MIBC samples revealed significant intrapatient mutational heterogeneity, with many mutations not shared between pre- and post-therapy samples.[73] Clonality analysis showed early branched evolution prior to metastasis and continued parallel mutational evolution of primary tumour and metastases. APOBEC signatures were enriched in post-treatment samples. Increased availability of single-stranded DNA (ssDNA) substrates for mutagenesis during repair of cisplatin-induced DNA damage may fuel mutational divergence in these samples. Overall, studies of MIBC indicate extensive evolution of the mutational landscape, both in space and time. Although in most cases the ancestral (or "truncal") mutations retained by the evolved lesion commonly include therapeutic targets, these studies emphasize the danger of missing or inappropriately selecting therapeutic targets based on the mutational profile of untreated primary tumour in the face of such extensive tumour evolution.

3.4 Basic Science on Bladder Cancer Chromatin Modifiers

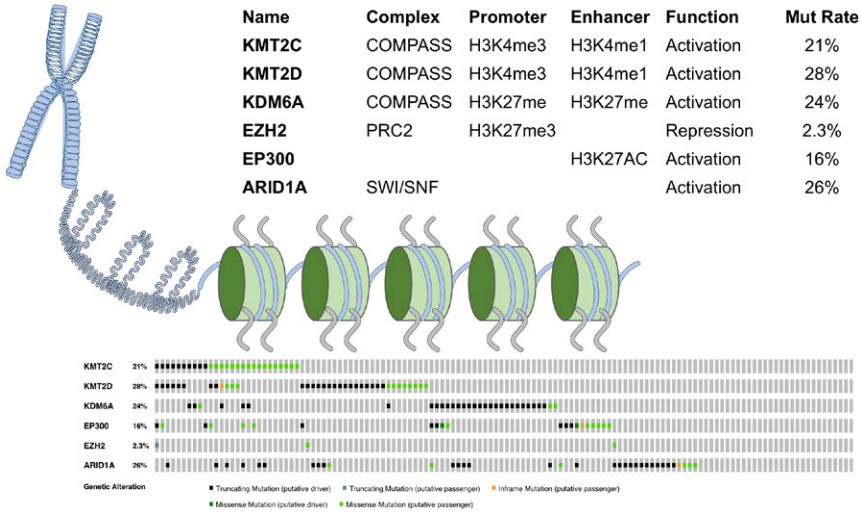
3.4.1 Introduction

Epigenetic dysregulation involving methylation of DNA, histone modification, and enzymatic nucleosome assembly is a hallmark of cancer.[90] While next-generation sequencing (NGS) has facilitated the identification of mutations in cancer, the post-translational modifications of histones found at both enhancers and transcriptional start sites across the genome remain a widely investigated field of study that is still in its infancy.[91] Chromatin modification genes have been implicated in both solid and hematologic malignancies, but the high frequency of mutations in genes associated with histone modification and chromatin structure are unique to bladder cancer.[92] Most of our knowledge of the function of chromatin modifiers is based on model organisms (*Drosophila*, *Saccharomyces cerevisiae*), with only recent work venturing into mammalian systems.[93] Unlike transcriptional activators that directly regulate gene expression, chromatin modifiers may influence promoter or enhancer activity on close genes (cis regulation) or distant genes (trans regulation). Therefore, the function of individual chromatin modifiers may be pleiotropic, depending on the cellular context. To investigate the role of chromatin modifiers in bladder cancer, we examine the mutation data from patients and contrast the mechanistic function predicted from studies of bladder cancer and other solid tumours.

3.4.2 Post-translational modification of histones

Advances in NGS and chromatin immunoprecipitation have facilitated the identification of genomic loci regulated by histone modification.[94] Double-stranded DNA is wrapped around histone octamers **(Figure 3–6)**.[95] The third histone (H3) of the octamer complex has a protein tail that may be enzymatically modified, affecting the steric folding of the other histones and adjacent proteins. These sites of gene regulation are controlled by three-dimensional factors in the nucleus and result in chromatin wrapped loosely and open to facilitate coactivator binding with exposed DNA or tightly condensed in a state of repression. The most commonly modified residue of the histone tail is the fourth lysine (K4) on H3, which is regulated by methylation (e.g. H3K4me1).[96] H3K4 can be mono-, di-, and tri-methylated depending on its location near the transcriptional start site. Promoters, for example, are often tri-methylated during activation (H3K4me3) and H3K4 is un-methylated when the transcriptional start site is inactive. At enhancers, H3K4 is mono-methylated (H3K4me1), resulting in activation of distant transcription. The second most modified lysine is the twenty-seventh (H3K27), which can be mono-, di-, or tri-methylated at either promoters or enhancers and is almost universally associated with gene repression. Dynamic regulation at enhancers occurs at H3K27, with K27 methylation associated with repression, while acetylation (K27Ac) is associated with gene activation. These histone modifications may be carefully orchestrated in the normal cell during homeostasis and development. Loss of function mutations in chromatin modifying genes are over-represented in the muscle invasive urothelial cancer TCGA cohort, including 10 of the top 39 genes (**Figure 3–6**).[97] In comparison to other tumour types, the only other malignancy with high rates of mutation in chromatin regulators are the lymphoid neoplasms.[92]

FIGURE 3–6
Chromatin Is Coiled Around an Octamer of Histones That Are Dynamically Modified



Enzymes that regulate histone structure can affect promoters and enhancers, depending on their proximity to target genes. Mutations of enzymes that regulate histones are common in urothelial carcinoma.

3.4.3 Histone writers

Chromatin modifiers with histone methyltransferase activity enzymatically add methylation marks to lysine tails and the mixed lineage leukemia (MLL) or lysine methyltransferase (KMT) family of methyltransferases are the most commonly affected in bladder cancer.[98] These genes include KMT2D (MLL2 or MLL4), KMT2C (MLL3), and KMT2A (MLL1) mutated in 28%, 18%, and 11% of muscle-invasive bladder cancers **(Figure 3–6)**.[97] Compared to other solid tumours, bladder cancer has the highest mutation rates of KMT2D and KMT2C among TCGA cancers.[99] KMT2D and KMT2C share the most homology and function in the same complex in *Drosophila* called trithorax-related complex due to its homology to the trithorax proteins.[100] Targeted deletion of the enzymatic SET domain of *Kmt2c* in mice results in ureteral cancers.[101] When the *Kmt2C* mutation was bred onto a *Tp53* mutant mouse, the rate of ureteral tumours increased from 40% to 100% of animals. Exome sequencing of n-butyl-n-4-hydroxybutyl nitrosamine (BBN)-induced bladder cancers identified combined loss of *Kmt2c* and *Tp53* in 80% of bladder cancers. These data mirror leukemia, in which loss of *KMT2C* or 7q, combined with TP53 loss, is associated with progression of leukemogenesis.[102] The molecular function of KMT2D in the bladder is under investigation, but murine experiments focused on cell metabolism suggest that KMT2D and KMT2C may regulate cell-fate determination.[103] In bladder cancer, mutations in KMT2C or KMT2D do not cluster in any locus, but are frequently nonsense mutations resulting in truncation of either protein with loss of the enzymatic SET domain located at the C-terminus.[92] Evaluation of tumour molecular subtypes suggests that loss of function mutations in KMT2C and KMT2D may occur more commonly in basal tumours in humans and BBN mice.[104] Research from both *Drosophila* and mammalian embryonic stem cells suggest that the enzymatic domains of KMT2C and KMT2D are nonessential for stem cell renewal.[105] Thus, the role of KTM2C/D in bladder cancer may be limited to protein interaction and scaffolding on the chromosome bringing other proteins of the COMPASS (complex of proteins associated with Set1) to specific loci.

Histone acetyltransferases (HATs) enzymatically add acetyl groups to H3K27 at enhancer sites, resulting in transcriptional activation. The two most commonly mutated HATs in bladder cancer are EP300 (15%) and CREBBP (12%).[97] Loss of HAT function could cause increased enhancer silencing due to increased H3K27me3 favouring polycomb-mediated repression.[106]

3.4.4 Histone erasers

Enzymes that remove methyl groups from histones are "erasers." The most commonly mutated histone demethylase in bladder cancer is *KDM6A* (*UTX)*.[97] KDM6A is located on the X-chromosome and is not dose-regulated by X-inactivation.[107] Thus, women may express twice the amount of KDM6A or require multiple mutations to affect its function. *KDM6A* was mutated in 26% of MIBC compared to 54% of noninvasive cancers, suggesting KDM6A loss may occur more commonly with early-stage cancers or *RAS/FGFR3* signalling, which are predominantly found in early-stage cancers.[108] Further support for the role of UTX in low-grade cancers is supported by the common occurrence of FGFR3 and *KDM6A* mutations, which were both mutually exclusivity of *RAS* mutations in non-invasive cancers.[108] There may be a gender association with *KDM6A* loss, as females with noninvasive tumours had *KDM6A* mutations in 74% of cancers, while men had mutations in only 35%. An alternative hypothesis is that the second copy of KDM6A in females is affected by the high mutation rate in bladder cancer with no functional consequence. While males have UTY with homology to UTX, UTY lacks the enzymatic domain of UTX and is not able to rescue UTX function *in vivo*.[109] In male mice with BBN-induced bladder tumours, we identified no occurrence of *KDM6A* mutations, which may be due to the association with female gender or the basal pathology of BBN cancers.

3.4.5 COMPASS/trithorax

The chromatin modifiers KMT2D, KMT2C, and KDM6A assemble with other proteins to form larger multi-protein complex called COMPASS[110] (**Figure 3–6**). COMPASS was originally identified in yeast, in which there was only one KMT enzyme (Set), with expansion of complexes in higher metazoans.[110,111] Coordinating COMPASS function to simultaneously demethylate histones (KDM6A) at some loci (e.g. H3K27me3) and add methyl groups at other loci (e.g. H3K4me) with histone methyltransferase, COMPASS can efficiently alter the transcriptional landscape of a cell. Mutual exclusion of mutations in KMT2D/KMT2C and KDM6A in the first TCGA cohort suggested that, while enzymatically distinct, KDM6A and KMT2D/C may cooperate to have similar function.[92] Further support for this hypothesis is the developmental Kabuki syndrome caused by germline mutations in either KMT2D or KDM6A.[112,113] One hypothesis may be that mutation of KDM6A or KMT2C/D is sufficient to destabilize the entire COMPASS complex. Due to the improved survival in patients with KMT2C mutation, we hypothesize COMPASS function may be recruited by oncogenic processes. In prostate cancer, for example, KMT2A binds Menin, and together they can activate the androgen receptor even with low levels of testosterone.[114]

3.4.6 Polycomb repressor complex

If the function of COMPASS is to activate gene transcription, the complementary activity of gene repression is mediated by the polycomb-repressor complex-2 (PRC2).[106] PRC2 is composed of the subunits EED and SUZ12, and the SET enzyme enhancer of zeste-2 (EZH2). EZH2 is the histone methyltransferase that binds H3K27 catalyzing H3K27me3, resulting in chromatin condensation and gene repression at both promoters and enhancer site.[115] Interestingly, there are no significant mutations in EZH2 in the bladder cancer TCGA cohort. Loss of function mutations of RB1 increase E2F, which has been shown to increase expression of EZH2.[116] Increased expression of EZH2 in bladder cancer is associated with invasive cancers, higher grade, epithelial-mesenchymal transition (EMT), and aggressiveness. Thus, EZH2 activity may play a central role in repression of urothelial cell differentiation. An alternative mechanism to H3K27me3 levels is increased EZH2 activity caused by loss of KDM6A function secondary to mutation. Pharmacotherapeutic studies investigating the use of EZH2 inhibitors (EZH2i) have demonstrated loss of KDM6A may increase the sensitivity of bladder cancers to EZH2i therapy.[117]

3.4.7 Chromatin remodellers

The adenosine triphosphate (ATP)-dependent activity of moving histones and nucleosomes during remodelling of chromatin structure is powered by the multi-protein SWI/SNF complex. The major enzymatic component of SWI/SNF is ARID1A, mutated in 25% of muscle-invasive bladder cancers.[97] Loss of function mutations in *ARID1A* were increased in luminal tumours (31%) compared to basal tumours (18%).[97] In NMIBCs, mutations of *ARID1A* have been associated with resistance to bacillus Calmette-Guérin (BCG) therapy.[118] As a possible therapeutic target, loss of function mutations in *ARID1A* in clear cell ovarian cancers resulted in synthetic lethality when these tumours were treated with EZH2i.[119] While not explored yet in bladder cancer, the interaction of SWI/SNF and PRC2 may be another possible therapeutic target.

3.4.8 Conclusion

Chromatin-modifying enzymes play a pivotal but complex role in shaping bladder tumour subtype and aggressiveness. While there is a greater understanding of gatekeeper and caretaker function in malignancy, the role of chromatin regulation will require further investigation. Targeting of chromatin modifying enzymes, potentially by combination with chemotherapy or immunotherapy, may offer therapeutic potential for treatment of patients with bladder cancer.

3.5 DNA Damage Response Gene Alterations

3.5.1 Introduction: DNA damage response genes that are altered in urothelial carcinoma

DNA damage response (DDR) gene alterations have been identified across numerous cancer types, including ovarian, prostate, endometrial, and breast cancers.[120] More recently, alterations in DDR genes have been detected in urothelial carcinoma.[92] ERCC1, a member of the nucleotide excision repair (NER) pathway, was explored as a prognostic marker in metastatic urothelial carcinoma.[121,122] Patients with low mRNA expression levels of ERCC1 were noted to have improved survival while in a separate study, patients receiving cisplatin-based chemotherapy for metastatic urothelial carcinoma

with high nuclear ERCC1 protein expression by immunohistochemistry were found to have an inferior median survival. TCGA of urothelial carcinoma, which sequenced 131 high-grade MIBCs, detected alterations in a number of DDR genes; notably, ERCC2 was one of the genes significantly mutated in this analysis (9%–12%), while additional genes, including BRCA1/2 and others, were altered at lower frequencies.[92,123]

3.5.2 ERCC2 mutations in urothelial carcinoma

Several findings have suggested that DDR gene alterations might serve as predictive biomarkers of chemotherapy sensitivity in urothelial carcinoma. An extreme phenotype approach was the first to identify an association between point mutations in the DNA helicase, ERCC2, and response to chemotherapy.[124] ERCC2 contributes to NER of damaged DNA and specifically plays a role in the repair of ultraviolet- and platinum-induced damage. This study analyzed 25 patients with MIBC who achieved pT0 or CIS responses to chemotherapy and 25 patients with residual pT2 or higher-stage disease at radical cystectomy following neoadjuvant cisplatin-based chemotherapy. An enrichment analysis was performed to identify mutated genes associated with responders or nonresponders. Only mutant ERCC2 was found to associate significantly with responding patients (9 of 25 vs 0 of 25 with mutations). The majority of alterations in ERCC2 identified in this study were point mutations that clustered within conserved helicase domains of the protein and were hypothesized to result in NER deficiency. In support of this, functional analysis revealed that ERCC2 point mutations were unable to rescue the cisplatin-sensitivity phenotype observed in a cell line with impaired NER function. The above results were validated in a separate MIBC cohort: 40% of responders were found to have ERCC2 mutations as compared to 7% of nonresponders. ERCC2 mutant responders receiving cisplatin-based chemotherapy also exhibited an improved survival compared to nonresponders.[125]

3.5.3 Mutation signatures of DNA damage response deficiency

Mutation signatures, delineated by specific base change patterns, have been characterized in urothelial carcinoma, including the APOBEC signature identified through the urothelial TCGA effort.[92] This signature is thought to be a main contributor to the high overall mutation burden observed in urothelial carcinoma tumours. ERCC2 mutations were also associated with a specific mutation signature (named "signature 5*" due to its similarity to signature 5 as defined by the COSMIC database) in three cohorts of bladder tumours, and this signature pattern may denote deficient NER function.[126]

3.5.4 Additional DNA damage response gene alterations in urothelial carcinoma as predictive biomarkers

In another study by Plimack *et al,* targeted sequencing was performed of 287 genes from patients with muscle-invasive bladder cancer who received two different cisplatin-based neoadjuvant chemotherapy (NAC) regimens.[127] Using both a discovery and validation cohort, the investigators found that mutations in any of three genes, RB1, FANCC, and ATM, were associated with chemotherapy sensitivity. These findings were similar to a study by Yap *et al* in which genomic characterization of pretreatment muscle-invasive bladder tumours showed that alterations in any one of six DDR genes were associated with improved recurrence-free survival (RFS) to perioperative chemotherapy.[128]

The correlative analyses from a phase 2 clinical trial testing the efficacy of neoadjuvant dose-dense gemcitabine and cisplatin for patients with muscle-invasive bladder cancer (NCT01589094) also identified alterations in DDR genes as predictive for response to therapy.[129] Specifically, pretreatment tumour tissue was sequenced using a targeted exon capture gene panel in 32 patients enrolled onto study who received at least three cycles of chemotherapy and who underwent radical cystectomy following chemotherapy. Twenty-nine genes associated with canonical DDR gene pathways were scrutinized for an association between alteration status and response to chemotherapy and, given that the majority of alterations in DDR genes are currently classified as variants of unknown significance, only alterations with a known functional impact (i.e. deleterious alterations) were selected for analysis. Of nine patients with deleterious DDR gene alterations, eight were identified within responders and one within a nonresponder. Four of eight alterations were ERCC2 point mutations previously reported in the literature, and additional deleterious alterations in BRCA2, ATR, and other genes implicated in diverse DDR pathways such as homologous recombination and DNA damage surveillance were also detected. The presence of DDR gene alterations has been correlated with a higher mutation burden in certain tumour types and, similarly, pretreatment tumours from patients with deleterious DDR gene alterations displayed a significantly higher overall mutation load compared to tumours without such alterations (median 15.2 mutations/Mb vs 5.8 mutations/Mb, $p<0.01$).

In a publication by Teo *et al,* DDR gene alteration status was correlated with response to chemotherapy in 100 patients with metastatic urothelial carcinoma.[130] This study identified an association between improved progression-free and overall survival (OS) in patients with DDR gene alterations receiving platinum-based chemotherapy.

MRE11 is a protein that plays an important role in homologous recombination, telomere maintenance, and DNA double-strand break repair via nonhomologous end joining. MRE11 protein expression has been examined for correlation with cause-specific survival in radiation-treated bladder cancer patients. Specifically, low levels of MRE11 by immunohistochemistry were associated with inferior survival, specifically in radiation-treated patients as compared to those managed with cystectomy.[131,132] In one study of patients treated with chemoradiotherapy, MRE11 protein levels did not correlate with clinical outcomes.[133] A trend towards improved RFS was observed in the presence of deleterious DDR gene alterations, including ERCC2, in this same patient cohort.

3.5.5 Future applications of DNA damage response

The therapeutic landscape of urothelial carcinoma has been transformed since the advent of ICB.[134,135] Data presented at the American Society of Clinical Oncology (ASCO) 2017 annual meeting indicated that deleterious DDR gene alterations were associated with improved overall response rate to ICB ($p<0.001$).[136] The therapeutic implications for DDR gene alterations in urothelial carcinoma are underscored by the recent US Food and Drug Administration (FDA) approval of pembrolizumab for patients with microsatellite instable high tumours due to deficient mismatch repair, agnostic of tissue type. This approval will allow patients with Lynch syndrome, a subset of which develop upper tract or, more rarely, bladder urothelial carcinoma to receive pembrolizumab following progression on standard of care therapy or for whom standard of care options are not possible. Additionally, retrospective analyses have indicated that patients with muscle-invasive bladder cancer who achieve a complete clinical response to neoadjuvant cisplatin-based chemotherapy could be managed with close cystoscopic surveillance and avoid a radical cystectomy.[137-139] Patients were selected based solely upon clinical stage but, with the findings above, an opportunity exists now to refine this process by basing patient selection upon both the presence of select DDR gene alterations and evidence of clinical downstaging to noninvasive disease. A clinical trial testing this approach is currently being designed through the cooperative group mechanism in the United States, with a primary endpoint of 3-year RFS in patients who elect a bladder-sparing approach following definitive chemotherapy.

3.6 Liquid Biopsy Analysis: Identifying the Next Generation of Biomarkers for Bladder Cancer Monitoring

3.6.1 Introduction

The concept of "liquid biopsy" refers to any minimally invasive body fluid sample such as blood, urine, saliva, cerebrospinal fluid, or seminal plasma. This concept has emerged as a result of the application of high-throughput technologies to "liquid" body fluids. Molecular analyses and patient monitoring using liquid biopsies represent an easy and alternative strategy to invasive tumour biopsies, since invasive tumour biopsies are not always available due to tumour location, patient safety, and costs. Additionally, liquid biopsies have the advantage of repetitive access for patient surveillance. The current growing trend towards exploring the use of liquid biopsies is justified by the ability of increasing and more sophisticated and efficient methods to extract circulating molecules from body fluids and amplification techniques to detect tiny amounts of, for example, circulating nucleic acids, other molecules, or cells. Multiple molecular markers can be measured in liquid biopsies. To date, these include: a) circulating tumour cells, b) circulating cell-free DNA (cfDNA), including ctDNA levels, integrity, methylation, rearrangements, and mutations; c) circulating RNAs (microRNAs [miRNA], lncRNAs, and mRNAs); d) cell-free proteins and peptides; and e) exosomes, among others.[140,141]

Circulating cfDNA represents the molecule easiest to extract in terms of quantity and quality for liquid biopsy analyses. Furthermore, measurements of patient-specific tumour-associated mutations make the analyses highly specific. cfDNA originates from cells undergoing apoptosis, necrosis, and, to a minor extent, active release.[142,143] cfDNA is highly fragmented, corresponding to fragments protected by the nucleosome core.[144] The half-life of tumour DNA in cfDNA (ctDNA) has been shown to be approximately 2 hours.[145] Consequently, analysis of ctDNA may provide real-time measurements of tumour burden and treatment efficacies. Total RNA has limitations in terms of available amounts and stability due to reduced half-life because of high amounts of RNAses in the majority of the bodily fluids.[140,146] Cells secrete miRNAs into vesicles within which they are either bound in a ribonucleoprotein complex or unbound as free molecules. Since miRNA secretion pathways are dysregulated in cancer and they are highly stable in several body fluids, miRNAs may represent attractive molecules for liquid biopsies analyses.[147] Cell free proteins and peptides display limitations depending on their quantity and stability. Exosomes represent an emerging exciting arena, with limitations in its difficulty for extraction and variable concentrations of their contents with limited amounts of nucleic acids and protein derived material.[148,149]

In this section, we will address the current stage of research, advantages, limitations, challenges, and potential clinical utility, with focus on cfDNA applications.

3.6.2 Blood and urine samples: advantages and limitations

The opportunity of liquid biopsy strategies in the context of bladder cancer management relates to the potential use of two types of samples in contact with tumour cells: urine and blood. Each sample type has its advantages and limitations for liquid biopsy analyses. Given the exophytic growth of nonmuscle invasive tumours versus the higher vascularization of muscle-invasive tumours, urine poses advantages for nonmuscle invasive disease, while blood may be the most optimal sample for advanced disease. Tumour-derived DNA fragments are often rare, and large volumes of blood may be required for obtaining the recommended nucleic acid amounts for current technologies, especially when detecting ctDNA of low concentration from, for example, minimally invasive cancers. Urine samples represent an alternative source for analyzing tumour-derived DNA. Samples can be obtained noninvasively, and relatively large volumes are available for analysis. It is relevant to highlight that, in the case of urine ctDNA, the nucleic acid content may originate from cancer cells reaching the urine that may release their contents in the sample and/or from the blood circulation. Renal clearance of the cfDNA into urine may increase ctDNA concentrations in urine compared to blood, but larger variability may occur due to the non-homeostatic sample type. Studies of various cancer types have identified ctDNA in urine samples, often in higher concentrations compared to plasma.[150-152] Several reports have previously focused on urinary cell pellets; however, in these studies, the DNA is less fragmented and originating from intact tumour or adjacent cells, and not from cellular release of DNA into urine (apoptosis, necrosis, or active release) or from renal clearance of blood. DNA from cell pellets may give an adequate representation of the tumour, if multiple cells are shed from the tumour. However, as tumour-associated cfDNA has been shown to be present at higher levels,[151] present in urine from cystectomized patients,[153] and typically originates from cellular processes associated with aggressive behaviour and therapeutic treatment (necrosis, apoptosis, and invasiveness), the prognostic and predictive value of cfDNA may be better compared to DNA from cell pellets. In analysis of urine cfDNA, the origin can be determined from the DNA fragment size, and analysis can be performed on fragmented DNA only, where longer DNA fragments can inform on contamination from intact cells.

3.6.3 Blood- and urine-based studies of cell-free DNA

One hallmark study on cfDNA from several major cancer types (including bladder cancer) documented that ctDNA was detectable in the majority of patients with advanced disease.[154] In a later methodological study, researchers compared DNA from tissue specimens, urine cell pellets, and urine supernatants in a series of 23 cases. Here, genomic analyses showed that alterations in DNA from urine supernatants better reflected tumour alterations compared to DNA from cell pellets.[155]

The first report of ctDNA for bladder cancer surveillance applied whole genome and whole exome sequencing of tumours for designing highly sensitive patient-specific droplet digital polymerase chain reaction (ddPCR) assays.[151] Patients with recurrent or progressive/metastatic disease were monitored using urine and plasma samples. One important observation in this study was that circulating tumour DNA was detectable already at early disease stages, before clinical documentation of progression and/or metastasis. Patients showing later progression also had detectable ctDNA despite no detectable tumour at cystoscopy. Furthermore, patients with progressive disease showed significantly higher levels of tumour DNA in plasma and urine before disease progression, compared with

patients with recurrent disease. A significant level of heterogeneity was observed for each patient; this could be due to tumour heterogeneity or assay performance based on the limited urine and plasma volumes available for the retrospective bio-bank study. A subsequent report focused on the presence of three hotspot mutations in FGFR3 and PIK3CA for disease surveillance for early diagnosis of disease progression in NMIBC and for early identification of metastatic disease following cystectomy. Large cohorts of patients were initially screened for hot-spot mutations in tumour samples. In total, 36% of the tumours from NMIBC patients and 11% of tumours from patients receiving cystectomy harboured at least one FGFR3 or PIK3CA mutation. Screening of DNA from serial urine supernatants from the NMIBC cohort revealed that high levels of ctDNA were associated with later disease progression. Furthermore, high levels of circulating tumour DNA in plasma and urine samples were associated with disease metastasis in the cystectomy cohort. A positive correlation between circulating tumour DNA levels in paired urine and plasma was observed (correlation coefficient 0.6). This study documented that clonal hot-spot mutations may be applicable for disease surveillance; however, especially in MIBC, hot-spot mutations are not frequently observed,[123] which makes predesigned assays less useful. This study was also based on bio-banked samples with limited volumes, which may have contributed to the observed heterogeneity in measurements.[152]

In a follow-up study, the same group performed a prospective evaluation of ctDNA in longitudinally collected plasma samples from patients with locally advanced bladder cancer. The aim was to detect local or metastatic relapse after cystectomy and measure treatment efficacy during NAC and chemotherapy for metastatic relapse. Exome sequencing of tumour and germline DNA from 24 patients with MIBC was applied for designing patient-specific ddPCR assays, and ctDNA from plasma samples obtained during treatment and follow-up were analyzed. Patients with metastatic relapse had significantly higher tumour DNA levels in plasma samples compared with disease-free patients. The median positive lead-time between tumour DNA detection in plasma and diagnosis of relapse was 101 days after cystectomy. Plasma ctDNA levels during chemotherapy showed a correlation to treatment response and progression data obtained from computed tomography (CT) scans.[153]

Sequencing of plasma cfDNA has been reported in two recent publications. In the first study, the authors used tagged amplicon and shallow whole genome sequencing to study plasma and urine samples from 17 patients undergoing NAC. A panel targeting frequently mutated genes was applied, and mutations were identified in 35.3%, 47.1%, and 52.9% of pretreatment plasma, urine cell pellets, and urine supernatants, respectively. Urine samples were found to contain higher levels of mutated DNA, in concordance with previous studies.[151] Interestingly, continued detection of mutated DNA during therapeutic treatment predicted disease recurrence.[156] Another group applied whole exome sequencing and targeted sequencing of 50 bladder cancer driver genes using plasma cfDNA from 51 patients with aggressive bladder cancer. Most patients with metastatic disease, but only 14% of patients with localized disease, had ctDNA levels above 2% of total cfDNA. In total, 95% of patients had deleterious alterations in TP53, RB1, or MDM2, and 70% had a mutation or disrupting rearrangement affecting chromatin modifiers. Consequently, direct sequencing of plasma cfDNA from especially metastatic patients may provide a cost-effective tool for identifying clinically informative somatic alterations and guide treatment selection.[157]

3.6.4 Current and future technologic strategies

Precision medicine with molecularly guided diagnostics, stratification, surveillance, and therapeutics is rapidly expanding in all subspecialties within oncology, including laboratory assays for patient monitoring in an individualized fashion. Advances in the field are mainly driven by development of efficient protocols for nucleic acid extraction, and development of ddPCR technologies, together with advances in sequencing applications. Initial studies have focused on identification of clonal mutations by sequencing technologies and tracking using ddPCR. These ddPCR techniques are highly sensitive but, because bladder cancer has been shown to have a heterogeneous molecular representation[88,89,123,158] and new mutations may appear due to treatment selection,[73] direct sequencing of ctDNA may also be an advantage for identification of novel actionable targets in a metastatic setting.[157] This has been performed successfully in other cancers and bladder cancer[157,159] but, for early detection where only small fractions of tumour DNA may be present, ultra-deep redundant sequencing is needed. For this to be economically feasible, smaller and more focused gene panels are needed, along with improved error correction using, for example, molecular barcodes to identify bona fide alteration.[160,161] However, it is still expensive compared to application of ddPCR assays for monitoring. As the technology becomes less expensive, a direct sequencing approach may also be applied for early diagnosis of bladder cancer in high-risk populations. One pitfall of this approach is, however, that people may harbour disease driver mutations without ever developing a cancer.[162]

Analysis of circulating cancer cells may also impact future clinical decision-making. Initial commercial automated systems such as CellSearch® used the epithelial cell adhesion molecule as the main candidate marker to identify epithelial cancer cells in several solid tumours, including bladder cancer. More recent studies have tested sialyl-Tn, a cancer-associated glycan antigen present in membrane glycoproteins, together with keratins (KRT4, 5, and 20) to improve the identification of bladder cancer cells using size-based microfluidic chips.[163] Analysis of exosome contents in plasma may also prove to be important for decision-making in the future and for understanding cancer biology. The exosomal nucleic acids originate mainly from live cells, which may better reflect tumour biology. In one study of 43 patients with advanced cancers, it was shown that analysis of DNA from exosomes detected mutations in KRAS, BRAF, and EGFR with higher sensitivity compared to analysis of archival tumour materials and cell-free DNA from plasma.[164] Remarkably, it has been shown that miRNAs can be profiled within urinary exosomes using microarray technologies, as well as individualized quantitative polymerase chain reaction (qPCR).[148]

Overall, recent data highlighted that it is necessary to perform proof of principle studies to prove that what is measured in each bodily fluid is mirroring matching tumours. Once such validation is performed, future analyses will use novel high-throughput genomic techniques to identify the highly recurrent alterations to be later optimized in multiplexed manner in more specific assays that can be tested in a high number of samples with limited free nucleic acid material.

3.6.5 Clinical implications

The utility of each body fluid sample differs along disease progression due to the number of cancer cells and, thus, nucleic acid and cell contents that may reach the bloodstream or the urine. In nonmuscle invasive disease, the urine is likely more enriched with cancer cell contents, and it offers

opportunities not only for diagnosis but also for surveillance, monitoring response to intravesical therapy, and potentially for disease screening in the near future. In muscle-invasive disease, tumour cell contents may easily reach the bloodstream, especially in highly vascularized tumours. This offers an opportunity for disease stratification, detecting disease progression, or monitoring chemotherapy or the novel immunotherapy strategies. Current studies are already showing the clinical usefulness of liquid biopsy analyses for disease classification and prediction of clinical behaviour (**Figure 3–7**).

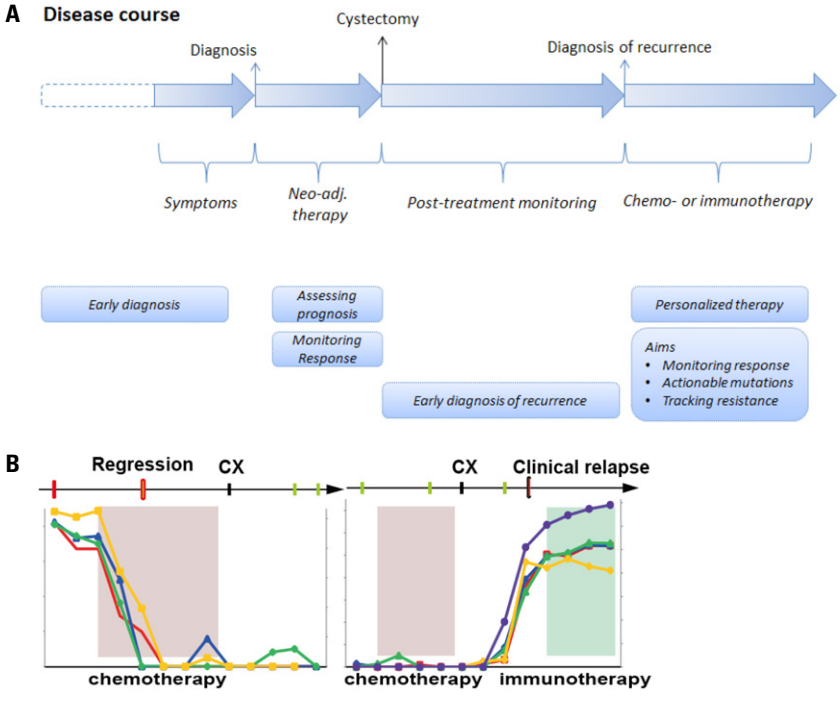


FIGURE 3–7
A Illustration of Areas Where Liquid Biopsy Analysis May Affect Future Clinical Decision-Making
B Two Examples of Disease Courses Are Shown

(A) This spans detection of early symptoms in, for example, high-risk populations before clinical diagnosis, monitoring NMIBC for recurrence and progression, predicting response to therapy before and after cystectomy, to personalized therapeutic treatment.

(B) The patient to the left shows response to chemotherapy with significant drop in four different mutations in plasma measured by ddPCR. Low levels of ctDNA are observed after cystectomy and CT scans are negative (green bars). The patient to the right is also monitored using four ddPCR assays against tumour-specific mutations. Here, low or no ctDNA during NAC is detected, but the levels of ctDNA increase after cystectomy and before metastasis is detected by CT scan. For this patient, the ctDNA levels indicate that the patient responds to immunotherapy and with a stable disease (SD) manifestation.

Results adapted from: Birkenkamp-Demtroder *et al*[153]

A major clinical problem in bladder cancer is the need for surveillance for many years following surgical therapy with cystoscopy. Since liquid biopsies represent an opportunity to obtain patient samples noninvasively at multiple timepoints during their treatment course without the need for obtaining invasive biopsies, they are representing samples for the next generation of surveillance markers. This is very convenient in nonmuscle invasive disease as an adjunct to personalized timing for cystoscopies during follow-up. This is also critical in a metastatic setting, to allow monitoring of the molecular evolution of the tumour during treatment, which should inform subsequent therapeutic decisions. Overall, the concept of liquid biopsies is leading us towards a highly specific precision medicine for patient monitoring and potentially for therapeutic selection.

3.6.6 Conclusions and take-home messages

The ability to extract minimal amounts of small tumour representative molecules in circulation or in other body fluids along with development of more sensitive and more efficient methods has opened the new field of liquid biopsies to identify biomarkers in a number of cancers, including bladder cancer. Liquid biopsies hold great promise for personalized medicine, due to their ability to provide multiple noninvasive global snapshots of tumours and their clinical behaviour. The data summarized in this chapter have shown a number of studies investigating how profiling of cfDNA in blood and urine is opening a new field for biomarker research. These studies suggest the role of cfDNA as promising noninvasive diagnostic, prognostic, and surveillance markers for bladder cancer. Methodological and analytical pitfalls exist and require further addressing to enable future translation of laboratory findings into biomarkers for clinical routine practice in bladder cancer.

The increasing potential of liquid biopsy analyses deserves excitement surrounding new sensitive and efficient technologies. However, studies are still required to determine to what extent these newly identified circulating molecules can provide a reliable and accurate picture of the tumour and patient behaviour. Studies are still required to select the most adequate candidates to have body fluids measured as the critical molecular drivers of cancer progression and/or clinical behaviour to inform treatment or patient management decisions. For example, it remains to be shown whether early detection of metastatic disease will improve survival by earlier therapeutic intervention, and not only improve progression-free survival (PFS). Furthermore, the fraction of patients that can actually be administered specific drugs based on actionable targets in plasma—and the survival benefit of this—is currently unknown. Most studies of bladder cancer patients are based on small patient cohorts; larger prospective studies are needed and should be followed by clinical intervention studies to prove survival benefit of liquid biopsy analyses.

3.7 Molecular Subtypes of Bladder Cancer

3.7.1 Introduction

Molecular subtypes can be defined as repeated observations of global patterns of gene or protein expression in a tumour type. This definition excludes rare outlier tumours, idiosyncratic expression profiles, and misclassification of other malignancies spread to the bladder. Using global analysis also ensures that identification of molecular subtypes is an unbiased process. The logic behind such a definition is simple: a frequently occurring phenotype is more relevant from biological and clinical perspectives than a rare one. Similarly, the more genes and proteins that are associated with a certain classification, the more relevant it is. For bladder cancer, molecular subtype classification has mostly been performed separately for NMIBC and MIBC tumours. Studies that have collected data from both NMIBC and MIBC suggest that molecular subtypes exist across tumour stages, although they may exist in different proportions and aggressiveness, depending on tumour stage.

Here, we review the literature to identify all studies that classify bladder tumours into molecular subtypes as defined above. We include only studies that published methods and data in sufficient detail to replicate the classification. Studies are grouped by research centre, and a summary of the most up-to-date work of each group is described. For each study, we briefly describe the study population, the tumour biology, and clinical or pathological associations. In the discussion section, we summarize the overlap of classification and what is known about the role in predicting treatment outcome.

3.7.2 The Cancer Genome Atlas (TCGA) classification

Within the TCGA project, fresh-frozen MIBC samples were collected and analyzed by global clustering of RNA sequencing (RNA-Seq) data. The first TCGA report on bladder cancer included 129 tumours, grouped by gene-expression data into four clusters (Clusters I–IV).[92] A new and updated analysis includes 408 tumours,[123] and the results from these analyses are summarized here. Unbiased non-negative matrix factorization consensus clustering of RNA-Seq data for 408 MIBC samples yielded five expression subtypes: luminal-papillary (n=142, 35%), luminal-infiltrated (n=78, 19%), luminal (n=26, 6%), basal-squamous (n=142, 35%), and neuronal (n=20, 5%). The subtypes were associated with OS ($p=4 \times 10^{-4}$), with neuronal showing the worst survival, luminal-papillary the best, and the other three being intermediate.

Most samples in the luminal subtypes showed high expression of UPKs (UPK2 and UPK1A) and urothelial differentiation markers (FOXA1, GATA3, PPARG). Differences in purity and in expression with respect to the "p53-like" EMT and stromal gene signatures contributed to their separation into different clusters.

The luminal-papillary cluster was enriched in tumours with papillary morphology (58% vs 20% in other subtypes; $p<10^{-13}$), lower stage (T2, 55% vs 23%; $p<10^{-8}$), and higher purity (median 0.84 vs 0.50 in other luminal subtypes). They were also enriched in FGFR3 mutations (42/57; $p<10^{-9}$), FGFR3

amplification (5/5; p=0.005), overexpression (4-fold vs median, 49/67; $p<10^{-11}$), and FGFR3-TACC3 fusions (8/10, p=0.004). These tumours also had low CIS expression signature scores and relatively high sonic hedgehog (SHH) signalling. These features suggest that many tumours in this cluster developed from a precursor papillary NMIBC.

The luminal-infiltrated subtype was distinguished from other luminal subtypes by lower purity (median 0.46 vs 0.68; $p<10^{-11}$), consistent with the presence of lymphocytic infiltrates, and by strong expression of smooth muscle and myofibroblast gene signatures. Thirty-six of 45 (80%) of the tumours in this subtype had features similar to the MD Anderson Cancer Center (MDA) subtype Tp53-like. The p53 signature score was inversely correlated with tumour purity (Pearson r=-0.4; p<.001), suggesting that smooth muscle and fibroblast cells in these tumours were the drivers of this signature. These tumours had increased expression of several immune markers, including CD274 (programmed death-ligand 1, or PD-L1) and PDCD1 (programmed cell death protein 1, or PD-1).

The luminal subtype had the highest expression levels of several UPKs (UPK1A, UPK2) and genes that are highly expressed in terminally differentiated urothelial umbrella cells (KRT20, SNX31), suggesting a transcriptional program that leads to an umbrella cell expression pattern.

The basal-squamous subtype was characterized by high expression of basal and stem-like markers (CD44, KRT5, KRT6A, KRT14) and squamous differentiation markers (TGM1, DSC3, PI3). The subtype included 37 of 45 tumours with squamous features ($p<10^{-11}$), was enriched in TP53 mutations (p=0.005), and was more common in females (33% vs 21% in other subtypes; p=0.024). Many tumours in this subtype also showed strong expression of CIS signature genes and loss of SHH signalling, suggesting that they developed from basal cells and CIS lesions. This subtype also showed the strongest immune expression signature, including T-cell markers and inflammation genes, consistent with relatively low purity (median 0.49) and the presence of lymphocytic infiltrates.

The neuronal subtype included three of four with neuroendocrine (NE) histology (p=0.005), and an additional 17 tumours that had no histopathological features suggestive of NE origin. All 20 showed relatively high expression of neuronal differentiation and development genes, as well as typical NE markers. Ten of 20 (50%) samples had mutations in both TP53 and RB1, or TP53 mutation and E2F3 amplification, consistent with inactivation of both pathways, as seen in small cell carcinoma in other sites including lung.

3.7.3 MD Anderson Cancer Center classification

The research group at MD Anderson Cancer Center (MDA) investigated RNA profiles of 73 MIBC tumours. Fresh frozen samples were analyzed and divided into three clusters termed Luminal-like, Tp53-like, and Basal-like.[48] Along with the University of North Carolina (UNC) group,[16] they first reported on the similarities between bladder and breast cancer molecular subtypes. Several markers (CD44, KRT5, KRT6, KRT14, and P-cadherin) identified basal cells in both epithelia, and are enriched in the Basal-like subtype of both tumour types. Conversely, Choi *et al* also identified markers down-regulated in Basal-like cases, and many known Luminal-like breast cancer subtype markers (CD24, FOXA1, GATA3, ERBB2, ERBB3, XBP1, and KRT20). These were highly expressed MIBC Luminal-like clusters. A third cluster was not Basal-like, or Luminal-like but was enriched for upregulated

genes in the Tp53-response pathway, hence termed Tp53-like. Although a minority of patients in the MDA cohort had received cisplatin-based NAC, those in the Tp53-like group were nonresponsive. In matched samples after chemotherapy, many previously Luminal-like tumours were classified as Tp53-like, indicating an increase in this subtype after chemotherapy treatment. Functional studies on bladder cancer cells identified TP63 and PPARG as likely drivers behind the Basal-like and Luminal-like transcriptional programs, respectively. Following this initial study, the group applied their classifier to micropapillary bladder tumours.[165] Tumours with micropapillary variant histology (n=43) were compared to stage-matched conventional bladder tumours (n=89), showing that this clinically aggressive histological variant was nearly exclusively of the Luminal-like or Tp53-like molecular subtypes. Importantly, the MDA classifier has been further developed since the original study, and compared to the other existing classifiers. Dadhania *et al*[49] compiled several data sets including data from TCGA, and performed a meta-analysis centred on the three subtypes described above. In addition to validating previous finding in much larger cohorts, the Tp53-like subtype was split up into Basal-like and Luminal-like subsets that could be identified by cancer-cell staining for GATA3 (Lum+) and KRT5 (Bas+). Immunohistochemistry revealed that strong expression signals from nontumour cells, including stromal and immune cells, were underlying the Tp53-like subtype. The meta-analysis also identified a minor GATA3/KRT5 "double-negative" subset, indicating further complexity beyond that captured by the MDA classifier.

3.7.4 University of North Carolina (UNC) classification

A research group at University of North Carolina (UNC) compiled data from four published cohorts into a meta data set (n=262) and performed clustering to identify molecular subtypes.[16] The work was performed on MIBCs only and included only high-grade cancer. This study and that of Choi *et al*[48] independently identified Basal-like (KRT14, KRT5, KRT6B, CD44) and Luminal-like (UPK1B, UPK2, UPK3A, KRT20) subtypes of bladder cancers that correspond to breast cancer counterparts. The authors defined a minimal subtype predictor signature of 47 genes that identifies this main Basal-like versus Luminal-like distinction of bladder tumours. The authors also identified a minor group of Claudin-low tumours, samples with low expression levels of CLDN3, CLDN7, CLDN14, CDH1, and the epithelial cell adhesion molecule and high expression of mesenchymal markers such as VIM, TWIST1, and SNAI2 (SLUG). In the context of the Basal-, and Luminal-like subtype-classifier, Claudin-low tumours were classified as Basal-like and the patients with Basal-like or Claudin-low tumours had similar poor outcomes. Since the initial study, a 40-gene classifier for detection of Claudin-low tumours has been published,[166] along with an in-depth characterization of this group that represents about 10% of MIBCs. Genomic data from TCGA revealed that Claudin-low samples more frequently contained RB1 and EP300 mutations and less frequently FGFR3 and KDM6A. These samples also had the highest levels of immune signatures, including a signature for immune suppression, indicating that Claudin-low samples contain immune cells and may be immunologically suppressed. Like the initial study, this analysis of TCGA data identified Basal-like and Claudin-low subtypes as poor prognosis compared to Luminal-like. Interestingly, the immune signatures that were elevated in the Claudin-low group did not show any strong prognostic effects. The authors also analyzed neoantigen burden, which was similar in tumours of the three different subtypes, and globally associated with better outcome for tumours with high neoantigen burden.

3.7.5 Genomic Subtyping Classifier classification

Seiler *et al*[17] investigated NAC response in the molecular subtypes by analyzing RNA profiles of 223 chemotherapy-naïve MIBC samples and comparing the results to the untreated TCGA cohort (*n*=397). The generated data set was classified by all available subtype-classification methods, proving good concordance of the different classifiers in external data. Guided by the differences in biology, but also outcome, a novel four-group classifier based on a single sample linear model fit was developed to optimally capture subtypes with prognostic or chemotherapy response predictive potential. The algorithm classified cases into Luminal, Basal, Claudin-low, and Luminal-Infiltrated in order of prevalence in the Seiler *et al* NAC cohort. As expected, when reapplied to the NAC-treated and to the TCGA cohorts, the pattern of OS differences remained significant. Basal-like samples showed poor outcome in the untreated TCGA cohort but a much better prognosis in the NAC-treated cohort. Interestingly, the improved outcome of cases with pretreatment Basal-like tumour was not reflected by increased proportion with pathological response. In their cohort, pathological response seemed to translate to improved OS for Basal-like tumours, but less so for the other subtypes.

3.7.6 Lund University (LundTax) classification

The Lund group was first to divide bladder tumours into molecular subtypes. An early study used mRNA profiling and array CGH to describe early- and late-stage tumours.[167] The increased size (*n*=308) of the subsequent taxonomy study on both NMIBC and MIBC[168] allowed robust detection of subtypes that were not limited by pathological stage. Sequential two-group splits of the RNA profiles resulted in five stable tumour classes. Subtypes were characterized by cancer signatures and parallel immunostainings and were termed Urobasal A (UroA), Urobasal B (UroB), Genomically Unstable (GU), Infiltrated, and Squamous-cell carcinoma-like (SCC-like). The nomenclature was later updated and "Urobasal" replaced with "Urothelial-like" (still using the *Uro* abbreviation). UroA tumours were enriched for low tumour stage and grade, FGFR3 and PIK3CA mutations, and depleted for TP53 mutations. Most early papillary tumours are thus placed in this molecular subtype, which is firmly on the Luminal-like side of bladder cancers. UroB tumours share with UroA some extent of retained urothelial-like stratification, with basal-cell markers limited to the stroma-adjacent basal cells of the tumour. For UroB, basal markers were less confined to the basal-cell layer and, despite their luminal nature, expression of basal markers sometimes resembled that of SCC-like tumours. GU tumours, while also Luminal-like, do not express FGFR3 and have frequent RB1 loss and CDKN2A (p16) overexpression, indicating genomic disruption of cell-cycle control. The SCC-like group showed overexpression of basal keratins, squamous signatures, consisted mainly of MIBCs, and was enriched for female gender. Recently, the Lund group updated the molecular taxonomy for MIBCs.[169] Carefully controlled sampling allowed analysis of mRNA and immunohistochemical (IHC) profiles of a large data set (*n*=307). The study reached two important conclusions: The minor (about 5% of MIBC each) subtypes Mesenchymal-like (Mes-L) and Small-cell/Neuroendocrine-like (Sc/NE-L) were described with corresponding mRNA and IHC markers for detection. Furthermore, distinct molecular subtypes defined by IHC were shown to sometimes converge in the same mRNA clusters. Evidence was presented that convergence was due to tumours with different cancer-cell phenotypes acquiring similar immune-, stromal-, or proliferation levels, bringing mRNA profiles of such tumours closer together.

3.7.7 Aarhus (UROMOL) classification

In Hedegaard *et al*,[170] 476 tumours were analyzed by RNA-Seq as part of the UROMOL multicentre prospective study on NMIBC. The study was dominated by pTa tumours, but also contained a large number of pT1 tumours. A small number of MIBC samples were included as well, which located to the same cluster (Class 2) as the NMIBCs with highest European Organisation for Research and Treatment of Cancer (EORTC) progression risk score. This cluster also included most of the T1 tumours in the study, whereas Classes 1 and 3 were dominated by pTa tumours. The three reported classes were significantly associated with technical factors, such as collecting centre and RNA quality, but also with both the UNC and Lund classifiers (Class 2 with Lund Genomically Unstable, Class 1 with Lund UroA, and Class 3 with UNC Basal-like subtypes). The strongest differences among the tumour classes were related to cell-cycle regulation. Early cell-cycle regulators, including CCND1, RBL2, ID1-3, and WEE1, showed highest expression in Class1, whereas late cell-cycle regulators, for example CCNA1, CCNB1-2, CCNE1, FOXM1, MYBL2, and PLK1, were relatively overexpressed in Class 2 samples. Furthermore, the Class 2 tumours had increased rate of progression and were enriched for concomitant CIS compared to Class 1 and Class 3. Being more aggressive, Class2 tumours were enriched for tumours from BCG-treated patients, but BCG response did not differ among classes. Finally, Hedegaard *et al* showed that an APOBEC-associated mutational signature was significantly increased in the more aggressive Class2 tumours.

3.7.8 Discussion and conclusions

Although there are differences among the classifiers reported, there is also clear and strong correspondence among these classifiers. This was reported in cross-comparison of the UNC, MDA, and Lund classifiers in an earlier TCGA data release (n=238),[171] and was confirmed in the current (n=408) TCGA cohort.[104] The approximate correspondence of the current subtypes between classification systems is shown schematically in **Figure 3–8.** Whenever global mRNA data are generated, it is recommended that investigators should apply many or all of the existing classifications, since the comparison may highlight the most relevant classification level for a study, with the added benefit of additional confidence in classification.

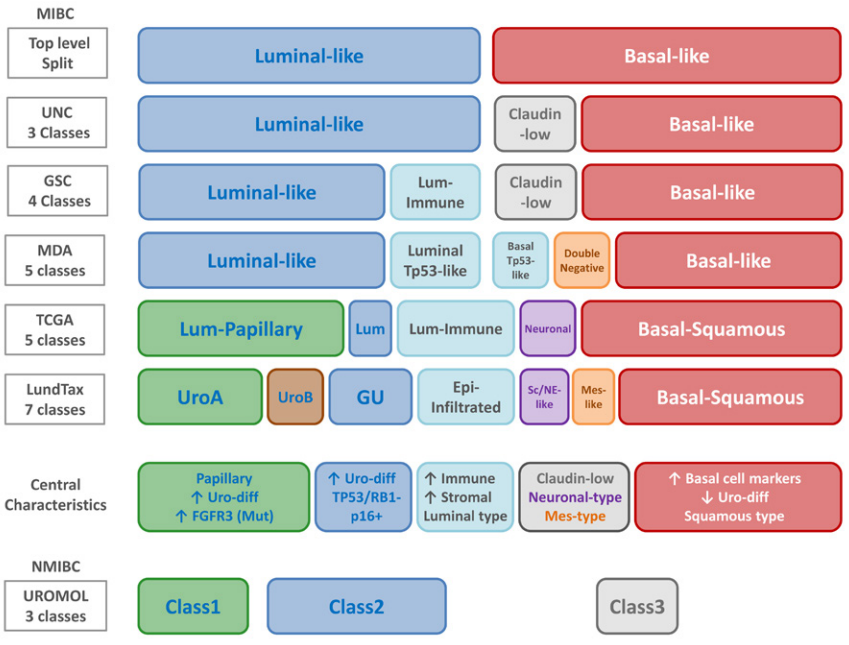


FIGURE 3–8
Schematic Map of Molecular Subtype Classification Studies of Bladder Cancer

On top is shown the main division (Top level split) into Luminal-like and Basal-like subtypes of MIBC. Thereafter follows the schematic, approximate overlap of the current most up-to-date versions of the five classification methods that apply to MIBC. Next, selected central biological characteristics are mapped to the subtypes. Finally, the UROMOL study that applies to NMIBC only is schematically mapped to the central characteristics in accordance with Hedegaard *et al.*

The different groups are collaborating, sharing classifiers, and encouraging projects aimed at consensus agreement. One effort resulted in a consensus statement regarding the existence of a Basal/Squamous-like (Ba/Sq) subtype, molecularly defined as KRT5/14 positive and GATA3/FOXA1 negative.[172] The label Ba/Sq reflects expression of basal markers (in urothelium or in Basal-like breast cancer), but also markers of squamous epithelia. The four-gene Ba/Sq definition is valid both in gene and protein expression data and is likely to be clinically relevant.[47] Further efforts are under way to develop consensus molecular subtypes for MIBC, similar to what has been done in colorectal cancer.[173]

The acid test for the molecular subtypes of bladder cancer is the potential to stratify patient populations in a clinically relevant manner. The studies described here all report significant differences in survival data among subtypes. It is clear that subtypes have a different natural history of disease. It remains to be shown whether these different subtypes should each be treated in a distinct manner, although that has been suggested in several studies (including TCGA).

The only publication reporting molecular subtypes and response to BCG is the UROMOL study,[170] but there was no significant difference in response among subtypes.

Two groups report differential response to cisplatin-based NAC. In Seiler *et al*,[17] the proportion of patients achieving downstaging in cystectomy specimen after NAC did not differ between molecular subtypes. There are data that the Tp53-like (MDA) subtype has a decreased rate of downstaging,[48] indicating relative resistance to NAC. The next critical issue to analyze is whether pathological response translates differentially to OS in the molecular subtypes. Here, data from both Seiler *et al* and data from MDA[174] indicate that patients with tumours classified by RNA profiling as Basal-like may have improved OS following cisplatin-based NAC, whereas for the other subtype this improvement was weaker. Taken together, better studies are needed, preferably combining RNA profiling and IHC classification. Such studies would allow us to resolve whether characteristics of cancer cells, subtype classification by RNA profile, or stromal/immune-cell content offer the best prediction of pathological downstaging, as well as subsequent OS.

There is evidence that the TCGA Cluster II (currently, luminal-immune) has improved response to immune-checkpoint inhibitors compared to the other subtypes.[135] It is puzzling why only the TCGA clusters were reported, especially since a positive but marginal enrichment of responders in one subtype was observed. It is also not known to what extent the predictive effect of molecular subtype is independent of other factors, for example mutational burden, tumour-infiltrating lymphocytes, and PD-L1 expression. A challenge for subtype classification in clinical trials for advanced bladder cancer is that biomarker analysis is performed on bladder tumours, whereas treatment is given for metastatic disease. Molecular classification of relapses, matched with bladder tumours before or after treatment, should be a priority for future studies.

3.8 Noncoding RNAs in Bladder Cancer

In this section, we discuss three types of noncoding RNAs: miRNAs, long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs).

3.8.1 microRNAs

3.8.1.1 Introduction

In this section, we first discuss normal function of miRNAs, outlining how miRNAs are expressed, processed, and act, and then noting interacting issues that indicate that miRNAs can participate in complex gene regulatory relationships and networks. Given this context, we outline how miRNAs can contribute to cancers by participating in dysregulated processes. Finally, we summarize recent review and research publications on miRNAs for diagnosis and prognosis in bladder cancers.

3.8.1.2 Normal function of microRNAs

miRNAs are small noncoding RNAs that are active in post-transcriptional, epigenetic gene regulation.[175,176]

miRBase (www.mirbase.org) offers reference information.[177] miRBase v21 reports ~2,500 human mature strands; how miRNA annotations develop may be influenced by large-scale analysis of small noncoding RNA-Seq data.[178]

Canonical miRNA biogenesis involves polymerase II-based expression of primary (pri)-miRNA transcripts with lengths from ~200 nucleotides to several kb.[179] Transcripts are processed in the nucleus to 60 to 70 nucleotides pre-miRNAs by Drosha, then exported to the cytoplasm by Exportin 5 and further processed to 5p/3p duplexes by Dicer. Each duplex typically yields a single ~22-nucleotides, 5p or 3p mature strand that hybridizes, within an RNA-induced silencing complex, to complementary sequences in "target" mRNAs. Which mRNA sequences a mature strand will target is strongly influenced by the strand's "seed" sequence, i.e. by bases 2 to 7;[180,181] however, noncanonical targeting also occurs.[182] Targeting may destabilize an mRNA, or may repress its translation to protein. Targeting destabilizes mRNAs more effectively when it occurs within 3'UTRs.[180, 183] Targeting can influence mRNA and/or protein levels, potentially reducing variation in mRNA or protein levels.[176] MiRNA mature strands can behave as oncogenes (oncomiRs) and as tumour suppressors.[184]

miRNAs that are relatively abundant are more likely to be influential, and relationships between miRNA concentrations and changes in targeted gene mRNA/protein levels may be nonlinear.[181,185] A mature strand's availability for targeting a gene or genes of interest can be reduced (i.e. titrated) by it binding to competitive endogenous RNAs (ceRNAs);[186-188] ceRNAs may be coding genes, pseudogenes, lncRNAs,[189] or circRNAs.[190] Recent work[191] addresses questions related to whether *in vivo* ceRNA effects are important generally, or only in certain dysregulated biological states.[189,192]

Computationally predicted and experimentally validated data are available for which mRNAs a mature strand will hybridize to, i.e. will target. Targeting data that have been validated by stronger or on weaker evidence types are available only for specific tested miRNAs, mRNAs, and biological systems.[193,194] While computationally predicted canonical and noncanonical target sites[176,183] are not subject to such experimental constraints, they may have algorithm-specific biases, which are typically addressed by combining predictions generated by more than one algorithm. Some sets of predictions assign scores to binding sites that reflect how effectively targeting should reduce levels of mRNA transcripts.[180,195,196]

A mature strand can target tens to hundreds of different mRNAs. A gene can be simultaneously targeted by more than one mature strand,[197] and in such combinatorial targeting, mature strands may act competitively or synergistically.[198]

Transcriptional regulation of miRNAs can be influenced by, for example, copy number, DNA methylation, and transcription factors. And while 30% of human miRNAs are intergenic, 70% are intragenic, i.e. are located within, so are transcribed with "host" genes.[179] An intragenic miRNA and its host gene may have independent promoters, and transcription of a miRNA and its host gene may be regulated independently.[199,200]

Groups of miRNAs that are in relatively dense genomic clusters may be transcribed together as polycistrons (e.g. hsa-mir-17 to -92a on 13q31.3).[200,201] Polycistron processing, and hence the relative abundances of the polycistron's miRNAs, can be influenced by the tertiary structure of the pri-miRNA.[202]

Similar mature strands' genomic sequences can be expressed from members of a miRNA family that are in different genomic locations (e.g. hsa-let-7a-5p can be expressed from -7a-1 at 9q22.32, -7a-2 at 11q24.1, and -7a-3 at 22q13.31); further, sets of mature strands from miRNA families or paralogous polycistrons can have identical seed sequences, and so similar mRNA targets.[175]

While miRBase catalogs "reference" miRNAs, mature strands can be expressed as isomiRs whose start or end locations differ from the reference locations.[178] As a mature strand's seed sequence is important for specific targeting and for "effective" mRNA destabilization,[180] targeting and its effects should be sensitive to 5' isomiR variation that changes the seed sequence. However, 3' variation may also be functionally important.[203]

For the biological effects of mature strands, it can be helpful to consider small networks, feedback/feedforward loops, transcription factor/miRNA co-regulatory loops, and regulatory circuits that support robust, switch-like transitions between alternative biological states.[175,204-206]

3.8.1.3 Roles of microRNAs in cancer

From the previous section, miRNAs can participate in complex gene regulatory relationships and networks, which can be dysregulated by genetic and epigenetic alterations. Such dysregulation can contribute to diverse processes in cancers, including epithelial-to-mesenchymal transitions, metastasis, chemoresponse, and chemoresistance.[184,207,208] For example:

- Alterations to biogenesis pathway genes can influence the abundance of many miRNAs.[209-211]
- The abundance of individual miRNAs, or smaller numbers of both intergenic and intragenic miRNAs, can be influenced by single nucleotide polymorphisms (SNPs), including those affecting polycistron transcript processing;[202] by copy number variation; and by DNA methylation and transcription factors.[211]
- The transcription of an intragenic miRNA that lacks an independent promoter(s) depends on its host gene being transcribed, and so on the transcriptional regulation of that host gene.[199,200]
- Target genes, and the 5p/3p strand ratio, can change when the sequence of a pre-miRNA or a mature strand is altered by isomiR variation, SNPs, or RNA edits.[178,212-214]
- Binding site sequences and secondary structures in mRNA 3' untranslated regions (UTRs) can be altered by SNPs and RNA editing,[213] and alternative polyadenylation can add or remove binding sites.[215]
- Expression may be dysregulated for ceRNAs that compete for and titrate mature strands.
- Mature strands that are influential may be differentially abundant between tumour and adjacent normal tissues, and between molecular subtypes.

3.8.1.4 The current state of the field for microRNAs in bladder cancer

Both NMIBC and MIBC are heterogeneous and have relatively high progression and recurrence rates. Applications for biomarkers, for which cost, invasiveness, complexity, and timeliness are considerations, in addition to sensitivity and specificity, involve: 1) population screening, particularly for early detection; and 2) for bladder cancer patients, diagnosis of disease types and subtypes, prediction of risk of progression, recurrence, or survival, and monitoring for progression or recurrence after treatment.[216]

As expressed stem-loops are rapidly transformed into biologically functional mature strands, biomarker assays typically measure the abundance of mature strands. Mature strands are more stable than mRNAs or proteins, so may be more appropriate for preserved (formalin-fixed paraffin-embedded [FFPE]) samples, and for noninvasive liquid biopsies involving blood, serum, or urine.[140] In urine, exosomes and extracellular vesicles may be isolated as a source of biomarkers.[217-220]

miRNAs in bladder cancer have been characterized using microarrays, short-read sequencing (NGS)[221] and quantitative reverse transcription PCR (qRT-PCR).[216] Given appropriate analysis methods, NGS can report mature strand variants like isomiRs, SNPs, RNA edits, and untemplated additions (see above). NanoString nCounter® assays have been reported for comparing FFPE-tumour tissue, plasma, and urine exosomes.[217]

Below, we summarize recent review and research publications. We note recent reviews.[179,216,221-225]

We do not discuss miRNA therapeutics.[179,184] We do not discuss subtyping bladder cancer cohorts with miRNAs, but note two reports using data from the TCGA muscle-invasive cohort,[123,226] and a discussion of bladder cancer subtypes as related coarser- and finer-grained sets.[227] We are unaware of publications that report miRNAs as biomarkers for immunotherapy.[228]

Tables 3–1 to **3-5** indicate current resources at the time of writing focused on the role of miRNA in bladder cancer.

TABLE 3–1 miRNA Expression and Target Genes

Type	Contents	Reference
Review	Table 1 reports a literature survey (2006 to 2017) for 118 miRNAs that are up- or downregulated (in at least two publications) in tissue, urine, blood, tissue and blood, or urine and blood.	Dong *et al*[211]
Review	From 374 publications to March 2016, Tables 1 and 2 report miRNAs (tumour-associated miRNAs, or oncomiRs, and tumour suppressors) that were differentially abundant between bladder cancer and normal tissue, and their validated direct target genes, and assign functions to sets of target genes. They note that all studies are retrospective, and that large prospective studies are needed.	Enokida *et al*[179]
Review	76 miRNAs differentially expressed in bladder cancer by at least two of 19 groups. Report miRNA-targeted biological processes.	Lee *et al*[222]
Review	NGS in tissues and biofluids. Technical issues, comparison with qRT-PCR. Limited consistency between results reported by different groups. Exosomes/microvesicles.	Matullo *et al*[221]

Abbreviations: miRNA, microRNA; qRT-PCR, quantitative reverse transcription polymerase chain reaction; NGS, next-generation sequencing.

TABLE 3–2 miRNAs as Diagnostic Markers for Bladder Cancer Detection

Type	Contents	Reference
Review	Table 3 lists two reports up to 2015 for tissue, 12 up to 2015 for urine, and five up to 2016 for blood. Note that panels of multiple miRNAs, rather than single miRNAs, will likely be needed.	Enokida *et al*[179]
Review	Publications to March 2016, 26 publications, 2753 patients. 12 publications satisfy meta-analysis criteria. Most are retrospective, some prospective. 6 miRNAs were identified by at least two of the 12 publications (Tables 1 and 2): miR-21, 143, 155, 200, 214, and 222.	Xie *et al*[223]

Abbreviation: miRNA, microRNA.

TABLE 3–3 miRNAs as Prognostic Biomarkers From Tumour Tissue

Type	Contents	Reference
Review	Table 4, 22 publications from 2009 to 2016, OS, RFS, PFS, DSS; cohorts with 18 to 202 cases.	Enokida *et al*[179]
Research	89 patients, FFPE tissue. qRT-PCR. miRNAs associated with cancer-specific survival, and progression to MIBC.	Lenherr *et al*[229]
Review	26 publications to March 2016, 2,753 patients. 12 publications satisfy meta-analysis criteria. Most are retrospective, some prospective. Tables 1 and 2: 6 miRNAs identified by at least two publications: miR-21, 143, 155, 200, 214, and 222. Early detection of progression or recurrence.	Xie *et al*[223]

Abbreviations: DSS, disease-specific survival; FFPE, formalin-fixed paraffin-embedded; MIBC, muscle invasive bladder cancer; miRNA, microRNA; OS, overall survival; PFS, progression-free survival; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RFS, recurrence-free survival.

TABLE 3–4 miRNAs as Biomarkers From Urine

Type	Contents	Reference
Review	Table 2, one serum miRNA study in MIBC. See also text on page 13.	Contreras-Sanz *et al*[224]
Research	276 bladder cancer patients and 276 controls. qRT-PCR on urine supernatant. Seven-miRNA panel with high diagnostic accuracy. Predict recurrence (RFS) in NMIBC.	Du *et al*[230]
Research	210 patients. NMIBC progression, qRT-PCR of 8 miRNAs previously described. miR-140-5p and 92a-3p were independent predictors of progression and of cancer-specific survival.	Ingelmo-Torres *et al*[231]
Research	803 patients. Surveillance for recurrence. Compared Cxbladder MonitorTM test to commercial urine markers (NMP22 Bladderchekâ, NMP22 ELISA, Cxbladder MonitorTM, UroVysion FISH) and to cytology	Lotan *et al*[232]
Review	Table 1 includes four prognostic publications on cell-free urine.	Xie *et al*[223]

Abbreviations: FISH, fluorescence in situ hybridization; MIBC, muscle invasive bladder cancer; miRNA, microRNA; NMIBC, nonmuscle invasive bladder cancer; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RFS, recurrence-free survival.

TABLE 3–5 Blood/Serum, Circulating Biomarkers

Type	Contents	Reference
Review	qRT-PCR. Recurrence in NMIBC. Table 2, one serum miRNA study. Section on epigenetic biomarkers includes miRNA.	Contreras-Sanz *et al*[224]
Review	Liquid biopsies. Table 3: diagnostic and prognostic miRNAs.	Di Meo *et al*[140]
Research	Serum from 207 MIBC patients, 285 NMIBC, 193 controls. Differential miRNAs by Illuminaâ Miseq NGS. qRT-PCR in independent cohorts, 40 MIBC, 40 NMIBC, 40 controls. Panel of 4 miRNAs to distinguish NMIBC from MIBC.	Jiang *et al*[233]
Review	Circulating biomarkers; circulating tumour cells, gene mutations, and methylation in cell-free DNA.	Nandagopal *et al*[234]
Review	Table 1 includes three prognostic publications on serum.	Xie *et al*[223]

Abbreviations: MIBC, muscle invasive bladder cancer; miRNA, microRNA; NGS, next-generation sequencing; NMIBC, nonmuscle invasive bladder cancer; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

3.8.2 LncRNAs

3.8.2.1 Introduction

The main types of shorter and longer noncoding RNA (ncRNA) are summarized in.[225] In this section we review long noncoding RNAs (lncRNAs). We first discuss the normal function of lncRNAs, outline how lncRNAs can contribute to cancers by participating in dysregulated processes, and then summarize recent review and research publications on lncRNAs for subtyping, diagnosis and prognosis in bladder cancers.

3.8.2.2 Normal function of lncRNAs

LncRNAs are mRNA-like molecules that are at least 200nt long that are typically subject to normal mechanisms of RNA polymerase II transcriptional regulation like 5' capping, splicing and 3' polyadenylation.[235,236] Relative to neighbouring coding genes, they may be intronic, intergenic or antisense. While mRNAs encode proteins, lncRNAs tend to lack robust open reading frames (ORFs) and therefore are generally not translated. LncRNA expression levels tend to be lower than those for coding RNAs. That their expression can be more specific to developmental stage, tissue, and cell type, compared to coding RNAs, may make lncRNAs suitable for subtyping and as biomarkers.[237]

Unlike protein-coding genes, which require translation to become functional molecules, lncRNAs exert their functions at the RNA level, by adopting complex secondary and tertiary structures that are analogous to folded proteins. Through these structures, lncRNAs can interact with proteins, metals, and other ligands. These structures can also reveal sequences that can be involved in specific base-pairing with other RNAs (i.e. miRNAs) or with specific DNA sequences (i.e. enhancers).

LncRNA functions are discussed in several recent papers.[235,238-240] Kopp and Mandell review *cis* and *trans* gene regulation by lncRNAs, including organization of nuclear architecture, and regulation of interacting proteins and RNAs; and discuss experimental approaches for investigating functional mechanisms.[238] Rebeiro, *et al* report computational results suggesting that lncRNAs act as protein scaffolds in many known protein complexes.[240]

3.8.2.3 LncRNAs in bladder cancer

Like protein-coding genes, lncRNAs can act as oncogenes, tumour suppressors or both. Coding genes and lncRNAs are subject to the same mechanisms of deregulation; e.g. copy number gain or deletion, alteration in DNA methylation patterns (hypomethylation/hypermethylation), chromatin remodelling, and changes in transcription factor expression. However, while mutations in protein coding genes can impact gene function, it is less clear whether this holds for lncRNA function. While some lncRNAs are highly evolutionally conserved with significant rates of compensatory mutations (e.g. the adenocarcinoma-associated *EVADR*), most do not show such conservation.[241-244] It is possible that mutations in lncRNAs are less likely to impact function due to relaxed base-pairing specificity (i.e. GU base-pairs) or reduced functional constraints on RNA secondary structure.

A recent review[225] discussed 32 lncRNAs that have roles in in bladder cancer formation (UCA1, MALAT1, H19, TUG1, MEG3, MIR31HG, Linc-UBC1 (BLACAT1), LOC572558, PANDAR, GHET1, ncRAN, GAS5, ANRIL, HIF1A-AS2, HOTAIR, HOXD-AS1, MDC1-AS1, PCAT-1, PVT1, SChLAP1, SPRY4-IT1, ZEB2-AS1, T-UCR 8+, NEAT1, and others). Similarly, functions and expression patterns in bladder cancer for 27 lncRNAs, and other cancers that these lncRNAs are involved in, were reviewed.[245] LncRNAs in urologic malignancies were reviewed by.[246] Below, we highlight lncRNAs in molecular subtyping, as ceRNA, and as biomarkers.

3.8.2.4 LncRNAs and molecular subtypes

Given their specific expression patterns, it is not surprising that lncRNAs show subtype-specific expression patterns in both NMIBC and MIBC.

For example, in a recent TCGA study of ~400 MIBC tumours, unsupervised clustering using the lncRNA transcriptome revealed a group of patients which was highly associated with the luminal papillary (mRNA) subtype with improved prognosis.[123] Interestingly, these tumours were enriched for FGFR3 mutations and depleted for p53 mutations, suggesting that these patients may be suitable candidates for therapies targeting FGFR3. These results have been replicated in an independent n=~350 cohort (Gibb *et al,* unpublished data).

In NMIBC, a recent study used gene expression profiling with total RNA for n=460 patients to report three molecular subtypes with basal- and luminal-like characteristics.[170] They assessed a 117-gene classifier in four independent data sets. Class 3 tumours had higher levels of lncRNA expression (e.g. MIR31HG, NEAT1, MALAT1), and a dormant tumour state, suggesting that lncRNAs may be enforcing this class of tumours to adopt a quiescent state.

3.8.2.5 LncRNAs as competitive endogenous RNAs

In our discussion of miRNAs we noted that a mature strand's availability for targeting a gene or genes of interest can be reduced (i.e. titrated) by its binding to ceRNAs,[186-188] that ceRNAs may be lncRNAs,[189] or circRNAs.[190] We noted recent work[191] addressing whether *in vivo* ceRNA effects are important generally, or only in certain dysregulated biological states.[189,192]

3.8.2.6 LncRNAs as biomarkers

Above, we noted considerations and potential applications for biomarkers in bladder cancer. Due to their enhanced specificity of expression for tissue, cell or tumour type, lncRNAs are promising biomarkers.

In a review comparing mRNAs, miRNAs and lncRNAs as biomarkers measured by qRT-PCR, the authors list lncRNAs that have been reported for diagnosis, and those that are potentially useful for predictive of survival, recurrence or progression in NMIBC or MIBC.[247]

Duan *et al* describe a three-lncRNA panel consisting of MEG3, SNHG16 and MALAT1 for detecting bladder cancer in serum, and MEG3 as an independent risk factor for RFS in NMIBC.[248]

Bao *et al* used lncRNA expression data for 234 TCGA MIBC cases to identify a prognostic set of four lncRNAs (AC005682.5, CTD-231H16.1, CTB-92J24.2 and RPLL-727F15.13) that were associated with survival.[249]

Terracciano *et al* review urinary lncRNAs as prognostic biomarkers in NMIBC, summarizing 19 lncRNAs from tissue, urine exosomes or urine sediments, then discuss oncogenic and tumour suppressor lncRNAs.[250]

Droop *et al* assessed validating 7 candidate biomarkers (GAS5, H19, link-UBC1, MALAT1, ncRNA, TUG1, UCA1) from 9 publications, using RT-qPCR on total RNA from n=106 tumour tissues, and from an mRNA-Seq–based lncRNA expression for n=252 TCGA MIBC cases.[251] They compared tumour versus tissue normal expression, and univariate and multivariate (stage, grade, lymph node metastasis and a high-low median split of either UCA1, TUG1, ncRNA or MALAT1) association with OS. They assessed differential expression between molecular subtypes in TCGA MIBC n=408 data, and between 3 subclasses reported for NMIBC.[170] We are unaware of lncRNAs being used as predictive biomarkers for response to immunotherapy in bladder cancer.[252,253]

3.8.3 Circular RNAs

3.8.3.1 Background

CircRNAs are covalently closed-loop, single-stranded RNAs that are generated by back-splicing of pre-mRNAs, can be more stable than linear RNAs, and can have complex, tissue- and developmental-stage-specific expression patterns.[254-258] They are understood to participate in regulatory networks as miRNA or RBP sponges (see ceRNAs in the miRNA and lncRNA sections), scaffolds, and transcriptional regulators.[255]

Because they are closed loops, they are best detected not from polyadenylated RNA, but from RNA depleted of either ribosomal RNAs or of both polyadenylated and ribosomal RNAs.[258] Biogenesis, classes, function, databases and detection tools, circRNAs in cancer, and roles as potential cancer biomarkers are reviewed by Bolha *et al*[259] Metge *et al* note four computational detection pipelines (DCC, CIRI, CIRCfinder, KNIFE), then describe a FUCHS pipeline that uses long (>150 bp) reads.[256]

Xia and colleagues list circRNA databases (Circ2Traints, CircBase, CircNet, circRNADb, CircInteractome), then describe a database of cancer-specific circRNAs, generated from 228 total RNA or polyA(-) RNAseq samples.[260] Maass *et al* describe a RiboMinus-based expression resource, generated for 20 clinically relevant human tissues using the[255] pipeline, and note that circRNAs can be differentially expressed in host, disease-associated genes.[254]

Russo *et al* include circRNAs with miRNAs and lncRNAs in a curated miRandola database of extracellular ncRNAs that may be useful as noninvasive biomarkers.[257]

3.8.3.2 Circular RNAs in bladder cancer

At the time of writing we are aware of only one study that used a large cohort. Noting that cirRNA's structural stability, specificity and accessibility make them potentially useful as biomarkers,[261] profiled circRNAs in total RNA from 457 NMIBC patients, identifying circHIPK3 and circDKYL as potential prognostic biomarkers for patients with early-stage NMIBC.

3.9 Cancer Stem Cells in Bladder Urothelial Carcinomas

Accumulating evidence highlights the importance of intratumoural cellular heterogeneity in human bladder urothelial carcinomas, which harbour functionally distinct cancer stem cells (CSCs, or tumour-initiating cells). These CSCs are enriched with tumour-initiating potential when engrafted *in vivo*. A panel of CSC markers has been reported, which includes, but is not limited to CD44, CD44v6, CD49f, CD90, CD133, 67LR, ALDH1, CD14, and KRT14.[262-269] These findings were reported by individual groups in their corresponding characterization of CSCs; therefore, the interrelationship of these markers within a single patient specimen remains undefined. One study utilized a combination of markers (CD90, CD44, and CD49f) to analyze CSCs, and found that such marker combination could isolate subpopulations of cancer cells representing stem, progenitor and differentiated stages of tumour differentiation.[266] Not all tumours contain such phenotypically distinct cell populations, and it is certainly expected that such marker expression does not necessarily tightly follow those during normal urothelial differentiation, likely reflecting the next level of tumour heterogeneity (i.e. inter-patient heterogeneity).[266,270] At least two distinct groups of bladder urothelial carcinomas have been identified: (1) those blocked at the early differentiation stage, and (2) those driven into a more differentiated state.[266,270] Such findings echo molecular subtyping studies by TCGA[92] and other groups[16,48] revealing a Cluster III/IV or basal subtype of tumours highly expressing CSC markers, e.g. CD44, CD49f, KRT14, and a Cluster I/II (or differentiated/luminal subtype) with a relatively lower expression of these markers. Basal tumours with high expression of CSC markers correlated with poor OS in a treatment-naïve setting, implicating their prognostic value.

State-of-the-art single cell exome sequencing of paired CD44(pos) cancer/normal stem cells versus CD44(neg) differentiated cells revealed the following interesting finding.[271] Depending on individual patients, phylogenic analysis of SNP/SNV sites implicated that CSCs could clonally arise from either normal stem cells (2 of 3 patients) or differentiated cells (1 of 3 patients).[271] Further functional analysis revealed that mutations in MLL2 cooperate with ARID1a and GRPC5a mutants, thereby significantly augmenting sphere-forming and tumour-initiating properties.[271] Another study revealed that a C228T mutation of the telomerase reverse transcriptase (TERT) promoter frequently occurred in CSCs but not in differentiated cells.[272] Such findings echo TCGA and other high-throughput analysis studies, as MLL2 (27%), ARID1a (25%), and TERT are amongst the most frequently mutated genes in human bladder urothelial carcinomas,[92,123,269,273,274] independently supporting the functional significance of CSCs in the pathogenesis of bladder cancer development. Molecular regulation of CSCs is emerging at multiple levels, including the transcriptional and miRNA/lncRNA levels. For instance, the transcription factor STAT3 was involved in the regulation of CSC expansion,[275] which corresponded with rapid development of CIS and invasive progression. A recent study connected a mechanistic link of KMT1A-GATA3-STAT3 in regulating self-renewal of bladder CSCs.[276] The histone methyltransferase KMT1A augmented H3K9me3 modification on the gene promoter of GATA3 in bladder CSCs, suppressing transcriptional activity of GATA3.[276] This in turn activates STAT3 activity, since GATA3 is a transcriptional suppressor of STAT3.[276] In additional to cell intrinsic regulation of CSCs, studies using elegant mouse models shed light on the crosstalk between stem cells and stroma during bladder cancer progression. Urothelial basal stem cell expression of SHH was found to

induce stromal-derived factors BMP4 and BMP5, which were paracrine inducers of urothelial differentiation that refrained invasive progression.[277] When SHH was lost during tumour progression, stromal-derived factors refraining invasion was also lost, therefore facilitating tumour invasion.[277]

CSCs have been implicated to be intrinsically less responsive to conventional therapies such as chemotherapy, due to several protective properties that CSCs shares with normal stem cells. These include higher expression of drug-efflux pumps, better DNA-repair capacity, and enhanced protection against reactive oxygen species. CSCs can also respond to chemotherapy-induced cell death and associated injury signals, by activating a wound response to "repopulate" residual tumours.[278] Recurrent repopulation of CSCs in consecutive chemotherapy treatment cycles led to increase in CSC content, and thus nonresponsive tumours.[269]

3.10 Bladder Cancer Metastases

3.10.1 Introduction

At diagnosis, 30% of bladder cancer patients will present with muscle-invasive disease, in which standard of care consists of a cystectomy and pelvic lymph node dissection.[279] Despite this surgery, half of the patients develop metastases and these patients have a 5-year survival rate of only 15%.[280] Preferential bladder cancer metastatic sites as part of a highly selective process between tumour cells and their microenvironment include regional lymph nodes, lungs, and bone.[281] Currently, two basic models of metastasis are accepted. Both state that the metastatic cascade is a multistep process enabling invasive growth, detachment from the primary tumour site, intravasation, migration, extravasation, and colonization of distant organs. The classic *linear progression model* states that metastases are clonally related to the primary tumour, will have comparable molecular characteristics, and that treatment response can be predicted by response of the primary tumour to a given treatment. The second model describes a *parallel progression* of early metastatic cells that acquire a fully metastatic profile at a distant site and thus have obtained different molecular characteristics than the primary tumour.[282] Metastases are the major contributor to bladder cancer mortality. Therefore, a better understanding of the molecular processes underlying metastasis and development of experimental metastatic models is essential in the identification of novel therapeutics to prevent, delay the appearance, or slow the growth of established metastasis. This can ultimately improve patient outcome. Here, we summarize the currently available metastatic models and their translational potential and functional biology of metastasis in bladder cancer.

3.10.2 Models used to study metastatic bladder cancer

Different molecular aspects of metastasis can be studied by using experimental models. An important limitation for development of these models is the lack of available tissue from human metastatic sites. To address this need, human and murine tumour cell lines with metastatic characteristics have been developed for *in vitro* and *in vivo* investigations. Most of these cell lines were derived by repeated *in vivo* passaging of metastatic nodules from parental invasive urothelial tumours and the route of passaging (bladder orthotopic, tail vein injections or intracardiac injections) determines the metastatic pattern. Currently, there are a number of models that exemplify this strategy using human bladder cancer cells in immunocompromised mice. For example, the human cell line 253J B-V, which metastasizes to lungs, was derived from a lymph node metastasis and passaged five times orthotopically before being established.[283] Furthermore, in mice, the murine cell line MB49 was inoculated subcutaneously and passaged multiple consecutive times to create MB49-I, which develops lung metastasis after orthotopic inoculation. A limitation of these models is that the spectrum of murine organ tropism does not typically resemble human organ tropism. Two models that have been used to study the organ preference of metastasis to lung and liver have used the T24T cell line, a poorly metastatic relative of the nonmetastatic T24 line.[284] The lung tropic model T24T/FL1-3 used repeated tail vein inoculations of T24T followed by harvests of the lung metastases to develop progressively more metastatic cell lines (FL1-3).[285] This approach was repeated for the poorly metastatic UMUC3 cell line

to derive lung metastatic models Lul1/2.[286] A similar approach was used to develop a liver "tropic" metastatic cell series T24T/SLT1-3.[287] Both models have been profiled by a variety of whole genome techniques and the genes associated with the metastatic phenotype to lung and liver identified.

There are two types of mouse models that can be used to study metastasis: carcinogen-induced and transgenic. Carcinogen-induced models of bladder cancer can robustly produce primary lesions and have been shown to metastasize.[288] Several carcinogen-induced tumours have been adapted to culture[289,290] and have generated cell lines that are widely metastatic, such as MB49.[291] In transgenic models, tumours arise *de novo* in the native tissue microenvironment as a result of experimentally induced genetic alterations. Multiple transgenic models have been developed, but only few result in a metastatic phenotype.[292-294] The first transgenic bladder cancer model was developed with expression of simian virus 40 (SV40) large T antigen under control of the uroplakin 2 (UPII) promotor (UPII-SV40T mice). The resulting transgenic mice developed CIS and invasive bladder cancer, with some mice having metastases.[295] Another model was developed by expression of SV40T under the cytokeratin 19 (CK19) promoter (CK19-SV40T mice) and resulted in CIS and invasive tumours with lung metastasis in some cases.[296] Conditional knock-down of p53 and phosphatase and tensin homolog (PTEN) resulted in mice developing CIS and invasive tumours, with metastasis in 60% of mice.[297] In summary, unfortunately there are limited bladder cancer cell lines and *in vivo* models available that result in a metastatic phenotype, and further effort should be devoted to optimizing and developing models. An overview of the currently available metastatic models is shown in **Table 3–6**.

TABLE 3–6 Animal Models Used to Study Bladder Cancer Metastasis

	Parental line	Origin	Development	Metastatic site	Reference
Human models					
253J-BV	253J	Lymph-node metastasis	Passaged 5x orthotopically	Lung	Harnden *et al*[5]
T24T	T24	Invasive G3 bladder tumour	Related to T24 cell line	Poorly metastatic to lung	Harnden *et al*[6]
T24T-FL1/2/3	T24T	Lung metastasis from T24T	Passaged multiple times by tail vein injection	Lung	Harnden *et al*[7]
SL1/2/3/4	T24T	Liver metastasis from T24T	Passaged multiple times by intrasplenic injection	Liver	Sun *et al*[9]
Lul1-2	UMUC3	Lung metastasis from UMUC3	Passaged multiple times by tail vein injection	Lung	Wu *et al*[8]
TSU-Pr1(T24)-B1/2	T24	Invasive G3 bladder tumour	Intracardiac inoculation, in vitro passaging, and repeat intracardiac inoculation	Bone	Hicks *et al*[20]

Abbreviations: CIS, carcinoma in situ; CK19, cytokeratin 19; IM, intramuscular; IV, intravenous; s.c., subcutaneous; SV40, simian virus 40; UPII, uroplakin 2.

continued on **page 231**

TABLE 3–6 Animal Models Used to Study Bladder Cancer Metastasis, *Cont'd*

	Parental line	Origin	Development	Metastatic site	Reference
Murine models					
Syngeneic					
BBN Carcinogen treated mice		Normal mouse urothelium		Lymph nodes, liver and lung in approximately 30%	
MB49-I	MB49	Carcinogen induced	Bladder tumour passaged 13x s.c., thereafter orthotopic inoculation	Lung	Günes *et al*[21]
MBT-2	MBT-2	Carcinogen induced	Intravenous or intramuscular injection of MBT-2	IV injection: low frequency of lung tumours, IM: high frequency of lung tumours	Varley *et al*[22]
Transgenic					
UPII-SV40T			SV40 large T antigen expressed under UPII promoter; CIS, and invasive urothelial carcinoma	Lymph nodes and liver in some cases	Seiler *et al*[17]
CK19-SV40T			SV40 large T antigen expressed under CK19 promoter; CIS, and invasive urothelial carcinoma	Lung in some cases	Limas *et al*[18]
p53-/-; Pten -/-			Conditional knock down of p53 and PTEN using Adeno-Cre; CIS, and invasive urothelial carcinoma	60%: lymph nodes, spleen, liver, and diaphragm	Marceau *et al*[19]

Abbreviations: CIS, carcinoma in situ; CK19, cytokeratin 19; IM, intramuscular; IV, intravenous; s.c., subcutaneous; SV40, simian virus 40; UPII, uroplakin 2.

3.10.3 Functional biology of metastasis

The biology and genetics of metastasis have been studied extensively in cancer.[298,299] Fewer studies have functionally examined the roles of specific genes in bladder cancer metastasis. Below we will provide some examples of these, which is by no means an exhaustive list.

Using a modified version of the mRNA differential display technique, five human bladder cancer cell lines from low-grade to metastatic were analyzed to identify differences in gene expression. A gene called missing in metastasis (MIM) was identified.[300] MIM was not expressed in the metastatic bladder cancer cell line TccSuP, the metastatic breast cancer cell line (SKBR3), or in metastatic prostatic cancer cell lines (LNCaP, PC3). Rho GDP-dissociation inhibitor 2 (RhoGDI2), which is a protein that in humans is encoded by the ARHGDIB gene, was identified as a suppressor of metastasis in

experimental bladder cancer models using the T24-T24T model.[301] Following research demonstrated that RhoGDI2 mRNA expression levels were inversely related to an invasive and metastatic phenotype in bladder cancer cell lines and, in patients, reduced RhoGDI2 protein expression was associated with poor clinical outcome.[302] RhoGDI2 downregulates expression of chemoattractant versican (VCAN) and chemokine (C-C motif) ligand 2 (CCL2). VCAN attracts macrophages to metastatic sites like the lung, promoting colonization and formation of tumour metastases. Correspondingly, high VCAN levels portended poor prognosis in patients with bladder cancer. Macrophage-derived osteopontin, which binds to receptor CD44 on bladder cancer tumour cells and hereby promoting tumour cell invasion, was identified as the key factor in the RhoGDI-axis.[303] RhoGDI2 loss was also shown to lead to increased endothelin 1 expression, which in turn drives metastasis.[291] This offers a potential therapeutic avenue in patients with low levels of RhoGDI2 expression, as there are clinically available endothelin receptor antagonists.[291] In an *in vivo* model knockdown of CD44, strongly suppressed lung metastases and overexpression of wild-type CD44 increased metastases levels.[303] As for the endothelin axis above, targeting the osteopontin-CDD44 pathway could be used to target development of metastases. In both cases, this work demonstrates that loss of tumour-suppressor genes drives metastasis and the usefulness of models discussed above in discovering them.

Activating transcription factor 3 (ATF3) was identified as a metastasis suppressor. Gene expression data showed that decreased ATF3 was associated with bladder cancer progression and poor outcome in patients. *In vitro* and *in vivo* studies demonstrated that ATF3 overexpression in metastatic bladder cancer cells decreased cell migration. Moreover, it was found that the ATF3 regulated cell migration through regulation of expression of the actin-binding protein gelsolin and gelsolin-mediated actin remodelling.[304] Overexpression of EGFR has been strongly associated with invasive bladder cancer.[305,306] Investigation of EGFR downstream pathways revealed a role of Ral GTPases in bladder cancer progression and metastasis *in vitro* in human T24 bladder cancer cells. Interruption of the Ral pathway resulted in reduced cellular motility.[307] Moreover, overexpression of Ral mRNA was associated with high tumour stage and high RalA and RalB protein expression was mainly found in invasive tumours, which correlated to poor patient outcome.[308] In a follow-up study, multiple drugs targeting RalA and RalB were developed that were able to inhibit anchorage-independent cell growth *in vitro* and human xenograft tumour growth.[309] These small molecular weight Ral inhibitors are promising research tools for the development of new targeting agents and are currently under investigation.[309]

CD24, a glycosyl phosphatidylinositol-linked sialoglycoprotein, was identified as a downstream target of the Ral signalling pathway and associated with metastasis in bladder cancer. Furthermore, CD24 protein expression was increased in metastatic tumours of bladder cancer patients and in an *in vivo* mouse model CD24 was found necessary for development of lung metastases.[286,310] Treatment of these lung metastases with anti-CD24 antibodies resulted in reduced tumour growth and prolonged survival, suggesting a role for CD24 as a therapeutic target.[310] Interestingly, it was found that the prognostic value of CD24 overexpression was limited to male mice and male patients. CD24 deficient mice had fewer metastases than wild-type mice, and only in male patients high CD24 expression was associated with a poor clinical outcome.[311] *In vivo a*ndrogen deprivation resulted in decreased tumour growth and CD24 expression, which could be rescued by exogenous CD24 overexpression.[311] These findings indicate that CD24 is androgen regulated and, if results are confirmed, antiandrogens could have a role in bladder cancer therapy in male patients.

Recently, it was shown that expression of E-cadherin, CD24, PD-L1, and vascular endothelial growth factor receptor-2 (VEGFR2) drive tumour formation at distant sites.[312] Fibroblast growth factor receptor (FGFR) signalling was also shown to be involved in EMT, which is required for epithelial cells to invade surrounding tissues and metastasize. Activated FGFR1 induced EMT in an *in vitro* bladder cancer model, and selective inhibition of FGFRs blocked the production of circulating tumour cells and formation of lymph node and distant metastases in a mouse model.[313,314]

3.10.4 Conclusion

Metastatic disease is still a major cause of morbidity and mortality in bladder cancer patients. Development of metastasis is a complex multistep process and, over the years, multiple *in vitro* and *in vivo* preclinical models have been developed to unravel different parts of the metastasis cascade, with the aim to identify novel therapeutic targets. Research progress is hindered by the limited availability of human metastatic tumour tissue. Despite these limitations, current models have increased our knowledge on the biology of metastatic bladder cancer. Improving currently available models, development of new models, and use of high-throughput genomics will increase knowledge of the process of metastasis and will allow for the discovery of diagnostic biomarkers and novel therapeutic targets.

3.11 Preclinical Human Models of Bladder Cancer

3.11.1 Introduction

The development of methods to propagate human tumours and tumour cells *ex vivo* resulted in a dramatic acceleration of progress in cancer research. Beginning with the isolation of HeLa cells from Henrietta Lacks' cervical carcinoma,[315] large collections of human cell lines have been established for nearly every malignancy, and whole genome approaches have recently been employed to deeply characterize their transcriptional and genomic properties, culminating in the massive Cancer Cell Line Encyclopedia project.[316] With respect to bladder cancer, over 40 different cell lines with associated gene expression, copy number, and mutation data are now publicly available,[317] and many of these lines have been used to generate subcutaneous and orthotopic human tumour xenografts in immunodeficient mice.[318] More recently, the potential advantages of directly implanting freshly harvested human tumours into immunodeficient mice have been recognized,[319] and successful efforts to generate these so-called patient-derived xenograft (PDX) models of bladder cancer have yielded attractive models for preclinical study.[320-322] Finally, some of the same motivations that drove the generation of PDX models have led to the development of methods to maintain and expand freshly isolated human tumour cells as "organoids,"[323] thereby maintaining primary tumour cellularity and architecture while completely avoiding the need to use mice as hosts at all. Here we will introduce these major types of human preclinical models, discuss the results of efforts to compare their properties to primary human tumours, and present some of the strengths and weakness of these models as compared with the carcinogen-induced and genetically engineered mouse models that are described in another section of this chapter.

3.11.2 Human cell lines

Over the last five decades, various investigators have successfully established cell lines from over 40 human urothelial cancers.[317,318,324] (The effort of a single team resulted in the isolation of 16 independent clones.[318]) The first large panel of human bladder cancer cell lines (the BLA40) was collected by Theodorescu's group, who used it to generate drug sensitivity and whole transcriptome gene expression profiling data that informed the CoXEN classifier for predicting chemosensitivity.[324] More recently, other groups have used whole transcriptome and whole genome copy number and mutation data to compare the cell lines to primary human tumours,[47,317,325] and they concluded that the cell lines can be grouped into the "basal" and "luminal" molecular subtypes and that they contain many of the major genomic alterations that are present in primary tumours.[317] Furthermore, human bladder cancer cell lines retain subtype-related differences in their sensitivities to targeted agents. For example, the subset of luminal cell lines that contain activating *FGFR3* mutations and fusions are selectively sensitive to FGFR inhibitors,[314,326,327] whereas sensitivity to EGFR inhibitors appears to be enriched in basal cell lines (discussed later in this chapter).[47] The preservation of the intertumoural molecular and biological heterogeneity observed in primary human tumours coupled with portability and ease of banking are probably the major strengths of these large panels of human cell lines.

Unfortunately, efforts to derive cell lines from NMIBCs have been largely unsuccessful; only RT4[328] and MGH-U3[329] are in widespread preclinical use. Another major concern with established cell lines is that they have become adapted to autonomous growth in two-dimensional culture. Although direct visualization of the transcriptional and genomic "drift" that has presumably occurred in them is generally not possible (because the parent tumours are no longer available), indirect comparisons do suggest that the cell lines display more genomic complexity and are less well-differentiated[317] than primary human tumours: many of the "luminal" cell lines express very high levels of "basal" biomarkers, and most of the "basal" cell lines express gene expression programs consistent with complete EMT (McConkey and Dinney laboratory, unpublished observations). Although it has been suggested that repeated passaging through the bladder might restore some of the original biological properties of the parent tumours,[283,330] direct genomic support for this conclusion is still, for the most part, lacking. Finally, studies with human bladder cancer cell lines were among the first to highlight the problem of cell line cross-contamination. For example, two independent groups discovered that the so-called "MGH-U1/EJ" and "MGH-U2" cell lines were actually T24 cells,[329,331] and "UM-UC2" was also subsequently also discovered to be T24.[332] Even more problematic was the discovery that the KU-7 cell line, which was originally fingerprinted and thought to be authenticated,[332] was later discovered to be HeLa.[333] Therefore, investigators working with conventional cell lines must perform regular DNA fingerprinting to ensure that cell lines do not become cross-contaminated. Statements verifying that this procedure will be followed are now mandatory in US NCI research grants.

3.11.3 Conventional xenografts

Interactions between tumour cells and their complex microenvironments have strong effects on tumour biology and hence response to therapies.[334] While it is possible that many of these interactions can be modelled *ex vivo*, assumptions must be made about the cell types involved. Established human cell lines can generally be propagated in immunodeficient mice, although "take rates" can vary markedly by cell line and by mouse strain, with increased take rates correlating with increased immunodeficiency. For example, a greater number of cell lines (and PDX models) can be maintained in non-obese diabetic/severe combined immunodeficiency mice that have been engineered to lack the interleukin (IL)-2 receptor/common cytokine receptor gamma chain (called NOD scid gamma mice, or "NSG mice") than can be grown in conventional nude mice. These differences may be due to the absence and presence of natural killer (NK) cells, respectively.[335]

A significant body of evidence suggests that implanting human bladder cancer cells in the correct organ microenvironment, as opposed to subcutaneous implantations, produces tumours that recapitulate important characteristics of primary human tumours.[336] Orthotopic implantation appears to be particularly important for studies focused on angiogenesis and/or metastasis.[336] Repeated passaging through the orthotopic site (termed "orthotopic recycling") has been reported to dramatically increase tumour metastatic potential,[283,330] linked to partial reversal of EMT ("MET").[330] The effects of orthotopic recycling require continued maintenance *in vivo* as enhanced metastatic potential (and associated MET) is lost after approximately 2 to 3 weeks in tissue culture.[330]

Although conventional xenografts appear to possess important biological advantages as compared to cell lines maintained in 2D tissue culture, they also carry with them some of the same important limitations. Subclonal heterogeneity is a now a well-established property of primary human bladder cancers,[73,89] and it seems likely that established human cell lines and the xenografts derived from them could lose a majority of these subclones as a consequence of continuous *in vitro* passage; single-cell sequencing studies are required to directly test this hypothesis. Conversely, it also seems likely that tissue culture introduces some new (and biologically significant) genetic alterations, and no *in vivo* orthotopic maintenance strategy will be able to reverse changes that are caused by irreversible changes to DNA. Potentially related to this is the fact that the value of xenograft studies to predict therapeutic clinical activity in cancer patients has been justifiably questioned—there are many examples of drugs that produced strong activity in xenografts but no activity in patients.[337] Finally, the histopathological appearance of the tumour-associated stroma appears to be less complex in conventional xenografts than in primary human tumours or the other preclinical *in vivo* models,[338] which probably also reduces their value as tools to predict therapeutic efficacy, since stromal cells play important roles in mediating sensitivity and resistance.[334] Given the importance of ICB in the clinical management of patients with bladder cancer, the lack of immune cells in these models may be the most significant stroma-associated problem. Investigators have had some success generating "humanized" adaptive immune systems in immunodeficient mice, and proof-of-principle studies have been performed with conventional xenografts that demonstrate T-cell–mediated tumour recognition (see below).[339] However, because the T cells in these models are not perfectly human leukocyte antigen (HLA)-matched, it seems likely that the T cells in these models are not killing tumours via recognition of tumour neoantigens.

3.11.4 Patient-derived xenografts

The first bladder cancer PDX models were developed in the 1980s, but their popularity is increasing with the recognition of the important limitations of conventional xenograft models combined with the potential to use them as patient-specific tools in precision medicine.[319] PDX models are established by implanting small pieces of freshly isolated human tumours directly into immunodeficient mice. As is true for conventional xenografts, take rates are higher in more severely immunodeficient mice, and implantation into the highly vascularized renal capsule may be more efficient than implantation subcutaneously.[321] (Orthotopic implantation of tumour chunks into the thin murine bladder wall is generally not feasible.) Early passage PDX models retain the histopathological and genetic properties of the primary tumours they were derived from, although the human stromal cells that were present are replaced with murine cells.[319-322] The availability of associated whole transcriptome and DNA sequencing and copy number data makes these models highly valuable as tools for preclinical tumour biology and drug development studies. Importantly, several of these models have been deposited at Jackson Laboratories, where they are publicly available to academic investigators and safer from loss and inventory exhaustion.

Some characterizations of the sensitivities of PDX models to conventional and investigational therapeutics have been performed. In one study, mice bearing six different PDX models were treated with cisplatin, gemcitabine, or both, revealing heterogeneous patterns of sensitivity to each of the single agents and the combination.[320] The authors also observed heterogeneous effects of targeted agents (lapatinib for ERBB2/3, BGJ398 for FGFRs, and BEZ235 for PIK3CA) that were not always coupled to target expression or mutation status.[320] Chan's group also studied the effects of combination chemotherapy with gas chromatography in PDX models and concluded that the patterns of response and development of acquired resistance were very similar to what is observed in patients.[269] Another group developed five different PDX models,[321] one of which contained an activating *FGFR3* S249C mutation that has been linked to FGFR3 dependency in human cell lines.[314,326] These tumours were sensitive to a blocking anti-FGFR3 antibody,[321] suggesting that they could prove valuable for studying the molecular determinants of FGFR3 dependency and potentially the development of acquired resistance.

In an effort to credential PDX models as appropriate preclinical tools to study the molecular mechanisms associated with sensitivity and resistance to ICB, the team at Jackson Laboratories transplanted NSG mice with human fetal liver-derived CD34+ hematopoietic progenitor/stem cells and used them as hosts for conventional and PDX.[339] They then compared the effects of the blocking antihuman PD1 antibody, pembrolizumab, on tumour growth in NSG mice that either were or were not reconstituted with human immune cells.[339] The results clearly demonstrated that the effects of pembrolizumab required $CD8^+$ human T cells and varied depending on the hematopoietic stem/progenitor cell donor. Although the molecular basis for T-cell–mediated tumour recognition was not identified, the authors speculated that alloreactivity was involved, which would not be involved in the responses to ICB observed in bladder cancer patients (although it would be relevant within the context of allogeneic bone marrow transplantation). Whether or not more sophisticated models could be developed by implanting a given patient's own tumour and hematopoietic stem cells into an NSG mouse remains to be seen.

3.11.5 Organoids

As described in the first section of this chapter, *ex vivo* cultures of primary urothelial cells have been used very effectively to study the transcriptional control of human urothelial differentiation, and they have used similar organoid techniques to study the effects of the stromal microenvironment on the behaviours of human cancer cell lines.[340] More recently, high profile studies from the Clevers[323] and Tuveson[341] laboratories have stimulated a new wave of strong interest in using them as potentially superior replacements for the other preclinical human cancer models. Organoids share many strengths with PDX models (i.e. lack of *in vitro* growth selection, presence of a complex stroma, genomic fidelity) but also have the added benefits of lower cost and higher fidelity of tumour-stromal interactions (which in PDX models must occur across species). Organoids can be expanded and frozen, which means that, in principle, they can be shared, although organoid sharing is still rare in practice. Their major disadvantage is that they are not in constant communication with an intact host microenvironment, so cells cannot traffic into and out of them, which is important to the generation of a normal immune response.

Several papers have documented the use of organoids derived from rodent bladder cancers,[29,342,343] and two groups recently reported on the successful generation of organoids from primary bladder cancers.[344,345] Significantly, in one of the studies most of the organoids were established from NMIBCs,[345] providing unique preclinical access to this understudied subset of tumours. The group also performed deep-panel (MSK-IMPACT) and whole exome sequencing on the primary tumours and various passages of the organoids, which revealed stability in truncal mutations with evolutionary gain or loss of subclones. They also performed immunofluorescence studies and RNA-Seq to assign the organoids to basal versus luminal subtype. Interestingly, two-thirds of the tumours underwent luminal-to-basal subtype switches when they were placed in organoid culture, but they reverted to the luminal subtype when the organoids were implanted orthotopically in immunodeficient mice.[345] Although they did not identify the mechanism(s) underlying this subtype switching, they speculated that it might have been caused by the lack of tumour-stromal interactions in the organoid cultures.

3.11.6 Summary

Bladder cancer researchers benefit from the availability of a relatively large number of human preclinical models. The new organoid models are particularly exciting because they provide the opportunity to perform preclinical mechanistic studies in models of NMIBC. These models possess different strengths and weaknesses. Because they are all established from frank cancers, they are probably not the best tools to study early carcinogenesis, and there are also limitations in using them to study the effects of immunotherapy. However, their human origins make them uniquely suitable for the study of human cancer heterogeneity, and ongoing optimization is making them even better models of human disease.

3.12 Mouse Models of Bladder Cancer

3.12.1 Introduction

Animal models are indispensable tools for cancer research. Across many types of human cancer, they have advanced the understanding of carcinogenesis and disease pathology, in addition to serving as a testing ground for novel therapeutic strategies. For cancers that may be categorized into distinct subtypes based on molecular profile and morphology, such as those occurring in the breast and bladder, a current challenge in basic research is the development of animal models for specific types of lesions observed in humans.

The task of developing mouse models for the unique subtypes of bladder cancer is dependent on advancements in the field mouse transgene technology. Namely, the ability to implement temporally controlled, site-specific genetic changes in bladder cells will be crucial for manipulation of signalling pathways that are involved in bladder carcinogenesis.[16,17,92,104,123,169,346]

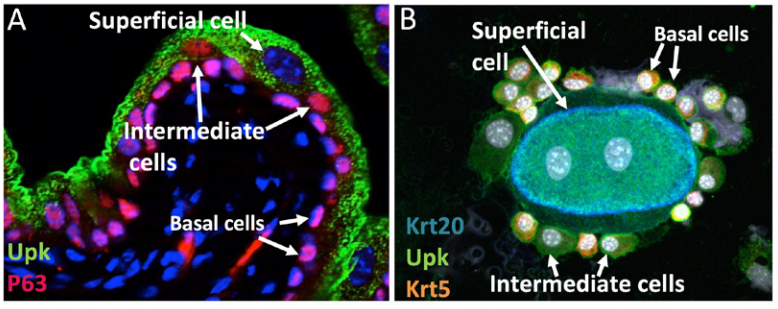
3.12.2 Urothelial cells give rise to bladder cancers

Bladder cancers can be subdivided into several classes with different clinical behaviours, morphologies, and mutational profiles. These lesions are derived from the urothelium, a specialized epithelial barrier that prevents exchange of substances between the urinary outflow tract and the blood. The adult urothelium is nearly quiescent but can quickly regenerate after acute injury from urinary tract infection or exposure to toxins, indicating that progenitors are present in adults that are capable of self-renewal and repair after acute injury.[347] The urothelium contains layers of basal cells, intermediate cells, and a luminal layer lined with superficial cells that are critical for producing and maintaining the urothelial barrier. At present, most urothelial markers are members of the UPK or keratin families and are not selective for a single urothelial subpopulation. To circumvent this problem, we have developed sets of markers that can be used to distinguish one population from another **(Figure 3–9)**.[348,349]

FIGURE 3–9
Urothelial Cell Types and Combinatorial Markers

A Section through an adult urothelium stained with UPK and P63.

B Wash from an adult urothelium stained with Krt20, Upk, and Krt5. The Table shows combinatorial markers used to distinguish cell types.



	Krt20	Upk	Krt5	Krt14	P63	Shh	DNA
Superficial							4n+4n
Intermediate							2n
Krt14-Basal							2n
Krt5-Basal							2n

Lineage analyses in mouse models of carcinogenesis suggest that basal cells are cells of origin of bladder cancer.[277,350,351] Basal cells account for about 80% of the urothelium. There are two known populations of basal cells in mice: K14-basal cells that make up about 20% of the basal population, and K5-basal cells that make up the rest of the basal population. K14-basal cells express P63, K14-, and K5, and are located exclusively in the basal layer **(Figure 3–9).** K5-basal cells, which express P63 and K5 but not K14, populate both the basal and suprabasal layers of the urothelium **(Figure 3–9).** Intermediate cells also produce tumours in mice.[351,352] They express P63 as well as UPKs, but not Krt20 or basal cell keratins **(Figure 3–9).** Superficial cells line the luminal layer of the urothelium. They are lined with an apical plaque, composed of UPK crystals, that is critical for function of the urothelial barrier. Superficial cells express Krt20 and Upk, but not P63 or basal cell keratins. Superficial cells, which are binucleated and have a DNA content of 8*n*, have traditionally been considered to be postmitotic, and hence not likely to form tumours. However, they are polyploid and thus have the ability to evade checkpoints that would normally induce apoptosis, features that may render them susceptible to transformation.[353]

While the human and mouse urothelium share many similarities, based on studies in other organs,[354] there also likely to be significant differences that could be important in studies of urothelial carcinoma. Several groups, including the GenitoUrinary Development Molecular Anatomy Project (www.gudmap.org) are currently identifying cell types and their expression profiles in the human and mouse urothelium using bulk and single-cell RNAseq. Clarifying the differences and similarities between the populations in mouse and human will be critical for developing new mouse models for bladder cancer studies.

3.12.3 Fate mapping and inducible Cre drivers

Fate mapping is a tool that is useful in evaluating cellular potential and behaviour. The approach depends on labelling cells with an indelible marker that will continue to be expressed in that cell and its daughters, independent of the differentiation state; it is a powerful tool that has been used in embryology for decades.[355-357] Cre-Lox recombination in the mouse is a common fate mapping technique used to identify progenitor populations and tumour cells of origin. In this model, mouse lines expressing a tamoxifen-inducible form of the Cre recombinase in a given cell type are bred with a second line harbouring a STOP sequence fused to a reporter (*Gfp*, Luciferase, or *LacZ* are common reporters). In cells expressing Cre recombinase, the STOP sequence is excised and the reporter will be expressed in the parent cell and its daughters. A critical factor in fate mapping is that marker expression is maintained regardless of the fate of the cell, and hence does not depend on transcription. This technique can also be used in combination with carcinogenic or genetic models to follow the fate of distinct cell populations during bladder cancer progression.

There are a number of Cre lines and reporters that can be used to target urothelial populations. Reporters that are useful in lineage analysis are commonly inserted in the *Rosa26* locus, which enables widespread expression during development and in the adult,[358] which are used in combination with Cre lines to target different urothelial populations. *K5CreERT2* and *K14CreERT2* drive tamoxifen-inducible recombination in K5-basal cells and K14-basal cells, respectively. The *Krt18CreERT*, *Upk3aGCE*, and *Upk2Cre* lines target both intermediate and superficial cells, and the *Krt20CreERT2* line selectively labels superficial cells. Technical issues with lineage studies include lack of specificity of promoter-driven Cre lines and resolution of reporter distribution in the urothelium. Since *LacZ* and fluorescent reporters are generally cytoplasmic or membrane-bound, analysis at the single-cell level can be challenging. Mouse lines harbouring nuclear reporters such as the *Rosa26;nTnG* line [(B6;129S6-Gt(ROSA)26Sortm1(CAG-tdTomato*,-EGFP*)Ees/) may help improve resolution in fate-mapping studies.

Cre-Lox recombination is also widely used to generate mouse models of bladder cancer for studies of tumourigenesis and metastasis. In this case, animal models can be constructed that express gain-of-function mutations, loss-of-function mutations, or copy number alterations to determine whether these genetic alterations produce bladder cancer (reviewed in: John and Said[359] and Indra *et al*[360]). There are a number of caveats of genetically-engineered mouse (GEM) models, including differences between the cell populations and biology in the mouse and human urothelium. In addition, Cre lines that drive recombination in the bladder almost always induce recombination in other tissues that can make it difficult to determine whether urothelial tumours are primary or secondary. For example, the K5CreERT2 line drives tamoxifen-inducible recombination in basal cells in the urothelium, as well as basal cells in the skin and numerous other epithelia.[361] Hence, expression of genes that induce bladder cancer using Krt5CreERT2 may produce tumours in the urothelium, as well as in in the skin and other tissues. These issues can be circumvented by directly introducing tamoxifen into the bladder to limit Cre-mediated recombination to the urothelium, instead of systemic tamoxifen treatment via injection or gavage.[297,362]

3.12.4 N-butyl-n-4-hydroxybutyl nitrosamine–induced carcinogenesis

BBN-induced bladder cancer has been an accepted model in rodents since the 1960s. BBN is a nitrosamine present in tobacco smoke that is metabolized to a number of carcinogenic derivatives in the liver and bladder. Once active, BBN metabolites bind covalently to DNA forming adducts that can induce DNA breaks by interfering with DNA replication and repair, leading to mutations. BBN is much more active in males compared to females for reasons that are not clear, inducing tumours that are similar to those in bladder cancer patients.[363] Based on gene expression analysis comparing BBN-induced tumours from mice rats with tumours from patients, tumours in BBN-treated animals are most similar to the basal-subtype of muscle invasive lesions in humans.[47,288,364] Our studies (see below) suggest that different lesions can be produced after BBN exposure, depending on cell types of origin and the mutational load.

3.12.5 Lineage studies using the n-butyl-n-4-hydroxybutyl nitrosamine model

Lineage studies using the n-butyl-n-4-hydroxybutyl nitrosamine model suggest that the genetic landscape and cell of origin are both important factors that determine the fate of tumour forming cells. There is considerable disagreement regarding which progenitor populations are important in urothelial homeostasis, which populations regenerate the bladder after injury, and which populations are cells of origin of bladder cancer subclasses. Reasons for these disparities most likely stem at least in part from differences in lineage and injury models employed by different groups. Fate mapping in wild type animals that uses the BBN mouse model of carcinogenesis and the *ShhCreERT2* or *Krt14CreERT2* lines to drive expression of reporters, identify basal cells as urothelial progenitors during regeneration that also produce bladder cancers.[277,350] However, mutations may alter the intrinsic behaviour of urothelial progenitors. In our studies, we used the *Upk2CreERT2* and the *Krt5CreERT2* lines to evaluate the potential of intermediate and basal cells, respectively, in the BBN model of carcinogenesis.[349,351] BBN was administered to *Upk2CreERT2* and *Krt5CreERT2* on a wild type background, or in animals heterozygous for *Trp53*, a tumour suppressor commonly mutated in bladder cancer.[104,123,365] We observed intermediate cell daughters populating lesions with papillary morphology, and basal cells contributing to CIS, MIBCs, and squamous lesions, suggesting that: (1) basal cells and intermediate cells can both produce tumours and (2) tumours originating from intermediate cells and basal cells may display differences in morphology and clinical behaviour.

We also observed differences in the types of lesions produced by basal cells in wild type mice compared to mice that were heterozygous for P53. Basal cells in BBN-treated wild type *Krt5CreERT* mice tended to produce SCC-like lesions, while basal cells in *Krt5CreERT;Trp53+/-* mice, tended to form CIS and MIBC. This plasticity in the basal population is likely to be a feature that contributes to tumour formation. It will be interesting to identify genetic and epigenetic pathways that drive alterations in the basal population that occur in response to mutations or carcinogen exposure, and to evaluate the differences in basal cell–derived tumours that arise in the bladder, skin, head and neck, and other tissues.

3.12.6 Genetic mouse models used to study tumourigenesis

Genomic and transcriptional analysis of MIBCs reveals that invasive tumours can be subclassified in terms of mutational load and marker expression into a number of subtypes, including the basal and luminal subtypes. A collection of mouse models has been generated, some of which produce tumours that are morphologically similar to those in human bladder cancer; however, in many instances mutations that are present in a large number of bladder cancers fail to produce tumours in mice. Somatic mutations in *Fgfr3* are common in bladder cancer, and several transgenic lines have been generated that express mutated forms of the Fgfr3 protein. However, expression of this mutation alone or even in combination with other mutations in *Kra*s and *Ctnnb1* produced tumours in skin and other tissues, but failed to produce bladder cancers.[366] Expression of the Cre-recombinase in these studies is driven by *Upk2* regulatory sequences, which drives selective and efficient recombination in intermediate and superficial cells from early stages of development through adulthood, but labels few, if any, basal cells.[348,351] It will be interesting to evaluate whether expression of the *Fgfr3* mutations in basal cells will produce urothelial carcinoma. Given the plasticity of basal cells observed in regeneration and bladder cancer, it would not be surprising if basal cells could generate lesions with both papillary and basal morphologies, depending on the types of mutations that are present.

3.12.7 Considering the temporal sequence of mutational events when generating mouse models

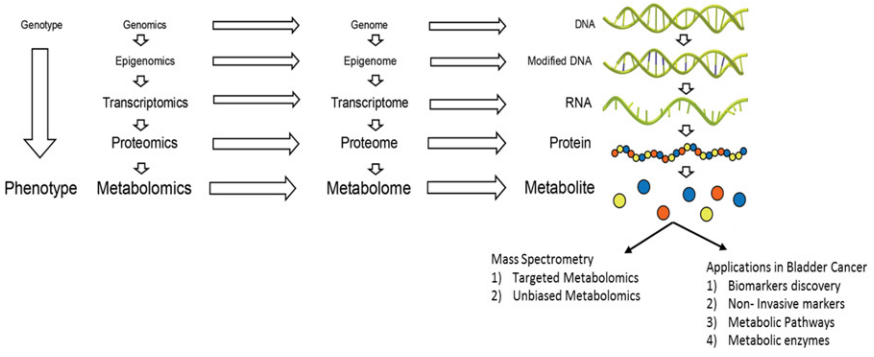
Recent studies have identified a large number of mutations that present in bladder cancers; however, the temporal sequence of mutations and their effects at different stages of tumourigenesis are not yet clear. Cells that form tumours acquire mutations that enable them to proliferate in the urothelium (which healthy urothelial cells rarely do), move through the basement membrane, establish colonies outside the urothelium, invade stroma, and migrate to muscle and to other tissues. Most mutational data at present are from analyses of MIBCs that have reached the muscle or metastasized. Whether these mutations exert different effects in cancer cell progenitors at different stages of cancer progression is an interesting question. Most signalling pathways are reused during development and in adult tissues, in some cases performing different functions at different stages in different cell types in the same organ. Given this kind of complexity, some classes of mutations are likely to exert different effects in cancer cells of origin, depending on the stage of tumour progression and the microenvironment. While studies in rodents have limitations, the ability to temporally follow tumour progression at the single-cell level is an advantage that is currently not available in other *in vivo* systems. The usefulness of this experimental tool, however, depends in large part on validation. Comparison of human bladder cancers and tumours produced in mice at the molecular level will be critical for identifying appropriate models for studying the etiology of bladder cancer.

3.13 Current Methods in Cancer Metabolomics

3.13.1 Metabolomics, metabolome, metabolite

Metabolites are endogenous or exogenous small low-molecular weight downstream intermediate or end products of genes and proteins in a living organism. The composition of all metabolites generated by a system in a living organism (e.g. cell, organ, tissue) constitute a metabolome. Metabolomics is the identification and quantification of all (nontargeted metabolomics profiling) or specified (targeted metabolomics profiling) metabolites in a biological sample (e.g. blood, urine) under a specified condition or disease, as well as identification of metabolic pathways and genes associated with the measured metabolites **(Figure 3–10).**

FIGURE 3–10
Metabolomics in Bladder Cancer



3.13.2 Major analytical techniques in metabolite detection and quantitation

Metabolomics utilizes analytical chemistry techniques and advanced computational methods to characterize complex biochemical mixtures. The diversity of the applications of metabolomics arises from the fact that it can be used to analyze a wide range of biological complexes, including solids (tissues), liquids (bio fluids), and gases (breath). Furthermore, metabolomics can be performed *in vivo* (using imaging or live cells), as well as *in vitro* (using extracts or bio fluids). Over the past 10 years, in bladder cancer research nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are commonly employed for metabolomics applications. Both techniques have their advantages and disadvantages and deliver broad coverage of many classes of biomolecules, including lipids, amino acids, sugars, biogenic amines, and organic acids. NMR is known for the analyses of multi-component mixtures, as sample preparation is rapid and nondestructive and provides highly reproducible results. NMR can detect 50 to 200 metabolites, depending on the type of sample used, and are limited to concentrations of ~1 μM. Peaks in the NMR spectra can be reliably assigned to specific metabolic species based on their chemical shifts and multiplet patterns, and thus NMR provides a wealth of information on the identity and quantity of a large number of metabolites in parallel from a single experiment.[367,368] On the other hand, the detection limit of MS (pM) is much

lower than that of NMR (μM), which makes it an important method for measuring metabolites in complex bio fluids, allowing the analysis of low-abundance metabolites. This may be the main reason why the major share of bladder cancer metabolomics studies has been based on liquid chromatography-MS. But MS analysis requires upfront separation of metabolites using chromatography, so the combination with separation techniques such as gas chromatography and liquid chromatography have been used in bladder cancer investigations.[369] Common types of mass spectrometers used in bladder cancer metabolomics include time-of-flight, quadrupole time-of-flight, quadrupole, and orbitrap to gain better separation or more structural information of metabolites.

Data obtained from MS and NMR experiments are generally complex, since they contain qualitative and quantitative information of several hundred metabolites. Multivariate statistical analyses are used for data reduction and, in particular, for differentiating cancer samples. A variety of statistical methodologies exist and many are now easily accessible via commercial or free online software like MetaboAnalyst. These methods provide extremely helpful tools for filtering the large amounts of data and for accessing the often subtle biochemical information.[369,370] In addition, these approaches are used to extract single or sets of biomarkers with the best properties for the assessment of disease status. Validation of such putative biomarkers is of great importance, as it is the biological understanding of the disease that can provide additional validation in the application of metabolomics.

3.13.3 Application of metabolomics in bladder cancer

Bladder cancer has profound metabolic alterations, which play a central role in tumour progression. Metabolomics helps us to understand relevant alterations in the impaired metabolic processes in bladder cancer through the identification of tumour-specific metabolic biomarkers with potential diagnostic, prognostic, or predictive value **(Figure 3–10).**

Tissue metabolite analysis identified differential metabolites between bladder cancer and benign bladder tissues. Among the perturbed metabolites were elevated levels of aromatic amino acids, namely tryptophan, phenylalanine, and histidine, aliphatic amino acids, serine, asparagine, and valine. There were also elevated levels of hydroxylated metabolites, 3-hydroxykyneurenine, 4-hydroxy phenyl lactic acid, and 5-hydroxy indoleacetic acid. Furthermore, levels of S-adenosyl methionine, which is a major methyl group donor for biological reactions, were also elevated in bladder cancer tissues. Apart from these metabolites, bladder cancer tissues also had higher levels of aniline, a xenobiotic compound known to be involved in bladder carcinogenesis, while levels of palmitic, lauric, and oleic acids were decreased in bladder cancer compared to adjacent benign tissues. Bladder cancer tissues showed multiple metabolic pathways and processes; namely, arginine and proline metabolism, tryptophan metabolism, lysine degradation, alanine metabolism, and glycerophospholipid metabolism were highly altered. A metabolite-derived gene expression study identified a signature that included xenobiotic enzymes of phase 1/2 metabolism. Cytochrome P450 1A1 (CYP1A1) and cytochrome P450 1B1 (CYP1B1), cytochrome P450 2E1 (CYP2E1), and glutathione S-transferase T1 were significantly reduced, whereas aromatic hydrocarbon receptor and cathechol-O-methyltransferase were higher in bladder cancer. Methylation plays a crucial role in regulating the expression of phase 1/2 metabolic enzymes in bladder cancer.[371] Metabolomic pathway enrichment analysis shows the aldehyde dehydrogenase family has been reported to be associated with shorter survival in bladder cancer. Importantly, ALDH7A1, ALDH2, and ALDH1B1 are present in the top enriched pathways. High

expression of guanidinoacetate N-methyltransferase, which is present in the arginine and proline metabolism pathway, has been associated with better prognosis of bladder cancer.[372] Expression of tryptophan 2,3-dioxygenase has been reported as a potential target in immunotherapy of multiple cancers. Bladder cancer tissues showed an increase in the levels glycerophospholipids, particularly phosphatidyl choline and phosphatidyl ethanolamine. Further analysis of metabolomics-derived gene enrichment studies characterized the six-gene signature and disclosed significant deregulation of glycerophospholipid metabolism and for glycerophospholipid biosynthesis. They were driven by genes such as phospholipase A2, group IVA (PLA2G4A) and glycerol-3-phosphate dehydrogenase 1 (GPD1).

Cigarette smoking is the most important risk factor for the development of bladder cancer, and the duration of smoking has been shown to greatly affect the grade and stage of the bladder cancer. At least 60 tobacco smoke compounds, belonging to different classes of chemicals compounds, are known to induce cancer in either laboratory animals or humans. Among these, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and benzo(a)pyrene are known to induce DNA adducts and mutations, thereby promoting tumour growth.[373,374] Smokers with bladder cancer have elevated levels of methylated metabolites, hexosamine biosynthetic pathway intermediates, acetylated metabolites, polycyclic aromatic hydrocarbons/their aromatic counterparts, and hydroxylated derivatives, and show the deregulation of DNA methylation, nicotine metabolism, glutathione metabolism, and nucleotide metabolism compared to nonsmokers. Bladder cancer smokers also exhibited higher DNA adduct formation and DNA damage that leads to more aggressive bladder cancer.[375]

Serum metabolic profiles of bladder cancer patients are distinctly different from healthy subjects. Evaluation and validation of these metabolic profiles and delineated metabolites have potential benefit in the understanding of the pathogenic process of bladder cancer for noninvasive diagnosis of the disease. Bladder cancer patients have decreased levels of aromatic amino acids (tyrosine, phenylalanine) but elevated levels of L-DOPA, which is synthesized from tyrosine, indicating that aromatic amino acids might play a critical role in the pathogenic process of bladder cancer. Levels of other amino acids are also altered in serum samples. The nonessential amino acid glycine, involved in production of DNA, phospholipids, and collagen, was decreased in tumours. Branched-chain amino acids isoleucine (Ile) and leucine (Leu) were also detected at low levels in serum samples from bladder cancer patients, indicating that branched-chain amino acids could exert a regulatory influence on proteolysis and thus could play an important role as building blocks. The levels of citrate and lactate, which are intermediates of glucose metabolism, were markedly lower in serum from bladder cancer patients.[376] However, these findings are strikingly different from the "Warburg effect" where, commonly, lactate levels are high in tumour tissues. Bladder cancer patients' serum also has a high level of very low-density lipoprotein (VLDL), which is related to lipogenesis, the origin of membrane biosynthesis, which is an essential requirement for cell growth and proliferation. In addition, products of lipid metabolism, ketone bodies such as acetoacetate, were observed with elevated serum levels in patients, indicative of increased lipogenesis.

Urinary metabolomics analyses discriminate bladder cancer patients from healthy ones. One of the most promising markers, the tetrapeptide GlyCysAlaLys, achieved a specificity of 82.61% with a sensitivity of 76.19% that results in an area under the curve at 0.834, while a simplified combination of GlyCysAlaLys, AspAspGlyTrp, and ureidosuccinic acid have an improved area under the curve of

0.919. This urinary metabolomics–based approach showed potential as an alternative or supplement diagnostic procedure to cystoscopy. Elevated levels of urinary nicotinuric acid and trehalose were identified in low-grade bladder cancer patients.[377] Nicotinuric acid is an endogenous end product of nicotinate and nicotinamide metabolism and is also a minor metabolite of fatty acid beta-oxidation. Inosinic acid (or inosine monophosphate, IMP) and ureidosuccinic acid were downregulated in the urine of bladder cancer patients and are involved in purine metabolism. It is converted by inosine-5'-monophosphate dehydrogenases (IMPDHs) in a rate-limiting step for the *de novo* biosynthesis of guanine nucleotides. IMPDHs have been shown to play vital roles in the development of malignancy such as myeloma, neuroblastoma, colorectal cancer, and prostate cancer. Urine metabolomics analysis also revealed the putative metabolite biomarkers related to bladder cancer, such as 3-amino-2-piperidone, pyroglutamic acid, N-acryloylglycine, O-phosphoethanolamine, hydroxyindoleacetic acid, dihydrolipoate, uridine, pseudouridine, N-acetylputrescine, N-acetylcadaverine, histamine, 1-amino-propan-2-ol, 3-diaminopropane, and L-prolyl-L-proline.[378] However, future research is required to validate these findings to clinical levels.

All the present studies have notable limitations. Most were performed on a single platform with a limited number of patient samples. Future large-scale retrospective or prospective studies are needed to test the true clinical utility of these metabolites as bladder cancer biomarkers. A metabolite-derived gene expression signature should ideally be validated in the same data set using gene expression data, as well as in independent data sets with long-term clinical follow-up data.

Currently, studies of tissues, serum, and urine samples from bladder cancer patients show distinct signatures of altered metabolite levels that are indicative of specifically disrupted metabolic pathways, including aromatic amino acid, glycolysis, citrate cycle, lipogenesis, nicotinamide, and xenobiotic metabolism. Integration and further validation of the results obtained from the metabolomics studies of tissue, serum, and urine samples is a next logical step. The trend in alteration of some metabolite levels is closely related to the aggressive cancers, suggesting that these characteristic metabolites might play a vital role in the pathogenesis of bladder cancer. These results might also provide new candidates for invasive detection and surveillance of bladder cancer and could be exploited as potential biomarkers for noninvasive diagnosis and treatment of bladder cancer.

3.14 Receptor Tyrosine Kinases in Bladder Cancer

3.14.1 Introduction

Receptor tyrosine kinases (RTKs) are transmembrane proteins located at the plasma membrane.[379] From the N to the C terminus, they consist of an extracellular domain responsible for ligand binding, a single-pass transmembrane domain, and an intracellular moiety with tyrosine kinase activity. The extracellular domain is the most variable part of the molecule, in terms of the amino-acid sequences of the various RTKs. In human, RTKs are encoded by 58 different genes from 20 families defined on the basis of sequence data. Alternative splicing events may result in the generation of several different isoforms from the same gene. RTKs convert an extracellular signal carried by a protein-ligand into various intracellular signals. Ligand binding to the receptor induces RTK dimerization or a change in conformation for RTKs from the insulin receptor family, which are already in dimer form. This dimerization and/or change in conformation of the receptor triggers its autophosphorylation and the activation of several signalling pathways. Heterodimerization between two different RTKs followed by activation can also occur, the best studied examples being heterodimerization within the EGFR/ERBB family. RTKs are involved in cell-to-cell communication. Their activation may up- or down-regulate cell proliferation, differentiation, survival, motility, and invasion. Due to the many different signalling pathways activated by a given RTK, which may differ between cell types, the consequences of RTK activation are context-dependent, making it difficult to extrapolate findings for one particular cell or tumour type to another. For example, in the urothelium, activating mutations of *FGFR3* are associated with low-stage, low-grade tumours that may subsequently progress to more aggressive tumours. The same mutations in the epidermis are associated with benign tumours (seborrheic keratosis) that will never progress, and identical mutations in chondrocytes are associated with the inhibition of bone growth.[380]

3.14.2 Genetic alterations of receptor tyrosine kinases in bladder cancer

RTKs can be activated by several mechanisms in cancers: overexpression of the receptor or its ligands, somatic activating mutations, or gene fusions. These mechanisms are not mutually exclusive, and overexpression can be associated with an activating mutation or gene fusion, for example. Stronger expression in tumours than in normal tissues is suggestive of the involvement of an RTK as a positive regulator of tumourigenesis, but does not provide absolute proof. Additional evidence is required to establish such a link. The existence of recurrent mutations or fusions provides genetic evidence for the involvement of an RTK. Other evidence is provided by functional studies *in vitro* or *in vivo* in preclinical models. Genetic alterations to a receptor are often (but not always) associated with treatment response (as for *human epidermal growth factor receptor 2 (HER2)/ERBB2* amplification in breast cancer and the response to trastuzumab, an antibody targeting this receptor).

The RTKs most frequently altered by mutations, fusions, and/or amplification in bladder cancers are FGFR3, epidermal growth factor (EGFR/ERBB1/HER1), HER2, and ERBB3. Recurrent activating mutations of *FGFR3* are very frequent in low-grade and low-stage tumours (more than 70% of mutations in TaG1 and TaG2) and less frequent in high-grade or muscle-invasive tumours.[381] These findings are consistent with the existence of different pathways of tumour progression in bladder cancer: the Ta low-grade pathway and the CIS/high-grade pathway.[382] Ta low-grade tumours often recur, but rarely progress to muscle-invasive disease, whereas the majority of muscle-invasive tumours are thought to originate from CIS or high-grade Ta tumours.[382] The most common *FGFR3* mutations are S249C, Y375C, R248C, and G372C, which together account for 93.5% of all *FGFR3* mutations (61%, 18.5%, 9%, and 5%, respectively) (http://cancer.sanger.ac.uk/cosmic). The S249C and Y375C mutations have been shown to be activating mutations *in vitro* and to be necessary for the maintenance of transforming properties in bladder tumour cell lines carrying these mutations.[383,384] Fusions involving *FGFR3* and, in most cases, its neighbouring gene *TACC3*, have been observed in 2% to 4% of MIBCs.[123,327] The fusion protein consisting of the N-terminus of FGFR3 (the extracellular domain, the transmembrane domain, and most of the intracellular domain, including the tyrosine kinase) and the C-terminus of TACC3 constitutively dimerizes due to the C-terminal coiled coil domain of TACC3, inducing autophosphorylation of the receptor.[327] This fusion protein has also been detected at low frequency in other cancers and has been shown to promote tumourigenesis in glioblastoma.[385]

HER2 is altered by amplification (5% of MIBC cases in a series of 1,005 patients)[386] or point mutations (8% of NMIBCs, mostly high-grade cases, and in 12% of MIBCs).[123,170] More frequent *HER2* amplifications have been reported in lymph node metastases compared to the matched primary tumours in a series of 150 cases (15.3% vs 8.7%, p=0.003) (Fleischmann *et al,* 2011). Activating HER2 mutations are particularly common on micropapillary urothelial carcinomas (40%).[387] One hotspot mutation, S310F, accounts for 29% of all mutations, the other mutations being less frequent (less than 5% of all *HER2* mutations) (http://cancer.sanger.ac.uk/cosmic). The functionality of several of these mutations has been assessed, and they have been shown to be activating mutations.[388-390] Relatively frequent *ERBB3* mutations have been reported in MIBC (10%), with no hotspot mutations.[123] Several of these mutations have been found to be activating and dependent on HER2 activity.[391] EGFR was one of the first RTKs identified as potentially involved in bladder cancer, as its overexpression was frequently detected in MIBC.[392] Recent studies have not identified recurrent *EGFR* mutations in bladder cancer, but have reported amplification in 5% to 10% of MIBCs.[47,123]

3.14.3 Involvement of genetically unaltered receptor tyrosine kinases in bladder cancer

Recurrent mutations, fusion genes, and amplifications provide genetic evidence for RTK involvement in cancer. Functional evidence for various cancers indicates that genetically unaltered RTKs may also be involved in tumourigenesis. There is little evidence for the involvement of FGFR3, HER2, or ERBB3 in tumours presenting no genetic alterations to these receptors. By contrast, studies using preclinical models have shown that EGFR can be protumourigenic even if not amplified. Based on transcriptome data, several teams have proposed a classification of MIBCs into different subtypes. These classifications differ in several ways, but all the teams responsible for their development agree that there is a basal-like or basal-squamous subtype.[172] This subtype, which accounts for about 25% of MIBC tumours, is aggressive, with most deaths occurring within a year of initial diagnosis.

Transcriptome analysis suggested that the EGFR pathway was activated in the basal-like subtype. Consistent with this finding, EGFR and its phosphorylated form were found to be overexpressed in this subtype. Preclinical basal-like models have been identified (human bladder tumour cell lines grown *in vitro* and as xenografts and a chemically induced mouse model of bladder tumours), and inhibitor studies in these models have shown that EGFR is protumourigenic.[47]

3.14.4 Expression of receptor tyrosine kinases by endothelial cells

In all the examples given above, RTKs were implicated in tumourigenesis through their expression in tumour cells. RTKs are also expressed by stromal cells. Angiogenesis (the formation of new blood vessels from pre-existing vessels) is generally required for tumour growth, as the nutrients and oxygen essential for tumour cell growth and survival are delivered by blood vessels. Vascular endothelial growth factor (VEGF) and its RTK receptors, VEGFR1 and VEGFR2, are required for angiogenesis. VEGF is expressed by tumour cells and VEGFR1 and VEGFR2 are expressed by endothelial cells. Blocking the activation of these receptors is, therefore, a possible therapeutic option. IHC analyses demonstrated that VEGF expression is associated with progression to muscle-invasive disease,[393] metastasis,[394] and shorter disease-specific survival (DSS)[395] in patients with bladder cancer. A causal connection between VEGF-VEGFR2 signalling and tumour growth was established in orthotopic 253J B-V xenografts treated with the antimouse VEGFR2 antibody, DC-101.[396,397]

3.14.5 Clinical trials of the use of receptor tyrosine kinases as therapeutic targets

3.14.5.1 FGFR inhibitors

The earliest tyrosine kinase inhibitors for FGFR3 were nonselective with wide-spectrum off-target inhibition against other tyrosine kinases. Dovitinib, which inhibits not only FGFR3 but also VEGFR3, FLT-3, and c-kit, showed evidence of biologic activity in a small pilot trial of patients with BCG nonresponsive NMIBC.[398] However, there was no evidence of clinical activity in either this trial or in a phase 2 clinical trial in previously treated metastatic FGFR3 mutant bladder cancer.[399]

More recently, selective inhibitors targeting the family of FGFRs are showing evidence of clinical activity in metastatic, previously treated urothelial cancer patients. BGJ398, which selectively inhibits FGFR1-3, has been shown to have an objective response rate (ORR) (complete response [CR] + partial response [PR]) of 36% in patients with FGFR3 mutations or translocations.[400] Despite treatment on an intermittent schedule 3 weeks out of 4, 42% of patients experienced grade 3/4 hyperphosphatemia. Erdafitinib, a pan-inhibitor of FGFR1-4, explored the impact of dose scheduling by randomizing previously treated metastatic urothelial cancer patients with FGFR3 mutations or translocations to an intermittent versus continuous dosing schedule.[401] The continuous dosing schedule was selected for the expansion cohort in this large phase 2 trial. Although results from this trial have not yet been reported, a small expansion cohort exploring continuous dosing on the erdafitinib phase 1 trial suggested an ORR as high as 54% with grade 3 hyperphosphatemia noted in 4% of patients.[402] Rogaratinib, another pan-inhibitor of FGFR1-4, is also showing evidence of clinical activity reporting an ORR of 30%, with a disease control rate (CR + PR+ stable disease [SD] >12w) of 75% in patients with either FGFR3 mutations or overexpression of FGFR3 mRNA.[403]

3.14.5.2 Vascular endothelial growth factor pathway inhibitors

The promising preclinical findings prompted the design of two clinical trials combining the anti-VEGF antibody bevacizumab (Avastin®) with cisplatin-based combination chemotherapy. A phase 2 single-arm clinical trial of bevacizumab combined with gemcitabine/cisplatin in the frontline metastatic setting produced an overall response rate of 72%, suggestive of clinical activity. A subsequent single-arm phase 2 neoadjuvant trial of bevacizumab with dose-dense methotrexate/vinblastine/Adriamycin/cisplatin (MVAC) produced pathological CRs and PRs in 38% and 53% of patients, respectively, also suggestive of clinical activity.[174] Importantly, patients with basal tumours had exceptionally good outcomes,[174] suggesting that the combination may have produced preferential benefit in basal tumours. This possibility is consistent with the presence of gene expression[48] and micro-RNA expression signatures[226] associated with the response to hypoxia in these tumours.

3.14.5.3 ERBB family inhibitors

Anti-EGFR and anti-HER2 treatments (cetuximab, lapatinib) have yielded no significant benefit in unselected patients with metastatic MIBC[404] or in patients selected for trials exclusively on the basis of expression of EGFR or HER2.[405] In one randomized multicentre phase 2 trial, patients were selected on the basis of *HER2* amplification in the tumour.[406] No benefit of treatment was observed. However, the number of patients included was relatively small, due to the initial overestimation of HER2 overexpression/amplification in bladder tumours (32 patients in the anti-HER2 treatment [trastuzumab and chemotherapy] arm, versus 29 patients in the control arm (chemotherapy alone). In a recent phase 2 trial, afatinib, an irreversible inhibitor of receptors of the EGFR family (EGFR, HER2, ERBB3, and ERBB4), was used in platinum-refractory metastatic urothelial carcinoma.[407] All patients regardless of *ERBB* status were permitted to enrol, but the authors also examined a prespecified hypothesis based on *EGFR*, *HER2*, *ERBB3*, or *ERBB4* genetic alteration status (mutations of *EGFR*, *HER2*, *ERBB3*, and *ERBB4*, or amplifications of *EGFR* and *HER2*). Results importantly indicated activity for afatinib in patients with *HER2* or *ERBB3* genetic alterations. The median time to progression/discontinuation was 6.6 months in patients with *HER2 or ERBB3* genetic alterations versus 1.4 months in patients without alterations ($p<0.001$).

One of the possible pitfalls of clinical translation of EGFR or HER2 as predictive biomarkers of treatment response is that there may be discordance between IHC assignment, FISH, qPCR, and genomic-level molecular characterization. This may explain in part the negative results of the large phase 3 study that examined lapatinib in urothelial cancer as a maintenance therapy after first-line chemotherapy.[35] Patients were selected by IHC analysis and patients were permitted to enrol if they carried 2+ or 3+ EGFR or HER2 by IHC. Such patients were coded as "EGFR/HER2 positive." This cohort therefore likely represents a heterogeneous group of patients who were truly *EGFR* or *HER2* amplified, and many who were not, making it difficult to discern if there was a signal of activity for truly *EGFR*- or *HER2*-amplified patients treated with lapatinib.

Future studies of the ERBB family in urothelial cancer should classify patients according to genomic molecular results, or should confirm HER2 classifications by IHC with a second method. The results of ongoing additional, exciting ERBB molecularly targeted studies that include urothelial cancer patients are awaited (e.g. NCT02780687; Bryce *et al,* JCO 35 [suppl 6S; abstract 348; 2017]).

3.14.6 Tumour heterogeneity and receptor tyrosine kinases

Individual patients may present diverse sources of tumour heterogeneity: heterogeneity between different tumours (synchronous or metachronous), between the primary tumour and the metastases, and within tumours (phenotypic or genotypic heterogeneity). Intratumoural heterogeneity has been observed for *HER2* amplification.[386] It has been shown that *FGFR3* mutation is not always the first event in *FGFR3*-mutated tumours, explaining why clonally related tumours with and without mutations can occur in a given patient.[408] Furthermore, within muscle-invasive tumours, *FGFR3* mutation may be detected in the superficial compartment, but not in deeper compartments (four of eight cases examined).[409] However, in the same study, the *FGFR3* mutation status of lymph node metastases was found to be systematically concordant with that of the primary tumour (primary tumour and lymph nodes *FGFR3*-mutated in 10 cases, primary tumour and lymph node metastases without *FGFR3* mutations in 191 cases). It will be important to assess in more detail the different types of heterogeneities and to take them into account when selecting treatments and predicting treatment responses. Treatment choice is usually based on a single biopsy of either the primary tumour or a single metastasis. Tumour heterogeneity could be assessed by deep sequencing. Circulating DNA may also be useful for the determination of RTK mutation/translocation/amplification status, as it originates from the various tumours/metastases of the patient.

3.14.7 Combinations of treatments

The inhibition of a single RTK is unlikely to be sufficient for a prolonged response, and treatment combinations are, therefore, required. The identification of additional treatments will be facilitated by preclinical and clinical studies investigating changes in the regulatory circuitry of the tumour cells upon anti-RTK treatment. In addition, as immunotherapy gradually becomes the standard treatment for advanced bladder cancer and, possibly, for other bladder cancers, one of the major goals for the next few years will be identifying optimal associations between immunotherapy and other treatments. These combinations of treatments must be tailored to each patient (personalized medicine) and, as a first step, to each different subtype of cancer (stratified medicine). Treatments targeting the RTKs expressed by tumour cells can indirectly influence the tumour microenvironment and, therefore, response to immunotherapy.[410]

3.15 Present and Future Immunotherapy for Bladder Cancer

3.15.1 Introduction

Until recently, there have been no major advancements in treatment for bladder cancer. First-line treatment for MIBC is cisplatin-containing combination chemotherapy: gemcitabine and cisplatin or methotrexate, vinblastine, adriamycin, and cisplatin.[411] These treatments prolong survival for about 15 months on average.[412,413] However, as many as 50% of patients will not tolerate cisplatin-containing chemotherapy regimens, due to poor Eastern Cooperative Oncology Group (ECOG) status (a measure of disease progression), diminished renal function, and/or other comorbidities that would put the patient at risk during the course of treatment.[411] As such, patients with advanced bladder cancer are in critical need of alternative therapeutics to improve prognosis. Recent advances in immunotherapy have shown promise for those with MIBC and represent one of the most significant treatment advances for these patients **(Figure 3–11).**

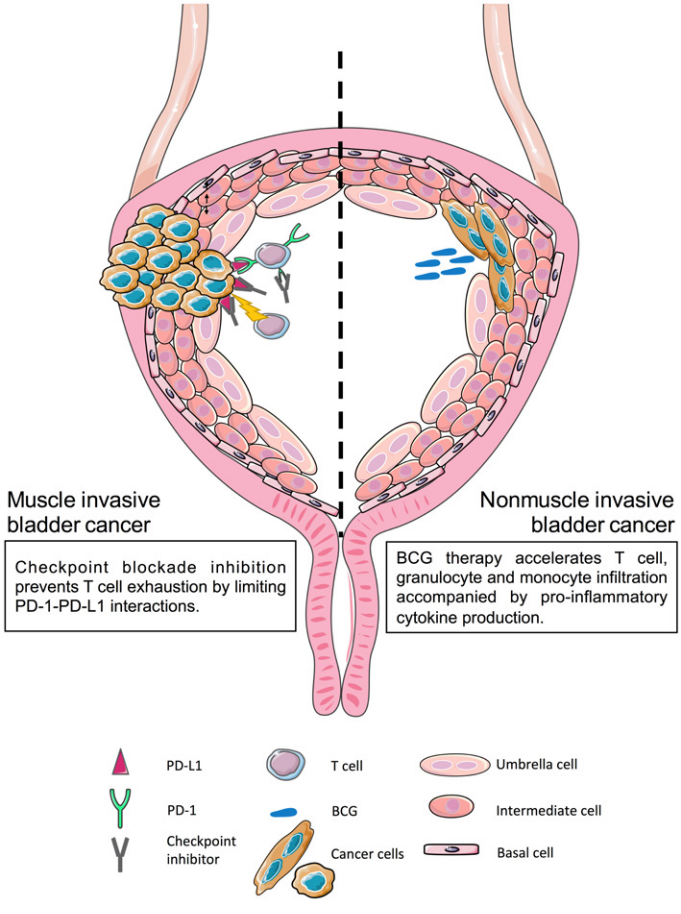


FIGURE 3–11
Immunotherapy for Bladder Cancer

On the left side, checkpoint blockade inhibitors, in the form of monoclonal antibodies, act to modify T-cell responses. α-PD-L1 binds to PD-L1 expressed on tumour cells, whereas α-PD-1 will bind PD-1 expressed on tumour-infiltrating lymphocytes. Subsequent disinhibition of T-cell function will boost T-cell proliferation, enhance cytotoxicity against cancer cells, and downregulate IL-10 production, thereby diminishing immunosuppression in the tumour microenvironment. On the right side, repeated intravesical instillation of BCG into the bladder results in enhanced infiltration of CD8+ T cells, granulocytes, and monocytes. Additionally, production of inflammatory cytokines is increased, promoting activation of immune cells and antitumour immune responses.

Similarly, therapies for NMIBC have remained largely unchanged since the mid-1970s, when Morales and colleagues first demonstrated that BCG (an attenuated form of *Mycobacterium bovis*) prevents recurrence and progression better than tumour resection alone **(Figure 3–11)**.[414] Given that NMIBC accounts for approximately 70% of bladder cancers, of which 20% to 40% will be tumours unresponsive to BCG therapy, and that NMIBC progresses to muscle invasive disease in approximately 10% to 20% of cases, new therapies are urgently needed.[415-417] Experimental approaches to modify BCG or administer BCG in combination with immune stimulating agents have now shown strong improvements over BCG alone.[418-420] However, early successes with immunotherapeutics, such as ICB in MIBC, suggest that we may be on the horizon of a breakthrough in the treatment of patients with bladder cancer.[421-424]

3.15.2 The original immunotherapy for bladder cancer: bacillus Calmette-Guérin

Manipulating the immune system for therapeutic benefit is not a new paradigm. Dramatic reduction in tumour burden in preclinical models treated with BCG led Morales and colleagues to test whether 6 weekly intravesical instillations of BCG, following tumour resection, had a positive therapeutic effect on the frequency of tumour recurrence and progression in NMIBC.[414,425-427] Remarkably, this regimen induces lasting tumour immunity in a large subset of patients and, as such, has remained largely unchanged over the last 40 years. More recent studies have shown that adding a schedule of maintenance BCG therapy, or additional BCG intravesical instillations, at regular intervals following the initial treatment confers increased protection against recurrence and progression.[428-431] To date, no other therapy has shown the same level of efficacy in clinical trials. Indeed, intravesical BCG therapy significantly reduces the risk of progression to muscle invasive disease, reduces recurrence rates by up to 65%, and is superior to other intravesical therapies used in NMIBC (mitomycin C, doxorubicin, epirubicin, and interferon [IFN]) in reducing rate of recurrence.[428,432-436] Accordingly, BCG therapy remains the standard of care treatment of NMIBC.

3.15.3 Mechanism of action of bacillus Calmette-Guérin

The mechanism by which BCG induces antitumour immunity is not entirely understood. Much of what we have learned is derived from animal models and *in vitro* observations, and the extent to which these observations reflect human responses is unclear. What we do understand can be summarized as follows.

Upon instillation, the proposed first step in therapy is BCG binding to fibronectin at sites of damage in the bladder, which are areas thought to have a greater amount of exposed extracellular matrix proteins.[437-439] Preclinical *in vivo* models demonstrated that animals exhibit more pronounced tumour outgrowth when treated with BCG and a peptide blocking the bacterial fibronectin binding protein or when BCG is coated with soluble fibronectin as compared to mice treated with BCG alone.[438,440] Following attachment, *in vitro* studies suggest that BCG may be internalized by cancer cells; however, strong evidence to support that this step occurs or is necessary in the context of therapy is lacking.[441] Indeed, different bladder cancer cell lines vary in their capacity to internalize BCG, depending on the presence of mutations in the Pak-1 dependent macropinocytosis pathway.[442] Furthermore, the differentiation state of immortalized cells appears to be a determinant of their capacity to internalize BCG,

in which undifferentiated cells are better able to take up bacteria than more differentiated cells.[443] Together, these data suggest BCG-induced antitumour immunity in humans may be dependent, in part, on the specific mutation(s) present in cancer cells. This may be one reason that 20% to 40% of NMIBC patients do not respond to BCG therapy. Indeed, internalization of BCG is essential for secretion of IL-2, IL-6, and IFN-γ from immortalized cancer cells.[443,444] Furthermore, BCG internalization is improved in cell lines following knockout of the tumour suppressor gene *PTEN*, strengthening the case that oncogenic mutations play a role in host response to BCG.[445] It is important to note, however, that BCG therapy occurs following transurethral tumour resection, when the majority of cancer cells have been removed. While it is unclear whether residual tumour cells remain in the bladder and if they play a role in BCG-mediated immunity, certainly the interaction of BCG with untransformed urothelial cells is likely an important determinant of response to therapy, and warrants study at both the preclinical and clinical level.[446]

Following exposure to BCG, *in vitro,* bladder cancer cells upregulate major histocompatibility complex class II and intracellular adhesion molecule 1.[447,448] Bladder cancer cells also secrete cytokines such as IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), and tumour necrosis factor (TNF)-α.[449-451] Following BCG instillation, NMIBC patients had measurable quantities of the cytokines IL-1, IL-2, IL-5, IL-6, IL-8, IL-10, IL-12, IL-18, TNF, IFN-γ, IP-10, monocyte chemoattractant protein-1, and regulated upon activation normal T cell expressed and secreted (RANTES) as well as a marked increase in granulocytes and monocytes, and subsequently lymphocyte populations in their urine.[452-458] Mouse studies, in which $CD4^+$ and $CD8^+$ T cells and NK cells were depleted, demonstrate that each of these cell types is indispensable for a successful response to BCG therapy.[459,460] Notably, however, additional studies suggest that NK cells may be dispensable for BCG therapy, as inhibition of NK cell activity does not impact BCG therapy efficacy in an *in vivo* bladder cancer model.[461] In an orthotopic bladder cancer mouse model, vaccination with BCG prior to intravesical therapy augments the innate immune and T-cell response to BCG therapy, leading to improved survival following tumour challenge.[462] This finding is supported by retrospective analysis revealing that patients with pre-existing BCG immunity, defined by a positive purified protein derivative test, had fewer tumour recurrences compared to patients who were purified protein derivative–negative.[462] Additionally, T-cell infiltration is linked to clinical response to treatment in patients.[463] Together, these findings suggest that BCG exposure, by vaccination, prior to BCG treatment could be used to improve patient response to BCG therapy in the clinic.[462] This concept is under investigation in a clinical trial in the United States (NCT02326168).

3.15.4 Fine-tuning bacillus Calmette-Guérin immunotherapy

A T helper (T_h) 1 response is required for BCG therapy efficacy.[464-466] The absence of IL-12 or IFN-γ in gene-deficient mice impedes the response to BCG therapy, whereas the absence of IL-10 improves immune responses and outcomes.[467] These observations have inspired efforts to modulate the T_h1 or T_h2 biased responses arising during BCG therapy. Recombinant BCG strains, engineered to produce IL-2, IFN-γ, IFN-α2b, or IL-18 have been tested *in vitro* and *in vivo* in mice. Thioglycollate-elicited mouse peritoneal cells express more IL-12, TNF, and IFN-γ and are more cytotoxic towards the mouse bladder cancer cell line, MBT-2, following treatment with recombinant IL-2-expressing BCG as compared to the parental BCG strain.[468] IFN-γ-producing BCG induces increased expression of major histocompatibility complex I on the mouse bladder cancer cell line MB49 compared to the

parental BCG strain. Furthermore, in an orthotopic mouse model of bladder cancer, instillation of this transgenic BCG strain promotes an enhanced recruitment of $CD4^+$ T cells into the bladder, local expression of IL-2 and IL-4 mRNA and significantly increases survival compared to treatment with the parental BCG strain.[469] Human peripheral blood mononuclear cell stimulation by a recombinant IFN-α2b-expressing BCG strain leads to enhanced cytotoxicity, mediated by NK and $CD8^+$ T cells, against human bladder cancer cell lines, such as T24, J82, 5637, TCCSUP, and UMUC-3.[470] Finally, treatment of mouse peritoneal cells with IL-18–expressing BCG promotes increased cytotoxicity against MBT-2 cells, dependent upon TNF-α.[471]

BCG administered with an anti–IL-10 receptor antibody significantly increases IFN-γ mRNA and protein in bladder and urine, respectively, in a dose-dependent manner, while decreasing mortality in an MB49 orthotopic bladder cancer model.[472] Using peripheral blood mononuclear cells from bladder cancer patients, it was observed that the combination of BCG plus IFN-α2b enhances the production of IFN-γ, IL-12, and TNF-α, while decreasing IL-10 expression, compared to stimulation with BCG alone.[473] This suggests that this combination of BCG and IFN-α2b biases immunity toward a T_h1 response. Notably, in the same study, BCG plus IFN-α2b induced equivalent levels of IFN-γ with one-third or even one-tenth of a standard BCG dose in two patients.[473] While these combination therapies have shown encouraging results in mice or *in vitro*, the concomitant instillation of IFN-α2b and BCG did not decrease tumour recurrence or improve BCG efficacy in patients.[474,475] Rather, concomitant instillation of IFN-α2b and BCG increased adverse events compared to BCG therapy alone.[474]

One new advance in BCG immunotherapy is the development of VPM1002BC, a genetically modified strain of BCG designed to be more tolerable and immunogenic than the clinically available BCG strains.[418] The modified strain expresses *Listeria monocytogenes*-derived listeriolysin, which induces higher levels of macrophage apoptosis, enhancing cross-priming, and a T_h1 and T_h17 cytokine response instead of a T_h1 response only.[418,419,476,477] This results in enhanced priming of $CD4^+$ and $CD8^+$ T cells, and expansion of $CD4^+$ central memory T cells in comparison to the parental BCG strain.[476,478] Phase 1 testing demonstrated that the new strain is well tolerated and safe, and a phase 1/2 trial is currently recruiting participants (NCT02371447).[479]

3.15.5 Immune checkpoint inhibition for bladder cancer

Immune checkpoint pathways are a way for the immune system to modulate its response to foreign antigen and self-antigen. By regulating the strength and duration of an immune reaction, immune checkpoint pathways protect host tissues from damage following an immune response.[480,481] However, it is now clear that checkpoint molecules are expressed in many cancers and their presence impedes antitumour immune responses.[482,483] Thus, targeting these molecules offers a promising approach to improve antitumour immunity. ICB works by specifically targeting, binding, and inhibiting known immune checkpoint molecules, their receptors, and ultimately their associated pathways.[484] The aim with these therapeutic molecules is to suppress the inhibitory signal, prolonging the effector function of tumour-specific T cells. ICB often comes in the form of monoclonal antibodies specific for one member of the receptor-ligand pair, or recombinant forms of the ligands or receptors.[481] The most studied pathway in bladder cancer is the programmed cell death pathway, composed of PD-1 and its ligand PD-L1. In 2016, the FDA approved atezolizumab (anti–PD-L1) for the treatment of metastatic

urothelial carcinoma, following promising ORRs of 15% in a phase 2 clinical trial.[135] Patients with high PD-L1 expression on tumour infiltrating immune cells had the highest objective responses of 26%.[135] A second checkpoint inhibitor, nivolumab (anti–PD-1), was FDA approved in February 2017 for the treatment of locally advanced or metastatic urothelial carcinoma following a clinical trial with an ORR of 24.4% across all participants.[485] Since these approvals, three additional checkpoint inhibitors, pembrolizumab (anti–PD-1), avelumab (anti–PD-L1), and durvalumab (anti–PD-L1), have been provisionally approved for the second-line treatment of metastatic bladder cancer.

3.15.6 PD-1/PD-L1 expression and mechanism of action

PD-1 is a cell surface receptor expressed on T cells that negatively regulates T-cell receptor signalling upon binding its cognate ligands PD-L1 and PD-L2.[480] This results in decreased cytokine production and proliferation and is a key component in T-cell exhaustion.[486,487] Its ligand PD-L1 is rarely expressed on the vast majority of human cells, but it is induced in cancer cells (and most nucleated cells) *in vitro* upon exposure to IFN-γ.[488,489] From IHC staining, we know that PD-L1 is expressed on the cell surface of many cancers, including melanoma, renal cell carcinoma, and bladder cancer.[490-492] Interestingly, in human melanocytic lesions, a significant positive correlation exists between PD-L1 expression and tumour-infiltrating lymphocytes.[493] This correlation is associated with local IFN-γ production at the interface between tumour-infiltrating lymphocytes and PD-L1 positive tumours. PD-L1 expression may represent a resistance mechanism of tumour cells in response to antitumour immunity and may explain how melanoma escapes immune destruction despite the presence of tumour-infiltrating lymphocytes and antitumour immunity.[493]

The binding of PD-1 to PD-L1 deregulates T-cell immunity in a number of ways.[489] First, tumours expressing PD-L1 induce apoptosis in activated T cells and promote expression of IL-10 in circulating T cells, thereby contributing to the maintenance of an immunosuppressive environment.[488,494] Second, PD-L1-PD-1 signalling in T cells induces T-cell anergy both *in vitro* and *in vivo*.[495-497] Finally, in a murine viral infection model, T-cell exhaustion induced by persistent antigen exposure can be reversed following anti–PD-L1 treatment.[498] Together, these findings suggest persistent PD-L1-PD-1 signalling exhausts tumour-infiltrating lymphocytes, thereby inhibiting their capacity to eradicate tumour cells. Importantly, very little information exists pertaining to the impact of PD-1 blockade on PD-L2-expressing cells.

3.15.7 Immune checkpoint inhibition in the clinic

Atezolizumab is currently the only approved drug targeting PD-L1 for the frontline treatment of advanced urothelial carcinoma. Atezolizumab was granted breakthrough status by the FDA in March 2016 following positive results in phase 1 and 2 clinical trials.[135,421] In the phase 1 trial, ORRs were up to 50% and correlated with PD-L1 expression on tumours and tumour infiltrating immune cells.[421] In addition, fewer adverse events were reported in response to treatment than typically reported in response to many other second-line treatments available for advanced bladder cancer.[421,499] The same pattern of response was observed in the phase 2 trial, with the highest response rate (26%) and longest median survival times observed in patients with higher PD-L1 positivity in their tumours. Of note, the response rate also strongly correlated with mutational load.[135] Taken together, these results suggest that PD-L1 expression and measures of mutational load may be useful biomarkers to predict response

to treatment. As the response rates and median survival time in patients treated with atezolizumab positively correlate with higher PD-L1 expression on tumours and tumour infiltrating cells, better patient stratification is necessary. Studies defining biomarkers that predict the response to treatment are needed to choose the best treatment option for the patients. It is important to note, however, that in May 2017 atezolizumab did not outperform chemotherapy in OS in the IMvigor211 phase 3 clinical trial evaluating patients with locally advanced or metastatic urothelial cancer whose disease progressed during or after chemotherapy.[134] The IMvigor211 trial has thus failed to meet its primary endpoint. One unexpected result, contributing to this outcome, was that chemotherapy performed better than anticipated (NCT02302807). Careful analysis will be needed to fully understand the final results from this trial.[135,500]

Nivolumab, targeting PD-1, was FDA approved in February 2017 for patients with locally advanced or metastatic urothelial carcinoma who undergo disease progression within 1 year following first-line platinum-containing chemotherapy, following positive interim results from a phase 1/2 multi-centre clinical trial (NCT01928394).[485] Tumour PD-L1 expression was determined retrospectively, with positive and low PD-L1 expression being defined as ≥1% and <1% of cells expressing the molecule, respectively. Researchers reported an ORR of 24.4%, with similar rates observed in patients at all levels of PD-L1 expression. A CR was observed in 16% of PD-L1 positive patients, and 2% of PD-L1 negative patients. Serious treatment-related adverse events were seen in 10% of patients and 3% stopped treatment as a result. Median OS was 16.2 and 7.0 months in the PD-L1 positive and low cohorts, respectively,[485] which is an improvement over the median survival of approximately 6 months in patients with disease progression/relapse following platinum-containing chemotherapy.[501]

Pembrolizumab, avelumab, and durvalumab have also shown demonstrable clinical success. Pembrolizumab was shown to be safe in the KEYNOTE-012 trial (NCT01848834), providing support for additional late-phase clinical trials.[502] In May 2017, pembrolizumab received FDA approval following the KEYNOTE-045 trial (NCT02256436), in which median OS for patients treated with pembrolizumab was greater than those treated with a paclitaxel, docetaxel, or vinflunine chemotherapy (10.3 months vs 7.4 months) regardless of PD-L1 expression status. The median duration of response for those receiving pembrolizumab was not reached in this trial, whereas patients treated with chemotherapy had a median duration of response of about 4 months.[503] Again in May 2017, avelumab was granted accelerated approval following the JAVELIN solid tumour trial (NCT01772004).[504] Avelumab was well tolerated with an ORR of 18%.[504] The phase 3 JAVELIN Bladder 100 trial (NCT02603432) is currently enrolling patients to evaluate avelumab in a first-line setting for urothelial carcinoma. Finally, in a trifecta of checkpoint blockade inhibitor approvals for bladder cancer, durvalumab received FDA approval in May 2017 following a phase 1/2 study (NCT01693562) demonstrating safety and efficacy. In this trial, the overall response rate was 31.0% in all patients and 46% in a PD-L1–positive subgroup of patients.[505] Notably, the VENTANA PD-L1 assay, a companion diagnostic, also received FDA approval. Similar to avelumab, accelerated approval of durvalumab is contingent upon completion of an ongoing clinical trial to confirm clinical benefit.

3.15.8 Immunotherapeutics on the horizon

Given the success of checkpoint inhibitors in treating advanced urothelial cancers, it is not unreasonable to suggest they may hold promise in treating NMIBC as well. Indeed, the lack of second-line options for NMIBC patients following BCG immunotherapy failure and the relatively high risk of treatment-associated side effects necessitates further investigation into novel treatment options.[506] In mouse models of NMIBC, antibodies targeting both cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and PD-L1, but not PD-1, demonstrated efficacy in reducing tumour burden and prolonging survival.[507,508] Based on preclinical evidence and the successes of checkpoint inhibitors against advanced and metastatic bladder cancer, several clinical trials are currently underway testing pembrolizumab and atezolizumab in BCG nonresponsive/refractory/relapsing NMIBC.[506] A phase 1/1B trial using the oral small molecule CPI-444, which prevents adenosine binding to the adenosine-A2A receptor leading to suppression of antitumour activity of T cells and other immune cells, is also undergoing testing alone or in combination with atezolizumab in advanced cancers, including bladder cancer (NCT02655822).

Antibodies targeting proteins other than immune checkpoint molecules are also under investigation. For example, an antibody targeting the protein tissue factor (HuMax-TF-ADC) is in a phase 1/2 to establish tolerability in patients with solid tumours, including bladder cancer (NCT02552121). An anti-FGFR type 3 antibody (B-701) is in a phase 1b/2, randomized, double-blind, placebo-controlled trial, in combination with docetaxel for the treatment of locally advanced or metastatic urothelial cell carcinoma in patients with recurrence or that are refractory to standard therapy (NCT02401542). An anti-VEGFR2 antibody (ramucirumab) is undergoing testing in a phase 3 trial to determine the efficacy of this antibody in combination with docetaxel in patients with urothelial cancer who have not responded to prior platinum-based therapy (NCT02628535).

Photoimmunotherapy takes advantage of antibodies, such as anti-EGFR, to carry particles or dyes specifically to cancer cells. While EGFR has a low expression on healthy urothelium, it is highly expressed on the surface of bladder cancer cells in approximately 75% of cases.[509] Thus, it is a highly suitable target for this particular immunotherapy. One such example is the use of gold nanorods conjugated to an anti-EGFR antibody. These nanorods bind EGFR-expressing bladder cancer cells and then are heated by infrared light, resulting in the induction of death in cells coated by nanorod-antibody conjugates.[510] Instillation of anti-EGFR antibody conjugated gold nanorods into a mouse orthotopic bladder cancer model once a week for 4 weeks resulted in reduced tumour size as measured by chemiluminescence.[511] Similar results were observed using a slightly different approach, in which an anti-EGFR antibody was conjugated to a photosensitizer/photoabsorber dye.[512] When the dye is activated by near infrared light, reactive oxygen species are generated and necrosis is induced only in cells that are bound by the anti-EGFR-photosensitizer dye compound.[512] Near infrared treatment of mice following administration of anti-EGFR-photosensitizer dye complex attenuated tumour growth in a xenograft model of bladder cancer expressing high levels of EGFR. The therapy had no effect on tumours with low levels of EGFR expression.[512] Photoimmunotherapy is a particularly promising approach, as the cell death is limited to a great extent only to cancer cells,[511,512] and potentially may provide dead tumour cells as antigen to induce a durable antitumour adaptive immune response.

Finally, strategies not relying on antibodies, such as oncolytic viruses, are also undergoing testing. CAVATAK, or coxsackievirus A21, induces tumour cell lysis and promotes antitumour immunity. It is currently in phase 1 evaluation for safety and tolerability alone or in combination with mitomycin C in patients with NMIBC (NCT02316171). Enadenotucirev, a group B adenovirus that selectively kill, tumour cells, is in a phase 1 trial for patients with metastatic or advanced epithelial cancers, including bladder cancer (NCT02636036). The CG0070 oncolytic virus causes tumour cell lysis and immunogenic cell death. CG0070 expresses the immune stimulatory cytokine GM-CSF that, in combination with the release of tumour antigens upon tumour cell lysis, can stimulate antitumour immune responses. CG0070 is also currently undergoing evaluation in a phase 2 trial for high-grade NMIBC patients not responding to BCG therapy and refusing cystectomy (NCT02365818). Finally, a phase 1 dose escalation study is currently investigating tumour-antigen–specific cytotoxic T cells to target solid tumours, including bladder cancer (NCT02239861).

3.15.9 Conclusion

Treatment options for bladder cancer were, until recently, limited to chemotherapy and cystectomy for MIBC and BCG immunotherapy after tumour resection for NMIBC. In the last few years, a significant number of molecules have been tested or are under investigation for the treatment of both MIBC and NMIBC. Some of them, such as the checkpoint blockade immunotherapies (CBIs), show promising results and beneficial outcomes for patients. Additional therapeutic strategies, such as the recently approved chimeric antigen receptor (CAR) T-cell gene therapy, in which the patient's own T cells are re-engineered to recognize leukemic cells and kill them, may also hold promise for bladder cancer patients. However, current[513] and future clinical trials are needed to improve upon these encouraging results and to determine whether combination therapy can induce even more potent long-lasting tumour immunity in patients with NMIBC and MIBC.

3.16 Summary of Recommendations

None of the genomic or proteomic markers outlined in the current chapter are recommended as diagnostic or prognostic tools in bladder cancer **[level of evidence (LOE) 3; grade of recommendation (GOR) C]**.

Presently, molecular subtypes of bladder cancer have no role in clinical practice **[LOE 3; GOR C]**.

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C4

Molecular Markers

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CONTENTS

Molecular Markers

4.1 Introduction: Why Do We Need Biomarkers?

Bladder cancer is a heterogeneous disease that presents diagnostic and treatment challenges for clinicians. The tools currently available to clinicians for diagnosis and staging require invasive procedures, such as cystoscopy and biopsy. Imaging by computed tomography and magnetic resonance often understage patients with non-organ-confined disease and are inadequate to predict which patients may have micro-metastatic disease. Furthermore, treatments such as chemotherapy and immunotherapy are given in a nonselective fashion, such that many patients receive treatments that are either unnecessary or ones they are unlikely to respond to.

Use of molecular markers in urine, tissue, or blood offers potential opportunities to improve our understanding of cancer biology that may help identify disease earlier, improve patient risk stratification and outcomes prediction, and help target therapy. While some urine-based tumour markers for bladder cancer detection and diagnosis have been more extensively studied and approved by the Food and Drug Administration (FDA), current guidelines have yet to embrace markers in any area of disease management. In this chapter, we will review the challenges of introducing markers into clinical care, and discuss urine-, tissue-, and blood-based markers for different stages of disease and a range of clinical scenarios.

4.2 Challenges of Marker Introduction Into Clinical Practice

The field of oncology is moving toward individualized approaches of cancer care regarding treatment, risk stratification, and prognostication, as well as follow-up and trial design. Biomarkers, especially tissue-, blood-, or urine-based biomarkers, have the potential to offer information regarding tumour behaviour and response to therapy that is independent of standard clinical and pathological information. However, despite significant research dedicated to enhancing our understanding of biological principles, cancer genetics, molecular pathways, and identification of possible biomarkers, no biomarkers are uniformly accepted or endorsed by clinical guidelines for bladder cancer (BC).[1–4] This is due to multiform challenges during biomarker evaluation, which will be discussed in this section.

Similar to drug-development studies, biomarker research can be categorized into initial preclinical exploratory studies, clinical assay development and validation studies, small clinical retrospective studies, external validation in larger cohorts (retrospective or prospective, usually multi-institutional), prospective clinical trials, and further post-approval studies, as well as possible expansion to other clinical scenarios and disease stages.[5] This should be a rigorous and complex process with expected low success rates for markers to be introduced into clinical practice, due to analytical as well as regulatory challenges (**Table 4–1**).[6] Unfortunately, much of the published biomarker research to date has not followed these stringent processes, contributing to the flood of "promising" biomarkers,

which are never further evaluated, reproduced, or validated. Typical shortcomings of marker studies are poorly defined study populations, including choice-of-convenience samples, sample size, nonstandardized or nonreproducible assays, and incomplete data. To improve design, analysis, and reporting of marker studies, a set of generally accepted reporting recommendations has been developed. These include the reporting REcommendations for tumour MARKer prognostic studies (REMARK) and the Tumor Marker Utility Grading System (TMUGS) (**Tables 4-2 and 4-3**).[7,8] The main goal of marker development is to identify a validated test that can improve clinical decision-making in a cost-effective way.

Theoretically, biomarkers can be applied to many different clinical scenarios in BC. They may be useful for screening for BC, risk-stratification for hematuria patients, diagnosis and surveillance of BC, as well as prognostication of outcomes and prediction of response to therapies. While the same assays may be used in different scenarios, their requirements may vary according to each specific clinical scenario. For example, if a marker is to be used for screening or diagnosis of BC, it should have a high specificity, as it is most important to avoid a high number of unnecessary work-ups of healthy individuals, while in other situations, like surveillance of high-grade BC, it might be of greater importance that the marker have high sensitivity to minimize the risk of missing cancer recurrence or progression.

However, while sensitivity and specificity are commonly reported in biomarker evaluation, clinicians usually make decisions based on positive and negative predictive values (PPV and NPV). Sensitivity and specificity are marker characteristics that are independent of disease prevalence, but PPV and NPV are directly affected by disease prevalence. This has a significant impact where the prevalence of disease is low, such as in patients with microhematuria or during screening, as even a highly sensitive and specific marker can have a low PPV.[9–11]

Many early marker evaluations used convenience samples that included a high proportion of cancer patients. While this is good in exploratory analyses, it skews marker performance, which can then decrease when applied to clinical practice. Also, the evaluation setting is very important; for example, incidence of cancer at a tertiary referral centre can differ significantly from the community. This highlights the necessity for biomarker validation in separate, multicentre cohorts.

Another consideration for marker evaluation is verification bias. In patients with obvious bladder lesions which are biopsied, there is no question the patients have cancer. On the other hand, in the setting where a marker is positive, but there is no obvious lesion, then there is a question of whether the marker was true or false-positive. Most marker studies are not designed to biopsy patients who have a normal cystoscopy with abnormal markers. As such, there is uncertainty about the accuracy of the marker. Enhanced cystoscopy has exposed the limitations of white-light cystoscopy, which may fail to detect both papillary lesions and carcinoma *in situ* (CIS).[12,13] In fact, the concept of anticipatory positive readings has arisen, as patients with an abnormal marker may have microscopic disease that is not identified cystoscopically but may indicate early disease recurrence.[14] In patients with a negative marker and cancer presence on biopsy, it is easy to determine a false-negative result; but if cystoscopy is also negative, then there is no way to determine if both the marker and cystoscopy missed a small cancer. Future trials will need to appropriately assess the marker while avoiding invasive procedures to validate the performance characteristics of the marker.

Biomarker measurements themselves can be manifold, including binary, categorical, quantitative, or multidimensional. However, most markers are evaluated by identification of a factor expressed in a different fashion by tumour cells than normal cells. Cutoff points are usually generated to optimize sensitivity and specificity, but unfortunately, cutoff points are not standardized. This has contributed to the wide variety of biomarker study outcomes and superior performance of one particular marker in the training or development set, rather than in external validation due to overfitting. While it is easier in clinical practice to have a marker that is either positive or negative to base clinical decisions upon, it is unrealistic to believe that a cutoff point is truly discrete for most markers. Risk is expressed on a continuum; therefore, biomarkers should be evaluated alongside that continuum as long as possible. Only in the final stages should a cutoff point need to be introduced to make the marker clinically applicable. An alternative to cutoff points is the assessment of predicted probabilities, where each level of the biomarker is converted to a probability of a given outcome. This can help individualize treatment decisions based on biomarker levels on an individual patient basis.

For a biomarker to be clinically valuable, it must demonstrate value in improving clinical decision-making. It is therefore insufficient to show only statistically significant independent association of the biomarker with the outcome investigated: it must also show improved prognostic or predictive accuracy of a multivariable model over clinical features alone. Ideally, the model should be improved with regard to discrimination, calibration, and decision analysis.[15] Many techniques have been described for model development, internal and external validation, as well as assessment of clinical utility. An example of such an approach is the development of a model based on clinical factors and a marker for detection of BC.[16] Equally important is the prospective validation of the model in a new multicentre cohort.[17]

Other approaches such as decision-curve analysis, net reclassification benefit assessments, nomogram development, and neuronal network integration are frequently used in this context. The key point of decision analysis lies in the inclusion of possible consequences of clinical decisions into the analyses by weighing the relative value of the benefits (true-positives) with the risks (false-positives).[18]

Finally, BC is a heterogeneous disease with some of the highest rates of mutational burden over different cancer types.[19] Consequently, it is unlikely that a single marker exists to adequately characterize this heterogeneous population and draw treatment conclusions. This has led many investigators to evaluate comprehensive pathways rather than single markers.[20,21] Marker panels including drivers from key pathways, in combination with clinical and pathological variables, may be the most promising approach for accurate risk stratification and clinical decision-making in this disease. Insights from The Cancer Genome Atlas (TCGA) project, which has already led to the detection of distinct molecular subtypes of BC, seem to be the tip-off to further developments in this area.[19]

TABLE 4–1 Proposed Structured Approach to Systematic Discovery, Evaluation, and Validation of Biomarkers (*Adapted from Bensalah et al.*)[6]

Phase	Goals/aims	Experimentation	Sample details
Preclinical testing	Exploratory; nominate and rank candidate biomarker profiles	Preclinical study for hypothesis generation	Possible bias: small size and convenience sampling
0	Develop an assay with clinically reproducible results	Reproducibility and robustness of assay; no assessment of benefit	
1	Test on small sample to determine benefit	Perform marker optimization, establish prediction rules, determine cutoffs	Sample population assay developed from candidate biomarker profile
2	Determine operating characteristics and perform internal validation	Retrospective design	Sample population should be the target population
3	Perform external validation	Retrospective or prospective; generalizability; impact on clinical decision-making	Multi-institutional, large study
4	Assess whether the biomarker reduces the burden of disease	Post-approval reporting and testing for other disease processes or disease stages	

TABLE 4–2 REMARK Criteria for Reporting Biomarker Studies (*Adapted from McShane et al.*)[7]

	Item
INTRODUCTION	
1	State the marker examined, the study objectives, and any pre-specified hypotheses.
MATERIALS AND METHODS Patients	
2	Describe the characteristics (e.g., disease stage or comorbidities) of the study patients, including their source and inclusion and exclusion criteria.
3	Describe treatments received and how chosen (e.g., randomized or rule based).
Specimen characteristics	
4	Describe type of biological material used (including control samples) and methods of preservation and storage.
Assay methods	
5	Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality-control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint.
Study design	
6a	State the method of case selection, including whether prospective or retrospective, and whether stratification or matching (e.g., by stage of disease or age) was used.

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TABLE 4–2 REMARK Criteria for Reporting Biomarker Studies (*Adapted from McShane et al.*)[7], *Cont'd*

	Item
6b	Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.
7	Precisely define all the clinical endpoints examined.
8	List all candidate variables initially examined or considered for inclusion in models.
9	Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size.
Statistical analysis methods	
10	Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.
11	Clarify how marker values were handled in the analyses; if relevant, describe methods used for cutoff determination.
RESULTS Data	
12	Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the number of patients and the number of events.
13	Report distributions of basic demongraphic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumour marker, including numbers of missing values.
Analysis and presentation	
14	Show the relation of the marker to standard prognostic variables.
15	Present unvariable analyses showing the relation between the marker and outcome, with the estimated effect (e.g., hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumour marker on a time-to-event outcome, a Kaplan-Meier plot is recommended.
16	For key multivariable analyses, report estimated effects (e.g., hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.
17	Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.
18	If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation.
DISCUSSION	
19	Interpet the results in the context of the pre-specified hypotheses and other relevant studies; include a discussion of limitations of the study.
20	Discuss implications for future research and clinical value.

TABLE 4–3 Levels of Evidence for Grading Clinical Utility of Biomarkers (TMUGS) (*Adapted from Hayes et al.*)[8]

Level of evidence (LOE)	Type of evidence
1	Evidence from a single high-powered study that is specifically deisgned to test marker, or evidence from a meta-analysis and/or overview of Level 2 or 3 studies. In the former case, the study must be designed so that therapy and follow-up are dictated by protocol. Indeally, the study is a prospective randomized trial in which diagnostic and/or therapeutic clinical decisions in one arm are determined based at least in part on marker results, and diagnostic and/or therapeutic clinical decisions in the control arm are made independently of marker results. However, may also include prospective but not randomized trials with marker data and clinical outcome as primary objective.
2	Evidence from study in which marker data is determined in relationship to a prospective therapeutic trial that is performed to test therapeutic hypothesis but not specifically designed to test marker utility (i.e., marker study is secondary objective of protocol). However, specimen collection for marker study and statistical analysis are prospectively determined in protocol as secondary objectives.
3	Evidence from large but prospective studies from which variable numbers of samples are available or selected. Therapeutic aspects and follow-up of patient population may or may not have been prospectively dictated. Statistical analysis for tumour marker was not dictated prospectively at time of therapeutic trial design.
4	Evidence from small retrospective studies that do not have prospectively dictated therapy, follow-up, specimen selection, or statistical analysis. May be matched case controls, etc.
5	Evidence from small pilot studies designed to determine or estimate distribution of marker levels in sample population. May include "correlation" with other known or investigational markers of outcome, but not designed to determine clinical utlity.

4.3 Biomarkers According to Clinical Stages

4.3.1 Urinary biomarkers for screening and hematuria

4.3.1.1 Molecular urinary markers for diagnosis of bladder cancer

The utility of molecular urinary biomarkers for detection of urothelial carcinoma has been the focus of intense debate in the last decade. So far, no molecular urinary marker has been broadly implemented in current international guidelines and clinical practice. Various studies have shown that a few molecular markers provide improved sensitivity compared to cytology, but in most cases, these markers do not reach the specificity of cytology. One of the main reasons why none of the molecular urinary biomarkers has made its way into current routine clinical practice is the lack of data from prospective randomized trials. Most studies on urinary markers have used a case-control design bearing important limitations.

In patients without a history of BC, there are several scenarios in which urinary biomarkers may play a role. One frequently discussed scenario is the use of biomarkers for screening purposes. So far, the low prevalence of BC in the general population has been a challenge for developing effective screening strategies.[22–25] Therefore, the use of biomarkers for screening high-risk populations has been suggested. However, data from recent trials indicate that even in patients with a high risk of developing BC, such as heavy smokers or workers with occupational exposure to agents known to increase the risk for BC, the incidence of BC is so low that screening cannot be clearly recommended. A second clinical scenario where urinary biomarkers may prove valuable is in risk-stratifying patients with asymptomatic microscopic hematuria (AMH). In patients with asymptomatic gross hematuria, previous studies have suggested a significant risk for BC (approximately 10%), necessitating a cystoscopic work-up.[26] In these patients, urinary biomarkers may be discussed as an adjunct tool to cystoscopy, but likely will not impact the decision to evaluate the patient. In patients with AMH, international guidelines on the optimal work-up differ significantly. This is because there is a high prevalence of AMH in the adult population, ranging as high as 10% to 18%, yet only 2% of referred populations have BC. As many patients, especially women, with AMH are not adequately evaluated, the use of risk-stratification strategies, particularly those incorporating a marker, is appealing.[17,26–31] Whereas many case-control studies on the use of specific markers have included information on hematuria status of the individuals included, only limited prospective data is available on the use of molecular urinary markers in patients presenting with AMH.

4.3.1.2 Existing markers for diagnosis of bladder cancer in patients with hematuria

4.3.1.2.1 Introduction

Several urinary markers enable noninvasive diagnosis of BC. Compared to cytology, many of these markers have shown improved sensitivity, but worse specificity.[32] Both cellular and soluble markers exist. An overview of the characteristics of commercially available molecular urinary markers for detection of BC is reported in **Table 4-4**.

TABLE 4–4 Overview of Characteristics of Commercially Available Molecular Urinary Markers for Detection of Bladder Cancer

Marker or test	Manufacturer	Description	FDA status
AssureMDx	MDxHealth	Mutation analysis of FGFR3, TERT, and HRAS; methylation analysis of OTX1, ONECUT2, and TWIST1	
BLCA4	Eichrom Technologies	ELISA for nuclear membrane protein BLCA4	
BTA stat	Polymedco	POC test for complement factor H–related protein and complement factor H	Approved for diagnosis and follow-up
BTA TRAK	Polymedco	Quantitative ELISA; same targets as BTA stat	Approved for diagnosis and follow-up
Cxbladder Detect	Pacific Edge Cancer Diagnostics	PCR assay for detection of mRNA expression of 5 genes (CDC2, HOXA13, MDK, IGFBP5, and CXCR2)	
CYFRA 21-1	Roche Diagnostics	Electrochemiluminescence immunoassay for CK19 fragments	
NMP22 BladderChek	Alere	POC detection system for NMP22	Approved for diagnosis and follow-up
NMP22 ELISA	Alere	ELISA for quantitative analysis of NMP22	Approved for follow-up
UBC	IDL Biotech	ELISA for fragments of CK8 and CK18	
UBC *Rapid*	IDL Biotech	POC system for detection of fragments of CK8 and CK18	
uCyt+	Scimedx	Immunocytochemical assay for detection of expression of CEA and BC-associated mucins	Approved for follow-up
UroVysion	Abbott Molecular	Multicolour FISH assay for detection of numerical aberrations of chromosomes 3, 7, 17, and locus 9p21	Approved for diagnosis and follow-up
Xpert Bladder Cancer	Cepheid	PCR assay for mRNA expression analysis of 5-gene panel (CRH, IGF2, UPK1B, ANXA10, and ABL1)	

Abbreviations: ABL1, ABL proto-oncogene 1; ANXA10, annexin A10; BC, bladder cancer; BLCA-4, bladder cancer 4; BTA, bladder tumour antigen; CDC2, cell division control 2; CEA, carcinoembryonic antigen; CK18, cytokeratin 18; CK8, cytokeratin 8; CRH, corticotropin-releasing hormone; CXCR2, C-X-C motif chemokine receptor 2; CYFRA 21-1, cytokeratin 19 fragment; ELISA, enzyme-linked immunosorbent assay; FGFR3, fibroblast growth factor receptor 3; FISH, fluorescence in situ hybridization assay; HOXA13, homeobox A13; IGF2, insulin-like growth factor 2; IGFBP5, insulin-like growth factor binding protein 5; MDK, midkine; mRNA, messenger RNA; NMP22, nuclear matrix protein 22; ONECUT2, one cut homeobox 2; OTX1, orthodenticle homeobox 1; PCR, polymerase chain reaction; POC, point of care; TERT, telomerase reverse transcriptase; UBC, urinary bladder cancer; UPK1B, uroplakin 1B.

4.3.1.2.2 Cellular markers

4.3.1.2.2.1 Cytology

Cytology has a high specificity, but limited sensitivity in low-grade tumours. Moreover, high interobserver variability limits the performance of cytology. Recently, an international attempt has been made to standardize cytology by introducing the Paris system for reporting urinary cytology.[33] (**Table 4-4**)

4.3.1.2.2.2 Immunocytology (ImmunoCyt, uCyt+)

The ImmunoCyt and uCyt+ assays allow the detection of BC-associated antigens in addition to standard cytology. Antibodies target a high molecular-weight form of carcinoembryonic antigen (CEA) and BC-associated mucins.[34] Following staining, more than 500 nuclei are examined to study immunofluorescent cells. In most studies, specimens with at least one cell showing expression of one of the antigens are considered positive. Due to the time required for processing and reading, costs are relatively high.[35] In general, immunocytology has shown improved sensitivity compared to cytology.[35–37] Quite consistently throughout the studies, sensitivity is especially improved in low-grade tumours compared to conventional cytology.[35,36]

4.3.1.2.2.3 Fluorescence *in situ* hybridization (UroVysion)

The multitarget fluorescence *in situ* hybridization assay (FISH) UroVysion (Abbott Molecular, Des Plaines, Illinois, USA) enables the detection of chromosomal aberrations associated with BC in urine specimens. The test uses probes for chromosomes 3, 7, and 17, and for the locus-specific identifier (LSI) 9p21 to detect numeric aberrations. Different criteria with varying performance characteristics have been introduced.[38] In general, at least 25 cells are required to be evaluated to provide a test result. A sample is considered positive when 4 or more cells have a gain of two or more of chromosomes 3, 7, or 17, or when 12 or more cells exhibit loss of the 2 copies of LSI 9p21.

The test appears promising, both in primary diagnosis and surveillance cohorts, and has demonstrated a broad variability of both sensitivity and specificity. The assay has been suggested for use in patients with inconclusive findings from cytology.[39] A significant rate of false-positive results (independent of the criteria applied) must be taken into consideration. Various reports exist discussing anticipatory positive test results,[40,41] which suggest that a tumour may be detectable molecularly before it is seen visually. Up to now, the clinical implications of these findings are unclear, as it is difficult to change management based on this type of information. Disadvantages of the assay include its relatively high costs compared to conventional cytology. Due to the technical and personnel requirements, this assay is restricted to specialized laboratories. To reduce personnel costs and save time, automated approaches for cell analysis have been introduced.[42] Approximately 10% of tests end up being noninformative.[35]

4.3.1.2.2 Protein markers

4.3.1.2.2.1 Introduction

Several commercially available tests use protein markers, such as nuclear matrix protein 22 (NMP22) and bladder tumour antigen (BTA), associated with BC or high cell turnover. Both point-of-care tests and quantitative enzyme-linked immunosorbent assays (ELISA) exist. In the instances where markers are not expressed exclusively by malignant cells, there is a high rate of false-positive test results, especially in patients with benign disease conditions, such as stones or urinary tract infection. Moreover, hematuria has been shown to have a significant influence on test results. Due to the high rate of false-positive test results, indications for performing a cystoscopy based on a single positive protein marker must be considered critically.

4.3.1.2.2.2 Bladder tumour antigen tests

The point-of-care BTA stat test and the quantitative BTA TRAK ELISA (Polymedco Inc., Cortlandt Manor, New York, USA) detect the human complement factor H–related protein and complement factor H in urine samples. Both tests are approved by the FDA as adjuncts to cystoscopy. Whereas the BTA stat assay is easy and fast to perform, the BTA TRAK assay requires equipment used for ELISA testing. The threshold for a positive BTA TRAK result is 14 U/mL, as recommended by the manufacturer. Wide ranges of sensitivities and specificities have been reported.[35–37,43] Sensitivity is significantly dependent on tumour stage and grade.[36] Both for BTA stat and BTA TRAK, sensitivities are higher in cohorts where the markers are used for detection when compared to surveillance, which is likely a result of larger tumour volumes and a higher rate of high-stage and high-grade tumours in detection cohorts, compared to surveillance cohorts.[35,36] Similar to other protein markers, the results of BTA test platforms are affected significantly by the presence of blood in the urine and infection, thereby limiting their specificity.[44–46] When using exclusion criteria (such as no signs of infection, no prior instrumentation), test performance can be improved.[47]

4.3.1.2.2.3 Nuclear matrix protein 22

Nuclear matrix proteins are essential components of the nucleus and contribute to the shape of the nucleus. Nuclear matrix protein 22 (NMP22) has been shown to be overexpressed in malignant cells compared with benign urothelium, and is released by apoptotic cells. The assessment of NMP22 concentrations in urine can be performed qualitatively, using a point-of-care test (BladderChek, Alere, Waltham, Massachusetts, USA), or quantitatively, using an ELISA platform (Alere, USA). Whereas both tests have been approved by the FDA for use in BC surveillance, the point-of-care test has also been approved for use in patients without a history of BC who possess high-risk features. Studies have shown sensitivity with these point-of-care tests compared to cytology, especially in low-grade tumours;[35–37] however, their specificity is clearly lower than cytology, and different factors have been identified as causing false-positive test results, such as infection, stones, and prior instrumentation.[48] Exclusion of patients with these conditions improves specificity.[47] In a recently performed prospective trial involving 1,303 patients assessing various clinical variables, as well as NMP22 levels and cytology, NMP22 was the strongest predictor of BC presence, compared to cytology and clinical variables. The addition of NMP22 to a model that included clinical risk factors significantly improved the predictive accuracy of the model.[49]

4.3.1.2.3 Other non–FDA-approved urine markers

Numerous other marker systems have been studied, but they have not been approved by the FDA so far.

Concentrations of fragments of cytokeratin 8 (CK8) and cytokeratin 18 (CK18) in the urine appear higher in patients with BC. To assess these proteins, a qualitative point-of-care test and a quantitative ELISA (UBC®, UBC® *Rapid*, IDL Biotech, Bromma, Sweden) exist. The suggested cutoff value for the ELISA is 12 µg/L. A meta-analysis including 623 patients showed an overall sensitivity for the UBC *Rapid* test of 59.3%, with a specificity of 86.1%.[35]

The cytokeratin 19 fragment (CYFRA 21-1) assay allows detection of cytokeratin 19 fragment by ELISA technique. A recent meta-analysis that included various case-control studies showed an overall sensitivity and specificity of 82.0% and 80%, respectively, when detected in urine.[50] A uniform cutoff for a positive test has not been determined so far.

Bladder cancer 4 (BLCA-4) is a nuclear membrane protein with high expression in BC. A recent pooled analysis of existing case-control studies showed a sensitivity and specificity of 93% and 97%, respectively, but validation studies are required before a statement can be made on its clinical usefulness.[51]

Survivin is an inhibitor of apoptosis (IAP) that is overexpressed in BC cells.[52] Several techniques are available to measure survivin levels in urine. A commercially available dot-blot assay (Fujirebio Diagnostics, Tokyo, Japan) measures protein levels of survivin. Most studies use messenger RNA (mRNA) expression analysis to measure survivin. However, so far, different polymerase chain reaction (PCR) assays and primers have been used, such that comparing the studies is not feasible.[53]

The analysis of gene alterations frequently observed in BC provides an alternative approach for detection of BC. Fibroblast growth factor receptor 3 (FGFR3) mutations have been shown to occur frequently, especially in low-grade nonmuscle-invasive bladder cancer (NMIBC). Several assays for detection of FGFR3 mutations in urine samples have been developed. Whereas a couple of studies have shown that FGFR3 mutation analysis in urine samples of patients during surveillance is feasible and appears promising, limited data is available on the role of FGFR3 analysis in patients without a prior history of BC.[54–56]

By using personalized PCR assays designed to detect specific mutations occurring in the primary tumour, in combination with digital droplet PCR, the analysis of cell-free DNA in the urine has been recently shown to provide a potential approach for surveillance in patients with BC.[57]

Various multiplex mRNA assays have been introduced for urine-based detection of BC. The Cxbladder Detect™ (Pacific Edge Cancer Diagnostics Company, Dunedin, New Zealand) is designed to analyze the expression of five genes (cell division control 2 [CDC2], homeobox A13 [HOXA13], midkine [MDK], insulin-like growth factor binding protein 5 [IGFBP5], and C-X-C motif chemokine receptor 2 [CXCR2]) associated with BC. A study including 485 patients presenting with gross hematuria revealed a sensitivity of 82% at a predefined specificity of 85%.[58]

A platform combining DNA mutation analysis of FGFR3, telomerase reverse transcriptase (TERT), and HRas proto-oncogene, GTAPase (HRAS), and methylation analysis of orthodenticle homeobox 1 (OTX1), one cut homeobox 2 (ONECUT2), and twist family bHLH transcription factor 1 (TWIST1) (AssureMDx, MDxHealth, Irvine, California, USA) has shown high NPV in patients with hematuria in a discovery and validation study, and may provide an option for reducing unnecessary cystoscopies in patients with hematuria.[56,59]

Another platform (Xpert Bladder Cancer, Cepheid, Sunnyvale, California, USA) using gene expression analysis for corticotropin-releasing hormone (CRH), insulin-like growth factor 2 (IGF2), uroplakin 1B (UPK1B), annexin A10 (ANXA10), and ABL proto-oncogene 1 (ABL1) showed superior sensitivity compared to cytology and UroVysion in a validation study including 895 subjects with hematuria.[60] A prospective trial using this platform is ongoing. **Table 4-5** reports the performance characteristics of FDA-approved urinary markers for detection of BC.

4.3.1.3 Combination of urinary markers and reflex testing

Combining several urinary markers may improve the accuracy of urine-based detection of BC. The influence of urine-marker combinations on the performance of an assay not only depends on the assay combination, but also on the interpretation algorithm for the data. If only one urinary marker has to be positive for a combination of markers to be considered positive, the combination will inevitably have improved sensitivity, but decreased specificity compared to the single tests. Therefore, the analysis of urine-marker combinations should always assess the optimal cutoff for an individual combination to be considered positive. This can be done by using receiver operating characteristics analysis defining an optimal threshold in a test model. A study including 808 patients without a prior history of BC evaluated cytology, FISH, immunocytology, and NMP22. The authors showed that expedient combinations of these markers can improve accuracy compared to the single markers. However, to improve accuracy, specific two-, three-, and four-test combinations with one positive marker (mostly NMP22) had to be considered as a negative test result.[61] Similar results were obtained by Wild *et al.* who assessed combinations of cytology, microsatellite analysis (MA), and FGFR3 mutation analysis in 119 patients with suspected BC.[62] Using expedient combinations of markers, the area under the curve (AUC) of the authors' diagnostic model could be improved.

An alternative to simultaneous testing of several markers is the so-called reflex testing in patients with negative or atypical cytology. The idea behind this concept is to save costs incurred by applying molecular markers by using cytology in the first setting. Several studies have shown that application of a molecular marker as a second step in patients with atypical cytology shows high sensitivity, and therefore, has the potential to reduce unnecessary cystoscopies in patients with symptoms suggestive of BC.[63,64]

4.3.1.4 Use of molecular markers in patients with asymptomatic microscopic hematuria

Due to the increased prevalence of BC in patients with gross hematuria, painless gross hematuria is generally considered as an indication for cystoscopy.[35] The management of AMH is more controversial and international evaluation guidelines vary widely.[65–67] As such, there is an unmet need to improve risk-stratification approaches in these patients. At this time, there is a lack of prospective trials focusing solely on patients with AMH, and many studies include patients with gross and microscopic hematuria, thereby introducing an important bias.

Cha *et al.* performed a retrospective analysis of 1,182 patients with hematuria, including 68% with AMH, and evaluated cytology, imaging, cystoscopy, and immunocytology in all patients. Using multivariate analysis, a nomogram predicting the risk for BC was constructed showing a predictive accuracy of 90.8%. Within this nomogram, a positive immunocytology was the strongest predictor of presence of BC. Other factors independently associated with BC and therefore included in the nomogram were age, smoking history, and gross hematuria.[68] A validation of this nomogram in an independent cohort has not been performed so far.

Lotan and coworkers validated a nomogram incorporating age, gender, ethnicity, smoking history, type of hematuria, cytology, and NMP22 BladderChek in a cohort of 381 subjects with hematuria.[17] All patients underwent cystoscopy. In total, 23 patients (6.0%) had BC. The predictive accuracy of the nomogram was 80.2%.

In a cohort including 86 patients with microscopic hematuria and 83 patients with gross hematuria, Beukers *et al.* used methylation analysis of odd-skipped related transcription factor 1 (OSR1), single-minded family bHLH transcription factor 2 (SIM2), OTX1, Meis homeobox 1 (MEIS1), and ONECUT2 for developing a model for prediction of BC.[69] The model also included clinicopathologic characteristics, such as type of hematuria, age, gender, and cytology results. The final model including cytology yielded a sensitivity and specificity of 85% and 87%, respectively, with an AUC of 0.89. Of note, the model performed better in patients with gross hematuria compared with those with microscopic hematuria.[65]

The degree of microscopic hematuria has been shown to have an important influence on broadly available markers such as UroVysion, NMP22, and immunocytology. In a retrospective cohort, including 2,365 individuals with hematuria, sensitivity of the tests increased with higher grades of hematuria, but specificity decreased.[70]

Summary of the use of molecular urine markers in patients with microscopic hematuria

Whereas previous studies suggest that the use of molecular markers in patients with AMH adds benefit to risk stratification based on demographic and clinical variables, prospective trials are lacking and urgently needed.

4.3.1.5 Use of urine markers as adjunct to cystoscopy

Current evidence of urinary markers is insufficient to replace cystoscopy. Guidelines therefore currently recommend urinary markers as an adjunct to cystoscopy.[1] In this context, urinary markers provide several advantages. Recent data suggests that at least in patients undergoing cystoscopy for surveillance purposes, the awareness of a positive urine test improves the detection rate of BC. A prospective, single-blind, randomized, multicentre trial included 448 patients undergoing surveillance for BC. In the intervention arm, urologists performing cystoscopy were informed about the results of urine testing (MA), whereas no information was available in the control arm. Whereas 42 recurrences were detected in 131 cystoscopies of patients with positive markers who underwent cystoscopy by a urologist aware of the positive marker, only six recurrences were observed in 120 cystoscopies performed in the control arm (without information on the positive test results). This approach would require the performance of the urine marker prior to cystoscopic evaluation.

The use of urinary markers can also help predict the aggressiveness of a bladder tumour before transurethral resection. In a retrospective study including 502 patients with BC tested by cytology, FISH, NMP22, and immunocytology before cystoscopy, the presence of both a positive cytology and NMP22 was associated with a 20-fold risk for a high-grade lesion or CIS, and a 9-fold risk for MIBC. Similar results were observed by Shariat *et al.* in a study including 302 subjects with a history of BC who were tested by NMP22 and cytology. The presence of a positive cytology and NMP22 test was associated with a 33-fold risk of an invasive tumour (≥pT1) and a 21-fold risk of a grade 3 tumour.[71]

4.3.1.6 Potential role of urinary marker–based screening for bladder cancer

The concept of screening is based mainly on the diagnosis of cancer in an asymptomatic population. An effective screening program should provide a wide variety of features.[72] First, screening strategies should enable earlier disease detection, compared to a symptom-orientated diagnosis. Earlier disease detection and treatment should be associated with improved overall outcome of the disease,

such as a decrease in disease-specific mortality and an increase in overall survival. Any discussion of screening strategies should always include the potential risks and benefits of cancer screening. False-positive test results may lead to unnecessary and invasive diagnostic procedures (in the case of BC, cystoscopy). Moreover, a positive test result has been shown to raise patient anxiety. The detection of nonaggressive tumours may lead to overtreatment of disease, which may be less relevant for BC, as most cases become symptomatic.

The effectiveness of a screening program is significantly affected by the incidence and mortality of a specific disease. Regarding the number of newly detected cancer cases in the US, BC accounted for 7% of cancers in men in 2015[73] and was responsible for 4% of estimated cancer-related deaths in men. The relatively low incidence, the high rate of low-grade tumours, and the low mortality associated with these tumours significantly impact the potential effectiveness of a screening program for BC. Therefore, it has been frequently discussed if screening for BC should be limited to a population with a high risk of developing BC. Various endogenous and exogenous risk factors have been identified for the development of BC.[74] Male gender and increasing age represent two of these risk factors.[1] Exogenous risk factors include occupational and nonoccupational factors. The most important nonoccupational exogenous risk factor is smoking. Therefore, a couple of the studies listed below included smokers. Occupational risk factors include exposure to polycyclic aromatic hydrocarbons and aromatic amines.

The urine-based tests that have been investigated most extensively for their performance in a BC screening context are dipstick testing for hematuria and cytology. **Table 4-5** summarizes data from prospective trials assessing exclusively the use of dipstick and cytology in different contexts (screening of general populations and populations with risk factors). One of the largest trials of a population without occupational risk factors has been performed by Messing *et al.*[75] This study included 1,575 men who carried out daily dipstick testing for hematuria for a period of 14 consecutive days; those patients who had no positive findings continued testing for another 14 days after 9 months. Men with positive tests were advised to undergo cystoscopy, cytology, and upper-tract imaging. Findings of this cohort were compared to those of a cohort of 509 men of the Wisconsin Cancer Reporting System. Long-term follow-up showed that none of the men who had a cancer detected through screening died of BC, whereas the proportion of BC-specific deaths was 20.4% in the control group (p=0.02).[76] The proportion of high-grade invasive BCs was lower in screened men (10%) than in unscreened men (60%; p=0.002). The proportion of low-grade (52.4% vs. 60.3%) and high-grade tumours (47.7% vs. 39.7%) did not significantly differ between the intervention and control groups. In a British screening study performed by Britton *et al.*, 2,356 male individuals underwent dipstick testing for a period of 10 weeks.[77] Of 474 patients with a positive dipstick result, 17 patients were found to have BC (nine of them with high-grade disease). Of note, no case of muscle-invasive disease was detected. However, long-term outcomes of this trial reported five patients with progression from high-grade NMIBC to MIBC, as well as three cancer-specific deaths.[78] The results of these and other trials have not led to the implementation of a dipstick-based BC screening program. A recent Cochrane review aiming to quantify the benefits and risks of screening with urinary dipsticks, both in general populations and hospitalized patients, could not find sufficient evidence for an adequate assessment of benefits and risks of screening with urinary dipsticks.[79]

TABLE 4–5 Performance Characteristics of FDA-approved Urinary Markers for Detection of Bladder Cancer (data from meta-analyses)

Marker	Reference	No. of patients	No. of studies	Context	Sensitivity	Specificity	No. of patients with tumours
Immunocytology	Chou *et al.*[36]	1,876	7	Primary diagnosis	85% (78–90%)	83% (77–87%)	401
	Mowatt *et al.*[37]	4,199	10	Mixed	84% (77–91%)	75% (68–83%)	NA
	Schmitz-Dräger *et al.*[35]	4,899	20	Mixed	81% (median)	75% (median)	1,252
UroVysion	Chou *et al.*[36]	651	2	Primary diagnosis	73% (50–88%)	95% (87–98%)	144
	Mowatt *et al.*[37]	3,321	14	Mixed	76% (65–84%)	85% (78–92%)	NA
	Schmitz-Dräger *et al.*[35]	2,852	21	Mixed	72% (median)	80% (median)	792
NMP22	Chou *et al.*[36] (quantitative)	1,313	9	Primary diagnosis	67% (55–77%)	84% (75–90%)	368
	Chou *et al.*[36] (qualitative)	1,816	2	Primary diagnosis	47% (33–61%)	93% (81–97%)	145
	Mowatt *et al.*[37]	13,885	56	Mixed	68% (62–74%)	79% (74–84%)	NA
BTA	Chou *et al.*[36] (quantitative)	96	1	Primary diagnosis	76% (61–87%)	53% (38–68%)	49
	Chou *et al.*[36] (qualitative)	1,021	8	Primary diagnosis	76% (67–83%)	78% (66–87%)	372
	Guo *et al.*[43] (qualitative)	3,175	13	Mixed	67% (64–69%)	75% (73–77%)	NA

Abbreviations: BTA, bladder tumour antigen; FDA, Food and Drug Administration; NA, not available; NMP22, nuclear matrix protein 22.

The number of trials applying molecular markers in a screening population is limited. To date, there are no randomized, controlled trials. Trials have been performed both in patients with and without exogenous risk factors (such as smoking or occupational exposure). **Table 4-6** summarizes studies that include molecular markers in a screening context. The lack of a control arm significantly limits previous studies.

TABLE 4–6 Summary of Studies Assessing Hematuria or Cytology for Screening Purposes in Different Populations

Reference	No. of patients	Test	Study population	Positive tests N, %	No. of cystoscopies performed	Tumours N, %
Davies *et al.*[301]	4,636	Urine microscopy	Men	84, 1.8	-	3, 0.06
Messing *et al.*[75]	1,575	Dipstick	Men >50 years	258, 16.4	258	21, 1.3
Britton *et al.*[77]	2,365	Dipstick	Men >60 years	474, 20.1	317	17, 0.7
Crosby *et al.*[412]	541	Cytology	Workers exposed to aromatic amines	64, 11.8	24	7, 1.3
Ward *et al.*[413]	385	Dipstick, cytology	Workers exposed to benzidine derivatives	60, 15.6	200	3, 0.78

One of the largest trials using molecular markers in a general population without predefined exogenous risk factors was carried out in The Netherlands.[80] In this trial, 1,747 men underwent dipstick testing on 14 consecutive days. Of these, 409 men (23.4%) had a positive dipstick result. Three hundred and eighty-five men underwent subsequent testing for a panel of molecular markers, including NMP22, MA, FGFR3 mutation, SNaPshot Multiplex System (Thermo Fisher Scientific, Waltham, Massachusetts, USA) assay, and a custom methylation-specific multiplex ligation–dependent probe amplification (MLPA) (MRC-Holland, Amsterdam, The Netherlands) test. Of the men being further tested, 75 (18.3%) had at least one positive test result and were further investigated by cystoscopy. Overall, 14 patients (3.6%) had a positive NMP22, 33 (8.6%) tested positive for MA, six (1.6%) tested positive for FGFR3, and 40 (10.4%) tested positive for the methyl group CH3 by MLPA. In total, four BC cases and one renal tumour were detected. Patients with positive markers and negative cystoscopy were asked to undergo rescreening 6 months later. One BC case occurring within a year of screening was missed by the screening protocol.[81] Sensitivity of NMP22, the most broadly used marker of this panel, was 25%, whereas specificity was 96.6%. Although the use of urinary markers could significantly reduce the number of cystoscopies performed, the low incidence of tumours questions the benefit of such a screening protocol in an unselected asymptomatic population.

In a smaller study from Scandinavia, 1,096 men between 60 and 70 years of age performed urine dipstick testing and the urinary bladder cancer (UBC) antigen test.[82] The International Prostate Symptom Score was also used to identify patients with increased risk for BC. Of the seven tumours detected, five had a positive dipstick result, whereas two had a positive UBC test, but no hematuria. All tumours were detected in smokers. Fluorescence cystoscopy performed in the framework of this study did not lead to detection of additional tumours not detected by white-light cystoscopy.

Due to the low incidence of BC in a nonselected cohort without risk factors, various trials have assessed the use of molecular markers in screening studies for patients with at least one exogenous risk factor for BC development.

Limited data is available from screening studies in smokers. Lotan *et al.* assessed the value of screening using the NMP22 test in a high-risk population including 1,175 men and 327 women at age 50 years, based on a history of at least 10 years of smoking or an occupational exposure of at least 15 years to known carcinogenic substances. Eighty-five (5.7%) subjects had a positive NMP-22.[11] Of 69 patients undergoing further evaluation, only three (3.5%) participants had abnormal findings (one pTa low-grade tumour, one pTa high-grade tumour, and one atypia). After a median follow-up period of 12 months, two of the 1,309 (0.15%) participants developed low-grade noninvasive bladder cancer (NIBC). The long-term follow-up of a subpopulation of 925 subjects included at the Veterans Affairs Hospital showed that nine additional patients were diagnosed with BC during a median follow-up of 78.4 months.[83] Of note, no patient developed muscle-invasive cancer. A positive NMP22 was not associated with worse overall survival.

In a study including 183 heavy smokers with at least 40 pack-years, Steiner *et al.* performed dipstick, cytology, NMP22, and FISH tests. In this cohort, 75 subjects (40.9%) had at least one positive marker. Five cases of urothelial carcinoma and 12 potential precancerous lesions were detected.[84]

The impact of occupational exposure to carcinogens appears to have decreased due to improved work safety in modern societies. However, in developing countries, where significant exposure levels are not as strictly regulated, a screening program can still be discussed. Previous studies have shown that even in patients with evident exposure to carcinogenic substances, the incidence of BC is relatively low.

So far, the largest trial using molecular urine markers in a screening population with occupational risk factors was carried out in Germany.[85–87] During a period of more than 6 years, 1,722 chemical male workers previously exposed to aromatic amines were enrolled. Men were tested using quantitative NMP22, UroVysion, FISH, and cytology. In the case of a positive marker, cystoscopy was recommended. BC was detected in 14 men. The results of this study confirm the low prevalence of BC even in patients considered high risk due to occupational exposure.

A summary of the main studies assessing molecular tests for screening purposes in different populations is reported in **Table 4-7**.

4.3.1.7 International recommendations on screening for bladder cancer

The European Association of Urology (EAU) notes that routine screening for BC is not recommended.[1] The US Preventive Services Task Force states that currently, there is insufficient evidence to evaluate the harms and benefits of screening.[88] BC is not included in the list of cancers with a recommendation for screening by the American Cancer Society.[89]

4.3.1.8 Summary on screening using molecular markers

Studies on screening programs using molecular urine markers have been performed both in the general population and in risk populations, showing low prevalence rates of BC. Most studies using screening did not include a control group. So far, no clear evidence exists showing that the application of molecular markers in a screening setting affects cancer-specific mortality.

4.3.1.9 Recommendations

Due to the low Levels of Evidence (LOEs) provided, urinary markers are currently not recommended for BC screening BC or in patients with microscopic hematuria.

TABLE 4–7 Summary of Studies Assessing Molecular Tests for Screening Purposes in Different Populations

Reference	No. of patients	Test	Study population	No. of patients with tumours	Comments
Roobol *et al.*[80] Bangma *et al.*[81]	1,747	Dipstick followed by NMP22, MA, FGRF3, and methylation MLPA	Men	4	409 patients (23.4%) with positive dipstick; 75 of 385 patients (18.3%) with positive molecular marker; 4 tumours detected in 71 cystoscopies; 1 tumour missed by markers
Hedelin *et al.*[82]	1,096	Dipstick, UBC test	Men 60–70 years	7	174 positive tests; 5 of 7 tumours detected by hematuria; 2 of 7 positive for UBC, but not hematuria
Lotan *et al.*[412]	1,502	NMP22	Smokers (≥10 years), occupational exposure	2 (plus 1 atypia)	85 positive results (8.5%); 3 of 69 cystoscopies revealed abnormal findings; after a median FU of 78.4 months, no case of MIBC in subpopulation of 925 patients
Steiner *et al.*[84]	183	Dipstick, NMP22, cytology, FISH	Smokers (≥40 pack-years)	5 (3 BC cases + 2 UTUC cases)	75 patients with at least 1 positive marker (40.9%); at least 1 positive marker in all tumour cases
Pesch *et al.*[85,87]	1,609	Dipstick, NMP22, FISH, cytology	Men with occupational exposure (chemical workers)	20 (including 3 PUNLMP cases)	493 positive tests; 8 cases detected by cytology

Abbreviations: BC, bladder cancer; FGFR3, fibroblast growth factor receptor 3; FISH, fluorescence in situ hybridization; FU, follow-up; MA, microsatellite analysis; MIBC, muscle-invasive bladder cancer; MLPA, multiplex ligation–dependent probe amplification; NMP22, nuclear matrix protein 22; PUNLMP, papillary urothelial neoplasm of low malignant potential; UBC, urinary bladder cancer; UTUC, upper tract urothelial carcinoma.

4.3.2 Urinary biomarkers for surveillance

4.3.2.1 Surveillance markers

Despite management, NMIBCs recur and progress in up to 80% and 20% of cases, respectively.[90,91] Once diagnosed with NMIBC, patients are subjected to lifelong follow-up. Although low-risk tumours do not pose a significant threat, early detection of high-grade recurrence is of utmost importance, as delay in the diagnosis may be life-threatening. Surveillance policies vary substantially according to the risk category of NMIBC, with cystoscopy being the mainstay tool. It is recommended that cystoscopy should be complemented with voided urine cytology (VUC) in patients with high-risk disease.[92] If VUC suggests cancer recurrence and cystoscopy fails to diagnose underlying disease, then random bladder biopsies—ideally, using enhanced cystoscopy and upper urinary tract check-up—are recommended. But cystoscopy and urine cytology have their limitations. The former is invasive

and may miss significant portions of cancer recurrences, whereas the latter has low sensitivity in low- to intermediate-risk NMIBC, and is biased by considerable inter- and intraobserver variabilities, especially in patients after bacillus Calmette-Guérin (BCG) immunotherapy.[93,94] Therefore, there is a need for novel marker development that would improve surveillance of patients with NMIBC. The implementation of a novel marker may be perceived in two major categories of potential applications: 1) as an adjunct to cystoscopy or 2) as a substitute to cystoscopy. Furthermore, the role of a marker in clinical decision-making would vary in patients with low- to intermediate-risk NMIBC and in those with high-risk NMIBC. In the former group, a negative test result would supplement cystoscopy; in the latter group, an abnormal test result would increase awareness of patients and physicians, identify those at risk for progression, facilitate the interpretation of indeterminate results of VUC, and assess response to BCG.[95,96]

It has been shown that positive results of a marker test can increase tumour detection in subsequent cystoscopies.[93] The phenomenon is not surprising and would be beneficial in both applications. Once informed of an abnormal test result, the treating physician will search more closely for a tumour during cystoscopy. To supplant cystoscopy, markers should have high sensitivity and NPVs to reassure NMIBC patients that there is no disease if the result is negative. However, simultaneous low PPVs may lead to unnecessary work-ups. Currently, abnormal VUC with negative assessment of the bladder and upper urinary tract should prompt random biopsies of the bladder and the prostatic urethra with fluorescence guidance, if available.[92]

White-light cystoscopy is currently the gold standard for outpatient surveillance, but this procedure may miss lesions, especially CIS. Current instruments are flexible and have lower morbidity compared with their rigid counterparts. It has been reported that cystoscopy may be replaced by a marker, but only if the marker has high sensitivity. According to a recent survey, sensitivity should be as high as 90% to 95%.[97] A thorough systematic review of protein urine-based markers suggests that only NMP22, BTA, UBC, and CYFRA 21-1 are well validated, although they are less sensitive than cystoscopy.[98] Comparison of NMP22 with other urine-based tests, including Cxbladder Detect, UroVysion, FISH, and cytology, revealed that Cxbladder Detect and cytology were more effective than the other markers regarding sensitivity and specificity, respectively.[99] Indeed, a newly published, multicentre study has shown that the Cxbladder Monitor is highly sensitive in the diagnosis of recurrent BC.[100] Furthermore, the rate of false-negative results of the test does not exceed 1.5%. Both metrics are believed to be prerequisites for the test to supplant cystoscopy. One of New Zealand's publicly funded healthcare providers (Waitemata District Health Board) has accepted the Cxbladder Monitor as a substitute for cystoscopy in all low-risk NMIBC patients.

To present potential urine-based markers that might be used in the follow-up of NMIBC, a literature search through the PubMed database was performed with the following terms: "bladder cancer," "urinary marker," "surveillance," and "follow-up." The search was limited to clinical trials that specifically addressed the role of urinary markers in the follow-up of BC patients. Studies with low numbers of patients (fewer than 20) were not included in the analysis. In many protocols, heterogeneous patient populations were included. However, only those with separate presentations of outcomes concerning cohorts subjected to follow-up after previous BC diagnoses and management were scrutinized. Results from the analysis of available papers with complete data on the diagnostic performance of urinary markers are shown in **Table 4-8**.

Results indicate the following sensitivity ranges: VUC, 7% to 84%; NMP22, 11% to 85.7%; BTA stat and BTA TRAK, 56% to 73.7% and 9.3% to 91%, respectively; ImmunoCyt, 50% to 81%; UBC, 12.1% to 80%; CYFRA 21-1, 71.4%; FISH, 13% to 76%; and the Cxbladder Monitor in BC detection recurrence, 91% to 93%. Specificity ranges were recorded as the following: VUC, 62% to 99%; NMP22, 49% to 98.4%; BTA stat and BTA TRAK, 67.6% to 85.7% and 54% to 88.6%, respectively; ImmunoCyt, 63% to 75%; UBC, 79.2% to 97.2%; CYFRA 21-1, 68.6%; and FISH, 63% to 94.3% (**Table 4-8**).

TABLE 4–8 Diagnostic Performance of Selected Urinary Markers in the Follow-up of Patients With Nonmuscle-invasive Bladder Cancer

Reference	No. of pts	Rate of recurrence (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Additional comments
VUC							
Raitanen *et al.*[101]	445	26.5	19.2	98.3	82.8	73.6	31 patients with BTA-positive test were excluded from final analysis (no follow-up)
Casetta *et al.*[102]	102	68.6	70.0	75.0	85.9	53.3	Each patient had bladder biopsy
Miyanaga *et al.*[103]	57	45.5	7.0	97.9	60.0	69.7	
Raitanen[46]	510	26.5	19.2	85.7	-	-	
Lahme *et al.*[104]	44	-	-	88.6	-	-	
Pode *et al.*[105]	162	35.2	22.0	96.3	72.6 - accuracy		
Nisman *et al.*[106]	154	13.6	61.9	96.2	28.6	94.3	High NPV; false-positive results much higher after BCG
Babjuk *et al.*[107]	88	57.9*	19.8	99.0	89.5	74.9	*The rate of "positive" cystoscopies
Doğan *et al.*[108]	49	24.5	25.0	97.0	75.0	80.0	Histologically confirmed recurrence
García-Peláez *et al.*[109]	98	24.5	36.5	97.8	93.5	63.4	
Lotan *et al.*[110]	803	-	22.0	-	-	87.0	
Hosseini *et al.*[111]	144	36.1	44.2	83.7	60.5	72.6	
Sullivan *et al.*[112]	100	24.0	21.0	97.0	71.0	78.0	

Abbreviations: BCG, bacillus Calmette-Guérin; BTA, bladder tumour antigen; FISH, fluorescence in situ hybridization; NMIBC, nonmuscle-invasive bladder cancer; NMP22, nuclear matrix protein 22; NPV, negative predictive value; PPV, positive predictive value; UBC, urinary bladder cancer; VUC, voided urinary cytology.

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TABLE 4–8 Diagnostic Performance of Selected Urinary Markers in the Follow-up of Patients With Nonmuscle-invasive Bladder Cancer, *Cont'd*

Reference	No. of pts	Rate of recurrence (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Additional comments
Horstmann *et al.*[113]	221	51.1	84.0	62.0	69.0	79.0	Cost analysis of marker panel performed
Messing *et al.*[114]	327	15.4	23.0	93.0	-	-	
NMP22							
Casetta *et al.*[102]	102	68.6	64.0	64.0	78.3	45.2	
Miyanaga *et al.*[103]	57	45.5	18.6–48.8	85.1–66.0	36.4–39.6	69.6–73.8	Results dependent on cutoff values
Soloway *et al.*[115]	90	12.2	51.5–69.7	78.5–91.1	57.5–70.8	81.8–86.1	Results dependent on cutoff values of the test; test done only once, no sooner than 5 days after resection
Lahme *et al.*[104]	44	-	-	65.9	-	-	Sensitivity increases with grade and stage of NMIBCa
Serretta *et al.*[116]	137	30.6	71.5	61.0	44.7	82.8	Previous grade or stage of NMIBC did not influence NMP22 levels; histologically confirmed recurrence of NMIBC
Coşkuner *et al.*[117]	95	-	44.4	98.4	80.0	92.6	
Doğan *et al.*[108]	49	24.5	33.0	76.0	31.0	78.0	Histologically confirmed recurrence of NMIBC; combination with VUC no better results
Lotan *et al.*[110]	803	-	11.0 (BladderChek) - 26.0 (ELISA)	-	-	86.0 (BladderChek) - 87.0 (ELISA)	
Hosseini *et al.*[111]	144	36.1	78.8	69.6	59.4	85.3	
Horstmann *et al.*[113]	221	51.1	68.0	49.0	57.0	60.0	Cost analysis of marker panel performed

Abbreviations: BCG, bacillus Calmette-Guérin; BTA, bladder tumour antigen; FISH, fluorescence in situ hybridization; NMIBC, nonmuscle-invasive bladder cancer; NMP22, nuclear matrix protein 22; NPV, negative predictive value; PPV, positive predictive value; UBC, urinary bladder cancer; VUC, voided urinary cytology.

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TABLE 4–8 Diagnostic Performance of Selected Urinary Markers in the Follow-up of Patients With Nonmuscle-invasive Bladder Cancer, *Cont'd*

Reference	No. of pts	Rate of recurrence (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Additional comments
Giannopoulos *et al.*[118]	95	52.6	56.0	81.5	-	-	
Shariat *et al.*[119]	2,871	36.4	57.0	81.0	64.0	77.0	At cutoff 10 U/mL
Grossman *et al.*[120]	668	15.4	49.5	87.3	-	-	
Gupta *et al.*[121]	145	38.6	85.7	77.5	70.6	89.6	
BTA stat							
Raitanen *et al.*[101]	445	26.5	56.0	85.7	63.1	81.7	The test was performed repeatedly before cystoscopy, in case of BTA-positive and VUC-negative results – upper tract control
Pode *et al.*[105]	162	35.2	73.7	67.6	69.8 - accuracy		Combined with VUC did not improve accuracy; sensitivity worse during surveillance; recurrent lesions smaller than primary lesions
Raitanen *et al.*[46]	510	26.5	56.0	85.7	42.5	81.4	9 tumours were found through the test; results were affected by a history of intravesical therapy
Giannopoulos *et al.*[118]	95	52.6	72.0	77.8	-	-	
Lokeshwar *et al.*[122]	70		60.7	74.1	87.9	37.7	
BTA TRAK							
Casetta *et al.*[102]	102	68.6	60.0	60.0	75.9	39.6	Each patient underwent bladder biopsy
Babjuk *et al.*[107]	88	57.9*	38.5–53.8	83.9–88.6	58.6	81.1	*The rate of "positive" cystoscopies

Abbreviations: BCG, bacillus Calmette-Guérin; BTA, bladder tumour antigen; FISH, fluorescence in situ hybridization; NMIBC, nonmuscle-invasive bladder cancer; NMP22, nuclear matrix protein 22; NPV, negative predictive value; PPV, positive predictive value; UBC, urinary bladder cancer; VUC, voided urinary cytology.

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TABLE 4–8 Diagnostic Performance of Selected Urinary Markers in the Follow-up of Patients With Nonmuscle-invasive Bladder Cancer, *Cont'd*

Reference	No. of pts	Rate of recurrence (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Additional comments
Miyanaga *et al.*[103]	57	45.5	9.3	86.2	23.5	67.5	Sensitivity depended on tumour size; recurrent lesions much smaller than primary lesions
UK (BTA, BARD UK)[123]	272	37.5	58.0	86.0	-	-	
Ianari *et al.*[124]	75	17.3	91.0	54.0	-	-	
Immunocyt							
Vriesema *et al.*[125]	104	25.6	50.0	73.0	39.0	81.0	18 patients excluded from the analysis (low cellularity of samples); significant interobserver variability
Sullivan *et al.*[112]	100	24.0	76.0	63.0	43.0	88.0	
Horstmann *et al.*[113]	221	51.1	73.0	72.0	72.0	74.0	Cost analysis of marker panel performed
Messing *et al.*[114]	327	15.9	81.0	75.0	38.0	95.0	
UBC test							
Mungan *et al.*[126]	101	28.7	20.7	79.2–84.7	28.6–35.3	71.3–72.6	Histologically confirmed recurrence
Sánchez-Carbayo *et al.*[127] (CK18)	104	29.8	77.4	86.3	76.8	81.9	
Babjuk *et al.*[107]	88	57.9*	12.1	97.2	64.8	72.3	*The rate of "positive" cystoscopies
Giannopoulos *et al.*[118]	95	52.6	80.0	88.9	-	-	
CYFRA 21-1							
Nisman *et al.*[106]	154	13.6	71.4	68.6	14.2	97.1	High NPV; rate of false-positive results much higher after BCG

Abbreviations: BCG, bacillus Calmette-Guérin; BTA, bladder tumour antigen; FISH, fluorescence in situ hybridization; NMIBC, nonmuscle-invasive bladder cancer; NMP22, nuclear matrix protein 22; NPV, negative predictive value; PPV, positive predictive value; UBC, urinary bladder cancer; VUC, voided urinary cytology.

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TABLE 4–8 Diagnostic Performance of Selected Urinary Markers in the Follow-up of Patients With Nonmuscle-invasive Bladder Cancer, *Cont'd*

Reference	No. of pts	Rate of recurrence (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Additional comments
FISH							
García-Peláez *et al.*[109]	98	24.5	64.2	89.9	85.0	73.9	
Lotan *et al.*[110]	145	-	33.0	-	-	92.0	
Sarosdy *et al.*[128]	176	-	71.0	65.8	53.0	80.7	Low rate of false-negative results in BCG-treated patients
Sullivan *et al.*[112]	100	24.0	13.0	90.0	33.0	72.0	
Horstmann *et al.*[113]	221	51.1	76.0	63.0	68.0	71.0	Cost analysis of marker panel performed
Gudjónsson *et al.*[129]	159	17.0	30.0	95.0	71.0	-	
Youssef *et al.*[130]	123*	13.8	23.5	94.3	40.0	88.5	100% sensitivity and 100% NPV in those with equivocal cytology* and negative cystoscopy (*atypical cytology only)
Cxbladder Monitor							
Lotan *et al.*[110]	803	-	91.0	-	-	96.0	High (100%) sensitivity maintained in patients after BCG
Kavalieris *et al.*[100]	763	15.1	93.0	-	-	97.0	Sensitivity not affected by BCG; low false-negative results in all patient subgroups

Abbreviations: BCG, bacillus Calmette-Guérin; BTA, bladder tumour antigen; FISH, fluorescence in situ hybridization; NMIBC, nonmuscle-invasive bladder cancer; NMP22, nuclear matrix protein 22; NPV, negative predictive value; PPV, positive predictive value; UBC, urinary bladder cancer; VUC, voided urinary cytology.

Not surprisingly, VUC has lower sensitivity than other markers in the diagnosis of low-grade lesions, but greater specificity than other tests. At the same time, other markers provide lower specificity in the diagnosis of high-grade lesions when compared to urine cytology (**Tables 4-4 and 4-5**). Recurrent tumours are usually smaller and of lower grade when compared to primary tumours. Therefore, trials with cohorts of patients with primary diagnosis and those subjected to surveillance clearly suggest that the markers have worse diagnostic accuracy when implemented in the latter group than in the former cohort.[108,131]

Adjuvant therapy commonly implemented in BC patients who have coexisting inflammation significantly hampers the performance of the majority of tests mentioned in **Table 4-8**.[106] FISH and Cxbladder Monitor accuracy was shown to be high, regardless of former BCG therapy.[100, 110, 128]

Investigators assessed the value of a panel of markers analyzed before cystoscopy instead of selecting only one marker. It has been shown that this approach—combining VUC with other tests—does not confer additional advantages, and the majority of patients with high-grade disease were diagnosed with both tests simultaneously.[105,108] Another approach toward improving surveillance protocols of NMIBC and optimizing costs is known as reflex testing. In patients with negative results from one test, follow-up accuracy is significantly increased by adding a subsequent, highly sensitive marker instead of simultaneous analysis of a panel of markers. Combining two among the four tests—VUC, immunocytology, FISH, and NMP22—results in sensitivity and NPV of no greater than 89.8% (ImmunoCyt and NMP22) and 92.1% (FISH and ImmunoCyt).[132] If VUC is supplemented with any of the four tests, corresponding values are no greater than 86.7% (NMP22) and 91.3% (immunocytology). Adding FISH to conventional urine cytology is associated with sensitivity of 80.5% (94.0% for high-risk tumours) and an NPV of 90.1% (98.8% for high-risk tumours).

TABLE 4–9 Sensitivity of Selected Urine-based Tests According to Tumour Grade in Follow-up of Patients With Nonmuscle-invasive Bladder Cancer

Reference	No. of patients	Sensitivity (%)
VUC		
Raitanen[46]		
Grade 1	48	12.5
Grade 3	3	100
Lahme *et al.*[104]		
Grade 1	-	20.0
Grade 3	-	66.7
Pode *et al.*[105]		
Grade 1	25	4.0
Grade 3	45	75.5
García-Peláez *et al.*[109]		
Low grade	31	16.0
High grade	53	51.0
Casetta *et al.*[102]		
Grade 1	-	53.3
Grade 3	-	80.6

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TABLE 4–9 Sensitivity of Selected Urine-based Tests According to Tumour Grade in Follow-up of Patients With Nonmuscle-invasive Bladder Cancer, *Cont'd*

Reference	No. of patients	Sensitivity (%)
Raitanen *et al.*[101]		
Grade 1	48	12.5
Grade 3	3	100
Sarosdy *et al.*[128]		
Grade 1	22	18.0
Grade 3	17	41.0
Hosseini *et al.*[111]		
Grade 1	6	0
Grade 3	16	93.8
Horstmann *et al.*[113]		
Grade 1	32	57.0
Grade 3	28	96.0
Messing *et al.*[114]		
Grade 1	28	7.0
Grade 3	6	67.0
NMP22		
Lahme *et al.*[104]		
Grade 1	-	25.0
Grade 3	-	100
Casetta *et al.*[102]		
Grade 1	-	53.3
Grade 3	-	74.2
Coşkuner *et al.*[117]		
Low grade	8	0
High grade	10	80.0
Hosseini *et al.*[111]		
Grade 1	6	68.8
Grade 3	16	81.3

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TABLE 4–9 Sensitivity of Selected Urine-based Tests According to Tumour Grade in Follow-up of Patients With Nonmuscle-invasive Bladder Cancer, *Cont'd*

Reference	No. of patients	Sensitivity (%)
Horstmann *et al.*[113]		
Grade 1	32	64.0
Grade 3	28	65.0
Grossman *et al.*[120]		
Grade 1	38	31.6
Grade 3	32	75.0
Gupta *et al.*[121]		
Low grade	27	81.1
High grade	14	84.6
Shariat *et al.*[119]		
Grade 3		75 (at 10 U/mL cutoff point)
BTA stat		
Raitanen *et al.*[46]		
Grade 1	48	47.9
Grade 3	3	100
Pode *et al.*[105]		
Grade 1	25	40.0
Grade 3	45	100
Raitanen *et al.*[101]		
Grade 1	48	47.9
Grade 3	3	100
Sarosdy *et al.*[128]		
Grade 1	22	27.0
Grade 3	18	72.0
BTA TRAK		
Casetta *et al.*[102]		
Grade 1	-	60.0
Grade 3	-	71.0

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TABLE 4–9 Sensitivity of Selected Urine-based Tests According to Tumour Grade in Follow-up of Patients With Nonmuscle-invasive Bladder Cancer, *Cont'd*

Reference	No. of patients	Sensitivity (%)
Immunocyt		
Horstmann *et al.*[113]		
Grade 1	32	62.0
Grade 3	28	72.0
Messing *et al.*[114]		
Grade 1	28	79.0
Grade 3	6	67.0
FISH		
García-Peláez *et al.*[109]		
Low grade	31	55.0
High grade	53	89.0
Sarosdy *et al.*[128]		
Grade 1	22	55.0
Grade 3	18	94.0
Horstmann *et al.*[113]		
Grade 1	32	54.0
Grade 3	28	83.0
Cxbladder Monitor		
Kavalieris *et al.*[100]		
Low grade	78	85.0
High grade	72	97.0

The EAU, the American Urological Association (AUA), and the Society of Urologic Oncology (SUO) do not recommend urinary markers in the routine surveillance of patients with NMIBC.[3,92] According to AUA/SUO Guidelines, "Clinicians may use biomarkers to assess response to intravesical BCG (UroVysion® FISH) and adjudicate equivocal cytology (UroVysion® FISH and ImmunoCyt®)."[3] Serial measurements of UroVysion FISH in patients undergoing BCG therapy revealed that abnormal test results at baseline (before BCG), at 6 weeks (before the sixth BCG instillation), and before 3 months' cystoscopy (before the first maintenance course) are significantly associated with both cancer recurrence and progression. Based on FISH results at 3 months' cystoscopy, cancer progressed after 2 years in half of those with positive test results and in only 3% of those who had normal results.[133] The authors proposed the term "molecular BCG failure" for patients with a negative cystoscopy with abnormal FISH findings, and suggested that these patients might be good candidates for clinical trials, as they had worse outcomes with repeated BCG therapy.

Immunotherapy is known to evoke inflammatory changes within the bladder, often making reliable assessment of lower urinary tract challenging. As such, accuracy of VUC used as an adjunct to cystoscopy to increase the detection of CIS or upper tract lesions is hampered by BCG. Atypical cytology creates a significant dilemma for both the patient and the physician when cystoscopy reveals no abnormal findings that would explain an equivocal result. In such cases, guidelines recommend random biopsies of the bladder or observation, but these options may miss high-grade cancer. Researchers investigated FISH and ImmunoCyt in patients with atypical cytology.[134,135] UroVysion FISH has demonstrated 100% sensitivity and 100% NPV in patients with negative cystoscopy, but equivocal VUC.[39] A cost analysis revealed that the decision to omit bladder biopsy when UroVysion FISH is negative in patients with atypical cytology, and negative or equivocal cystoscopy, is cost-effective, and may benefit the American healthcare system.[136] ImmunoCyt was found to have 73% sensitivity in detecting recurrent bladder tumours in patients with atypical cytology with a corresponding NPV of 80%.[63] Interestingly, the test performance did not differ significantly in patients with a history of low- versus high-grade disease. Both tests are recognized by the AUA and SUO as potential reflex markers to adjudicate atypical cytology and avoid unnecessary work-up.

There are multiple published studies addressing the potential role of novel urine-based markers in the detection of BC recurrence. Many new candidates remain under evaluation, but the great majority require validation, and the presentation of entire panels expands beyond the limits of this section. Furthermore, to change the surveillance paradigm and revolutionize its pattern, the role of these markers should be verified in randomized trials. The first such trial investigated the possibility of MA to reduce cystoscopy rate in low- to intermediate-risk NMIBC.[93] The results were disappointing, but provided an example that is worth following.

Among markers analyzed, Cxbladder Monitor has the potential to replace cystoscopy in low- to intermediate-risk NMIBC. No marker has been evaluated sufficiently to reduce the frequency of cystoscopies in high-risk cancers. However, FISH provides prognostic information in patients undergoing BCG, and together with ImmunoCyt, is shown to be helpful for patients with atypical cytology. Neither the Cxbladder Monitor nor FISH performance is affected by immunotherapy.

4.3.2.2 Recommendations

In the surveillance of patients with NMIBC, urinary markers should not be used to replace cystoscopy. Urinary markers can be used to assess the response to intravesical immunotherapy and as a reflex test for equivocal urinary cytology (Expert Opinion).

4.3.3 Tissue biomarkers for nonmuscle-invasive bladder cancer

4.3.3.1 Introduction

Bladder cancer is burdened by the highest per-person lifetime treating cost of all cancers. This is due mainly to its high recurrence rate, and the consequent need for frequent and long-term follow-up schedules. Intensity of follow-up varies, depending on the risk profile of each patient: for high-risk NMIBC, international guidelines currently recommend cystoscopy and VUC every 3 months for 2 years, then every 6 months for 5 years, then yearly.[92] This leads to a non-negligible morbidity rate and decreased quality of life.

Tissue biomarkers can theoretically be used in NMIBC to predict oncological outcomes, such as recurrence-free survival (RFS) and progression-free survival (PFS), as well as the response to intravesical BCG, and they may be used to improve the predictive accuracy of currently existing risk-stratification systems. Ideally, tissue biomarkers could improve individualized treatment and surveillance based on risk of recurrence and progression. Moreover, they can be useful for identifying the proportion of high-risk NMIBC that will progress to invasive disease, thus leading them to early cystectomy.

Markers associated with pathways important for tumour growth and spread have been evaluated, including cycle-cell regulators; angiogenesis, apoptosis, and signalling proteins; and hormonal receptors. However, to date, there is insufficient evidence to recommend use of tissue markers in clinical practice, and more research is needed to determine their role in improving the predictive accuracy of currently available tools.

The detailed role of each tissue biomarker in NMIBC is reported and summarized in **Tables 4-10 to 4-17**. For the purposes of this chapter, a literature review was performed and only studies exclusively focusing on NMIBC patients were used.

4.3.3.2 Cycle-cell regulation

The cell cycle is a regulated and coordinated pathway that can be arrested at several points as the result of cell stress, in order to prevent carcinogenesis. These steps are regulated mainly by cyclins, proteins that activate cyclin-dependent kinases. Cyclins are responsible for retinoblastoma phosphorylation, which is a key factor in the progression of the cell cycle from grade 1 to S phase. Inhibitors of cyclin-dependent kinases, such as p21 and p27, prevent progression through the cell cycle.[137] As a "guardian of the genome," p53 induces cell-cycle arrest in response to cell stress.[138] Mutations in cycle-cell genes are the most common alterations in the majority of cancers and one of the first steps in cell carcinogenesis.

4.3.3.2.1 p53

The protein p53 is the product of the TP53 gene, the most common oncosuppressor gene mutated in all human cancers. The p53 protein can be activated by many different stress signals, such as oncogene activation, genotoxic and ribosomal stress, and DNA damage. This leads to an irreversible exit from the cell cycle or activation of cell death, in order to prevent cancer transformation.[139] TP53 mutations lead to a protein loss of function, and can be detected with PCR or through immunohistochemistry (IHC) as protein overexpression.

Different groups of clinical researchers have documented a significant correlation between altered patterns of p53 expression and poor outcomes in BC patients[140–147] (**Table 4-10**). Moreover, p53 mutations appear to be related to features of tumour aggressiveness at presentation, such as high stage, high grade, and lymphovascular invasion (LVI). Interestingly, p53 is also found to be mutated in normal mucosa of patients with NMIBC who experience recurrence, probably due to premalignant alterations in tumour-surrounding areas.[148] However, several other studies have not identified an association between p53 status and oncological outcomes of NMIBC.[149–156] In 1999, Liukkonen *et al.* reported the findings of the first randomized study investigating the role of p53, MIB1, mitotic index, and epidermal growth factor receptor (EGFR) in 207 patients

with NMIBC. At multivariable analyses, MIB1 and mitotic index, but not p53, were associated with progression.[157] These discrepancies may be related to nonstandardized immunohistochemical techniques, such as variability in the antibodies used, stratification criteria, and inconsistencies in specimen handling.

Prognostic markers such as p53 could be particularly helpful in selecting for early cystectomy high-risk NMIBC patients who will progress to muscle-invasive disease. p53 has shown the ability to identify the most aggressive T1G3 cancers.[146] Moreover, when examining the role of p53 combined with the level of lamina propria invasion in T1 patients, it was found that p53 status and T1c stage were the only independent predictors of survival, and that patients with T1c stage, and those with T1b and p53 mutation should be considered for immediate radical cystectomy.[144] However, these promising findings need validation in larger prospective trials.

TABLE 4–10 Studies Evaluating the Role of p53 Mutation in Patients With Nonmuscle-invasive Bladder Cancer

Reference	No. of patients	Population	% of cells with mutations	Cutoff used	Outcomes investigated	Method used
Sarkis *et al.*[140]	43	T1	58	20%	Association with Prog	IHC
Sarkis *et al.*[141]	54	Ta	22	20%	Association with Prog and CSM	IHC
Serth *et al.*[142]	69	Ta-T1	20	20%	Association with Prog	IHC
Kuczyk *et al.*[143]	41	Ta-T1	20	20%	Association with Prog	IHC
Tetu *et al.*[150]	265	Ta-T1	15	50%	No association with Prog	IHC
Lacombe *et al.*[158]	98	Ta-Tis-T1	-	-	Association with Prog and predictor of BCG response	IHC
Burkhard *et al.*[151]	46	Ta-T1	78 35	20% 50%	No association at both cutoffs	IHC
Çalişkan *et al.*[159]	30	Ta-T1	20	20%	Predictor of BCG response	IHC
Hermann *et al.*[144]	143	T1	42	20%	Association with survival	IHC
Pages *et al.*[163]	43	T1	63	10%	Not a predictor of BCG response	IHC
Zlotta *et al.*[164]	47	Ta-Tis-T1	45	10%	No association with Rec or Prog in patients treated with BCG	IHC
Tzai *et al.*[216]	100	Ta-T1	7	-	Not a predictor of intravesical CT response	IHC

Abbreviations: BCG, bacillus Calmette-Guérin; CSM, cancer-specific mortality; CT, chemotherapy; DFS, disease-free survival; IHC, immunohistochemistry; MVA, multivariable analysis; Prog, progression; Rec, recurrence.

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TABLE 4–10 Studies Evaluating the Role of p53 Mutation in Patients With Nonmuscle-invasive Bladder Cancer, *Cont'd*

Reference	No. of patients	Population	% of cells with mutations	Cutoff used	Outcomes investigated	Method used
Ye *et al.*[190]	43	Ta-T1	26	-	Association with Rec in patients treated with intravesical CT	PCR
Lebret *et al.*[165]	35	T1G3	-	Incre-mental	Not a predictor of BCG response	IHC
Pfister *et al.*[160]	60	Ta-T1	27	-	Predictor of BCG response	PCR
Liukkonen *et al.*[157]	207	Ta-T1	-	20%	No association with Prog	IHC
Pfister *et al.*[149]	244	Ta-T1	19	5%	No association with Rec	IHC
Wu *et al.*[152]	93	Ta-T1 LG	70	20%	No association with Rec	IHC
Llopis *et al.*[145]	80	T1	-	-	Association with survival	IHC
Gontero *et al.*[153]	192	Ta-T1	13	20%	No association with Rec	IHC
Shariat *et al.*[154]	36	T1	72	10%	No association with Prog or survival	IHC
Friedrich e*t al.*[217]	40	Ta-T1	25 70	- 5%	Association with Rec only in IHC	PCR IHC
Wolf *et al.*[146]	30	T1G3	-	-	Association with DFS	IHC
Peyromaure *et al.*[166]	29	T1G3	62	20%	No association with Rec or Prog in patients treated with BCG	IHC
Gil *et al.*[155]	67	High risk Ta-T1	60	20%	No association with Prog or survival	IHC
Shiraishi *et al.*[218]	70	Ta-T1	23	-	Predictor of intravesical CT response	IHC
Saint *et al.*[161]	102	High risk Ta-T1	24	20%	Association with Rec and predictor of BCG response	IHC
López Beltrán *et al.*[156]	159	Ta-T1	34	6%	No association with survival	IHC
Hitchings *et al.*[147]	78	Ta-T1	45	20%	Association with Rec in T1	IHC
Esuvaranathan *et al.*[167]	80	Ta-T1	39	50%	No association with BCG response	IHC
Oderda *et al.*[219]	192	Ta-T1	-	20%	Inverse association with Rec in BCG-treated patients	IHC

Abbreviations: BCG, bacillus Calmette-Guérin; CSM, cancer-specific mortality; CT, chemotherapy; DFS, disease-free survival; IHC, immunohistochemistry; MVA, multivariable analysis; Prog, progression; Rec, recurrence.

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TABLE 4–10 Studies Evaluating the Role of p53 Mutation in Patients With Nonmuscle-invasive Bladder Cancer, *Cont'd*

Reference	No. of patients	Population	% of cells with mutations	Cutoff used	Outcomes investigated	Method used
Hegazy *et al.*[162]	88	Ta-T1	50	20%	Association with Rec and Prog after BCG	IHC

Abbreviations: BCG, bacillus Calmette-Guérin; CSM, cancer-specific mortality; CT, chemotherapy; DFS, disease-free survival; IHC, immunohistochemistry; MVA, multivariable analysis; Prog, progression; Rec, recurrence.

The ability of p53 mutational status to predict response to BCG is controversial. While some studies suggest that p53 mutational status can predict response in high-risk NMIBC,[158–162] the majority of recently published trials do not confirm these findings.[163–167]

Finally, two meta-analyses summarizing the role of p53 in NMIBC have recently been published.[168,169] Du *et al.* focused on the role of p53 in T1 patients and reported that p53 overexpression predicts progression in this group of patients, even if heterogeneity of the included studies and limitations related to IHC limited the results.[168] Zhou *et al.* reviewed the literature of patients treated with BCG and confirmed that p53 mutation is not associated with any oncological outcomes in NMIBC patients treated with BCG.[169] Therefore, to date, p53 should not be used to test susceptibility to BCG in NMIBC patients.

4.3.3.2.2 Retinoblastoma

Retinoblastoma (Rb) tumour suppressor is of fundamental importance for the cell cycle. It plays a role in stem-cell maintenance, tissue regeneration, differentiation, and developmental programs.[137] Rb negatively regulates cell-cycle progression from G1 to S phase by interacting with the E2F family of transcription factors, and with chromatin remodellers and modifiers, thus contributing to the repression of genes important for cell-cycle progression. Rb is mutated or functionally inactivated in the majority of human cancers.[170] Rb alterations are detected at IHC as an absence of Rb expression or strong overexpression.[171]

Only a few studies have reported on the role of Rb-altered expression in NMIBC patients. These are summarized in **Table 4-11**. Altered Rb expression has been reported in more than 30% of BC patients[172,173] and is observed in 10%[172] to 22%[173] of Ta-T1 tumours. Tetu *et al.* [150] analyzed the Rb profile in 74 specimens of Ta-T1 BC patients, but failed to show an association between altered Rb expression and oncological outcomes, such as recurrence and progression. Conversely, other studies showed an association between Rb loss of expression and outcomes,[174] even if this was mainly observed only at univariable analyses and not confirmed after adjusting for classical prognosticators.[175] More recently, it has been shown that Rb could have predictive value for BC recurrence and progression, but only when combined with other biomarkers, such as p53 and p27,[171] in a panel of biomarkers predictive tool.

TABLE 4–11 Studies Evaluating the Role of Retinoblastoma-altered Expression in Patients With Nonmuscle-invasive Bladder Cancer

Reference	No. of patients	Population	% of cells with mutations	Cutoff used	Outcomes investigated	Method used
Tetu *et al.*[150]	74	Ta-T1	16	<10% or >50%	No association with Rec or Prog	IHC
Cordon-Cardo *et al.*[174]	59	Ta-T1	19	0%	Association with Prog and OS	IHC
Grossman *et al.*[220]	45	T1	42	Absent or strongly homogeneous	Association with Prog	IHC
Korkolopoulou *et al.*[175]	118	Primary BC	78	<50%	Association with OS only at UVA, not at MVA	IHC
Hitchings *et al.*[147]	78	Ta-T1	30	0% or >50%	No association with outcomes	IHC
Shariat *et al.*[171]	74	Ta-Tis-T1	39	0% or >50%	Association with Prog only at UVA, not at MVA	IHC
Esuvaranathan *et al.*[167]	80	Ta-T1	40	<6% or >50%	Underexpression associated with nonresponse to BCG + IFN	IHC
Cormio *et al.*[176]	27	T1G3	52	0% or >50%	Association with Rec and Prog in BCG-treated patients	IHC
Sato *et al.*[177]	27	CIS	41	5% or >50%	Association with nonresponse to BCG	IHC

Abbreviations: BC, bladder cancer; BCG, bacillus Calmette-Guérin; IHC, immunohistochemistry; IFN, interferon; MVA, multivariable analysis; OS, overall survival; Prog, progression; Rec, recurrence; UVA, univariable analysis.

The role of Rb-altered expression in predicting response to BCG is inconclusive. One study found that Rb underexpression was associated with an impaired response to BCG and interferon therapy, but not to BCG alone, while other unvalidated studies found that Rb status could predict BCG response.[176,177]

In conclusion, the predictive value of Rb alone is questionable, but it may have some value in combination with other biomarkers in selected patient cohorts, such as those with BCG-treated, high-risk NMIBC.

4.3.3.2.3 p21$^{WAF1/CIP1}$

The protein p21 is the product of the CDKN1A gene, and acts as a cell-cycle regulator by binding and inhibiting the activity of CDK2/4 complexes, thus controlling cell-cycle progression at the G1 checkpoint. Moreover, p21 regulates cell proliferation by blocking DNA replication. Therefore, cells lacking p21 may fail to arrest the cycle in response to DNA damage. Usually, lack of p21 is associated with p53 abnormalities, even if p21 expression is independent from that of p53.[164]

Loss of p21 is a relatively frequent event in NMIBC carcinogenesis and is usually related to p53 alteration. However, p21 expression seems to be regulated by p53-independent pathways.[164] Loss of p21 may be weakly associated with recurrence in NMIBC patients, but association is not independent of other risk factors.[164] Moreover, loss of p21 cannot predict progression or survival outcomes (**Table 4-12**). Interestingly, a positive association with recurrence and progression was found in patients with primary CIS.[178] In these patients, a contemporaneous loss of p21 and alteration of p53 led to impaired survival.

TABLE 4–12 Studies Evaluating the Role of p21 Mutation in Patients With Nonmuscle-invasive Bladder Cancer

Reference	No. of patients	Population	% of cells with mutations	Cutoff used	Outcomes investigated	Method used
Zlotta *et al.*[164]	47	Ta-T1	49	10%	Association with Rec only at UVA; no association with Prog in BCG-treated patients	IHC
Pfister *et al.*[149]	244	Ta-T1	16	5%	No association with Rec	IHC
Chow *et al.*[221]	89	Ta-T1	36		No association with outcomes	IHC
Migaldi *et al.*[222]	96	Ta-T1	71	5%	Association with OS	IHC
Liukkonen *et al.*[223]	207	Ta-T1	75	5%	No association with Prog	IHC
Wolf *et al.*[146]	30	pT1G3	-	-	No association with Prog	IHC
Shariat *et al.*[178]	39	CIS	31	10%	Association with Rec and Prog	IHC
López Beltrán *et al.*[156]	159	Ta-T1	45	10%	No association with survival	IHC

Abbreviations: BCG, bacillus Calmette-Guérin; CIS, carcinoma in situ; IHC, immunohistochemistry; OS, overall survival; Prog, progression; Rec, recurrence; UVA, univariable analysis.

4.3.3.2.4 $p27^{Kip1}$

Like $p21^{WAF1/CIP1}$, the protein $p27^{Kip1}$ is a member of the family of cyclin-dependent kinase inhibitors that bind CDK2, arresting the cycle in G1 phase. The protein acts similarly to p21 as a tumour suppressor, and its loss of function could lead to carcinogenesis. The studies investigating the role of p27 in NMIBC are summarized in **Table 4-13**.

Loss of p27 is associated with features of tumour aggressiveness in NMIBC, such as high-stage and high-grade disease.[179–181] The few studies that have investigated the role of p27 loss of function in NMIBC patients discovered a possible association with impaired survival outcomes.[179,182] One study showed a possible role of p27 in predicting survival in patients with T1G3.[182] However, these findings were not validated.[183]

TABLE 4–13 Studies Evaluating the Role of p27 Mutation in Patients With Nonmuscle-invasive Bladder Cancer

Marker	Reference	No. of patients	Population	% of cells with mutations	Cutoff used	Outcomes investigated	Method used
Survivin	Gazzaniga *et al.*[187]	30	Ta-T1	30	-	Not associated with Rec	PCR
	Ku *et al.*[225]	88	Ta-T1	58	20%	Association with DFS	IHC
	Karam *et al.*[226]	74	Ta-Tis-T1	53	10%	Association with Rec and Prog	IHC
	Fristrup *et al.*[185]	283	Ta-T1	-	10%	Association with Prog, DFS, and OS	IHC
	Sun *et al.*[227]	78	Ta-T1	67	-	Association with Rec	IHC
	Xi *et al.*[189]	72	Ta-T1	85	10%	Association with Rec	IHC
	Wang *et al.*[188]	138	Ta-T1	71	10%	Association with Rec	IHC
	Senol *et al.*[228]	115	Ta-T1	37	10%	Association with Rec and Prog	IHC
	Breyer *et al.*[211]	233	Ta	60	-	Association with Prog	IHC
BCL2/BAX	Gazzaniga *et al.*[187]	30	Ta-T1	53	>1	Association with Rec	PCR
	Ye *et al.*[190]	43	Ta-T1		>1	Association with Rec	WB
BCL2	Ajili *et al.*[191]	28	Ta-T1	71	15%	Association with Rec in BCG-treated patients	IHC
	Tzai *et al.*[216]	100	Ta-T1	12	-	No association with Rec	IHC
	Wu *et al.*[152]	93	Ta-T1	11	1%	No association with Rec	IHC
	Wolf *et al.*[146]	30	pT1G3	-	-	Association with Rec	IHC
BAX	Ajili *et al.*[191]	28	Ta-T1	43	2.5%	Association with Rec in BCG-treated patients	IHC
	Wolf *et al.*[146]	30	pT1G3	-	-	No association with Rec	IHC
Caspase-3	Wang *et al.*[188]	138	Ta-T1	51	10%	Association with Rec	IHC
Livin	Gazzaniga *et al.*[187]	30	Ta-T1	23	-	Association with Rec	PCR
	Wang *et al.*[188]	138	Ta-T1	65	10%	Association with Rec	IHC
	Xi *et al.*[189]	72	Ta-T1	75	10%	Association with Rec	IHC

Abbreviations: BCG, bacillus Calmette-Guérin; DFS, disease-free survival; IHC, immunohistochemistry; OS, overall survival; PCR, polymerase chain reaction; Rec, recurrence; Prog, progression; WB, Western blot.

4.3.3.3 Apoptosis markers

Apoptosis is a complex and highly regulated process that leads to programmed cell death. Alterations in pro- and antiapoptotic pathways allow malignant cells to survive, resist a variety of stressors, and proliferate. Therefore, these modifications are important steps in carcinogenesis.[137] The studies investigating apoptosis markers are summarized in **Table 4-14**.

One of the more widely investigated apoptotic markers is survivin. It inhibits apoptosis by blocking the activity of caspases, and induces mitotic progression and expression of genes involved in tumour-cell invasion. Survivin is overexpressed in a variety of human tumours, including breast, colon, pancreas, and prostate carcinoma, neuroblastoma, melanoma, and non-Hodgkin's lymphoma.[184]

In NMIBC, survivin is reported to be overexpressed in 30% to 85% of cases. Almost all the published studies are in agreement that survivin can predict oncological outcomes such as recurrence, progression, and survival. In the largest series, Fristrup *et al.* analyzed the expression of survivin in 283 NMIBC patients and reported a strong association with progression, disease-free survival (DFS), and overall survival (OS).[185] Recently, a meta-analysis of 14 studies reported that, in NMIBC, the pooled hazard ratio (HR) was statistically significant for recurrence (pooled HR, 1.81; 95% confidence interval [CI], 1.30–2.52), progression (pooled HR, 2.12; 95% CI, 1.60–2.82), cause-specific survival (pooled HR, 2.01; 95% CI, 1.32–3.06), and overall survival (pooled HR, 1.53; 95% CI, 1.02–2.29).[186]

Livin, like survivin, is an apoptotic marker belonging to the IAP family. The literature on livin is scarce, but some studies have found that livin can predict recurrence in NMIBC.[187–189]

TABLE 4–14 Studies Evaluating the Role of Cell Apoptosis Markers in Patients With Nonmuscle-invasive Bladder Cancer

Reference	No. of patients	Population	% of cells with mutations	Cutoff used	Outcomes investigated	Method used
Sgambato *et al.*[179]	96	Ta-T1	39	25%	Association with DFS and OS	IHC
López Beltrán *et al.*[156]	159	Ta-T1	54	30%	No association with survival	IHC
López Beltrán *et al.*[182]	51	T1G3	61	40%	Association with survival	IHC
Schrier *et al.*[224]	41	Ta-T1	-	25%	Association with better OS	IHC
Park *et al.*[183]	61	T1G3	37	30%	No association with Rec or Prog	IHC

Abbreviations: DFS, disease-free survival; IHC, immunohistochemistry; OS, overall survival; Prog, progression; Rec, recurrence.

The BCL2 family includes antiapoptotic and proapoptotic members, such as BCL2 and BAX, respectively. The BAX gene may form homodimers and heterodimers with BCL2 that oppose BCL2 function and contribute to cell death. It has been proposed that the ratio of BCL2:BAX governs the relative sensitivity response of cells to apoptotic stimuli.[190] While studies of BCL2 and BAX report contrasting results, the few trials investigating the role of BCL2:BAX show an association with recurrence when this ratio is <1.[146,191]

In conclusion, apoptosis markers, particularly survivin, appear to predict outcomes in NMIBC patients. However, prospective trials investigating the role of these tissue markers among standard prognosticators are needed to clarify their utility in clinical practice.

4.3.3.4 Cell-signalling pathway markers

Signalling proteins are mainly represented by tyrosine kinase receptors. Their effectors promote genetic changes and their overstimulation leads to a malignant transformation. They act as oncogenes and have dominant effects on cell phenotype. Moreover, due to their position in cell anatomy, they could represent particularly effective therapeutic targets. Studies investigating the role of cell-signalling pathway markers in NMIBC are summarized in **Table 4-15**.

Human epidermal growth factor receptor 2 (HER2) may be the best-known member of the ErbB family (EGFR, HER2, HER3, and HER4). It is overexpressed in many human cancers and could also represent a target for therapy. The few studies exploring the role of HER2 in NMIBC report a possible association with oncological outcomes such as recurrence and progression.[162,192,193]

Among members of the FGFRs, which regulate cellular processes such as growth, differentiation, and angiogenesis, overexpression of FGFR3 has been found in up to 60% of low-grade, low-stage NMIBC tumours, which rarely progress.[194,195] Several published studies demonstrate that FGFR3 is inversely associated with recurrence and progression, and directly associated with disease-free survival, thus identifying a subgroup of patients with a good prognosis and allowing a better risk-stratification profile among standard prognosticators.[196–199] The same authors report that the combination of FGFR3 and a marker of worse prognosis, such as Ki-67, seems to confer an even more accurate prediction. Based on the levels of these markers, a new molecular grade was developed. This has proved to be a reliable tool for assessing progression in NMIBC and is more reproducible than the standard pathologic grade.

TABLE 4–15 Studies Evaluating the Role of Cell-signalling Pathway Markers in Patients With Nonmuscle-invasive Bladder Cancer

Marker	Reference	No. of patients	Population	% of cells with mutations	Cutoff used	Outcomes investigated	Method used
HER2	Tetu *et al.*[150]	256	Ta-T1	8	10%	No association with Rec or Prog	IHC
	Janane *et al.*[192]	84	T1	68	3+ (HT)	Association with Rec	IHC
	Hegazi *et al.*[162]	88	Ta-Tis-T1	50	20%	Association with Rec or Prog	IHC
	Lim *et al.*[193]	141	Ta-T1	4	2+/3+ (HT)	Association with Rec or Prog	IHC
				3	-		PCR
	Breyer *et al.*[229]	302	T1±CIS	-	-	Association with Prog	PCR
FGFR3	van Rhijn *et al.*[196]	246	Ta-T1	67	-	Inverse association with Prog	PCR
	Hernández *et al.*[408]	772	Ta-T1	50	-	Association with DFS; inverse association with Rec and Prog	PCR
	Burger *et al.*[198]	221	Ta-T1	64	-	Inverse association with Prog	PCR
	van Rhijn *et al.*[197]	230	Ta-T1	67	-	Inverse association with Prog	PCR
AR	Nam *et al.*[200]	169	Ta-Tis-T1	37	10%	Inverse association with Rec	IHC
	Kim *et al.*[202]	118	Ta-T1	-	30%	No association with Prog	IHC
	Sikic *et al.*[203]	296	T1	-	-	Inverse association with Rec or CSM	PCR
ER	Nam *et al.*[200]	169	Ta-Tis-T1	31	10%	No association with Rec or Prog	IHC
	Han *et al.*[201]	42	Ta-T1	81	10%	Inverse association with Rec	IHC

Abbreviations: AR, androgen receptor; CSM, cancer-specific mortality; DFS, disease-free survival; ER, estrogen receptor; FGFR3, fibroblast growth factor receptor 3; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; HT, HercepTest™ (Agilent Technologies, Santa Clara, California, USA); PCR, polymerase chain reaction; Prog, progression; Rec, recurrence.

Approximately 13% of BC tumours harbour a mutation in one of the genes of the RAS–mitogen-activated protein kinase family (HRAS, NRAS, KRAS2).[137] However, to date, no studies concerning the prognostic role of RAS mutations in NMIBC have been published. Similarly, PI3K pathway genes have not been evaluated.

Hormonal receptors, such as androgen receptors and estrogen receptors, are members of the family of nuclear receptors that act as transcriptional factors by nuclear traslocation after ligand binding. Their overexpression seems to be related to improved RFS and PFS in NMIBC patients, but the evidence is limited due to insufficient data.[200–203]

4.3.3.5 Angiogenesis markers

Angiogenesis is a key step in carcinogenesis, and is related to invasion and progression of solid tumours. Historically, angiogenesis has been evaluated by measuring microvessel density (MVD). However, several other molecular markers have been shown to be associated with angiogenesis (**Table 4–16**).

Vascular endothelial growth factor (VEGF), MVD, and hypoxia inducible factor 1 alpha (HIF1α) are overexpressed in a non-negligible percentage of BC cells. Their overexpression has been associated with grade and stage. Unfortunately, the majority of published trials do not report any association with recurrence, progression, or both.[204–207] Conversely, thrombospondin-1 (TSP1) is a potent inhibitor of angiogenesis, and its reduced expression appears to be associated with outcomes of MIBC and with progression in NMIBC patients.[208]

TABLE 4–16 Studies Evaluating the Role of Angiogenesis Markers in Patients With Nonmuscle-invasive Bladder Cancer

Marker	Reference	No. of patients	Population	% of cells with mutations	Cutoff used	Outcomes investigated	Method used
VEGF	Chow *et al.*[204]	185	Ta-T1	19	-	No association with Rec	IHC
	Stavropoulos *et al.*[205]	127	Ta-Tis-T1	54	25%	No association with Rec or Prog	IHC
	Theodoropoulos *et al.*[206]	140	Ta-T1	-	Semiquantitative	No association with Rec or Prog	IHC
	Agrawal *et al.*[207]	90	Ta-T1	36	20%	No association with Rec	IHC
	Chen *et al.*[230]	72	Ta-T1	46	Semiquantitative	Association with Rec	IHC
	Sun *et al.*[227]	78	Ta-T1	69	30%	Association with Rec	IHC
MVD	Stavropoulos *et al.*[205]	127	Ta-Tis-T1	14	Median based	No association with Rec or Prog	IHC
	Theodoropoulos *et al.*[206]	140	Ta-T1	-	Semiquantitative	No association with Rec or Prog	IHC

Abbreviations: HIF1α, hypoxia inducible factor 1 alpha; IHC, immunohistochemistry; MVD, microvessel density; Prog, progression; Rec, recurrence; TSP1, thrombospondin-1; VEGF, vascular endothelial growth factor.

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TABLE 4–16 Studies Evaluating the Role of Angiogenesis Markers in Patients With Nonmuscle-invasive Bladder Cancer, *Cont'd*

Marker	Reference	No. of patients	Population	% of cells with mutations	Cutoff used	Outcomes investigated	Method used
HIF1α	Theodoropoulos *et al.*[206]	140	Ta-T1	53	Semiquantitative	No association with Rec or Prog	IHC
TSP1	Goddard *et al.*[208]	220	Ta-T1	-	Semiquantitative	Association with Prog	IHC

Abbreviations: HIF1α, hypoxia inducible factor 1 alpha; IHC, immunohistochemistry; MVD, microvessel density; Prog, progression; Rec, recurrence; TSP1, thrombospondin-1; VEGF, vascular endothelial growth factor.

4.3.3.6 Tumour-cell invasion markers

E-cadherin and N-cadherin are members of the cadherin superfamily, and are responsible for cell–cell interaction and intercellular adhesion in epithelial tissues. Dysregulation of cadherins has been linked to tumour spread in various malignancies. In normal tissues, epithelial and mesenchymal cells mainly express E-cadherin and N-cadherin, respectively; however, downregulation of E-cadherin, up-regulation of N-cadherin, or both are observed in cancer transformation, as a result of E-cadherin to N-cadherin switch. N-cadherin overexpression has been associated with features of tumour aggressiveness, such as high-stage and high-grade BC. Moreover, it has been associated with recurrence, but not progression, in NMIBC patients.[209,210] Conversely, high levels of E-cadherin expression are associated with better RFS and PFS rates.[201,210–212] To better understand the significance of invasion markers as predictors of intravesical recurrence, Liu *et al.* tested the expression level of 13 tissue markers in 161 NMIBC patients. N-cadherin, E-cadherin, matrix metalloproteinase 9 (MMP9), and TWIST were independently associated with RFS at multivariable analyses. Furthermore, there were significant differences in RFS, according to positive numbers of these five independent risk factors (i.e., positive for 0 or one factor vs. positive for two factors vs. positive for three or more factors).[213] The studies investigating the role of invasion markers in NMIBC are summarized in **Table 4–17**.

TABLE 4–17 Studies Evaluating the Role of Invasion Markers in Patients With Nonmuscle-invasive Bladder Cancer

Marker	Reference	No. of patients	Population	% of cells with mutations	Cutoff used	Outcomes investigated	Method used
E-cadherin	Han *et al.*[201]	42	Ta-T1	50	5%	Inverse association with Rec	IHC
	Muramaki *et al.*[210]	115	Ta-T1	54	90%	Inverse association with Rec	IHC
	Khorrami *et al.*[212]	180	Ta-T1	44	-	Inverse association with Rec	IHC
	Breyer *et al.*[211]	233	Ta	39	-	Inverse association with Prog	IHC
N-cadherin	Muramaki *et al.*[210]	115	Ta-T1	42	>0%	Association with Rec	IHC
	Abufaraj *et al.*[209]	827	Ta-Tis-T1	40	-	Association with Rec	IHC
P-cadherin	Wang *et al.*[231]	110	Ta-T1	49	50%	Association with Prog	IHC

Abbreviations: IHC, immunohistochemistry; Prog, progression; Rec, recurrence.

4.3.3.7 Molecular marker panel

One can conclude from the current literature that none of the evaluated tissue biomarkers alone can be used to predict oncological outcomes during routine clinical practice. Therefore, it has been postulated that a panel of biomarkers may improve the predictive value of standard tools, such as the European Organisation for Research and Treatment of Cancer (EORTC) and the Club Urológico Español de Tratamiento Oncológico (CUETO) risk group calculators. However, even in this setting, results are conflicting. Zlotta *et al.*[214] evaluated the prognostic value of the EORTC risk calculator and several proapoptotic, antiapoptotic, proliferation, and invasiveness molecular markers in predicting outcome of NMIBC patients treated with BCG. The tested biomarkers were p53, p21, BCL2, cyclin D1, and MMP9. Combining EORTC results with marker expression (MMP9, BCL2, cyclin D1, and p21 for recurrence, and MMP9 and p21 for progression) led to an improved predictive accuracy. Recently, a panel of cell-cycle markers has been tested in combination with EORTC and CUETO models.[215] Some 131 patients with high-grade NMIBC were enrolled in this study and immunohistochemical staining of five biomarkers (p21, p27, p53, KI-67, and cyclin E1) was performed. Markers were not significant predictors of recurrence or progression, and their addition to prediction models only marginally improved their discrimination, resulting in very little clinical benefit.

In conclusion, as BC develops along multiple molecular pathways, the inclusion of different molecular markers in predictive tools could improve the accuracy of these tools. The use of multiple molecular markers could represent the future of risk stratification to guide precision therapies, patient

counselling, and decision management. However, to date, due to the low number and quality of published trials, and to the contrasting reported findings, no recommendation on the routine use of tissue biomarkers in NMIBC can be given.

4.3.3.8 Recommendations

Due to the low LOE and the contrasting reported findings, the use of tissue biomarkers in BC is not recommended, as it does not contribute to clinical decision-making.

4.3.4 Blood and tissue biomarkers for muscle-invasive bladder cancer

4.3.4.1 Tissue biomarkers in muscle-invasive bladder cancer

The development of muscle-invasive urothelial carcinoma of the bladder (MIUCB) involves alterations in multiple homeostatic pathways with profound deregulations within a complex molecular circuitry. The net effect of such deregulations on key cellular processes establishes the ultimate fate of a tumour (**Table 4–18**). Therefore, these alterations often serve as predictors of patient outcomes, and they may also act as therapeutic targets.[232–235]

TABLE 4–18 Alterations in Tissue Markers Associated With Muscle-invasive Urothelial Carcinoma of the Bladder and Their Prognostic Impact *(Adapted from Mitra et al. 2016[235])*

Marker	Function	Association of alteration with prognosis		
		Recurrence probability	Recurrence probability	Other
Cell cycle				
p53 [a]	Inhibitd G1-S progression	↑	↓	
p21 [b]	Cyclin-dependent kinase inhibitor	↑	↓	
Mdm2 [c]	Mediates proteasomal degradation of p53			Advanced stage/grade
Rb [d]	Sequesters E2F; inhibits cell-cycle progression	↑	↓	
p27 [b]	Cyclin-dependent kinase inhibitor	↑		
Apoptosis				
Caspase-3 [b]	Promotes apoptosis	↑	↓	
Survivin [c]	Inhibits apoptosis	↑	↓	

[a]Altered, [b]Underexpressed/lost, [c]Overexpressed/increased, [d]Lost/hyperphosphorylated, [e]Overactivated, ↑Increased, ↓Decreased, * Conflicting data

Abbreviations: AR, androgen receptor; bfGF, basic fibroblast groth factor; ICAM1, intercellular adhesion molecule 1; MMP, matrix metalloproteinase; Rb, retinablastoma protein; STAT, signal transducer and activator of transcription; TSP-1, thrombospondin-1; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor 2.

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TABLE 4–18 Alterations in Tissue Markers Associated With Muscle-invasive Urothelial Carcinoma of the Bladder and Their Prognostic Impact *(Adapted from Mitra et al. 2016[235]), Cont'd*

Marker	Function	Association of alteration with prognosis		
		Recurrence probability	Recurrence probability	Other
Bcl-2 [c]	Inhibits caspase activation		↓	
Bax [b]	Releases cytochrome c from mitochondria; promotes apoptosis		↓	
Apaf-1 [b]	Promotes apoptosis		↓	
Cell signalling				
ErbB-1 [c]	Epidermal growth factor receptor; transmits growth signals	↑	↓	
ErbB-2 [c]	Epidermal growth factor receptor; transmits growth signals		↓*	
Estrogen receptor-β [c]	Sex hormone receptor; regulates transcription			Advanced stage/grade
AR [b]	Sex hormone receptor; regulates transcription			Advanced stage/grade*
STAT3 [c]	Regulates gene expression; increases Bcl-2 expression	↑	↓	
Angiogenesis				
Microvessel density [c]	Histologic marker of angiogenesis	↑*	↓*	Nodal metastasis
VEGF [c]	Promotes angiogenesis through nitric oxide synthase	↑	↓	Advanced stage/ grade, lymphovascular invasion,nodal metastasis
VEGFR2 [c]	VEGF receptor; transmits angiogenic signals			Advanced stage, nodal metastasis
bFGF [c]	Growth factor stimulating angiogenesis	↑		Advanced stage, lymphovascular invasion,nodal metastasis
TSP-1 [b]	Inhibits angiogenesis	↑		
Invasion				

[a]Altered, [b]Underexpressed/lost, [c]Overexpressed/increased, [d]Lost/hyperphosphorylated, [e]Overactivated, ↑Increased, ↓Decreased, * Conflicting data

Abbreviations: AR, androgen receptor; bfGF, basic fibroblast groth factor; ICAM1, intercellular adhesion molecule 1; MMP, matrix metalloproteinase; Rb, retinablastoma protein; STAT, signal transducer and activator of transcription; TSP-1, thrombospondin-1; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor 2.

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TABLE 4–18 Alterations in Tissue Markers Associated With Muscle-invasive Urothelial Carcinoma of the Bladder and Their Prognostic Impact *(Adapted from Mitra et al. 2016[235]), Cont'd*

Marker	Function	Association of alteration with prognosis		
		Recurrence probability	Recurrence probability	Other
E-cadherin [b]	Mediates intercellular adhesion	↑	↓	
MMP-2 [c]	Degrades extracellular matrix	↑	↓	Advanced grade
MMP-9 [c]	Degrades extracellular matrix		↓	Advanced grade
a6b4 integrin [d]	Links collagen VII to cytoskeleton; transduces regulatory signals		↓	
ICAM1 [c]	Binds integrins			Nodal metastasis

[a]Altered, [b]Underexpressed/lost, [c]Overexpressed/increased, [d]Lost/hyperphosphorylated, [e]Overactivated, ↑Increased, ↓Decreased, * Conflicting data

Abbreviations: AR, androgen receptor; bfGF, basic fibroblast groth factor; ICAM1, intercellular adhesion molecule 1; MMP, matrix metalloproteinase; Rb, retinablastoma protein; STAT, signal transducer and activator of transcription; TSP-1, thrombospondin-1; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor 2.

4.3.4.1.1 Cell-cycle markers

The most extensively characterized cellular process in MIUCB involves pathways that control cell-cycle progression.[236] The cell cycle is primarily controlled by the p53 and Rb pathways that closely interact with mediators of apoptosis, signal transduction, and DNA repair. Encoded by the TP53 tumour-suppressor gene located on chromosome 17p13.1, the p53 protein inhibits cell-cycle progression at the G_1-S transition by transcriptionally activating $p21^{WAF1/CIP1}$.[237] While MIUCB is generally characterized by loss of a single 17p allele, mutation in the remaining allele can lead to TP53 inactivation and loss of its tumour-suppressor function.[238] Loss of heterozygosity on chromosome 17 is often seen in MIUCB and is associated with an aggressive phenotype. The half-life of wild-type p53 is <30 minutes, which prevents accumulation of the protein in the nucleus.[239] However, TP53 mutations result in an altered protein that is resistant to ubiquitin-mediated degradation, leading to increased intranuclear protein accumulation that can be detected by IHC.

Several retrospective studies have reported that nuclear accumulation of p53 is prognostic in MIUCB, especially in patients treated with radical cystectomy.[140,142,240–243] The rate of p53 alterations also increases progressively as MIUCB metastasizes to lymph nodes.[244–246] An analysis of high-grade MIUCB specimens by TCGA Research Network identified TP53 mutations in nearly half of the samples, which were mutually exclusive in their relationship with amplification and overexpression of MDM2; hence, TP53 function was noted to be inactivated in 76% of samples.[19] However, at this time, the use of p53 as a prognostic marker in MIUCB is still not clinically established, despite more than 100 studies evaluating its utility. Indeed, discordance in p53 nuclear accumulation and TP53 mutations has been noted.[247] A meta-analysis of 117 studies that examined the role of p53 in BC noted that observational discrepancies may be related to the different types of antibodies used in immunohistochemical assays, variability in interpretation, stratification criteria, and other technical and

specimen-handling inconsistencies.[248] A phase 3 trial designed to evaluate the benefit of stratifying organ-confined invasive BC patients, based on their p53 status for adjuvant cisplatin-based chemotherapy, could not confirm the prognostic value of the protein alteration or any association with chemotherapeutic response.[249] However, this trial had several limitations, including high patient-refusal rate, a lower-than-expected event rate, and patient failure to receive assigned therapy that compromised the study's power.

The $p21^{WAF1/CIP1}$ gene encodes for the p21 cyclin-dependent kinase inhibitor (CDKI) protein, which is transcriptionally regulated by p53. Loss of p21 expression is a potential mechanism by which p53 alterations influence MIUCB progression.[250] Loss of p21 expression is an independent predictor of progression in MIUCB, and maintenance of its expression appears to abrogate the deleterious effects of altered p53.[251] In patients with MIUCB, p21 is an independent predictor of recurrence and cancer-specific mortality.[246] The prognostic value of p21 may be most useful in patients with pT2-3N0 disease, especially in combination with other markers.[244]

MDM2 is involved in an autoregulatory feedback loop with p53, thus controlling its activity. Increased p53 levels lead to promoter transactivation and upregulation of MDM2, and the translated protein mediates proteasomal degradation of p53. The resulting lower levels of p53 then reduce the levels of MDM2. MDM2 amplification has been noted in BC, and its frequency increases with increasing tumour stage and grade.[252]

Encoded on chromosome 13q14, the Rb protein interacts with other regulatory proteins involved in the G_1-S transition. Dephosphorylated Rb sequesters the E2F transcription factor. Upon phosphorylation of Rb by cyclin- or cyclin-dependent kinase complexes, E2F is released, leading to transcription of genes required for DNA synthesis. Inactivating Rb mutations resulting in loss of protein expression have been noted in BC.[253] In conjunction with other cell-cycle regulatory proteins, Rb has been shown to be prognostic in MIUCB.[242,254] Negative regulation of cyclin-dependent kinases is achieved by CDKIs such as p21 and p27, which act as tumour suppressors. p27 alterations have also been linked to shortened disease-free survival and overall survival in BC.[181] Combined immunohistochemical assessment of p53, p21, Rb, cyclin E1, and p27 has been shown to yield predictive accuracies superior to that of any single molecular marker in patients with BC treated with radical cystectomy, and can improve risk stratification.[255,256]

4.3.4.1.2 Apoptosis markers

Apoptosis is a highly regulated process of programmed cell death, comprising a series of coordinated steps that occur throughout normal development and in response to a variety of initiation stimuli. The intrinsic apoptotic pathway is mediated by mitochondria, whereas the extrinsic pathway involves activation of cell-surface death receptors. Both pathways activate caspases that cleave cellular substrates and lead to the characteristic apoptotic changes. Decreased caspase-3 expression has been associated with higher probability of disease recurrence and cancer-specific mortality (CSM).[257] Survivin is a member of the IAP family and partly inhibits apoptosis by blocking downstream caspase activity. In a study of 226 BC patients, survivin overexpression was present in 64% of participants, and was associated with higher probability of disease recurrence and cancer-specific mortality.[257] In another trial, the proportion of specimens with survivin overexpression increased progressively from

NMIBC to MIUCB, and to metastatic lymph-node tissue.[258] In a large multicentre validation study, addition of survivin improved the accuracy of standard clinicopathologic features for prediction of disease recurrence and cancer-specific survival in a subgroup of patients with pT1-3N0M0 disease.[259]

The BCL2 family of proteins is involved in the intrinsic apoptotic pathway; it includes antiapoptotic members such as BCL2, as well as proapoptotic members such as BAX and BAD. Increased BCL2 expression has been associated with poor prognosis in patients with BC treated with radiotherapy or synchronous chemoradiotherapy.[260,261] In patients with advanced BC undergoing radiotherapy, BCL2 may also serve as a marker for patients who may benefit from neoadjuvant chemotherapy.[262] However, other findings suggest that BCL2 overexpression confers worse all-cause survival and lower response rates to chemotherapy.[263] A prognostic index using MDM2, p53, and BCL2 has also been proposed where alterations in all three markers correspond to the worst survival probability in MIUCB.[264] On the other hand, BAX expression is an independent predictor of a more favourable prognosis in patients with invasive BC.[265–267] BAX mediates its proapoptotic role through the activation of apoptotic peptidase activating factor 1 (APAF1).[268] Decreased APAF1 expression is associated with higher mortality in patients with BC.[269]

4.3.4.1.3 Markers in cell-signalling pathways

Several receptors on the cell surface modulate signals from external cues and transmit them via transduction pathways to the nuclei of urothelial cells that regulate gene expression. Aberrations in these receptors, the transmitted signals, or both can lead to uncontrolled cellular proliferation and cancer progression. Among members of the FGFRs, activating mutations of FGFR3 are noted in nearly 60% to 70% of low-grade papillary Ta tumours.[270] Such activation results in downstream signalling through the Ras–mitogen-activated protein kinase pathway. Expression of HRAS, a candidate member of this pathway, has also been associated with NIBC recurrence at initial presentation.[271]

ErbB1 and ErbB2 (HER2/neu) are members of the EGFR family, which are overexpressed in invasive BC.[272–274] ErbB1 overexpression is associated with higher probability of progression and mortality.[157,275] Similarly, ErbB2 overexpression is also associated with aggressive tumours and poor disease-specific survival.[276–279] However, other studies indicate that ErbB2 expression is not associated with prognosis.[280,281] While a combined expression profile of ErbB1 and ErbB2 has been suggested as a better predictor of outcome than individual markers alone, this has not been corroborated.[282,283]

Variable expression of sex steroid hormone receptors has been proposed as a possible cause for differential BC behaviour between genders, although direct evidence is currently lacking.[284] Across both genders, decreased expression of estrogen receptor-β has been associated with better PFS in patients with noninvasive urothelial carcinoma.[285] A meta-analysis of 2,049 patients with BC showed that estrogen receptor-β–positive rates were significantly higher in high-grade and muscle-invasive tumours.[286] The androgen receptor (AR) is a nuclear receptor and ligand-dependent transcription factor that mediates biologic effects of androgens. Its expression is inversely correlated with pathological stage: a study noted that 75% and 21.4% of NMIBC and MIUCB, respectively, expressed AR.[287] Another study noted that loss of AR expression was associated with higher grade and invasive tumours; however, no association was found with patient outcomes.[285] In contrast, a study of 472 patients with urothelial bladder carcinoma failed to find any association between AR protein expression and BC stage, grade, or outcomes.[288]

Janus kinase represents a family of tyrosine kinases that is activated by cytokine and growth receptors, and mediates multiple signalling pathways. Following Janus kinase activation, the best-characterized event is activation of the signal transducer and activator of transcription (STAT) pathway, which controls transcription of several important genes. STAT1 can reduce BCL2 expression, while STAT3 has the opposite effect.[289] In combination with other markers, STAT3 expression can predict risk for recurrence and survival in patients with BC.[290]

4.3.4.1.4 Angiogenesis markers

Tumour cell–derived factors can interact with stromal elements to recruit endothelial cells to the site of cancerous growth and establish a vascular supply. This process of angiogenesis helps ensure the delivery of required nutrients for tumour-cell growth. Angiogenesis may be histologically quantified by MVD, which has been reported to be associated with disease recurrence and decreased overall survival in invasive BC.[291] MVD quantification may also provide additional prognostic information in patients with p53-altered tumours.[292] While the prognostic association of MVD has not been confirmed by other studies, it has been shown to be higher in patients with lymph node metastasis.[293,294]

VEGFs are angiogenesis-promoting signalling proteins that stimulate cellular responses by binding to their receptors (VEGFRs). In a study of 204 patients treated with radical cystectomy and bilateral pelvic lymphadenectomy, VEGF was overexpressed in 86% of patients, supporting its role in bladder tumourigenesis and identifying it as a potential target for therapy.[294] This study also showed that basic fibroblast growth factor (BFGF), a downstream proangiogenic molecule, was associated with established features of biologically aggressive disease, including higher pathologic stage, LVI, lymph-node metastasis, and disease recurrence. VEGF overexpression has also been associated with these aggressive pathologic features and shorter disease-free survival.[295,296] VEGFR2 (KDR/Flk-1) mediates most of the known cellular responses to VEGF. Expression of this protein has been associated with advanced BC stage and muscle invasion.[297] Another study has also shown that VEGFR2 expression may be an important determinant for nodal metastasis in BC.[298]

In addition to regulating the cell cycle, p53 upregulates TSP1, a potent inhibitor of angiogenesis. Tumours with p53 alterations are associated with decreased TSP1 expression, and such tumours demonstrate higher MVD.[299] Decreased TSP1 expression has been associated with lower probabilities of RFS and overall survival in BC. A combination of angiogenesis-related biomarkers, including VEGF, BFGF, and TSP1, has been associated with established clinicopathologic features of biologically aggressive disease in patients who underwent radical cystectomy for MIUCB.[294] On multivariable analyses that adjusted for standard pathological features, BFGF and TSP1 were identified as independent predictors of disease recurrence and cancer-specific mortality.

4.3.4.1.5 Markers of tumour-cell invasion

The ability of cancer cells to invade blood vessels and lymphatics determines their potential to spread and metastasize. Cadherins are ubiquitous tissue molecules that are prime mediators of intercellular adhesion. E-cadherin is a prototypic member of the cadherin family, and it plays a critical role in epithelial cell–cell adhesion. Decreased E-cadherin expression has been correlated with disease recurrence and progression, as well as with shorter survival in patients with BC.[269,300]

A tumour's ability to degrade the matrix and invade the basement membrane is facilitated by the actions of proteases, such as MMPs. Increased MMP2 and MMP9 expression has been associated with higher tumour grade.[301] MMP2 overexpression can also predict poor relapse-free survival and disease-specific survival.[302] MMP9:E-cadherin ratio has also been reported to be prognostic for disease-specific survival.[303]

Integrins are transmembrane glycoprotein receptors for collagen and adhesion molecules, which, when altered, can promote tumour progression, invasion, and metastasis. In normal urothelial cells, the α6β4 integrin is in close relationship with collagen type VII and it restricts cell migration. Loss of polarity of α6β4 expression has been noted in nonmuscle-invasive disease, and MIUCB shows either a loss of α6β4, collagen type VII expression, or both, or a lack of colocalization of the two proteins.[304] Patients with tumours that exhibit weak α6β4 immunoreactivity have better outcomes than those with either no expression or strong overexpression.[305] Intercellular adhesion molecule 1 (ICAM1) is a member of the immunoglobulin superfamily that binds to certain integrin classes. Immunohistochemical studies indicate that ICAM1 expression is closely associated with an infiltrative histological phenotype.[306] ICAM1 is a member of multimarker models that can predict nodal status in BC.[307]

4.3.4.1.6 Tissue-based genomic analyses in muscle-invasive bladder cancer

Pathogenesis and ultimate clinical behaviour in MIUCB is influenced by the combined alterations in several molecular pathways. Analyzing these alterations in combination may therefore provide greater insight into the disease pathobiology, while also generating marker panels that may be able to better predict patient outcome and treatment response. Tissue-based gene- and transcriptome-level profiling has been used to identify markers that characterize and can potentially predict prognosis in MIUCB.[308] Supervised learning approaches have been used to distinguish between NMIBC and MIUCB, based on their genomic signatures.[309–311] Another effort uses oligonucleotide arrays and support vector machine algorithms to develop prognostic panels with reported 90% accuracy for predicting overall survival in MIUCB.[312] Patients with node-positive disease and poor survival were found to have a 174-probe signature. While several signatures have been reported across various stages of BC, none have thus far been adopted in clinical practice, and the clinicopathologic determination of disease stage remains the gold standard. This is attributable, in large part, to the potential for false discovery that hampered early efforts. Furthermore, imperfect genomic coverage across older generations of microarray platforms and other customized platforms impacted data reproducibility.[313] This often led to different molecular classifiers constructed in comparable clinical populations that showed few common markers.

More recent studies have focused on technologies that can assess multiple markers in a reliable, efficient, and cost-effective fashion for development of prognostic panels. Several studies have quantified finite numbers of molecular targets across several BC-associated cellular pathways in an attempt to define prognostic signatures.[314] This strategy has been used in a report to identify molecular alterations associated with progression across all pathologic stages, which could potentially supplement disease staging in predicting clinical outcome.[290] The expressions of 69 genes involved in different cancer pathways were assessed on primary tumours to identify a panel of four markers (JUN, MAP2K6, STAT3, and ICAM1) that were associated with disease recurrence and overall survival. Five-year probabilities for recurrence and survival, based on a favourable versus unfavourable profile

using this panel, were 41% versus 88%, and 61% versus 5%, respectively (both, $p<0.001$). The prognostic potential of this panel was confirmed on an independent external dataset (disease-specific survival, $p=0.039$).

The advent of newer microarray technology that can interrogate the entire coding region of the human genome, while also accounting for splice variants and non–protein-coding transcripts, has broadened the realm of transcriptomic profiling in MIUCB.[315] Researchers from Chungbuk National University, South Korea, have adopted this methodology to describe a panel comprising IL1B, S100A8, S100A9, and EGFR, important mediators of MIUCB progression.[316] Another study using whole genome mRNA expression profiling identified three unique molecular subtypes of MIUCB that share some genetic features with established subtypes of breast cancer.[317] The study authors designated these subtypes as basal, luminal, and p53-like muscle-invasive tumours. The basal subset shared biomarkers with basal breast cancers, and it was characterized by p63 activation and more aggressive disease at presentation. Tumours in the luminal subset contained features of active PPARγ and estrogen receptor transcription, and KRT20 upregulation, and were enriched with activating FGFR3 mutations. The p53-like tumours were consistently resistant to neoadjuvant methotrexate, vinblastine, doxorubicin, and cisplatin chemotherapy, and all chemoresistant tumours adopted a p53-like phenotype after therapy.

Comprehensive characterization of the genomic landscape of BC through efforts of TCGA Research Network resulted in identification of four expression clusters of high-grade MIUCB.[19] Tumours in cluster I had papillary-like morphology with increased FGFR3 expression, mutations, and copy number gain, thereby suggesting that these patients may respond to FGFR inhibitors or their downstream targets. These tumours also showed decreased miR-99a and miR-100 expression, which, in turn, downregulates FGFR3 expression.[318] Tumours in clusters I and II also showed features similar to those of luminal A breast cancer, with high expression of luminal breast differentiation markers, including GATA3 and FOXA1. These tumours also showed increased expression of UPKs, E-cadherin, and members of the miR-200 family. Increased expression of ERBB2 and ERβ by these tumours also suggested that these two proteins may serve as potential targets for hormonal therapies. The expression signature of tumours in cluster III ("basal/squamous-like") were similar to those of basal-like breast cancers and squamous cell cancers of the head, neck, and lung, and characterized by overexpression of epithelial lineage genes. These findings suggest the presence of distinct molecular subtypes of MIUCB with characteristic expression signatures, which may impact prognosis and may be candidates for unique therapeutic approaches.[319]

Decision models based on clinicopathologic metrics can provide reasonable prognostic value to influence clinical management.[320–322] Recent studies have focused on combining such clinical models with genomic biomarkers to improve prognostic performance. A large transcriptome-wide profiling effort to discover and validate a prognostic signature in MIUCB resulted in the identification of a 15-feature genomic classifier that had a prognostic value of 77% on blinded independent validation.[323] The genomic classifier also uniquely reported on the prognostic potential of certain non–protein-coding transcripts, which have been shown to play important regulatory roles in cancer development.[324] While the prognostic accuracy of a model that comprised clinicopathologic variables alone was 78% in the validation set, it improved to 86% when the genomic classifier was added. Prognostic potential of the genomic classifier was also validated on four independent datasets.

A similar approach was also adopted to identify a 51-feature classifier that could identify lymph-node metastasis in MIUCB.[325] In a validation set, the classifier achieved an AUC of 0.82, with an odds ratio for nodal metastasis of 2.65 for every 10% increase in score (p<0.001). This data suggests that genomic assessment can yield robust validated prognostic biomarker panels that can identify subsets of MIUCB patients with varying outcomes. The performance of these biomarker panels may be enhanced in combination with clinicopathologic variables, thereby identifying candidates who may need more aggressive management.

4.3.4.2 Blood-based markers in muscle-invasive bladder cancer

Assessment of biomarkers in the blood offers several advantages over tissue samples, including ease of procurement, a minimally invasive collection method, and higher sample homogeneity.[326] However, the clinical applicability and value of these markers in the setting of MIUCB remains to be tested and proven in large multi-institutional studies. Akin to their tissue-based counterparts, blood-based markers can reflect alterations in normal homeostatic pathways that are responsible for tumour formation and progression (**Table 4–19**).

TABLE 4–19 Alterations in Blood-based Markers Associated With Muscle-invasive Urothelial Carcinoma of the Bladder and Their Prognostic Impact

Marker	Function	Association of alteration with prognosis		
		Recurrence probability	Recurrence probability	Other
Cellular proliferation				
IGFBP-3 [a]	Regulates cell proliferation, transformation, apoptosis	↑	↓	Nodal metastasis
TGF-b1 [b]	Regulates cell proliferation, chemotaxis, differentiation	↑	↓	Advanced stage, lymphovascular invasion, nodal* and distant metastasis
Inflammation and immune modulation				
IL-6, IL-6sR [b]	Lymphocyte proliferation, production of acute phase proteins	↑	↓	Advanced stage, lymphovascular invasion, nodalmetastasis
CRP [b]	Promotes phagocytosisby macrophages, activates complement system		↓	
Angiogenesis				
VEGF [b]	Promotes angiogenesis through nitric oxide synthase		↓	Advanced stage/grade
uPA [b]	Degrades extracellular matrix	↑	↓	Nodal metastasis

[a]Decreased, [b]Increased, ↑Increased, ↓Decreased, *Conflicting data

Abbreviations: CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CRP, C-reactive protein; CTCs, circulating tumour cells; ICAM1, intercellular adhesion molecule 1; IGFBP-3, insulin growth factor binding protein-3; IL-6, interleukin-6; IL-6sR, IL-6 soluble receptor; MMP, matrix metalloproteinase; TGF-b1, transforming growth factor–b1; uPA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor.

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TABLE 4–19 Alterations in Blood-based Markers Associated With Muscle-invasive Urothelial Carcinoma of the Bladder and Their Prognostic Impact, *Cont'd*

Marker	Function	Association of alteration with prognosis		
		Recurrence probability	Recurrence probability	Other
Invasion				
ICAM1 [b]	Binds integrins			Advanced grade, large tumours
CA 19-9 [b]	Unknown	↑	↓	
CEA [b]	Mediates intercellular adhesion		↓	
MMP-7 [b]	Degrades extracellular matrix	↑	↓	
MMP-9 [b]	Degrades extracellular matrix			Advanced stage/grade, distant metastasis
CTCs [b]	Early surrogate indicator of metastasis	↑	↓	

[a]Decreased, [b]Increased, ↑Increased, ↓Decreased, * Conflicting data

Abbreviations: CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CRP, C-reactive protein; CTCs, circulating tumour cells; ICAM1, intercellular adhesion molecule 1; IGFBP-3, insulin growth factor binding protein-3; IL-6, interleukin-6; IL-6sR, IL-6 soluble receptor; MMP, matrix metalloproteinase; TGF-b1, transforming growth factor–b1; uPA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor.

4.3.4.2.1 Cellular proliferation

Insulin-like growth factor binding proteins play a central role in regulating cellular growth, proliferation, and transformation. Insulin-like growth factor binding protein 3 (IGFBP3) is a member of this family that also has its own proapoptotic effects.[327] Lower preoperative plasma levels of IGFBP3 are a significant predictor of lymph-node involvement in patients undergoing radical cystectomy, independent of clinical stage and grade.[328] Low IGFBP3 levels also portend a significantly increased risk of disease recurrence and cancer-specific mortality in the preoperative setting, after adjusting for stage and grade.

Transforming growth factor beta 1 (TGFB1) is a polypeptide cytokine encoded by the TGFB1 gene that exerts its function by modulating cellular proliferation, chemotaxis, cellular differentiation, immune response, and angiogenesis. Loss of response to the antiproliferative effects of TGFB1 is associated with the progressive stages of carcinogenesis. Overexpression of TGFB1 is associated with loss of expression of TGFB1 receptors. While tissue overexpression of TGFB1 is associated with disease progression, elevated preoperative plasma levels of this protein have been associated with LVI, and nodal and distant metastasis.[329,330] However, this association with nodal metastasis has not been corroborated by another study.[331] Results from a subanalysis of 41 patients with MIUCB also indicate that high TGFB1 plasma levels are predictive of disease recurrence and death from BC.[330] A single nucleotide polymorphism in a TGFB1 receptor (TGFBR1-rs868) has also been significantly associated with disease-specific mortality in MIUCB.[332]

4.3.4.2.2 Inflammation and immune modulation

Interleukin-6 (IL6) is a cytokine that modulates the immune system via proliferation and activation of cytotoxic T cells, proliferation and differentiation of B cells, and production of acute-phase proteins. IL6 signalling is initiated when the protein binds to its non-signalling receptor IL6R, which also exists in soluble form (IL6sR). Elevated IL6 and IL6sR levels were associated with advanced pathological stage, LVI, and nodal metastases in a prospective study of 51 BC patients.[333] Both biomarkers were also independent predictors of disease recurrence and cancer-specific mortality, after adjusting for stage and grade.

C-reactive protein (CRP) is an acute-phase protein of hepatic origin whose levels increase following IL6 secretion by macrophages and T cells. As the most widely studied serum marker for inflammation in BC, elevated CRP levels have been consistently associated with adverse outcomes.[334] Although variables for adjustment have varied among studies, CRP has been shown to be an independent prognostic factor for cancer-specific and overall mortality.[335–339]

4.3.4.2.3 Tumour angiogenesis

In line with tissue-marker observations, elevated levels of serum VEGF have also been reported in BC. VEGF stimulates nitric oxide synthase, which in turn stimulates nitric oxide formation and tumour vascularization. High serum levels of VEGF are associated with high BC stage and grade, vascular invasion, metastases, and poor disease-free survival.[340]

VEGF also induces the formation of urokinase-type plasminogen activator (uPA), which degrades the extracellular matrix, thereby facilitating endothelial cell migration and invasion. Preoperative plasma uPA levels have been associated with LVI, nodal metastasis, disease progression, and death from UCB.[341]

4.3.4.2.4 Tumour-cell invasion

The secreted and soluble counterparts of several biomarkers associated with the invasive potential of tumour cells have been studied in the serum of patients with MIUCB. A study analyzed serum ICAM1 levels across all stages of BC, and found associations with the presence, grade, and size of bladder tumours.[342] However, preoperative levels were not significantly different between superficial and invasive tumours.

The protein carbohydrate antigen 19-9 (CA 19-9) is a common tumour marker for pancreatic cancer. Carcinoembryonic antigen describes a set of highly related glycoproteins involved in cell adhesion. Elevated precystectomy serum levels of CA 19-9 and CEA have been shown to be independent predictors of worse overall survival in patients with BC.[343] Elevated serum CA 19-9 levels have also been associated with poor recurrence-free survival.

Elevated serum MMP7 levels have been associated with metastatic disease, and are predictors of metastasis-free, disease-specific, and overall survival in BC.[344] These findings have been validated in an independent cohort.[345] Increased MMP9 serum levels have also been found in patients with advanced-stage and advanced-grade disease, and distant metastasis.[346]

The presence of circulating tumour cells (CTCs) in peripheral blood may represent an early step in the metastatic progression of BC. With the exception of several early reports using reverse transcription PCR-based approaches, nearly all studies in BC to date have used the CELLSEARCH® CTC Test (Menarini Silicon Biosystems, Inc., Huntingdon Valley, Pennsylvania, USA) for CTC detection.[347] In an evaluation of 55 patients undergoing radical cystectomy for BC, 30% of patients with localized disease had ≥1 CTC/7.5 mL of blood (median, 1; range, 1–11). By contrast, all five patients with metastatic disease had detectable CTCs (median, 2; range, 1–372). The study noted that patients with ≥1 CTCs had shorter times to disease recurrence and cancer-specific death than those with <1 CTC.[348] These findings were later validated in a larger cohort of 100 patients with nonmetastatic disease undergoing radical cystectomy.[349]

4.3.4.3 Recommendations

The use of blood and tissue biomarkers as predictive tools and to guide decision-making in MIBC is currently not recommended due to the weak strength of evidence of published studies.

4.3.5 Response to systemic chemotherapy

4.3.5.1 Introduction

This section assesses biomarkers that can predict response to chemotherapy. In this context, "response" indicates meaningful clinical evidence of drug activity, as evidenced by tumour shrinkage on imaging (usually assessed by RECIST criteria) or the prolongation of survival (usually measured by DFS, PFS, or OS). "Chemotherapy" denotes any drug therapy, targeted or otherwise, that is not primarily an immunotherapy. In BC, chemotherapies can be administered intravesically (usually for nonmuscle-invasive cancer) or systemically (usually for muscle-invasive or metastatic cancer), and both are considered here, though few biomarkers of response exist for intravesical chemotherapies. Key biomarkers of chemotherapy response that have been tested in BC are found in **Table 4–20**, and each class of biomarkers is briefly described below.

TABLE 4–20 Biomarkers of Chemotherapy Response

Molecular pathway	Biomarker(s)	Method	Drug(s)	N	Results	Reference
Cell cycle and proliferation	Cyclin D1	IHC	Cis	63	High levels predict better chemo response	Seiler *et al.*[361]
	CCDN1	FISH	Cis	63	Does not predict chemo response	Seiler *et al.*[361]
	Ki-67	IHC	CMV, MVAC	99	Does not predict chemo response	Siu *et al.*[362]
		IHC	MVAC	94	High Ki-67 associated with better OS (HR, 0.74)	Grossman *et al.*[363]
Apoptosis and survival	p53	IHC	MVAC	114	Does not predict chemo response (phase 3 RCT)	Stadler *et al.*[249]
		DNA seq	aMVAC	44	Does not predict chemo response	Plimack *et al.*[364]
		IHC	MVAC	90	Nuclear overexpression (>20%) associated with OS (HR, 3.1)	Sarkis *et al.*[365]
		IHC	M-CAVI	35	Does not predict chemo response	Ribas *et al.*[366]
		IHC	MC, MEC CMV, MVAC	83	Does not predict chemo response	Qureshi *et al.*[367]
		IHC	CMV, MVAC	99	Does not predict chemo response	Siu *et al.*[362]
		IHC	MVAC	94	Does not predict chemo response	Grossman *et al.*[363]
		IHC	MMC	43	Overexpression associated with higher recurrence rate	Ye *et al.*[190]
		IHC	CISCA, MVAC	25	Overexpression associated with worse response	Kong *et al.*[263]
	BCL2	IHC	Cis	51	Low BCL2 predicts better response to chemoradiation	Cooke *et al.*[262]
		IHC	MMC	43	BCL2:BAX ratio >1 associated with higher recurrence rate	Ye *et al.*[190]
		IHC	CISCA, MVAC	25	Overexpression associated with worse response	Kong *et al.*[263]
	Telomere length	qPCR	aMVAC	44	Does not predict chemo response	Plimack *et al.*[364]

Abbreviations: aMVAC, accelerated methotrexate, vinblastine, doxorubicin, cisplatin; Cis, cisplatin; CISCA, cisplatin, doxorubicin, cyclophosphamide; CMV, cisplatin, methotrexate, vinblastine; ddGC, dose-dense gemcitabine, cisplatin; DNA seq, DNA sequencing; FISH, fluorescence in situ hybridization assay; GC, gemcitabine, cisplatin; GCb, gemcitabine, carboplatin; GCT, gemcitabine, cisplatin, Taxol; IHC, immunohistochemistry; MC, methotrexate, cisplatin; M-CAVI, methotrexate, carboplatin, vinblastine; MEC, methotrexate, epirubicin, cisplatin; MMC, intravesical mitomycin C; MVAC, methotrexate, vinblastine, doxorubicin, cisplatin; MVEC, methotrexate, vinblastine, epirubicin, cisplatin; NGS, next-generation sequencing; PCR, polymerase chain reaction; qPCR, quantitiative PCR; RCT, randomized controlled trial, SNPs, single nucleotide polymorphisms; WES, whole exome sequencing.

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TABLE 4–20 Biomarkers of Chemotherapy Response, *Cont'd*

Molecular pathway	Biomarker(s)	Method	Drug(s)	N	Results	Reference
DNA repair	BRCA1	IHC	Cis	104	Does not predict chemo response	Mullane *et al.*[368]
		rtPCR	CMV, GC	57	Low BRCA1 predicts better chemo response and survival	Font *et al.*[369]
		rtPCR	GC, GCT	57	Does not predict chemo response	Bellmunt *et al.*[370]
	BRCA2	IHC	Cis	104	Does not predict chemo response	Mullane *et al.*[368]
	RAD51	IHC	Cis	104	High RAD51 expression associated with worse survival	Mullane *et al.*[368]
	PAR	IHC	Cis	104	High PAR expression associated with worse survival	Mullane *et al.*[368]
	PARP1	IHC	Cis	104	Does not predict chemo response	Mullane *et al.*[368]
	ERCC1	IHC	Cis	104	High ERCC1 expression associated with worse survival	Mullane *et al.*[368]
		rtPCR	GC, GCT	57	High ERCC1 expression associated with worse survival	Bellmunt *et al.*[370]
		IHC	aMVAC	39	Does not predict chemo response	Van Allen *et al.*[371]
	ERCC2	WES	Cis	55	ERCC2 mutations associated with better response and survival	Liu *et al.*[356]
		WES	Cis	50	ERCC2 mutations associated with better response but not survival	Van Allen *et al.*[371]
		WES	MVAC, GC, GCb	94	Does not predict chemo response	Groenendijk *et al.*[372]
	ATM/RB1/FANCC	NGS	aMVAC, ddGC	58	Altered ATM, RB1, or FANCC associated with better response and survival	Plimack *et al.*[355]
	RRM1	rtPCR	GC, GCT	57	Does not predict chemo response	Bellmunt *et al.*[370]

Abbreviations: aMVAC, accelerated methotrexate, vinblastine, doxorubicin, cisplatin; Cis, cisplatin; CISCA, cisplatin, doxorubicin, cyclophosphamide; CMV, cisplatin, methotrexate, vinblastine; ddGC, dose-dense gemcitabine, cisplatin; DNA seq, DNA sequencing; FISH, fluorescence in situ hybridization assay; GC, gemcitabine, cisplatin; GCb, gemcitabine, carboplatin; GCT, gemcitabine, cisplatin, Taxol; IHC, immunohistochemistry; MC, methotrexate, cisplatin; M-CAVI, methotrexate, carboplatin, vinblastine; MEC, methotrexate, epirubicin, cisplatin; MMC, intravesical mitomycin C; MVAC, methotrexate, vinblastine, doxorubicin, cisplatin; MVEC, methotrexate, vinblastine, epirubicin, cisplatin; NGS, next-generation sequencing; PCR, polymerase chain reaction; qPCR, quantitiative PCR; RCT, randomized controlled trial, SNPs, single nucleotide polymorphisms; WES, whole exome sequencing.

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TABLE 4–20 Biomarkers of Chemotherapy Response, *Cont'd*

Molecular pathway	Biomarker(s)	Method	Drug(s)	N	Results	Reference
Drug resistance	**MDR1**	PCR		34		Hoffman *et al.*[410]
	P-glycoprotein (MDR1)	IHC	CMV, MVAC	99	Does not predict chemo response	Siu *et al.*[362]
		IHC	CMV	25	Does not predict chemo response	Sandlow *et al.*[373]
	Caveolin-1	rtPCR	GC, GCT	57	Does not predict chemo response	Bellmunt *et al.*[370]
	CTR1	IHC	Cis	47	CTR1 expression associated with better chemo response	Kilari *et al.*[411]
	Nucleoside transporters	IHC	GC	62	Nucleoside transporters (hENT1, hCNT3, dCK) do not predict chemo response	North *et al.*[374]
Growth factors	**FGFR3**	WES	Pazopanib	1	Partial response with FGFR3 mutation	Palma *et al.*[375]
		WES	Pazopanib	3	Partial response with FGFR3 mutation	Pinciroli *et al.*[376]
	ERBB2 (HER2)	WES	MVAC, GC, GCb	94	ERBB2 mutations/amplification associated with better chemo response	Groenendijk *et al.*[372]
		WES	Cis	50	Does not predict chemo response	Van Allen *et al.*[371]
		WES	Pazopanib	3	ERBB2 mutations associated with better response	Pinciroli *et al.*[376]
		IHC	Lapatinib	116	Does not predict chemo response	Powles *et al.*[377]
		IHC	Lapatinib	34	Does not predict chemo response	Novara *et al.*[378]
	HER1	IHC	Lapatinib	116	Does not predict chemo response	Powles *et al.*[377]
	EGFR	IHC	Lapatinib	34	Increased EGFR expression associated with response	Novara *et al.*[378]
	VEGF	Serum	Sunitinib	26	Does not predict chemo response	Grivas *et al.*[379]
		Serum, IHC	Pazopanib	18	Does not predict chemo response	Pili *et al.*[380]
	HIF1α	IHC	Pazopanib	18	Does not predict chemo response	Pili *et al.*[380]

Abbreviations: aMVAC, accelerated methotrexate, vinblastine, doxorubicin, cisplatin; Cis, cisplatin; CISCA, cisplatin, doxorubicin, cyclophosphamide; CMV, cisplatin, methotrexate, vinblastine; ddGC, dose-dense gemcitabine, cisplatin; DNA seq, DNA sequencing; FISH, fluorescence in situ hybridization assay; GC, gemcitabine, cisplatin; GCb, gemcitabine, carboplatin; GCT, gemcitabine, cisplatin, Taxol; IHC, immunohistochemistry; MC, methotrexate, cisplatin; M-CAVI, methotrexate, carboplatin, vinblastine; MEC, methotrexate, epirubicin, cisplatin; MMC, intravesical mitomycin C; MVAC, methotrexate, vinblastine, doxorubicin, cisplatin; MVEC, methotrexate, vinblastine, epirubicin, cisplatin; NGS, next-generation sequencing; PCR, polymerase chain reaction; qPCR, quantitiative PCR; RCT, randomized controlled trial, SNPs, single nucleotide polymorphisms; WES, whole exome sequencing.

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TABLE 4–20 Biomarkers of Chemotherapy Response, *Cont'd*

Molecular pathway	Biomarker(s)	Method	Drug(s)	N	Results	Reference
RNA expression signatures	Takata	Microarray, qPCR	MVAC	49	Expression of 14 genes predicts chemo response	Takata *et al.*[381] Takata *et al.*[382]
	Kato	Microarray, qPCR	GC	37	Expression of 12 genes predicts chemo response	Kato *et al.*[383]
	COXEN	Microarray	MVAC	59	Score predicts chemo response and survival	Williams *et al.*[384]
	Basal vs. luminal vs. p53-like tumours	RNAseq	MVAC, aMVAC	100	p53-like tumours resistant to chemo	Choi *et al.*[317]
	McConkey	Microarray	ddMVAC	85	Basal tumours sensitive to chemo and p53-like tumours resistant to chemo	McConkey *et al.*[385]
	DeCypher	Microarray	MVAC, GC	305	Basal tumours sensitive to chemo and p53-like tumours resistant to chemo	Seiler *et al.*[386]
MicroRNA	Bellmunt	rtPCR	MVAC, GC	83	Increased miR-21, miR372, and E2F1 associated with chemo response and survival	Bellmunt *et al.*[387]
DNA markers	Germline SNPs	Microarray	MVAC, GC	205	Does not predict chemo response	O'Donnell *et al.*[388]
		Microarray	Cabazitaxel	45	SNPs predicted chemo response and toxicity	Duran *et al.*[389]
		Microarray	Cis	210	SNPs predicted chemo response	Gallagher *et al.*[390]
	Tumour DNA ploidy	Flow cytometry	MVEC	24	Does not predict chemo response	Türkölmez *et al.*[391]
		Flow cytometry	CMV	25	Does not predict chemo response	Sandlow *et al.*[373]
	Tumour S-phase fraction	Flow cytometry	MVEC	24	High S-phase fraction predicts better response and survival	Türkölmez *et al.*[391]
Immune markers	Lymphocyte count	Automated	GC, GCb, MVAC	55	High count predicts better response	Leibowitz-Amit *et al.*[392]
	IL-8	Luminex xMAP	Sunitinib	38	Low IL-8 associated with better time to progression	Bellmunt *et al.*[393]

Abbreviations: aMVAC, accelerated methotrexate, vinblastine, doxorubicin, cisplatin; Cis, cisplatin; CISCA, cisplatin, doxorubicin, cyclophosphamide; CMV, cisplatin, methotrexate, vinblastine; ddGC, dose-dense gemcitabine, cisplatin; DNA seq, DNA sequencing; FISH, fluorescence in situ hybridization assay; GC, gemcitabine, cisplatin; GCb, gemcitabine, carboplatin; GCT, gemcitabine, cisplatin, Taxol; IHC, immunohistochemistry; MC, methotrexate, cisplatin; M-CAVI, methotrexate, carboplatin, vinblastine; MEC, methotrexate, epirubicin, cisplatin; MMC, intravesical mitomycin C; MVAC, methotrexate, vinblastine, doxorubicin, cisplatin; MVEC, methotrexate, vinblastine, epirubicin, cisplatin; NGS, next-generation sequencing; PCR, polymerase chain reaction; qPCR, quantitiative PCR; RCT, randomized controlled trial, SNPs, single nucleotide polymorphisms; WES, whole exome sequencing.

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TABLE 4–20 Biomarkers of Chemotherapy Response, *Cont'd*

Molecular pathway	Biomarker(s)	Method	Drug(s)	N	Results	Reference
Other	**Metal-lothionein**	IHC	CMV, MVAC	99	High levels associated with worse survival	Siu *et al.*[362]
	GDPD3 + SPRED1	IHC	GC	37	High GDPD-3 and low SPRED-1 predict better chemo response	Baras *et al.*[394]
	Maspin	IHC	Cis	62	High levels associated with better survival	Chen *et al.*[395]

Abbreviations: aMVAC, accelerated methotrexate, vinblastine, doxorubicin, cisplatin; Cis, cisplatin; CISCA, cisplatin, doxorubicin, cyclophosphamide; CMV, cisplatin, methotrexate, vinblastine; ddGC, dose-dense gemcitabine, cisplatin; DNA seq, DNA sequencing; FISH, fluorescence in situ hybridization assay; GC, gemcitabine, cisplatin; GCb, gemcitabine, carboplatin; GCT, gemcitabine, cisplatin, Taxol; IHC, immunohistochemistry; MC, methotrexate, cisplatin; M-CAVI, methotrexate, carboplatin, vinblastine; MEC, methotrexate, epirubicin, cisplatin; MMC, intravesical mitomycin C; MVAC, methotrexate, vinblastine, doxorubicin, cisplatin; MVEC, methotrexate, vinblastine, epirubicin, cisplatin; NGS, next-generation sequencing; PCR, polymerase chain reaction; qPCR, quantitiative PCR; RCT, randomized controlled trial, SNPs, single nucleotide polymorphisms; WES, whole exome sequencing.

4.3.5.2 Cell cycle and proliferation

The urothelium that lines the lumen of the urinary tract, from the renal papillae to the urethra, is the most impenetrable epithelium in the human body, and, under normal circumstances, turns over every 3 to 6 months.[350] Urothelial turnover is tightly controlled, and cell-cycle regulators are crucial for maintaining this homeostasis. A hallmark of cancer, including BC, is dysregulation of the cell cycle, which results in the sustained signal for proliferation required for cancer development.[351] Though many molecules have a role in controlling the cell cycle, the main players are the cyclins and the cyclin-dependent kinases.[352] Cell-cycle regulators and markers of proliferation are known to be important prognostic markers in BC,[242,256] and several have been tested as predictors of chemotherapy response in cyclin D1, CCDN1, and Ki-67.

4.3.5.3 Apoptosis and survival

A second hallmark of cancer cells is their ability to resist cellular death signals that are generated during cellular proliferation.[351] Indeed, the ability to escape apoptosis, a process controlled by caspases, is crucial for cancer-cell survival.[353] Caspases, in turn, are regulated by several molecules involved in the detection of DNA or mitochondrial damage, including p53 and BCL2. The protein p53 has been extensively tested in BC, both as a prognostic marker and as a predictor of treatment response, and, while initial retrospective studies were promising, randomized trial results showed no role for p53 as a predictor of chemotherapy response.[240,249]

4.3.5.4 DNA damage repair

Many chemotherapy agents work by causing DNA damage; if the cancer cell's DNA integrity is sufficiently disrupted, the cancer cell cannot replicate.[354] Several chemotherapy agents used in BC cause DNA damage, including alkylating agents that crosslink DNA strands (mitomycin C, cisplatin); antimetabolites that mimic normal pyrimidine bases (gemcitabine, 5-fluorouracil) or that inhibit DNA synthesis (methotrexate); and anthracyclines that intercalate DNA base pairs and inhibit the topoisomerases that uncoil DNA for replication (doxorubicin, epirubicin, valrubicin). Cancer cells that have deficient DNA damage repair mechanisms are unable to fix the damage induced by these

chemotherapy agents and are therefore more susceptible to being killed by the agents. Proteins involved in DNA damage detection and repair that play a role in BC chemotherapy response include BRCA1, BRCA2, RAD51, PARP1, ERCC1, ERCC2, ATM, RB1, and FANCC. These have been the most promising markers so far in studies of DNA repair genes. In the study by Plimack *et al.*, 22 of 58 patients (38%) in the combined discovery and validation cohorts of patients who underwent neoadjuvant platinum-based chemotherapy had an alteration in ATM, RB1, or FANCC, of which 91% had pathologic stage T1 or lower at time of cystectomy.[355] Similarly, a validation study of ERCC2 in a cohort of patients with muscle-invasive disease found an ERCC2 mutation in 10 of 48 patients (21%), and 80% of the marker-positive patients had pathologic stage T1 or lower at time of cystectomy after neoadjuvant chemotherapy.[356]

4.3.5.5 Drug transport and activation

For most chemotherapies to work, the agents must enter the cancer cell and disrupt a biological process. This implies that proteins that affect drug transport into the cancer cell or that alter drug metabolism could have important effects on chemotherapy efficacy. In BC, several such examples exist, including CTR1, a copper transporter that helps cisplatin enter the cell; p-glycoprotein, a protein that pumps foreign substances, like chemotherapy drugs, out of the cell; the nucleoside transporter ENT1, which equilibrates intracellular and extracellular nucleosides like gemcitabine; CNT3, which transports nucleosides into the cell in a Na^+- and H^+-dependent manner; and metabolic enzymes, like dCK, which phosphorylates nucleosides into nucleotides, a key step in DNA synthesis.

4.3.5.6 Growth factors

It has been known for decades that the mitogenic signals deriving from the binding of growth factors to their receptors are crucial for cancer development.[357] Many growth factors and growth-factor receptors signal into the cell via transmembrane tyrosine kinases, and these kinases are the targets of several new systemic therapies in oncology. Several tyrosine kinase inhibitors have been tried in BC, including lapatinib (inhibits EGFR and HER2/neu pathways), sunitinib (inhibits platelet-derived growth factor [PDGF] and VEGF pathways), and pazopanib (inhibits FGF, PDGF, and VEGF pathways). While these three drugs appear to have limited activity in BC, the possibility of biomarker enrichment for response has been assessed.

4.3.5.7 RNA expression signatures

RNA expression profiling, also known as transcriptomics, assesses the relative quantity of a large number of RNA molecules (usually thousands of mRNA molecules) in cancer specimens.[358] RNA expression is assessed using either microarrays or RNA sequencing technologies. Microarrays measure the abundance of known RNA transcripts by their hybridization to complementary sequences immobilized on miniaturized chips (e.g., Affymetrix high-density arrays [Thermo Fisher Scientific, Waltham, USA]; NanoString [NanoString Technologies, Seattle, USA] is a new method related to microarrays). With RNA sequencing, next-generation sequencing instruments are used to determine the exact sequence and quantity of the RNA fragments in the cancer cell. Bioinformatic methods are then used to take the vast amount of RNA expression data generated by these technologies and convert them into a signature. Each signature is a combination of individual RNA transcript expression patterns and can contain the expression patterns of a few RNA molecules to the patterns of several hundred molecules per subject. Some RNA expression signatures are calculated from

human tumour samples (e.g., DeCypher, TimeLogic, Carlsbad, California, USA), while others were initially derived from cell lines exposed to chemotherapies (e.g., COXEN). The COXEN approach is currently being evaluated in a large cooperative group trial.

More recently, microRNAs (small noncoding RNA molecules 20–25 base pairs in size) have been shown to regulate several cellular processes.[359] Only preliminary assessments of microRNAs as predictors of chemotherapy response have been carried out.

4.3.5.8 DNA mutations and alterations

Both germline (patient) and somatic (tumour) DNA have been assessed for alterations that might predict the response to chemotherapy agents, a field known as pharmacogenomics.[360] In BC, germline single-nucleotide polymorphisms (single base-pair changes that occur in a patient's genes), DNA ploidy (the DNA content within a cell, normally two copies of every gene), and S-phase fraction (a proliferative index) have been tested as predictors of chemotherapy response.

4.3.5.9 Immunologic factors and other molecules

Several other factors have been assessed for their ability to predict chemotherapy response, including immunological factors. In some cases, these factors have been arrived at using a logical discovery process (like proteomics), while in other cases, the rationale behind the biomarker is less clear. Several of these factors are summarized in **Table 4–20**.

4.3.5.10 Recommendations

Few biomarkers have shown potential as predictors of response to chemotherapy, due to contrasting findings and to the low LOE. Consequently, their biomarker use is currently not recommended to predict the response to intravesical chemotherapy, systemic chemotherapy, or both in BC.

4.3.6 Response to systemic immunotherapy

4.3.6.1 Introduction

Over the past several years, immunotherapies have exhibited unprecedented activity in urothelial cancer after failure of cisplatin-based therapies. Inhibition of immune checkpoints has been demonstrated for several agents targeting programmed cell death-1 (PD-1) receptor or its ligand, programmed cell death ligand-1 (PD-L1), and cytotoxic T-lymphocyte antigen 4 (CTLA-4).

Two classes of drugs have recently been approved by the FDA for treatment of urothelial cancer. Atezolizumab,[396] a PD-L1–targeting agent, as well as the PD-1–blocking monoclonal antibodies nivolumab[397] and pembrolizumab,[398] have proven activity as second-line agents, while atezolizumab[399] and pembrolizumab[400] have also been approved as first-line treatment for cisplatin-ineligible patients. Durvalumab[401] and avelumab[402] have also been granted breakthrough therapy designation by the FDA for patients with locally advanced or metastatic urothelial cancer.

Nevertheless, the majority of patients do not respond to treatment,[396–399,401–404] resulting in a significant financial burden and potential treatment-related side effects to patients who do not benefit from therapy. Therefore, biomarkers are needed to predict those patients most likely to benefit from checkpoint-targeting therapy.

Clinical trials have explored several different biomarkers. No recommendations can be made at this point regarding testing for a specific biomarker prior to treatment, as a significant proportion of patients do still respond to treatment, despite testing negative for a biomarker. Moreover, FDA approvals for checkpoint inhibitors in urothelial cancer are independent of a biomarker status.

Many potential biomarkers have been explored in different tumour entities and show promising results, either as a single marker or in combination with others, while the following are the best described potential biomarkers for immunotherapy in urothelial cancer.

4.3.6.2 Programmed cell death ligand-1

Detection of PD-L1 on tumour samples with IHC has been used by several clinical trials to evaluate the feasibility of PD-L1 expression as a predictive biomarker. As testing for PD-L1 is not standardized, the evaluation of PD-L1 has several limitations. There have been variations in antibodies used in assays (SP142,[396,399] 28-8,[397,403] 22C3,[398,400] SP263, and 73-10[402]) and staining platforms, the decision to stain tumour cells or immune cells, and which cutoffs to use to define positivity. Therefore, evaluation of the predictive value of PD-L1 positivity is difficult, and the correlation of PD-L1 positivity with response to treatment or survival varies between trials.

In the IMvigor 210 trial, the cisplatin-pretreated arm (cohort 2) revealed an objective response rate (ORR) of 15% (95% CI, 11–19) in all patients undergoing atezolizumab treatment. The PD-L1 expression status on infiltrating immune cells (ICs) in the tumour microenvironment was defined by the percentage of PD-L1–positive ICs: IC0 (<1%), IC1 (≥1%, but <5%), and IC2/3 (≥5%). The following are the objective response rates for each prespecified IC group: IC2/3: 27% (95% CI, 19–37), $p<0.0001$; IC1/2/3: 18% (95% CI, 13–24), $p=0.0004$; and in all patients, 15% (95% CI, 11–20), $p=0.0058$. With longer follow-up (data cutoff September 14, 2015), by independent review, objective response rates were 26% (95% CI: 18–36) in the IC2/3 group, 18% (95% CI, 13–24) in the IC1/2/3 group, and 15% (95% CI, 11–19) overall in all 310 patients.[396] In the cisplatin-ineligible arm, the ORR was 23% (95% CI, 16–31), independent of PD-L1 status.[399]

Results from the CheckMate 032 trial indicated no significant difference in ORR between patients with PD-L1 expression <1% (26.2%, 13.9–42.0) and patients with PD-L1 expression ≥1% (24.0%, 9.4–45.1),[403] In CheckMate 275, which evaluated nivolumab in metastatic urothelial carcinoma after platinum therapy, confirmed objective response was achieved in 28.4% (95% CI, 18.9–39.5) with PD-L1 expression ≥5%, 23.8% (95% CI, 16.5–32.3) of patients with PD-L1 expression ≥1%, and 16.1% (95% CI, 10.5–23.1) in the group with <1% PD-L1 expression.[397]

In KEYNOTE-045, PD-L1 expression was evaluated by the IHC 22C3 pharmDx assay (Agilent Technologies, Santa Clara, USA) and categorized as the PD-L1 combined positive score, defined as the percentage of PD-L1–expressing tumour and infiltrating ICs, relative to the total number of tumour cells. In this trial, the benefit of pembrolizumab appeared to be independent of PD-L1 expression on tumour cells and infiltrating ICs.[398]

4.3.6.3 The Cancer Genome Atlas subtype

Molecular subtypes of muscle-invasive BC have recently been categorized based on gene expression. TCGA[405] describes four subtypes of BC based on cluster analysis of mRNA. Tumour samples from recent clinical trials have been analyzed based on these mRNA subtypes and correlated with treatment response.

The exploratory analyses from the cisplatin-pretreated arm of the IMvigor 210 trial showed TCGA subtypes to be independently predictive for response to atezolizumab treatment.[396] PD-L1 IC prevalence was highly enriched in the basal subtype versus the luminal subtype (60% vs. 23%, $p<0.0001$), with IC2/3 expression of 15% in the papillary-like luminal cluster I, 34% in cluster II, 68% in the squamous-like basal cluster III, and 50% in the basal cluster IV subtype. Elevated PD-L1 tumour-cell expression was almost exclusively seen in the basal subtype (39% in the basal subtype vs. 4% in luminal subtype, $p<0.0001$) and did not correlate with ORR. Response to atezolizumab occurred in all TCGA subtypes, but was significantly higher in the luminal cluster II subtype than in other subtypes, demonstrating an ORR of 34% ($p=0.0017$).[396] For cisplatin-ineligible patients, responses were seen across all subtypes and were more frequent with the luminal II subtype.[399]

In CheckMate 275, all four urothelial carcinoma molecular subtypes were represented: luminal 1 ($n=66$), luminal 2 ($n=55$), basal 1 ($n=23$), and basal 2 ($n=33$). Basal 1 subtype contained the highest proportion of responders.[397]

4.3.6.4 Mutational load

High mutational load may be associated with better response to immunotherapy, particularly for checkpoint inhibitors, with some trials showing a correlation between patients with a higher mutational burden and better responses to immunotherapeutic agents. However, mutational load alone may not have enough influence on response to affect treatment decision-making.

In the IMvigor 210 trial, cohort 1 (cisplatin-ineligible patients) mutational load was associated with overall survival; patients with the highest mutational load had significantly longer survival. In cohort 2 (cisplatin-pretreated patients), the median mutational load was significantly increased in responders compared with nonresponders ($p<0.0001$). The relationship between mutational load and response was unrelated to TCGA subtype ($p=0.22$) or IC subgroup.[396]

4.3.6.5 Interferon-γ gene signature

Gene-expression profiling may predict response to immunotherapy. Genetic markers associated with response to immunotherapy are addressed as they pertain to the tumour genomic landscape.[406]

CheckMate 275 found that higher values of the 25-gene interferon-γ signature were associated with a greater proportion of responders to nivolumab and higher PD-L1 expression. As well, patients with high interferon-γ signature were more likely to respond to nivolumab than those with low interferon-γ signature ($p=0.0003$).[397] The strongest interferon-γ signature was noted in patients with basal 1 subtype. These patients were more likely to have a high interferon-γ signature score than patients with the other subtypes.[397]

4.3.6.6 Chemokines and CD8+ T-cell infiltration

The tumour microenvironment is the primary location of interaction between tumour cells and the host immune system. Different IC subsets are recruited into the tumour microenvironment. Complex interactions occur between chemokines and chemokine receptors, and these populations have distinct effects on tumour progression and therapeutic outcomes.[407]

CheckMate 275 found that the highest CXCL9 or CXCL10 expression occurred in nivolumab responders, in basal 1 subtype, and in the subgroup of patients with PD-L1 expression of ≥5%, with CXCL9 and CXCL10 expression at least three times higher than in other subgroups. Additionally, 12-chemokine signature was highly enriched in tumours from nivolumab responders. The highest CD8 expression was associated with nivolumab responders and basal 1 subtype.[397]

In the IMvigor 210 cohort 2 of cisplatin-pretreated patients, responses to atezolizumab were most closely associated with high expression of the two interferon-γ–inducible T helper 1 (T_H1)–type chemokines, CXCL9 (p=0.0057) and CXCL10 (p=0.0079).[396] Consistent with increased T-cell trafficking chemokine expression, tumour centre CD8+ T-cell infiltration was also associated with both PD-L1 ICs (p<0.0001) and response to atezolizumab (p=0.0265). Consistent with PD-L1 IC2/3 expression, CD8 T-effector gene expression was elevated in luminal cluster II and basal cluster III/IV, but not in luminal cluster I.[396]

4.3.6.7 Recommendations

Despite the encouraging findings, the LOE is insufficient to recommend the use of biomarkers for predicting response to systemic immunotherapy in BC.

4.4 Summary of Recommendations

- Biomarkers are not recommended for the screening and the diagnosis of bladder cancer. (Grade of Recommendation **[GOR] C, LOE 3**)
- In the surveillance of patients with nonmuscle-invasive bladder cancer, biomarkers should not replace cystoscopy. Biomarkers can be used in the surveillance of intermediate- to high-risk patients to assess their response to intravesical immunotherapy, and as a reflex test for equivocal urinary cytology. (**GOR C, LOE 3**)
- Biomarkers are not recommended for the assessment of response to intravesical or systemic chemotherapy. (**GOR C, LOE 3**)
- Biomarkers are not recommended for the assessment of response to systemic immunotherapy. (**GOR D, LOE 2**)

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C5

Management of Nonmuscle-invasive Bladder Cancer

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CONTENTS

Management of Nonmuscle-invasive Bladder Cancer

Committee 5

5.1 Introduction

In this section, we cover the entire spectrum of nonmuscle-invasive bladder cancer (NMIBC). Focused topics include: (1) prognostic factors of recurrence and progression; (2) risk stratification of NMIBC (clinical, pathological, molecular); (3) staging workup (primary assessment of NMIBC); (4) management of positive urine cytology with negative white-light cystoscopy (WLC); (5) indications of bladder and prostatic urethral biopsies; (6) management of prostatic urethral involvement; (7) management of primary and recurrent low-grade Ta (TaLG); (8) management of primary and recurrent high-risk tumours (TaHG, T1, carcinoma *in situ* [CIS]); (9) impact of bacillus Calmette-Guérin (BCG) strain and host on outcomes of NMIBC; (10) management of complications of intravesical therapy; (11) role of alternative therapies (radiation, Electromotive Drug Administration [EMDA]/mitomycin C [MMC], chemohyperthermia [CHT]); (12) indications for early cystectomy: balancing risks and benefits; (13) surveillance strategies (urine markers, imaging, cystoscopy, etc); and (14) new treatment strategies from ongoing and future clinical trials. Where appropriate, we have embedded levels of evidence **[LOE]** and grades of recommendation **[GOR]** for various aspects within the management of NMIBC.

5.2 Staging Workup (Primary Assessment of Nonmuscle-invasive Bladder Cancer)

Evidence acquisition

A detailed review of the literature was performed focusing on original high-level of evidence articles addressing staging and initial assessment aspects of non-muscle-invasive bladder cancer from the past 10 years including Medline and Cochrane databases. The scientific evidence available was classified when possible using the Oxford method and the summary of the recommendations was graded based on the Oxford Centre for Evidence-based Medicine system.[1]

5.2.1 Screening

Screening with the objective of identifying early stages of disease has not yet been proven beneficial in bladder cancer. There are no studies comparing benefits of treatment in patients diagnosed through screening versus without screening.[2-4] Therefore, the recommendation on screening for bladder cancer remains unchanged from the previous 2012 International Consultation on Bladder Cancer guidelines.

5.2.2 Presentation

Hematuria is the cardinal clinical symptom of bladder cancer. Usually painless, it can be associated with irritative storage or voiding symptoms in the presence of CIS.

The prevalence of asymptomatic hematuria in general ranges from 0.19% to 21% and seems to increase with age depending on the population studied.[5,6] The incidence of bladder cancer ranges from 17% to 18.9% in patients with macroscopic hematuria, while it ranges from 4.8% to 6% in patients with microscopic hematuria[7-9] **[LOE 2]**.[7-9] In men over 60 years of age, the prevalence of microscopic hematuria is 23%, and the risk for bladder cancer detection on subsequent investigation is 5%[5] **[LOE 2]**. There is no evidence supporting that patients with bladder cancer detected after microscopic hematuria work-up have better oncological outcomes after treatment.

Other signs and symptoms in NMIBC are unspecific and the physical exam is mostly unremarkable.

5.2.3 Initial investigation

The mainstay of the initial investigation in a suspected bladder cancer is cystoscopy. It can be performed utilizing flexible or rigid instruments, either in the office setting or in the hospital surgical suite. In cases with an obvious lesion identified through imaging methods, or with indication for clot evacuation and fulguration of bleeders, diagnosis through office-based flexible cystoscopy can be omitted and these patients can be taken directly to transurethral resection (TUR).

The incidence of upper urinary tract tumour in patients evaluated for hematuria ranges from 0.2% to 0.7% **[LOE 2]**.[5,6,8-10] Although these rates are small, during hematuria evaluation, in addition to the cystoscopy for assessment of the lower urinary tract, the initial mandatory investigation should also include upper-tract imaging **[LOE 4]**.

Among the different imaging modalities, the possible options are ultrasonography, IV urography, computed tomography urography (CTU), magnetic resonance imaging (MRI), and retrograde pyelography. Ultrasonography and IVU seem to have similar sensitivity for upper-tract disease **[LOE 2]**.[10] When assessing the bladder, ultrasonography has reported sensitivity rates ranging from 63% to 98%, and specificity rates of 99% **[LOE 2]**.[7] Thus, in the presence of positive ultrasonography findings, the diagnostic office-based cystoscopy can be safely omitted.

CTU is considered the gold standard for evaluation of upper urinary tract. For the detection of upper urinary tract lesions, it has reported sensitivity rates ranging from 88% to 100% and specificities ranging from 93% to 100%. A meta-analysis performed on this subject yielded a pooled sensitivity and specificity of 86% and 99%, respectively **[LOE 2]**.[11] In patients with poor renal function or intravenous (IV) contrast allergy, the use of alternative modalities, including MRI urography and retrograde pyelography, during the cystoscopic evaluation are equally acceptable.

Urine cytology is another critical component of the initial investigation and consists of cytopathological assessment of the morphologic features of urothelial cells. The combination of cystoscopy with cytology has been considered the gold standard method for diagnosis and surveillance of bladder cancer and is superior to cystoscopy alone in the detection of high-grade urothelial lesions. Cytology is limited in the detection of low-grade tumours due to low sensitivity and negative predictive values, but it has the highest specificity and negative predictive values for high-grade disease, since it is based on direct cytopathological identification of atypical and dysplastic cells. Voided specimens are useful and easily collected without the need for invasive methods but, at the same time, have a lower diagnostic yield than washing samples obtained during cystoscopy. Moreover, the diagnostic yield of urine cytology is increased when more than three samples are analyzed **[LOE 2]**.[12] A pooled analysis of urine cytology revealed a sensitivity and specificity ranging from 29% to 77% and 71% to 100%, respectively, with most studies observing specificities greater than 90% for both low- and high-grade tumours **[LOE 2]**.[13-19] Several urinary biomarkers are commercially available, including UroVysion (fluorescence *in situ* hybridization [FISH], microsatellite analysis, ImmunoCyt or uCyt+, nuclear matrix protein 22 (NMP22), BTA (bladder tumour antigen) stat, BTA TRAK, and cytokeratins. These tests in general have better sensitivity but lower specificity than cytology and are not routinely utilized or recommended because of inferior performance when compared to cystoscopy and cytology.[17,20-22] There are no commercially available urinary biomarkers that perform better than cystoscopy alone or in combination with cytology and, thus, there is no consensus about the utilization of urinary biomarkers for the diagnosis or the initial evaluation of bladder cancer.

5.2.4 Staging and risk stratification

The clinical staging classification of bladder cancer utilizes the 2010 American Joint Committee on Cancer/Union Internationale Contre le Cancer (AJCC/UICC) tumour-node-metastasis (TNM) system 7th edition, which was recently updated in 2017 (8th edition) without changes to the bladder cancer staging classification.[23,24] The final staging is given by a combination of the three key elements: the pT-stage on the TUR specimen, the cT-stage from the examination under anesthesia (EUA), and the imaging findings. Detailed pathology discussion of bladder tumours is in the scope of another chapter, but it is important to note that pathological evaluation of the TUR specimen provides critical information, such as the histological type of the tumour (and presence of more aggressive variants), depth of invasion (which determines the T classification), tumour grade, and presence of important prognostic risk factors (such as lymphovascular invasion). [LVI][25,26]

The grading system changed from a three-tier classification (1973 World Health Organization [WHO]) to a 2-tier classification (2004 WHO/International Society of Urological Pathologists [ISUP]).[27-29] While both systems are still in use nowadays, especially with a grade 2 score, it is recommended that the 2004 WHO/ISUP classification is also mentioned to better define between a low- versus high-grade tumour. An update of the 2004 WHO/ISUP classification has been recently published but has maintained the same grading system.[28] The 1973 and the 2004 grading systems are not directly interchangeable. The 1973 WHO grade 1 carcinomas are reassigned to the 2004 WHO papillary urothelial neoplasia of low malignant potential (PUNLMP) and low-grade carcinoma categories, while the 1973 WHO grade 2 carcinomas are reassigned to the 2004 WHO low-grade and high-grade carcinoma categories.[30] All 1973 WHO grade 3 tumours are assigned to the high-grade carcinoma category.[30]

The nonmuscle-invasive bladder tumours are also stratified based on stage and grade, number, and size of lesions, as well as history of recurrences. Therefore, the combination of the staging procedures, along with the elements of the cystoscopic evaluation and cytology listed previously, are paramount for the initial evaluation and treatment decision-making in bladder cancer. The risk-group stratification not only defines prognostic risks of recurrence and progression, but also dictates different management strategies.

The European Association of Urology (EAU) simplified version of the European Organisation for Research and Treatment of Cancer (EORTC) risk stratification classification has been developed.[31-34] A limitation of the original EORTC risk tables was the lack of inclusion of patients who had undergone a repeat transurethral resection of the bladder tumour (TURBT); no patient received a single postoperative instillation of chemotherapy. No maintenance BCG treatment was given, and BCG induction was not used in about 20% of patients.[31,35] Recently, the EORTC has updated the risk tables to include these patients, and these scoring models can also be assessed in the Club Urológico Español de Tratamiento Oncológico (CUETO; i.e., Spanish Urological Club for Oncological Treatment) tables.[36,37]

5.2.5 Technical aspects of the transurethral resection of the bladder tumour and examination under anesthesia

The TUR is performed after a detailed cystoscopic evaluation and is followed by the bimanual examination of the bladder under anesthesia. These combined procedures serve both diagnostic and therapeutic purposes in the management of bladder cancer. The diagnostic role would include the identification and localization of the lesions in the bladder, the determination of histological subtypes, the definition of the clinical staging and grade of the tumours, and also the identification of histological prognostic markers of more aggressive disease that would matter for clinical decision-making. The therapeutic role is defined by removal of all nonmuscle-invasive lesions in preparation for subsequent intravesical therapies. Additional therapeutic benefits would include mitigation of bleeding, relieving of both voiding and storage urinary symptoms caused by the presence of tumour, inflammation, or bladder outlet obstruction, and resolution of upper urinary tract obstruction caused by ureteral orifice invasion and/or obstruction.

The procedure can be performed under different forms of anesthesia, varying from general to regional neuraxial (spinal or epidural). Probably one of the biggest advantages of general anesthesia is the ability to perform neuromuscular blockade, which is the most efficient way to prevent stimulation of obturator nerve reflex during the resection of lateral wall tumours. The use of obturator nerve block is a possible alternative, but rarely utilized these days **[LOE 4]**.

A barbotage urine sample can be obtained at the beginning of the procedure using normal saline irrigation through the cystoscope or resectoscope sheath after an initial complete inspection of the bladder internal mucosal surface. The initial inspection is important to distinguish mucosal lesions caused by the barbotage or rough scope manipulation inside the bladder during the procedure.

The cystoscopic evaluation should utilize both 30- and 70-degree lenses for optimal assessment of the bladder neck. Alternatively, a retroflexion maneuver using a flexible cystoscope can also be utilized. The anterior wall and the dome of the bladder are better visualized during concomitant suprapubic pressure to depress and stretch these areas. Optimal bladder distention should also be observed, so no excessive folding occurs in the empty bladder and, contrarily, there is no excessive hydro-distension. This is also important during the TUR portion of the procedure to avoid inadvertent perforation. The discussion on the utilization of enhanced cystoscopic imaging technologies with fluorescence cystoscopy for photodynamic diagnosis (PDD), narrow-band imaging (NBI), optical coherence tomography, confocal laser endomicroscopy, and others is not in the scope of this section and will be discussed elsewhere in these guidelines.

There are basically two different techniques of bladder tumour resections: the stage resection and the en bloc resection. No formal prospective studies exist comparing these techniques.

Stage resection can be applied to any size of lesions and consists of resection of the lesion in different layers or levels. Initially, the tumour is resected completely in multiple pieces from the most prominent portion until the base is reached. Then, the base is resected deeply to muscle to provide adequate staging information.[38,39] To avoid cautery artifact, some authors would even recommend sampling the base of the tumour with cold cup biopsy forceps.

En bloc resection is usually employed for small lesions (<3 cm).[40-42] The purported advantages would include more accurate pathological staging, since the orientation of the tumour is preserved and there is less cautery artifact, and less tumour cell shedding, since the tumour is not cut in several pieces.

The choice of energy (monopolar vs. bipolar) does not seem to impact the quality of resection or the incidence of obturator reflex, but some evidence exists supporting less cautery artifact formation and better pathological assessment in specimens after bipolar resection.[43]

Diffuse CIS-suspected lesions must be sampled during the TUR or biopsied for diagnosis, but extensive resections are not beneficial. Instead, these lesions are better treated with immunotherapy with BCG intravesical instillations **[LOE 1]**.[44]

Although coagulative energy should be avoided around the ureteral orifices, tumours that obstruct or involve the ureteral orifices can be resected under pure cutting settings. In this situation, ureteral stenosis is uncommon, and a temporary ureteral stent placement (2–6 weeks) can further help prevent it **[LOE 4]**. Unblocking ureteral orifices must always be attempted during the TUR, since the effective systemic chemotherapy in muscle-invasive disease is based on cisplatin-based regimens for which an appropriate renal function is a necessary requirement.

Tumours in the anterior wall are challenging to resect and require suprapubic pressure and abdominal compression to aid in bladder distension, as well as proper resectoscope angles. Lesions in the anterior wall and dome may be better resected using open-angled loops **[LOE 4]**. The lateral wall tumours expose patients to obturator nerve stimulation during the resection, increasing the risk for bladder perforations. Obturator nerve reflex can be avoided by neuromuscular blockade during general anesthesia, direct obturator block, avoidance of bladder overdistension, use of intermittent current, and lower current settings **[LOE 4]**.

Tumours within bladder diverticula are also challenging to manage and properly stage because of the thin wall and lack of muscle layer in the diverticula. Small and limited low-grade lesions can be carefully resected or fulgurated. Because of the increased risk for perforation, in large and/or high-grade lesions located inside of a bladder diverticulum, the recommendation is for diverticulectomy or partial cystectomy, when feasible, or radical cystectomy (RC) **[LOE 4]**.

Random bladder biopsies usually involve removal of bladder specimens with a cold cup forceps, sampling mostly mucosa and submucosa layers with the intention to identify CIS in cystoscopically normal-appearing areas. Despite the lack of evidence supporting strong recommendations, these biopsies are indicated in patients with positive cytology and negative cystoscopy, or to further investigate the presence or the extent of CIS involvement in patients with high-grade nonmuscle-invasive disease. Because this sampling is not randomly taken but instead directed to lateral, anterior, and posterior walls, trigone, and dome of the bladder, some authors prefer to call these site-directed biopsies. There is no strong evidence to support the routine recommendation of random or site-directed biopsies on every TURBT. The incidence of positive random or site-directed biopsies in areas of normal mucosa ranges from 1.5% to 14.5% **[LOE 3]**.[45-49] These biopsies seem to influence and change management in 8% of patients **[LOE 3]**.[49]

Prostatic urethra biopsy is another important component of the initial staging and should be performed in patients with high-grade disease, during assessment of the extent of CIS, and/or in the presence of positive cytology and negative cystoscopy. It should be performed with the resection loop including the prostatic sinuses bilaterally at 5 and 7 o'clock positions, since these areas carry the largest concentration of prostatic ducts **[LOE 4]**. If there is confirmed presence of Tis or T1 urothelial disease in the prostatic urethra, these patients should undergo a formal transurethral resection of the prostate (TURP) for more accurate staging, and so these areas can also benefit from exposure to intravesical immunotherapy instillations with BCG **[LOE 4]**. If prostatic stroma invasion is identified, the disease is upstaged to *c*T4 (if primary from the bladder) or cT2 (if primary from the prostatic urethra), and thus managed as locally invasive disease **[LOE 4]**.

In all T1 and/or high-grade tumours, especially if muscle was not identified in the previous TUR specimen, the standard of care recommendation is for a repeat TURBT to be performed within 4 to 6 weeks. Further details are discussed in another section.

Although the examination under anesthesia (EUA) can be performed just before or at the beginning of the case, the formal EUA evaluation for staging purposes must be done at the end of TURBT, with the bladder drained completely empty, and without catheters in place. One hand should depress the suprapubic region, while the other hand should assess the anatomic features through rectal exam in men or vaginal exam in women. Thickening of bladder wall suggests muscle-invasive disease, while tridimensional palpable and mobile mass define *c*T3 disease. Fixed masses felt during the EUA define *c*T4b disease. There may be additional pelvic organ involvement, and assessment of the prostate and seminal vesicles in men should also be performed **[LOE 4]**.

5.2.6 Imaging modalities for initial staging

Cross-sectional imaging assessment of patients with bladder cancer is another critical component of initial evaluation and staging. It can provide important information about local extent of the disease or invasion of perivesical tissues and neighbour organs, involvement of local, regional, and distant lymph nodes, and presence of distant metastases. Moreover, complete assessment of all other possible urothelium sites for disease in both upper and lower urinary tract is mandatory before establishing overall treatment plans, especially in high-grade cancers. Additional benefit would also include information about kidney obstruction and impact of the bladder tumour by the observation of hydroureter and hydronephrosis. This information is important for definition of the clinical T-stage and for clinical decision-making. The presence of a hydroureter and/or hydronephrosis associated with the presence of a bladder mass on imaging tests alone suggest *c*T3 disease. Since systemic chemotherapy on more advanced stages of disease is centred around cisplatin-based regimens, which rely on the presence of good renal function, the presence of upper urinary tract obstruction needs to be addressed and resolved promptly during the initial assessment.

The final clinical staging information should be a combination of (1) the cystoscopic examination with pathological information of depth of invasion in the bladder wall and grade, (2) EUA findings, and (3) cross-sectional imaging findings **[LOE4]**.

A computed tomography (CT) scan of the abdomen and pelvis is recommended in all cases where there is cystoscopic identification of a solid lesion, appearing high grade or suggesting invasion into the muscle **[LOE 4]**. CT can also be used in combination with positron emission tomography (PET) in the detection of local or distant disease.

The reported sensitivity and specificity of multidetector CT in the diagnosis and staging of bladder cancer range from 89% to 91% and 92% to 95%, respectively **[LOE 3]**.[50,51] Ideally, CT should be performed before or 7 days after the TURBT to avoid false-positive results due to postoperative inflammation, perivesical swelling, or fluid infiltration **[LOE 3]**.[50]

MRI has high reported detection rates of 98% to 100% utilizing diffusion-weighted sequences.[52-54] Staging sensitivity and specificity rates are similar to the CT, ranging from 68% to 80% and 90% to 93%, respectively.[55,56] There is some evidence that MRI can predict grade and tumour features.[57,58]

Lymph node metastases are detected by CT with sensitivity and specificity ranging from 31% to 50% and 68% to 100%, respectively **[LOE 3]**.[59-61] Although MRI has better overall detection rates of lymph nodes than CT, particularly for nodes smaller than 5 mm, its ability to identify malignant disease within normal or slightly enlarged lymph nodes is limited.[62]

PET scanning in bladder cancer has mostly been utilized with ^{18}F-fluorodeoxyglucose (18-F FDG) as the radiopharmaceutical contrast agent. Because FDG is excreted in the urine, the application for detection and staging of early disease in the bladder or upper urinary tract are very limited and not useful. PET, especially when combined with CT, has better applications in more advanced disease and identification of nodal or distant organ and bone metastatic sites.

5.2.7 Conclusion

Hematuria is the most common sign and symptom of bladder cancer, especially for nonmuscle-invasive disease. Initial assessment should involve cystoscopic evaluation with urine sample collection for cytology, associated with imaging of the upper urinary tract. Once the presence of the lesion in the bladder is confirmed, patients should undergo bladder biopsy and TURBT, associated with EUA to achieve final diagnosis, staging, and even therapeutic goals, depending on the stage of the lesion. It is important to observe the need for combining the physical examination, pathological examination of TURBT specimen, and cross-sectional imaging assessment for a complete and final clinical staging. Adequate imaging test application and interpretation and attention to technical details during in the endoscopic evaluation of these tumours are very important for disease management and require training and expertise. Finally, accurate staging, grading, and risk stratification are critical determinants of the management and outcomes of these patients. The summary of recommendations discussed in this section is listed as follows.

Summary of the Recommendations for Staging Workup (Primary Assessment of NMIBC)

Summary of main recommendations	Grade
Cystoscopy and cytology are the gold standard for diagnosis and surveillance of bladder cancer.	B
Barbotage washing cytology sample provides better diagnostic yield than a voided sample.	B
CTU is the standard imaging evaluation of the upper urinary tract, but IVU, MRI urography, ultrasonography, and retrograde pyelography are acceptable alternatives.	C
Timing of cross-sectional imaging should be before or 1 week after TURBT to avoid artifacts.	C
A CT scan of abdomen and pelvis is recommended in all cases where there is solid lesion, HG, or suggestion of muscle invasion.	C
Abdominal and pelvic CT or MRI is not better than cystoscopy in diagnosis, but both complement staging by assessing the tissues around the bladder, the upper urinary tract, local regional and distant lymph nodes, and possible distant visceral and bone metastasis.	B
The EUA should always be performed in association with the TURBT, ideally at the end.	C
General anesthesia with muscle relaxation or obturator nerve blockade, among other techniques, must be used to prevent nerve stimulation and accidental inadvertent bladder perforations when resecting lateral wall tumours.	C

Abbreviations: CTU, computed tomography urography; EUA, examination under anesthesia; HG, high grade; IVU, intravenous urography; MRI, magnetic resonance imaging; TURBT, transurethral resection of bladder tumour.

5.3 Prognostic Factors of Recurrence and Progression: Risk Stratification of Nonmuscle-invasive Bladder Cancer

5.3.1 Introduction

Bladder cancer is the ninth most commonly diagnosed malignancy worldwide.[63,64] In 2012, there were an estimated 430,000 new cases of bladder cancer globally.[65] The incidence of bladder cancer is highest in developed regions, which account for 60% of cases, and it is the 13th leading cause of cancer mortality worldwide.[63,65] Incidence is highest in Europe, followed by the United States, Northern Africa (due to endemic *Schistosoma haematobium*), and Western Asia, which has the highest rates of bladder cancer mortality.[65] Males have a three-fold greater likelihood of developing bladder cancer as compared to females, and the average age of diagnosis is 73 years.[64]

At the time of diagnosis, 75% of bladder tumours fall into the category of NMIBC.[66] NMIBC is composed of noninvasive papillary carcinoma (Ta), CIS (Tis), and tumour invading the subepithelial connective tissue only (T1). High rates of disease recurrence, ranging from 30% in Ta to 70% in T1 tumours, as well as progression to invasive disease, represent a substantial challenge in the management of NMIBC.[67] To achieve reductions in bladder cancer recurrence and progression rates, it is vital to identify prognostic factors that can guide therapy plans based on individual patient risk factors and pathology. Candidate prognostic factors range from patient-specific to tumour- and treatment-specific characteristics. Although much work is currently being done to develop disease modelling and prognostication strategies for use in daily clinical practice, there is as of yet no one standardized prognostic model for NMIBC. The most widely used risk stratification tools are those put forth by EORTC and the CUETO.[68,69] These tools, which will be discussed in this chapter, provide valuable information on the management of NMIBC, but are limited in applicability to current practice and have had variable results from attempts at external validation.

Our aim is to provide an overview of the currently available prognostic factors for NMIBC, in which the focus is on patient, tumour and treatment characteristics, molecular markers, and available predictive models.

5.3.2 Patient-related factors

5.3.2.1 Age

Age may be useful for stratifying patients at highest risk for disease recurrence and progression after initial treatment. CUETO evaluated a cohort of 1,062 patients treated with BCG for NMIBC. Data were collected prospectively from four randomized trials comparing different intravesical treatments for NMIBC, and a risk stratification model developed using multivariate regression. They stratified subjects by age (<60; 60–70; and >70 years) and concluded that age was an independent predictor of

recurrence and progression after BCG administration (hazard ratio [HR], 1.17; 95% confidence interval [CI], 1.02–1.34 and HR, 1.29; 95% CI, 1.04–1.61, respectively) **[LOE 2]**.[68,69] It had been suggested that age may decrease immune response to intravesical therapy. An earlier series lead by the EORTC evaluated 2596 NMIBC patients from seven randomized controlled trials (RCTs) evaluating intravesical therapy after TURBT and found that age (≥65 years) was an independent predictor of progression (HR, 1.36; p=0.012) but not of recurrence **[LOE 2]**.[68,70] This study was limited, as only 361 out of 2,596 subjects received BCG as their post-TURBT intravesical therapy. Both the CUETO and EORTC studies used age as a component of broader predictive models; however, they likely overestimate the risk for both progression and recurrence.[70] More recently, Cambier *et al.* evaluated patients in two EORTC randomized, phase 3 trials with intermediate- and high-risk NMIBC treated with 1 to 3 years of BCG after TURBT to identify high-risk patients. They found that both increased age and grade of disease are associated with risk for progression and recurrence in patients treated with BCG maintenance **[LOE 2]**.[71] This study served as an update to the prior EORTC risk stratification tables by including BCG therapy. Gontero *et al.* found that, amongst patients with high-grade T1 disease treated with BCG who were over 70 years of age with tumour size over 3 cm and CIS, bladder cancer–specific mortality was significantly higher at 10 years of follow-up **[LOE 3]**.[72]

5.3.2.2 Gender

Men are four times more likely to develop bladder cancer than women, yet women present with more advanced disease.[73] Female gender is associated with larger, multifocal tumours, higher tumour grade, higher rates of recurrence, variant histology, and increased mortality after treatment **[LOE 3]**.[74,75] Numerous gender-specific risk factors have been proposed to explain this disparity, including the role of androgen and estrogen receptors in bladder tumour biology, differences in environmental toxin exposures, varying metabolic clearance of carcinogens, and delayed presentation and evaluation of hematuria in women.[76] Data regarding outcomes in females with NMIBC are conflicting. Higher cancer-specific mortality amongst women has been reported in national cancer registry databases; however, other studies suggest that treatment with BCG nullifies this effect of gender on outcomes.[77,78] The study establishing the CUETO scoring model found an increased risk for disease recurrence in women treated with BCG (HR, 1.69; 95% CI, 1.26–2.30) **[LOE 2]**.[69] A meta-analysis of 15,215 patients conducted by Martin-Doyle and colleagues investigated patients with high-grade T1 bladder cancer who underwent early RC and found a higher risk for disease progression amongst women, but no impact of female gender on recurrence or cancer-specific survival **[LOE 2]**.[79] Country of residence may also have an impact on disease-specific survival (DSS) by gender.[76]

Gender likely plays a role in NMIBC outcomes and should be considered when discussing management strategies. Further investigation is necessary to determine the impact of each of the gender-specific risk factors and differences in treatment outcomes.

5.3.2.3 Race

In the United States, a higher mortality rate for bladder cancer has been reported amongst African Americans as compared to their white counterparts.[80] Hollenbeck *et al.* found that black patients with NMIBC have a significantly higher risk for death from bladder cancer when compared to white patients, even when accounting for differences in treatment intensity and provider effect (HR, 1.22;95% CI, 1.06–1.42), with no difference in disease severity at the time of diagnosis **[LOE 3]**.[81] Other studies have similarly shown worse overall survival (OS) amongst black patients with bladder

cancer **[LOE 3]**.[82] Proposed factors for increased mortality include more aggressive tumour phenotype, socioeconomic status, differing rates of comorbidity, differing exposure and metabolic response to carcinogens, and later stage at time of diagnosis. It remains unclear what role race plays specifically in progression and recurrence of NMIBC.

5.3.3 Environmental factors

5.3.3.1 Carcinogens

The major risk factor for developing bladder cancer is smoking.[83] Former and current smokers have a two- to three-fold increased risk of developing bladder cancer as compared to nonsmokers.[83] Much of the observed difference in global incidence of bladder cancer and cancer-specific mortality is attributable to variation in tobacco consumption.[65] Smoking is an independent risk factor for both disease recurrence and progression in NMIBC, with resultant worse survival **[LOE 3]**.[84,85] Differences in tobacco exposure may also account for the higher incidence of bladder cancer in men. The population-attributable risk for bladder cancer for tobacco use is estimated to be around 50% for both men and women.[86]

Occupational or therapeutic exposure to carcinogens is another risk factor for bladder cancer **[LOE 3]**.[87] Notable carcinogens include aromatic amines, arsenic, polycyclic hydrocarbons, and chlorinated hydrocarbons.[88] Workers in textiles, agriculture, trucking, and chemical manufacturing are at increased risk for exposure to these substances. An example of an environmental carcinogen that is a cause for concern is arsenic. A meta-analysis evaluating the exposure to arsenic in drinking water and the risk for bladder cancer showed that cancer risk doubled with exposure to 50 **µ**g/L of arsenic and tripled with exposure to 150 **µ**g/L arsenic.[89]

5.3.3.2 Infection and inflammation

Infection and inflammation have also been implicated in bladder cancer. Chronic cystitis is associated with squamous cell carcinoma (SCC) of the bladder in patients with chronic indwelling catheters.[88] *Schistosoma haematobium*, a parasitic flatworm endemic to Africa and the Middle East, represents another form of carcinogen that induces an inflammatory response leading to dysplasia, and there is evidence that exposure is related to SCC of the bladder **[LOE 3]**.[90] It is estimated that 3% of bladder cancer cases worldwide are related to *Schistosoma haematobium*, although confounding factors such as high rates of exposure to other carcinogens make it difficult to calculate its true impact.[91] Other infections, such as with human papillomavirus, have been suggested to contribute to bladder cancer risk, but early data are conflicting and limited **[LOE 3]**.[88]

5.3.3.3 Medication

Several therapeutic agents have been implicated in bladder cancer. Phenacetin, an analgesic similar in structure to aniline dyes, was found to increase the risk for bladder cancer in the 1970s and was subsequently removed from the market.[92] Cyclophosphamide is an alkylating agent commonly used for treatment of autoimmune diseases and malignancy, the use of which increases the risk for bladder cancer nearly six-fold if used without 2-mercaptoethanesulfonate **[LOE 3]**.[93] More recently, a large population-based study of patients receiving the antidiabetic drug pioglitazone found an HR of 1.63 for developing bladder cancer (95% CI, 1.22–2.81).[94] The risk was even higher for patients receiving pioglitazone for more than 2 years (HR, 1.78; 95% CI, 1.21–2.64) **[LOE 3]**.

5.3.3.4 Ionizing radiation

Ionizing radiation to the lower pelvis is a risk factor for developing bladder cancer. In a large observational study, prostate cancer patients exposed to external-beam radiation therapy (EBRT) had a 1.5-fold higher risk of being diagnosed with a subsequent bladder cancer than patients who underwent a radical prostatectomy alone **[LOE 3]**.[95] Another study reported an overall risk for 1.63 (95% CI, 1.44–1.84) for developing bladder cancer after EBRT **[LOE 3]**.[96] Moreover, it has been shown that radiation-induced bladder tumours are more likely to be of nonurothelial origin (6.4% vs. 3.8%, p=0.004), to be located in the trigone (6.9% vs. 5.4%, p=0.012), and to have concomitant CIS (9.2% vs. 7.0%, p<0.001).[97]

5.3.3.5 Family history

Epidemiologic data suggests that family history is associated with a two-fold increase in bladder cancer risk, which is not fully explained by environmental exposure.[98] An Italian case-control study by Turati and colleagues demonstrated an odds ratio of 2.13 (95% CI, 1.02–4.49) for bladder cancer development with a positive family history **[LOE 3]**.[99] Similar findings have been demonstrated in the United States and Iceland **[LOE 3]**.[100,101] Family history does not appear to have an impact on the prognosis of NMIBC specifically **[LOE 3]**.[102]

5.3.4 Tumour-related factors

5.3.4.1 Tumour size and location

Both the EORTC and CUETO studies developed a model to predict the probability of recurrence and progression in the NMIBC patient.[68,70] In the EORTC study, tumour size was a risk factor for developing recurrence and progression (HR, 1.34 and 1.94, respectively; p<0.001). A tumour size of 3 cm was selected as a cut-off value based on an earlier series that showed that size over 3 cm increases the risk for recurrence, progression, and disease-specific mortality (OR, 1.65; 95% CI, 1.3–2.0; p=0.00001) **[LOE 2]**.[103,104] Using tumour size over 3 cm as a metric for higher-risk disease has been subsequently validated by other groups **[LOE 2]**.[79,105,106] Whether the location of the tumour in the bladder is related to risk for recurrence or progression is less clear, although tumours located in the trigone or prostatic urethra have a poorer prognosis and are associated with synchronous tumours in the upper urinary tract **[LOE 3]**.[107,108]

5.3.4.2 Multifocality and synchronous upper-tract tumours

In addition to tumour size, patients with multifocal tumours are at increased risk for recurrence and progression. In the EORTC data, multifocality imparted an HR of 1.96 and 1.86 (p<0.001) for recurrence and progression, respectively **[LOE 2]**. In the CUETO study, multifocality imparted an HR of 1.283 (95% CI, 1.102–2.493; p<0.001) for recurrence without impact on progression **[LOE 2]**.[68,69] In contrast to their original findings, the updated EORTC data demonstrated no impact of multifocality on progression, but the increased risk for recurrence persisted.[71] Of note, multiple tumours are often reported to be a single tumour, so the contribution of multiplicity to the risk for recurrence and progression may be underestimated.[109] Multiplicity is also related to synchronous upper urinary tract tumours (relative risk [RR], 2.7; 95% CI, 1.06–6.84; p=0.0365), although the presence of synchronous upper urinary tract tumour is uncommon (incidence varies from 1.8% to 2.6%) **[LOE 3]**.[110,111] When a bladder tumour is located at the trigone, the risk for a synchronous upper urinary tract tumour is even higher (RR, 5.8; 95% CI, 2.18–15.9; p<0.0005) **[LOE 3]**.[111]

5.3.4.3 Recurrence rate

Tumour recurrence is both an important endpoint, as well as a significant prognostic factor. The original EORTC data demonstrated that a first recurrence conferred an HR of 1.19 (p=0.027) for progression and 1.42 (p<0.0001) for recurrence **[LOE 2]**.[68] The updated EORTC data, which evaluated those who received BCG therapy for 1 to 3 years of maintenance, included a prior recurrence rate of over 1 per year as a significant factor in predicting early recurrence **[LOE 2]**.[71] The CUETO group also demonstrated that recurrent tumour, defined as recurrent tumour detected after 3-month cystoscopy, increased risk for both recurrence (HR, 2.01; 95% CI, 1.61–2.51) and progression (HR, 1.92; 95% CI, 1.36–2.72) **[LOE 2]**.[69] Stratification by time to recurrence and number of recurrences further informs prognostic risk, as demonstrated by a study of 616 patients with T1G2 disease in whom recurrence at 3 months was the principle prognostic factor for predicting disease progression (RR, 4.0; 95% CI, 1.2–13.3) **[LOE 2]**.[112] Patients with recurrent disease following BCG therapy demonstrate an increased risk for progression.[113]

5.3.4.4 Transurethral resection

Cystoscopic resection of the bladder tumour itself plays a role in determining recurrence and progression. Sufficient resection depth, resection of all visible tumour, and appropriate timing of re-resection are all critical components in the management of NMIBC **[GOR A]**. Please refer to the section on treatment for further discussion.

5.3.5 Pathologic-related factors

5.3.5.1 Tumour stage

NMIBC comprises tumour stages Ta, T1, and Tis, and approximately 75% of new bladder cancer patients have a nonmuscle-invasive tumour.[114] Tumour stage is an independent prognostic factor for recurrence and progression, but accurate staging is hampered by interobserver variability **[LOE 2]**.[114] CUETO found that T stage (Ta, T1) is an independent prognostic factor for tumour progression (HR, 2.35; 95% CI, 1.36–4.08) **[LOE 2]**.[69] In the initial EORTC cohort, T1 disease increased risk for both recurrence (HR, 1.21; 95% CI, 1.32–1.80) and progression (HR, 2.19; 95% CI, 1.67–2.86) **[LOE 2]**.[68]

5.3.5.2 Tumour grade

Along with invasiveness, tumour differentiation is an important prognostic indicator. For many years, the 1973 WHO grading system was used for tumour differentiation, whereby tumours could be assigned a grade of 1, 2, or 3. Grade 1 indicated a low degree of cellular anaplasia, whereas grade 3 indicated a high degree of anaplasia. Grade 3 NMIBC has been reported to confer a significantly increased risk for tumour progression **[LOE 2]**.[103] The original EORTC data demonstrated an HR of disease progression of 2.67 (95% CI, 1.99–3.59; p<0.0001) in the case of grade 3 disease **[LOE 2]**.[68] The grading system has since been modified, and the 2004 WHO grading system created low- and high-grade categories. When comparing the original and the revised grading systems in risk prognostication and reproducibility, they seem to be equal in strength **[LOE 2]**.[115]

5.3.5.3 Carcinoma *in situ*

CIS, which is a flat, high-grade lesion confined to the mucosa, can be subdivided into primary (isolated, no previous or concurrent tumours or CIS), secondary (detected during follow-up), or concurrent (in the presence of any other urothelial tumour) CIS.[116] CIS is always high grade and

reported to be concurrent in 72% of Ta/T1 high-grade bladder tumours.[117] CIS is associated with an increased risk for recurrence and also progression. CIS within the prostatic urethra in men with T1G3 bladder cancer is associated with shorter time to first recurrence (HR, 2.30; 95% CI, 1.25–4.22), progression (HR, 4.35; 95% CI, 1.65–11.50), and bladder cancer–specific mortality (HR, 3.53; 95% CI, 1.40–8.89) **[LOE 3]**.[108] Amongst patients with T1G3 NMIBC treated with BCG, concomitant CIS conferred an increased risk for disease progression (HR, 1.46; 95% CI, 1.17–1.82) **[LOE 3]**.[72]

5.3.5.4 Lymphovascular invasion

LVI is considered to be a poor prognostic sign in muscle-invasive bladder cancer (MIBC), as the prevalence of LVI increases with pathological stage (pT1 9.0%, pT4 78%) and grade. However, in NMIBC, LVI has been associated with a poor clinical outcome **[LOE 3]**.[118-120] In a multi-institutional study of 958 patients who underwent RC, 101 had final pathological stage T1N0 disease and, in these patients, LVI conferred increased risk for both recurrence and cancer-specific mortality (HR, 4.9; 95% CI, 1.40–16.50 and HR, 6.7; 95% CI, 1.50–30.30, respectively) **[LOE 3]**.[121] In a smaller series on newly diagnosed T1 NMIBC patients treated by TURBT, the presence of LVI was associated with an increased risk for recurrence (HR, 2.016; 95% CI,1.114–3.903; p=0.029) and progression (HR, 3.065; 95% CI, 1.233–7.620; p=0.016) **[LOE 3]**.[119] Concerns about poor diagnostic reproducibility in diagnosing LVI have been raised.[122]

5.3.5.5 Tumour substaging

Substaging of pathological stage T1 has been proposed. One model for substaging creates T1a and T1b classifications, which stratifies invasion as above (a) or beyond (b) the muscularis mucosae.[123] Other substaging models focus on tumour size; one model assigned a cut-off value of 0.5 mm for invasiveness (pT1a <0.5 mm, pT1b >0.5 mm) and another model proposed 1 mm (pT1a <1 mm, pT1b >1 mm).[124,125] Substaging hasn't been standardized into routine clinical practice and so there are limited data on prognostic usefulness. The WHO 2016 guidelines recommend against T1 substaging; however, this may provide prognostic value in the future.[116]

5.3.5.5 Prostatic urethra tumours

The incidence of prostatic urethra involvement in high-grade T1 NMIBC is around 10%.[108] In a series of 146 such patients, multivariate analyses found that prostatic involvement was correlated with a higher risk for recurrence (HR, 2.40; 95% CI, 1.16–4.95; p=0.02), progression (HR, 4.35; 95% CI, 1.65–11.50; p=0.003), and death due to bladder cancer (HR, 3.53; 95% CI, 1.40–8.89; p=0.004) **[LOE 2]**. with bladder neck tumour, CIS, positive cytology without evidence of tumour in the bladder, and abnormal-appearing prostatic urethra, multiple biopsies of high-grade nonmuscle-invasive bladder tumours of the prostatic urethra are recommended **[GOR C]**.

5.3.5.7 Aberrant histology

It is estimated that 75% to 80% of all NMIBC derives from urothelial cells, with mixed histological features present in 20% to 25% of the cases.[126,127] Variant differentiations include adenocarcinoma, SCC, small cell carcinoma, glandular carcinoma, inverted tumours, micropapillary tumours, nested tumours, sarcomatoid carcinoma, lymphoepithelial tumours, and plasmacytoid tumours. Squamous (32% and 40%) and glandular (18% and 13%) differentiation are the most frequently reported in

published studies. Micropapillary, plasmacytoid, sarcomatoid, nested, and squamous features correspond to a higher incidence of high-grade, invasive tumours with disease progression, and so require a nuanced management strategy **[LOE 3]**.[127]

5.3.6 Molecular-related factors

Significant progress is being made in identifying molecular determinants of tumour behaviour in cancer therapy for a myriad of entities. Treatment decisions still heavily rely on classic staging and grading, however, and tumour-specific genomic alterations are not yet taken into account in clinical decision-making. To address the risk for recurrence and progression in NMIBC, tumour-specific molecular alterations that carry prognostic significance might play an important role in the future. The majority of data currently available are limited to muscle-invasive and metastatic disease, and the impact of markers on clinical outcomes remains unclear.

5.3.6.1 Urine markers

Fluorescence *in situ* hybridization (FISH) and urine cytology are tools in bladder cancer diagnosis and surveillance. UroVysion is a test that employs FISH in order to identify chromosomal abnormalities, specifically aneuploidy of chromosomes 3, 7, or 17 or loss of the 9p21 locus.[128] The primary use of UroVysion has been in improving bladder cancer detection, but the test may play a role in predicting recurrence and progression of NMIBC in patients undergoing BCG therapy. Urine cytology may similarly predict BCG failure. In a retrospective review by Whitson *et al.* of 42 patients with high-risk NMIBC who received intravesical BCG therapy, either induction or maintenance after resection, positive UroVysion and cytology after completion of treatment was predictive of disease recurrence **[LOE 2]**.[129] A prospective study by Savic *et al.* of 68 patients with NMIBC treated with BCG found that positive cytology and FISH after treatment were associated with disease recurrence, with FISH outperforming cytology **[LOE 2]**.[130] NMP22 is a scaffolding protein involved in mitosis regulation that is overexpressed in certain urothelial tumours, making it a potential candidate for a urinary biomarker. A large, prospective study of 2,222 patients with confirmed NMIBC and negative urine cytology found that NMP22 levels are significantly associated with both recurrence and progression of bladder cancer **[LOE 2]**.[131]

5.3.6.2 Somatic mutations and chromosomal rearrangement

Genomic mutations in the genes fibroblast growth factor receptor 3 (*FGFR3*) and *HRAS* and in components of the mammalian target of rapamycin (mTOR) pathway are the most frequently reported molecular alterations in NMIBC.[132] In a recent study, FGFR3 mutations corresponded with a favourable outcome in terms of progression in T1 NMIBC (HR, 2.203; 95% CI, 1.010–4.805) **[LOE 3]**.[133] The tumour suppressor genes *TP53* and *RBL1* are thought to be involved in the progression of NMIBC to MIBC, and chromosome 9 alterations occur frequently at an early stage of NMIBC **[LOE 3]**.[134] Spruck *et al.* described a more common loss of heterozygosity of chromosome 9 in NMIBC as compared to CIS (34% vs. 12%, p=0.04), and the proportion of *p53* mutations in CIS (65%) and muscle-invasive tumours (51%) was much higher than in NMIBC (3%) **[LOE 3]**.[135] Hartmann *et al.*, however, found deletion of chromosome 9 in 86% of CIS and 75% of dysplasia lesions **[LOE 3]**.[136] The tumour-suppressor gene *CDKN2A* is located on chromosome 9 and is inactivated in 30% to 50% of the urothelial tumours. Microsatellite instability represents another tumour biomarker of clinical utility, with data showing improved treatment response with pembrolizumab when used for a range

of malignancies demonstrating microsatellite instability **[LOE 3]**.[137] Gene signatures may also serve as prognostic markers for response to pembrolizumab, and current candidates include interferon (IFN)-gamma (10-gene) and an "expanded immune" (28-gene) signature, which are associated with progression-free survival (PFS) **[LOE 3]**.[138]

5.3.6.3 Genetic subtyping

Gene expression analyses have recently revealed the existence of different molecular subtypes of bladder cancer. Similar to breast cancer, urothelial cancer can be subdivided into luminal and basal subtypes. Five molecular subtypes were reported by Sjodahl *et al.*, analogous to those in breast cancer: urobasal A, genomically unstable, urobasal B, highly infiltrative, and squamous cell carcinoma (SCC)-like. Urobasal A correlated with a good prognosis and the majority were nonmuscle-invasive with low-grade histology **[LOE 3]**.[139,140] These tumours were characterized by the elevated expression of FGFR3, CCND1, and TP63. In contrast to urobasal A, genomically unstable tumours are high grade and frequently have *TP53* mutations. The SCC-like subtype, which was more frequently found in female patients, corresponded with a poor outcome. Stratifying tumours into urobasal, genomically unstable, and SCC-like subtypes, Patschan *et al.* tried to assess the risk for progression in T1 NMIBC.[141] Genomically unstable and SCC-like subtypes were associated with high-grade T1 tumours, while urobasal tumours where more likely to be low grade **[LOE 3]**. Descotes *et al.* used gene expression profiling to classify T1 tumours into T1a and T1b tumours with high accuracy.[142] PD-L1 expression is another avenue of investigation, in that determining the degree of expression may predict response to immunotherapay with PD-L1 blockade **[LOE 4]**. The usefullness of PD-L1 as a marker is unknown at this time, as significant variation exists in defining cut-off values, creating assay standardization, and correlating PD-L1 expression to improvemed outcomes with immunotherapy.[138]

5.3.6.4 Methylation and mutational load

Mutational load has been proposed as a surrogate for response to immunotherapy.[138] The IMvigor210 study, which evaluated the role of atezolizumab for treatment of advanced and metastatic urothelial carcinoma in patients ineligible for conventional chemotherapy, found that mutational load was associated with treatment response in the form of improved OS **[LOE 3]**.[143] DNA promoter hypermethylation, which results in silencing of genes responsible for cell-cycle control, may correlate with tumour grade and invasiveness. López *et al.* found that hypermethylation of *SOX1, PITX2,* and *CSPG2* confers a worse cancer-specific survival **[LOE 3]**.[144] Bilgrami and colleagues reported that hypermethylation of *RASSF1A, APC*, and *MGMT* was more prevalent in MIBC as compared to NMIBC **[LOE 3]**.[145]

5.3.6.5 Novel techniques

There is active, ongoing investigation into other potential molecular markers for disease detection, prognostication of recurrence and progression, and prediction of response. Novel techniques include evaluation of MicroRNA up- and down-regulation, circulating cell-free tumour DNA, and mitochondrial DNA variation.[128] (See **Table 5-1** for summary of prognostic factors for nonmuscle-invasive bladder cancer.)

TABLE 5-1 Summary of Major Prognostic Variables for Nonmuscle-invasive Bladder Cancer

	Factor	Recurrence	Progression	LOE
Patient-related factors	Age >65	+	+	2
	Female gender	+		2
	African American		+	3
Environmental factors	Tobacco use	+	+	3
Tumour-related factors	Size (>3 cm)	+	+	2
	Multifocality	+	+/-*	2
	Prior recurrence	+	+	2
Pathology-related factors	Tumour stage (T1)	+/-	+	2
	Tumour grade (HG or G3)		+	2
	Concurrent CIS	+	+	2
	LVI	+	+	3
	Prostatic urethra involvement	+	+	2
Molecular factors	Urine marker positivity	+		2
	Somatic mutations (absence of FGFR3 mutation)		+	3

Abbreviations: CIS, carcinoma in situ; FGFR3, fibroblast growth factor receptor 3; HG, high grade; LOE, Level of Evidence; LVI, lymphovascular invasion.

+ indicates increased risk for either recurrence or progression.

+/- indicates conflicting level 2 data.

*See references Sylvester *et al.*[68] Fernandez-Gomez *et al.*[69]

Empty space indicates no well-demonstrated association.

5.3.7 Risk grouping

The American Urological Association (AUA) and the Society of Urologic Oncology (SUO), as well as the European Association of Urology (EAU) have created risk stratification groups based on the literature of known risk factors.

5.3.7.1 American Urological Association/Society of Urologic Oncology guidelines

The AUA/SUO guidelines[146] (**Table 5-2**) represent the panel's consensus on the likelihood of developing recurrence and progression, and are based on the sum of available data and not on any specific risk stratification tool (such as the EORTC or CUETO data) or meta-analysis. The panel's objective was to provide a general framework for clinical practice. The panel also sought to incorporate prior BCG failure into their recommendations, something not previously done. The risk groups have not undergone any validation analysis **[GOR C]**.

TABLE 5-2 American Urological Association/Society of Urologic Oncology Risk Stratification for NMIBC

Low risk	Intermediate risk	High risk
Low-grade solitary Ta ≤3 cm	Low-grade Ta recurrence within 1 year	High-grade T1
	Low-grade solitary Ta >3 cm	High-grade Ta recurrence
	Low-grade, multifocal Ta	High-grade Ta >3 cm or multifocal
	High-grade Ta ≤3 cm	Any CIS
	Low-grade T1	Any BCG failure in high-grade disease
		Any variant histology
		Any LVI
		Any high-grade prostatic urethral involvement

Abbreviations: BCG, bacillus Calmette-Guérin; CIS, carcinoma in situ; LVI, lymphovascular invasion.

Source: Recreated with permission from Chang SS, Boorjian SA, Chou R, *et al.* Diagnosis and treatment of non-muscle invasive bladder cancer: AUA/SUO guideline. *J Urol.* 2016;196(4):1021–1029.146

5.3.7.2 European Association of Urology guidelines

The EAU guidelines[116] (**Table 5-3**) were created with a combination of panel consensus and original and updated EORTC risk tables.[68,71] The EORTC risk calculator, even after being updated, is limited by lack of patients with CIS and routine re-resection. The update does, however, include patients who received BCG maintenance. Numerous studies have attempted to validate both the EORTC and CUETO data in other patient populations and have found modest discriminatory ability **[GOR B]**.[70,147]

TABLE 5-3 EAU Risk Stratification for NMIBC

Low risk	Intermediate risk	High risk
Low-grade, primary, solitary Ta <3 cm without CIS	All tumours not otherwise defined as low or high risk	T1 tumour
		High-grade tumour
		Any CIS
		Multiple, recurrent, and low-grade Ta >3 cm

Abbreviations: EAU, European Association of Urology; CIS, carcinoma in situ; NMIBC, nonmuscle-invasive bladder cancer.

Source: Recreated with permission from Babjuk M, Bohle A, Burger M, *et al.* EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016. *Eur Urol.* 2017;71(3):447–461.[116]

5.3.8 Summary

NMIBC remains a common and challenging malignancy to manage. Improvements in risk factor stratification, identification of high-risk patient and tumour characteristics, and development of new technologies will further guide clinical management in the future. Current tools for risk stratification are limited but informative and should be used in clinical practice when determining diagnosis, surveillance, and treatment of NMIBC.

Summary of Recommendations

Age over 65 years is associated with increased risk for disease recurrence **[LOE 2]** and progression **[LOE 2]**.

Female gender is associated with increased risk for disease recurrence **[LOE 2]**.

African Americans have a higher risk for disease progression **[LOE 3]**.

Tobacco use is associated with increased risk for disease recurrence **[LOE 3]** and progression **[LOE 3]**. Patients should be advised to quit tobacco use **[GOR B]**.

Tumour size over 3 cm is associated with increased risk for recurrence **[LOE 2]** and progression **[LOE 2]**.

Tumour multifocality is associated with increased risk for recurrence **[LOE 2]**. Evidence is conflicting on the relationship between tumour multifocality and risk for progression.

Prior tumour recurrence is associated with increased risk for recurrence **[LOE 2]** and progression **[LOE 2]**.

Clinicians should consider tumour size, focality, and prior recurrence when advising patients on risks for recurrence or progression **[GOR B]**.

Tumour stage of T1 is associated with increased risk for disease progression **[LOE 2]**. Evidence is conflicting on the relationship between tumour stage and risk for recurrence.

High-grade tumours are associated with a higher risk for progression **[LOE 2]**.

Concurrent CIS is associated with increased risk for disease recurrence **[LOE 2]** and progression **[LOE 2]**.

Tumour LVI is associated with increased risk for disease recurrence **[LOE 3]** and progression **[LOE 3]**.

Tumour involvement of the prostatic urethra is associated with increased risk for disease recurrence **[LOE 2]** and progression **[LOE 2]**.

Clinicians should consider tumour stage, grade, presence of CIS, LVI, and prostatic urethral involvement when advising patients on risks for recurrence or progression **[GOR B]**.

Urine FISH and urine cytology are predictive of disease recurrence **[LOE 2]**.

Somatic mutations in tumour suppressor genes may be associated with disease progression **[LOE 3]**.

At present, there is insufficient evidence to recommend routine testing for tumour somatic mutations or genetic subtyping **[GOR D]**.

Clinicians should consider use of risk stratification tables, such as those from the AUA/SUO **[GOR C]** or the EAU **[GOR B]**.

5.4 Management of Primary and Recurrent Low-grade Ta Bladder Cancer

5.4.1 Introduction

Low-grade noninvasive bladder cancers (LGTa) form up to 50% of all new bladder cancers.

The initial management and the diagnosis of this cancer is the same as those for other categories of bladder cancer, and are therefore addressed in other chapters.

With a 5-year risk for recurrence of up to 50%,[148] surveillance strategies and risk stratification in LGTa cancers form the cornerstone in its management. Surveillance in LGTa, in the absence of reliable biomedical markers, is essentially reliant on cystoscopy. Since the evaluation of the accuracy of urine-based markers is being addressed elsewhere, we have not covered the topic in this section.

In this section, we have elaborated on the specific areas that allow for a unique approach in LGTa cancers.

5.4.1.1 Definitions and risk groups

The standard bladder cancer grading systems recommended are the WHO 1973 and 2004 classifications. The low-grade cancer (based on the 2004 system) included all of the grade 1 cancers and some of the grade 2 cancers from the 1973 system. Whilst both classifications are in use, for ease and standardization of description across this consultation, we adopt the 2004 classification. Low-grade cells are defined by specific pathological criteria (please refer to the chapter on pathology).

Using the AJCC TNM classification[149] for tumour stage, Ta refers to a noninvasive papillary cancer, i.e. confined to the epithelial layer with no invasion to the lamina propria.

Based on the natural history (discussed in Section 5.3: Prognostic Factors of Recurrence and Progression; Risk Stratification of NMIBC), these LGTa cancers can be stratified on account of recurrence risk. There are risk categories based on nomograms[150,151] recommended by the major guidelines groups: EAU,[152] AUA/SUO,[153] National Comprehensive Cancer Network (NCCN),[154] and National Institute for Health and Care Excellence (NICE).[155] Subgroups that apply to the LGTa cancers are low risk (new, single, less than 3 cm in size) or intermediate risk (new multifocal or new single tumours larger than 3 cm or recurrent LGTa cancers).

5.4.2 Diagnosis and initial treatment

The diagnosis of bladder cancer (covered in section 5.2: Staging Workup) relies almost exclusively on cystoscopy, which is considered the gold standard by major guidelines.[152-155] Noninvasive assessment of patients with visible hematuria (for example, with ultrasound) may detect a small proportion of bladder cancers (please refer to the chapter on diagnosis of NMIBC).

Urine-based biomarkers have a higher sensitivity (when compared with urine cytology) but lower specificity in detecting bladder cancers, and therefore have not been recommended as a diagnostic tool (please refer to the chapter on biomedical markers).

5.4.3 Initial treatment of the new low-grade Ta cancer

All major bladder cancer guidelines consider that the gold standard initial treatment of low-grade Ta bladder cancer (and the means to the initial diagnosis) is with TURBT.[152-155] Whilst this is covered in another chapter, it is important to emphasize the need to achieve a complete resection of the tumour (when possible) and to obtain detrusor muscle in the resected specimen for accurate staging,[152-155] especially as this has quality implications on tumour clearance and subsequent recurrence rates[156,157] and survival implication in all grades of cancers.[158] Other factors that must be noted at the time of the TURBT are the number and location of the tumour(s), preferably using a bladder diagram/map and a measure of the tumour size; these are essential features adopted for risk stratification.[152]

5.4.4 Upper-tract imaging in low-grade Ta urothelial carcinoma

Patients with bladder cancer are susceptible to developing urothelial tumours within the entire urothelium, as a result of the field change. However, as the incidence of upper-tract urothelial carcinoma is small, the value of routine imaging of the upper tracts at the time of diagnosis of bladder cancer remains controversial.[159] CT urography (CTU) has almost replaced the traditional IVU as the modality of choice for imaging the upper tracts.[160-162] CTU has been described as having a higher sensitivity and specificity than IVU.[163] In the study by Jinzaki *et al.*, the sensitivity of CTU and IVU were 94% and 80%, respectively, while the specificity was 95% and 81%, respectively. The CTU has the added benefit of being able to identify other intra-abdominal pathology, while being able to stage the cancer by detecting lymph node and liver metastasis.

Whilst the incidence of synchronous and metachronous upper-tract tumours is somewhat higher in patients with bladder cancers affecting the trigone,[164] the incidence of this occurring in low-grade Ta cancers is small. In a large retrospective analysis of 1,529 patients, Palou and colleagues identified 1.1% of patients with noninvasive urothelial carcinomas as having synchronous upper-tract tumours.[164] Others have not been able to demonstrate the presence of synchronous upper-tract urothelial carcinoma in Ta bladder cancers.[159] Conversely, Bajaj and colleagues found synchronous upper-tract tumours in 3.3% of patients (4 out of 120 patients) with noninvasive bladder cancers.[165] Across all tumour categories, the risk for synchronous upper-tract urothelial carcinoma appears to be higher in multifocal bladder cancers, further reinforcing the effect of this field change.[166]

5.4.5 Follow-up surveillance and upper-tract imaging during surveillance

In the absence of reliable biomarkers or imaging modalities, the surveillance for NMIBC is mainly dependent on cystoscopy. The role of alternative surveillance methods is covered in another section (5:12: Surveillance strategies).

Based on the natural history, major guidelines[152-155] recommend the first-check cystoscopy be carried out at 3 months following the initial resection of the tumour, as this is an accepted independent predictor of subsequent recurrence.[167] While continued cystoscopic surveillance is important and affords an opportunity to allay patient anxiety, as tumour recurrence in LGTa patients is almost always low grade and noninvasive,[168] small papillary LGTa are not life-threatening and therefore early detection is not essential.[152] Considering the contemporary recurrence rates for LGTa cancers appear lower, with the advent of better visual aids,[169,170] cystoscopic surveillance schedules and the duration of follow-up could possibly be relaxed in favour of a shorter overall timescale.[155,167] As surveillance and management of bladder cancer make this cancer type one of the most (if not *the* most) expensive cancer to manage within most health care systems,[171,172] due consideration has to be made to balance resource and disease.[173] The roles of office fulguration in the recurrence and expectant management or active surveillance fall very much into this pragmatic approach of managing LGTa cancers and is covered in detail later in this chapter.

In relation to surveillance of the upper tracts in LGTa bladder cancer patients, the aspects that remain controversial are the cost effectiveness of imaging the upper tracts; the duration and frequency of surveillance; and whether the early detection of upper-tract tumours (by virtue of surveillance) improves the prognosis.[174-177] The incidence of upper-tract urothelial carcinoma developing during surveillance of patients with noninvasive cancer (from sub-group analyses) ranged from 0.3% to 3.4%.[174,178-181] Other have also identified the association between the development of upper-tract urothelial carcinoma and variables such as vesical-ureteric reflux[182] and tumour multiplicity.[164,166,178] These aspects are covered in another chapter. Despite the fact that approximately 50% of upper-tract tumours identified during surveillance of noninvasive bladder cancers were high grade, it must be noted that detection by virtue of surveillance was not associated with a superior outcome.[182]

5.4.6 Treatment of recurrent low-grade Ta bladder cancer

Whilst recurrent bladder cancers should be, in principle, managed no differently than the primary lesion, i.e. TURBT, the surgical rigour of carrying out a formal TURBT can be avoided (in favour of an office fulguration, for example) in the vast majority of recurrent LGTa consequent to the recurrent lesions being small and noninvasive in most instances.[150,183-185] In managing these lesions, several patient and tumour characteristics must be taken into account. A TURBT is not without risks, either.[186,187] Additionally, when formulating the strategy for dealing with a recurrent bladder tumour, consideration should be made as to the risk for progression to either a higher grade or stage. In the LGTa tumours, the recurrences are often of a low grade and noninvasive (pTa), with the risk for progression to a higher grade or stage being infrequent to rare.[168,188,189] This assumption, whilst holding true in most instances, requires a certain amount of experience in being able to discern the likelihood of a recurrent tumour being low grade and noninvasive[190] and, certainly, the addition of urine cytology

to the surveillance armamentarium adds to the diagnostic accuracy of the cystoscopic appearance, if anything, to reinforce the absence of a high-grade tumour.[191] The absence of MIBC can quite reliably be determined by the cystoscopic appearance.[192,193] Reassuringly, these papillary-appearing tumours have been demonstrated in animal models to have a more indolent behaviour.[194]

The risk for mortality from LGTa tumours is also quite negligible[183] and it is indeed critical to weigh this against the non-negligible risks of putting an elderly patient with a small recurrent LGTa tumour through a general/regional anesthetic to have the lesion removed.[195]

One caveat that must be borne in mind when attempting to adopt a more conservative, nonresection approach in the recurrent LGTa cancer is that the pathological evaluation of the recurrent tumour grade and stage with a TURBT or biopsy is necessary to allow for more accurate risk stratification. This is particularly true when defining intermediate risk NMIBC on the basis of a LGTa recurrence, which would then make the recommendation of a course of intravesical chemotherapy[152,155] or the development of a high-risk NMIBC (on the basis of tumour features and frequency of recurrence) where intravesical BCG would be the more effective adjuvant treatment.[196] We will cover the role of intravesical treatment in a later section in this chapter.

Therefore, in selected patients with LGTa recurrences, taking into account the caveat and principles highlighted above, a more *surgically* conservative approach[152,153] could be adopted and, in this section, we elaborate the various options available. We have attempted to evaluate each option (where data are available) in relation to (a) clinical effectiveness; (b) cost–effectiveness and patient experience health-related quality of life (HR-QoL) aspects.

5.4.6.1 Office fulguration with diathermy (also referred to as cystodiathermy)

This approach involves destruction of the recurrent tumour with diathermy through a flexible scope under local anesthetic in the out-patient (office) setting. This procedure and its principles, in particular, are not new and, in fact, the pioneering work by Edwin Beer with cauterizing papillary tumours through a cystoscope paved the way and revolutionized how we manage NMIBC in the modern era.[197,198] Herr and colleagues were first to describe cystodiathermy with the modern flexible cystoscope on 185 patients with recurrent bladder tumours, followed up over 2 years.[199] This was followed by German and colleagues demonstrating the safety and tolerability of cystodiathermy being carried out with the modern flexible cystoscope in 17 patients with small bladder tumours in the United Kingdom.[200]

As office fulguration/cystodiathermy is carried out as soon as the recurrence is identified, i.e. at the time of the surveillance flexible cystoscopy, this could potentially allay patients' anxiety by being able to deal with the recurrence in a timely manner, as opposed to being brought back for removal under general or regional anesthetic. In most public health care systems, this would mean being placed in a separate queue or list, further compounding the patients' experience.

Whilst most published descriptions do not include a biopsy sample of the recurrent tumour, some authors have included a biopsy, and certainly this would be quite feasible, although it would be a small sample, through a flexible cystoscope.

Whilst observational studies have demonstrated the pragmatic approach to carrying out office fulguration to reduce the potential economic burden and improve the patients' quality of life, there are very sparse data around these aspects to date. Additionally, the longer-term oncological benefit, particularly survival, has not been explored either.

Data from the studies evaluating fulguration of recurrent LGTa tumours are summarized in **Table 5-4**.

Adequacy of clearance by fulguration is an important aspect to consider and has not been evaluated directly; conversely, it could be measured by using subsequent recurrence close to or at the site of a previous tumour as a surrogate. Where reports of this parameter were available, recurrence at this site was found in 6%[201] to 12.6% of patients,[202] suggesting adequate initial clearance. Most recurrences, with no specification of the location, were able to be dealt with by fulguration or cystodiathermy.[167,201-203]

DSS following office fulguration was evaluated by Donat and colleagues.[203] There was no difference noted between patients who underwent TURBT and those who underwent office fulguration for recurrent LGTa cancers.

Since the underlying principle in performing minimal interventions is to reduce the financial burden and improve the patients' experience, it is essential to measure the cost effectiveness and HR-QoL of office fulguration/cystodiathermy. HR-QoL has not been evaluated in any of the published literature that was accessed. Authors from New York constructed a Markov model to calculate cost of managing LGTa tumours following the initial TURBT, based on the presence or absence of recurrence.[204] Office fulguration was found to be more clinically effective on the basis of quality-adjusted life-years (as being dominant) and cost-effective when evaluated over the patients' long-term follow-up. Green and colleagues found that it was more cost-effective to have office fulguration as one of the treatments available for recurrent bladder cancer, either on its own or in combination with TURBT during the patients' journey.[205] The authors concluded that the most cost-effective way of treating recurrent low-risk NMIBC was with office fulguration without intravesical chemotherapy.

While the safety and efficacy of office fulguration is evident from the available literature, heterogeneity of methodology and selection criteria make deriving a consensus recommendation for patient selection quite difficult. Following a review of published observational studies comparing the risk for recurrence/progression following office fulguration and TURBT/cystodiathermy with biopsy, the NICE guidelines recommend office fulguration (without biopsy) when the following criteria are met: (a) no previous bladder cancer that was intermediate or high risk; (b) a disease-free interval of at least 6 months; and (c) solitary papillary recurrence and a tumour diameter of 3 mm or less.[155]

It must be emphasized that whilst office fulguration is quite easy to carry out and safe, it should not be carried out if there is any doubt as to the stage or grade of the recurrent tumour. We would also advise clinicians to err on the side of caution and biopsy or formally resect the tumour if in doubt[167] or when histology would be essential in formulating further treatment.

TABLE 5-4 Office Fulguration/Cystodiathermy for Recurrent LGTa Tumours

Study	Design	Inclusion criteria	Number of patients	Recurrence following fulguration	Progression following fulguration	Pain score
Wedderburn *et al.*[202]	Observational	"All patients with superficial Ta recurrence"	103	49.5%	N/A	Minimal on the visual analogue score
Donat *et al.*[203]	Prospective observational with defined selection criteria	Patients with recurrent low-grade Ta cancer who had a recurrence after 6 months of being recurrence-free. Patients underwent fulguration if the tumours were <5 mm in size, less than 5 in number, cytology negative and "patient desired" fulguration	267	46.0% over a median 2.6-year follow-up	Similar to patients with recurrent LGTa having TURBT	N/A
Davenport *et al.*[201]	Prospective audit	Recurrence of low-grade Ta cancer that are solitary and less than 10 mm	48	37% at a median 15-week follow-up	No progression	88% tolerated the procedure well
Park *et al.*[206]	Retrospective matched comparison	Less than 10 mm and 3 or less recurrence Ta cancers	42 fulguration and 42 matched to TURBT	28.5% (fulguration), 26.2% (TURBT)	N/A	N/A

Abbreviations: LGTa, low-grade Ta; N/A, not available; TURBT, transurethral resection of the bladder tumour.

5.4.6.2 Laser treatment

Lasers have been advocated as an alternative to diathermy as outpatient or office procedures for the removal of bladder tumours, particularly small noninvasive ones. Preceded by canine bladder experiments[207] on the safety of neodymium-doped yttrium aluminum garnet (Neo:YAG, or Nd:YAG) lasers, the first North American description was from 1994, when the holmium:YAG (Ho:YAG) laser was used to treat 52 tumours in 15 patients with minimal discomfort and without complication.[208] Although 73% of the patients had recurrences at 3-month follow-up, only 3 of 11 patients with recurrence had tumours at the original tumour site(s).

From the United Kingdom, Syed *et al.*[209] reported on 41 patients with 71 recurrent noninvasive tumours treated using the Ho:YAG laser between 1994 and 1997. In comparing with a historical cohort having cystodiathermy in their centre, the rates of local recurrence with laser treatment and cystodiathermy were 10% and 32%, respectively. Patient satisfaction and pain scores were evaluated and found to be satisfactory.

In a prospective study, Gao and colleagues evaluated the efficacy of the thulium laser in recurrent bladder tumours.[210] The authors found no residual cancer on biopsies taken from the tumour site and random locations after treatment. In the 32 patients treated, nine (28.1%) had recurrences, with location of recurrences being at or separate from the initial tumour location in three and six patients, respectively.

Following their initial study,[209] Syed and colleagues prospectively evaluated longer-term outcomes in 151 patients undergoing 444 procedures. With a median follow-up of 24 months, local recurrences in only LGTa tumour patients was 4%, with the overall median time to local and distant recurrence being 12 and 25 months, respectively. The mean pain score (on a scale of 0 to 10, with 10 being the worst) was 1. Complications reported included dysuria (4.2%), hematuria (1.9%), and urinary frequency (1.5%). There were no bladder perforations in this large series.

Jønler and colleagues evaluated the surgeon and the patient experience qualitatively, and the cost efficiency in treating 52 patients with recurrent bladder tumours managed with the laser.[211] Eighty-six percent of patients had no pain, with all patients saying that they would have the procedure again, compared to TURBT. Using a qualitative analysis, the five surgeons found the procedure easy in 78% of patients and difficult in 6% of patients, with the cost of office fulguration using the laser being less than with the alternatives.

In a prospective evaluation of office laser ablation (OLA) of recurrent tumours in elderly patients (with more than half having three or more comorbidities), Wong and colleagues compared outcomes between patients who had white-light OLA with PDD-assisted OLA.[212] Apart from one patient with hematuria, there were no other complications. Early recurrence (at 3 months) was evident in 10.6% and 4.3% in white-light and PDD-assisted OLA, respectively, while the recurrences at 1 year were 65.1% and 46.1%, respectively. This suggests an improved detection of recurrent tumours (allowing for a better clearance) with PDD. Tolerability of the procedure was good.

The safety and efficacy of the green-light (or potassium-titanyl-phosphate [KTP]) laser has been assessed by several authors in new and recurrent NMIBC, revealing fewer complications and reduced hospital stay when compared with TURBT.[213-215]

Kramer and colleagues reviewed the use of lasers in the management of recurrent bladder cancers and concluded from the evaluation of available observational studies that Ho:YAG and Thulium-doped YAG (Tm:YAG) lasers were suitable for removal of recurrent LGTa tumours, but that Nd:YAG had no role to play in the management of bladder tumours.[216]

In terms of the cost and clinical effectiveness of laser treatment of recurrent tumours, Wong and colleagues were the first to use Markov modelling to work out cost-efficacy in patients undergoing OLA.[212] In comparing with in-patient cystodiathermy (under general or regional anesthesia), the authors found a savings of British Pound Sterling (GBP) 936 in favour of OLA and, using thresholds set by NICE, there was an 82% probability that OLA was cost effective. The authors also measured the quality-adjusted life-years and found OLA to be more clinically effective than in-patient

cystodiathermy. As there was no comparison made with TURBT (which was considered the gold standard), the NICE guidelines group could not draw any conclusive recommendation in terms of cost effectiveness for laser treatment of LGTa tumours based on the available evidence.[155]

5.4.6.3 Chemo-resection (or chemo-ablation)

In 1994, Popert and colleagues from King College, London, reported a 46% complete ablation of papillary bladder tumours (marker lesion) at 3 months following treatment with only intravesical epirubicin (using two dosages, 1 mg/mL or 2 mg/mL) in 81 patients.[217] Chemical cystitis and bladder irritability occurred in 15% of patients. Extending the study to 122 patients with a longer follow-up period, the group carried out an RCT comparing standard (1 mg/mL) and high (2 mg/mL) dosages of epirubicin.[218] The authors followed patients for a minimum 12 months with 3-monthly cystoscopies; they found a response rate of 46% and 42% in the standard and high-dose groups, respectively, after 5 courses of chemotherapy. The authors proved the concept was feasible without specifically using the LGTa tumours alone (although the vast majority of patients had grade 1 and grade 2 tumours and were Ta/T1) and concluded that the higher dosage was not superior to the standard dose of epirubicin.

There is currently a UK phase 2 RCT comparing intravesical chemotherapy and standard surgical ablation (TURBT or cystodiathermy) in patients with recurrent low risk (EORTC recurrence risk score ≤6) that is recruiting patients (CALIBER).[219]

5.4.6.4 Expectant management (also known as active surveillance)

As there may be a risk for overtreatment in recurrent LGTa cancers, a parallel could be drawn with the active surveillance strategy in low-risk Gleason 3+3 prostate cancer, where regular prostate-specific antigen (PSA) testing, clinical prostate examination, and/or MRI are carried out as part of a follow-up strategy. This is done with a view to intervention if and when the risk-benefit balance of nonintervention tilts in favour of risk, when radical treatment (or, in the case of recurrence of LGTa, a TURBT or fulguration) is proposed. Similar strategies have been adopted for small renal masses to avoid potentially unnecessary treatment.[220] "Active surveillance" could be another term used for this concept, drawing a parallel with the management of low-risk prostate cancer.[173] The National Cancer Institute (NCI) defines active surveillance as "a treatment plan that involves closely watching a patient's condition but not giving any treatment unless there are changes in test results that show the condition is getting worse. Active surveillance may be used to avoid or delay the need for treatments such as radiation therapy or surgery, which can cause side effects or other problems. During active surveillance, certain exams and tests are done on a regular schedule."[221] It must be kept in mind that overzealous follow-up and intervention in the low-risk NMIBC patient can have a negative impact on the quality of life.[167]

Soloway and colleagues first recommended this approach in 2003, having closely monitored 32 patients with Ta and T1 tumours, with none of the patients progressing to muscle-invasive disease on subsequent biopsies.[222] The underlying principle, as the authors emphasized,[223] was to not expose patients to harm. Carrying out a linear cystoscopic evaluation, the authors calculated the mean growth rate in Ta/T1 tumours as being 1.7 mm per month and the mean time to a new recurrence as 13.4

months—very reassuring and potentially helpful for patient counselling and formulating a follow-up protocol. Since then, over the past decade or so, several groups have published their experiences in this area, including information on long-term outcomes. The results are summarized in **Table 5-5**.

TABLE 5-5 The Study Design and Outcome of Active Surveillance

Study	Inclusion criteria of active surveillance	No. of patients	Surveillance cystoscopy	Criteria of surveillance termination	No. of treatment interventions	Pathology of recurrent tumour
Soloway *et al.*[222]	History of Ta or T1; small (undefined); papillary appearance	32	Every 3–6 months	Significant tumour growth; change in tumour appearance; gross hematuria	28 (50%) underwent TURBT; unknown number of patients underwent fulguration	No. of TURBT: 28; T1G1–G2 in 21; TaG3 with CIS in 4; T1 in 3
Gofrit *et al.*[225]	History of G1–G2 Ta; small (<10 mm); papillary appearance; asymptomatic; negative urine cytology	28	Every 3 months for 2 years; every 6 months thereafter	Tumour size >10 mm; change in tumour appearance; tumour-related symptoms	30 (79%); all underwent TURBT	No. of TURBT: 30; TaG1 in 23; T1G2 in 7
Pruthi *et al.*[226]	History of low-grade Ta	22	Every 3 months for 2 years; every 6 months during 3–5 years; every 12 months thereafter	Made on a case-by-case basis	7 (32%); 4 underwent TURBT; 3 underwent fulguration	No. of TURBT: 4; low-grade Ta in 2; high-grade Ta in 1; high-grade T1 in 1
Gofrit *et al.*[227]	History of G1–G2 Ta; small (<10 mm); papillary appearance; asymptomatic; negative urine cytology	31	Every 3 months for 2 years; every 6 months thereafter	Tumour size >10 mm; change in tumour appearance; tumour-related symptoms; patient's request	35 (81%); 34 underwent TURBT; 1 underwent fulguration	No. of TURBT 34; TaG1 in 22; TaG2 in 11; T1 in 1
Hernandez *et al.*[228]	History of G1–G2 Ta or T1; small (<10 mm); no. of tumours <5; papillary appearance; asymptomatic; negative urine cytology	64	Every 3–4 months	Significant tumour growth; increase in number of tumours; tumour-related symptoms; gross hematuria; positive urine cytology; patient's request	45 (64%); all underwent TURBT	No. of TURBT 45; 3 progressed in grade (from G1–G2 to G3/CIS); 3 progressed in stage (from Ta to T1)

Abbreviations: CIS, carcinoma *in situ;* MIBC, muscle-invasive bladder cancer; TURBT, transurethral resection of the bladder tumour.

Adapted with permission from Miyake M, Fujimoto K, Hirao Y. Active surveillance for nonmuscle invasive bladder cancer. Investig *Clin Urol.* 2016;57(suppl 1):S4–S13.[224]

continued on **page 413**

TABLE 5-5 The Study Design and Outcome of Active Surveillance, *Cont'd*

Study	Inclusion criteria of active surveillance	No. of patients	Surveillance cystoscopy	Criteria of surveillance termination	No. of treatment interventions	Pathology of recurrent tumour
Hernandez *et al*.[229]	History of G1–G2 Ta or T1; small (<10 mm); no. of tumours <5; papillary appearance; asymptomatic; negative urine cytology	186	Every 3–4 months for 2 years; every 6 months thereafter	Significant tumour growth; increase in number of tumours; tumour-related symptoms; gross hematuria; positive urine cytology; patient's request	203 (81%); 198 underwent TURBT; 5 noncancer related deaths or lost to follow-up	No. of TURBT: 198; 15 progressed in grade (from G1–G2 to G3/CIS); 23 progressed in stage (from Ta to T1); 4 progressed to MIBC (from T1 to T2)

Abbreviations: CIS, carcinoma *in situ;* MIBC, muscle-invasive bladder cancer; TURBT, transurethral resection of the bladder tumour.

Adapted with permission from Miyake M, Fujimoto K, Hirao Y. Active surveillance for nonmuscle invasive bladder cancer. Investig *Clin Urol.* 2016;57(suppl 1):S4–S13.[224]

In the absence of prospective randomized trials to compare expectant management and the alternatives (office fulguration, biopsy and cytodiathermy, TURBT) the LOE in favour of active surveillance/expectant management is low, with all of these being observational cohort studies and many having retrospective analyses. The selection criteria (based on patient and tumour features), triggers for intervention, and surveillance frequency for active surveillance were heterogeneous. This makes the development of consensus/guidance in formulating a surveillance strategy/regime, in the absence of precise selection criteria within the studies, quite difficult. Furthermore, the observed changes or the absence of changes are quite subjective and would, intuitively, require an experienced clinician carrying out the surveillance in these patients—a strategy that may be impractical in most public health care systems and training centres. Whilst the overall principle of expectant management appears to be safe and intuitive, it must be adopted into clinical practice where there are governance processes in place, with thorough patient counselling (ideally with written information) being a mandatory requirement. Reassuringly, the study by Hernandez and colleagues demonstrated a patient preference to delay surgical removal of the recurrent tumour for as long as possible.[228] In general, the accepted triggers to moving from active surveillance to surgical ablation of the tumour should be a rapid increase in the size of the tumour, an increase in the number of tumours, hematuria, and positive urine cytology. Notwithstanding this, Miyake *et al.* have proposed an algorithm for active surveillance in LGTa bladder cancers (see **Figure 5-1**).[224]

From the available literature, there doesn't appear to be any cost effectiveness or HR-QoL evaluation carried out in patients undergoing active surveillance/expectant management for LGTa bladder cancers.

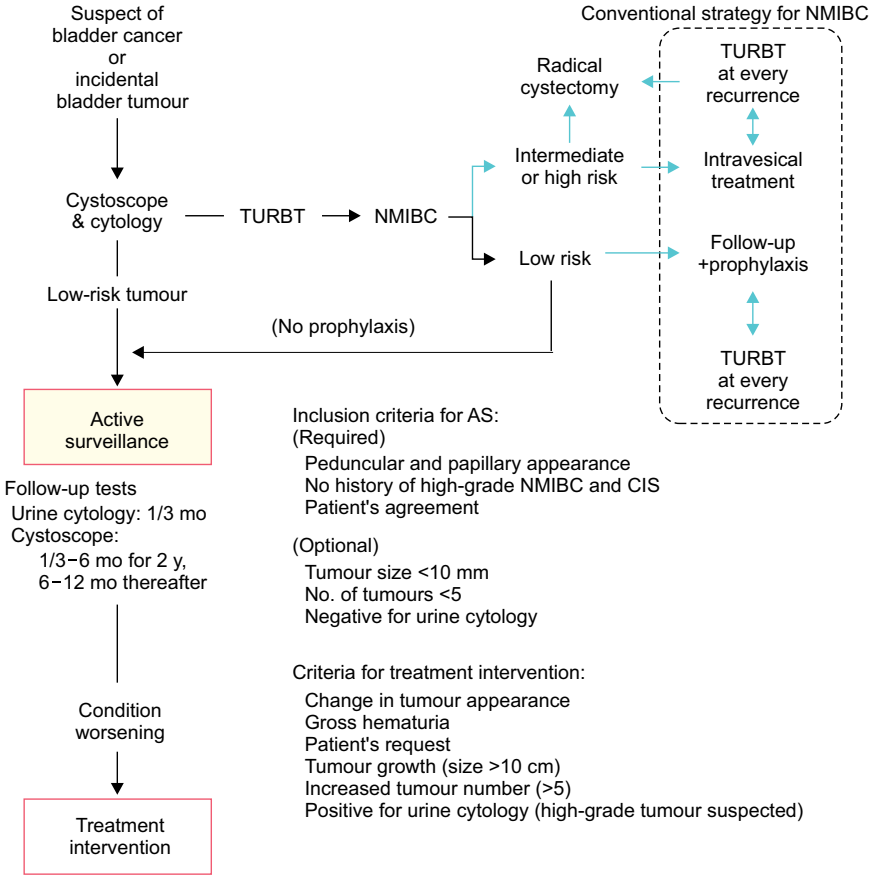
It must be mentioned that smoking cessation should form an integral part of the management of new and recurrent LGTa bladder cancers. The effects of smoking and the benefits of smoking cessation are being addressed in another chapter.

For future development, it would appear beneficial for an RCT to be carried out comparing diathermy, laser ablation, and expectant management using standardized endpoints.[196]

FIGURE 5-1
Proposed Algorithm for Expectant Management/ Active Surveillance

Abbreviations: AS, active surveillance; CIS, carcinoma in situ; NMIBC, nonmuscle-invasive bladder cancer; TURBT, transurethral resection of the bladder tumour.

Reproduced with permission from Miyake M, Fujimoto K, Hirao Y. Active surveillance for nonmuscle invasive bladder cancer. Investig Clin Urol. 2016;57(suppl 1):S4–S13.[224]



5.4.7 Intravesical treatment of low-grade Ta bladder cancer

There are two approaches traditionally used in cases of additional intravesical therapy in LGTa tumours: an immediate single-dose intravesical instillation (SI) of chemotherapy agents following transurethral resection of the bladder (TURB) or adjuvant regimens using full courses of chemotherapy or immunotherapy agents, with or without maintenance.

Modern, evidence-based clinical practice should adjudicate the adjuvant treatment to balance risks and benefits of such intervention. Risk-adapted therapy for NMIBC, based on the EORTC[150] and NCCN[154,230] risk tables, allows us to discriminate among different risk groups, each subsidiary of different effective adjuvant approaches. The CUETO risk tables,[151] better reflecting the impact of BCG therapy in high-grade NMIBC,[231] are less relevant to this section, which is focused on low-grade pTa tumours. When referring to low-grade pTa disease, the rationale of any adjuvant intervention will be the reduction of disease recurrence, since the risk for progression is negligible. For example, a primary, single, <3 cm in size, pTa G1 tumour (EORTC Score 0) will carry a 31% (range, 24%–37%)

risk for recurrence and only a 0.8% (range, 0%–1.7%) risk for progression at 5 years. However, do not forget that even in the case of pTa G1 disease, the presence of multi-focality, previous high recurrence rate, and large tumour size may translate into recurrence and progression rates of up to 78% (range, 73%–84%) and 17% (range, 14%–20%) at 5 years, respectively.[150]

The role of single immediate instillation after TURB has been assessed in several trials and reviewed by four meta-analyses, representing an LOE of 1a.

In 2004, Sylvester *et al.*[148] performed a first meta-analysis of published data from seven randomized trials (1,476 patients) comparing TURB alone versus TURB plus SI of chemotherapy (epirubicin, MMC, thiotepa, or pirarubicin), concluding that single immediate instillation of chemotherapy post-TURB is the treatment of choice in patients with a single, low-risk papillary tumour.

In a more recent analysis published by Abern *et al.*,[232] in 2013, the authors systematically reviewed RCTs comparing a single immediate postoperative dose of intravesical chemotherapy agents versus placebo (within 24 hours of TURB) and conducted a meta-analysis using a random-effects model to predict the pooled RR of tumour recurrence. A total of 18 RCTs (3,103 patients) were included. The recurrence rate in patients receiving a single immediate instillation post-TURB was 37% versus 50% in the TURB-alone group. The pooled RR of recurrence for intravesical chemotherapy (IVC) and TUR was 0.67 (95% CI, 0.56–0.79), corresponding to a 13% absolute reduction and a number needed to treat of 7.2. A single dose of IVC administered within 24 hours of TUR of NMIBC was found to result in a reduction in tumour recurrence (RR, 0.67; 95% CI, 0.56–0.79).

A contemporary systematic review (SR) and meta-analysis, conducted by Perlis *et al.*,[233] aimed to assess the impact of immediate postoperative SI chemotherapy on recurrence and to explore the quality of evidence by means of risk for bias assessment (Cochrane Collaboration risk-of-bias tool) and the Grading of Recommendations Assessment, Development, and Evaluation system. A total of 13 studies (2,548 patients) were included. Immediate SI of chemotherapy prolonged the recurrence-free interval (RFI) by 38% (HR, 0.62; 95% CI, 0.50–0.77; $p<0.001$; I[2], 69%) and recurrence was 12% less likely in the intervention group (absolute risk reduction [ARR], 0.12; 95% CI, -0.18 to -0.06; $p<0.001$; I[2], 0%), with a number needed to treat of 9. However, high risk for bias was present in 12 of 13 publications, demonstrating that the quality of evidence was low.

In 2016, Sylvester *et al.*[234] published an updated SR and individual patient data (IPD) meta-analysis of RCTs comparing the efficacy of a single instillation after TURB versus TURB alone in pTa pT1 patients, aiming to identify which patients benefit from a single immediate instillation. Thirteen studies met eligibility criteria. IPD were obtained for 11 studies randomizing 2,278 eligible patients (1,161 to TURB and 1,117 to a single instillation of epirubicin, MMC, pirarubicin, or thiotepa). A single immediate instillation reduced the risk for recurrence by 35% (HR, 0.65; 95% CI, 0.58–0.74; $p<0.001$) and the 5-year recurrence rate from 58.8% to 44.8%. Importantly, single immediate instillation did not reduce recurrences in patients with a prior recurrence rate >1 recurrence per year or in patients with an EORTC recurrence score ≥5. Furthermore, it did not prolong either time to progression or death from bladder cancer and it is not effective or recommended in patients with high-risk disease, as previously demonstrated in a prospective, randomized, multicentre Swedish trial published in 2009 by Gudjonsson,[235] also included in this latest meta-analysis.

Based on this robust evidence, the EAU NMIBC guidelines panel recommends a single, immediate, postoperative instillation of chemotherapy, with an LOE 1a and a GOR A.[152] Also, the AUA recommends the use of immediate instillation in low-grade pTa disease.[153]

The next obvious question is: how could we improve our results even further? The answer will come, on the one hand, by further research on the development of novel intravesical agents or combinations of such agents and, on the other hand, by optimization of currently available drugs and instillation protocols. In this regard, an effort must be made to perform the instillation within 6 hours of the TURB. Nevertheless, as demonstrated by the published literature, instillation is effective within 24 hours. The optimal duration of instillation has not been fully defined, but Giesbers *et al.*,[236] in a prospective randomized trial, demonstrated the superiority of 1-hour versus 30-minute instillation.

Furthermore, Au *et al.*[237] demonstrated improved outcomes when using MMC in an optimized protocol. In this study, patients in the optimized-treatment arm (n=119) received a 40-mg dose of MMC in 20 cc plus fluid restriction and urine alkalinization. Patients in the standard-treatment arm (n=111) received a 20-mg dose without pharmacokinetic manipulations or urine alkalinization. Both treatments were given weekly for 6 weeks. In an intent-to-treat analysis, patients in the optimized arm showed a longer median time to recurrence (29.1 vs. 11.8 months) and a greater 5-year recurrence-free survival (RFS) (41.0% vs. 24.6%) than those in the standard group (p=0.005). Improvements were found in all risk groups, with statistically significantly enhanced efficacy. These findings, however, could be biased by the different MMC doses used in the two study arms and will require further validation.

A completely new approach aimed to improve the outcomes of a single, immediate instillation of MMC is the pre-TURB administration by means of EMDA. In a prospective trial by Di Stasi *et al.*,[238] patients were randomly assigned to receive TURB alone (n=124), immediate post-TURB passive diffusion MMC (n=126) or immediate pre-TURB EMDA MMC (n=124). With a median follow-up of 86 months, patients assigned to receive EMDA MMC pre-TURB demonstrated a lower rate of recurrence (38%) than those assigned to passive post-TURB MMC (59%) and TURB alone (64%) (p<0.0001). Patients assigned to receive EMDA mitomycin before TURBT also had a higher disease-free interval (52 months) than those assigned to passive MMC post-TURB (16 months) or TURB alone (12 months) (p<0.0001) **[LOE 1b]**.

An important question remaining is if, following single immediate instillation, further intravesical chemotherapy could even further decrease the recurrence rate in low-risk patients. This has been investigated in three separate phase 3 trials using thiotepa, MMC and epirubicin. Although there is a trend toward improved recurrence rates for patients, the differences did not reach statistical significance in any of the trials.[239-241] In summary, adding further intravesical chemotherapy instillations to one immediate instillation does not seem to be effective in low-risk patients. Consequently, following a single instillation of intravesical chemotherapy, both AUA and EAU guidelines do not recommend further treatment for low-grade pTa. The AUA guideline[153] clearly states that, "in a low-risk patient, a clinician should not administer induction intravesical therapy. (Moderate Recommendation; Evidence Strength: Grade C)". Equally, in patients with low-risk disease, defined as primary, solitary Ta, G1/PUNLMP, LG, <3 cm with no CIS, the EAU guidelines panel recommends one immediate instillation of intravesical chemotherapy after TURB" **[LOE 1a; GOR A]**.

Nevertheless, even low-grade (G1) pTa tumours may become tumours of intermediate risk, as seen above. In this clinical scenario, adjuvant intravesical therapy with either BCG or chemotherapy is recommended in intermediate risk (IR) by all international bladder cancer guidelines. However, the recommendation varies due to the lack of robust evidence for this cohort of patients and the absence of a clear, broadly accepted definition of IR. The AUA[153] recommends administration of a 6-week course of induction intravesical chemotherapy or immunotherapy **[GOR B]**. Moreover, the EAU guidelines[152] do recommend, in patients of intermediate-risk disease and previous low recurrence rate (less than or equal to one recurrence per year) and expected EORTC recurrence score <5, one immediate instillation of intravesical chemotherapy after TURB **[LOE 1a; GOR A]**. In all patients, the recommendation is for either 1-year full-dose BCG treatment (induction plus 3-weekly instillations at 3, 6, and 12 months), or instillations of chemotherapy (the optimal schedule is not known) for a maximum of 1 year **[LOE 2a; GOR A]**. The NCCN guidelines (version 5.2017)[840] includes observation, intravesical chemotherapy with MMC, or, preferably, intravesical BCG. The Canadian Urological Association (CUA) recommends induction chemotherapy followed by 1-year maintenance for IR tumours.[242] The International Bladder Cancer Group (IBCG) recommends BCG induction plus maintenance or intravesical chemotherapy after complete TURB, where adjuvant chemotherapy should not exceed 12 months.[243]

A cycle of instillations with a chemotherapeutic agent, with or without some form of maintenance, is able to reduce the short-term risk for recurrence in patients with intermediate-risk NMIBC. For this, the most-used drugs are MMC and epirubicin. A combined analysis of EORTC and Medical Research Council (MRC) trials involving 2,535 patients with pTa and pT1 disease demonstrated that adjuvant chemotherapy after TURB significantly improves disease-free survival (DFS) compared to TURB alone, but it had no effect on progression.[244] Similarly, Lamm *et al.*, while reporting a 14% (range -3 to +43%) reduction in tumour recurrence, did not demonstrate any impact on progression among 2,011 randomized patients (progression occurred in 7.5% of those receiving chemotherapy vs. 6.9% of those treated by TURB alone).[245]

At the present time, still no consensus exists regarding the optimal schedule and duration of treatment. Based on the evidence from the SR by Sylvester *et al.*, it appears that a short, intensive schedule of instillations within the first 3 to 4 months after an immediate instillation (i.e., SI) may be as effective as longer-term treatment schedules. Instillations during ≥1 year in IR patients seem advisable only when an SI was not given.[246]

Novel chemotherapeutic agents are currently being evaluated, although clinically relevant data are still lacking.

Gemcitabine appears to have minimal toxicity when used intravesically in doses up to 2,000 mg/50 mL for 2 hours.[247] The clinical effectiveness and toxicity of intravesical gemcitabine in NMIBC has been evaluated by a Cochrane systematic review published by Jones *et al.* in 2012. A total of 704 patients from 6 RCTs are included. One study compared an SI of intravesical gemcitabine with placebo and found no significant difference in the recurrence rates (28% vs. 39%, respectively) or RFS. The rate of progression to invasive disease was greater with gemcitabine (2.4% vs. 0.8%). A further trial compared gemcitabine with MMC and demonstrated that the rates of recurrence (28% vs. 39%) and progression (11% vs. 18%) were lower with gemcitabine but did not reach statistical significance. The

global incidence of adverse events (AEs) was significantly less with gemcitabine (38.8% vs. 72.2%, p=0.02). In untreated intermediate-risk patients (primary Ta-T1, no CIS) one trial demonstrated that gemcitabine and BCG were comparable in terms of recurrence (25% vs. 30%, p=0.92) and overall progression (p=1.0) rates, while gemcitabine was better tolerated.[248]

Apaziquone (EO9, Qapzola™), a novel indole quinone derivative of MMC, is a prodrug that is activated to DNA damaging species by oxidoreductases (particularly NQO1) and has the ability to kill aerobic and/or hypoxic cancer cells. Intravesical apaziquone demonstrated good activity against NMIBC when administered to marker lesions and was well tolerated, with no systemic side effects. However, the two phase 3 clinical trials, SPI-611 and SPI-612 **[LOE 1b]**, did not reach statistical significance for the primary endpoint of 2-year recurrence in apaziquone versus placebo in low-risk NMIBC patients. A total of 1,614 patients were enrolled across 152 centres in the United States, Poland, and Canada. The studies were analyzed individually and neither met their primary endpoint. However, when the results of the two trials were pooled, statistically significant differences were obtained in both the 2-year recurrence rate (apaziquone 38.8% vs. placebo 45.5%) and time to recurrence (apaziquone 18.2 months vs. placebo 16.8 months). A further phase 3 study is ongoing to test the hypotheses generated in the unsuccessful phase 3 studies conducted to date.[249,250]

Taxanes, potential candidates for intravesical therapy, exert their action via the inhibition of microtubule depolymerization, leading to cell cycle arrest and cell death. So far, promising results have been observed in high-grade NMIBC.[251-253] However, its potential role in the low- and intermediate-risk setting has not been formally assessed.

Available clinical evidence indicates that intravesical chemotherapy instillations, MMC and epirubicin being the standard, are safe and effective in reducing tumour recurrence in the short term. Nevertheless, there is only marginal long-term efficacy. Novel delivery systems and drugs are interesting and already have some promising results, but robust clinical evidence with long-term follow-up is still limited or even lacking.

5.4.8 The role of bacillus Calmette-Guérin in primary and recurrent low-grade Ta nonmuscle-invasive bladder cancer

The therapeutic strategy in patients with low-grade pTa tumours requires striking a balance between efficacy and tolerability, as well as an evaluation of its potential impact on the quality of life. In this context, the role of BCG is questionable, due to the higher toxicity of the BCG and the low risk for progression of these tumours. Nevertheless, an elevated recurrence rate, requiring multiple resections and courses of chemotherapy, will also negatively impact the quality of life. Consequently, in this clinical scenario, one should also evaluate the potential benefits of BCG.

In the absence of randomized trials comparing BCG versus intravesical chemotherapy as adjuvant therapy in patients with low-grade pTa tumours, we need to extrapolate results from subgroup analyses of randomized studies and meta-analyses, and consequently have a lower LOE.

When assessing the efficacy of BCG therapy in this cohort of patients, we generally identify the recurrence rate as the primary endpoint, due to the very low rate of progression, which makes it unlikely that any pharmacological intervention could demonstrate a significant impact on progression in low-grade pTa disease. In a published meta-analysis by Han *et al.*,[254] assessing the efficacy of BCG versus any other form of therapy different from BCG, BCG was superior only when administrated in maintenance for at least 1 year (OR, 0.47; 95% CI, 0.28–0.78; p=0.004), an effect that was also seen in papillary tumours (OR, 050; 95% CI, 0.33–0.75; p=0.0008), whereas no differences were identified in the absence of maintenance (OR, 0.97; 95% CI, 0.52–1.56; p=0.71). However, this meta-analysis did not stratify patients by risk groups and, consequently, the real impact in the subgroup of LGpTa cannot be assessed.

More specifically, in a meta-analysis conducted by Bohle *et al.*,[255] including 7 randomized and nonrandomized studies comparing MMC and BCG, patients were stratified in high-risk and low-to-intermediate–risk groups, as well as into those who received maintenance and those who did not. In high-risk patients, BCG significantly reduced recurrence when compared to MMC when patients received a maintenance BCG schedule. In a nonrandomized series, patients who did not receive maintenance still demonstrated a reduction of recurrence, although this did not reach statistical significance. However, in patients with intermediate risk, no differences were observed between BCG and MMC without maintenance and, although some benefit was observed with maintenance, the evidence is supported only by three nonrandomized series, hence, with a low LOE. This hypothesis is further supported by Malsmtröm *et al.*[256] in a randomized trial, that demonstrated superiority of the BCG with maintenance when compared to MMC in the overall cohort, but no differences in recurrence were observed in patients with papillary tumours (p=0.22) and those with G1 disease (p=0.97), suggesting that BCG may not be superior to MMC in patients with low-grade NMIBC. In another randomized trial[257] including a high proportion of low-risk patients (Ta, 63% and G1–G2, 84%), the authors observed that, in the absence of concomitant CIS, induction of BCG alone was not superior to MMC at reducing recurrence (p=0.354). This is further supported by a contemporary meta-analysis published by Shelley *et al.*[258] concluding that BCG was not superior to MMC (p=0.76). Indeed, BCG was only superior to MMC at reducing recurrence rates in high-risk patients and independently of the use of maintenance.

Regarding the superiority of BCG compared to other intravesical adjuvant agents, published evidence has demonstrated that BCG is superior to doxorubicin. In a phase 3 trial[259] enrolling predominantly pTa (70.8%) and G1–G2 (91.1%) tumours, Lamm *et al.* demonstrated the superiority of BCG in terms of RFS (p=0.015) in the absence of CIS. In another randomized study by Martínez-Piñeiro *et al.*,[260] BCG was more effective than doxorubicin (p=0.002) and thiotepa (p=0.0037) in low- and intermediate-risk tumours.

Similarly, in a randomized trial conducted by the EORTC,[261] BCG significantly improved the time to the first recurrence when compared to epirubicin in intermediate risk (OR, 0.59; 95% CI, 0.45–0.76; p<0.001). Whether this benefit is also real in low-risk tumours can only be speculated, since this outcome was not analyzed in this cohort. Nevertheless, the significant number of primary tumours (45.3%), stage pTa (63.6%), grades G1–G2 (87.5%), and tumour size ≤1cm (50.6%) would suggest that this benefit would also be applicable to low-grade tumours.

The potential role of BCG in the context of rescue therapy for LGpTa NMIBC has not been formally assessed. However, in a meta-analysis published by Huncharek *et al.*,[262] where patients were stratified into those receiving previous chemotherapy and those who were chemo-naïve, BCG demonstrated a benefit in the earlier group, when assessing the 2-year (OR, 0.51; 95% CI, 0.40–0.65) and 3-year (OR, 0.43; 95% CI, 0.34–0.55) recurrence rates, with no significant differences in recurrence observed in the chemo-naïve patients at 2 years (OR, 0.1.21; 95% CI, 0.93–1.58) and with even worse 3-year outcomes for those treated with BCG in this group (OR, 0.1.67; 95% CI, 1.29–2.17). Unfortunately, this study did not sub-stratified by risk groups, although one can assume that, since progression of high-risk tumours was mostly rescued with cystectomy, many of these patients were indeed of low and intermediate risk. This has been further supported by the findings of the Malmström meta-analysis[263] demonstrating superiority of BCG in those patients previously treated with intravesical chemotherapy, but only when using maintenance (p=0.0264). Notably, this benefit was observed in all risk groups, but the cohort of low-risk patients (n=92) was very small.

In addition to the previously discussed limitations of the available series, such as absence of specific randomized trials and lack of stratification by risk group in many studies and meta-analyses, other confounding factors and potential biases include: the use of different BCG strains, with published evidence suggesting that they may play a role in the outcomes observed; the comparison with variable doses of MMC (ranging from 20 to 40 mg); and the use of widely variable BCG maintenance protocols among different published series.

In summary, there is no robust evidence that BCG is superior to MMC at reducing recurrence in low-risk patients. Although maintenance appears to improve the efficacy of BCG, with a clear benefit in high-risk cases, its impact on low-risk patients is not well defined. Considering the higher toxicity demonstrated by the BCG, BCG is not recommended in low-risk disease. However, in patients with intermediate-risk disease and those patients in whom previous first- or second-line intravesical chemotherapy has failed, BCG may represent a therapeutic alternative and its use, acknowledging its higher toxicity, may be considered.

5.4.9 International Consultation on Urologic Diseases proposed recommendation

While the management of low-risk (SI alone) and high-risk (BCG plus maintenance) disease appears clear, the best treatment option for IR patients remains undefined. Indeed, some patients from the IR cohort, the "low-intermediate" patients, will be better treated with intravesical chemotherapy instillations and maintenance. On the other hand, patients with a more aggressive profile, the "high-intermediate" cohort, will be better managed with BCG and maintenance for 1 year, as in the high-risk category.

When considering the revision of current ICUD guidelines, the authors took into account all the available clinical evidence and, in line with other international guidelines (EAU and IBCG), recommends a risk-stratified approach of low risk and further stratifying the intermediate-risk group into "low-intermediate" and "high-intermediate" subgroups (algorithm is presented in **Figure 5-2**). Ofude *et al.* retrospectively analyzed the data of 469 TURB cases for NMIBC and found that, with an

increase in EORTC score, the efficacy of intravesical BCG therapy became prominent compared to intravesical chemotherapy. The overall RFS rate at 1 and 3 years was 59.1 and 40.3%, respectively. Of the total, 424 TURB cases (90.4%) had an EORTC score of 1 to 9. In patients with an EORTC score of ≥5, in particular, intravesical BCG therapy was superior to chemotherapy at preventing recurrence (HR, 2.425; 95% CI, 1.068–5.506; $p<0.034$) **[LOE 3]**.[264] In concordance with Kamat *et al.*, when using the classical IBCG and AUA definition of IR (multiple or recurrent low-grade Ta tumours), advise a risk-adapted strategy, taking into account four key factors (tumour size, tumour multiplicity, timing and frequency of recurrences, and previous treatment). Patients with none of these factors are at low risk for disease recurrence and progression and, therefore, can be treated like low-risk patients. For those with one or two factors, both intravesical chemotherapy and BCG maintenance are appropriate options. IR patients with three or more factors are at the highest risk for recurrence and progression and, therefore, will benefit most from BCG maintenance therapy.[265]

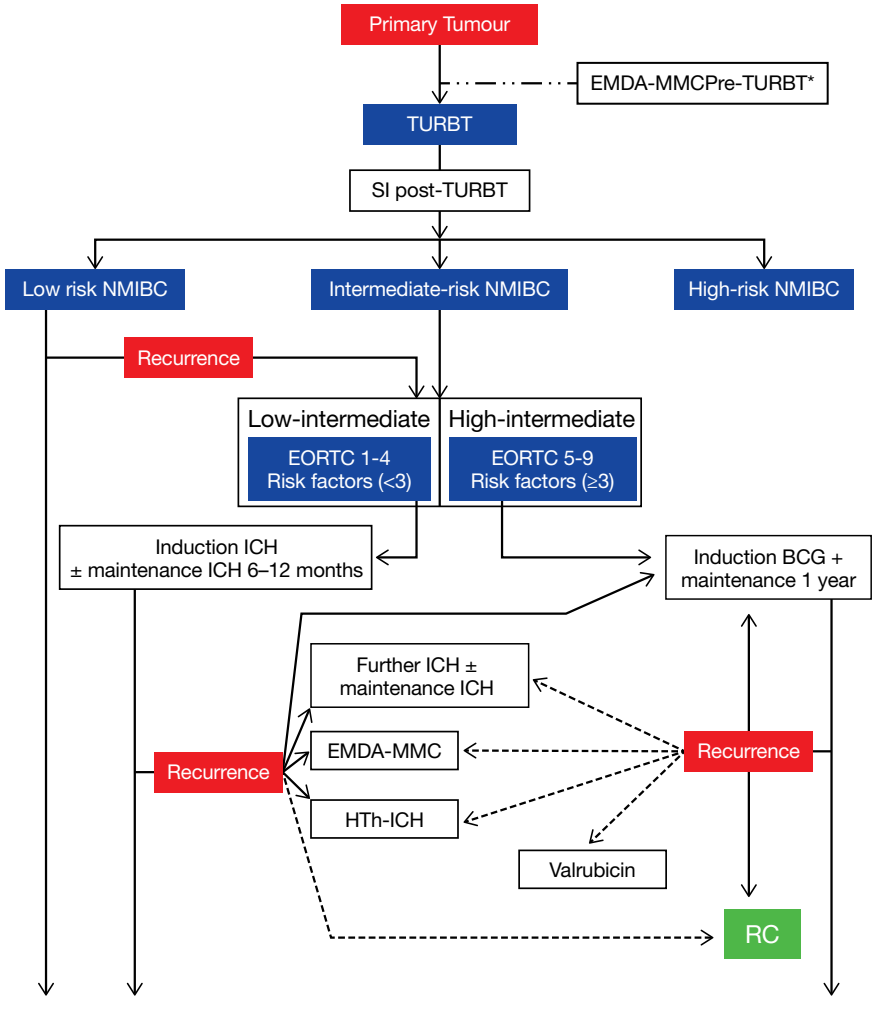
Consequently, the ICUD recommendations for the intravesical therapy of low-risk and intermediate-risk NMIBC will be summarized as:

- For **low-risk disease,** a single-dose instillation post-TURB (SI) is sufficient and no further adjuvant treatment is recommended.
- For **low-intermediate–risk disease,** a single-dose instillation post-TURB (SI) followed by induction chemotherapy followed by maintenance (6–12 months) is recommended.
- For **high-intermediate–risk disease,** a single-dose instillation post-TURB (SI) followed by induction with full-dose BCG instillations and maintenance for 1 year is recommended.

FIGURE 5-2
ICUD Recommendation Algorithm for the Use of Adjuvant Intravesical Therapy in Low- and Intermediate-Risk NMIBC as Supported by Relevant Clinical Evidence and Integrating EAU and IBCG Recommendations

Abbreviations: BCG, bacillus Calmette-Guérin; EAU, European Association of Urology; EMDA-MMC, electromotive drug administration–mitomycin C; EORTC, European Organisation for Research and Treatment of Cancer; HTh-ICH, thermochemotherapy–intravesical chemotherapy; IBCG, International Bladder Cancer Group; ICH, intravesical chemotherapy; NMIBC, nonmuscle-invasive bladder cancer; RC, radical cystectomy; SI, single instillation; TURBT, transurethral resection of the bladder tumour.

*EMDA-MMC pre-TURBT is supported by a single phase 3 trial and is still considered experimental.



Summary of Recommendations

1. Upper-tract imaging in LGTa urothelial carcinoma

Recommendation	LOE	GOR
Routine imaging of the upper tracts is not recommended in patients with LGTa bladder cancer	3	B
Upper-tract imaging could be carried out in patients with multi-focal tumours and those with tumours centred in the trigone	3	B

2. Follow-up surveillance and upper-tract imaging during surveillance

Recommendation	LOE	GOR
Routine imaging of the upper tracts is not recommended for surveillance in patients with LGTa bladder cancer	3	B

3. Treatment of recurrence of low-grade Ta bladder cancer

a. Office fulguration with diathermy (also referred to as cystodiathermy)

Recommendation	LOE	GOR
Office fulguration or cystodiathermy can be carried out in patients with small (<10 mm) recurrent LGTa with no previous history of high-grade cancer or CIS	2	B

b. Laser treatment of recurrent LGTa bladder cancers

Recommendation	LOE	GOR
Ho:YAG laser could be used to fulgurate recurrent LGTa bladder cancers	2	B

c. Chemo-resection (or chemo-ablation) in recurrent LGTa bladder cancers

Recommendation	LOE	GOR
Chemo-resection is not recommended for routine use in recurrent LGTa bladder cancers outside the setting of a clinical trial	3	B

d. Expectant management (also known as active surveillance) in recurrent LGTa bladder cancer

Recommendation	LOE	GOR
Expectant management or active surveillance can be adopted in patients with established recurrent LGTa bladder cancers	3	B
The ideal tumour(s) for expectant management or active surveillance are those that are small (5 mm or less), papillary in appearance, and three or fewer in number	3	B
If a strategy of expectant management/active surveillance is adopted, a clear protocol (with criteria for intervention) must be followed	3	B
Examination of urine cytology must be a part of the expectant management or active surveillance protocol	3	B

4. Intravesical treatment for primary and recurrent LGTa bladder cancer

a. For **low-risk disease**, a single-dose instillation post-TURB (SI) is sufficient and no further adjuvant treatment is recommended.	1a	A
b. For **low-intermediate–risk** disease, a single-dose instillation post-TURB followed by induction chemotherapy and maintenance (6–12 months) is recommended	2a	B
c. For **high-intermediate–risk** disease, a single-dose instillation post-TURB (SI) followed by induction with full dose BCG instillations and maintenance for 1 year is recommended	1a	A

5.5 Management of Primary and Recurrent High-risk Disease (High-grade Ta, T1, Carcinoma *in situ*)

5.5.1 Introduction

Among patients with NMIBC, 20% to 40% will present with tumours that have a high risk for recurrence and progression.[266,267] While there is some variability in the definition of high-risk NMIBC, patients with histological high-grade disease have the highest risk for recurrence and progression and are considered high risk by all professional guidelines.[268-270] There are additional factors that impact recurrence and progression that should also be taken into consideration when risk stratifying patients, including tumour size, multiplicity, recurrent tumours, stage, presence of CIS, variant histologies, LVI, relapse after treatment with intravesical therapy, and prostatic urethral involvement.[267,271-274] Although patients with T1 disease or CIS have the highest risk for progression, high-grade Ta tumours also have a significant risk for progression and should be managed as high risk.[275] This section considers patients with any high-grade Ta, T1, or CIS to be high risk. This section of the Guideline is organized according to 12 important clinical questions about the management of high-risk NMIBC.

5.5.2 Guideline questions

1. What is the role of TURBT in the diagnosis and treatment of NMIBC?

a. **Guideline statement 1:** A thorough examination should be performed on all patients with bladder tumours at the time of TURBT, including a bimanual exam and cystoscopic assessment of tumour characteristics **[LOE 2; GOR B]**.

b. **Guideline statement 2:** A complete TURBT should be performed on all patients with bladder tumours when safe and feasible and when bladder preservation is planned **[LOE 3; GOR C]**.

c. **Discussion:** A thorough and complete TURBT is the first step in the management of all patients with a bladder tumour and is critical for disease risk stratification, staging, and treatment response. Surgeons should evaluate and document several disease characteristics at the time of cystoscopy that are important for risk stratification, including tumour size, number, appearance, location, and any areas of concern for CIS.[267,276,277] Bimanual EUA is an important part of clinical staging and should be performed at the time of TURBT.

The goal of a TURBT is to completely resect all tumour if bladder preservation is planned. However, prior studies have demonstrated significant variation in the quality of TURBT, likely due to differences in surgical quality.[278] In addition, grossly incomplete resections have been reported in as high as 70% of patients.[279] Therefore, work is needed to improve the quality and consistency of how this procedure is performed.

Enhanced cystoscopy, either with fluorescence or NBI, has been shown to improve cancer detection rates.[280,281] In particular, fluorescence cystoscopy during TURBT has been shown to increase cancer detection, improve completeness of resection, and decrease recurrence rates in several randomized clinical trials.[281,282] Surgical checklists may also help improve TURBT quality.[277]

In addition to resecting all visible disease, surgeons should resect a margin of visually normal tissue around the edges of the tumour. The high risk for early recurrence at the site of initial TURBT is likely due to unresected microscopic disease.[283,284] Although unresected microscopic disease can be found at the resection base, there is a significant risk for residual disease in the lateral margins.[285]

A proxy for the adequacy of a resection of a high-risk tumour is the presence of detrusor muscle in the specimen. Patients who have detrusor sampled are more likely to be staged accurately, and will have fewer intravesical recurrences and improved survival.[286,287] Among contemporary cohorts of patients with high-risk NMIBC, 40% to 70% of patients will have muscle in the initial resection specimen.[277,286,288,289] More experienced surgeons are more likely to obtain muscle in the specimen.[286,290] Patients with a more thorough resection have a significantly improved response rate to intravesical BCG and chemotherapy.[291,292] Importantly, adjuvant intravesical therapy cannot compensate for an inadequate tumour resection.

2. When should a repeat TURBT be performed?

a. **Guideline statement 3:** A repeat TURBT should be performed within 6 weeks of initial resection for all patients with an incomplete initial resection and for patients with T1 high-risk disease after a complete initial resection **[LOE 2; GOR B]**.

a. **Guidelines statement 4:** A repeat TURBT should be considered within 6 weeks of a complete initial resection for patients with high-grade Ta tumours, particularly for patients with large or multifocal tumours **[LOE 3; GOR C]**.

a. **Discussion:** The success of intravesical therapy for high-risk NMIBC depends on proper patient selection and maximal resection. However, after an initial complete TURBT, a significant percentage of patients will have residual or understaged disease, thus prompting the need for a repeat TURBT.

At repeat TURBT, around 50% of high-risk patients will have residual disease, most often at the site of the initial resection.[283,284,289,291-294] For patients with T1 disease, the risk for residual disease can be upward of 80%.[293,295] Understaging is also problematic, and is seen in upward of 20% of patients with high-risk tumours on repeat TURBT.[284,288,289,291,295,296] Patients with clinical T1 disease have a risk for understaging of upward of 30% to 50%, particularly if no muscle is obtained in the specimen.[294,297-299] The adequacy of initial TURBT is critical in establishing clinical stage, since the ability of the surgeon to sample detrusor is highly related to the risk of understaging.[289,294,297] Patients who have residual T1 disease on repeat TURBT are at substantial risk for progression and may benefit from early cystectomy.[300,301]

The role of repeat TURBT is unequivocally important for patients with T1 tumours, given the significant risk of understaging; however, its role in patients with Ta tumours is less clear. One series observed a risk for residual disease of more than 50% repeat TURBT for patients with HGTa tumours, but with very low rates of upstaging, especially if detrusor was obtained in the initial resection.[289] Furthermore, patients with HGTa

tumours who were selected to have a repeat TURBT had a lower risk for early recurrence than those who did not have a repeat TURBT. Therefore, repeat TURBT may be considered for patients with HGTa disease, particularly those with high volume or multifocal tumours in whom the initial resection may have been incomplete.

Some have suggested that patients with completely resected high-risk tumours who had detrusor muscle adequately sampled may not need a repeat TURBT.[293] If a thorough and complete initial TURBT down to detrusor muscle was performed by an experienced surgeon, a repeat TURBT may have less benefit in certain cases. In addition, many of these studies that demonstrated the importance of repeat TURBT are from academic centres whose patients were referred after their initial TURBT or bladder biopsy was done elsewhere, and the quality and extent of the initial resection was not known. Thus, a repeat TURBT may not be needed in all high-risk cases. Still, at least half of patients with high-risk disease will not have detrusor sampled at the initial TURBT, and the risk for residual disease and understaging is significant even for patients who have detrusor sampled.[297,298] Therefore, a repeat TURBT should be considered for patients with high-risk NMIBC.

In addition to improved staging and risk stratification, repeat TURBT improves response to intravesical therapy.[283,292,302] One trial randomized 210 patients with newly diagnosed completely resected T1 tumours to induction MMC or repeat TURBT followed by induction MMC.[291] Patients who had a repeat TURBT had significantly higher 5-year RFS and PFS, and were less likely to die from bladder cancer. A large retrospective trial demonstrated that repeat TURBT substantially improved the response to BCG.[292] This effect was likely due to the resection of persistent disease after the first TURBT.

Up to one-quarter of TURBT specimens will demonstrate variant histology, and these tumours can behave more aggressively than pure urothelial carcinoma, have an increased risk of understaging, and may be less amenable to bladder sparing treatments.[273,303] If a bladder sparing treatment is considered in a patient with variant histology, repeat TURBT should be performed.[304]

3. Do patients with high-risk NMIBC who receive BCG benefit from an immediate dose of intravesical chemotherapy after TURBT?

a. **Guideline statement 5:** Patients with high-risk NMIBC who are treated with intravesical BCG do not benefit from an immediate dose of intravesical chemotherapy **[LOE 2; GOR B]**.

b. **Discussion:** A single dose of intravesical chemotherapy within 24 hours of TURBT is effective at decreasing intravesical recurrences for patients with a low risk for recurrence.[305] The use of an immediate postoperative instillation of intravesical chemotherapy has also been studied among patients at higher risk who eventually are treated with intravesical BCG.

A group of 161 patients with NMIBC were randomized to an immediate dose of postoperative epirubicin followed by induction BCG versus induction BCG alone.[306] Epirubicin was not associated with improved recurrence rates. Another trial randomized 51 patients with intermediate- or high-risk NMIBC who had TURBT to a single dose of MMC followed by BCG induction or BCG induction alone.[307] Patients given chemotherapy had a lower recurrence rate (36% vs. 19%) at 41 months follow-up, but this was not statistically significant (p=0.052).

Several retrospective studies have also examined the effectiveness of intravesical chemotherapy before induction BCG. One retrospective series suggested a possible benefit to an immediate dose of MMC prior to induction BCG, but this was not statistically significant on multivariable analysis.[308] Another retrospective series suggested that a single dose of epirubicin after TURBT may result in improved 5-year RFS among patients treated with induction BCG (55% vs. 66%), although this was not statistically significant (p=0.149).[309]

Due to the risk for toxicity and uncertain benefit, if a patient is likely to require induction BCG then a single dose of chemotherapy should not be given.

4. What is the importance of variant histology and adverse pathological features?

a. **Guideline statement 6:** Patients with variant histology, LVI, or deeply invasive T1 tumours are at an increased risk for progression and may not be candidates for bladder sparing treatments **[LOE 3; GOR C]**.

b. **Discussion:** Variant histological subtypes of urothelial carcinoma include squamous, nested, micropapillary, glandular, plasmacytoid, neuroendocrine, and sarcomatoid. These subtypes are increasingly recognized by pathologists and may have a more aggressive disease biology compared to pure urothelial carcinoma.[310] Variant or mixed histology may be difficult to diagnose on TURBT, but is seen in up to 25% of patients with high-risk NMIBC.[273,303] Variant histology on TURBT is an adverse pathological finding that is associated with higher rates of understaging, progression, and metastasis compared to pure urothelial carcinoma.[273,303,304,311-313] It is currently unclear how the percentage of variant histology in the TUR specimen impacts risk and how reliably the percentage of variant histology can be reported. For further details, refer to the pathology section within the guidelines.

There have been several retrospective studies addressing the impact of variant histology on response to intravesical therapy. An initial report of patients with micropapillary NMIBC treated with intravesical BCG observed high rates of progression and metastases, and suggested early cystectomy should be the treatment of choice.[311] Others have challenged this assertion and suggested that BCG is appropriate for well-selected patients.[304] For example, responses to BCG for patients with glandular and squamous differentiation and nested variants have been reported.[313-315] Select small tumours with squamous or glandular features, nested and micropapillary variants, that have been properly staged and maximally resected may be considered for intravesical BCG.[316] Alternatively, small cell carcinoma, adenocarcinoma, pure squamous carcinoma, sarcomatoid carcinoma, and plasmacytoid carcinoma are generally not responsive to intravesical therapy and should not be managed with bladder-sparing treatments.

LVI is found in 9% to 36% of patients with high-risk NMIBC at the time of TURBT, more commonly in patients with T1 tumours.[271,274,317-320] Although LVI can be difficult to identify on TURBT specimens and not prospectively validated in NMIBC, it is an adverse prognostic factor if found.[274,317,318,320-323] One study of 118 patients with T1 disease treated with intravesical therapy found that LVI was significantly associated with disease recurrence (HR 2.0) and progression (HR 3.1).[274] This finding was confirmed in a large meta-analysis.[271] If identified in a patient with high-risk NMIBC, LVI may predict failure of intravesical therapy and early cystectomy should be considered.

Finally, some pathologists have attempted to quantify the extent of lamina propria invasion for T1 tumours, such as according to absence (T1a) or presence (T1b) of muscularis mucosa invasion.[324] Quantifying the extent of lamina propria invasion is subject to the quality of the specimen submitted, the experience of the pathologist, and the ability to identify muscularis mucosa in the specimen. Therefore, the extent of T1 invasion may not be reliably reported on TURBT specimens.[324] However, if identified, the presence of extensive lamina propria invasion portends a worse prognosis with higher rates of recurrence and progression.[271,303,325,326] Among 587 patients with T1 disease, two-thirds had T1a disease and the rest had T1b.[324] Patients with T1b disease had a significantly increased risk for recurrence (OR 1.3) and progression (OR 1.9). Based on a large, systematic review and meta-analysis of patients with T1 disease, T1 substage was significantly associated with disease progression (HR 3.3) and mortality.[271] Because substaging according to depth relative to muscularis mucosa can be difficult and sometimes impossible, others have suggested quantifying T1 tumours according to the depth of lamina propria invasion in millimetres.[327] When stratified by T1e (≤0.5 mm deep) or Tm (>0.5 mm deep or multiple sites of focal invasion), tumours that invaded more deeply had a worse prognosis.[328] In all, lamina propria invasion should be quantified and reported by pathologists if possible, although there is currently no standard method for reporting. However defined, patients with T1 disease who have more extensive lamina propria invasion should be considered for early cystectomy.[325]

5. What is the role of adjuvant induction intravesical therapy after TURBT?

a. **Guideline statement 7:** Patients with high-risk NMIBC for whom bladder sparing is desired should be offered induction intravesical BCG after a complete TURBT **[LOE 1; GOR A]**.

b. **Guideline statement 8:** Patients with high-risk NMIBC for whom bladder sparing is desired who are ineligible to receive intravesical BCG may be offered induction intravesical chemotherapy after a complete TURBT **[LOE 2; GOR B]**.

c. **Discussion:** Patients with high-risk NMIBC have a significant risk for recurrence and progression.[329] Using prediction models, the risk for recurrence within 5 years of TURBT is as high as 78%, and up to 45% of patients will progress.[267] Intravesical BCG and chemotherapy have been extensively studied as adjuvant therapy for high-risk patients or as treatment for CIS.[330-332]

BCG is a live, attenuated strain of Mycobacterium bovis that has been utilized as an intravesical treatment for bladder cancer since the 1970s.[333] Intravesical BCG is an immunotherapy that creates a local antitumour effect through upregulation of T cells and cytokines. BCG has proven to be the most effective intravesical agent for treatment of high-risk NMIBC.

There have been several randomized trials investigating the effectiveness of adjuvant BCG following TURBT compared to observation alone.[334-336] Meta-analyses of these randomized trials demonstrate that, compared with observation, adjuvant intravesical BCG is associated with a 44% to 65% decreased risk for intravesical recurrence.[330,331,337] In addition, adjuvant intravesical BCG is associated with a 60% decreased risk for progression compared to observation.[330] Induction BCG is given as a once-weekly dose of full-dose BCG for 6 weeks with an intravesical retention

time of up to 2 hours. After induction therapy, approximately 60% to 70% of patients with high-risk NMIBC will have a complete response (CR) at the 3- or 6-month evaluation based on cystoscopy and cytology.[332,336,338-341]

Recurrent Ta or persistent CIS following an initial induction course of BCG is an adverse prognostic factor.[342,343] However, assuming no disease progression or recurrent T1 disease, up to 50% of these patients will ultimately have a CR at 6 months after a second induction course or first maintenance dose.[344,345]

For patients with a positive cytology following induction BCG, approximately 25% will have a CR at 6 months with no additional therapy.[345]

IFN alpha is another intravesical immunotherapy that has been studied as monotherapy or in combination with BCG in the first-line setting. IFN is associated with a decreased risk for recurrence compared with observation, but BCG has a lower recurrence rate when compared to IFN.[330] In the first-line setting, combining IFN with BCG for induction therapy increases toxicity without improving response rates.[346]

Various intravesical chemotherapies have also been studied as adjuvant treatment after TURBT, including doxorubicin, MMC, epirubicin, docetaxel, gemcitabine, and thiotepa. Compared with TURBT alone, induction intravesical chemotherapy with MMC, doxorubicin or epirubicin is associated with a 20% to 40% lower risk for recurrence.[330] These medications have been studied either as an immediate postoperative dose or as induction therapy followed by maintenance.[347] There is no evidence to support use of thiotepa or gemcitabine as initial induction treatment.[330] There is no evidence that intravesical chemotherapy reduces disease progression compared to observation alone.[330]

Importantly, there have been several randomized trials and meta-analyses comparing intravesical chemotherapy to BCG.[330,348,349] When maintenance therapy is given, intravesical BCG is associated with a 20% to 30% lower recurrence risk compared to chemotherapy.[330,347-352] Without maintenance, BCG may have a similar or higher risk for recurrence compared to chemotherapy, specifically MMC.[330,347] There is no strong evidence that BCG decreases the risk for progression compared to MMC, but it does have more side effects.[330,348,349]

Some have attempted to reduce the toxicity or improve the effectiveness of intravesical therapy by combining BCG and chemotherapy. Although two meta-analyses observed a benefit to sequential therapy, they were based on several randomized trials with significant heterogeneity in regards to patient selection, induction and maintenance protocols, delivery method, and medications.[353-355] The observed benefit of sequential therapy in one meta-analysis did not apply to patients with high-risk tumours.[353] Given the possibility of increased toxicity with sequential therapy but with unclear benefit, sequential therapy is not recommended at this time.

6. Should patients be given maintenance intravesical therapy after an induction course?

a. **Guideline statement 9:** Patients who respond to induction intravesical BCG should be offered up to 3 years of maintenance **[LOE 2; GOR B]**.

b. **Guideline statement 10:** Patients who respond to induction intravesical chemotherapy should be offered maintenance therapy for 1 year **[LOE 2; GOR C]**.

c. **Discussion:** Patients who respond to induction BCG still have a significant risk for recurrence and progression.[332,345] After an induction course of BCG, the immune response in the bladder will wane after 3 to 6 months, thus providing the rationale for repeated instillations.[333]

There are two randomized trials that demonstrated the importance of maintenance BCG and have influenced how maintenance therapy is administered. First, Southwest Oncology Group (SWOG) 8507 randomized 550 patients with high-risk NMIBC and no evidence of disease after induction BCG to observation alone or maintenance BCG.[345] Maintenance BCG was given as full-dose BCG weekly for 3 weeks at months 3, 6, 12, 18, 24, 30, and 36. This maintenance schedule was based on prior research demonstrating that the optimal immune stimulation from BCG occurs after 3 weeks.[356] Patients in the maintenance arm had a higher 5-year RFS (60% vs. 41%, p<0.0001) than patients in the observation arm, although only 16% of the maintenance arm received all 8 scheduled courses.

The second trial was EORTC 30962, which was designed to determine if lower BCG doses or a shorter maintenance duration were as effective as full-strength BCG and maintenance for 3 years.[357] This trial randomized 1,355 patients with NMIBC to one-third dose BCG with 1 year of maintenance, full-dose BCG with 1 year of maintenance, one-third dose BCG with 3 years of maintenance, and full-dose BCG with 3 years of maintenance. Approximately 40% of these patients were high-risk and nearly 50% did not complete their maintenance treatment. This was rarely due to toxicity, but predominantly due to recurrent disease. Compared to 1 year of maintenance therapy, the 5-year disease-free rate was higher for 3 years of maintenance therapy (63.4% vs. 56.6%), but this difference was not statistically significant (p=0.059). However, on sub-group analysis, high-risk patients treated with 3 years of full-dose maintenance BCG had a significantly higher DFS compared to 1 year of full-dose maintenance therapy (HR 1.61, 95% CI: 1.13–2.30, p=0.0087). There was no significant increase in toxicity with 3 years of maintenance compared with 1 year.[358] Finally, the meta-analyses examining the effectiveness of intravesical BCG versus chemotherapy found that BCG was superior only if maintenance therapy was used.[330,332,347,351,359]

There have been several negative trials on maintenance BCG, which used a variety of different maintenance schedules, such as quarterly, monthly, and repeated 6-week instillations.[342,360-363] While it is possible that some of these trials were too small to demonstrate a benefit or that no benefit exists, it may be that the SWOG maintenance schedule is required for effectiveness.

Although there is some controversy about the relative benefit,[342,364,365] there is level 1 evidence to support the use of maintenance BCG for reducing the risk for intravesical recurrence; this practice is also recommended by several professional guidelines.[268-270] Patients with high-risk NMIBC who respond to induction BCG should be offered 3 years of full-dose maintenance BCG in accordance with the SWOG schedule.

Intravesical chemotherapy is not the recommended first-line treatment for high-risk NMIBC, but if it is utilized and patients have a CR, then maintenance therapy should be offered. Many of the randomized trials that observed a benefit to intravesical chemotherapy included maintenance therapy for up to 1 to 2 years.[366-369] There have been several randomized trials that have specifically examined the effectiveness of maintenance intravesical chemotherapy.[370-372] Among patients with intermediate- or high-risk NMIBC who respond to a 6-week intravesical induction course of MMC, those treated with

maintenance therapy for up to 3 years had a significantly higher RFS compared to patients treated with observation only (69% vs. 86%; HR, 0.38; 95% CI, 0.21–0.69; p=0.0012).[370] Another randomized trial observed that compared to induction intravesical epirubicin followed by observation, induction therapy followed by maintenance had a significantly higher RFS (85% vs. 64%; HR, 0.39; 95% CI, 0.18–0.82; p=0.138).[372] While the benefit of maintenance chemotherapy has not been consistently demonstrated,[373-379] it is generally a well-tolerated treatment that may be beneficial and therefore should be considered.

7. Is dose reduction appropriate for patients who experience BCG toxicity?

a. **Guideline statement 11:** For patients who have intolerable side effects from BCG, dose reduction may be considered **[LOE 2; GOR B]**.

b. **Discussion:** The majority of patients treated with BCG will experience local toxicity, such as cystitis, hematuria, and dysuria. While these can often be treated symptomatically (refer to the section of this guideline on management of intravesical complications), some patients may be unable to complete their treatment.[357,358,380] As a result, dose-reduction for induction therapy has been considered as a means of reducing toxicity and improving compliance without compromising effectiveness.

Based on trial data from the CUETO group, one-third dose BCG has been shown to have similar effectiveness to full-dose BCG and with fewer side effects.[381] Although high-risk tumours were more likely to recur with reduced dose BCG (37% vs. 30%), this difference was not statistically significant (p=0.14). A complementary trial randomized patients with high-risk NMIBC to full dose and one-third dose BCG and similarly found a higher risk for recurrence with reduced-dose BCG (39% vs. 45%), but this difference was not statistically significant (p=0.4).[382] Another CUETO trial randomized patients with intermediate-risk NMIBC to one-sixth dose BCG, one-third dose BCG, and MMC, and found that both MMC and one-sixth dose BCG were less effective than one-third dose BCG, and with similar toxicity between both BCG arms.[383] Therefore, one-third dose BCG appears to be the minimum effective dose.

EORTC 30962 examined treatment efficacy and side effects between one-third dose and full-dose BCG.[357,358] For the efficacy analysis, the 5-year DFS for the full-dose and one-third dose arms was 59% and 62%, respectively, which was not significantly different (p=0.092).[357] Interestingly, the side effects between full-dose and one-third dose BCG were similar. Over 60% of patients experienced local side effects and 30% had systemic side effects, and dose reduction did not appear to improve the ability to tolerate treatment. Therefore, while a dose as low as one-third may have similar effectiveness as full dose, it may not have a more favourable toxicity profile.

While several trials support the effectiveness of reduced-dose BCG, there are conflicting data. A randomized trial was unable to demonstrate noninferiority of half-dose BCG, but did observe that it was associated with fewer side effects.[384] A CUETO trial suggested a possible benefit to full-dose BCG for patients with multifocal and high-grade tumours,[381] and EORTC 30962 observed that full-dose BCG with 3 years of maintenance was the optimal treatment for high-risk patients on subgroup analysis.[357] Others have shown that BCG dose is an important factor in treatment effect[385,386] and that lower doses of BCG may be associated with a higher risk for recurrence.[359] Therefore, full-dose BCG is recommended for

high-risk tumours; however, dose reduction to as low as one-third strength is an option, albeit with uncertain toxicity advantages.[357] It is currently unknown if dose-reduction during maintenance therapy is a reasonable alternative for patients who experience severe symptoms with full-strength induction therapy.

8. How is BCG failure defined?

a. **Guideline statement 12:** BCG-refractory NMIBC is either 1) disease progression after one induction cycle of BCG, or 2) persistent or worsening disease after two induction cycles or one induction cycle and one maintenance dose **[LOE 3; GOR B]**.

b. **Guideline statement 13:** BCG-unresponsive NMIBC includes both BCG refractory and BCG relapsing high-risk disease within 6 months of last BCG exposure **[LOE 4; GOR C]**.

c. **Discussion:** There has historically been significant heterogeneity in how response, or lack of response, to BCG was defined. Such definitions are essential to identify patients at highest risk for progression and in whom additional BCG can be given, as well as to improve clinical trial study design.[387,388]

Patients with **BCG-refractory** NMIBC have progressive disease after a single induction cycle (at 3 months) or persistent or progressive disease after two induction cycles or an induction cycle and a 3-week maintenance dose (at 6 months).[360] Patients should have been exposed to adequate BCG during this period, which has been defined as at least five of six induction doses and at least two of three maintenance doses.[388] Patients who do not respond to two induction cycles have a high rate of progression, and additional BCG should not be administered given low response rates.[389] While they do not strictly meet the definition of BCG refractory, patients with recurrent T1 disease after induction BCG have a very high likelihood of progression and may behave similarly to BCG-refractory NMIBC.[390]

Patients with **BCG-relapsing** NMIBC have a CR to BCG by 6 months, and then recur following a disease-free period. Although most patients who relapse after BCG will still have high-risk disease, each recurrence should be risk stratified and treated according to the risk category at the time of recurrence.[391] BCG relapsing disease generally has a better prognosis than BCG refractory disease.[392] Still, patients who relapse **early** (less than 12 months from last BCG exposure) tend to have a more aggressive disease course compared to those who relapse **late** (more than 12 months from last BCG exposure).[393,394] Patients who recur more than 12 months from their last BCG treatment may be offered an additional BCG induction course, while patients with early recurrences may be less likely to benefit from additional BCG.[388] **BCG-unresponsive** NMIBC includes both very early relapsers within 6 to 9 months of BCG exposure and BCG-refractory patients.[387,395] This definition is particularly relevant for clinical trial design.

Some patients who have residual tumour or a positive cytology after induction BCG will ultimately have a CR and are considered **BCG resistant**. Up to 50% to 70% of patients with persistent disease at 3 months after a BCG induction course will have no evidence of disease at 6 months with or without additional BCG.[344,345,360] Although persistent disease at 3 months is an adverse prognostic factor, disease status at 6 months is most predictive of long-term outcomes.[343,360,396-398] Because many patients with persistent disease

at 3 months can be converted to complete responders at 6 months, persistent, nonprogressive disease at 3 months is not equivalent to BCG failure.

Some patients are unable to complete induction therapy due to severe symptoms and are considered **BCG intolerant**. At this time, there is no common definition of BCG intolerance, which presents problems for clinical trial design and enrolment. Around 50% of patients will experience local or systemic symptoms during BCG induction, but very few are unable to complete their treatments.[358] Most patients with bothersome symptoms from BCG can be managed with analgesics, anticholinergics, treatment interruptions, or dose-reduction and are able to complete induction therapy. Patients who have severe symptoms but have not had efforts to manage these symptoms should not be considered BCG intolerant, with the exception of those who experience BCG sepsis. Based on expert opinion, patients should have attempted at least 3 induction treatments with every effort to manage symptoms prior to being considered BCG intolerant. Objective measures of symptom severity may also be needed to further improve the definition of BCG intolerance. At this time, patients who are truly BCG intolerant may be treated with intravesical chemotherapy or entered in a clinical trial. These definitions have demonstrated clinically relevant differences and have improved selection criteria for clinical trials.[392,399,400]

9. How do advanced diagnostic tests impact the definition of BCG failure?

a. **Guideline statement 14:** Further research is required to determine how the increased sensitivity of enhanced cystoscopy or urinary FISH impacts the definition of BCG response **[LOE 4; GOR D]**.

b. **Discussion:** Enhanced cystoscopic techniques, such as fluorescence cystoscopy and NBI, allow for improved detection and decreased risk for recurrence of NMIBC.[280-282,401] As emphasized in Guideline statement 1, a thorough and complete TURBT is critical for optimal treatment response with intravesical therapy. It is likely that some patients fail BCG due to incomplete resection, and are more surgically refractory than BCG refractory. Therefore, it is uncertain how the definition of BCG refractory should incorporate the use of enhanced cystoscopic techniques that increase the sensitivity for disease detection.[281] For instance, has a patient failed intravesical BCG if they did not have a complete resection using enhanced cystoscopy prior to starting treatment? If a patient has recurrent disease at 6 months after BCG induction that is detected only on fluorescence cystoscopy but not WLC, are they considered BCG refractory? Further research is needed to determine the definition of BCG responsiveness and how enhanced cystoscopy should be integrated into the management of patients treated with intravesical therapy.

Similarly, it is uncertain how new urinary biomarkers should be incorporated into response to intravesical therapy. Urinary FISH is one such marker that uses fluorescent probes to detect cellular chromosomal alterations consistent with urothelial carcinoma in voided urine samples. It has been most extensively studied for surveillance of patients with a history of bladder cancer, but recently has been investigated as a marker of response to BCG. Among 126 patients treated with BCG for NMIBC, patients with a positive urinary FISH at 3 months after the start of induction BCG who did not have evidence of clinical recurrence were significantly more likely to recur (58% vs. 15%) and progress (25% vs. 7%) compared to patients with a negative FISH.[402] Thus, urinary FISH may help identify patients at high risk for

BCG failure before an abnormal cystoscopy or cytology. Based on this finding, a patient with no clinical evidence of viable cancer but who has a positive urinary FISH at 3 months after the initiation of BCG induction have been described as having a "molecular BCG failure."[403] Such patients may be eligible for clinical trials for salvage intravesical therapies, similar to those with BCG unresponsive NMIBC. Further research is needed to determine the utility of urine FISH in evaluating BCG response and clinical trial eligibility.

10. What are the treatment options for BCG-refractory high-risk NMIBC?

a. **Guideline statement 15:** RC is the gold-standard treatment for patients who have BCG-refractory high-risk NMIBC **[LOE 3; GOR C]**.

b. **Guideline statement 16:** Patients with BCG-refractory high-risk NMIBC who are unfit for or refuse cystectomy should be offered a clinical trial of salvage intravesical therapy **[LOE 4; GOR C]**.

c. **Guideline statement 17:** Patients with BCG-refractory high-risk NMIBC who are unfit for or refuse cystectomy for whom a clinical trial is unavailable may be offered salvage intravesical chemotherapy or immunotherapy **[LOE 2; GOR C]**.

d. **Discussion:** Patients with BCG-refractory NMIBC are at significant risk for progression and metastasis, and should be offered an RC.[404,405] Although cystectomy is overtreatment for some patients, currently available intravesical salvage therapies have had limited effectiveness, and there is a pressing need to identify more effective salvage options. Patients with BCG-refractory high-risk NMIBC who are unfit for or unwilling to have cystectomy should be referred to centres with clinical trials in this area. There are multiple ongoing clinical trials evaluating novel treatments, including systemic immunotherapies with or without intravesical agents, for BCG-unresponsive NMIBC.[406]

If a clinical trial is not available, there is a variety of intravesical chemotherapies that have been tested as salvage agents, including valrubicin, docetaxel, paclitaxel, and gemcitabine.[407-411] At this time, valrubicin is the only approved treatment for BCG-refractory CIS in the United States. This drug was approved based on a phase 2 trial of 90 patients with recurrent CIS despite at least one course of BCG.[411] Patients had a CR rate of approximately 20%, but only 8% were disease-free at 2 years. Other intravesical chemotherapies have similar effectiveness, with CRs of less than 50% and at least 80% of patients recurring within 2 to 3 years.[407-410]

Other immunotherapies have also been investigated in this setting, namely IFN and mycobacterial cell wall nucleic acid complex (MCNA). Combination BCG and IFN alpha-2B was tested in a phase 2 trial of patients with NMIBC, of whom 46% (N=467) had previously failed BCG.[412] Most BCG failures were either BCG refractory or relapsing within 12 months. At 2 years, 45% of BCG failure patients were free of disease, and only 34% of BCG-refractory patients.[393] MCNA is an immunomodulatory and cytotoxic agent derived from *Mycobacterium phlei* that has been studied in patients with BCG-refractory NMIBC. A phase 2 study treated 129 patients with BCG-refractory or relapsing high-risk NMIBC with induction and maintenance MCNA.[413] The 1-year DFS was 25%, but many of those who responded at 1 year had a durable response.

In general, most intravesical therapies that have been studied in the salvage setting have demonstrated modest short-term and poor long-term response rates. At this time, there is no clear first-line salvage therapy. Patients with BCG-refractory disease may also benefit

from device-assisted therapy, if available. Additional research is needed to determine the effectiveness of device-assisted therapy for BCG-refractory NMIBC. Refer to the section 5.10 on "Role of Alternative Therapies" of this Guideline for additional information.

11. What are the treatment options for BCG-relapsing high-risk NMIBC?

a. **Guideline statement 18:** Patients with early relapsing high-risk NMIBC within 12 months of induction therapy plus maintenance or two induction courses should be managed as BCG-refractory NMIBC **[LOE 3; GOR C]**.

b. **Guideline statement 19:** Patients with late-relapsing high-risk NMIBC after 12 months from induction therapy may be offered additional intravesical therapy **[LOE 3; GOR C]**.

c. **Discussion:** The timing of relapse from the most recent BCG exposure is an important predictor of overall prognosis and response to additional BCG for patients who experience a high-risk recurrence after induction BCG.[396,414] Some patients with relapsing high-risk NMIBC can be managed with additional induction BCG.[415] Based on a large phase 2 study of BCG and IFN, patients who failed BCG but relapsed more than 12 months from their most recent BCG exposure had similar response to intravesical treatment as patients who were BCG naïve.[393] Therefore, patients who relapse over 12 months from their last BCG treatment may be offered additional BCG. Conversely, patients with an early relapse should be managed more aggressively.[387]

An important caveat in the management of patients with an early relapse is that they should have been exposed to sufficient BCG.[388] Patients treated with a single induction course and no maintenance who relapse within 12 months may be considered for additional BCG. While patients who respond to induction BCG and experience a late relapse can be treated with repeat induction therapy, further research is needed to determine if patients who respond to induction BCG and relapse while on maintenance therapy should be managed similarly. Disease of patients who relapse while on maintenance therapy may behave more like BCG-refractory disease and may be better served with RC, a clinical trial, or intravesical salvage therapy.[388]

In addition to categorizing relapses according to time since last BCG, patients should also be categorized and treated according to their risk group at the time of relapse. While patients treated with BCG who experience a relapse of high-risk NMIBC require additional BCG, salvage intravesical therapy, or RC, patients treated with BCG who relapse with low-grade tumours have a favourable prognosis and can continue BCG treatment.[391] Patients with low-risk relapses should not be considered BCG refractory, as their prognosis is favourable.

12. When should upper-tract or prostatic urethral biopsies be considered for patients who fail BCG?

a. **Guideline statement 20:** Patients with a persistently positive cytology after intravesical therapy with no evidence of intravesical recurrence should have prostatic urethral biopsies and an upper urinary tract evaluation **[LOE 3; GOR C]**.

b. **Discussion:** The bladder, upper urinary tract, and prostatic urethra are all lined by urothelium. Based upon the field-defect hypothesis of urothelial carcinoma and its propensity for multifocality, patients with urothelial carcinoma of the bladder are at risk for involvement of the upper tracts and prostatic urethra. At the time of diagnosis, 2% to 4% of patients with high-risk NMIBC will have synchronous tumours of the upper tracts,[416,417] and over 20% of patients will develop upper-tract disease over time.[417,418] While prostatic urethral biopsies are not routinely performed on all patients with newly diagnosed high-risk NMIBC, between 10% and 25% are found to have involvement of the prostatic urethra.[272,419] Although urothelial carcinoma can invade the prostatic ducts, acini, and stroma, synchronous or recurrent prostatic urethral involvement is most commonly in the form of CIS. Prostatic urethral involvement significantly increases the risk for recurrence and progression for patients treated with BCG, and is an adverse prognostic factor.[272,420] Patients with noninvasive prostatic urethral involvement should have an aggressive TURP prior to being given additional intravesical therapy. Refer to the section 5.7.2 on "Management of prostatic urethral involvement" of this Guideline for additional information.

The upper urinary tract and prostatic urethra are common sites of recurrence for patients with high-risk NMIBC after treatment with intravesical therapy. Among patients treated with BCG who relapse, up to 30% will experience a noninvasive prostatic urethral recurrence, and as many as 25% will experience an upper-tract recurrence.[418,421] Among a selected group of 110 patients who experienced BCG failure, over half recurred in the upper tracts or prostatic urethra.[422] Extravesical recurrence is more common in patients with bladder CIS at the time of diagnosis.

The upper urinary tract and prostatic urethra may be sites of incompletely resected and untreated disease leading to a persistently positive urine cytology. Patients who have a persistently positive urine cytology after intravesical therapy but no evidence of disease in the bladder after treatment with BCG should have biopsies of the prostatic urethra and an upper-tract evaluation.[422-424] If cross-sectional upper-tract imaging with CT or MR urography does not identify an upper-tract lesion, selective upper-tract cytology and ureteroscopy may be required.

5.5.3 Conclusion

High-risk NMIBC has a significant risk for recurrence and progression. Several tumour characteristics are associated with disease aggressiveness, including size, multifocality, variant histology, LVI, extent of lamina propria invasion, and associated CIS. The foundation of effective treatment for high-risk NMIBC is a thorough and complete TURBT. Patients with high-risk NMIBC should be treated with adjuvant intravesical BCG and up to 3 years of maintenance therapy. Patients who progress during induction BCG or do not respond by 6 months may require RC, salvage intravesical chemotherapy, or enrolment in a clinical trial.

Summary of Recommendations

Recommendation	LOE	GOR
A thorough examination should be performed on all patients with bladder tumours at the time of TURBT, including a bimanual exam and cystoscopic assessment of tumour characteristics.	2	B
A complete TURBT should be performed on all patients with bladder tumours when safe and feasible and when bladder preservation is planned.	3	C
A repeat TURBT should be performed within 6 weeks of initial resection for all patients with an incomplete initial resection and for patients with T1 high-risk disease after a complete initial resection.	2	B
A repeat TURBT should be considered within 6 weeks of a complete initial resection for patients with high-grade Ta tumours, particularly for patients with large or multifocal tumours.	3	C
Patients with high-risk NMIBC who are treated with intravesical BCG do not benefit from an immediate dose of intravesical chemotherapy.	2	B
Patients with variant histology, LVI, or deeply invasive T1 tumours are at an increased risk for progression and may not be candidates for bladder-sparing treatments.	3	C
Patients with high-risk NMIBC for whom bladder sparing is desired should be offered induction intravesical BCG after a complete TURBT.	1	A
Patients with high-risk NMIBC for whom bladder sparing is desired who are ineligible to receive intravesical BCG may be offered induction intravesical chemotherapy after a complete TURBT.	2	B
Patients who respond to induction intravesical BCG should be offered up to 3 years of maintenance.	2	B
Patients who respond to induction intravesical chemotherapy should be offered maintenance therapy for 1 year.	2	C
For patients who have intolerable side effects from BCG, dose reduction may be considered.	2	B
BCG-refractory NMIBC is either 1) disease progression after one induction cycle of BCG, or 2) persistent or worsening disease after two induction cycles or one induction cycle and one maintenance dose.	3	B
BCG-unresponsive NMIBC includes both BCG refractory and BCG relapsing high-risk disease within 6 months of last BCG exposure	4	C
Further research is required to determine how the increased sensitivity of enhanced cystoscopy or urinary FISH impacts the definition of BCG response.	4	D
RC is the gold-standard treatment for patients who have BCG-refractory high-risk NMIBC.	3	C
Patients with BCG-refractory high-risk NMIBC who are unfit for or refuse cystectomy should be offered a clinical trial of salvage intravesical therapy.	4	C
Patients with BCG-refractory high-risk NMIBC who are unfit for or refuse cystectomy for whom a clinical trial is unavailable may be offered salvage intravesical chemotherapy or immunotherapy.	2	C
Patients with early relapsing high-risk NMIBC within 12 months of induction therapy plus maintenance or two induction courses should be managed as BCG refractory NMIBC.	3	C
Patients with late relapsing high-risk NMIBC after 12 months from induction therapy may be offered additional intravesical therapy.	3	C
Patients with a persistently positive cytology after intravesical therapy with no evidence of intravesical recurrence should have prostatic urethral biopsies and an upper urinary tract evaluation.	3	C

5.6 Management of Positive Urine Cytology With Negative White-light Cystoscopy

The management of positive urine cytology with negative WLC involves 1) confirmation of urine cytology through a second opinion review or repeat cytology **[LOE 3]**, 2) utilization of PDD or fluorescent cystoscopy to identify occult lesions in the bladder **[LOE 2a]**, and 3) ruling out occult disease in the prostatic urethra and the upper urinary tracts **[LOE 2b]**.

Urine cytology can be falsely positive if it is obtained within a week following bladder resection due to cauterization artifact.[425] Similarly, acute inflammation from a urinary tract infection (UTI) (e.g. candida, bacteria, or virus) or stone may occasionally be misread as being positive for malignant cells. However, positive cytology after recent bladder resection should not be ignored, as it has been shown that, if elevated in the first 3 days after resection, it may be associated with increased risk for tumour recurrence.[426] Therefore, it may be helpful to have the urine cytology reviewed by another cytopathologist to confirm the presence of malignant cells but, unfortunately, urine cytology is plagued by poor inter- and intraobserver concordance among pathologists, even within those with specialist training in cytopathology.[427] However, most of the discordance is seen when interpreting an atypical cytology rather than one that is unequivocally positive. If the original cytology was from a voided specimen, a bladder barbotage specimen that has a higher reported specificity than the interpretation of a voided sample may be collected.[428]

After confirming the positive cytology, the next step is to look for occult disease in the bladder, upper tracts, and the prostatic urethra in men. PDD using hexaminolevulinate (fluorescent cystoscopy) can be useful to identify occult sites of disease in the bladder, and complement random bladder biopsies. In a study of 348 patients with a negative WLC, 77 patients with a positive urine cytology were further investigated with fluorescent cystoscopy.[429] Fluorescent cystoscopy identified bladder pathology in 63 (82%), which included 18 with moderate dysplasia, 27 with CIS, and 18 with pTa-T1/G1–G3 tumours. Thus, fluorescent cystoscopy can be informative in the setting of a positive cytology with negative WLC.

It is also critical to rule out extravesical sources of the positive cytology, especially if fluorescent cystoscopy fails to identify an occult bladder lesion. The precollicular area of the prostatic urethra (5 o'clock and 7 o'clock positions) should be sampled to rule out involvement of the prostatic ducts and stroma.[430] Upper urinary tract tumours can be identified by a site-specific cytology or barbotage[431] but, in general, urine cytology has poor sensitivity for detecting upper urinary tract tumours.[432] Instrumented specimens of the upper urinary tract are at risk for contamination from the bladder and therefore CTU prior to cystoscopy is recommended, as it has greater than 95% sensitivity and specificity for the diagnosis of upper urinary tract tumours.[433] If cytology remains positive despite negative upper-tract imaging and cancer is not detected in the prostatic urethra or bladder, diagnostic

ureteroscopy and renoscopy should be considered. Suspicious lesions can be biopsied. Selective cytology may be obtained directly from renal pelvis by aspiration of fluid through the ureteroscope, but, again, possible contamination from the bladder remains a concern.

If the workup is negative, patients with a positive cytology and an initial negative WLC need to be informed that they have a 76% chance of developing bladder cancer within 1 year[434] and that intense surveillance is required for at least the next year. UroVysion/FISH is a consideration, but it is plagued by anticipatory positive results. Therefore, this test must be ordered with a full understanding of its implications.

A nice algorithm for management of positive voided urine cytology was recently suggested, and we have modified it as shown below.[435] The role of random bladder biopsies and selective ureteral cytologies are prone to subjectivity in the former and contamination in the latter, and so these are left to the discretion of the treating urologist.

Summary of Recommendations

1. Upper-tract imaging (CTU or IV pyelogram and renal ultrasound, MRI) and cystoscopy **[GOR C]**
    a. If findings are negative, perform cytological review and/or repeat cytology with bladder barbotage
2. Cytological review and/or repeat cytology **[LOE 3]**
    a. If positive, consider fluorescent cystoscopy
3. Fluorescent cystoscopy **[LOE 2a]**
    a. If positive, biopsy abnormal areas
    b. If negative, consider prostatic urethral biopsies at the same time **[LOE2b]**
4. Bilateral retrograde ureteropyelograms with bilateral ureteroscopy, biopsies, and selective urine sampling **[GOR C]**.
5. Repeat cytology in 3 to 12 months if no abnormality found **[GOR D]**.

5.7 Indications of Bladder and Prostatic Urethral Biopsies and Management of Prostatic Urethral Involvement

5.7.1 Indications of bladder and prostatic urethral biopsies

5.7.1.1 Role of bladder biopsies in newly diagnosed or suspected bladder cancer

The initial evaluation and management for patients with suspected bladder cancer involves TUR of visible tumour and an assessment of the entire bladder and urethra for the presence of concomitant CIS. CIS left undetected and untreated can result in early tumour recurrence and progression to muscle-invasive disease.[436] Since it is often difficult to distinguish CIS from benign inflammatory changes, cold-cup or TUR biopsies should be performed on abnormal-appearing areas within the bladder or urethra **[LOE 3]**. Unfortunately, CIS might not be visible on cystoscopy and present as cytology positive–only disease.[437] The use of enhanced cystoscopy may help overcome some of the challenges clinicians face with a positive cytology in the setting of a negative WLC, and is discussed at length elsewhere in this book[438] **[LOE 3]**.

The use of random biopsies of normal-appearing urothelium to detect occult CIS is controversial. In an analysis of 393 patients with low-risk Ta-T1 tumours in EORTC trial 30863 who underwent a random biopsy of normal-appearing urothelium, only 6 patients (1.5%) were found to have CIS.[439] The authors of that study also evaluated 602 patients with intermediate- and high-risk Ta-T1 tumours on EORTC trial 30911 who had multiple random biopsies taken from normal-appearing urothelium, and only 21 patients (3.5%) were found to have CIS.[439] While the incidence of occult CIS is relatively low overall in patients with newly diagnosed bladder cancer, numerous reports show that the risk for detecting occult CIS increases in the presence of high-risk tumour and positive cytology.[436,440] This suggests a possible role for random biopsies in patients at highest risk of harboring concomitant CIS[436] **[LOE 3]**. In a retrospective study of 173 patients with newly diagnosed NMIBC undergoing random biopsies, concomitant CIS was seen in 1 (12.5%) of 8 low-risk, 18 (24.7%) of 73 intermediate-risk, and 41 (59.4%) of 69 high-risk cases in normal-appearing urothelium.[440] A positive cytology had the strongest association with concomitant CIS on multivariable analysis in that study.[440] Several other studies have also supported random biopsies of normal-appearing mucosa when cytology is positive[441,442] **[LOE 3]**. In a study of 234 consecutive patients undergoing random biopsies at time of initial bladder cancer diagnosis or at first relapse, occult concomitant CIS was identified in 34 (14.5%). In that study, a positive cytology before TUR had a sensitivity of 50.0%, specificity of 91.7%, positive predictive value of 56.8%, and negative predictive value of 89.3% for predicting CIS on the random biopsies.[442]

Multifocal disease is also a risk factor for concomitant CIS **[LOE 3]**. In a study assessing the results of random biopsies on normal-appearing mucosa of 100 bladder cancer patients, no concomitant CIS was detected in 72 patients with solitary tumour, pedunculated tumours, or negative urinary cytology, but occult concomitant CIS was found in 5 of 28 (17.9%) patients with multiple broad-based tumours, and three additional high-grade Ta tumours were found.[443] In a large study that excluded patients with small, primary, solitary tumours and focused on performing multiple random biopsies only in high-risk NMIBC patients, occult urothelial carcinoma was identified in 126 (12.4%) of 1,033 consecutive patients, including CIS in 74, Ta in 41, T1 in 12, and T2 in 1.[444] Due to random biopsies, 70 (6.8%) patients had a change in treatment **[LOE 3]**.[444] Treatment changes included a switch from intravesical chemotherapy to BCG in 45, performing a restaging TUR in 48, and RC in 15 patients.[444] Despite random biopsies potentially changing management for some patients, random biopsies have not improved recurrence or progression rates.[445-447] **[LOE 3]**

5.7.1.2 Role of bladder biopsies after prior treatment

The role of biopsies on follow-up surveillance cystoscopy and after intravesical therapy is also controversial. Prior TUR and intravesical therapy can result in developing areas of increased vascularity or erythema changes that appear to be CIS but are only inflammatory changes.[448] Thus, there can be a high false-positive biopsy rate in previously treated bladders.[448,449] In a meta-analysis of 740 patients from seven studies of biopsies after BCG, positive biopsies were found in 73 of 107 (68%) suspected tumours, 20 of 125 (16%) erythematous lesions, and 44 of 738 (6%) random biopsies of normal mucosa.[449] The predictive value of positive urinary cytology for a positive biopsy was 70%.[449] The positive predictive value for the combination of a positive cytology and an erythematous lesion was 56%, and for positive cytology and suspected tumour it was 89%.[449] The combination of negative cytology and normal cystoscopy was associated with negative biopsy in 94% of cases.[449] The authors of that meta-analysis argued against routine TUR biopsies after BCG, instead recommending a tailored approach[449] **[LOE 2]**.

Given the profound implications of persistent CIS after intravesical therapy, biopsy of any suspicious lesion for cancer is certainly justifiable; however, in the absence of a positive cytology there is little evidence to support random biopsies of normal-appearing mucosa or even of all erythematous-only changes **[LOE 3]**. The use of enhanced cystoscopy techniques may be more helpful than random biopsies in the routine assessment of response to intravesical therapy treatment **[LOE 3]**. For example, hexylaminolevulinate fluorescence blue light cystoscopy (BLC) has been reported to detect clinically significant occult disease in 6% (2/32) patients after BCG, albeit with a 63% false-positive rate.[450,451] Restricting biopsies to only patients with a positive cytology likely improves the specificity of BLC.[450,451] Given the high rates of false-positive rates, it is recommended to wait at least 6 weeks from completing BCG to perform BLC. This might not be needed after intravesical mitomycin treatment, as the specificity of BLC does not appear to be effected.[450,452]

5.7.2 Management of prostatic urethral involvement

5.7.2.1 Staging and incidence

The prostatic urethra and ductal system are lined by the same transitional cell urothelium as the bladder and are exposed to the same carcinogens. Isolated urothelial carcinoma of the prostate (UCP) is rare, as UCP is usually associated with multifocal disease, most commonly with tumours in the bladder.[453] Unlike the bladder, there is no muscularis propria layer in the prostate, so the urothelial lining of the prostatic urethra, ducts, and terminal acini are separated from the prostatic stroma by only a thin basement membrane. Prior to 2010, the AJCC staging system had any prostate involvement classified as tumour stage T4 disease. This has since changed into two separate AJCC TMN staging systems to reflect the different mechanisms of prostatic involvement that are associated with differing prognoses[454-457] **[LOE 3]**. The most common mechanism of UCP is a Pagetoid transurethral spread via the mucosa lining of the prostatic urethra (Ta/T1, Tis pu), the prostatic ducts (Tis pd), or invading into the prostate stroma from the prostatic urethra (PrT2). The AJCC staging system now reserves tumour stage T4a disease for true bladder primary tumours invading the prostatic stroma through direct extension.[457]

The true incidence of UCP is unknown, as most data are from cystoprostatectomy specimens that are biased toward MIBC and treatment-refractory NMIBC **[LOE 3]**. UCP incidence rates vary by detection method used (cystoscopic visualization, cold-cup biopsy, TUR, or cystoprostatectomy).

Even the degree to which a cystoprostatectomy specimen is evaluated can alter the reported rates of prostatic stromal invasion from 21% to 36%, and prostatic urethral CIS from 12% to 28%.[458,459] UCP incidence also varies by the clinical state (initial diagnosis, after intravesical treatment, positive cytology with negative cystoscopy, etc) and by the presence of risk factors.

Integrating UCP into the treatment and management of a patient with bladder cancer can often result in confusion for some clinicians, but UCP can have profound implications for management. Prostatic urethral CIS is a major risk factor for recurrence, progression, and death in high-risk NMIBC patients[436] **[LOE 3]**. The prostatic urethra is also a common site of relapse after NMIBC treatment[460-462] and failing to intervene before prostatic stromal invasion occurs can be fatal[460,463] **[LOE 3]**. Thus, the risk for UCP needs to be assessed in all patients at initial diagnosis and throughout follow-up, particularly in the setting of recurrent high-grade disease[460] **[LOE 3]**.

5.7.2.2 Role of prostatic urethral biopsies in newly diagnosed bladder cancer

For patients with newly diagnosed bladder cancer, the incidence of UCP has been reported to be as high as 11.7% in primary HGT1,[436] but there are sparse data on the role random biopsies have in newly diagnosed bladder cancer patients when their prostatic urethral mucosa appears normal. In one series that routinely performed random cold-cup biopsies in newly diagnosed NMIBC, UCP was reported to occur in 21 (6.2%) of 340 male patients on cystoscopy, of which 12 (3.5%) had visible disease and 9 (2.7%) tumours were found on random biopsy of visibly normal-appearing prostatic urethral urothelium.[464] In that series, UCP was associated with multiple bladder tumours, as well as higher grade and stage.[464] Additional risk factors for UCP include multiple bladder tumours, multifocal bladder CIS, and trigonal/bladder neck tumours.[459,465,466] In a study comparing cystoscopic tumour

characteristics to subsequent radical cystoprostatectomy specimens, the authors reported that 31.3% of patients with bladder CIS had UCP, while only 4.5% without bladder CIS had UCP.[465] Similarly, 34.7% with multifocal tumours had concomitant UCP, while only 4.2% with solitary tumour had UCP.[465] Yet, UCP was grossly visible for the vast majority of these cases.[465] So while careful inspection of the entire urethra is always necessary on cystoscopy, random prostatic urethral biopsies of normal mucosa are usually not indicated in newly diagnosed bladder cancer **[LOE 3]**.

5.7.2.3 Role of prostatic urethral biopsies after prior treatment

Over time, the risk for high-grade NMIBC patients developing secondary UCP increases to 10% to 15% at 5 years and up to 20% to 40% at 10 years.[460,461] In one study of 186 high-risk NMIBC patients, 72 (39%) developed UCP after a median follow-up of 28 months (range 3–216), including 45 (24%) with noninvasive prostatic tumour and 27 (14.5%) with stromal invasion.[460] In another study of NMIBC patients treated with one or more courses of BCG and followed for a minimum 10 years, 21 of 98 (21.4%) patients developed UCP.[461] Patients who experience multiple bladder tumour recurrences are also at increased risk of developing UCP.[466] In a retrospective study of 110 patients whom multiple courses of BCG had failed, 24 (21.8%) patients were found to have UCP.[462] The risk of developing stromal invasion significantly increases in NMIBC patients whose intravesical therapy has failed.[460,463,467]

Thus, urologists must be cognizant that the prostatic urethra is a common site of disease relapse[462] but, in the absence of bladder tumour recurrence or a positive cytology after intravesical treatment, there is no existing evidence to support randomly sampling the prostatic urethra on follow-up cystoscopy if mucosa appears normal **[LOE 3]**. If either recurrent NMIBC or positive cytologies do develop after intravesical therapy, there is a greater risk for ductal and stromal involvement than in newly diagnosed NMIBC patients[463] **[LOE 3]**. Since cold-cup biopsies under-sample the prostatic ducts and stroma,[468,469] TUR loop biopsies from bladder neck to proximal verumontanum should be considered over cold-cup biopsies **[LOE 3]**. Detecting stromal invasion on TUR biopsies might affect the decision for neoadjuvant chemotherapy prior to cystectomy[470] **[LOE 3]**.

5.7.2.4 Management of high-grade noninvasive disease of the prostatic urethral mucosa (Ta/T1, Tis pu) and involving the prostatic ducts (Tis pd)

Historical recommendations were radical cystoprostatectomy for UCP even if noninvasive prostatic disease was present[456,471] **[LOE 3]**. Since the prostatic urethra is an extravesical site of disease, there was concern that intravesical agents would be ineffective as they only make contact with the prostatic urothelium during voiding **[LOE 4]**. However, there are several retrospective studies supporting the use of bladder preservation options for prostatic urethral mucosal disease. Intravesical chemotherapy has been reported, but appears be less effective when compared to BCG in small nonrandomized series, so BCG has been the predominant agent used.[471,472] One series found 7 of 12 (58%) patients treated with BCG had CRs compared to only 2 of 7 (28.5%) treated with epirubicin for prostatic urethral disease.[472] In another small series, patients with prostatic urethral disease treated with either mitomycin or Adriamycin® achieved a CR rate of only 37.4%, with 22.2% of nonresponders progressing to prostatic stromal invasion.[473] Some have argued that, since contact with the prostatic urothelium is so limited, BCG has an advantage over chemotherapy in that it actively binds to cells.[472,474]

Yet there is concern that BCG does not adequately penetrate the prostate. Indirect evidence for the penetration of intravesical BCG therapy into the prostate comes from the presence of granulomatous prostatitis being found in 75% to 100% of post-BCG cystoprostatectomy specimens and prostate biopsies,[475,476] as well as a transient rise in PSA after BCG therapy.[477] Transient elevations in PSA levels are even greater if TURP is performed before BCG.[478] Many argue for TUR of the bladder neck and prostate prior to intravesical treatment to increase the chance of prostatic penetration **[LOE 3]**. Cystographic evaluation suggests that TUR of the bladder neck and prostate allows greater amounts of reflux into prostatic ducts.[471] Further support for performing TURP prior to intravesical treatment can be seen in a study by Donat *et al.* where 45% (36 of 80) of men who were found to have UCP on TUR biopsy had no evidence of UCP on cystoprostatectomy, suggesting that TUR alone might have therapeutic effects in limited disease.[479]

Intravesical BCG only without TURP has been reported to achieve CRs in 54% to 80% of select cases of prostatic urethral disease.[464,480-483] Gofrit *et al.* reported a CR in the prostatic urethra in 18 of 20 (90%) of their own patients with BCG after TURP.[482] When their data were combined with other published series, they report a CR in the prostatic urethra of 65.7% for BCG-only treated patients, which increased to 95.3% when TURP is performed prior to BCG.[482] However, they reported similar CR rates for BCG-only and BCG-plus TURP for the combined bladder and prostate urethral recurrence rates (46.5% and 51.8%, respectively).[482] TURP prior to BCG appears to help reduce overall tumour burden and improve contact with tumour cells, which are two important requirements for BCG effectiveness.[484,485]

Management of UCP involving the prostatic acini and ducts is controversial, as there are very limited data to guide management. Radical cystoprostatectomy should be strongly considered whenever there is extensive ductal involvement due to the risk of understaging and potentially lethal consequences for missing stromal invasion[479] **[LOE 3]**. Cystectomy outcomes for patients with Tis pd appear similar to Tis pu, but the relative numbers of patients in these series are small.[486,487] In one retrospective series, the 3-year survival rate for 29 patients with ductal involvement was 52% compared to 59% for 74 patients with Tis pu disease.[486] Survival dropped to 17% for the 21 patients with stromal invasion.[486]

The alternative to cystoprostatectomy for prostatic ductal involvement is attempted bladder preservation with TURP and BCG; however, there are limited data on this approach and results do not appear as promising as for prostatic urethral mucosa–only involvement **[LOE 3]**. Most data are from single-centre experiences on just a handful of patients. CRs to BCG and TURP have been reported in 4 of 7 (57%), 2 of 3 (66%), and 3 of 4 (75%) patients.[483,488-490] In a series of 10 patients with the prostatic ductal CIS treated with BCG with a mean follow-up of 40 months, 8 patients had CRs in their prostatic urethra and 2 patients underwent cystoprostatectomy for prostatic recurrence: one for recurrent ductal CIS and the other for progression to stromal invasion.[480] An additional patient died from metastatic disease 8 months after intravesical treatment without evidence of local recurrence.[480] So while bladder preservation with BCG and TURP for ductal CIS is possible, the evidence demonstrating safety is only from a few small series, and patients need to be monitored closely with cystoscopy, cytology, and urethral biopsies **[LOE 3]**.

There are also limited data on how to manage prostatic recurrences after BCG. Radical cystoprostatectomy with concurrent urethrectomy is considered the standard treatment and should be strongly considered for any recurrences in the prostatic urethra **[LOE 3]**. While TUR and another course of BCG have been reported, conservative management of prostatic recurrence should be approached cautiously, as the risk for stromal invasion is considerable[460,463,485] **[LOE 3]**.

5.7.2.5 Management of stromal invasion

Stromal invasion should be treated as MIBC with a poor prognosis and high rate of nodal metastasis. Primary bladder tumours invading into the prostatic stroma (T4) have a 5-year survival rate of 6% to 22% with nodal metastasis rates of at least 40% to 50%.[454-456,486,487,491] Noncontiguous stromal invasion arising from the prostatic urethra (stage PrT2) has a 5-year survival rate of 43% to 57% and node-positive disease in at least 22%.[454,455,487,491]

The high rate of nodal metastases might be due to the absence of a muscularis propria layer in the prostate, as a few series have reported the rate of nodal metastasis from stromal invasion to be as high as 74%[486,492] **[LOE 4]**. Nodal mapping studies in patients with stromal invasion have demonstrated involvement of multiple nodal sites, including the common iliac and presacral packets as the only site of disease in 7%.[493] The 5-year OS after cystectomy and extended lymph node dissection for pT4a disease is 44% if node negative and 26% if node positive.[494] Although still lacking level 1 evidence, extended lymph node dissection might be beneficial in the setting of stromal invasion **[LOE 4]**.

Radical cystoprostatectomy with or without concomitant urethrectomy is the preferred treatment for locoregional control **[LOE 3]**. Prostatic involvement is the most important risk factor for urethral recurrence following cystectomy.[495-497] The risk for urethral recurrence is less than 10% for prostatic urethral mucosa disease, 10% to 25% for ductal involvement, and as high as 30% to 67% for stromal invasion.[495-497] For patients with extensive prostatic urethral involvement, a concomitant urethrectomy at the time of RC is a reasonable consideration, given the recurrence risk in the defunctionalized urethral remnant. Alternatively, if urethrectomy is not performed at the time of RC and there's no overt distal urethral involvement, close surveillance of the retained urethra with regular examination and washings followed by delayed urethrectomy in those that recur can be considered. Most urethral recurrences identified in patients that undergo regular urethral surveillance are identified at the CIS, Ta, or T1 stages and should not affect long-term survival after delayed urethrectomy.[498]

Patients with localized disease who are able to receive cisplatin should be strongly considered for neoadjuvant chemotherapy based on level 1 evidence in MIBC, the high incidence of lymph node involvement, and the overall poor prognosis for stromal invasion[499,500] **[LOE 1]**. Neoadjuvant chemotherapy might provide as much as a 25% to 30% improved survival advantage over cystectomy alone for T4a disease at 5 years.[499,500] There are insufficient data on adjuvant chemotherapy in prostatic stromal invasion, as the numbers of patients have been limited in randomized trials.[501,502] A few retrospective studies have suggested adjuvant chemotherapy might be beneficial for stromal invasion.[503,504]

Data on chemoradiation for prostatic stromal invasion are lacking. A pooled analysis of six Radiation Therapy Oncology Group (RTOG) bladder-preservation studies included 468 patients treated from 1988 to 2007, but only 18 (3.9%) patients had T4a disease.[505] Similarly, only 28 (8.1%) of 348 treated with chemoradiation from 1986 to 2006 at a single high-volume centre had T4a disease.[506] It is not possible to draw any conclusions at this time regarding chemoradiation for UCP.

5.7.3 Conclusions and recommendation

Selective use of random biopsies may detect more occult CIS, particularly in certain high-risk groups **[LOE 3]**. However, random biopsies have not been shown to improve recurrence or progression outcomes and their impact on subsequent therapy decisions remains unclear **[LOE 3]**. Thus, random biopsies in newly diagnosed patients should be restricted to high-risk patients whose management might be altered by the detection of occult disease **[LOE 3]**.

The potential benefits of random biopsies after intravesical therapy needs to be weighted against the increased risk for false positive. Restricting biopsies to only those with positive cytology may be one strategy to improve specificity **[LOE 2]**. The use of enhanced cystoscopy techniques may be another way to improve the detection of occult disease compared to the use of random biopsies **[LOE 3]**.

It appears safe to initially manage high-grade noninvasive disease of the prostatic urethral mucosa (Ta/T1, Tis pu) with TURP and BCG **[LOE 3]**. More caution is needed for high-grade disease involving the prostatic ducts (Tis pd), but TURP and BCG can be considered if ductal involvement is properly staged and found to be limited to the superficial ducts without invasion **[LOE 3]**. Extensive ductal involvement or prostatic urethral recurrence after failed conservative treatment should be managed with radical cystoprostatectomy, preferably with concurrent urethrectomy **[LOE 3]**.

Patients with stromal invasion should be considered for cisplatin-based neoadjuvant chemotherapy followed by radical cystoprostatectomy, preferably with concurrent urethrectomy **[LOE 1]**. Extended pelvic lymph node dissection should also be strongly considered, given the rates of node-positive disease when stromal invasion is present **[LOE 4]**.

Summary of Recommendations

Recommendation	LOE	GOR
1. Indications of bladder and prostatic urethral biopsies		
Lesion directed cold-cup or TUR biopsies should be performed on abnormal-appearing areas within the bladder or urethra.	3	C
Random biopsies of normal-appearing mucosa can be considered in patients at high risk for concomitant CIS, such as a positive cytology, but in the absence of a positive cytology there is little evidence to support random biopsies.	3	C
The use of enhanced cystoscopy may help overcome some of the challenges clinicians face with a positive cytology in the setting of a negative WLC.	3	B
2. Management of prostatic urethral involvement		
The risk for UCP needs to be assessed in all patients at initial diagnosis and throughout follow-up, particularly in the setting of recurrent high-grade disease.	3	C
If either recurrent NMIBC or positive cytologies do develop after intravesical therapy, there is a greater risk for UCP with ductal and stromal involvement than in newly diagnosed NMIBC patients, so consideration of TUR loop biopsies over cold-cup biopsies should be made to avoid under-sampling the prostatic ducts and stroma.	3	C
Management of high-grade noninvasive disease of the prostatic urethral mucosa (Ta/T1, Tis pu) and involving the prostatic ducts (Tis pd):		
For conservative treatment of UCP, intravesical BCG appears superior to chemotherapy, and performing TUR of the bladder neck and prostate prior to starting intravesical treatment should be done to increase the likelihood of prostatic penetration and improve contact between BCG and tumour cells.	3	B
Radical cystoprostatectomy with concurrent urethrectomy should be considered the standard treatment for recurrent UCP after prior BCG.	3	C
Radical cystoprostatectomy should be strongly considered whenever there is extensive ductal involvement due to the risk of understaging and potentially lethal consequences for missing stromal invasion.	3	C
Management of stromal invasion:		
For patients with localized prostatic stromal invasion, radical cystoprostatectomy with or without concomitant urethrectomy is the preferred treatment for locoregional control.	3	C
An extended pelvic lymph node dissection at the time of cystectomy should be considered in the setting of stromal invasion, given the high incidence of lymph node involvement.	3	C
Patients with stromal invasion who are able to receive cisplatin should be strongly considered for neoadjuvant chemotherapy based on level 1 evidence in MIBC, the high incidence of lymph node involvement, and the overall poor prognosis for stromal invasion.	1	A

5.8 Impact of Bacillus Calmette-Guérin Strain and Host

Precise mechanisms underlying response to BCG are not fully elucidated. This has limited our ability to predict response to BCG. In addition, most studies assessing predictive markers were retrospective and included patients with different risk profiles, or insufficiently specified risk profiles, and different treatment schedules (e.g. maintenance vs. no BCG maintenance). However, predicting response to BCG is important because potentially curable disease may later progress to incurable disease in patients who fail to respond to BCG. Many intrinsic factors have been implicated in determining response to BCG. Here, we review the role of BCG strains and host variations and their potential influence on BCG treatment outcome.

5.8.1 The influence of bacillus Calmette-Guérin strain on treatment response

BCG was released in 1921 as an attenuated live vaccine for tuberculosis (TB), distributed worldwide, and maintained by continuous serial passaging.[507] As a result of continuous passaging under different conditions in various laboratories throughout the world, BCG began to diverge genetically, until the introduction of freeze-dried seed lots in the 1960s. This resulted in extensive genomic diversity, with mutations including large deletions and duplications.[508] Starting from 1976, BCG was increasingly used for the prevention of recurrence and progression of NMIBC.[509] Currently, there are more than eight different strains used for the treatment of NMIBC[507] and, while they are considered to be bio-similar agents, it is debated among vaccination specialists and urologists as to whether BCG strain differences impact efficacy and/or adverse effects secondary to treatment.[510,511]

In some human and *in vivo* studies, genetically distinct strains have been associated with differences in elicited immune responses, including reactogenicity and immunogenicity;[512,513] however, it is not known if such changes influence BCG's efficacy in the treatment of bladder cancer (see also following sections on the influence of the host immune system).

Studies comparing BCG strains in head-to-head trials suggest that strains can influence clinical outcomes.[514-518] Except for one trial indicating Connaught to be superior to TICE (HR 0.4),[513] there were only few prospective trials with small patient numbers. Therefore, most of these trials lacked statistical power to reliably assess effects related to strain differences. Likewise, in a systematic review and network meta-analysis including 10 different BCG strains used in clinical studies (Connaught, Pasteur, TICE, Tokyo, RIVM, Danish 1331, Armand-Frappier, Moreau, Glaxo, and Evans), no specific strain was found to be superior to another but, as outlined above, data were challenged by the relatively small numbers of prospective randomized trials. Thus, additional investigation is warranted and planned in order to compare different BCG strains (Tokyo vs. TICE; NCT03091660) and genetically improved BCG (NCT02371447).

5.8.2 The influence of the host on treatment response to bacillus Calmette-Guérin

5.8.2.1 Patient characteristics influencing treatment outcome

5.8.2.1.1 Gender

In a review of 1,021 patients treated with BCG, similar outcomes were observed between men and women, when 78.4% of men and 82.6% of women had no evidence of disease at 6 months after BCG treatment (p=0.14). In addition, there was no difference in disease recurrence or progression following BCG across gender in multivariable analysis.[519] Similarly, in a cohort of 84 patients treated with Tokyo 172 strain for CIS of the bladder, gender was not associated with response.[520] Among 204 patients treated with BCG, females exhibited an improved response to BCG (63% vs. 83%, p=0.046).[521] In another single-institution experience with 146 patients with T1HG treated with BCG, female gender was associated with increased risk for disease relapse (p=0.003) and progression (p=0.001). The CUETO study examined data from 1,062 patients treated with BCG and found that female gender (HR 1.71) conferred a higher risk for disease relapse but a similar progression rate.[522] In conclusion, while female gender may confer a higher risk for disease relapse, there is no convincing evidence that women do not respond to BCG.

5.8.2.1.2 Influence of aging

There is evidence that aging could influence response to BCG. Decrease in the activity of the immune system at older age is thought to be the mechanism behind this. However, the association behind older age and a decreased response to BCG is not conclusive and has not considered competing risks due to aging. In an analysis of 231 patients with CIS treated with BCG plus IFN alpha-2b, age greater than 70 was associated with a trend toward increased risk for relapse (HR, 1.48; p=0.057); no difference was seen in female versus male (p=0.82).[523] In a study of 1,106 patients treated with intravesical BCG plus IFN alpha-2b for bladder cancer, older patients had increased relapse rates following BCG compared to younger patients.[524] For example, of patients aged 61 to 70 years old versus patients older than 80, cancer-free survival was 39% versus 61%, respectively, at a median follow-up of 24 months corresponding to an adjusted HR of 1.564 (95% CI, 1.065–2.296; p=0.02). In a cohort of 204 patients treated with BCG, patients aged <65 had improved response to BCG compared to patients ≥65 (73% vs. 59%, p=0.006).[521] In a large (n=805) cohort of patients with bladder cancer, age was found to have a small but measurable association with response to BCG therapy.[525] In a cohort of 238 patients with bladder cancer treated with BCG, age was an independent risk for disease progression; the 2-year PFS was 87% among patients <75 years of age compared to 65% among patients >75 years of age (p<0.001).[526] An important observation comes from a large clinical trial comparing BCG with or without IFN alpha versus epirubicin for the treatment of NMIBC.[527] Consistent with previous reports, age was adversely associated with outcome among patients treated with BCG as patients >70 had a shorter time to progression (p=0.028), cancer-specific survival (p=0.049), and OS (p=0.028) after adjustment for disease risk scores.[527] In the CUETO study (n=1,062 patients treated with BCG), age was categorized as <60, 60 to 70, and >70 years of age and evaluated as a predictive factor for response to BCG.[528] In multivariable models, increased age was associated with a higher risk for disease recurrence (HR, 1.12; p=0.03) and progression (HR, 1.29; p=0.023) after BCG therapy.[528] The influence of competing risk for death from other causes and selective surgical management of elderly patients versus immune effects cannot be completely accounted for from these studies, and a conclusion regarding the influence of age on BCG antitumour activity is not reached. Nevertheless, there

is a consistent and significant association of diminished response for aged individuals across these studies. In addition, as BCG is standard of care immunotherapy and bladder cancer patients tend to be older than for many other cancer types, these data merit follow-up.

5.8.2.1.3 Influence of genetic variations

Variations in host genetics have been reported to be associated with response to BCG in bladder cancer. Alterations such as single-nucleotide polymorphisms, single-nucleotide variations, gene mutations, and copy number alterations have been associated with response to BCG (reviewed by Zhang *et al.*[529]). In 204 patients treated with BCG, genetic polymorphisms in 38 genes predicted to be involved in BCG's mechanism of action were examined as predictive factors.[521] Loss of heterozygosity on the IFN-alpha locus was associated with response to BCG.[530] Ke and collegues[531] identified several genetic variants in the glutathione pathway that were associated with response to BCG. Four SNPs were significantly associated with bladder cancer recurrence after BCG therapy. In addition, they observed a cumulative effect, as increased number of these unfavourable genotypes was associated with increased risk for disease recurrence. Multiple SNPs in oxidative-stress genes were associated with risk for disease relapse in 421 BCG-treated patients.[532] In a separate study, five polymorphisms involved in antigen presentation were studied. Patients carrying SNP for intercellular adhesion molecule 1 (ICAM-1) presented a two-fold risk for relapse after BCG treatment (p=0.032). Additional polymorphisms showing association with response to BCG included Fas ligand, tumour necrosis factor–related apoptosis-inducing ligand receptor 1, interleukin (IL)-2 receptor alpha, and IL17A.

5.8.2.1.4 Smoking

In two separate cohort studies, smoking was associated with worse outcome for patients treated with BCG.[533,534] Among 81 patients treated with BCG, smoking intensity was associated with increased risk for disease relapse (p=0.012).[534] In patients with <60 pack-years, BCG failure was observed in 20/68 (29%) and in patients with ≥60 pack-years, BCG failure was observed in 9/13 (69%).[534] Separately,[533] smoking status was associated with disease progression among 2,043 patients with NMIBC, with current smokers having the highest cumulative incidence in disease progression. Importantly, smoking cessation >10 years reduced the risk for disease recurrence and progression. While one observational cohort[535] review of 623 patients treated with BCG did not find an association of smoking status with outcome, the outcomes of this study were questioned due to the lack of standard receipt of BCG maintenance therapy[536] and, as a result, their outcomes were not comparable to outcomes in populations using standard of care maintenance therapy. These cumulative findings support a strong recommendation for smoking cessation and prevention for patients with NMIBC, but there is no evidence that tobacco use influences response to BCG.

5.8.2.1.5 Concomitant comorbid conditions

5.8.2.1.5.1 Immunologically compromised conditions

Issues concerning administration of BCG in the context of immunosuppression include the potential decreased efficacy, as BCG's antitumour efficacy is dependent on the immune response, and the potential increase in side effects and risks, as infectious complications can occur during BCG administration. Traditionally, BCG has been withheld for patients with immunosuppressive conditions. However, with increasing experience in using BCG in these high-risk situations, this is no longer considered an absolute contraindication. In a review of 45 immunosuppressed patients, including 12 with organ transplants, 23 undergoing systemic chemotherapy, and 10 taking immunosuppressive

agents, response rates were favourable and well tolerated, with no patients developing bacterial or BCG sepsis.[537] Among 14 patients with prior organ transplant on immunosuppression treated with BCG, disease recurrence and progression was observed in 63% and 13%, respectively, and 70% had no side effects.[538] Of note, 42% of patients did not receive prophylactic antibiotics.[538] Additional small case series support the use of BCG in transplant patients given the relatively low risk for side effects and potential for bladder preservation.[539-541] In conclusion, BCG can be used to treat patients with immunocompromised conditions but should be used carefully with close monitoring due to potential for more serious consequences in the event of infection.

5.8.2.1.5.2 The use of nonsteroidal agents

Given the association of bladder cancer with age and tobacco use, many patients with bladder cancer consume Aspirin (acetylsalicylic acid) or other anti-inflammatory agents for cardiovascular disease protection. These agents have immunomodulatory properties and their concomitant use with BCG has been studied, but results are not conclusive. Among 43 patients with HG NMIBC,[542] Aspirin was associated with an improved RFS, suggesting that Aspirin could improve outcomes for patients treated with BCG. On the other hand, in a separate report of 99 patients with HG NMIBC who received at least one induction course of BCG,[543] anti-inflammatories (including statins [65%], Aspirin [63%], or non-Aspirin nonsteroidal anti-inflammatory drugs [NSAIDs] or cyclooxygenase [COX] inhibitors [26%]) and anti-inflammatory use were not significantly associated with any outcome.

Summary and Recommendations

1. Definitive conclusions regarding effectiveness across BCG strains are not reached, and additional studies are warranted. However, some of these could influence antitumour immune responses, as suggested by clinical studies comparing BCG strains **[LOE 3]**.
2. Host variability, including age **[LOE 2]**, genetics **[LOE 3]**, and smoking **[LOE 3]** will influence response to therapy.
3. There is most support for the notion that age could influence response to BCG. However, studies with appropriate accounting for competing risks would be needed to make more substantiated conclusions regarding the association of age and BCG response.
4. The influence of gender and concomitant medication remains inconclusive **[LOE 3]**.
5. In the absence of higher evidence for any of these potentially influencing factors, it is recommended that no patient be denied BCG, regardless of specific strain availability or host features.

5.9 Management of Complications of Intravesical Therapy

5.9.1 Introduction

With an overall recurrence rate approaching 70% after surgical treatment alone, NMIBC is among the most difficult to eradicate and most costly of all human cancers.[544,545] To reduce recurrence and repetitive surgery, adjuvant topical therapy in the form of instillation of either cytotoxic chemotherapeutic or immunotherapeutic agents directly into the bladder (intravesical administration) has become a major part in the treatment algorithm. TURBT is still the standard initial treatment for bladder tumours. It serves to establish the diagnosis, stage, and grade, and to remove the tumour. This, however, should be followed by a single postoperative instillation of chemotherapy in the low-risk patients and maintenance adjuvant intravesical BCG for high-risk patients.[546] BCG has been shown to delay recurrence, reduce rate of progression, and improve OS.[547] Studies showing survival benefit of other intravesical chemotherapeutics are currently lacking. However as many patients are either not candidates for or are unwilling to undergo cystectomy, there is increasing interest in salvage therapy for BCG-refractory patients.[548] Since topical treatment can lead to a variety of local and/or systemic complications, it is incumbent on the administering physician to fully understand the potential toxicity of this therapy to make the best decision on its appropriate use and, when side effects do result from intravesical therapy, it is crucial that the prescribing urologist be well versed in their management.

5.9.2 Intravesical chemotherapy: general principles related to efficacy and toxicity

The scientific rationale behind the use of intravesical chemotherapy is to introduce a cytotoxic drug at relatively high concentration directly to the tumour cells, while minimizing systemic exposure. Two general formats have been widely used. A single dose of perioperative chemotherapy has been advocated on the basis of Class A medical evidence demonstrating a 39% relative reduction in the odds of tumour recurrence.[549] The reputed basis of efficacy is prevention of tumour cell reimplantation shortly following TURBT, and destruction of small tumour remnants not seen during resection. The more commonly used format is repetitive, usually weekly, chemotherapy over 6 to 8 weeks, occasionally followed by further (usually monthly) maintenance treatments, of which the utility of the latter is still under some debate.[550,551] Urothelial tumour–drug contact is very important in this whole process. For this reason, best results are nearly always obtained with the most complete prior tumour resection possible. For any remaining residual disease, whether microscopic or not, the administered drug must penetrate into the full depth of the tumour to be effective. The variables affecting tumour eradication include the nature of the drug (mechanism of action), concentration at the tumour site, ability to penetrate, contact time with the tumour, and stability in the urine.[552] Since dwell time in the bladder is limited by bladder capacity (typically 2 hours) and ongoing urine production (with progressive drug dilution over time), the drug usually has to be administered several times over a certain period of time to produce an efficient anticancer response. Unfortunately, local toxicity (usually in the form of cystitis) is also directly related to effective drug exposure (time and concentration), as

well as the drug's intrinsic irritability on normal urothelium or exposed resected stroma. Systemic toxicity depends on drug absorption and the unique properties of the agent. Factors affecting drug absorption include not only effective drug exposure but also its molecular weight and the integrity of the bladder wall. A large, deep tumour resection will expose a larger thin surface area facilitating absorption, while an inflamed bladder from a coexistent UTI can do the same. Worse yet, an unrecognized bladder perforation or traumatic catheterization can allow direct extravasation of most of the administered dose.

Cytotoxic drugs that have been used for intravesical chemotherapy are listed in **Table 5-6** and can be catalogued by the mechanistic class of agent to which they belong. Systemically, all have the potential for inducing myelosuppression, as well as a variety of other side effects. Until recently, class-specific drugs were limited to topoisomerase inhibitors, primarily anthracyclines, and alkylating agents, primarily thiotepa and MMC. Although gemcitabine and taxanes have been used extensively against metastatic bladder cancer, intravesical clinical experience with gemcitabine in phase 1 or 2 trials was first reported in 2002 and with docetaxel in 2006. There are a few drugs, such as cisplatin, mitoxantrone, and methotrexate, that have been used intravesically but have lost favour due to reduced efficacy (for example, mitoxantrone and methotrexate) and/or toxicity (for example, cisplatin, which caused anaphylactic shock). Another important feature of chemotherapeutic drugs is their tendency for venous irritation and tissue damage after inadvertent extravasation during IV drug administration. This property of intrinsic local tissue reactivity has been well studied and has allowed drugs to be catalogued according to the level of contact toxicity.[553] Vesicant agents are those that are destructive to local tissues and can cause extensive tissue necrosis, sometimes requiring skin grafting and resulting in permanent disability. For this reason, they are usually delivered via central access lines. Nonvesicant agents can still be highly irritating but are seldom destructive. The relative categorization of some of the major chemotherapeutic drugs according to their vesicant/irritant status is provided in **Table 5-7**. The category of the agent has significant relation to the side-effect profile. It is noteworthy that the two most commonly used chemotherapeutic drugs for intravesical therapy, that is, most of the anthracyclines (doxorubicin and epirubicin but not valrubicin) and MMC, belong to the vesicant subclass. At the same time, the most common dose-limiting side effect of these intravesical agents is local in origin and manifests as irritable cystitis with urgency, frequency, dysuria, bladder pain, and/or hematuria.

TABLE 5-6 Classification of Intravesical Agents

Biologic class	Subtype	Drugs	Molecular weight (Da)	Systemic toxicity
Topoisomerase inhibitors	Anthracyclines	Doxorubicin (Adriamycin)	580	Cardiomyopathy, myelosuppression, mucositis
		Epirubicin	580	Less cardiotoxicity
		Valrubicin	724	Even less cardiotoxicity
	Anthracenediones	Mitoxantrone	444	Myelosuppression, nausea, vomiting, mucositis
Alkylating agents	Ethyleneimine	Thiotepa	189	Myelosuppression, nausea, vomiting, mucositis
	Bioreductive alkylator	MMC	334	Myelosuppression, nausea, vomiting, mucositis, dermatitis, asthenia, fibrosis, congestive heart failure, hemolytic uremic syndrome, hypersensitivity
	Platinum analogues	Cisplatin	300	Renal, neuropathy, nausea, vomiting, myelosuppression, electrolyte disturbances, anaphylactoid reactions
Antimetabolites	Antifolate	Methotrexate	454	Myelosuppression, mucositis, renal, pneumonitis, hepatic fibrosis
	Pyrimidine analogues	Gemcitabine	300	Myelosuppression, nausea, vomiting, flu-like symptoms
Mitotic Spindle Inhibitors	Taxanes	Docetaxel	862	Myelosuppression, cardiac arrhythmia, alopecia, neuropathy, capillary leak, hypersensitivity

Abbreviation: MMC, mitomycin C.

TABLE 5-7 Classification of Chemotherapeutics by Vesicant/Irritant Status

Vesicant	Irritant	Minimal	None
All anthracyclines (excepting valrubicin)	Cisplatin (standard dose)	Methotrexate	Bleomycin
All vinca alkaloids	Carboplatin	Mitoxantrone	Gemcitabine
MMC	Etoposide	Pemetrexed	Cytarabine
Cisplatin (high dose)	Ifosfamide	Thiotepa	5-Fluorouracil
Paclitaxel	Docetaxel	–	–
	Busulfan	–	–

Abbreviation: MMC, mitomycin C.

5.9.2.1 Mitomycin C

MMC is a bioreductive alkylating agent isolated from *Streptomyces caespitosus* that requires intracellular enzymatic reduction by quinone reductase to become activated.[554] While not cell-cycle specific, increased susceptibility is seen during late G1 and early S phases of DNA synthesis.[555] MMC's major mechanism of action is through DNA cross-linking, but generation of reactive oxygen species also contributes to its activity.[556] MMC has a molecular weight of 334 Da and is typically used in doses of 20 to 40 mg in 20 to 40 cc of water or saline. MMC's larger size is assumed to contribute to its limited systemic absorption. Indeed, numerous pharmacodynamic studies of absorption of MMC in animal and human bladders under various conditions have consistently demonstrated little systemic absorption, typically <1% of the administered dose (corresponding to plasma levels of T3 under 40 ng/mL).[557-559] Under conditions of extensive resection, active inflammation or infection, or prior radiation, levels of three to five times higher have been reported.[560,561] Methods to increase the depth of penetration of MMC by using higher concentrations (e.g., 40 mg in 20 cc) or coincident microwave hyperthermia (HT) or electromotive therapy have also resulted in two to five times higher serum levels.[562-564] However, because the level of MMC required for myelosuppression is estimated to be >400 ng/mL (10 times the typical levels), bone marrow toxicity is only very rarely observed (average 2% incidence).[565] Serious systemic side effects, including severe bone marrow suppression and death after intravesical mitomycin, have occurred but have almost uniformly been reported in cases of suspected bladder perforation. Therefore, in cases of suspected bladder perforation, administration of perioperative MMC is contraindicated.[566,567] Necrosis of the glans penis and urethral sloughing following MMC administration were also found after traumatic catheterization and are consistent with the known strong vesicant properties of MMC.[568,569] However, during normal use of intravesical mitomycin, either after TURBT or as adjuvant intravesical treatment, local toxicity in the form of chemical cystitis is the most frequent side effect, occurring in about 18% of patients (range 12%–26%).[570,571] Importantly, chemical cystitis must be distinguished from bacterial cystitis that also occurs with a similar frequency in these patients. Milder manifestations of frequency or dysuria are even more common in 42% and 35% of the patients, respectively. Hematuria is found in 16% and pain in 10%, while actual incontinence is rare (1%) (see **Table 5-8**). Treatment interruption or discontinuation occurs in about 10% of patients, largely due to these local effects. As with other agents, there is some suggestion that these side effects are dose exposure–related. The more serious side effect of bladder contracture, the end result of severe chemical cystitis, appears highest with mitomycin than with any other agent, at approximately 5%, with rates as high as 23% reported for patients treated for 2 years.[572] This may be a function of MMC's strong vesicant nature, allergic/hypersensitivity, and/or fibrosis potential. Eosinophilic infiltrates and even inflammatory mass lesions have been reported to be a result of MMC therapy.[573]

Many case reports of intravesical stones in the wall of formerly resected transurethral bladder tumours with instillation of postoperative mitomycin have been reported (**Figure 5-3**). The stones are commonly associated with symptoms of urgency, frequency, and dysuria.[574,575]

TABLE 5-8 Summary of Toxicity Reported for Common Intravesical Agents

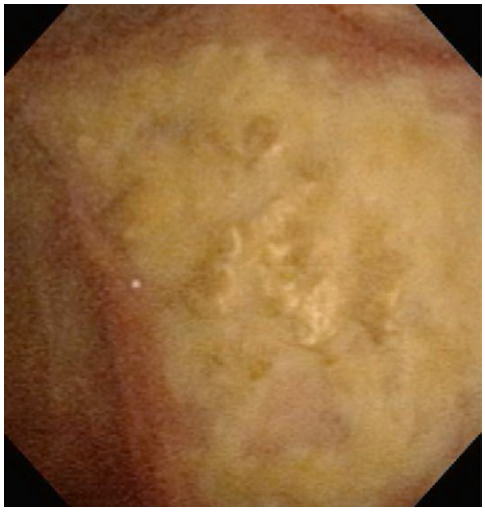
Toxicity	MMC	Doxorubicin	BCG
Local (%)	NR	NR	NR
Frequency/nocturia (%)	42 (26–59)	27 (23–32)	63 (48–76)
Dysuria (%)	35 (30–41)	20 (8–39)	75 (64–84)
Irritative symptoms (%)	18 (12–26)	21 (13–30)	Extremely variable
Pain/cramps (%)	10 (6–14)	12 (4–25)	12 (7–18)
Hematuria (%)	16 (7–28)	19 (12–29)	29 (22–36)
Incontinence(%)	1 (0.4–4)	9 (3–18)	4 (3–6)
Bladder contracture (%)	5 (2–11)	3 (0.8–6)	3 (2–5)
Systemic (%)	NR	NR	NR
Flu-like (%)	11 (4–23)	7 (3–13)	24 (18–31)
Fever/chills (%)	4 (1–10)	4 (2–9)	27 (22–32)
Arthralgias (%)	NR	1 (0.1–5)	5 (1–13)
Myelosuppression (%)	13 (8–19)	0.8 (0.2–2)	1 (0.1–4)
Nausea/vomiting (%)	9 (0.8–31)	8 (4–13)	9 (6–14)
Skin rash (%)	2 (0.4–4)	2 (0.5–6)	6 (3–10)
Infectious (%)	NR	NR	NR
Bacterial cystitis (%)	20 (17–23)	6 (2–12)	20 (13–28)
Epididymitis, prostatitis, urethritis (%)	4 (2–9)	2 (0.1–7)	5 (4–8)
Pneumonia (%)	0.2 (0–2)	NR	1 (0.2–3)

Abbreviations: AUA, American Urological Association; BCG, bacillus Calmette-Guérin; MMC, mitomycin C, NR, not reported.

Adapted from Smith et al. Bladder cancer clinical guidelines panel summary report on the management of nonmuscle invasive bladder cancer (stages Ta, T1 and TIS).

The American Urological Association. J Urol. 1999;162(5):1697–1701.[570]

FIGURE 5-3
Intravesical Bladder Calcification From Mitomycin



Due to the inflammatory origin of the mitomycin side effects, the first form of treatment is to withhold therapy at the first sign of severe cystitis (i.e. moderate to severe symptoms persisting beyond 1 week). One successfully described algorithm for mitomycin-induced cystitis starts with holding further mitomycin treatments and obtaining an immediate urinalysis (UA) and urine culture in all patients who present with any new-onset urinary symptoms. If UA and urine culture are normal, a cystoscopy should be obtained. If any intravesical stones are visualized, complete stone removal has been advocated, as the stone may become a nidus for infection and prevent appropriate healing of the urothelium. Stones can be removed with a combination of cold-cup biopsy and loop resection. Care must be taken to avoid perforation of the bladder, as the area will likely be thin since it is a prior resection site. We recommend careful removal with judicious use of loop resection and an attempt to minimize excessive cautery. After the entirety of the stone is removed, the bladder wall will usually epithelialize, provided ample viable tissue remains. Any suspicious lesion after removal of the stone should be biopsied, as it could represent a recurrence of malignancy.[576,577]

If UA and cystoscopy are completely normal and symptoms are mild, starting with conservative measures such as timed voiding should be performed. If this is unsuccessful, an oral antihistamine can be attempted for mild symptoms. If this is unsuccessful, anticholinergic medications and alpha blockers are attempted (Expert Opinion).

For more severe unremitting symptoms, we have observed more success with moving directly to treatment with oral prednisone taper, typically: 40 mg x 4 days, 20 mg x 4 days, 10 mg x 7 days, 5 mg x 7 days, and 2.5 mg x 7 days. Others have suggested 60 mg of prednisone for 2 weeks followed by 40 mg for 1 day, 30 mg for 1 day, 20 mg for 1 day, and 10 mg for the last day. This is taken alongside an antihistamine such as oral (PO) Benadryl®. If this is unsuccessful, a 4-week course of 60 mg of prednisone can be attempted prior to the same 4-day taper[578] **[GOR C]**.

Another significant side effect associated primarily with MMC is a desquamative, eczematous rash most commonly appearing on the palms, soles, chest, face, and genitals in approximately 13% of treated patients.[570] This rash is often associated with coincident chemical cystitis. The origin of this

rash is not completely clear, but is suspected to be the result of a delayed hypersensitivity reaction, possibly exacerbated by contact sensitivity in certain areas (palms and genitals).[579] Evidence for the hypersensitivity phenomenon includes its occurrence only after prior MMC exposure, association with eosinophils, skin patch recall, and occurrence with systemic MMC therapy (hand-foot syndrome).[580,581] These rashes usually respond to cessation of further therapy and institution of either topical or systemic steroid taper such as that described above.[582] Minor rashes responding to treatment do not necessarily require cessation of further treatment. Avoiding inadvertent extended patient skin contact with the drug, and thorough washing upon exposure are recommended during instillation and after voiding.

5.9.2.2 The anthracyclines (doxorubicin, epirubicin, and valrubicin)

The anthracycline family includes doxorubicin and all its derivatives, including epirubicin, pirarubicin, and valrubicin (among others). All the members of the anthracycline family are relatively large molecules with molecular weights exceeding 500 Da. Doxorubicin and epirubicin (the racemic version of doxorubicin) are both 580 Da, while pirarubicin (853 Da) and valrubicin (724 Da) are even larger due to side-chain modifications that affect solubility and biodistribution. Doxorubicin hydrochloride (Adriamycin) was originally isolated from *Streptomyces caesius*. Its mechanism of action primarily involves inhibition of topoisomerase II, but intercalation of the drug between adjacent base pairs of the DNA double helix and free radical formation also contribute to its efficacy.[583] There is also evidence of direct cytotoxicity through interaction with the cell membrane.[584] Doxorubicin is relatively non–cell cycle–specific, but most active in the S phase of DNA replication.[585] The common dose for most of the anthracyclines is 50 mg in 50 cc of water or saline, but higher concentrations of 80 to 90 mg in 50 cc have also been described. Because of their larger molecular weights, systemic absorption of the anthracycline drugs is very low and systemic side effects very rare.[586,587] Indeed, myelosuppression occurs in <1% of patients.[570] Allergic reactions, primarily skin rash, have been reported in 2% of patients treated with doxorubicin, but have also been documented with epirubicin and valrubicin.[588-590] These allergic reactions are usually treated according to their symptoms, mainly by using antihistaminic drugs and supportive measures. Fever (4%) and nausea/vomiting (1%–2%) have also been reported.[570] As with all intravesical drugs, integrity of the bladder wall is important in limiting absorption, with lower levels of absorption found during later instillations. One severe local reaction with doxorubicin and three with epirubicin (and one death) from bladder perforations have been reported.[591,592] Because classic signs of peritonitis may not always be present, a CT scan (preferably CT cystogram) should be performed for all suspected perforations after transurethral resection of the bladder (TURB) with perioperative drug instillation. In one documented case of valrubicin leakage, no accompanying local untoward effects were observed.[590] Valrubicin has also been used for intraperitoneal chemotherapy of ovarian disease and is the only family member classified as a nonvesicant.[593] Not unexpectedly, given their vesicant profile, local side effects are more commonly seen with all anthracyclines administered intravesically. Valrubicin is solubilized in an irritant castor oil/ethanol base and also causes cystitis. Chemical cystitis (urgency, frequency, and dysuria) has been reported in about one-quarter of the patients (range 8%–39%) treated with doxorubicin, with hematuria in 19%.[570] These side effects are generally mild and self-limiting with time. A direct comparative study by Eto *et al.*[594] with low doses of epirubicin and doxorubicin yielded similar results, with cystitis as the most common side effect.

Valrubicin was approved in the United States for intravesical use in patients with CIS who failed BCG treatment. Valrubicin intravesical therapy is most commonly associated with localized AEs exceeding those of the other anthracyclines. In patients with an intact bladder, some studies have only shown bladder irritation.[595] Chemical cystitis and hematuria are found in the majority of patients. More serious systemic effects were found with postoperative therapy.[595] One patient with a perforated bladder developed neutropenia 2 weeks after the treatment. He also presented with moderate anemia and mild thrombocytopenia that were probably related to the treatment. Another patient experienced mild postinfusion contact dermatitis, having a rash in the groin area. The third patient had a new diagnosis of cancer unrelated to valrubicin, and the fourth patient had an exacerbation of his chronic obstructive pulmonary disease unrelated to the therapy. Despite the extensive use of valrubicin, only mild AEs have been reported. These are generally well managed with anticholinergic medications as needed for chemical cystitis. In one study by Cookson *et al.* looking at 113 patients undergoing intravesical valrubicin treatments for NMIBC, only 5/113 discontinued valrubicin due to AEs.[596]

Well-organized protocols are lacking for the rare patient who suffers from unrecognized bladder perforation and instillation of intravesical anthracyclines. Small-scale case reports of three patients and two patients with unidentified perforation of the bladder and instillation of intravesical epirubicin have been published.[592,597] These cases demonstrate the importance of having a high level of suspicion of extravasation of the intravesical agent. Signs and symptoms that may suggest extravasation include abdominal pain, peritonitis, and ileus. The perforation may be identifiable on a CT cystogram with contrast instilled by gravity. First-line therapy is conservative management, with placement of a large-bore (24 French, if possible) Foley catheter to maximize bladder drainage, placement of nasogastric (NG) tube, and initiation of total parenteral nutrition (TPN). If a patient does not begin to respond quickly and very well to conservative management, exploratory laparotomy with bladder repair, placement of intraperitoneal drains, running of the small bowel, and examination of the colon/rectum with possible need for a diverting colostomy are indicated. Duration of Foley catheter was variable between cases, although in cases managed conservatively, a minimum of 2 weeks of continuous indwelling catheterization was performed. Cystogram may be useful prior to removing catheter. One of the five patients died, and all had prolonged hospitalizations, emphasizing the importance of avoiding instillation of postoperative chemotherapy if perforation is suspected and rapid recognition of extravasation of chemotherapy if it does happen.

5.9.2.3 Gemcitabine

Gemcitabine is a pyrimidine antimetabolite, analogous to cytosine arabinoside, with a molecular weight of 300 Da. Its mechanism of action involves incorporation of the pyrimidine base analog into DNA by one of the metabolites gemcitabine triphosphate (dFdCTP), resulting in chain termination.[598] In addition, gemcitabine inhibits ribonucleotide reductase, an enzyme necessary for DNA synthesis.[599] It was first approved in the United States to treat pancreatic cancer,[600] but has since been found to be effective in other tumours such as non–small-cell lung cancer, leiomyosarcoma, and ovarian cancer.[601] Phase 3 clinical trial data, as well as level 1 evidence, have shown similar survival rates in patients but reduced toxicity with metastatic urothelial cancer treated with gemcitabine plus cisplatin versus the conventional treatment with methotrexate, vinblastine, doxorubicin, and cisplatin.[602,603] The dose of gemcitabine most commonly being used for intravesical therapy is 1,000 mg, 1,500 mg, or 2,000 mg dissolved in 50 to 100 cc of water or saline. Buffering has been used

by some investigators to raise its pH from 2.5 to a more physiologic range by adding 50 to 100 mEq of bicarbonate, but precipitation may occur in some cases.[604] However, there is no evidence that this affects efficacy and/or toxicity. Studies of the intravesical pharmacodynamics of gemcitabine in the non-postsurgical state have revealed the low serum absorption (0.5%–5.5%) expected based on its 300-Da molecular weight. This corresponds to an absorption of between 10 mg and 110 mg from the bladder, well below the dose typically used systemically, >1,500 mg.

Furthermore, plasma levels of the active metabolite 2',2'-difluorodeoxyuridine (dFdU) have also been recorded in the low micromolar range.[605] **Table 5-9** summarizes the clinical trials using gemcitabine as the intravesical agent, with a total of 153 patients. It should be noted that, in these studies, rather strict criteria for assessing toxicity were used based on NCI and WHO grading scales. Toxicities are graded from 1 to 5: 1, mild side effects; 2, moderate side effects; 3, severe side effects; 4, life-threatening or disabling side effects; 5, fatal. Rare interruption of the treatment was reported in these nine cohorts of patients during the treatment, and all toxicities were short term and reversible with discontinuation of the drug. Most were grade 1 (mild) or grade 2 (moderate). No studies reported grade 4 (life threatening) or 5 (fatal) toxicities. Most authors concluded that gemcitabine was well tolerated with regard to local cystitis. This may be related to the nonvesicant activity of the drug. This has also been the authors' personal experience, accepting worse local toxicity in patients with baseline bladder irritability, where pH buffering of the acidic solution may be helpful. Their experience has been that patients who take 1,300 mg of sodium bicarbonate the evening prior to a treatment and 1,300 mg of sodium bicarbonate the morning of a treatment. Alkalization of the urine helps minimize irritation of gemcitabine, as gemcitabine solution has a pH of 2.5. Naproxen (250 mg) 2 hours prior to instillation and 250 mg in the evening after instillation will significantly lessen cystitis symptoms (Expert Opinion). Transient nausea and occasional vomiting occurring usually 24 hours after instillation is the other most common side effect that responds well to antiemetic drugs such as 4 to 8 mg of oral ondansetron (Zofran®) given to susceptible patients at the time of treatment and repeated 8 hours later (Expert Opinion). Urinary frequency responds well to oxybutynin 5 mg prescription ordering direct (POD) three times a day as needed and may be given 1 hour of pretreatment along with relative dehydration for those who have difficulty retaining the drug for the recommended 1.5 to 2 hours (Expert Opinion).[606]

TABLE 5-9 Toxicity From Intravesical Gemcitabine

Study	Patients	Induction dose (mg)	Dose frequency	Chemical cystitis (%)	Hematuria (%)	Other AEs
Dalbagni *et al.*[604]	18	500–2,000	2x/wk x 6 wk (repeat cycle with 1-wk break)	39	29	UTI (1), myelosuppression (1), hand-foot syndrome (2), asthenia (4), nausea (4), vomiting (1)
Laufer *et al.*[607]	15	500–2,000	Q wk x 6 wk	67	67	
Witjes *et al.*[608]	10	1000–2,000	Q wk x 6 wk	40	NR	Headaches, fatigue, and heavy legs (30%)
De Berardinis *et al.*[605]	12	500–2,000	Q wk x 6 wk	1 patient	NR	
Serretta *et al.*[609]	27	500–2,000	Q wk x 6 wk	11	NR	Nausea (11%), fatigue (4%)
Palou *et al.*[610]	10	1500–2,000	Post-TURB x1	1 patient	NR	Liver toxicity (2)
Di Lorenzo *et al.*[611]	40	2,000	2x/wk x 6 wk, then 1x wk at 3, 6, 12 months	10	5	Fever (1), myelosuppression (1), dermatitis (2), nausea-vomiting (2)
Perdonà *et al.*[612]	20	2,000	2x/wk x 6 wk, then 1x wk at 3, 6, 12 months	15	15	Thrombocytopenia (1), fever (1), dermatitis (1), nausea-vomiting (3)
Bounedjar *et al.*[613]	60	2,000	Q wk x 6 wk	5	2	Leukopenia (3), nausea-vomiting (1)

Abbreviations: AE, adverse event; NR, not reported; TURB, transurethral resection of the bladder; UTI, urinary tract infection.

5.9.2.4 Docetaxel

Docetaxel is a taxane that exerts its anticancer effects via mitotic spindle inhibition.[614] This is done specifically by the inhibition of microtubule depolymerization, leading to cell-cycle arrest and cell death.[615] Various small-scale reports have shown promising long-term rates of cancer control.[614] Intravesical docetaxel is a generally very well-tolerated treatment with no reported systemic toxicity when administered in doses of up to 75 mg in 100 mL of normal saline, with an intravesical dwell time of up to 2 hours. In one small study of 33 patients, no grade 3 toxicities were reported and only 2/33 patients had a grade 2 toxicity. Twelve of the 33 patients experienced grade 1 or 2 side effects. All 33 patients were able to complete the planned six-dose treatment cycle, with the two patients having grade 2 toxicity having their treatment cycle extended by 1 week. Only one patient during one treatment was not able to complete the planned 2-hour dwell time. Median follow-up for the 33 patients was 2.9 years.[616,617] Our experience with docetaxel will be further detailed in the gemcitabine/docetaxel section. However, it should be noted that, in our experience, all patients who cannot tolerate sequential gemcitabine-docetaxel therapy could tolerate single-agent docetaxel therapy, so we believe it to be a great choice for patients who are particularly sensitive to the irritable effects of intravesical therapy.

5.9.3 Combination intravesical chemotherapy

While multi-agent chemotherapy has become the norm in the systemic treatment of most human malignancies, this strategy is just beginning to find a place in topical intravesical use against bladder cancer. Attempts at using mitomycin (20 mg on day 1) in close sequence with Adriamycin (40 mg on day 2) have shown significant activity, e.g. 81% CR to CIS, but at the expense of moderate to severe chemical cystitis in over half of the treated patients, one-third of whom had to terminate therapy prematurely.[618,619] This may be attributable to the overlapping known vesicant properties of both agents.

5.9.3.1 Gemcitabine/mitomycin

Sequenced gemcitabine followed immediately by mitomycin was first described in 2006 by O'Donnell and colleagues and subsequently reported by other investigators.[620-622] The order of instillation was chosen based on mechanistic and practical considerations. Of the two agents, gemcitabine is better tolerated, thus given first to facilitate treatment completion. Also, gemcitabine works best in the S phase of DNA replication, while mitomycin is relatively cell-cycle nonspecific. As gemcitabine requires DNA synthesis, we believe giving it first is likely to be beneficial, since theoretically mitomycin could block or reduce the amount of cell-cycle progression, making subsequent gemcitabine potentially less effective.

Administration routinely involves instilling the gemcitabine (1 gram in 50 cc saline) through an indwelling Foley catheter that is clamped for 90 minutes and then drained. Mitomycin is then instilled, whereupon the Foley catheter is either removed for a 1.5- to 2-hour void or clamped for 1.5 to 2 hours.[621] Six weekly treatments are given for induction therapy followed by non-obligatory monthly maintenance for up to 1 year. Side effects with this regimen have been reported in 47 patients: 10 (21%) developed mild to moderate cystitis symptoms, 3 developed a rash, and 1 patient developed pericarditis after completing the planned 6-week induction course. Only 4 patients (9%) were unable to complete the induction course due to side effects (rash and dysuria were the causative side effects.) In all of these cases, gemcitabine monotherapy was able to be performed for a total of six weekly intravesical treatments, suggesting that the majority of side effects were mitomycin related.[623] In an independent study of 27 prior intravesical therapy failures, eight patients reported side effects/AEs.[622] The most common was irritative voiding and bladder spasms, which occurred in six patients (22%). Anemia occurred in two patients, and was thought to be secondary to systemic absorption of gemcitabine. One patient developed acute renal failure during therapy. Four patients (15%) received incomplete courses of therapy, one for acute renal failure and three secondary to irritative voiding symptoms.

Given the rarity of serious side effects, further studies enrolling many more patients will have to be undertaken to understand appropriate treatment/prevention of side effects with combination gemcitabine/mitomycin therapy. Until that time, it seems prudent that methods used to prevent and treat the side effects of each agent individually are acceptable for the agents in combination.

5.9.3.2 Gemcitabine/docetaxel

In response to the mitomycin shortage in 2009, these authors transitioned to using docetaxel in place of mitomycin for sequenced intravesical chemotherapy. As discussed previously, gemcitabine is a deoxycytidine nucleoside analog that inhibits DNA synthesis, leading to cell apoptosis. As docetaxel inhibits tubulin disassembly that prevents cell division, gemcitabine was administered prior to docetaxel because gemcitabine seemingly would require active DNA synthesis to be most effective. As before, 1 gram of gemcitabine in 50 cc of saline is instilled for 90 minutes followed immediately by bladder drainage and instillation of 37.5 mg of docetaxel dissolved in 50 cc of saline for 1.5 to 2 hours. Patients are given 1,300 mg of oral sodium bicarbonate both the evening before and the morning before a treatment to minimize the acidic effects of gemcitabine. In all patients who are not contraindicated to received NSAIDs, 200 mg of Naprosyn® may be given 1 to 2 hours prior to administration. Prophylactic ondansetron (8 mg PO) is given to any patient who reports nausea after any previous treatment. An additional 8 mg is given 8 hours later. This has greatly reduced nausea and vomiting. In our initial experience, only 5/45 (11%) patients were unable to complete a planned 6-week induction course of the previous regimen. Frequency (4 patients), hematuria (4 patients), and dysuria (2 patients) were reported for the five patients who did not complete the planned 6-week course. For the 45-patient group, the most common side effects were dysuria (15/45=33%), mild urgency/frequency (15/45=33%), hematuria (5/45=11%), and nausea (3/45=7%).[624] Further clinical trials are needed on gemcitabine/docetaxel to determine appropriate treatment of side effects, but these heavily pretreated patients invariably consider this regimen more tolerable than past treatments. In the rare case when intractable nausea or cystitis has developed despite the above mechanisms, we have discontinued gemcitabine and treated with docetaxel monotherapy very successfully. Monthly maintenance therapy for 2 years is very well tolerated, with only occasional transient mild tiredness for 1 to 3 days in a minority of patients.

5.9.3.3 General measurements

In patients who have difficulty holding intravesical medications due to bladder size or bladder irritability, we employ several steps to ensure adequate dwell time. Patients are instructed to avoid caffeine the morning of the treatment and minimize fluid consumption prior to treatment. After the last treatment is voided out, we encourage oral fluids as tolerated (Expert Opinion). In patients who struggle with bladder spasms despite anticholinergics, we premedicate with 2 tablets of Percocet® (each tablet contains 5 mg oxycodone/325 mg acetaminophen) and 10 mg of Valium® approximately 1 hour prior to treatment. For patients with small-capacity bladder, we employ split-dosing of all drugs. We will instill half the medication for half the time, drain the bladder, and instill the remaining half of the medication for the remaining half of the time. For instance, instead of 50 cc of gemcitabine for 90 minutes, we will use 25 cc for 45 minutes, drain the bladder, and then use the remaining 25 cc for 45 minutes (Expert Opinion). For patients with significant pain and spasticity associated with instillation, we will use 40 cc of 2% lidocaine mixed with 4 cc of sodium bicarbonate 8.4% instilled 10 to 15 minutes prior to instilling the first drug. We then drain the lidocaine-sodium bicarbonate mixture immediately before administering the first drug (Expert Opinion).

5.9.4 Bacillus Calmette-Guérin

BCG remains the standard of care for patients with high-risk NMIBC and for those with intermediate risk failing conventional intravesical chemotherapy.[570] It is a live attenuated cow TB (*Mycobacterium bovis*) vaccine. Although its exact mechanism of action remains unknown, it remains the only agent that has been shown to reduce the risk for progression to muscle-invasive disease.[625]

5.9.4.1 Local toxicity associated with bacillus Calmette-Guérin

For patients previously naïve to BCG or TB, it is very unusual to have much in the way of local toxicity or bladder irritability during the first few weekly doses of BCG. Thereafter, patients commonly begin to experience frequency, urgency, and dysuria beginning shortly after the first 2-hour void that escalates over the ensuing 6 to 12 hours. These symptoms usually resolve by 24 hours initially but, with increasing retreatment, tend to become more intense more quickly, with a longer time (3–7 days) to completely dissipate. The local toxicity situation with BCG/TB-exposed patients is more accelerated. Using a validated questionnaire, Bohle *et al.* addressed the symptoms during the course of 6-week instillations of BCG.[626] Even after the first instillation, 50% of the patients complained of dysuric episodes. During subsequent instillations, there was an increase of up to 80% of patients with dysuric complaints. In a study by Saint *et al.*, cystitis of 2- to 48-hour duration was noted in 46% of patients, 48 hours to 7 days in 38% of patients, and >7 days duration in 12% of patients.[627] Increased duration was seen after the fourth induction treatment. Along with this increased intensity of irritable symptoms, the likelihood of gross hematuria also increases such that up to one-third of patients suffer from this side effect (29%). The recorded incidence of these varied symptoms is listed in **Table 5-8** and is notably greater for BCG than for any of the cytotoxic chemotherapeutics. Lamm *et al.* reported that only 16% of patients randomized to a miniseries of 3 weekly maintenance treatment actually received all their scheduled doses, presumably due to toxicity.[628] Saint *et al.* reported a similar 19% completion rate for all maintenance doses in a smaller trial of similar design.[627] Furthermore, 57% had dose reduction for toxicity and 39% had treatment discontinuation. Even if maintenance therapy is associated with higher local toxicity, the clinical significance of this is uncertain, as most side effects are reversible.

The histological changes found in the bladder after BCG therapy imply a generalized inflammatory process with pronounced mononuclear inflammatory infiltrate and epithelial sloughing.[629] Granulomas are present in roughly one-quarter of the cases. Visual abatement of most bladder inflammation occurs after 6 weeks, but full resolution of granulomatous changes may take 6 months or longer.[630]

After irritative symptoms, the most common side effect is asymptomatic prostatitis, which is estimated to occur in up to 40% of male patients and is often associated with an abnormal digital rectal examination (DRE), but does not require specific therapy.[631] However, because it may be difficult to distinguish the abnormal DRE from the nodularity associated with prostate cancer, irregularity persisting over 3 months may require biopsy.[632] Prolonged symptomatic BCG cystitis and/or prostatitis (estimated incidence <5%) can be troublesome during therapy and in the post-BCG observation period.[570] This is particularly more likely to occur during retreatment or prolonged maintenance therapy. This situation is best avoided by withholding BCG treatment until all significant symptoms from the prior instillation have subsided. A 1- to 2-week delay has not been shown to reduce BCG efficacy

in such a setting.[633,634] Reinstitution of BCG at a lower dose or premature termination of further treatment for this cycle may also be appropriate. Reduction of dose may reduce local symptoms without compromising treatment efficacy, especially during the maintenance phase.[634,635] If localized severe cystitis does occur and conservative symptomatic treatment measures fail, this condition can be treated with oral fluoroquinolones (3–12 weeks) or oral isoniazid. An oral steroid taper sandwiched between antibiotic coverage has also been shown to be helpful in refractory cases. These patients may require a taper of oral prednisone over 6 to 12 weeks and is similar to that described earlier for chemical cystitis. Successful tapers for refractory BCG cystitis have been described with doses starting at 20 mg daily for 3 weeks with a 3-week taper to 0 mg. Higher-dose tapers have been described for more difficult-to-treat cases.[635,636]

5.9.4.2 Systemic side effects of bacillus Calmette-Guérin

Systemic side effects of BCG occur in one of two major forms, infectious and noninfectious. Fever/chills and a flu-like illness are reported in roughly one-quarter of patients receiving BCG (see **Table 5-8**) and have actually been associated with an improved cancer prognosis.[637]

Later studies have refuted the benefits of improved cancer control in patients suffering systemic side effects. Sylvester in 2003 showed that the side effects of BCG did not predict BCG efficacy. However, a longer treatment period has been associated with both more toxicity and better efficacy[638] In roughly 3% of patients, body temperature exceeds 39.5°C.[639] Not all fevers are a sign of BCG infection but, rather, may be the result of spillover of BCG-induced pyogenic inflammatory cytokines from the bladder into the bloodstream.[640] Unfortunately, in the acute setting it is very difficult to distinguish an infectious event from a noninfectious event. At a minimum, patients with fevers after BCG instillation should be evaluated, and many will require hospitalization for observation. A fluoroquinolone antibiotic should be considered, since it will treat the majority of non-BCG bacterial UTIs and has reasonable antimycobacterial activity until the patient declares him/herself symptom free. Patients with self-limiting fevers <48 hours may be retreated with NSAID prophylaxis prior to next BCG treatment (e.g. ibuprofen 600 mg q 6 hours × 3 beginning 2 hours prior to therapy) and a reduced dose of BCG.[641] Clinical signs that suggest BCG-osis (systemic BCG infection) include exaggerated manifestations of the above-mentioned systemic effects, particularly if they occur within 2 hours after BCG instillation, or in the setting of traumatic catheterization, or too soon after TURB. In the extreme case, a picture resembling gram-negative bacterial sepsis may emerge with rapid and sequential appearance of skin mottling, chills, rigors, high temperatures (often over 39.5°C), and hypotension likely as a result of high levels of cytokines released directly into the bloodstream (the so-called cytokine storm).[642,643] The estimated incidence of this life-threatening event may be as high as 0.4%, and several deaths have been reported.[640,644,645] Fevers that persist more than 48 hours, or relapse in a diurnal pattern (usually in the early evenings) following the cortisol cycle are more indicative of BCG infection than a noninfectious process. Prompt fluid resuscitation measures should be instituted, and antipyretics, anti-TB antibiotics, and systemic steroids have been shown to be life-saving in such instances.[643,646] These patients should undergo treatment with rifampin 600 mg PO daily, isoniazid 300 mg PO daily, pyridoxine 50 mg PO daily, ethambutol 1,200 mg PO daily, and prednisolone 40 mg IV daily that is tapered over a 2- to 3-week period after the sepsis has resolved. Ethambutol is continued for 2 months and rifampin, isoniazid, and pyridoxine are continued for 6 months.

TB drugs should be continued for 3 to 12 months, depending on the severity of the presenting illness. Liver enzyme monitoring is required for isoniazid (INH) and rifampin. Other noninfectious systemic side effects of BCG may be related to an immune hypersensitivity state. Minor examples include arthralgias and skin rashes accruing in 5% to 6% of patients.[570] These are typically self-limiting and provider judgement should be used in deciding whether treatments should continue. However, more severe cases involve polyarthritis, Reiter's syndrome (urethritis, arthritis, conjunctivitis), and frank anaphylactic reactions.[640,647,648] These require immediate and permanent cessation of further therapy, along with steroid therapy.

5.9.4.3 Methods to prevent or minimize bacillus Calmette-Guérin complications

The serious infectious side effects of BCG are best prevented by careful adherence to prescribed technique. Several mechanisms to reduce the risk for BCG complications are well known. First, catheter placement must be atraumatic, and treatment should be withheld in the event of any gross blood or severe pain. At least 1 week but preferably 2 to 3 weeks should elapse after TURB before initiation of BCG. Urethral dilation should not be performed immediately prior to BCG instillation. BCG should never be administered under high pressure, but ideally dripped into the bladder under gravity. Caution should be exercised in treating immunosuppressed patients with BCG. Patients on low-dose oral or inhaled steroids have been successfully treated, as have a few transplant patients on stronger antirejection medications.[649,650] However, there have been documented cases of reactivation TB or BCG sepsis in immunocompromised patients.[651,652] Patient with cystitis symptoms should be investigated with UA and/or culture. To reduce the risk of inducing a sustained BCG cystitis, BCG should be delayed if bacteriuria is present or if symptoms are moderately severe.[653]

Adjustments to the BCG regimen may help reduce its local and systemic toxicity. Dose reduction of BCG has been studied to various levels down to one-sixth of standard dose. Results are mixed—in some studies a 50% to 75% reduction in BCG dose results in a 30% to 50% drop in serious morbidity without a significant impact on anticancer efficacy.[654,655] With further dose reduction to one-sixth of standard dose, a significant suboptimal cancer control has been observed and the morbidity improvement with the dose reduction from one-third dose is not significant.[656] Some studies have shown a lack of reduction in morbidity with dose reduction; however, these studies may represent patients in BCG-naïve populations in North America during initial therapy for high-grade papillary disease or CIS.[657,658] However, dose reduction may be useful during reinduction and/or maintenance therapy, when dropout rates from toxicity are higher. Validation studies have not yet been performed; however, small studies have been published regarding reduced dwell time to 30 minutes or spreading out treatments to every other week.[659,660] Prophylactic INH has not been shown to diminish either the associated symptomatology or the incidence of serious BCG infection, but it has been shown to transiently elevate liver function enzymes. Therefore, prophylactic INH is not recommended.[661] Administering 200 mg of ofloxacin 6 and 18 hours after each BCG treatment, however, significantly decreased by 18.5% the incidence of moderate and severe AEs resulting in better compliance with full BCG treatment.[662] What is unclear is the long-term effect on BCG efficacy, as well as the long-term safety of recurrent doses of prophylactic ofloxacin.

5.9.4.4 Side effects of interferon alpha therapy

IFN alpha is a large protein with a molecular weight close to 20,000 Da; therefore, minimal absorption occurs with intravesical instillation. Doses in the range of 50 to 100 million units (MU) are administered on a weekly schedule. With IFN alpha no dose-limiting toxicity has been seen, even with doses as high as 1,000 MU.[663] Systemic side effects include fever and a flu-like syndrome of fatigue that occurs in under 15% of patients. No therapy is described for management of side effect of IFN alpha. Symptoms are self-limiting and resolve upon cessation of therapy.

5.9.4.5 Combined bacillus Calmette-Guérin plus interferon alpha therapy

A theoretical advantage of combined BCG plus IFN alpha exists in that IFN alpha may elicit a more productive cell-mediated T-helper type 1 immune response.[664] While the theoretical advantage of BCG plus IFN alpha is present, it has not played out in the limited clinical data available at this point. A four-armed trial designed to evaluate the efficacy of megadose vitamins, as well as BCG versus BCG with IFN alpha in BCG-naïve patients did not show any advantage to adding IFN alpha. After a period of 24 months, median RFS was similar in all four groups. The groups getting BCG with IFN alpha did have higher incidence of fever and constitutional symptoms, which is consistent with the known side effects of IFN alpha.[665] Another trial looked at tolerability and toxicity data of 490 patients, comparing the half that was BCG-naïve who received standard-dose BCG plus IFN alpha (50 MU) to prior BCG-failure patients who received one-third dose BCG plus IFN alpha (50 MU).[666] This trial showed a very low rate of treatment delay (4%) and a very low dropout rate (3%). Oncological outcomes did not seem to be compromised. Therefore, low-dose BCG (one-third the standard dose of BCG) when added to IFN seems to be an acceptable treatment strategy for patients not tolerating standard BCG treatments. It may allow some patients who are not able to tolerate full-dose BCG therapy to undergo maintenance therapies, who would otherwise not tolerate maintenance.

5.9.5 Summary

Intravesical therapy with cytotoxic chemotherapy or immunotherapeutics share several common features of inducing local toxicity in the form of chemical or inflammatory cystitis. In the chemotherapy group, this is more prevalent with known vesicant agents and during long-term therapy with high drug concentrations. Unrecognized bladder perforation can exaggerate these toxicities, leading to deadly consequences. In the case of BCG, attention must be given to avoiding serious infections associated with improper catheter placement and patient selection. Prompt recognition and specific therapy are required to avoid potentially lethal septic complications. Additional vigilance is required to recognize hypersensitivity immune reactions and in preventing local toxicity from escalating to serious levels. Having knowledge of the management of these side effects will help maximize the utility of intravesical therapies.

Summary of Recommendations

1. Recommendations for treatment of mitomycin side effects:
   a. If UA and cystoscopy are completely normal and symptoms are mild, starting with conservative measures such as timed voiding should be performed. If this is unsuccessful, an oral antihistamine can be attempted for mild symptoms. If this is unsuccessful, anticholinergic medications and alpha-blockers are attempted (Expert Opinion).
   b. For cases of moderate to severe unremitting symptoms, we have observed more success with moving directly to treatment with oral prednisone taper, typically: 40 mg x 4 days, 20 mg x 4 days, 10 mg x 7 days, 5 mg x 7 days, and 2.5 mg x 7 days. Others have suggested 60 mg prednisone for 2 weeks followed by 40 mg for 1 day, 30 mg for 1 day, 20 mg for 1 day, and 10 mg for the last day. This is taken alongside an antihistamine such as PO Benadryl. If this is unsuccessful, a 4-week course of 60 mg of prednisone can be attempted prior to the same 4-day taper **[GOR C]**.
   c. If any intravesical stones are visualized, complete stone removal should be performed, as it may become a nidus for prevention and prevent healing of urothelium (Expert Opinion).
2. Recommendations for treatment of unrecognized bladder perforation and instillation of intravesical anthracyclines:
   a. Maximize bladder drainage with a large (24 French, if possible) Foley catheter (Expert Opinion).
   b. If patient does not respond quickly to conservative management, exploratory laparotomy with bladder repair, placement of intraperitoneal drains, running of the small bowel, and examination of the colon/rectum with consideration of diverting colostomy are indicated (Expert Opinion).
   c. Perform cystogram prior to removal of Foley catheter (Expert Opinion).
3. Recommendations for treatment of gemcitabine side effects:
   a. Alkalization of the urine helps minimize irritation of gemcitabine, as the gemcitabine solution has a pH of 2.5 (Expert Opinion).
   b. Naproxen (250 mg) 2 hours prior to instillation and 250 mg in the evening after instillation will significantly lessen cystitis symptoms (Expert Opinion).
   c. At the time of treatment and 8 hours later, 4 to 8 mg of oral ondansetron (Zofran) are given to all patients with a history of nausea or vomiting with previous intravesical gemcitabine treatments (Expert Opinion).
   d. Oxybutynin 5 mg three times a day PRN is given for patients who develop urinary frequency while undergoing gemcitabine treatments (Expert Opinion).
   e. In patients who have difficultly retaining the treatment for the recommended 1.5 to 2 hours, they are instructed to take 5 mg of oxybutynin 1 hour prior to their planned treatment time and to minimize fluid intake during that hour (Expert Opinion).
6. Recommendations for general intravesical side effects:
   a. Patients are instructed to avoid caffeine the morning of the treatment and to minimize fluid consumption prior to the treatment (Expert Opinion).
   b. In patients with bladder spasms despite taking anticholinergics, we premedicate 1 hour prior to treatment with 2 tablets of 5 mg oxycodone/325 mg acetaminophen and 10 mg of Valium (Expert Opinion).

c. For patients with a small-capacity bladder, we employ split-dosing of all drugs. Thus, we instill half the medication for half the time, drain the bladder, and instill the remaining half of the medication for the remaining half of the time (Expert Opinion).

d. For patients with significant pain and spasticity associated with instillation, we will use 40 cc of 2% lidocaine mixed with 4 cc of sodium bicarbonate 8.4% instilled 10 to 15 minutes prior to instilling the drug. We then drain the lidocaine-sodium bicarbonate mixture immediately before administering the first drug (Expert Opinion).

5.10 Role of Alternative Therapies

NMIBC has a high prevalence resulting in frequent therapies.[667] Alternatives to the standard intravesical treatments have been developed and evaluated to increase bladder preservation and quality of life. This paragraph focuses on the most promising alternative device-assisted techniques.

5.10.1 Hyperthermia

HT can be radiofrequency (RF)-induced—either externally or intravesically—or achieved by heat conduction using recirculation of extracorporeally heated fluid.[668]

5.10.1.1 Intravesical radiofrequency-induced chemohyperthermia

The majority of the available evidence for CHT is based on the intravesical RF-induced chemohyperthermia technique. Currently, the only device that applies this technique is the Synergo® system (see **Figure 5-4**).[669]

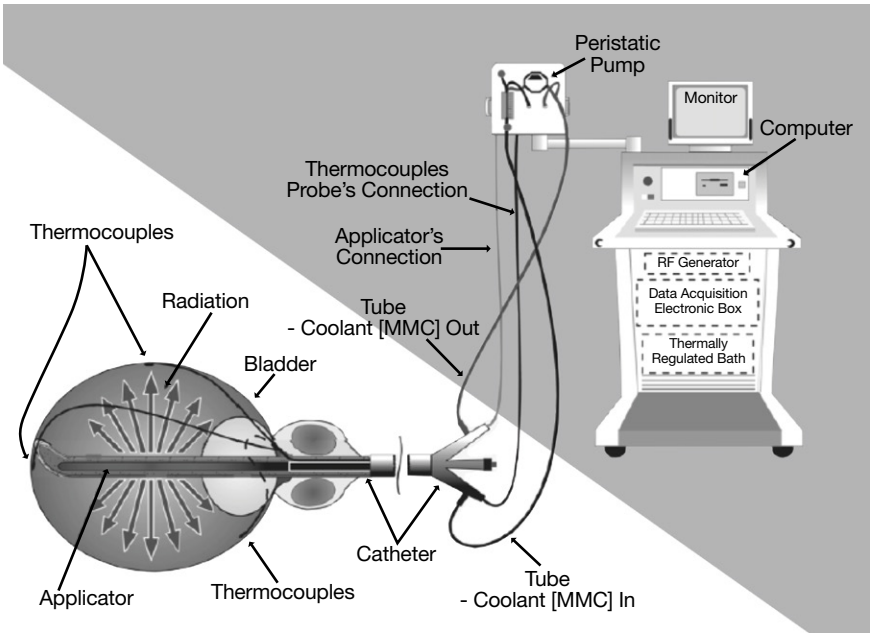
Pooled efficacy results of two prospective studies, one retrospective study in intermediate-high–risk NMIBC patients, and one retrospective study in low-risk patients show a recurrence rate of 28% (26/93) in intravesical RF-induced CHT versus 68% (67/99) for conventional cold MMC instillations (median follow-up >24 months).[670,671] Meta-analysis based on these four studies showed an overall risk ratio of 0.41 (95% CI: 0.290–0.579), meaning the risk for recurrence after intravesical RF-CHT is 59% lower compared to cold MMC.[670] CR rates in the two retrospective studies have shown to be 66% (38/58) and 22% to 28% (10/36 and 5/23) for RF-CHT and cold MMC, respectively.[672,673] After a median follow-up of 90 months in the two prospective studies, the bladder preservation rate was 86% in RF-CHT treated patients (n=83), whereas progression was seen in 6% of patients (79% and 8% for cold MMC, respectively, p>0.05 in both). The OS was 83% for RF-CHT and 78% for cold MMC (p>0.05).[674,675]

FIGURE 5-4
Schematic of the SB-TS 101 Synergo System

A 915-MHz intravesical RF applicator delivers HT by direct bladder wall irradiation. The bladder mucosal temperature is measured by a thermocouple set. Two additional thermocouples measure the temperature in the proximal urethra.

Abbreviation:
HT, hyperthermia; MMC, mitomycin C; RF, radiofrequency.

Reprinted, with permission from S. Karger AG (Basel), from Colombo R, Brausi M, Da Pozzo L, et al. Thermo-chemotherapy and electromotive drug administration of mitomycin C in superficial bladder cancer eradication. a pilot study on marker lesion. Eur Urol. 2001;39(1):95–100.[672]



In an RCT comparing intravesical RF-CHT with BCG in 190 intermediate-high–risk NMIBC patients, the 24-month RFS was 82% in RF-CHT compared to 65% in BCG (p=0.02).[676] Progression was described in <2% in both groups (p>0.1).

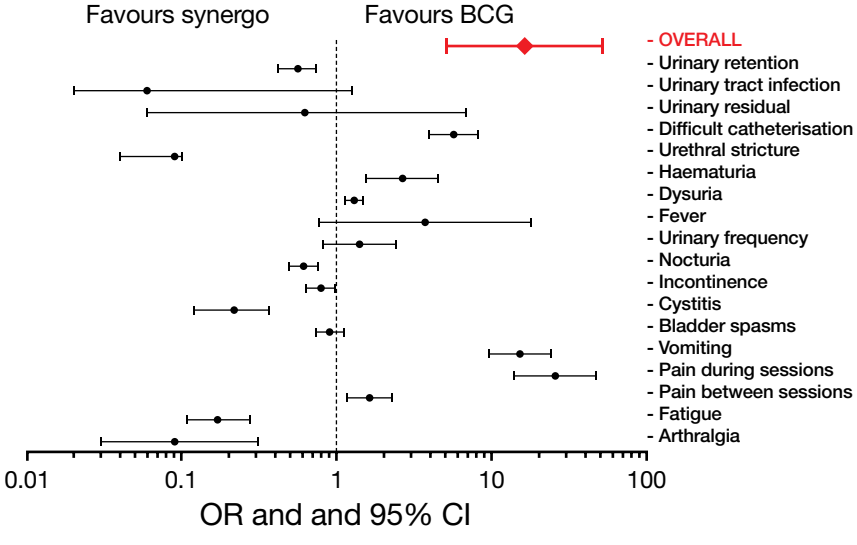
Foremost pain in the pelvic region during treatment and asymptomatic intravesical posterior wall thermal effects after treatment are seen in RF-CHT. Compared to BCG treatment, the type of AEs differ, but not the incidence (see **Figure 5-5**).[676]

FIGURE 5-5
Forrest Plot of Transient Adverse Events

ORs and 95% CIs of adverse events on a log10 scale.

Abbreviations: BCG, bacillus Calmette-Guérin; CI, confidence interval; OR, odds ratio.

Reprinted, with permission from Elsevier, from Arends TJ, Nativ O, Maffezzini M, et al. Results of a randomised controlled trial comparing intravesical chemohyperthermia with mitomycin C versus bacillus Calmette-Guerin for adjuvant treatment of patients with intermediate- and high-risk non-muscle-invasive bladder cancer. Eur Urol. 2016;69(6):1046–1052.[676]



5.10.1.2 External radiofrequency-induced chemohyperthermia

In external RF-induced CHT technique, the RF is applied to the bladder from outside of the body (deep external HT, e.g. AMC 70 MHz system and BSD-2000 device).[668]

No comparative studies with either MMC alone or BCG exist. Efficacy is based on two pilot studies showing a 2-year RFS of 78% in intermediate- and high-risk NMIBC (n=18) using the AMC 70 MHz system, and a 15-month RFS of 33% using the BSD-2000 device in BCG-refractory patients (n=15).[668,677,678]

The most common AEs reported were local abdominal and skin pain due to heat treatment (24%–33%), discomfort due to treatment position (20%), and bladder spasms and irritative urinary symptoms (21.7%–27%).[668]

5.10.1.3 Conductive chemohyperthermia

Conductive CHT consists of circulation of fluid that is heated outside the body, such as with the UniThermia[679] or Combat BRS systems.[680]

As with external RF-CHT, no randomized comparative trials exist. An extended pilot study in intermediate- and high-risk NMIBC patients showed a CR of 62.5% with a 4-year RFS of 79.2% using the Combat BRS device.[680] In the adjuvant treatment group, a 2-year RFS of 87.5% was reported. The UniThermia system was used in two studies: 1) a phase 1/2 study[679] in 34 patients with Ta-T1, G1-G2 NMIBC recurrence after BCG induction showed an RFS of 65% after 41 months of follow-up, and 2) a retrospective study[681] in 40 patients with high-risk NMIBC undergoing adjuvant conductive CHT (excluding CIS or intravesical therapy <1 year) showed an RFS of 61% after 24 months of follow-up.

The most common adverse events were irritative lower urinary tract symptoms (40%), bladder spasms (32.5%), genitourinary pain (27.5%), hematuria (22.5%), and UTI (22.5%), and were comparable for both systems.[680]

5.10.2 Electromotive drug administration

EMDA is another method to enhance drug delivery through the bladder urothelium by creating an electrical gradient between chemotherapeutic agent and bladder wall (see **Figure 5-6**).[672]

Di Stasi *et al.* reported three studies on EMDA. In the first study MMC only, EMDA-MMC, and BCG was compared in patients (n=108) with multifocal CIS, including 98 with T1 tumours. They found a CR in 31%, 58%, and 64%, respectively.[682] Median times to recurrence were 20, 35, and 26 months, respectively. Side effects in EMDA patients were more than in MMC-only patients, but fewer than in BCG. Peak plasma MMC was significantly higher following electromotive MMC than after passive MMC (43 vs. 8 ng/mL).

In the second study, an RCT in stage T1 patients, maintenance BCG (n=105) was compared to maintenance BCG/EMDA-MMC (n=107).[683] Patients treated with BCG/EMDA-MMC had a higher disease-free interval (69 vs. 21 months, p=0.001), lower recurrence rate (42% vs. 58%, p=0.001), and lower progression rate (9% vs. 22%, p=0.004) than patients treated with BCG.

The third study randomized 374 patients with primary NMIBC (Ta-T1) between TURBT alone (n=124), a single post-TURBT MMC (n=126), and pre-TURBT EMDA-MMC (n=124). The disease-free interval was 12, 16, and 52 months, respectively (p<0.0001).[684] Recurrence rates after 86 months of follow-up were 64%, 59%, and 38%, respectively (p<0.0001). Authors concluded that pre-TURBT EMDA-MMC was very effective and a safe procedure.

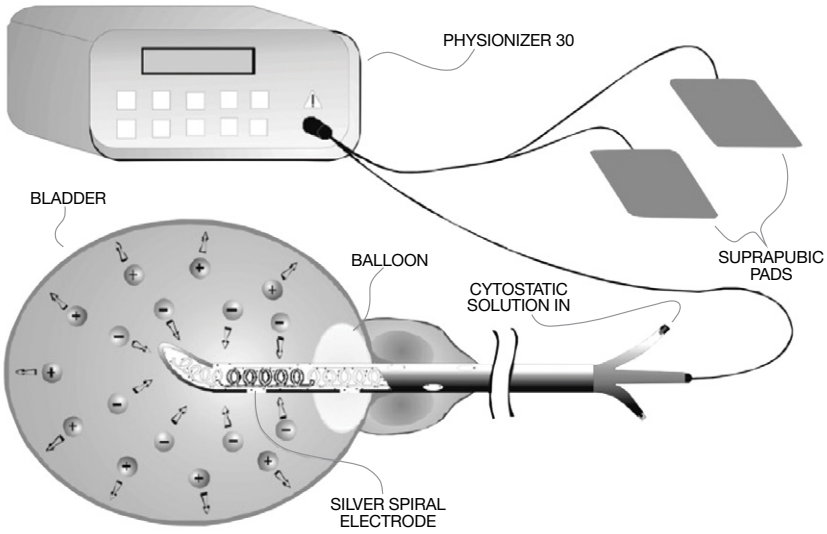


FIGURE 5-6
Schematic representation of the EMDA administration system.

Transurethral catheter containing a spiral silver electrode (anode) and suprapubic electrode pads (cathode). Current source battery powered (12 V) Physionizer 30.

Reprinted, with permission from Elsevier, from Colombo R, Brausi M, Da Pozzo L, et al. Thermo-chemotherapy and electromotive drug administration of mitomycin C in superficial bladder cancer eradication. a pilot study on marker lesion. Eur Urol. 2001;39(1):95–100.[672]

5.10.3 (Chemo)radiation

Radiotherapy, especially when combined with chemotherapy, seems promising in selected patients with T1G3 NMIBC or minimally muscle-invasive disease (i.e. stage T2). Typically, external radiotherapy is used, either alone or in combination with systemic chemotherapy.[685] However, no published reports on efficacy of (chemo)radiation after BCG in NMIBC patients exist.

A phase 3 randomized trial in 210 BCG-naïve T1G3 patients divided those with single tumours after TURBT (n=77) over radiation monotherapy (n=39) versus observation, and those with multiple tumours (n=133) over radiation monotherapy (n=65) or intravesical BCG or MMC.[686] No significant differences were found for RFS (HR, 1.07), PFS (HR, 1.35) or OS (HR, 1.32), although methodology was suboptimal and radiation was used without chemotherapy.[685] Another group reported on 141 high-risk patients (60% T1G3, 40% TG1/2 and CIS, multifocality, size >5cm, or multiple recurrences) after radical TURBT and subsequent platinum-based chemoradiation as primary treatment (n=113).[687] About 20% received radiotherapy only (n=28). A CR rate at 6 weeks by restaging TURBT of 87% versus 82% was reported (p=0.97). Salvage cystectomy was performed in case of failure (n=4/16) or relapse (n=16/49), resulting in a 5-year progression rate of 37% for radiotherapy and 14% for chemoradiation (p=0.09), which is comparable to rates after BCG.[667] The 5-year DSS was 68% versus 85%, respectively (p=0.03). No numbers were reported for OS after radiotherapy versus chemoradiation separately. The 10-year DSS and OS rates of all 141 patients taken together were 73% and 51%, respectively, similar to many series of primary cystectomy.[685,687] Based on reviewed

literature and reanalyzed data in a later paper from the same authors, cisplatin-based chemoradiation seems to result in better local control rates (57% at 5 years, n=93) compared to radiation or carboplatin-based chemoradiation (37% at 5 years, n=48, p=0.02).[688]

Regarding AEs, (chemo)radiation seems to be a safe treatment.[685-687,689] Radiation combined with chemotherapy has shown to have low rates of late grade 3 pelvic toxicity (7% at 5 years in one study), and appears to have low impact on long-term bladder function and quality of life.[685]

5.10.4 Summary

Intravesical RF-CHT is an effective and promising treatment option in BCG-unresponsive and highly recurrent intermediate- and high-risk NMIBC patients **[LOE 1b]**. For external RF-CHT and conductive CHT, limited evidence is available. However, current literature **[LOE 3]** suggests that these techniques are safe and effective.

EMDA-MMC is an effective alternative treatment option in BCG-naïve NMIBC patients **[LOE 1b]**. It appears to be safe and can be given pre-TURBT or combined with BCG in a maintenance schema to improve disease-free intervals and recurrence rates **[LOE 1b]**.

Although chemoradiation seems to be a good and safe option for high-risk NMIBC patients **[LOE 1B]**, no studies yet have been performed in BCG-treated patients. Thus, at this point it can only be considered as an alternative treatment option in patients unfit or unwilling to undergo RC if BCG is not available or is contraindicated **[LOE 1b]**. Radiation monotherapy proved not superior to other conservative treatment strategies **[LOE 1b]**.

Summary of Recommendations

Recommendation	GOR
Offer RF-induced CHT to NMIBC patients who failed on BCG treatment and are unfit or unwilling to undergo RC.	B
Offer treatment with RF-induced CHT or EMDA in patients with intermediate- to high-risk NMIBC if BCG is not available.	B
Offer chemoradiation in patients with high-risk NMIBC who are unfit or unwilling to undergo RC, only if BCG is not available or is contraindicated.	B

5.11 Indications for Timely Cystectomy: Balancing Risks and Benefits

As previously outlined, NMIBC presents with a wide range of disease aggressiveness and risk for progression and metastasis. The treatment goal of any malignancy is to optimize cancer control while limiting detrimental side effects that may limit patient quality of life. These goals are especially prescient in the treatment of NMIBC, given the variable risk for disease progression coupled with a broad range of therapeutic treatment options. While RC is the treatment of choice for MIBC,[690] its use in cases of NMIBC has the potential to provide excellent cancer control, accurate staging, and improved survival when deployed in a timely manner. When appropriately balanced with risks associated with an invasive and potentially morbid operation along with long-term changes in quality of life, early RC has a clear role in the management of NMIBC, though its ideal and proper deployment remains difficult.

NMIBC unfortunately has an estimated 10% to 20% chance of progressing to muscle-invasive disease during follow-up.[691] Multiple studies demonstrate that increased depth of invasion, LVI, concomitant CIS, increased size, and variant histologies place patients at higher risk for recurrence, progression, and disease-related mortality.[692–697] Thankfully, early cystectomy is potentially curative in patients with high-grade T1 disease, as 5-year cancer specific survival rates approach 90%.[695] Furthermore, 5-year OS of patients with organ-confined disease on pathological review following RC is approximately 80%.[690]

While no randomized trials exist evaluating early cystectomy versus intravesical treatment with delayed cystectomy, multiple retrospective studies (with varying degrees of bias) have demonstrated improved survival rates with early RC. Herr *et al.* retrospectively evaluated 90 patients with high-grade NMIBC who ultimately underwent RC. Fifteen-year cancer-specific survival was improved in patients who had RC within 2 years of BCG initiation (69%) compared to those who had it after 2 years (26%).[698] In a comparison of the same historical cohort with a more contemporary cohort, in which immediate RC was utilized in 50% of patients with recurrent high-grade T1 NMIBC, 5-year DSS was improved in the contemporary group (72% vs. 52%, respectively).[699] Denzinger *et al.* evaluated 105 patients with high-risk disease who were offered early RC (compared to delayed RC after recurrence). Fifty-four patients (51%) opted for early RC, and the 10-year cancer-specific survival for this group was 78% compared to 51% for the delayed group.[700] Hautmann *et al.* evaluated 175 patients with high-risk disease who underwent RC immediately following TURBT compared to 99 who ultimately underwent RC for recurrent T1 disease. They noted a 79% cancer-specific survival rate at 10 years in immediate RC patients compared to 65% in those with delayed intervention.[701] Stöckle *et al.* examined similar populations and showed a 90% 5-year cancer-specific survival rate with immediate RC for T1 disease compared to 62% for delayed RC.[702] Jäger *et al.* demonstrated that patients who underwent RC within 6 to 12 months of initial TUR had improved 10-year survival (79%) compared to those who had cystectomy a year after resection (61%).[703] Notably, two retrospective studies did not demonstrate survival benefits when comparing high-risk NMIBC patients who underwent early cystectomy to patients managed conservatively.[704,705] In both studies, however,

the conservative management group included patients who responded to BCG and did not undergo delayed cystectomy, thereby decreasing the power of the studies to detect differences seen between immediate and delayed cystectomy.

The above studies focus on patients who are at increased risk for disease progression; however, it is worth noting that even patients with low-risk NMIBC who progress to invasive disease may have a poor prognosis. In a study of 699 patients with low-grade Ta bladder cancer, Linton *et al.* found that 2.4% of patients died of bladder cancer at a median 61-month follow-up, including 13 of 14 patients who progressed to muscle-invasive disease.[706] This is similar to the cohort described by Rieken *et al.*, in which 5-year cancer-specific mortality was estimated to be 2% in patients with Ta grade 1 bladder cancer. Advanced age, prior recurrence, and male sex were associated with cancer-specific mortality in this population.[707]

In addition to potential survival benefits, early RC offers the advantage of improved NMIBC staging. The determination of muscle invasion on pathological specimens is not straightforward, and muscularis mucosa can be scattered and/or discontinuous in over 80% of specimens.[708] In fact, studies suggest that over 20% of patients diagnosed with T1 NMIBC are incorrectly staged.[704,690] Similarly staged patients have a high rate of upstaging at RC, with 25% to 50% harboring T2 or higher disease.[690,709,710] High-risk patients are additionally at risk for lymph node metastases, with up to 15% of patients found to have lymph node–positive disease after extirpative therapy.[690,711,712] Improved staging is particularly important in patients with pathological variants, such as micropapillary disease, where understaging and occult metastatic disease can occur more frequently.[712,713] Many of these variants, including sarcomatoid,[714] plasmacytoid,[715] and micropapillary,[713] present with locally advanced disease and metastases. Therefore, consideration of cystectomy at a time when cure may be possible is essential.[716] Given the benefits in terms of staging and cancer control seen with early cystectomy, it is surprising that treatment is often delayed in patients with high-risk disease. In one survey, 80% of American urologists would not recommend RC to a patient who had NMIBC that was refractory to two treatments of induction intravesical therapy.[717]

Much of this hesitation likely stems from risks associated with RC, regardless of timing. A major downside of RC for NMIBC is the exposure of patients to an aggressive, invasive treatment that may alter quality of life when compared to conservative management. It is well established that RC is a morbid operation, with published contemporary series demonstrating a 30% or higher rate of short-term complications and approximate 3% risk for 30- to 90-day mortality following surgery.[718,719] A historical series examining RC in patients with NMIBC only showed similar rates of morbidity and mortality.[720] Regarding quality of life, initial surveys of patients focusing on quality of life in those managed with intravesical strategies compared to RC revealed a lack of sexual activity and worsened physical condition in patients who underwent RC.[721] While many of the quality of life changes associated with RC are likely relegated to the immediate postoperative period and are expected to improve over time, patients continue to report poor urinary and sexual function when compared to the general population during long-term follow-up.[722] These differences may be mitigated, though, through the use of continent diversion techniques, particularly given that patients who receive these types of urinary diversions report improved emotional function and body image compared to those with incontinent diversion.[722] In fact, one study demonstrated that measures of well-being were similar in patients who underwent orthotopic neobladder diversion compared to a matched control

population who had not undergone RC (despite increased prevalence of urinary leakage, need for catheterization, and UTI).[723] Advanced modelling techniques even suggest that young patients with aggressive NMIBC may benefit in terms of quality-adjusted life expectancy with immediate cystectomy compared to conservative treatment with surveillance, intravesical therapy, and delayed cystectomy.[724] While the challenge to optimize quality of life after RC remains, improvements such as those realized by advanced surgical techniques and innovations in perioperative management (such as the use of enhanced recovery protocols[725]) only further limit the downsides of early RC for NMIBC.

Therefore, while the downsides of potential morbidities, mortality, and changes in quality of life associated with RC give pause to those considering early cystectomy for NMIBC, there is a clear role for its use in providing disease control for this population, particularly in those at high risk for progression. While no evidence demonstrating clear superiority of RC compared to intravesical therapy and delayed RC exists, there are enough data to suggest potential mortality benefits in patients with aggressive NMIBC that consensus exists to consider early cystectomy with lymph node dissection for these patients.

Summary of Recommendations for Timely Cystectomy

Recommendation	LOE	GOR
1. In patients who are operative candidates and have T1 disease with high-risk features (e.g. concomitant CIS, LVI, deep lamina propria invasion) or persistent/recurrent T1 disease at re-resection, clinicians should consider initial RC.	3	C
2. In patients with recurrent high-grade T1 disease after a single course of induction BCG, clinicians should offer RC.	3	C
3. In patients with pure variant histology (micropapillary, sarcomatoid, or plasmacytoid), clinicians should offer initial RC.	3	C

5.12 Surveillance Strategies for Nonmuscle-invasive Bladder Cancer

While patients with NMIBC have favourable survival outcomes, the risk for disease recurrence and progression to MIBC necessitate timely and appropriate surveillance strategies. NMIBC is composed of a clinically heterogeneous group of cancers and is consequently associated with a wide range of recurrence and progression risks that depend on several clinical and pathological factors. For example, low-grade Ta lesions recur at a rate of 31% in 5 years, while high-grade T1 lesions recur in up to 78% of patients and progress to muscle-invasive disease in 17% of patients within 5 years.[726–728]

The probabilities of recurrence and progression can also vary according to use of adjuvant intravesical therapies. As a result, risk-adjusted surveillance strategies that reflect an individual patient's risk for recurrence and progression should be utilized.

Surveillance for NMIBC has historically relied on the diagnostic combination of cystoscopy and urinary cytology. Most protocols include this combination every 3 to 6 months for 2 years after the initial diagnosis, then every 6 to 12 months for the following 2 years, and then annually thereafter, resetting the clock with each newly identified tumour.[726,727,729] More recently, novel urinary markers and enhanced cystoscopy techniques have also been evaluated to augment these conventional methods. In this chapter, we evaluate current and emerging surveillance strategies for NMIBC.

5.12.1 Surveillance cystoscopy and enhanced cystoscopy techniques

Office-based cystoscopy allows for diagnostic visualization of bladder urothelium and identification of papillary lesions. While there has been a recent emergence of new tumour markers and enhanced endoscopic techniques, cystoscopy can readily identify the site and characteristics of most tumours and remains the hallmark of surveillance. There is a high positive predictive value with cystoscopy, as most lesions believed to be malignant are subsequently proven so pathologically.[726] Combined with the use of intraurethral local anesthetic lubricant, cystoscopy allows for diagnostic evaluation of the majority of patients' bladder and urethra with minimal discomfort and is the gold standard in cancer surveillance.[730]

While enhanced cystoscopy is being utilized in the detection of malignant tumours, its use in surveillance of urothelial carcinoma is limited in practice. Data on both fluorescence cystoscopy and NBI in the surveillance setting are limited. Both methods of enhanced cystoscopy are also discussed in additional length in another chapter of this publication.

5.12.1.1 Fluorescence cystoscopy

Cystoscopy and TURBT are conventionally performed using white light. The use of WLC, however, can lead to missing lesions that are present but not visible or indistinguishable from inflammation, such as CIS, under white-light. Use of PDD, or fluorescence-guided cystoscopy, can help improve the detection of malignant tumours, particularly CIS, compared to conventional procedures[731,732] **[LOE 2a]**. PDD is performed using violet light after intravesical instillation of 5-aminolevulinic acid (ALA) or hexaminolevulinate acid (HAL). 5-ALA is converted to protoporphyrin IX, a precursor of heme, which tends to accumulate in malignant cells. As a result, cancers exhibit strong fluorescence and may be identified more easily.

Using this technology, both small papillary lesions and almost one-third more cases of CIS that are overlooked by cystoscopy can be identified.[726,733–735] In one phase 3 trial of 146 patients with suspected tumours, 96% of all tumours were detected with HAL imaging compared with 77% using standard WLC.[736] Detection was improved for dysplasia (93% vs. 48%), CIS (95% vs. 68%), and papillary tumours (96% vs. 85%).

Fluorescence cystoscopy using HAL has also been shown to decrease the risk for bladder cancer recurrence. A meta-analysis based on raw data of prospective trials reported an increase in detection of malignant lesions in HAL arms and an absolute reduction of <10% in recurrence rates within 1 year (35% vs. 45%; RR, 0.761; p=0.006)[737] **[LOE 1a]**. The benefit was independent of the baseline level of risk for recurrence and was evident in patients with primary or recurrent Ta, T1, and CIS lesions. As a result, clinicians may consider using fluorescence cystoscopy to increase detection of bladder lesions **[GOR B]**. The value of fluorescence cystoscopy for improvements in relation to progression rate and survival remains to be demonstrated.[729]

The use of PDD is covered in additional length in another chapter of this publication.

5.12.1.2 Narrow-band imaging

The use of NBI enhances the contrast between normal urothelium and hypervascular malignant tissue to improve the detection of urothelial carcinoma **[LOE 2b]**.[729] A meta-analysis by Zheng *et al.* found that NBI is an effective method for the identification of cancerous lesions, with a pooled sensitivity and specificity of 0.943 (95% CI, 0.914–0.964) and 0.848 (95% CI, 0.803–0.885), respectively.[738] A recent prospective, randomized, multicentre study compared recurrence rates following resection with either NBI-assisted TURBT or WLC TURBT. At 12 months, recurrence rates were not different between the groups (27.1% in WLC group vs. 25.4% in NBI group [p=0.585]). In patients with low risk for disease recurrence, however, the 12-month recurrence rates were significantly lower (27.3% in WLC group vs. 5.6% in NBI group [p=0.002]).[739] As a result, clinicians may consider using NBI to increase detection and decrease recurrence **[GOR C]**.

The use of NBI is also covered in additional length in another chapter of this publication.

5.12.2 Urine cytology

In addition to cystoscopy, use of urine cytology plays an important role in the surveillance of NMIBC. The use urine cytology involves the microscopic evaluation of voided urine or bladder-washing specimens for exfoliated cancer cells. It has been found to have a high sensitivity for detecting high-grade lesions (84%) but low sensitivity for low-grade (LG) tumours (16%).[740,741] The sensitivity for CIS detection is 28% to 100%[741] **[LOE 2b]**. Urine cytology also has a high specificity (>90%) for both low- and high-grade tumours, including CIS.[726,742] As a result, a positive reading, regardless of cystoscopic or radiographic findings, suggests the existence of malignancy in the vast majority of patients. One study found that even in the setting of negative diagnostic evaluation (cystoscopic and upper-tract evaluation), 41% of patients with persistently positive cytology were found to have a genitourinary cancer within 24 months, with a mean time to diagnosis of 5.6 months.[743] Cytology is therefore useful, particularly as an adjunct to cystoscopy, if HG/CIS malignancy is present. A positive voided urinary cytology can indicate the presence of a malignant lesion in the urinary tract; negative cytology, however, does not exclude the presence of a tumour.[726] The management of specific situations such as a positive cytology with negative cystoscopy and indications for prostatic urethral biopsies are discussed in other sections of this chapter.

Urine cytology, however, also has several drawbacks. Unlike tumour markers, urine cytology is not a laboratory test—it is a pathologist's interpretation of the morphologic features of exfoliated urothelial cells. As a result, cytology is often associated with a lack of interobserver consistency and a wide range of readings (e.g. atypical, atypical-suspicious, nondiagnostic).[727] As a result, the accuracy of cytology is dependent on the level of expertise of the pathologist.[742] In addition, cytologic evaluation can be hampered by low cellular yield, and inflammation secondary to infection, stones, or intravesical instillations can affect the accuracy of urine cytology.[727]

5.12.3 Urinary biomarkers

Several novel urinary biomarkers have been developed and investigated over the last three decades to complement or replace urine cytology. Current urinary markers have been developed to detect tumour-associated antigens, blood group antigens, growth factors, cell cycle/apoptosis, and extracellular matrix proteins. Several of these markers have been approved by the US Food and Drug Administration (FDA) and are commercially available in the United States.[744] The NMP22® and BTA® tests are protein-based, while UroVysion FISH and are cell-based. While most biomarkers have demonstrated adequate sensitivity, they are associated with poor specificity that can result in substantial false-positive readings and thus create the need for further diagnostic testing. The pooled sensitivity, specificity, and positive and negative likelihood ratios for current biomarkers are listed in **Table 5-10**.[727]

TABLE 5-10 Performance Characteristics of Commonly Used and FDA-Approved Urinary Markers

Marker	Sensitivity	Specificity	Pos. likelihood ratio (95% CI)	Neg. likelihood ratio (95% CI)
NMP22 quantitative*			3.05 (2.28–4.10)	0.40 (0.32–0.50)
Overall	69%	77%		
Diagnosis	67%	84%		
Surveillance	61%	71%		
NMP22 qualitative*			4.89 (3.23–7.40)	0.48 (0.33–0.71)
Overall	58%	88%		
Diagnosis	47%	93%		
Surveillance	70%	83%		
BTA quantitative*			2.52 (1.86–3.41)	0.47 (0.37–0.61)
Overall	65%	74%		
Diagnosis	76%	53%		
Surveillance	58%	79%		
BTA qualitative*			2.80 (2.31–3.39)	0.47 (0.30–0.55)
Overall	64%	77%		
Diagnosis	76%	78%		
Surveillance	60%	76%		
UroVysion FISH*			5.02 (2.93–8.60)	0.42 (0.30–0.59)
Overall	63%	87%		
Diagnosis	73%	95%		
Surveillance	55%	80%		
ImmunoCyt*			3.49 (2.82–4.32)	0.29 (0.20–0.41)
Overall	78%	78%		
Diagnosis	85%	83%		
Surveillance	75%	76%		
Cxbladder	82%	85%	5.53 (4.28–7.15)	0.21 (0.13–0.36)

Abbreviations: BTA: bladder tumour antigen; CI: confidence interval; FISH: fluorescence in situ hybridization; NMP22: nuclear matrix protein 22.

*FDA-approved urinary biomarkers for bladder cancer.

Adapted from Chang SS, Boorjian SA, Chou R, et al. Diagnosis and treatment of non-muscle invasive bladder cancer: AUA/SUO guideline. J Urol. 2016;196(4):1021–1029.[146]

5.12.3.1 Protein-based urinary biomarkers

The NMP22 BladderChek® test (Matritech, Inc., Newton, MA) identifies NMP22, part of the mitotic apparatus released from urothelial nuclei upon cellular apoptosis. The protein is elevated in urothelial carcinoma and is released in dying urothelial cells. Similarly, the BTA test (Polymedco, Inc., Cortlandt Manor, NY) is a protein-based marker that identifies a basement membrane antigen that is related to complement factor H and is present within urine at higher levels in patients with bladder cancer. Like NMP22, the BTA test is available in both qualitative and quantitative formats. Both protein-based markers are FDA approved for the diagnosis and surveillance of bladder cancer. Some benign conditions, however, such as infection, inflammation, hematuria, and cystoscopy, can cause false positives, resulting in lower specificity than urine cytology.[727]

5.12.3.2 Cell-based urinary biomarkers

UroVysion (Abbott Molecular, Chicago, IL) is a cytology-based test that uses FISH of DNA probes to identify aneuploidy in chromosomes 3, 7, and 17 and alterations to the chromosome 9p21 locus. Cumulative data from comparative studies demonstrated sensitivity for cytology compared with FISH of 35% versus 64% for Ta, 66% versus 83% for T1, and 76% versus 94% for muscle-invasive carcinoma.[745] Notably, cytology detected only 67% of cases with CIS versus 100% detection by FISH. UroVysion has the highest specificity of the available tumour markers, and is not affected by hematuria, inflammation, or other factors that can cause false-positive readings with some tumour markers; this makes it useful as a marker of BCG response.[726,746]

ImmunoCyt (DiagnoCure, Inc., Sainte Foy, Canada) is a hybrid of cytology and an immunofluorescent assay. The test identifies three cell surface glycoproteins that are present on the membrane of cancer cells and can be used in conjunction with cytology to enhance the sensitivity of cytology.[727] In one study, the sensitivity of cytology for stages Ta, T1, T2 and over, and for Tis tumours was 12%, 67%, 47%, and 50%, while it reached 78%, 83%, 79%, and 100% when combined with ImmunoCyt.[747] It has not been shown to be affected by benign conditions, but interpretation is complex and operator dependent.

The Cxbladder™ (Pacific Edge Ltd., Dunedin, New Zealand) test is a laboratory-developed test and, as a result, does not require approval by the FDA. It identifies the presence of five mRNA fragments in the urine that are expressed at high levels in patients with bladder cancer.[748] One such fragment, CXCR2, is an inflammatory marker that helps discriminate against false-positive cases. This test appears to be able to distinguish between low- and high-grade tumours and may perform better than protein-based markers, such as NMP22 and BTA.[727] In one study, Cxbladder was found to have a sensitivity of 82%, including 97% for high-grade tumours and 100% for tumours stage I or greater.[748]

The role of urinary biomarkers as adjuncts to cystoscopy continues to be evaluated. Comprehensive literature analysis shows that cytology is highly specific and sensitive for detecting high-grade urothelial carcinoma.[749] While many urine markers exhibit promising sensitivity, particularly for lower-grade tumours, their specificity is still lower than that of urine cytology.[726,727] Furthermore, lack of prospective data to support the impact of urinary biomarkers on patient prognosis has limited their widespread adoption at this time. As result, and given the uncertainty in their sensitivity and specificity, urinary biomarkers cannot be used to replace cystoscopy **[GOR B]**.

5.12.4 Risk-adjusted surveillance strategies

NMIBC is composed of a clinically heterogeneous group of cancers and, due to the risk for disease recurrence and progression to MIBC, timely surveillance strategies are necessary. The frequency and duration of cystoscopy and imaging, however, should reflect the individual patient's degree of risk (see **Table 5-11; see Table 5-12 for risk-stratification for NMIBC**).

TABLE 5-11 Risk-Adjusted Surveillance Strategies

Risk category	Surveillance strategies following negative 3-month surveillance cystoscopy
Low risk	Cystoscopy 6–9 months later, and annually thereafter Consider cessation following 5 recurrence-free years No upper-tract imaging necessary unless hematuria present
Intermediate risk	Cystoscopy with cytology every 3–6 months for 2 years Every 6–12 months during years 3 and 4 Annually for lifetime thereafter Upper-tract imaging every 1–2 years
High risk	Cystoscopy with cytology every 3 months for 2 years Every 6 months during years 3 and 4 Annually for lifetime thereafter Upper-tract imaging every 1–2 years

TABLE 5-12 Risk Stratification for NMIBC

Risk category	Definition
Low risk	Solitary LGTa ≤3 cm PUNLMP
Intermediate risk	Solitary LGTa >3 cm Multifocal LGTa Solitary HGTa ≤3 cm*

Abbreviations: BCG, bacillus Calmette-Guérin; CIS, carcinoma in situ; HG, high grade; LG, low grade; LVI: lymphovascular invasion; PUNLMP, papillary urothelial neoplasm of low malignant potential.

*Considered high-risk in some clinical guidelines.

Adapted from Chang SS, Boorjian SA, Chou R, et al. *Diagnosis and treatment of non-muscle invasive bladder cancer: AUA/SUO guideline.* J Urol. *2016;196(4):1021–1029;*[146] *and Babjuk M, Bohle A, Burger M, et al. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016.* Eur Urol. *2017;71(3):447–461.*[116]

Risk category	Definition
High risk	HGTa >3 cm (or multifocal)
	Recurrent HGTa
	HGT1
	CIS
	BCG failure
	Any variant histology
	Any HG prostatic urethral involvement
	Any LVI

Abbreviations: BCG, bacillus Calmette-Guérin; CIS, carcinoma in situ; HG, high grade; LG, low grade; LVI: lymphovascular invasion; PUNLMP, papillary urothelial neoplasm of low malignant potential.

*Considered high-risk in some clinical guidelines.

Adapted from Chang SS, Boorjian SA, Chou R, et al. *Diagnosis and treatment of non-muscle invasive bladder cancer: AUA/SUO guideline.* J Urol. *2016;196(4):1021–1029;*[146] *and Babjuk M, Bohle A, Burger M, et al. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016.* Eur Urol. *2017;71(3):447–461.*[116]

The first surveillance cystoscopy for patients with NMIBC should be performed 3 months following TURBT, as it is an important prognostic indicator of recurrence and progression[729,750–753] **[LOE 1a; GOR A]**. Several studies have found that tumour status 3 months following resection is an important predictive factor for recurrence and progression. In a study by Palou *et al.* on 616 patients following TURBT with T1G2 bladder cancers, the principal prognostic factor to progression to muscle-invasive disease was recurrence at 3 months, with an RR for 4.0 (95% CI, 1.2–13.3).[750] In addition to providing prognostic information, visualization of the bladder following a short interval allows the treating urologist to verify that the initial resection was complete.[727] In a combined analysis of seven EORTC randomized trials (without re-TURBT), early tumour recurrence was observed in 13.1% (6.7%–40%) of 2,410 patients analyzed.[754] Thus, early surveillance cystoscopy also provides important prognostic information irrespective of initial risk grouping and can allow for prompt management of potential disease recurrence/progression.

5.12.4.1 Low-risk surveillance

For patients with low-risk disease and a negative initial surveillance cystoscopy, clinicians should perform subsequent surveillance cystoscopy 6 to 9 months later, and then annually thereafter **[GOR C]**. The risk for recurrence after 5 recurrence-free years is low, and further surveillance after 5 years in the absence of recurrence should be based on shared decision-making between the patient and clinician[727] **[LOE 3]**. A study by Mariappan *et al.* found that, of 115 patients with LGTa disease who did not have recurrence in 5 years, 98.3% remained tumour-free for 20 years.[753] In a study of 262 patients with nonmuscle-invasive bladder tumours without evidence of tumour recurrence for more than 5 years, however, Matsumoto *et al.* found disease recurrence in 39 tumours (14.9%), irrespective of risk grouping.[755] The median follow-up interval was 10 years in the study. After a 5-year tumour-free period, low-, intermediate-, and high-risk patients had the same degree of late-timepoint disease recurrence. This suggests that longer follow-up may be required, even for low-risk patients.

While data evaluating different surveillance regimens for low-risk disease are limited, one prior randomized study found no difference in the risk for recurrence or progression between a more frequent follow-up regimen (every 3 months for 2 years, every 6 months in year 3, then annually thereafter) compared to a less frequent one (every 6 months for year 1, then annually thereafter).[756] For patients with low-risk disease, less frequent surveillance that is limited to 5 recurrence-free years also has significant quality of life and cost implications. By limiting the number and duration of surveillance cystoscopies, patients are subject to less anxiety, discomfort, and risk for infection associated with the procedure.[727]

For patients with sub-centimetre papillary tumours found on surveillance cystoscopy, clinicians may offer office-based fulguration to decrease the therapeutic burden of resection under anesthesia[727,729,757-759] **[LOE 3]**. Alternatively, Soloway *et al.* presented the initial series of expectant management for these lesions and reported the feasibility and safety of this concept in patients with low-risk bladder cancer in 2003. In the study, 3 of 45 (6.7%) patients had tumour progression from a low-grade, noninvasive (TaG1 or G2) to a high-grade Ta or T1 tumour; there was no disease progression to muscle invasion of studied tumours.[760] Prospective, randomized trials comparing office-based fulguration to formal TURBTs under anesthesia have not been published.

5.12.4.2 Intermediate-risk surveillance

For patients with intermediate-risk disease and a negative initial surveillance cystoscopy, clinicians should perform subsequent surveillance cystoscopy with cytology every 3 to 6 months for 2 years, then every 6 to 12 months for the third and fourth years, and then annually thereafter[727,729] **[GOR C]**. There are no prospective studies comparing different cystoscopic surveillance regimens for patients with intermediate-risk disease and, given the increased risk for recurrence and progression, a more frequent surveillance regimen has been advocated.[727] In tumours with intermediate or high risk, recurrences after 5 recurrence-free years are not unusual, and lifelong surveillance is also consequently recommended[752,761] **[LOE 3; GOR C]**. A study by Holmäng *et al.*, for example, found that in 204 of 542 patients treated with BCG who were tumour-free for 5 years, 22 (10.8%) had evidence of late disease recurrence.[761] As a result, extended surveillance strategies are warranted in this category of patients. The management of specific situations, such as a positive cytology with negative cystoscopy and indications for prostatic urethral biopsies, are discussed in other sections of this chapter.

5.12.4.3 High-risk surveillance

For patients with high-risk disease and a negative initial surveillance cystoscopy, clinicians should perform subsequent surveillance cystoscopy with cytology every 3 months for 2 years, then every 6 months until year 5, and then annually thereafter[727,729] **[GOR C]**. As with intermediate-risk patients, there are no prospective studies that have evaluated whether less stringent surveillance regimens can be utilized without significantly affecting oncological outcomes in these patients.

5.12.4.4 Upper-tract imaging

A number of patients with NMIBC are at risk of developing upper-tract urothelial carcinoma; in patients with higher-grade, recurrent, or stage T1 disease, the rate of upper-tract recurrence is as high as 10%[762,763] **[LOE 3]**. A review by Hurle *et al.* of 591 patients with median follow-up of 86 months found upper-tract recurrence in 0.9% of low-risk patients (solitary, low-grade, low-stage Ta/T1), 2.2% in patients at intermediate risk (recurrent or multifocal disease), and 9.8% in high-risk patients

(high-grade, CIS, and intravesical chemotherapy failures).[763] Patients with high-risk disease treated with BCG can also experience upper-tract recurrence of up to 13%.[764] Furthermore, although infrequent, the appearance of upper-tract disease is associated with mortality rates of 40% to 70%.[726]

As a result, most reviews and guidelines have found that patients with intermediate- or high-risk disease should undergo upper-tract surveillance, even following adjuvant treatments. Proposed intervals of surveillance imaging with CT urography range from every 1 to 2 years[726,727] **[GOR C]**. Alternative options can include MR urography, retrograde pyelography, renal ultrasound, or foregoing upper-tract imaging, depending on the patient's comorbidities.

The table below lists recommendations for surveillance of NMIBC.

Summary of Recommendations for Surveillance of Patients With NMIBC

Recommendation	GOR
In a patient with NMIBC, clinicians should not use urinary biomarkers in place of cystoscopic evaluation.	B
For patients with low-risk disease and a negative initial surveillance cystoscopy, clinicians should perform subsequent surveillance cystoscopy 6 to 9 months later, and then annually thereafter for 5 years.	C
For patients with intermediate-risk disease and a negative initial surveillance cystoscopy, clinicians should perform subsequent surveillance cystoscopy with cytology every 3 to 6 months for 2 years, then every 6 to 12 months for the third and fourth years, and then annually thereafter.	C
For patients with high-risk disease and a negative initial surveillance cystoscopy, clinicians should perform subsequent surveillance cystoscopy with cytology every 3 months for 2 years, then every 6 months until year 5, and then annually thereafter.	C
For patients with intermediate- or high-risk disease, regular (every 1 to 2 years) upper-tract imaging is recommended.	C

5.13 New Treatment Strategies From Ongoing and Future Clinical Trials

5.13.1 Introduction

Of all incident bladder cancers, 75% first present as NMIBC. The majority of these tumours are low grade, thus making the low- and intermediate-risk categories of NMIBC amongst the most prevalent cancers managed by urologists.[765] High-grade NMIBC, on the other hand, is highly recurrent, with at least 50% of patients experiencing a relapse despite BCG therapy.[766] The management of BCG failures is complex and treatment options short of RC are limited. In fact, over the last 30 years, only three drugs have been approved for NMIBC by the FDA: TICE® BCG Connaught, and valrubicin.[767] Given the lack of new therapies in high-risk disease and the burden of therapy in low/intermediate-risk disease, unmet clinical needs exist for additional therapies for NMIBC patients. To address these needs, a number of novel clinical trials and treatment strategies are being explored in the NMIBC space.

5.13.2 Methods

Active and future clinical trials were determined via a search of ClinicalTrials.gov using the disease search terms "non-muscle invasive bladder cancer," "non muscle invasive bladder cancer," and "NMIBC." This yielded 103 trials at the time of writing this manuscript. Trials were then filtered to include those "not yet recruiting," "recruiting," "active, not recruiting," "enrolling by invitation," and "completed," resulting in 85 trials for possible inclusion.[768] Trials were categorized into those focused on low/intermediate-risk NMIBC and those focused on high-risk NMIBC, with high-risk BCG-naïve and BCG-failure strategies serving as the priority of this review. Only those studies evaluating new treatment strategies (as opposed to diagnostic or prognostic tests) were included. Omitted were those studies assessing radiation, thermotherapy/HT, and EMDA, as these have been addressed in a preceding section of this chapter. Conference abstract proceedings from recent (2016–2017) international meetings (e.g. EAU, AUA, Société Internationale d'Urologie [SIU], American Society of Clinical Oncology Genitourinary Cancers Symposium [ASCO GU], American Society of Clinical Oncology [ASCO]) were also searched for nonregistered studies. Only studies with the most relevance, clinical potential, and supporting data from human clinical trials are highlighted in this section. The ClinicalTrials.gov trial number is provided where possible. A summary of all trials identified is provided in **Figure 5-7**.

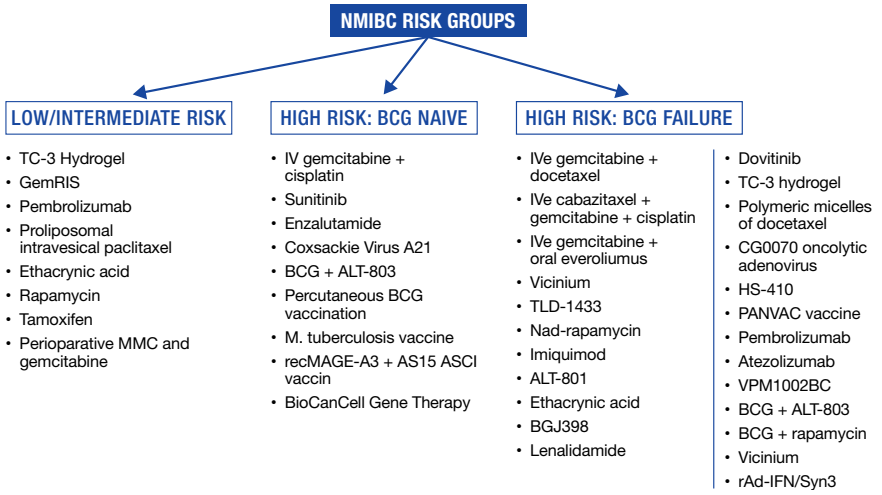


FIGURE 5-7
Risk-Adapted Novel Treatment Strategies in NMIBC

Abbreviations: ASCI, antigen-specific cancer immunotherapeutic; BCG, bacillus Calmette-Guérin; GemRIS™, gemcitabine-releasing intravesical system; IFN, interferon; IV: intravenous, IVe, intravesical, MMC: mitomycin C, NMIBC, nonmuscle-invasive bladder cancer.

5.13.3 Levels of evidence and grade recommendations

The majority of the trials listed below are not randomized studies, with most in the early phases. As a result, the levels of evidence supporting this section are 3b (case series without control groups) or 4 ("bench to bedside" or phase 1/2 clinical trials). Consequently, all agents and approaches come with a GOR D (no recommendation possible). As additional trial data become available, more definite recommendations will emerge.

5.13.4 Low- and intermediate-risk nonmuscle-invasive bladder cancer trials

At least 11 different early-phase clinical trials in the low and intermediate-risk bladder cancer populations are active or pending results. As low- and intermediate-risk trials are not the primary focus of this chapter, the majority of trials in this category are simply highlighted in **Table 5-13**. Two of the most novel and promising therapies, however, are detailed below.

1. TC-3 Hydrogel (Vesigel, Urogen Pharma) (NCT02891460, NCT01803295): TC-3 hydrogel is a thermoreversible gel that is semi-solid at body temperatures and liquid at cooler temperatures. When mixed with MMC and instilled into the bladder, it enables sustained release of MMC, as the gel slowly reverts from solid to liquid with urine contact. For low-grade tumours (low- and intermediate-risk bladder cancer), the chemoablative properties of TC-3 hydrogel are being studied as an alternative to TURBT. Recent 1-year data (n=64) from the Optimized Instillation of Mitomycin for Bladder Cancer (OPTIMA) trial comparing TC-3 hydrogel plus MMC at concentrations of 0.06% (40 mg in 64 cc gel) and 0.12% (80 mg in 64 cc gel) to MMC 0.1% in water (40 mg in 40 cc) have been reported.[769] Following 6 weekly instillations, response at cystoscopy at 2 to 4 weeks after the final instillation demonstrated CR rates of 45.0%, 86.4%, and 69.6%, respectively. Amongst patients experiencing a CR, the RFS was similar across groups, suggesting that the main impact of TC-3 hydrogel plus MMC arises from early chemo-ablation. A larger

phase 2 trial (OPTIMA II) is currently being designed for launch in 2018 to corroborate the encouraging early OPTIMA findings.[770]

2. GemRIS™ (TAR-200, Taris Biomedical) (NCT02720367): GemRIS is a drug-delivery system that facilitates the slow release of gemcitabine into the bladder over a span of 7 days. The TAR-200 system consists of a silicone gemcitabine impregnated tube that is cystoscopically inserted into the bladder. Controlled release of gemcitabine occurs from days 0 to 7, after which the tube is removed cystoscopically. As per protocol, a new tube is then reinserted on days 21 to 28 to complete the treatment. A safety, tolerability, and preliminary efficacy (phase 1b) study of GemRIS 225 mg in low- and intermediate-risk disease is currently recruiting.

TABLE 5-13 Novel Trials Involving Patients With Low- or Intermediate-Risk NMIBC

Trial name	Design	Primary endpoint(s)	NMIBC study population
NCT03167151 (PemBla)	Phase 1/2 randomized: intravesical vs. IV pembrolizumab	Safety and AEs at 90 days	Intermediate risk
NCT02075060 (IPOI vs. IPOP)	Phase 2 randomized: preoperative vs. postoperative MMC	PFS at 12 months	Low or intermediate risk
NCT03081858	Phase 1/2 cohort: proliposomal intravesical paclitaxel (TSD-001)	MTD and marker lesion response rates	Low or intermediate risk
NCT02852564	Phase 1 cohort: ethacrynic acid (Edecrin) oral	Urine concentrations of ethacrynic acid	Low, intermediate, or high risk
NCT03298958	Phase 3 randomized: oral sirolimus (rapamycin) vs. placebo	RFS at 2 years	Low, intermediate, or high risk
NCT02070120 (CALIBER)	Phase 2 randomized: TURBT vs. MMC	CR rate with chemoresection	Low or intermediate risk
NCT02197897 (BCTamoxifen)	Phase 2 cohort: tamoxifen	Marker lesion response	Low or intermediate risk
NCT03058757	Phase 2 randomized: preoperative MMC vs. standard of care	RFS	Low, intermediate, or high risk (not specified)
NCT02695771	Phase 3 randomized: postoperative MMC vs. postoperative gemcitabine vs. standard of care	Safety and AEs	Low, intermediate, or high risk (not specified)

Abbreviations: AE, adverse event; CR, complete response; IPOI, immediate preoperative instillation; IPOP, early postoperative instillation; IV, intravenous; MMC, mitomycin C; MTD, maximal tolerated dose; PFS, progression-free survival; RFS, recurrence-free survival; TURBT, transurethral resection of bladder tumour.

Source: US National Library of Medicine. ClinicalTrials.gov. Available: https://clinicaltrials.gov/ct2/results?cond=non+muscle+invasive+bladder+canc r&term=&cntry1=&state1=&recrs=; Accessed: September 10, 2017.

5.13.5 High-risk nonmuscle-invasive bladder cancer

The gold standard treatment for high-risk NMIBC is TURBT plus adjuvant BCG induction and maintenance.[771] Thus, trials in the high-risk NMIBC setting can be divided into two categories based on BCG treatment status: (i) BCG naïve and (ii) BCG failures. The former are required because BCG as an initial adjuvant therapy is only effective at preventing recurrence in half of patients, implying

that therapies superior to upfront BCG are required. For the latter, after an initial BCG failure, the efficacy of further BCG therapy diminishes. The majority of novel and innovative trials in NMIBC are for those who fail BCG, as this group is extremely complex to manage. Ongoing trials for each of these two broad patient populations will be discussed separately below.

5.13.6 High-risk nonmuscle-invasive bladder cancer: bacillus Calmette-Guérin–naïve trials

5.13.6.1 Chemotherapy

5.13.6.1.1 Gemcitabine + cisplatin (NCT02716961)

A randomized phase 3 trial originating from China is investigating the role of adjuvant IV gemcitabine plus cisplatin chemotherapy in NMIBC. A total of 208 intermediate- and high-risk patients are being randomized to either epirubicin adjuvant induction and maintenance intravesical chemotherapy alone (8-week induction, plus 8 instillations every other week followed by monthly instillations for 6 months) or epirubicin induction/maintenance plus IV gemcitabine (1,000–1,200 mg/m^2) and cisplatin (70 mg/m^2) for one cycle. The IV chemotherapy will be given on days 1 and 8, beginning 5 days after TURBT. The primary endpoints of this trial are PFS and safety. Although intravesical chemotherapy (epirubicin) as first-line adjuvant therapy in high-risk disease is not guideline recommended, this trial will add insight into the efficacy and tolerability of established IV chemotherapy agents known to be active in the metastatic setting as adjuvants in NMIBC.

5.13.6.2 Novel agents

5.13.6.2.1 Sunitinib (SU11248; Sutent®; Pfizer Inc) (NCT00794950)

Sunitinib is a multitargeted tyrosine kinase inhibitor with activity against vascular endothelial growth factor receptors 1, 2, and 3 (VEGFR1/2/3); platelet-derived growth factor receptors (PDGFR-alpha/beta); the stem cell receptor c-kit; the Fms-like tyrosine kinase-3 receptor FLT3; and the ret proto-oncogene, among others. Established in advanced renal cell carcinoma, sunitinib's role in urothelial carcinoma stems from observations of VEGF/PDGF axis activation in this tumour type. Specifically, the downstream product hypoxia inducible factor-1alpha is associated with an aggressive phenotype in NMIBC and VEGFRs are present and activated in numerous bladder cancer cell lines.[772,773] Furthermore, *in vitro* models suggest that sunitinib may enhance BCG-mediated cytotoxicity.[774] Data from phase 2 trials in metastatic urothelial carcinoma suggest sunitinib exerts antitumour effects, albeit without robust results in the second-line metastatic setting.[775] Nevertheless, a phase 2 trial of sequential BCG induction-recovery-sunitinib (6 weeks/2 weeks/4weeks) in patients naïve to BCG within 12 months of enrolment has completed and is expected to report in the near future.

5.13.6.2.2 Enzalutamide (Xtandi®, Astellas Pharma Inc) (NCT02605863)

Enzalutamide is a potent androgen receptor antagonist with established safety and efficacy in castrate-resistant prostate cancer. Preclinical data implicate androgen receptor signalling as a mediator of cell growth and progression in bladder cancer, with oral enzalutamide therapy in mouse xenograft models leading to tumour involution.[776] Furthermore, gemcitabine-resistant bladder cancer cell lines demonstrate upregulation of the androgen receptor and thus enzalutamide sensitivity, with subsequent cell cycle arrest, transcriptional downregulation, and impaired tumour cell proliferation.[777] A phase 1/1b study of enzalutamide with gemcitabine and cisplatin chemotherapy in

metastatic urothelial carcinoma is currently underway.[778] A phase 2 trial in patients with intermediate-risk NMIBC and high-risk NMIBC who have never experienced a BCG failure is also recruiting. In both cohorts, oral enzalutamide 160 mg daily will be provided for 1 year, in addition to standard of care.

5.13.6.3 Immune therapy

5.13.6.3.1 Coxsackie virus A21 (CVA21, CAVATAK®, Viralytics) (NCT02316171)

Coxsackie virus A21 is an oncolytic common cold virus that exhibits tumour specificity via adherence to ICAM-1, a molecule expressed in abundance on cancer cells. After cellular uptake, the virus causes cell lysis and release of replicated viral particles that adhere to and kill adjacent tumour cells. Results of the phase 1/2 CANON (CAVATAK in NON-muscle invasive bladder cancer) trial have been reported preliminarily.[779] In this study, patients with *de novo* NMIBC, the majority of which were high grade, were treated preoperatively with dose escalated intravesical CAVATAK monotherapy followed by TURBT 7 to 10 days later (n=9). A further six patients treated in a similar manner with CAVATAK plus MMC were then evaluated.[780] Molecular proof of concept of viral targeting, replication, and tumour cell death were established, as was the safety of the regimens, with no grade 2, 3, or 4 AEs.

5.13.6.3.2 Bacillus Calmette-Guérin + ALT-803: (IL-15N72D/IL-15R alpha Su-Fc, Altor BioScience Corp) (NCT02138734)

IL-15 is a potent mediator of natural killer (NK) and cytotoxic T-cell development, maturation, and activation. ALT-803 is a fusion protein of a mutant IL-15 superagonist and the soluble domain of the IL-15R (IL-15 receptor) alpha protein, which makes the complex 25 times more potent than wild-type IL-15 alone. Coupled with BCG in early rat models, ALT-803 demonstrated potent antitumour activity above and beyond BCG alone.[781] A phase 1b trial report demonstrated an excellent safety profile for intravesical ALT-803 and BCG with limited AEs and 1-year durable CRs in all 9 patients.[782,783] Based on these data, the FDA granted fast track status to ALT-803 to accelerate development and review of the drug.[784] A phase 1b/2 trial (QUILT-2.005) of intravesical BCG in combination with ALT- 803 in BCG-naïve patients has been initiated. Phase 1b is a dose escalation/safety study to determine the maximum tolerated dose and will consists of BCG plus ALT-803 induction and 1 year of maintenance, as per SWOG 8507 protocol.[785] During the phase 2 expansion study, patients will be randomized to either ALT-803 + BCG (50 mg) or BCG (50 mg) alone. QUILT-2.005 will complete recruitment by the end of 2018.

5.13.6.4 Vaccine therapy

5.13.6.4.1 Percutaneous Bacillus Calmette-Guérin vaccination (NCT02326168, NCT03091660)

To harness the adaptive immune response and boost the efficacy of intravesical BCG therapy, intradermal percutaneous BCG vaccination prior to intravesical instillation has been proposed. Clinical data supporting priming the immune response with BCG vaccination arise from a retrospective study in which patients with reactivity to purified protein derivative (PPD) had significantly improved RFS compared to PPD-unreactive patients.[786] In 2014, a pilot phase 1 trial evaluating the safety and early efficacy BCG vaccination approximately 19 to 31 days prior to induction BCG was initiated. This early phase 1 study (PRIME, NCT02326168) was the predecessor of SWOG 1602 (also called PRIME, NCT03091660), which is large, phase 3 trial aimed at assessing the impact of BCG vaccination and

strain on NMIBC outcomes. In PRIME, BCG-naïve, PPD-negative patients (*n*=969) will be randomized to TICE BCG (50 mg/dose), BCG Tokyo-172 (80 mg/dose) or intradermal vaccination with Tokyo strain BCG plus BCG Tokyo-172 (80 mg/dose). The comparison between TICE and Tokyo is aimed at testing a noninferiority hypothesis, whereas BCG vaccination plus intravesical BCG is hypothesized to be superior to intravesical BCG alone. This trial opened to accrual in 2017.

5.13.6.4.2 Mycobacterium TB vaccine (RUTI®, Archivel Farma S.L.) (NCT03191578)

RUTI is a polyantigenic vaccine used for the treatment of latent TB infection.[787] Manufactured from nonreplicative fragments of *Mycobacterium tuberculosis*, RUTI elicits an immune response that is hypothesized to be cross-reactive with intravesical BCG. Building on the intradermal BCG vaccination model established by PRIME (discussed in previous section), RUTI is being tested in a phase 1 trial whereby high-risk BCG-naïve patients are randomized to two subcutaneous RUTI injections or placebo prior to traditional induction and maintenance BCG. Outcomes being assessed include the local and systemic immune response, in addition to usual clinical parameters (e.g., RFS, PFS, toxicity). This trial is listed as open to accrual in 2017.

5.13.6.4.3 recMAGE-A3 + AS15 ASCI vaccine (GlaxoSmithKline, GSK Vaccines) (NCT01498172)

The *MAGE-A3* (Melanoma Antigen) gene belongs to the cancer/testis gene family, which is only expressed during embryogenesis or exclusively in tumour cells.[788] Since it is not expressed in adult somatic cells, the MAGE-A3 protein is a true tumour-specific antigen.[789] In urothelial carcinoma, MAGE-A3 is found in approximately 50% of tumour specimens. Recombinant MAGE-A3 is often formulated in the AS15 adjuvant, which is a strong immunostimulant for the induction of T cells. Together, recMAGE-A3 + AS15 is termed an antigen-specific cancer immunotherapeutic (ASCI). In a recently published phase 1 trial in low-, intermediate-, and high-risk NMIBC patients, all patients received intramuscular recMAGE-A3 + AS15 vaccine every 3 weeks for 5 total doses and intravesical induction oncoTICE® (BCG, Strain TICE®) except low-risk patients, who only received vaccine).[790] The combination of vaccine plus BCG was found to be safe, without exacerbation of BCG side effects and few serious adverse events (SAEs), thus setting the stage for a future phase 2 trial.

5.13.6.5 Gene therapy

5.13.6.5.1 BioCanCell gene therapy (BC-819/PEI, BioCanCell Therapeutics Ltd.) (NCT01878188)

BC-819 is a recombinant, double-stranded DNA plasmid containing the H19 promoter and the diphtheria toxin A gene sequence. Present only in malignant cells and quiescent in mature somatic cells, H19 expression drives diphtheria toxin A production.[791] The toxin thus selectively kills tumour cells by inhibiting protein translation. The lack of the diphtheria toxin B subunit in the plasmid prevents migration of toxin A to adjacent healthy cells, leading to tumour-specificity. A phase 2b marker lesion trial in intermediate-risk disease demonstrated a 33% CR rate.[792] Based on these data, a second phase 2 trial in high-risk patients was launched in 2013, with the BC-819 plasmid given along with BCG (either alternating, sequential, or biweekly) intravesically. The 3-month and 1-year RFS in 38 patients were reported as 89% and 68% respectively.[793] Final results from this study are still pending. Two further trials in BCG-unresponsive patients with BC-819 given as monotherapy (phase 2 Trial 204, *n*=140) and in single BCG-failure patients with randomization to BC-819 plus BCG versus second-induction BCG (phase 3 Trial 301, *n*=247) are being planned for 2018.

5.13.7 High-risk nonmuscle-invasive bladder cancer: trials of bacillus Calmette-Guérin failures

5.13.7.1 Chemotherapy

Although single-agent chemotherapeutic regimens have been investigated in the setting of BCG failure, most have been plagued by generally poor results. Recurrence rates of 80% to 90% over short time frames of 1 to 2 years demonstrate both limited efficacy and durability.[794,795] Nevertheless, recent trials utilizing combination therapy or novel drug pairings have shown some promise.

5.13.7.1.1 Gemcitabine + docetaxel

A single-institution retrospective series of 33 patients receiving gemcitabine and docetaxel has been reported.[796] For the 28 patients with initial high-grade tumours, 1- and 2-year high-grade recurrence-free survival (HG-RFS) was 51% and 34%, respectively. The median HG-RFS was 15.7 months and the median time to cystectomy (7 patients) was 14.9 months. The sequential regimen was well tolerated, with an acceptable side effect profile.

5.13.7.1.2 Cabazitaxel + gemcitabine + cisplatin (NCT02202772)

A phase 1 trial of cabazitaxel, gemcitabine, and cisplatin instilled intravesically for 2 hours each, weekly for 6 weeks is currently recruiting, with a target accrual of 24 patients. Although oncological outcomes from this trial are still pending, early safety results from 9 patients were recently reported.[797] With escalating doses of cabazitaxel and cisplatin (gemcitabine at a constant dose of 2,000 mg), no Common Terminology Criteria (CTC) grade 3 AEs were reported. Although 7 of the 9 patients had local side effects (grade 1/2), the combination regimen was well tolerated and 7 patients were able to proceed to maintenance therapy.

5.13.7.1.3 Gemcitabine + everolimus (NCT01259063)

Investigators at Memorial Sloan Kettering Cancer Center have recently investigated the safety and efficacy of intravesical gemcitabine and oral everolimus in patients with CIS who failed at least one induction BCG.[798] In the phase 2 component of the trial, gemcitabine biweekly for 3 weeks with 1 week off was combined with 10 mg of everolimus. The 1-year RFS was only 20%, and 10 of 19 patients experience grade 3 or higher AEs, ultimately leading to premature closure of the trial. The investigators concluded that toxicity of this combination regimen was rate limiting and that everolimus did not add significantly to the efficacy of monotherapy intravesical gemcitabine.

5.13.7.2 Novel agents in bladder cancer

A number of novel agents and tumour ablative strategies are being developed with the premise that additional BCG and existing chemotherapeutic regimens are not effective in the BCG failure population. Established antineoplastic agents with demonstrated utility for other tumour types have also been adapted for investigation to elucidate their cross-reactivity with bladder cancer.

5.13.7.2.1 Oportuzamab monatox (VB4-845, Vicinium™, Eleven Biotherapeutics) (NCT02449239)

A recombinant fusion protein of Pseudomonas exotoxin A and a monoclonal antibody that binds epithelial cell adhesion molecule (EpCAM), oportuzamab monatox's mechanism of action is via targeted delivery of exotoxin A to tumour cells overexpressing EpCAM.[799,800] When given

intravesically, the EpCAM on tumour cells localizes the fusion molecule and exotoxin A is internalized to exert its cytotoxic effect. Phase 2 data in CIS patients with at least one BCG failure have demonstrated an overall CR rate of 44% (20 of 45 patients), with the majority (18 patients) occurring at 3 months.[801] Although the 1-year RFS rate was 16%, given the encouraging safety profile of the treatment (i.e. no discontinuation of study drug or series AEs), these results were deemed clinically meaningful and a phase 3 trial in BCG-unresponsive patients was launched, is currently recruiting, and has results due in 2019.

5.13.7.2.2 TLD-1433 (Theralase Inc.) (NCT03053635)

A phase 1b study of TLD-1433, a photodynamic compound that responds to different wavelengths of light, is currently under way at the University of Toronto.[802,803] The wavelength of light applied determines tissue penetration, distinguishing TLD-1433 from historic photosensitizers that resulted in excess bladder fibrosis. Administered for 1 hour preoperatively as an intravesical instillation, TLD-1433 is preferentially taken up by tumour cells via transferrin-mediated uptake. Intraoperative illumination by green light laser is hypothesized to result in drug activation and cell death. Safety and efficacy results are to be reported in 2018 after accrual of 9 target patients.

5.13.7.2.3 Nab-Rapamycin (ABI-009, Celgene Corp.) (NCT02009332)

Activation of the mTOR pathway appears to be associated with NMIBC recurrence and progression.[804] It follows, then, that the mTOR inhibitor rapamycin may exhibit an antitumour benefit in NMIBC. In a preclinical murine model, Seager and colleagues demonstrated that rapamycin is capable of suppressing tumourigenesis when instilled intravesically.[805] Since rapamycin is relatively water insoluble, binding to nanoparticle albumin facilitates albumin-mediated endocytosis by tumour cells.[806] With these data, this same group has reported preliminary results of a phase 1/2 clinical trial evaluating the safety and efficacy of intravesical nab-rapamycin in patients failing BCG.[807] In 13 patients with doses escalating from 100 to 400 mg, only grade 1 or 2 AEs were noted with no systemic toxicity, thus supporting future clinical trials.

5.13.7.2.4 Imiquimod (TMX-101, Vesimune, Urogen Pharma) (NCT01731652)

TMX-101 is a novel liquid form of imiquimod, a toll-like receptor 7 (TLR7) agonist with proven benefit in the management of nonmelanoma skin cancers.[808] Toll-like receptor 7 activation stimulates the innate immune system and enhances production of antigen specific T cells.[809] The safety of TMX-101 has been established in phase 1 trials,[810] and results of the phase 2 study in patients with CIS have recently been reported.[811] A total of 12 patients, 9 of whom had received prior BCG, were enrolled and received weekly TMX-101 0.4% solution for a 6-week induction. Only 2 of 10 evaluable patients were disease free at first cystoscopic assessment. One patient experienced a grade 3 AE (severe UTI) and many experienced local, mild AEs. The utility of TMX-101 in the management of BCG failures thus requires further evaluation.

5.13.7.2.5 ALT-801 (c264scTCR-IL2, Altor BioScience Corp) (NCT01625260)

ALT-801 is an immunotherapeutic fusion protein consisting of IL-2 linked to a single-chain, p53-specific, T-cell receptor domain.[812] The linkage modifies the functional activity of IL-2, theoretically increasing its potency. The soluble ALT-801 fusion protein can thus target p53 over-expressing tumour cells, enabling localization of IL-2 immunostimulatory and cytotoxic activity at

the intracellular cancer cell level. A phase 1b/2, multicentre trial of IV ALT-801 combined with IV gemcitabine in patients with BCG failures is underway and has completed accrual. Endpoints for the study (safety, tolerability, and efficacy) are due in 2018.

5.13.7.2.6 Ethacrynic Acid (Edecrin, Merck & Co., Inc.) (NCT02852564)

Ethacrynic acid is an established loop diuretic that has purported antitumour properties. It functions by inhibiting both glutathione S-transferase P1-1 (GSTP1-1) and WNT (i.e., wingless-type MMTV [mouse mammary tumor virus] integration site) activity.[813] Glutathione S-transferase is recognized as an important mediator in cell detoxification, while the WNT signalling pathway is ubiquitously activated in bladder cancer cell lines.[814] A phase 1 trial of ethacrynic acid prior to TURBT has launched to determine the levels of urinary ethacrynic acid and its conjugates after a 50-mg oral dose. Outcomes of this trial will guide future clinical trial development in the pre- and post-BCG settings.

5.13.7.2.7 BGJ398 (NVP-BGJ398, Novartis Oncology) (NCT02657486)

BGJ398 is a potent pan-fibroblast growth factor receptor (FGFR) tyrosine kinase inhibitor with anti-angiogenic and antineoplastic properties. FGFR-activating mutations are found in approximately 10% to 20% of bladder cancers, making the FGFR pathway a potential therapeutic target for select NMIBC patients.[815] In a phase 1 trial, investigators at Memorial Sloan Kettering Cancer Center are assessing the initial efficacy of BGJ398 in the BCG-failure setting in a tumour marker study. Patients will take BGJ398 125 mg PO (3 weeks on, 1 week off) with cystoscopic and cytologic assessment at 7 weeks to determine the CR rate of the marker lesion.

5.13.7.2.8 Lenalidomide (Revlimid®, Celgene) (NCT01373294)

Oral lenalidomide is a derivative of thalidomide. It exerts its anticancer properties via numerous mechanisms, including antiangiogenic, anti-inflammatory, apoptotic, and immunomodulatory actions.[816] Murine bladder cancer models have demonstrated improved tumour shrinkage when lenalidomide was coadministered with BCG compared with BCG therapy or lenalidomide therapy alone.[817] A phase 2 nonrandomized trial post-BCG failure in which patients will either receive intravesical BCG and oral lenalidomide or BCG alone is ongoing. PFS is the primary endpoint, with secondary outcomes being safety and correlative cytokine studies.

5.13.7.2.9 Dovitinib (TKI-258, Novartis Pharmaceuticals Corp) (NCT01732107)

Dovitinib is a potent inhibitor of fibroblast growth factor receptor 3 (FGFR3). It also exhibits inhibitory activity with other molecules in the receptor tyrosine kinase super family, including FLT3, c-Kit, FGFR1, VEGFR1, and VEGFR3. In bladder cancer, FGFR3 mutations lead to activation of the FGFR signalling cascade, with resultant proliferation, cell survival, and angiogenesis. Although the only reported trial of dovitinib in urothelial carcinoma, in the heavily pretreated metastatic space, was essentially negative,[818] FGFR3-activating mutations cluster at a very high rate (60%–70%) in NMIBC,[819] suggesting that targeted FGFR3 inhibition may be an effective strategy in this patient population. To this end, the Hoosier Cancer Research Network has initiated a phase 2 trial in BCG-refractory bladder cancer assessing the 6-month CR rate and toxicity profile of 500 mg oral dovitinib (5 days on/2 days off per cycle) in FGFR3-mutated NMIBC. Closed to accrual, this study is in the follow-up phase.

5.13.7.3 Novel delivery methods

To increase the intravesical dwell time of new and existing agents and/or to increase the concentration of drug that is delivered to the bladder, a number of unique mechanisms are being investigated.

5.13.7.3.1 TC-3 hydrogel (Vesigel, Urogen Pharma) (NCT02307487)

As discussed above, most data pertaining to TC-3 hydrogel are from preliminary studies in low-grade bladder cancer. With an excellent safety profile, use in the BCG failure setting is being explored. A dose-escalation study (up to 160 mg MMC in 60 cc gel) including patients with asymptomatic BCG failure (greater than 6 months since last BCG administration) has just been completed with data presentation pending.

5.13.7.3.2 Polymeric micelles of docetaxel (mPEG-PDLLA, Nanoxel-M®, Samyang Biopharmaceuticals Corp.) (NCT02982395)

In preclinical nonbladder cancer studies, a polymeric micelle delivery system for encapsulating docetaxel has led to less toxicity than standard docetaxel.[820,821] As a result, a phase 3 clinical trial comparing polymeric micelle docetaxel to MMC in patients who have failed at least one induction course of BCG is currently recruiting, with a planned accrual of 88 patients. Although randomized, the generalizability of this trial hinges on the perceived adequacy of the MMC control group and thus may be limited.

5.13.7.4 Immune therapy

Ever since the discovery of BCG, manipulation of the immune system to treat bladder cancer has long been recognized as a potentially effective therapeutic research avenue. Today, numerous lines of investigation that aim to exploit the power of the immune system are being considered. Studies using treatments such as radiotherapy, thermotherapy, or photodynamic therapy to induce immunogenic cell death (i.e. facilitate release of tumour-associated antigens) and thus potentiate immune targeted therapies are in the design or early phases and have been discussed in prior accompanying sections on those topics. Trials using oncolytic viral therapy, vaccination as a means of augmenting or priming the BCG response, immune modulators, and combination therapy with multiple immune targeted agents are discussed below.

5.13.7.4.1 CG0070 oncolytic adenovirus (Cold Genesys Inc.) (NCT02365818)

Oncolytic viral therapy is mediated by viruses that infect and replicate preferentially within tumour cells, thus causing lytic cell death.[822] CG0070 is a genetically engineered oncolytic, granulocyte macrophage colony-stimulating factor (GM-CSF)–modified common cold adenovirus that is being investigated in NMIBC.[823] As implied, there are two purported mechanisms of action for CG0070. The first is direct tumour lysis after uptake and replication within tumour cells. The second is via a cancer promoter (E2F1 [E2F transcription factor 1])-driven GM-CSF transgene that results in GM-CSF overexpression and release during cell lysis, with subsequent systemic antitumour immune stimulation. Bladder tumour cells are targeted via a defective retinoblastoma tumour-suppressor gene pathway. Intravesical CG0070 has been evaluated in phase 1 and phase 2 clinical trials with promising results.[824,825] With a tolerable safety profile (only one grade 3 AE), interim results from the phase 2 open-label single-arm BOND2 trial involving patients failing BCG demonstrated a 6-month

CR of 47%. Unfortunately, no T1 patient had a 6-month CR. Encouragingly, the 6-month CR for CIS patients was 58%, suggesting that this patient group may be the prime beneficiaries of oncolytic viral therapy. Final results of the BOND2 trial are expected in 2019.

5.13.7.4.2 Vesigenurtacel-L (HS-410, Heat Biologics Inc.) (NCT02010203)

HS-410 is a vaccine consisting of live, irradiated bladder tumour cells that are injected intradermally to induce an adaptive immune response.[826] The injected cells secrete heat shock protein gp96 and tumour-associated antigens, which improve antigen presentation to cytotoxic T lymphocytes (CTLs) and ultimately enhance the CTL response to the endogenous bladder tumour.[827] HS-410 has been investigated in a combination phase 1/2 trial.[828,829] HS-410 was reported as safe and tolerable (no grade 3/4/5 AEs). The phase 2 trial included both BCG-naïve and BCG-relapsing (i.e. last BCG more than 12 months ago) patients. In patients scheduled for additional BCG therapy (induction and maintenance), patients were randomized to low-dose vaccine ($1x10^6$ cells), high-dose vaccine ($1x10^7$ cells), or intradermal placebo; in those for whom BCG was not planned, high-dose vaccine alone was administered. Overall, there was no difference in DFS or RFS across the trial arms, although the DFS rate was over 70%. Additional study of HS-410 is thus warranted.

5.13.7.4.3 PANVAC vaccine (NCT02015104)

PANVAC is a pox viral vector-based vaccine that induces a T-lymphocyte immune response to the tumour-associated antigens carcinoembryonic antigen (CAE) and mucin-1 (MUC-1), which are expressed in higher concentrations on bladder tumour cell surfaces.[830] PANVAC is also genetically modified to secrete three T-cell costimulatory molecules (B7-1, ICAM-1, and leukocyte function-associated antigen-3 [LFA-3]) that may enhance the immune response. When coadministered with intravesical BCG, PANVAC is theorized to augment the BCG response. Currently, a randomized, phase 2 trial in patients with BCG failure is recruiting, comparing PANVAC plus BCG versus BCG alone, with a primary endpoint of 1-year DFS.

5.13.7.4.4 Pembrolizumab (MK-3475, Keytruda®, Merck Sharp & Dohme Corp) (NCT02625961, NCT02808143)

Pembrolizumab is a humanized monoclonal anti-PD-1 antibody that blocks the interaction between programmed cell dealth-1 (PD-1) and its ligands, programmed cell death-ligand 1 and 2 (PD-L1 and PD-L2). As an immune checkpoint inhibitor, pembrolizumab functions to potentiate the T-cell response against tumour cells by removing the inhibitory PD-1–PD-L1/L2 signal. The safety and tolerability profile of pembrolizumab has been established in multiple cancer settings, including a phase 3 trial involving patients with advanced urothelial carcinoma.[831] A phase 2 open-label, single-arm trial (KEYNOTE-057) of pembrolizumab in the BCG-unresponsive NMIBC setting is currently recruiting (NCT02625961).[832] The trial consists of 200 mg IV pembrolizumab instillations every 3 weeks for up to 2 years or until disease recurrence, progression, or unacceptable toxicity. Pembrolizumab is also being assessed as an intravesical instillation in a separate phase 1 clinical trial (NCT02808143). In this dose-escalation study in BCG-unresponsive patients, pembrolizumab will be given intravesically 2 weeks prior to repeat BCG induction and then in parallel with BCG induction and maintenance. Safety and maximum-tolerated dose are the primary endpoints of this currently recruiting study.

5.13.7.4.5 Atezolizumab (MPDL 3280A, Tecentriq®, Hoffmann La-Roche) (NCT02844816, NCT02792192)

Like pembrolizumab, atezolizumab is an immune checkpoint inhibitor. It is a humanized monoclonal anti-PD-L1 antibody that inhibits the interaction of PD-L1 on tumour cells with PD-1 and B7-1 receptors on CTLs. Adaptive PD-L1 expression on tumour cells facilitates inactivation of T cells and prevents subsequent tumour cell death, which atezolizumab counters. Two studies with atezolizumab are ongoing. The first is a SWOG phase 2 trial (SWOG1605) in patients with BCG-unresponsive disease (NCT02844816).[833] Patients will receive 1,200 mg IV atezolizumab as monotherapy every 3 weeks for up to 51 weeks. Endpoints of the trial are CR, event-free survival, and PFS. The second nonrandomized phase 1b/2 trial assesses the feasibility and safety of coadministering atezolizumab IV and intravesical BCG in BCG-unresponsive patients (NCT02792192). Three cohorts are planned for this study, with the first 2 involving BCG-failure patients (BCG unresponsive and BCG relapsing). This trial is currently recruiting, with an anticipated end date of 2021.

5.13.7.4.6 VPM1002BC (*Mycobacterium bovis* BCGΔureC::Hly+, Serum Institute of India Ltd.) (NCT02371447)

VPM1002BC is a genetically modified *Mycobacterium bovis* engineered to deliver broader activation of the immune system with improved tolerability. Specifically, VPM1002BC has been modified to express the bacterial toxin listeriolysin, which leads to pore formation in infected cells and thus destabilization of cell membranes, apoptosis, improved antigen presentation, and subsequent improved stimulation of CTLs.[834,835] This improved version of BCG is currently being tested in a phase 1/2 clinical trial (SAKK 06/14 trial) in patients whose disease recurs after initial BCG therapy. Phase 1 will be a dose-escalation study and phase 2 will assess efficacy in 45 planned patients, with results reporting in 2022.

5.13.7.4.7 Bacillus Calmette-Guérin + ALT-803: (IL-15N72D/IL-15R alpha Su-Fc, Altor BioScience Corp) (NCT03022825)

As introduced above (see section 5.13.6 on high-risk NMIBC: BCG-naïve trials), ALT-803 is an extremely potent IL-15 that potentiates the immune-mediated response to cancer. A current phase 2 trial (QUILT-3.032) in BCG-unresponsive patients is currently recruiting. Patients will receive concomitant BCG and ALT-803 as an induction and with maintenance therapy for 1 year with endpoints of CR, PFS, OS, time to cystectomy, and quality of life.

5.13.7.4.8 Bacillus Calmette-Guérin + rapamycin (Sirolimus) (NCT02753309)

Given the preclinical data supporting the mTOR inhibitor rapamycin as an antineoplastic agent in NMIBC (see section on rapamycin above), the combination of BCG and rapamycin is being studied in an early phase 1 study. Patients will take either 0.5 mg or 2.0 mg of rapamycin PO daily for 28 days in addition to BCG therapy. Both BCG-naïve and BCG-failure patients can enroll, and the main outcome measures are peripheral T-cell counts and function.

5.13.7.4.9 Oportuzamab monatox (VB4-845, Vicinium, Eleven Biotherapeutics) + durvalumab (MEDI4736, Imfinzi®, AstraZeneca) (NCT03258593)

Under the hypothesis that oportuzamab monatox may potentiate the action of immuno-oncology agents, a phase 1 study of oportuzamab and durvalumab (a PD-L1 checkpoint inhibitor) is planned in BCG-unresponsive patients.[836] Patients will receive 1,500 mg IV durvalumab and intravesical Vicinium for 2 years, with safety being as assessed as the primary endpoint.

5.13.7.5 Gene therapy

5.13.7.5.1 rAd-IFN/Syn3 (SCH 721015/SCH 209702, Instiladrin®, FKD Therapies Oy) (NCT01162785, NCT01687244, NCT02773849)

The effectiveness of intravesical IFN for the treatment of NMIBC is felt to be limited because of the short duration of exposure and low urothelial IFN concentrations achieved when delivered in a simple, intravesical manner. Increased IFN exposure is possible when the gene is delivered to the urothelium through a recombinant adenovirus that constitutively expresses IFN gene product (rAd-IFN).[837] Syn3 is a small molecule that improves viral-mediated transduction through the urothelium, further facilitating IFN gene production. The tolerability of rAd-IFN/Syn3 has been previously established,[838] with no dose-limiting toxicity. In a recently reported phase 2 trial evaluating the efficacy of rAd-IFN/Syn3 in BCG-refractory or relapsed NMIBC, patients were randomized to either low-dose (1 x 1,011 viral particles/mL) or high-dose (3 x 1,011 viral particles/mL) arms.[839] The 12-month high-grade RFS was 35%, with comparable results regardless of dose. While 39/40 (97.5%) of patients experienced an AE and 5 patients experienced an SAE, there were no treatment discontinuations related to AEs and the drug was well tolerated. Overall, the authors concluded that rAd-IFN/Syn3 demonstrated encouraging efficacy in this difficult-to-treat patient population, justifying the ongoing phase 3 high-dose expansion trial in BCG-unresponsive patients.

5.13.8 Conclusions

The treatment of high-risk NMIBC is complex, and very few agents are approved in this treatment setting. Compounding this issue is the fact that very few trials have historically moved beyond phase 1 or 2. Nevertheless, with the numerous new treatment modalities being explored, the possibility of true advances in the field is high.

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C6

Localized Muscle-invasive Bladder Cancer

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Committee 6

CONTENTS

Localized Muscle-invasive Bladder Cancer

6.1 Introduction

This chapter, produced by a panel of an international multidisciplinary group of experts in this field, assesses an evidence-based and updated management of patients with muscle-invasive, presumably node-negative bladder cancer. It describes treatment options and their outcomes, including radical surgery, neoadjuvant and adjuvant treatment modalities, bladder-sparing approaches, and treatment of secondary urothelial recurrences, after a clinical diagnosis of a circumscribed disease was made based on the guidelines in previous chapters. Specific recommendations are also given for follow-up strategies after curative-intent therapy. The level of evidence (LOE) and grade of recommendation (GOR) given are based on the guidelines of the Oxford Centre for Evidence-Based Medicine.[1]

6.2 Indication and Algorithm of Treatment

About 20% to 40% of patients with newly diagnosed urothelial carcinoma of the bladder exhibit or progress to muscle-invasive or locally advanced disease.

Prior to any local treatment for muscle-invasive bladder cancer (MIBC), locoregional and systemic staging has to be performed via computed tomography (CT) or magnetic resonance imaging (MRI) of the chest, abdomen, and pelvis to rule out significant lymphadenopathy or visceral metastasis. Assessment of the local extent of the disease, especially extravesical extension, cannot be performed adequately by the above-mentioned studies due to their low sensitivity. Sensitivity and specificity to identify lymph node, visceral, and/or bone metastases can be improved by the use of fludeoxyglucose (FDG) positron emission tomography (PET).

Once systemic spread has been ruled out, various treatment options are available that need to be discussed with the patient based on his/her performance status, biologic age, and pre-existing comorbidities. If patients are aged >75 years or if patients present with an Eastern Cooperative Oncology Group (ECOG) performance status ≥2, geriatric assessment should be performed.[2,3]

According to the most recent European Association of Urology (EAU) guidelines, neoadjuvant cisplatin-based chemotherapy followed by radical cystectomy (RC), pelvic lymphadenectomy, and urinary diversion represent the standard treatment of choice.[4] Alternatively, patients with muscle-invasive disease might undergo organ-preserving treatment using the trimodality approach consisting of radical transurethral resection of the bladder tumour (TURBT), radiation therapy, and concurrent systemic chemotherapy. External beam radiation monotherapy can also be administered in patients ineligible for chemotherapy.

Neoadjuvant chemotherapy (NAC) with 4 cycles of gemcitabine and cisplatin (GC) represents the guideline-recommended approach due to the only 50% 5-year survival rate in patients with MIBC and NAC's life-prolonging efficacy.[4] Despite its potential drawbacks, NAC has been demonstrated to be associated with an overall survival (OS) benefit of 5% to 6% after 10 years.[5-7] In patients with an impaired renal function between 40 to 60 mL/min/1.73 m^2, a split dose of cisplatin[8] can be delivered with a similar oncological efficacy.[9-11] However, carboplatin should not be administered in the neoadjuvant setting due to the uncertain benefit and the concomitant delay in definitive surgery. If patients are scheduled for chemoradiation, cisplatin can be replaced by the combination of 5-fluorouracil (5-FU) and mitomycin C (MMC) according to the data of a large, prospective, randomized trial.[12]

RC with an extended pelvic lymphadenectomy followed by an incontinent or continent urinary diversion is the standard therapy. The choice of the urinary diversion depends on performance status, comorbidities, patient wish, previous surgeries, and, to a lesser extent, age. Even in elderly patients aged above 80 years, 5-year cancer-specific survival (CSS) rates as high as 50% have been reported.[13]

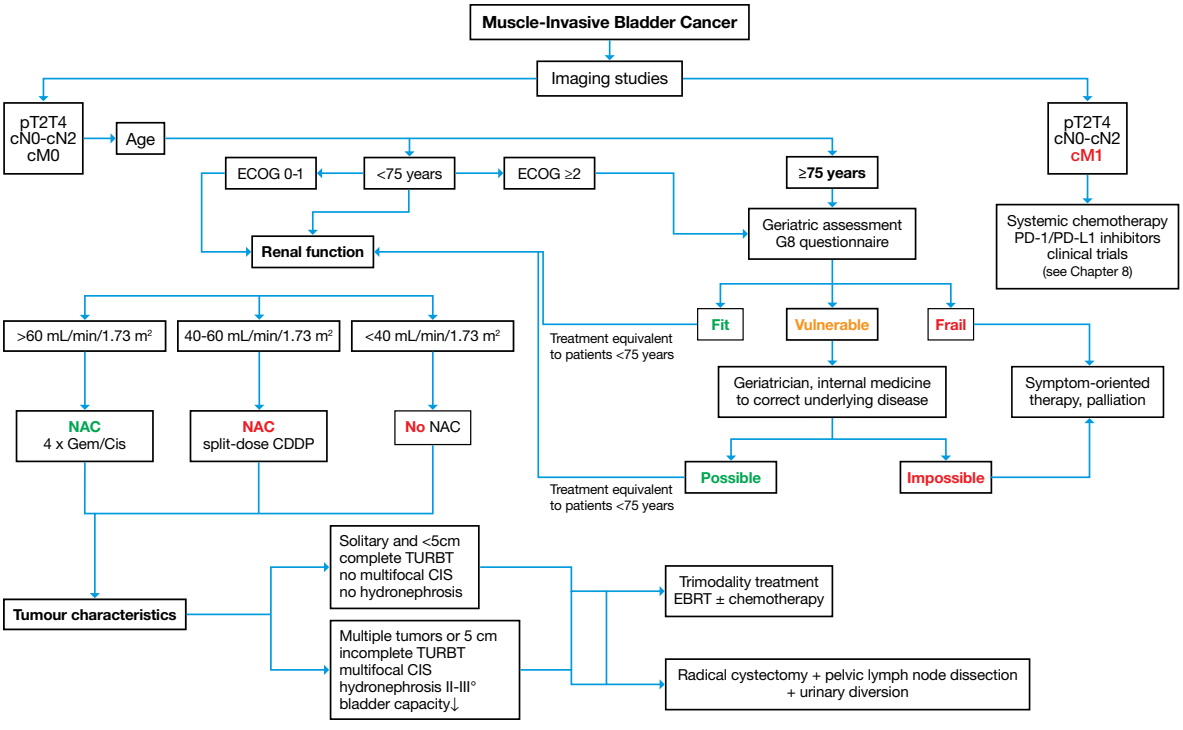
Trimodality therapy consisting of radical TURBT, external beam radiation therapy (EBRT), and concurrent systemic chemotherapy, represents an alternative treatment option in MIBC. Radiation therapy without chemotherapy clearly results in inferior relapse-free survival and OS rates as compared to chemoradiation.[14] Bladder preserving trimodality therapy approaches resulted in similar relapse-free survival and OS rates as radical cystectomy if patients were well selected.[15-17]

In a recent retrospective comparative evaluation of trimodality therapy (n=1,257) versus RC (n=11,586) in patients derived from the National Cancer Database (NCDB), Seisen *et al.*[18] found that the trimodality therapy is associated with a worse long-term OS becoming evident 2 years after delivery of treatment. The adverse treatment effect of trimodality therapy versus RC decreased with increasing age (hazard ratio [HR], 0.99; 95% confidence interval [CI], 0.98–0.99; p=0.03), whereas no significant impact of gender or cT stage was identified. Based on these data, RC is to be preferred in patients with a substantial life expectancy.

Another study performed a propensity score analysis of RC and trimodality treatment in 112 patients.[19] Patients either underwent RC with NAC or adjuvant chemotherapy (AC), or patients underwent debulking TURBT followed by radiation therapy and concurrent radiosensitizing cisplatin-based chemotherapy. Selection criteria for trimodality therapy included a small, solitary tumour without multifocal carcinoma *in situ* (CIS) or hydronephrosis. Considering the selection bias, the median OS of 6.61 years was identical between both groups, as was the disease-specific survival. There was a significant difference in terms of overall recurrence rates, which were 38% and 59% for RC and trimodality therapy, respectively. Considering the age categories, there was significant difference in oncological outcome for patients aged £60 years and >60 years. Based on these data, trimodality therapy is as effective as RC in small, solitary MIBC, which is worth considering especially in the elderly population.

A potential treatment algorithm is shown in **Figure 6–1**.

FIGURE 6–1
Potential Treatment Algorithm



Abbreviations: CDDP/Cis, cisplatin; CIS, carcinoma in situ; EBRT, external beam radiation therapy; ECOG, Eastern Cooperative Oncology Group; Gem, gemcitabine; NAC, neoadjuvant chemotherapy; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1; TURBT, transurethral resection of the bladder tumour.

6.3 Imaging in Muscle-invasive Bladder Cancer

The role of imaging in patients with MIBC has traditionally been to identify the extent of local invasion, to evaluate for upper tract lesions or hydroureteronephrosis (requiring intervention prior to treatment), and to identify any metastatic disease that would preclude definitive local therapy. Appropriate treatment selection is incumbent on accurate disease staging, primarily performed with cross-sectional imaging. Similar to the modalities discussed in Chapter 1, Section 1.5 for the general diagnosis and evaluation of bladder cancer, the mainstay of imaging in MIBC includes CT, MRI, and PET scans. Given the risk of aggressive and metastatic disease with MIBC, additional imaging studies, such as dedicated bone and head imaging, may also be considered in certain situations.

The standard-of-care treatment for patients with MIBC is currently RC, pelvic lymph node dissection (LND), and urinary diversion.[20] However, novel bladder-sparing treatment strategies for specific patients with lower-risk MIBC or for patients who may be too frail to undergo major surgery are being investigated in an effort to reduce pursuant morbidity.[21] Novel imaging modalities will play an increasingly important role in selecting patients who are most likely to remain disease-free following treatment with bladder-sparing approaches. To date, imaging has been primarily used as a static assessment prior to definitive therapy. If integrated effectively in a dynamic fashion, though—quantifying treatment response over time—imaging may help predict response to neoadjuvant systemic therapy, evaluate for an ongoing response, and assess the need for definitive consolidative local therapy (i.e., cystectomy). To accomplish this goal, innovative imaging strategies are needed.

Herein, we summarize current guidelines for imaging in patients with MIBC; discuss the status of traditional and novel imaging modalities to detect non-organ confined disease; and discuss the current literature that supports the role of novel imaging modalities in delineating tumour biology and predicting response to systemic chemotherapy and immunotherapy.

6.3.1 Guideline recommendations

Three major professional organizations, including the American Urological Association (AUA), the European Association of Urology (EAU), and the National Comprehensive Cancer Network (NCCN), provide guidelines on imaging in patients with MIBC, with only slight differences among them (**Table 6–1**).[20,22,23] Abdominal and pelvic cross-sectional imaging with either CT or MRI is universally agreed upon for the staging of MIBC. The AUA and NCCN guidelines recommend that this should be performed prior to TURBT in tumours suspicious for being high grade or muscle invasive. However, this is not specified in the EAU guidelines. The majority of patients with bladder cancer will present with gross hematuria and will therefore have already undergone delayed contrast-enhanced imaging (CT or MRI urography). The NCCN and EAU guidelines recommend chest imaging with noncontrast CT for all patients, while the AUA guidelines consider a posterior-anterior and lateral chest x-ray as being adequate in nonsmokers. The AUA limits chest CT to smokers, given their additional risk of pulmonary malignancy.[24]

TABLE 6–1 Current Guidelines for Imaging Patients With Muscle-invasive Bladder Cancer

	AUA/ASCO/ASTRO/ SUO23	EAU22	NCCN20
Abdomen/pelvis	CT urogram Use MRI if unable to give iodinated contrast, with gadolinium if possible Noncontrast imaging with retrograde pyelograms if contrast unable to be given	CT urogram CT urography preferred over MR urography	CT urogram Use MR urogram in patients with iodinated contrast allergy or abnormal renal function with GFR >30 CT without contrast, renal ultrasound, and retrograde pyelograms in patients unable to receive iodinated or gadolinium contrast
Chest	CT scan in smokers, given risk of second primary lung malignancy May perform PA/lateral chest x-ray in nonsmokers	CT scan for evaluation of pulmonary metastases	CT with contrast if undergoing contrast-enhanced A/P imaging Otherwise, CT without contrast PA/lateral chest x-ray is acceptable but perform CT if equivocal, abnormal x-ray, or in select high-risk patients
Bone	Bone scan indicated only if patient symptomatic or if alkaline phosphatase is elevated	Imaging only if patient symptomatic MRI preferred over bone scan	Imaging only if patient is symptomatic, high risk, or has an elevated alkaline phosphatase MRI, PET-CT, or bone scan
Brain	No recommendations	No recommendations	Imaging only if patient is symptomatic or is high risk MRI with and without gadolinium or CT with iodinated contrast if unable to undergo MRI

Abbreviations: A/P, anteroposterio (from front to back); ASCO, American Society of Clinical Oncology; ASTRO, American Society for Radiation Oncology; AUA, American Urological Association; CT, computed tomography; EAU, European Association of Urology; GFR, glomerular filtration rate; MR, magnetic resonance; MRI, magnetic resonance imaging; NCCN, National Comprehensive Cancer Network; PA, posterioranterior (from back to front); PET, positron emission tomography; SUO, Society of Urologic Oncology.

Variable recommendations exist regarding PET imaging in the work-up of patients with MIBC. The AUA guidelines state that PET imaging should be reserved for patients with abnormal chest, abdominal, or pelvic imaging findings, or in patients with potentially metastatic lesions that cannot be biopsied. The NCCN guidelines suggest that PET, specifically PET-CT, may be beneficial in patients with ≥cT3 disease. The EAU guidelines state that there is insufficient evidence to recommend PET imaging for nodal staging, but do not comment on this modality for evaluation of extranodal metastases.

Finally, given the high rate of advanced disease in MIBC, imaging of additional sites of metastasis may be warranted. All three guidelines recommend bone imaging only if alkaline phosphatase is elevated or if the patient has specific symptoms suggestive of bony metastases. The recommendation is for a bone scan, although the EAU states that MRI may be more sensitive and specific for bone

metastases than a bone scan. In a prospective series of 48 patients with locoregional or metastatic bladder cancer, PET-CT was more sensitive and specific than bone scan for detection of skeletal metastases.[25] Brain imaging is also only warranted if clinical suspicion is high.

Despite clear guidelines set forth by the AUA, EAU, and NCCN, utilization of chest, body, and bone imaging in patients with MIBC is variable, likely secondary to the limitation of any one modality to provide high-accuracy staging of MIBC.[26-28] Adherence to guidelines, specifically with chest and bone imaging, is known to be associated with improved CSS and OS in population-based studies.[26] Strategies to improve compliance with guidelines would likely benefit clinical outcomes and should be addressed.

6.3.2 Identifying locally advanced and subclinical metastatic disease

The discrepancy between clinical and pathological stage in bladder cancer is well known.[29] Up to 36% of patients with clinically localized MIBC will have pathological evidence of extravesical extension and/or lymph node–positive disease at the time of RC. Clearly, improvements in preoperative imaging are needed to accurately identify those patients who would be most likely to benefit from NAC. To this end, many centres are evaluating MRI and PET imaging as alternatives to traditional contrast-enhanced CT for improved staging prior to definitive therapy. **Table 6–2** lists the ranges of sensitivities and specificities for CT, MRI, and PET imaging to detect the primary lesion, extravesical extension (≥T3 disease), and lymph node–positive disease.

TABLE 6–2 Sensitivities and Specificities of Abdominal Imaging Modalities in the Staging of Bladder Cancer

		CT	MRI*	PET†
Primary lesion	Sensitivity	77%–95% (Lodde *et al.*[37], McKibben and Woods[60])	80%–100% (McKibben and Woods[60])	85%–87% (Lodde *et al.*[37]; Harkirat *et al.*[61])
	Specificity	28%–71% (Lodde *et al.*[37], McKibben and Woods[60])	78%–91% (McKibben and Woods[60])	25%–100% (Lodde *et al.*[37]; Harkirat *et al.*[61])

Abbreviations: ^{18}F-FDG, fluorine-18 2-fluoro-2-deoxy-D-glucose; A/P, anteroposterio (from front to back); CT, computed tomography; MRI, magnetic resonance imaging; PA, posterioranterior (from back to front); PET, positron emission tomography.

* Includes multiparametric functional studies (diffusion weight imaging, dynamic contrast enhancement).

† ^{18}F-FDG-PET-CT unless otherwise indicated.

continued on **page 551**

TABLE 6–2 Sensitivities and Specificities of Abdominal Imaging Modalities in the Staging of Bladder Cancer, *Cont'd*

		CT	MRI*	PET†
≥T3 disease	Sensitivity	83%–89% (Baltaci *et al.*[62], Oz *et al.*[64])	80%–100% (Ghafoori *et al.*[32], Kim *et al.*[33], Daneshmand *et al.*[34], Takeuchi *et al.*[35])	Limited data available
	Specificity	63%–100% (Baltaci *et al.*[62], Oz *et al.*[64])	47%–97% (Ghafoori *et al.*[32], Kim *et al.*[33], Daneshmand *et al.*[34], Takeuchi *et al.*[35])	Limited data available
N+ disease	Sensitivity	27%–85% (Lodde *et al.*[37], Swinnen *et al.*[39], Pichler *et al.*[40], McKibben and Woods[60])	0% (Jensen *et al.*[65]) 76%–83% (McKibben and Woods[60])	33%–78% (Soubra *et al.*[38]; Pichler *et al.*[40])
	Specificity	67%–100% (Lodde *et al.*[37], Swinnen *et al.*[39], Pichler *et al.*[40], McKibben and Woods[60])	80%–98% (McKibben and Woods[60], Jensen *et al.*[65])	81%–100% (Soubra *et al.*[38]; Pichler *et al.*[40])

Abbreviations: ^{18}F-FDG, fluorine-18 2-fluoro-2-deoxy-D-glucose; A/P, anteroposterio (from front to back); CT, computed tomography; MRI, magnetic resonance imaging; PA, posterioranterior (from back to front); PET, positron emission tomography.

* Includes multiparametric functional studies (diffusion weight imaging, dynamic contrast enhancement).

† ^{18}F-FDG-PET-CT unless otherwise indicated.

MRI combines high spatial resolution with additional functional sequences that improve detection of muscle-invasive and locally advanced bladder cancer.[30] In multiparametric MRI (mpMRI) protocols, dynamic contrast-enhanced MRI (DCE-MRI) evaluates tumour vascularity, while diffusion-weighted MRI (DW-MRI) characterizes the tumour microenvironment. In a meta-analysis of over 1,700 patients by Woo *et al.*, mpMRI demonstrated a sensitivity and specificity of 94% and 95%, respectively, in identifying MIBC.[31] This analysis did find that 3T magnets (compared with 1.5T) plus at least one functional technique (DCE-MRI or DW-MRI) improved the accuracy of staging. Furthermore, several studies have used MRI to distinguish extravesical extension on preoperative imaging, albeit with a fair degree of variability.[32,33] In a prospective study of 122 patients by Daneshmand *et al.*, dynamic gadolinium (contrast)-enhanced MRI yielded a sensitivity and specificity of 87% and 47%, respectively, in identifying extravesical extension.[34] However, in a smaller study of 52 patients (only 10 with ≥pT3 disease) using DW-MRI and DCE-MRI, the sensitivity and specificity of differentiating ≤T2 tumours from ≥T3 tumours was 80% and 97%, respectively.[35] Factors such as the receipt of NAC, protocol differences, and radiologist experience have been cited as potential causes of the high degree of variability seen. Despite the potential improvements in staging locally advanced disease, the potential limitations of routine mpMRI include the availability of this imaging

modality at nonreferral centres, the requirement of experienced radiologists, the time required to perform the study, and the associated costs. While this continues to be evaluated, the utilization trends and financial impact on the health care system is unknown.

PET imaging is standard in the evaluation of many cancers. As reviewed in Chapter 1, Section 1.5 multiple PET tracers are available, each with specific advantages. Currently, the most commonly used tracer is fluorine-18 2-fluoro-2-deoxy-D-glucose (^{18}F-FDG).[30] The major disadvantage of ^{18}F-FDG as a tracer is the urinary excretion, which often obscures primary lesions and locoregional lymph nodes. Protocols such as voiding prior to imaging, diuresis, and others have been used in an effort to improve imaging quality. Tracers such as ^{11}C-choline, ^{11}C-acetate, and ^{11}C-methionine are of interest, given their minimal urinary excretion and enhanced ability to evaluate intraluminal lesions.[36] The major disadvantage of ^{11}C-choline is its short half-life (20 minutes), which requires an onsite cyclotron for generation of this tracer, while ^{11}C-methionine suffers from high noise background, which obscures weakly positive lesions. Nevertheless, there are data to suggest that PET imaging may be better able to preoperatively stage patients with MIBC, although studies are conflicting.[37-40] In one prospective study, ^{18}F-FDG-PET-CT performed prior to pathological confirmation of lymph node metastasis (biopsy or LND) had a sensitivity and specificity of 70% and 94%, respectively.[41] In a retrospective institutional study and meta-analysis, Soubra *et al.* evaluated 78 patients with cT2 disease with PET-CT for nodal metastasis and found that the sensitivity and specificity was 56% and 98%, respectively. The authors then performed a meta-analysis and pooled the sensitivity and specificity of seven additional studies, all judged to be of sufficient quality for analysis by the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool. The pooled sensitivity and specificity was 57% (range 33%–78%) and 95% (range 86%–100%), respectively. The authors suggest that a PET-CT would be most useful in patients at low risk for node-positive disease in whom a positive PET-CT would steer a patient towards systemic, rather than local, therapy. This would be especially true for low-risk patients with imaging that is equivocal for lymph node–positive disease. On the other hand, Swinnen *et al.* found an equivalent sensitivity (46%) and only modest improvement in specificity (97% vs. 92%) when PET-CT was compared with conventional CT.[39] Similarly, Pichler *et al* found that the modest improvement in sensitivity of PET-CT over conventional CT was dependent on the cut-off criteria for a positive lymph node (>8 mm vs. 10 mm) and did not warrant PET-CT if a threshold of >8 mm was used.[40] Nevertheless, PET-CT represents an improvement in the preoperative staging of patients with MIBC and may alter management in patients found to have distant disease not identified on conventional imaging.

Combination PET imaging and MRI is a newer modality that has been recently evaluated. In a small study by Rosenkrantz *et al.*, the value of PET-MRI was assessed in 22 patients based on this modality's ability to change the level of suspicion of bladder tumour, nodal metastasis, and extranodal metastasis compared with MRI alone.[42] They found that the addition of PET changed the level of suspicion in 36%, 52%, and 9% of the bladder, lymph node, and extranodal pelvis sites, respectively. Theoretically, patients with tumours cystoscopically suspicious for muscle-invasive disease or pathologically confirmed MIBC at TURBT could benefit from a single PET-MRI study compared with separate PET and MRI studies for optimal preoperative staging.

Finally, advanced technologies are always at risk of being adopted before sufficient evidence supports their use. In contrast to the relatively stable use of MRI in the management of bladder cancer, it is known that PET imaging has increased dramatically in the workup of bladder cancer. In a study by Huo *et al.*, use of PET-CT was found to have increased 16-fold from 2004 to 2011 in Medicare beneficiaries with nonmetastatic bladder cancer, corresponding to nearly $12 million in excess spending compared with conventional CT imaging alone.[43] One potential limitation of this study is that it is unknown whether PET-CT is replacing MRI or conventional CT in the standard staging evaluation of patients with bladder cancer. It is possible that PET-CT is being used in lieu of these other imaging modalities, for example in patients with chronic renal insufficiency to void intravenous contrast or in those with allergy to contrast agents. Clearly, however, for both MRI and PET imaging, potential improvements in preoperative staging will have to be linked with clinically meaningful benefits in order to justify the costs.

6.3.3 Role of imaging in novel treatment strategies in muscle-invasive bladder cancer

Traditionally, the standard-of-care treatment for MIBC has been RC, LND, and urinary diversion.[20] Given the known morbidity of this operation in a patient population that often suffers from many other significant comorbidities, several bladder-sparing treatment strategies are being evaluated.[21] Currently, the most common and well-studied bladder-sparing treatment is concurrent chemoradiation therapy.[44] In more fragile patients or those who refuse RC, maximal endoscopic resection followed by systemic chemotherapy has been shown to provide reasonable outcomes.[45-47] The key to any bladder-sparing treatment is a thorough assessment of the stage of disease, which is known to be incomplete with current imaging modalities.[29] Patients in whom a complete resection is not possible are not ideal candidates for bladder-sparing treatments. Furthermore, identification of systemic chemotherapy-nonresponsive disease and prediction of aggressive disease would help sway patients and providers from bladder-sparing treatments and towards more aggressive options, such as multimodal therapy with cystectomy. For this reason, novel imaging strategies warrant investigation for patients with MIBC.

One important consideration is the completeness of the endoscopic resection.[48] Current techniques used to ensure maximal endoscopic resection include enhanced cystoscopy, such as photodynamic diagnosis and narrow band imaging, rebiopsy of the resection bed, and urinary cytology.[49] MRI has also shown promise in evaluating the intraluminal lesion. Specifically, diffusion kurtosis imaging (DKI) has been evaluated as a method to distinguish postendoscopic resection or inflammation following NAC from recurrent bladder tumours.[50] In this study, 50 patients with bladder tumours following endoscopic resection or NAC underwent DWI-MRI with DKI. Inflammation versus tumour was assessed on restaging endoscopic resection or cystectomy. With DKI, the area under the curve was 93%, with a sensitivity and specificity of 91% and 92%, respectively. Patients in whom a recurrent tumour is identified would warrant more aggressive definitive local treatment.

NAC has become a quality-of-care indicator in the management of MIBC.[20,22,23] In addition to being associated with an improvement in OS, patients whose tumours completely respond to NAC experience the greatest improvement in outcomes.51 On the other hand, nearly three-quarters of patients who receive NAC do not completely respond and, thus, may experience unnecessary toxicity and

delays in definitive treatment.[51] While several biomarkers are being evaluated, none have made it into clinical practice as of yet.[52] In a study by Seiler *et al.*, response to NAC was stratified by molecular subclass, showing that basal tumours had the greatest benefit from systemic therapy prior to surgery.[53] Similarly, imaging may be able to identify and elucidate the biology of disease. In a proof-of-concept study looking at patients with MIBC, the apparent diffusion coefficient, a parameter calculated from the DWI-MRI sequence that characterizes tissue micro-cellularity, was able to distinguish responders from nonresponders based on a combination of pathological data and Response Evaluation Criteria In Solid Tumors (RECIST) criteria (≤ypT1 or ypT2 with a RECIST criteria response).[54] Out of the 20 patients included in the study, the 15 patients who responded to NAC had statistically higher calculated uniformity and lower entropy compared with nonresponders, suggesting that heterogeneous tumours have a poorer response to NAC. NAC nonresponders would potentially benefit from expedited RC, and responders would benefit from a full course of systemic therapy prior to local therapy. Prospective studies evaluating this imaging technique and strategy, potentially in combination with molecular data from biopsies, are warranted.

One of the most important advances in the treatment of bladder cancer in the last several decades has been the discovery of checkpoint inhibitors (CPI), now approved for metastatic and advanced disease.[55] While not yet approved for localized disease, the tide is quickly turning and clinical trials evaluating CPI as neoadjuvant systemic therapy are under way.[56] Traditional radiographic (i.e. RECIST) criteria to identify treatment response may not apply to patients being treated with CPI, given the known pseudo-progression experienced in approximately 10% of patients, and therefore several modifications to response criteria have been made for patients treated with immunotherapy.[57,58] Several novel PET tracers have also been developed that may be more specific for responses seen in patients treated with immunotherapy, such as CPI.[58] 18F-fluoro-ethyl-tyrosine is absorbed by neoplastic cells and is used in imaging of brain malignancies to circumvent the pseudo-progression often seen secondary to cerebral edema following treatment. Tracers specific for T cells, the target of several immunotherapeutic agents, such as 1-(2'-deoxy-2'-[18F]fluoroarabinofuranosyl) cytosine (18F-FAC) and 2'-deoxy-2'-[18F]fluoro-9-b-Darabinofuranosylguanine (18F-F-AraG), are being tested in early clinical studies. 18F-F-AraG PET is being evaluated as part of a clinical trial (ClinicalTrials.gov number: NCT03007719) in patients with locally advanced bladder cancer undergoing neoadjuvant atezolizumab therapy or those with locally advanced or metastatic bladder cancer receiving primary treatment with atezolizumab. Investigators will be evaluating the change in standardized uptake values between pre- and post-treatment imaging, as well as determining whether these changes correlate with downstaging or clinical response. These tracers offer improvements over 18F-FDG in their specificity for neoplastic cells and may become more common, especially as immunotherapy becomes more routine.

To date, molecular biomarkers from pathology specimens, such as the percent immunohistochemical staining of programmed cell death-1 protein (PD-1) and programmed cell death ligand-1 (PD-L1) on tumour and immune cells, have resulted in incomplete and conflicting prognostic information.[59] Differences in the immunohistochemical assays utilized and heterogeneity within bladder tumours have been cited as potential confounders to reliable prognostic value of PD-1/PD-L1 staining. Another possibility is that expression of these biomarkers may change at varying time points along the treatment pathway (e.g., treatment naïve vs. pretreated tumour). Therefore, a noninvasive method of assessment that does not require tissue could potentially prove useful. Immuno-PET imaging is

being used in both animal models and humans to characterize the expression of cell surface immunologic receptors involved in response to CPI.[58] Several clinical trials are evaluating 89Zr-labelled anti-PD-L1 antibodies to quantify and locate PD-L1 in patients with multiple cancer types, including bladder cancer (ClinicalTrials.gov number: NCT02453984). Ultimately, immuno-PET may be able to provide the real-time granular biological data necessary to inform treatment with CPI and improve responses.

6.3.4 Summary

Imaging plays a critical role in the workup of patients with MIBC, especially in identifying patients who would benefit from RC. Furthermore, the successful treatment of patients with MIBC with bladder-sparing approaches is incumbent on thorough staging and assessment of tumour response, which can be enhanced with novel imaging, such as MRI and PET scans. Novel tracers for PET scans will also begin to play an important role in patients treated with immunotherapy, especially as this treatment moves into the neoadjuvant space. Finally, methods to improve compliance with guidelines for imaging and thorough cost analyses should be performed to optimize the benefit of novel imaging techniques.

6.4 Radical Surgery

6.4.1 Removal of the tumour-bearing bladder and regional lymph nodes

6.4.1.1 Introduction

RC is the standard treatment for MIBC in most countries worldwide.[66] In the precystectomy era, patients with muscle-invasive disease rarely exceeded 5-year survival rates of more than 3%, and performing radical surgery was associated with a considerably increased perioperative morbidity and mortality.[67] In the last decades, advances in the surgical technique as well as perioperative anesthesiological care have significantly decreased the complication rates associated with this procedure, and today RC is considered the mainstay of treatment in muscle-invasive disease.

In the last decade, increased interest in quality-of-life issues, however, has increased the trend toward bladder preservation with trimodality TURBT followed by chemoradiation therapy.[6,68,69] Also, performance status and age were formerly reported to influence the choice of therapy, with cystectomy being reserved for younger patients without concomitant disease and better performance status.[70,71]

To better define old age, the terms "patients older than 80 years" or "octogenarians" were used instead of the terms "elderly patients" or "old patients."[72] The feasibility and safety of radical surgery in octogenarians has thus been demonstrated.[73,74]

The value of assessing overall health before recommending and proceeding with surgery is emphasized because of the association between comorbid illness, adverse pathological findings, and survival outcome following RC.[69] Similar to these results, a recent analysis from the Surveillance, Epidemiology and End Results (SEER) registries evaluated the impact of comorbidities on cancer-specific and other-cause mortality in a population-based competing risk analysis of more than 11,260 patients. Age was found to confer the highest risk for other-cause mortality, but not for increased cancer-specific death, whereas locally advanced tumour stage was the strongest predictor for decreased CSS.[75] Frailty assessment is a further strong selection criterion for elderly and old patients. In a validation study, a score from a modified frailty index predicted postoperative outcome.[76,77]

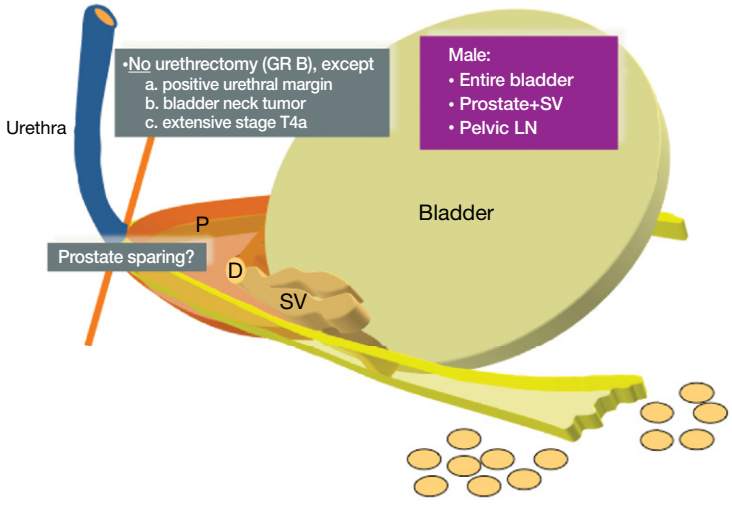
Stratifying elderly patients according to their individual risk-benefit profile as well as frailty within a multidisciplinary teamwork might therefore help to select those who might benefit most from radical surgery and to optimize their outcomes, regardless of sex or age.

6.4.1.2 Radical cystectomy technique

In male patients, the literature over the last two decades has set the standard of surgical limits for curative RC as complete removal of the bladder with all macroscopically visible and resectable bladder perforating tumour extensions, removal of the adjacent distal ureters, and the lymph nodes corresponding to the tumour-bearing bladder (**Figure 6-2**).

FIGURE 6–2
Extent of Cystectomy in Male Muscle Invasive Bladder Cancer

Abbreviations: LN, lymph node; SV, seminal vesicle.



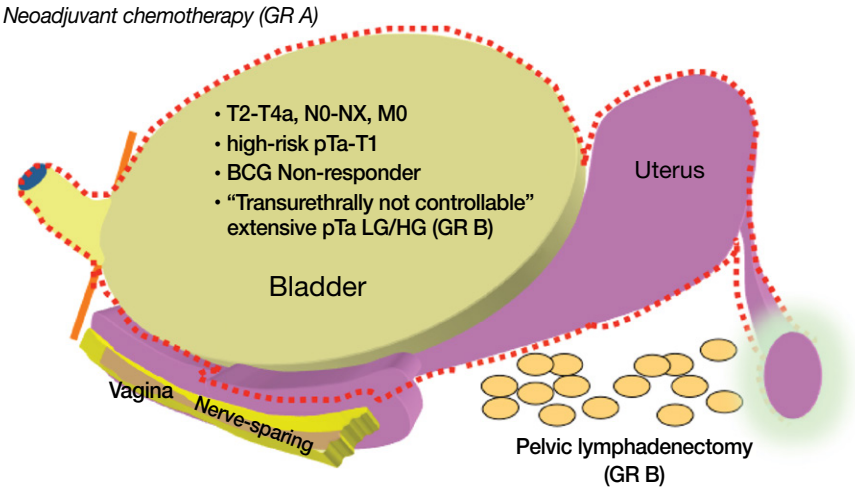
Preservation of the anterior and membranous urethra, including rhabdosphincter in order to enable an orthotopic neobladder (NB); parts of the prostate and seminal vesicles for reasons of fertility, potency, and continence; and intrapelvic autonomic and sensory nerves to enhance potency and continence, are all technical variations to this standard that may improve patients' quality of life (QoL) but must be attentively judged against possible oncological risks[78] **[LOE 3; GOR C]**.

If leaving parts of the prostatic gland during the resection, the hazard of an unsuspected adenocarcinoma may be as high as 23% to 54%, of which up to 29% may be clinically significant, leading to local recurrence or even metastasis.[79-81] Furthermore, urothelial carcinoma may be present in the prostate, and in some series up to 27% of patients undergoing cystoprostatectomy had prostate cancer.[82] Another technical variation is deliberately leaving the seminal vesicles and prostatic capsule in order to better preserve the surrounding autonomic nerves. The results regarding potency versus oncological risk in small series of selected patients are encouraging but need long-term confirmation in larger series.[83,84] To date, these technical modifications have not been documented to improve continence and they remain highly controversial regarding oncological safety. It remains to be seen whether there is a functional or oncological difference when leaving the seminal vesicles while dissecting the bladder and prostate and removing them separately at the end of the ablative surgery.

In *female* patients, standard anterior pelvic exenteration includes the entire urethra, adjacent vagina, uterus, distal ureters, and respective lymph nodes (**Figure 6-3**) **[LOE 3; GOR C]**. Unless the primary tumour is located at the bladder neck or in the urethra, a major part of the functioning female urethra and—provided a complete tumour resection is possible—its supplying autonomous nerves can be preserved in case of a planned orthotopic NB[68,85-88] **[LOE 3; GOR C]**.

FIGURE 6–3
Extent of Cystectomy in Women

Abbreviations: BCG, bacillus Calmette-Guérin; HG, high grade; LG, low grade.



New data also question the necessity of removing the uterus or any portion of the vagina, arguing for a better anatomical support of the NB and better preservation of surrounding autonomous nerves.[89]

In *both sexes*, the length of the distal ureteral segment to be removed with the bladder has not been specified and depends on oncological issues such as tumour extension or presence of CIS, and type of subsequent urinary diversion. In one recent study, frozen sections of the distal ureteral margins had a sensitivity of 74% and a specificity of 99.8%, resulting in an overall accuracy of 98.3%.[90] With a serial sectioning strategy, most initially positive ureteral margins can be converted into negative final margins. Those patients were also at decreased risk of developing upper urinary tract recurrent disease.[91,92]

6.4.1.3 Pelvic lymph node dissection for bladder cancer

Pelvic lymphadenectomy is part of an oncologically indicated RC to control locoregional disease and to potentially improve CSS. Survival after RC is usually predicted by pathological tumour stage, status of surgical margins, and involvement of lymph nodes.[93]

Although earlier studies have already demonstrated a prognostic benefit to extended pelvic lymphadenectomy as compared to a limited lymphadenectomy, the anatomically adequate extent of LND to obtain reliable staging results is still controversial. In light of a recent prospective, multidisciplinary, randomized study, the benefit of an extended lymphadenectomy is also controversial.[94]

In a recent prospective, randomized phase 3 trial on the clinical efficacy of NAC plus cystectomy,[5] it was shown that surgical factors, including the extent of LND and the individual surgeon's experience, have a major impact on the therapeutic outcome and OS[95] [**LOE 1**]. This trial's data also indicate that chemotherapy was more likely to be beneficial if patients received high-quality surgery from an experienced surgeon. It was concluded that it is extremely important to develop universally accepted standards for RC and pelvic lymph node dissection (PLND) in patients with invasive bladder cancer in order to improve outcome.[96]

Lymph node metastases are found in 20% to 25% of patients who undergo RC and pelvic lymphadenectomy for bladder cancer, and are the most important prognostic factor in these patients, predicting significantly decreased recurrence-free survival (RFS) and OS compared to patients without node metastasis.[97-100] In node-negative patients, the total number of lymph nodes removed and the anatomical extent of the node dissection are both useful measures in evaluating the proper extent of surgery and in predicting outcome. In patients with nodal metastasis, the number of nodes removed, and the number and percent of positive nodes, may both be independent predictors of recurrence and survival.[101-104]

Regarding the number of lymph nodes needed for accurate staging, Capitanio *et al.*[105] evaluated the likelihood of finding one or more positive lymph nodes according to the number of lymph nodes removed at RC **[LOE 2]**. A total of 731 assessable patients underwent RC and bilateral pelvic lymphadenectomy at three different institutions. Receiver operating characteristic (ROC) curve coordinates were used to determine the probability of identifying one or more positive lymph nodes according to the total number of removed lymph nodes. One hundred and seventy-four patients had lymph node metastases (23.8%). The mean (median, range) number of lymph nodes removed was 18.7 (17, 1–80). The ROC coordinate-based plots of the number of removed lymph nodes and the probability of finding one or more lymph node metastases indicated that removing 45 lymph nodes yielded a 90% probability. Conversely, removing either 15 or 25 lymph nodes indicated, respectively, 50% and 75% probability of detecting one or more lymph node metastases. These data indicate that removing 25 lymph nodes might represent the lowest threshold for the extent of lymphadenectomy at RC. In a similar approach, Koppie *et al.* reported a study designed to determine if there was a minimum number of lymph nodes analyzed above which there was no improvement in survival (LE: 2).[106] The cohort included 1,121 patients from Memorial Sloan Kettering Cancer Centre (MSKCC) accrued over a 14-year period. The investigators determined that there was no plateau in the dose-response curve with an increasing number of nodes up to ≤23 nodes, as very few had 24 or more nodes removed. The authors did not indicate the percent of patients who underwent an extended node dissection and, in fact, 13% of patients had no nodes identified in the pathology report. The median number of nodes removed was nine.

6.4.1.4 Impact of extent of pelvic lymph node dissection on outcome

The anatomical extent of PLND and the minimum number of lymph nodes to be retrieved for an accurate staging still have to be defined. The crossing of the ureters with the common iliac vessels may be regarded as the most cranial limit for a standard LND[107] (9), whereas extended lymphadenectomy is the extension up to the aortic bifurcation. It is generally agreed that the more lymph nodes are removed, the higher the number of patients with positive lymph nodes[102] **[LOE 3]**. Furthermore, it has been demonstrated that survival after RC is predicted by the pathological stage of the primary bladder tumour and pelvic nodes. Leissner *et al*[105] suggested that a significant survival benefit was maintained if more than 16 lymph nodes were removed. Stein *et al*[104] reported that survival in patients with positive lymph node disease was better if more than 15 pelvic lymph nodes had been retrieved. On the other hand, Abdel-Latif and coworkers[108] and Herr[101] could not reproduce the relationship between survival and number of dissected lymph nodes by using multivariate statistical analysis. In this context, it has to be underlined that the number of retrieved lymph nodes can be influenced by many factors, such as the specifics of the surgeon,[105] the extent of lymphadenectomy,[102,103,107] presentation of the pathological specimen,[109] and pathohistological work-up and techniques of analysis.[105] The

clinical reality, however, is significantly different: an evaluation of the SEER database in 2003 demonstrated that the majority of patients in a population-based analysis had 4 or fewer nodes removed with cystectomy.[110,111]

In a prospective, randomized, multicentre, phase 3 trial by the Association of Urologic Oncology (AUO) of the German Cancer Society in patients with locally resectable T1 high-grade or muscle-invasive urothelial bladder cancer (T2–T4a, cM0) comparing limited (defined as obturator, internal, and external iliac nodes) versus extended (defined as additional deep obturator, common iliac, presacral, paracaval, interaortocaval, and para-aortal nodes up to the inferior mesenteric artery), lymphadenectomy did not show any benefit of extended lymphadenectomy regarding 5-year RFS, CSS, and OS. Patients with NAC or radiotherapy (RT) were excluded; AC was allowed.

In 401 patients, the median number of dissected nodes was 19 in the limited and 31 in the extended arm. Clavien grade ≥3 lymphoceles were more frequently reported in the extended LND group within 90 days of surgery (ClinicalTrials.gov number: NCT01215071) [**LOE 1; GOR A**]. This is in contrast to older, nonrandomized studies having demonstrated a significant impact of the technique of PLND with regard to therapeutic outcome. Poulsen *et al.*[112] were one of the first groups to compare the prognostic significance of limited versus extended pelvic lymphadenectomy in a retrospective analysis of 194 patients undergoing RC [**LOE 3**]. Limited pelvic lymph node dissection (LPLND) began at the iliac bifurcation, including the lymph nodes along the external and internal iliac artery and the obturator fossa; extended pelvic lymph node dissection (EPLND) began at the aortic bifurcation and included the common, external, and internal iliac artery and the obturator fossa. The authors observed a substantial improvement of 5-year RFS in patients with tumours confined to the bladder wall (85% vs. 64%; $p<0.02$) and without lymph node involvement (90% vs. 71%; $p<0.02$). Five-year probability for locoregional (7% vs. 2%) and systemic recurrences (21% vs. 10%) were reduced substantially in patients with bladder cancer confined to the bladder wall in the EPLND group, however, without reaching statistical significance.

In a retrospective analysis of 484 patients undergoing RC and pelvic lymphadenectomy, Leissner *et al.*[105] demonstrated that the total number of lymph nodes retrieved had a significant impact on RFS ($p<0.01$) [**LOE 2**]. The 5-year RFS was 25% and 53% in patients with ≤14 and ≥15 lymph nodes being removed, respectively. Furthermore, the surgeon had a significant impact on the prognosis, as it was shown the number of lymph nodes dissected ranged between 10.6 and 25.7 and differed significantly between the 11 different surgeons being involved in the study. These data are further corroborated by a recent paper on the standardization of RC and pelvic lymphadenectomy.[96] However, the authors did not demonstrate a significant OS and CSS advantage for patients undergoing EPLND as compared to those undergoing limited pelvic lymphadenectomy (LPLA) only. The authors further evaluated the concept of EPLND in a prospective clinical trial comprising 290 patients.[113] The cranial limit of EPLND was the inferior mesenteric artery, the lateral border was the genitofemoral nerve, and the caudal limit was the pelvic floor. A mean number of 43.1 ± 16.1 lymph nodes were removed, with 27.9% of the patients demonstrating positive lymph nodes. Although the group identified a preferred pattern of metastatic spread, they were not able to identify a well-defined sentinel lymph node or lymph node area.

These data are in contrast to the recently published prospective trial of Bochner *et al.*[114] on the evaluation of lymph node count and lymph node mapping [**LOE 3**]. One hundred and forty-four consecutive patients were included in this monocentric evaluation, with 56 and 88 patients undergoing standard pelvic lymph node dissection (PLND) and EPLND, respectively. Standard PLND included the nodal regions of the external iliac, hypogastric, and obturator fossa, with the iliac bifurcation representing the cranial limit of LND. EPLND included the lymph nodes at the aortic bifurcation to no more than 2 cm cranially to the bifurcation and the nodal regions of standard PLND. Although the median number of positive lymph nodes differed significantly between both groups (22.5 vs. 8), there was no difference with regard to the percentage of positive nodes, which was 21% in both groups. Interestingly, all patients with positive nodes above the aortic bifurcation also had positive nodes detected in the lower packages, indicating that only extensive locoregional metastatic disease might involve the retroperitoneal areas associated with a dismal prognosis. Including the lymph nodes along the common iliac artery above the iliac bifurcation, however, appears to be of prognostic value and of clinical significance. In the study of Bochner *et al.*,[114] four patients had unexpected micrometastatic lymph node disease at the common iliac region only. Reflecting the survival data of patients exhibiting micrometastatic lymph node disease at time of RC, most of these patients are expected to have a relatively favourable outcome. Morbidity of pelvic lymphadenectomy is not increased by including the common iliac region in routine PLND, so this area should be removed as a standard part of staging lymphadenectomy.

Further evidence to include the common iliac region derives from the prospective multi-institutional study published by Leissner *et al.*[113] **[LOE 2]**. Eighty-one (27.9%) patients demonstrated lymph node involvement, and 35% of all positive lymph nodes derived from above the iliac bifurcation. Furthermore, 20 patients (6.9%) were shown to harbour positive lymph nodes above the bifurcation of the common iliac artery only. Although no data with regard to the prognostic significance in terms of CSS or progression-free survival (PFS) are available, these data strongly support the idea of including the lymph nodes of the common iliac region up to the aortic bifurcation in the routine LND technique for MIBC.

In another study, Abol-Enein *et al.*[115] evaluated the locoregional distribution of positive pelvic lymph nodes in 200 consecutive patients undergoing RC **[LOE 3]**. The authors also attempted to identify the probability of lymph node clearance with increasingly wide fields of node dissection. In their investigation, extended pelvic lymphadenectomy included the lymphatic tissue up to the inferior mesenteric artery, the common, external, and internal iliac region. A mean number of 50.6 lymph nodes were retrieved per patient, with 48 (24%) patients exhibiting positive nodes. More than one-third (39.6%) of these patients demonstrated bilateral involvement. A single positive lymph node was identified in 22 (45.8%) patients. The authors demonstrated that close to 80% of all positive nodes could be cleared completely of the field of PLND, including all lymphatic tissues along the common, external, and internal iliac region. Metastases outside the true pelvis were only detected in multinodal disease, and these metastatic deposits were always associated with metastases at the obturator fossa and/or the internal iliac region. Therefore, the authors conclude that standard lymphadenectomy in bladder cancer should always include all lymphatic tissues in the true pelvis; LND might be extended up to the inferior mesenteric artery if frozen section examination exhibits positive lymph nodes in the sentinel region of the true pelvis.

Dhar and coworkers[98] evaluated the impact of limited and extended pelvic lymphadenectomy in a cohort of 336 and 322 patients, respectively, who were treated at two different institutions **[LOE 3]**. The overall lymph node–positive rate was 13% for patients with LPLND and 26% for those who had EPLND. The authors identified a significantly better RFS for patients who underwent extended pelvic lymphadenectomy. These figures held true for both organ-confined and locally advanced disease. The 5-year RFS of patients with lymph node–positive disease was 7% for LPLND and 35% for EPLND. The 5-year RFS for pT2N0 cases was 67% for limited and 77% for EPLND, and the respective percentages for pT3N0 cases were 23% and 57% ($p<0.0001$). The 5-year RFS for pT2N0-2 cases was 63% for LPLND and 71% for EPLND, and for pT3N0-2 cases the respective figures were 19% and 49% ($p<0.0001$). These data confirm that EPLND allows for more accurate staging and improved survival of patients with nonorgan-confined and lymph node–positive disease.

In another single institution analysis, the clinical importance of dissecting all lymphatic tissue up to the aortic bifurcation became evident when the outcome of 336 patients was analyzed. The patients underwent RC and extended pelvic lymphadenectomy, including the lymph nodes, the common and external iliac lymph nodes, and the periaortic, presacral and obturator fossa nodes[116] **[LOE 3]**. The lymphatic tissue removed above and below the bifurcation of the common iliac vessels was submitted separately for histopathological analysis. Overall, 64 patients (19%) had lymph node metastases, of whom 22 (34.4%) had lymph node involvement above the bifurcation of the common iliac vessels outside the template of the standard LND. The median number of retrieved lymph nodes was 27 (range 7–78) and, in those with lymph node metastases, 27 (range 11–49) included 8 (range 0–17) above the bifurcation and 18 (range 8–41) below the bifurcation of the common iliac vessels in the true pelvis. Lymph node involvement proved a significant adverse prognostic factor, with a 5-year probability of survival of 39% versus 76%. The overall 5-year survival rates were similar in patients with lymph node involvement above the bifurcation of the common iliac vessels (37%) compared to the entire population with lymph node metastasis (41%) and to those with lymphatic metastases in the true pelvis below the bifurcation of the common iliac vessels (42%). The survival rate was significantly higher in patients with 5 or fewer involved lymph nodes (50% vs. 13%; $p<0.002$) and in those with a lymph node density (number of lymph nodes involved/total number of lymph nodes removed) less than 20% (25% vs. 47%; $p<0.05$), but it did not relate to the total number of retrieved lymph nodes. These data underscore the contention that extended dissection not only provides the most accurate staging, but also offers the patient the best chance of survival. Following RC patients can be stratified into risk groups according to tumour stage, lymph node involvement, number of metastatic nodes, and lymph node density. The results of Steven *et al.*[116] support the idea that the benchmark for RC should include EPLND with anatomical boundaries including the common iliac and presacral nodes.

6.4.1.5 Critical issues in anatomical pelvic lymph node dissection for bladder cancer

Pathohistological examination of dissected lymph node specimens in these studies has been done more thoroughly and extensively than in other studies, concentrating on issues such as OS, CSS, and regional versus distant failure. Lymphatic tissues dissected from different areas should be sent separately instead of en bloc for pathohistological evaluation, since it has been demonstrated that the yield of lymph nodes increases significantly, thereby increasing the frequency of micrometastatic

deposits.[109] Intrainstitutional standardization of pelvic lymphadenectomy appears to be of utmost importance to generate reliable and reproducible results, since staging lymphadenectomy is extremely surgeon-dependent, as has been demonstrated by Leissner *et al.*[105]

6.4.1.6 Recommendations

- Preservation of the anterior and membranous urethra, including parts of the prostate and seminal vesicles for reasons of fertility, potency, and continence are technical variations to the nerve-sparing approach, which may improve patients' QoL but must be attentively judged against possible oncological risks **[LOE 3; GOR C]**.
- In female patients, standard anterior pelvic exenteration includes the entire urethra, adjacent vagina, uterus, distal ureters, and respective lymph nodes **[LOE 3; GOR C]**. The urethra-supplying autonomous nerves can be preserved in case of a planned orthotopic NB **[LOE 3; GOR C]**.
- RC in patients with MIBC should be performed within 3 months after initial diagnosis of stage T2 to T4 disease **[LOE 3; GOR B]**.
- The more lymph nodes are removed, the higher the probability of detecting at least one positive lymph node. However, there is no real threshold of the numbers of lymph nodes that need to be removed **[LOE 2; GOR B]**.
- Although there is some evidence from retrospective and prospective analyses that an extended pelvic lymphadenectomy might be associated with an improvement in 5-year PFS the most recent prospective randomized multicentre study did not show any evidence but did show an increased incidence of lymphoceles ($p<0.01$) **[LOE 2; GOR B]**.
- LND should include all lymphatic tissues around the common iliac, external iliac, and internal iliac groups, as well as the obturator group bilaterally, since up to one-third of all positive nodes are located around the common iliac artery **[LOE 3; GOR B]**.

6.4.2 Minimally invasive approach: Laparoscopic and robotic-assisted radical cystectomy

6.4.2.1 Introduction

Minimally invasive approaches to RC have continued to evolve since 1993.[117] Several meta-analyses have highlighted that minimally invasive techniques compared with open RC decrease blood loss and transfusion rates, have a shorter time to normal diet, and reduce length of stay.[99,118,119] Additional potential advantages of a minimally invasive approach are reduced postoperative pain, resulting in less opioid requirement and quicker return to normal day-to-day activities.[120,121] Robotic-assisted radical cystectomy (RARC) has also been found to be advantageous in patient groups susceptible to complications, such as the elderly,[122] resulting in laparoscopic and robot-assisted approaches being increasingly performed as an alternative to open radical cystectomy (ORC) over the past decade.[99] The robotic approach is similar to the laparoscopic approach, with the added benefits of the improved range of motion of instruments and three-dimensional vision affording a less steep learning curve. Although the number of RARCs performed in the United States is steadily increasing, <20% of RCs are currently performed robotically,[123] and reporting of long-term oncological outcome results is currently limited to a few individual centres and cumulative series.[124,125]

6.4.2.2 Patient selection

Care should be taken in patient selection. The selection process includes preoperative investigation to ensure fitness for surgery, as well as specific counselling about laparoscopic and robotic technology. Patients with decreased pulmonary compliance who cannot tolerate prolonged Trendelenburg positioning are not candidates for the robotic-assisted technique. Furthermore, if the patient has a history of previous extensive abdominal surgery, RARC may be contraindicated. Relative contraindications include patients aged >75 years, body mass index (BMI) >30, and those with bulky disease; such cases should be avoided early in the operative learning curve. Patients with clinical T3 and T4 disease should be carefully selected for this approach following consideration for NAC.[120,126]

Table 6–3 summarises the indications and relative contraindications for a minimally invasive approach.

TABLE 6–3 Indications and Contraindications for Minimally Invasive Approach to Radical Cystectomy

Indications for RC	Relative contraindications for minimally invasive approach
Patients with T1 tumours at high risk for progression	Patients with high BMI
T1 patients failing intravesical therapy	Salvage cystectomy following chemotherapy and radiation treatment
Patients with MIBC T2-T4a, N0-Nx, M0-1	Patients with clinical lymphadenopathy
Patients with high-risk and recurrent superficial tumours, BCG-resistant Tis, T1G3, as well as extensive papillary disease that cannot be controlled by TURBT and intravesical therapy alone	Patients with clinically advanced disease or large bulky tumours (T3/T4)
Salvage cystectomy is indicated for nonresponders to conservative therapy, recurrence after bladder-sparing treatment, and nonurothelial carcinoma	Previous history of surgery, e.g., previous prostatectomy, abdominoperineal resection, low anterior resection surgery, or with multiple previous lower abdominal surgeries
As a purely palliative intervention, including in fistula formation, for pain or recurrent hematuria	Patients with previous history of pelvic radiation for malignancy, e.g., prostate or rectal cancer

Abbreviations: BCG, bacillus Calmette-Guérin; BMI, body mass index; MIBC, muscle-invasive bladder cancer; TURBT, transurethral resection of the bladder tumour; RC, radical cystectomy.

6.4.2.3 Surgical margins

Despite advantages with magnified three-dimensional vision and precision achieved by the endo-wrist instruments in robotic interface, the lack of tactile feedback has created concerns about the adequacy of wider excision in advanced disease and avoiding soft tissue surgical margins. Positive surgical margins (PSMs) at cystectomy are a measure of disease burden and a predictor of outcome, being associated with high local recurrence and resulting in poor OS.[95,127] An MSKCC series of 1,589 patients who underwent ORC reported a PSM rate of 4.2%. Risk factors for PSMs were female sex, higher pathological stage, vascular invasion, mixed histology, and lymph node involvement. Patients with PSMs had a reduced 5-year CSS of 32%.[127] Another large multi-institutional analysis of 4,400 ORC patients reported the overall incidence of PSMs was 6.3%, and rates by stage were 2.3% for pT2,

7.6% for pT3, and 24% for pT4 disease.[128] Open expert consensus in 2004 recommended <10% of all cases and <15% for bulky tumours as acceptable PSM rates in RC.[129] Data from the International Laparoscopic Cystectomy Registry (ILCR) in 2008 revealed a soft tissue surgical margin rate of 2%.[130]

A recent cumulative analysis of the literature demonstrated that PSMs are uncommon in RARC series and rare for pT2 disease, with no significant difference in PSM rate found when comparing between RARC and ORC.[131] The review, however, highlighted the high variability of PSMs across studies (range 0%–26%), which suggested significant heterogeneity in the series regarding cancer characteristics, patient selection, and surgical technique and experience. In the cumulative analysis, the average PSM rate in RARC series was 5.6%, which is comparable to the large ORC series.[131] The effects of the learning curve as institutions adopted RARC and patient selection toward earlier-stage disease likely affected reported margin rates. Higher reported PSM rates in pT3/4 disease in some earlier multi-institutional RARC series suggest that, early in the surgeon's learning curve, caution should be used in patient selection of higher-stage disease.[132] This is supported by series that have shown no significant increase in the PSM rate despite an increasing proportion of patients with pT3/4 stage disease.[133,134]

6.4.2.4 Lymph node yield

Bilateral extended pelvic lymphadenectomy is a crucial part of RC. The incidence of positive nodes at the time of RC is in excess of 20%.[95] An LPLND including only the external region and obturator fossa will remove only 50% of all primary lymphatic landing sites compared to 90% clearance with an EPLND.[135] In a relatively recent survival analysis of two academic centres, 5-year RFS was significantly improved with EPLND compared to LPLND in patients with ≤pT3pN0-2 bladder cancer (49 vs. 19%, respectively).[98]

Early concerns about whether an EPLND could be adequately performed robotically achieving the same quality of resection as ORC appear unsubstantiated.[136,137] A prospective, randomized, noninferiority study by Nix *et al.* demonstrated a mean lymph node yield of 19 in the RARC group versus 18 in the ORC group.[138] Second look (open) lymphadenectomy by a different experienced open surgeon showed minimal additional lymph node yield (range 0–8) compared to the previous median 43 lymph nodes removed by a robot-assisted approach.[139]

Systemic reviews and meta-analyses have concluded that robot-assisted LND achieves similar lymph node yields to those of open LND.[131,136,137] There is also evidence that yield is related to expertise, with high-volume surgeons more likely to perform extended LND, reflecting a correlation between the surgeons' growing experience and increased comfort with advanced vascular dissection.[137] The number of lymph nodes retrieved depends on node viability, method of submission (en bloc or separately), and the processing technique. In conclusion, a thorough anatomical dissection around the pelvic vessels and complete clearance of all nodal tissue within the anatomical boundaries using minimally invasive approach is advocated and shown to be achievable.

6.4.2.5 Complications

Despite preoperative optimization, advances in surgical techniques, and postoperative care, RC remains a morbid operation with complication rates of 26% to 64% and mortality rates of 1% to 7%.[140-143] RC readmission rates are high, with bowel-related and urinary infection complications

being most common.[144] One of the major attractions of the minimally invasive approach to RC is that the morbidity of this procedure could possibly be reduced.[120] A recent cumulative analysis of the literature concluded that RARC can be performed safely. Assessing all modalities of RC, the authors found that, while the risk of intraoperative complications is low, postoperative complications and readmission are common.[99] The analysis revealed operative time was shorter with ORC, whereas blood loss and transfusion rates were significantly lower with RARC than with ORC. Conversely, rates for any-grade and grade 3 complications at 90 days were slightly lower with RARC than with ORC. Similarly, transfusion rates were lower with RARC than with LRC, as were any-grade and grade 3 complication rates. A prospective, randomised, controlled trial comparing open with robot and laparoscopic cystectomy (CORAL trial) reported that the 30-day complication rates varied by type of surgery and were significantly higher in the ORC arm than the LRC arm. There was no significant difference in 90-day Clavien-graded complication rates between the three arms.[100]

The International Robotic Cystectomy Consortium (IRCC) published their accumulated data complication rates.[145] In all, 41% (n=387) and 48% (n=448) of patients experienced a complication within 30 and 90 days of surgery, respectively; 29% had grade 1 to 2 complications and 19% had grade 3 to 5 complications. Gastrointestinal (GI), infectious, and genitourinary complications were most common (27%, 23%, and 17%, respectively). On multivariable analysis, increasing age group, NAC, and receipt of blood transfusion were independent predictors of any and high-grade complications. The 30- and 90-day mortality rates were 1.3% and 4.2%, respectively.[145] In a further paper, the IRCC looked at the difference in postoperative complications between patients undergoing extracorporeal urinary diversion compared with intracorporeal urinary diversion. No difference in the re-operation rates at 30 days was noted between the groups. The overall 90-day complication rate was not significantly different between the groups, but a trend favouring intracorporeal urinary diversion was noted (41% vs. 49%; p=0.05). GI complications were significantly lower in the intracorporeal urinary diversion group ($p \leq 0.001$). Overall, patients with intracorporeal urinary diversion were at a lower risk (32%) of experiencing a postoperative complication at 90 days (odds ratio [OR], 0.68; 95% CI, 0.50–0.94; p=0.02),[146] indicating a potential advantage of a totally minimally invasive approach that minimises bowel handling and exposure.[120]

6.4.2.6 Oncological outcome

Despite recent advances, approximately 50% of all patients undergoing RC experience recurrence and subsequent mortality.[66,125] Recognised early indicators of oncological efficacy include PSM rates and lymph node yields.[147,148] The relationship between the quality of surgery and oncological efficacy is a key issue.[149] Standards for surgical quality were proposed by Herr and the Bladder Cancer Collaborative Group for ORC in 2004, and included acceptable PSM rates of <10% and lymph node yields of ≥14.[129] Recent cumulative analyses have concluded that these indicators of oncological efficacy, namely PSM rates and PLND yields, are comparable to the ORC series.[125,150] Long-term RFS and avoidance of bladder cancer–related death are the primary measures of treatment efficacy following extirpative surgery for bladder cancer by any approach. There has been some debate as to whether cystectomy via minimally invasive cystectomy techniques negatively impacts early recurrence patterns because of inadequate resection or the pneumoperitoneum.[151,152]

Recurrence following RC often occurs early, with >80% of recurrences occurring within the first 2 years.[148] The EAU Robotic Urology Section scientific working group recently reported on early recurrence patterns among 717 patients who underwent RARC with intracorporeal urinary diversion at nine different institutions with a minimum follow-up of 12 months.[150] Clinical, pathological, radiologic, and survival data at the latest follow-up were collected. RFS at 3, 12, and 24 months was 95.9%, 80.2%, and 74.6%, respectively. Distant recurrences most frequently occurred in the bones, lungs, and liver, and pelvic lymph nodes were the most common site of local recurrence. They identified five patients (0.7%) with peritoneal carcinomatosis and two patients (0.3%) with metastasis at the port site (wound site), concluding that early recurrence rates and sites of recurrence appear similar to those for ORC series. Positive lymph nodes, non–organ–confined disease, and PSMs were associated with early recurrences, indicating that early recurrences following RARC are primarily related to tumour biology and not the modality of surgical treatment. This conclusion is supported by long-term oncological outcome data, which also failed to identify differences in oncological outcome between minimally invasive and open techniques.[125]

6.4.2.7 Randomized control trials

To date, there have been 4 published randomized control trials (RCT) that have compared minimally invasive surgery with ORC. Difficulties in performing RCTs have included low sample sizes with associated underpowering, and the potential confounding of surgeon experience, such that the RCTs may be examining individual surgeons' skills and expertise as much as the approach they employ.

Nix *et al.* reported the first RCT in 2010.[138] This was a single-centre noninferiority study comparing ORC versus RARC. The study recruited only 41 patients, with 21 randomized to the robotic approach and 20 to the open technique. The study concluded that RARC was noninferior to ORC with regards to the LND, and that the robotic approach showed significant improvements over the ORC group with regard to estimated blood loss, time to flatus, time to bowel movement, and use of inpatient morphine sulfate equivalents. There was no significant difference between the groups in overall complication rate or length of hospital stay.

Bochner *et al.* designed an RCT to compare the incidence of complications after RARC and ORC. The study successfully randomised 118 patients and reported that the RARC group had lower mean intraoperative blood loss (p=0.027) but significantly longer operative time (p<0.001) compared to the ORC group. Pathological variables, including PSMs and lymph node yields, were similar. The study failed to identify any significant advantages with respect to complication rates for RARC over ORC. Similar 90-day complication rates, hospital stay, pathological outcomes, and 3- and 6-month QoL outcomes were observed regardless of surgical technique.[153]

The CORAL study was a three-arm RCT comparing ORC with LRC and RARC.[100] A total of 164 patients requiring RC were invited to participate, with an aim of recruiting 47 patients into each arm. Of 93 patients who were suitable for trial inclusion, 60 (65%) agreed to randomization and 33 (35%) declined. Mean operative time was significantly longer in RARC compared with ORC or LRC. ORC resulted in a slower return to oral solids than RARC or LRC. The 30-day complication rates varied by type of surgery and were significantly higher in the ORC arm than the LRC arm. There was no

significant difference in 90-day Clavien-graded complication rates between the three arms. There were no significant differences in QoL measures. The authors concluded that the study was limited by small sample size and potential surgeon bias.

The latest updates from the RAZOR trial were reported at the annual AUA meeting in 2017.[154] This is the first phase 3, multicentre, prospective randomized trial comparing an open to robotic approach for any organ site. The RAZOR trial aimed to compare ORC versus RARC using oncological, perioperative, functional, and QoL endpoints. Patients with biopsy-proven bladder cancer were recruited from 15 participating US institutions: clinical stage T1–T4, N0–N1, M0 or bacillus Calmette-Guérin (BCG)–refractory CIS. The trial was designed and powered as a noninferiority comparison, with RARC being considered inferior if the 2-year PFS was >15% lower than ORC. A total of 350 patients were recruited. After exclusions, 151 in the RARC and 156 in the ORC arms were analyzed. RARC was associated with lower estimated blood loss (mean 363 vs. 829; $p<0.001$), less frequent intraoperative transfusions (13.6% vs. 33.6%; $p<0.001$), fewer postoperative transfusions (25.6% vs. 41.0%), longer operative time (425 vs. 361 minutes; $p<0.001$), shorter median hospital stay (6.5 vs. 7 days; $p=0.023$), more patients staying ≤5 days (28.6% vs. 18.7%), and a higher PSM rate (10.6% vs. 4.5%; $p=0.042$) compared to ORC. There was no difference in extent of LND, complication rates, or final pathology between the two arms. There was no difference in 2-year PFS or OS (80.2% vs. 79.1%; HR, 0.80; $p=0.31$).

6.4.2.8 Recommendations

- RARC is a surgical option for locally advanced bladder cancer with oncological outcomes similar to those of open series **[LOE 2; GOR B]**.
- High-volume centres with dedicated minimally invasive surgical teams have shown better results than smaller centres **[LOE 2; GOR C]**.
- Difficult cases should be avoided early in the surgeon's learning curve; see **Table 6–3** for relative contraindications **[LOE 2; GOR C]**.

6.4.3 Surgical outcome: morbidity and mortality

6.4.3.1 Introduction

Despite major strides to improve perioperative care and an overall trend towards reduced mortality,[155] RC continues to be one of the most morbid cancer-related operations.[99] RC-related complications may arise due to pre-existing patient comorbidities, the surgical procedure itself, the bowel anastomosis, sarcopenia, or the urinary diversion. Factors such as hospital volume, case mix, and surgeon skill and experience can influence the rate, type, and severity of surgical complications. Other aspects, including the availability and breadth of consultative, diagnostic, and ancillary services, can all influence the association between cystectomy and surgical outcomes.[156]

When reporting surgical complications during cystectomy, regardless of the technique, a standardized and reproducible classification system of surgical complications should be applied. The modified Clavien system is one such paradigm and has been used to evaluate complications in more than 6,300 different surgical procedures.[157] Recently, complications of both ORC[158] and LRC[121] have been reported using this modified Clavien system. When possible, overall mortality in the perioperative period

should be captured and reported for both the 30- and 90-day postoperative periods.[159] Morbidity occurring within 90 days of operation should be considered an early complication, while complications arising 90 days or later should be considered late-onset.[121,158] Despite improvements in surgical techniques, anesthetic delivery, and perioperative care, RC is associated with a high rate of morbidity. In the 30 days following cystectomy, the rate of adverse events of any grade is 58% and mortality rate is 3.9%.[158,160-162] The following sections will detail the rates of morbidity and mortality associated with cystectomy and urinary diversion, as well as candidate quality of care indicators for the treatment of bladder cancer. Moreover, recommendations from the literature to minimize complications will be discussed. **Table 6–4** includes a comprehensive list of possible RC-related complications.

TABLE 6–4 Complications Following Radical Cystectomy Using Standardized Reporting Methodology

Complication type	Possible complications
GI*	Ileus, small bowel obstruction, emesis, peptic ulcer, anastomotic bowel leak, enterocutaneous fistula, ascites, GI bleed, diarrhea, *Clostridium difficile*
Infection*	Fever of unknown origin, pelvic/retroperitoneal abscess, urinary tract infection, pyelonephritis, cellulitis other than incisional, peritonitis, diverticulitis, cholecystitis, sepsis
Wound	Dehiscence, wound seroma, wound infection, cellulitis
Cardiac	Myocardial infarction, arrhythmia, congestive heart failure, hypotension,
Genitourinary	Acute renal failure, hydronephrosis, ureteral stricture, urinary leak (anastomosis or pouch), urinary fistula to bowel or skin, urinary retention, bladder neck contracture, urinary ascites, parastomal hernia, stomal stenosis, venous congestion/ischemia stoma
Pulmonary	Atelectasis, pneumonia, acute respiratory distress syndrome, dyspnea, pneumothorax, pleural effusion, empyema
Bleeding	Anemia requiring transfusion, significant (≥1 L) intraoperative or postoperative hemorrhage, flank hematoma, wound hematoma, scrotal hematoma, disseminated intravascular coagulopathy
Thromboembolic	Deep venous thrombosis, pulmonary embolus, superficial phlebitis, subclavian vein thrombosis
Neurologic	Nerve palsy, paralysis, loss of consciousness, agitation, delirium, cerebrovascular accident, vertigo
Miscellaneous	Psychiatric illness, tendonitis, dermatitis, acidosis, thrombocytopenia without bleeding, foot ulcer, lymphocele, decubitus ulcer
Surgical	Incisional hernia, vascular injury, retained drain, rectal injury, obturator nerve injury, enterotomy

Abbreviations: GI, gastrointestinal.

* Most common complications.

Adapted from Maffezzini M, Campodonico F, Capponi G, et al. Fast-track surgery and technical nuances to reduce complications after radical cystectomy and intestinal urinary diversion with the modified Indiana pouch. Surg Oncol. 2012;21(3):191–195.

6.4.3.2 Morbidity after radical cystectomy

RC-related 30-day complication rates are of grave concern, with readmission rates as high as 25% to 40%, even in high-volume centres.[159,162,164] Historically, the surgical procedure itself has been the main focus of research, with a relative paucity of information regarding any risk factors or patient attributes that are associated with readmission. This issue remains a "black box" in the bladder cancer

treatment arena, as there are many links between RC and readmission, yet the underlying cause is not yet fully understood. For instance, examination of patients' preoperative characteristics does not reliably predict post-RC readmission, as patient demographics such as age, BMI, American Society of Anesthesiologists (ASA) classification of the patient fitness before surgery, race, or gender appear to have little influence on readmission rates. In other words, trying to identify patients who are at high risk of readmission prior to surgery by conventional metrics is futile. However, some aspects of the patients' postoperative recovery are potential red flags and can be indicative of subsequent readmission. For instance, an individual struggling to maintain oral intake over a period of 2 to 3 days who makes direct contact with the emergency department rather than seeking telephone- or clinic-based advice is at an increased risk for readmission. This is not unusual, since the majority of morbidity following RC is related to the return of normal gut function. A study by Krishnan *et al* exploring the post-discharge period identified the readmission rate 30 days after RC as 23%. Readmitted patients had a greater likelihood of using the emergency department due to initial concerns in comparison to the non-readmitted patients.

Higher likelihood of readmission was also found in patients who reported infection and failure to thrive as concerns. The researchers established that, with a better understanding of the pre-readmission interval, it is possible to optimize postdischarge practices.[165] Likewise, recent studies have focused on the involvement of the readmitting institution on OS and clinical outcomes.[166] Indeed, patients admitted to centres other than the treating institution have a significantly increased risk of mortality. Under certain circumstances, readmission can be avoided, particularly if the patient had been offered a senior review by a specialist at an earlier time point.

Comorbidities greatly increase the risk of RC-related complications. Prior abdominal surgery, extravesical disease, and prior RT are all risk factors that increase RC morbidity.[162] Furthermore, advanced age[167,168] and, even more, so physiological age, as well as female gender increase the risk for development of complications due to RC. Moreover, elevated BMI is associated with increased rate of wound dehiscence and hernia. Unfortunately, the majority of studies evaluating RC do not include indices of morbidity in patient evaluations. Suffice to say, patients with pre-existing neurologic disease, cardiopulmonary compromise, renal insufficiency, autoimmune disease, and bowel disease experience higher rates of complication.[162] Therefore, comorbidities should be carefully assessed pre-RC in order to better identify patients who might be at a higher risk of complications due to secondary health conditions.

Greater precision in identifying patients at risk of readmission would allow improved counselling, focused patient information, and targeted interventions. Avoiding unplanned readmissions would have benefits for both the patient and health care provider. In addition, a designated readmission pathway directly to the treating surgical team could allow for earlier intervention by personnel who are well-seasoned in managing complications arising from major pelvic surgery. A timely targeted approach would ultimately improve the quality of care and overall clinical outcomes following RC. Furthermore, a hands-on approach that preempts readmission with a preplanned approach for management of surgical outcomes is highly likely to be associated with an overall reduction in the health care costs.

6.4.3.3 Perioperative complications

6.4.3.3.1 General perioperative complications

Complications surrounding the surgical procedure are associated with significant perioperative and long-term morbidity.[162] Complications are reported in 58% to 77% of patients within the first 30 days of RC.[163,169]

Acute blood loss that may require a transfusion and injury to adjacent organs are amongst the most common intraoperative RC complications. Acute bleeding is typically associated with ligation of the bladder pedicles or dorsal vein of the prostate in men and excision of the anterior vagina in women. The development of bipolar cautery devices, surgical staplers, and improved understanding of the prostatic anatomy have helped to minimize blood loss during cystectomy. Regardless, average blood loss during the procedure ranges from 600 mL to 1,700 mL[134,160,170] and transfusion rates can be as high as 66%.[163] Injuries to associated structures, such as the rectum, may occur in as many as 1.7% of procedures.[171]

Perioperative complications comprise a large proportion of the morbidity experienced by post-RC patients. These complications include thromboembolic, cardiac, pulmonary, infectious, and renal adverse events. The rates of deep venous thrombosis (DVT) and pulmonary embolus are up to 5%.[172] Low-molecular-weight heparin prophylaxis has been shown to reduce the rate of both DVT and pulmonary embolus.[173] Cardiac events, such as congestive heart failure, arrhythmia, and myocardial infarction, occur in as many as 7% of patients.[174] Pulmonary compromises in the form of acute respiratory distress, reintubation, or pneumonia complicate the postoperative course of up to 7.8% of cystectomy patients.[175] As many as 13% of RC patients suffer from infectious complications such as pyelonephritis, sepsis, wound infection, or urinary tract infection.[173] It is quite common for patients to demonstrate a colonized urine specimen. However, symptomatic urinary tract infections and pouchitis require treatment with appropriate antibiotics. Lastly, renal insufficiency requiring dialysis may occur in as many as 7% of patients.[142]

Surgical complications may arise from the cystectomy procedure, PLND, bowel anastomosis, or urinary diversion. Paralytic ileus is quite common during the postoperative course, plaguing as many as 22.7% of patients.[176] Fortunately, true small bowel obstructions or anastomotic leaks are less common, but can occur in up to 8.7% of patients.[142] Lymphocele rates vary based on the degree of PLND. Bear in mind that even an appropriate LND may carry a risk of symptomatic lymphocele in up to 5% of patients.[171] Rates of wound infection, incisional hernia, pelvic hematoma, and fascial dehiscence are widely variable, but may occur in as many as 9% of patients.[160]

6.4.3.3.2 Complications related to urinary diversion

Complications arising from urinary diversion vary depending on the type of diversion and may occur either early or late post-RC. Early complications may manifest in the form of urine leak, pouch leak, excessive mucus, and ureteroenteric stricture. Urine leak from either a pouch or ureteroenteric anastomosis is noted in as many as 7.7% of patients.[177] Typically, prevention involves the placement of suction drains or ureteral catheters until adequate time for healing has been provided. However, the necessity and duration for routine use of ureteral catheters is under debate. Ureteral stricture–related complications can occur early or late post-RC and have been reported in up to 14% of patients in some series.[178] The stricture may be benign or, of greater concern, a malignant recurrence. Treatment

options include percutaneous nephrostomy with antegrade stenting, ureteroscopic balloon dilation, or open revision. The type of anastomosis (Bricker vs. Wallace), does not appear to effect ureteroenteric stricture incidence.[179,180]

Urinary diversion may be the underlying cause of a variety of late complications in RC patients. It may occur due to a scar from ischemia, a technical error, or from a recurrent tumour. Stomal stenosis has been described in 1.7% of patients and is likely related to ischemia of the conduit. Para stomal hernia is more common, occurring in at least 5.2% of cases, but is likely underreported.[176] Both complications may require open revision; the latter may require transfer to the contralateral side or reinforcement with mesh. Hydronephrosis and worsening renal function may occur in as many as 50% of patients 15 years post-RC.[177] This late-onset complication underlines the importance of long-term follow-up, serial imaging, and laboratory assessments. Urinary diversions are accompanied by metabolic changes in up to 3% of patients. These metabolic alterations include vitamin B-12 deficiency, metabolic acidosis, and electrolyte derangements. Metabolic changes may result in concomitant urinary stone disease. Furthermore, chronic bacterial colonization, mucous production, urinary retention, and enteric hyperoxaluria may exacerbate stone formation in patients with urinary diversions. Rates of stone formation may approach 30% in some cases.[177]

6.4.3.3.3 Gastrointestinal complications

RC-related GI complications are quite common and can occur in as many as 25% of RC patients.[181] Minor complications include persistent nausea, a need for nasogastric tube (NGT) placement, postoperative ileus (POI) or partial bowel obstruction (PBO), a need for total parenteral nutrition, diarrhea, and infections (e.g., *C. difficile*). Major complications can include complete bowel obstruction, GI bleeding, bowel leakage, and fistula involving the bowel.[142,161,182,183] The most common GI complication reported after RC is POI/PBO.[142,144,161,168,182,184] POI is generally defined as oral intake intolerance that persists beyond day 5 after surgery or by nausea and emesis accompanied by abdominal distention requiring GI rest (NPO, NGT, or total parenteral nutrition) at any time postoperatively.[142,182] POI accounts for the majority of extended hospital stays and the attendant increases in financial cost.[182,185,186]

6.4.3.4 Enhanced recovery after surgery

An enhanced recovery program (ERP) describes a standardized multimodal perioperative care pathway that aims to minimize the physiological and psychological stress effects of elective surgery. ERPs are also known as enhanced recovery after surgery (ERAS) or fast-track surgery programs. The concept of ERAS was first introduced in the 1990s in elective colorectal surgery as a means to improve postoperative recovery and shorten length of stay.[155]

Despite improvements in care, RC continues to be associated with significant levels of surgical morbidity, with high complication rates and prolonged length of stay.[99,156] The goal of a modern ERP is to positively impact patient care from diagnosis through treatment to return to normal function. However, there remains a lack of high-level evidence for ERPs following RC, with much of the evidence coming from the management of patients in colorectal care.[157]

There is increasing evidence from open colorectal surgery series that implemented ERPs can successfully reduce complication rates, length of stay in hospital, and the time taken to get back to normal activities following major pelvic surgery.[158] It is also recognised that minimally invasive surgery reduces the surgical stress response compared to open surgery.[121] RARC aligns itself with the original stated principles of enhanced recovery that minimally invasive surgery is advantageous in aiding quicker patient recovery.[159]

Although there is a growing body of evidence to support the use of ERPs in cystectomy patients, the uptake of enhanced recovery protocols for RC patients has been slow. A recent survey of surgeons with a specialist interest in RC found that 64% of respondents classified themselves as proponents of ERPs, but that only 20% were practising all interventions proposed in ERAS Society guidelines.[187]

A recent UK audit of enhanced recovery protocols found that good compliance with an ERP was associated with a 3-day reduction in median length of stay in urological patients. However, the audit revealed that there were large variations in ERPs between individual hospitals, leading the authors to conclude that changes in process, resulting from protocol-driven pathways, may be as important in reducing length of stay as any individual element of the ERPs taken in isolation.[188]

Overall, the quality of studies currently available is low **[LOE 3b]**. Considering multimodal interventions, 16 articles were identified reporting results of their ERAS protocols for RC, four of which incorporated RARC (see **Table 6–5**). Consistency throughout these protocols was variable. Commonly employed elements of an ERP included:

- Preoperatively: avoidance of mechanical bowel preparation and carbohydrate loading
- Intraoperatively: epidural anesthesia, opioid-sparing analgesia, avoiding hypothermia, and careful fluid management
- Postoperatively: avoidance or early removal of NGT in recovery with early mobilization and early oral feeding

TABLE 6–5 Current Published Series on Enhanced Recovery After Surgery Protocols for Radical Cystectomy

Study	Year published	No. patients (No. receiving ERP)	Comparative control group included	Number of ERAS recommendations included	RARC included	Additional elements to ERAS recommendations	LOE
Arumainayagam *et al.*[160]	2008	112[189] (56)	Y	6	N	–	3
Pruthi *et al.*[161]	2010	362 (362)	N (evolved ERP)	7	N	–	3
Shah *et al.*[162]	2011	30 (30)	N	10	Y	–	3
Maffezzini *et al.*[163]	2012	68 (68)	N	6	N	–	3
Mukhtar *et al.*[164]	2013	77 (51)	Y	12	N	–	3
Saar *et al.*[165]	2013	63 (31)	Y	9	Y	–	3
Karl *et al.*[166]	2014	101 (62)	RCT (2:1)	5	N	–	3
Dutton *et al.*[167]	2014	165 (165)	N (evolved ERP)	19	N	Rectus sheath analgesia catheter; intraoperative cell salvage; telephone contact given	3
Daneshmand *et al.*[168]	2014	110 (110)	Y (historical)	7	N	Para-incisional subfascial catheter	3
Smith *et al.*[190]	2014	133 (64)	Y	–	N	Rectus sheath catheter; intraoperative cell salvage; 24-hr ERP telephone helpline	3
Guan *et al.*[191]	2014	115 (60)	Y	7	N	Laparoscopic approach	3
Cerruto *et al.*[192]	2014	31 (31)	N	17	N	–	3
Persson *et al.*[193]	2015	70 (31)	Y	13	N	–	3
Koupparis *et al.*[194]	2015	270 (102)	Y	10	Y	Intracorporeal urinary diversion	3
Xu *et al.*[169]	2015	205 (124)	Y	17	N	–	3
Collins *et al.*[134]	2016	221 (135)	Y	20	Y	Intracorporeal urinary diversion	3

Abbreviations: ERAS, enhanced recovery after surgery; ERP, enhanced recovery program; RARC, robotic-assisted radical cystectomy; RCT, randomized control trial.

Recently, the European Association of Urology Robotic Urology Section (ERUS) published a consensus view on an ERP to guide standardised perioperative management of robotic cystectomy patients.[170] The project was carried out in phases: a systematic literature review of current evidence for ERPs in RARC, laparoscopic radical cystectomy (LRC), and ORC, surveys sent to ERUS Scientific Working Group members, and Internet- and panel-based consensus findings using the Delphi process to agree

on and formulate guidance. Consensus was reached in multiple areas of an ERP for RARC. The key principles include patient education, optimization of nutrition, RARC approach, standardised anesthetic, analgesic, and antiemetic regimens, and early mobilization. A summary of the consensus view on an ERP for patients undergoing RARC can be seen in **Table 6–6**.

TABLE 6–6 Consensus Statement on Structured ERP for RARC Patients (Preoperative, Perioperative, and Postoperative Care)

Consensus View on an ERP for Patients Undergoing RARC
Out-patient assessment
Preoperative counselling and education: verbal and written information supplied on operation and urinary diversion options and planned ERP
Preparation for surgery
Preoperative medical optimization
Preoperative nutritional optimization
Seen by stoma nurse specialist: advice on stoma and NB care
Cardiopulmonary exercise testing if indicated
Advice and support for cessation of smoking
Social issues addressed and discharge planning
Day before RC
No bowel preparation
Carbohydrate loading[160,162]
Day of RC: Day 1
Solids up to 6 hours and clear fluids up to 2 hours pre-op, including carbohydrate loading[160,162]
Avoidance of long-acting sedatives
Thrombosis prophylaxis; compression stockings and low–molecular weight heparin
Limited antimicrobial prophylaxis and skin preparation with chlorhexidine–alcohol (or equivalent solution)
Standard anesthetic protocol to attenuate surgical stress response: intraoperative maintenance of hemodynamic control, central and peripheral oxygenation, muscle relaxation, optimized depth of anesthesia with spinal, and appropriate analgesia, avoiding opiates with peripheral action
RARC approach
Goal-directed fluid management, with judicious use of fluid restriction[141]
Prevention of hypothermia (Bair Hugger™)
Removal of NGT in recovery
Days 2–4
Prevention of postoperative nausea and vomiting: regular antiemetics may be of benefit (metoclopramide)
Chewing gum[195,196]
Unrestricted diet
Drain fluid routinely sent for creatinine day 2 and drain removed day 2 if drain fluid indicates serum creatinine levels
Thrombosis prophylaxis; compression stockings and low molecular weight heparin
Regular analgesia: standardized poly-pharmacological opioid-sparing analgesia to include paracetamol
Early mobilization
Daily nutritional supplements with nutrition goal 900 Kcal/day
Fluid/electrolyte (30 mL/kg/day)
Encourage self-care (catheter care/flushing if NB, and stoma bag care if IC)
Day 4 onwards
Continue as previously; increase daily nutritional goal to 1,500 Kcal/day
Pain adequately controlled
Independently mobile
Regular diet/normal bowel function
Competent with NB or stoma care
Post-discharge
Stents out day 10 (no stentogram)
Removal of clips on day 10
Contact with specialist nurse via telephone
Audit cycle of compliance and outcomes

Abbreviations: ERP, enhanced recovery program; IC, ileal conduit; NB, neobladder; NGT, nasogastric tube; RARC, robotic-assisted radical cystectomy; RC, radical cystectomy.

The ERAS protocol is an evidence-based process that was originally developed for patients undergoing colectomy, with the goal of optimizing perioperative care and recovery and decreasing length of stay without increasing complication or readmission rates.[197,198]

The ERAS process has now been adopted for RC patients in some institutions.[168] The ERAS protocol described by Djaladat *et al.* decreased median length of stay following surgery to 4 days, without increasing 30-day readmission rate.[168,184] No significant difference in overall, minor, or major complications between the ERAS and control groups were found.[181] The most common complications in both groups (ERAS and traditional) were infection and GI related; however, these complications were significantly lower in the ERAS group. Indeed, the ERAS protocol was associated with a reduction in GI complications of at least 50%, and of 70% compared to traditional perioperative care (**Table 6–7**). Several components of ERAS are effective at accelerating GI recovery and decreasing length of stay, including the use of alvimopan, a m-opioid receptor antagonist that decreases the rate of POI and shortens length of stay, as demonstrated in multiple double-blind randomized studies.[182,184,199,200] A recent meta-analysis of 13 distinct ERAS studies revealed a lower overall complication rate (especially minor Clavien grades) and faster return of bowel function in the ERAS group.[201]

TABLE 6–7 Overall and Gastrointestinal Complications in Patients on ERAS Protocol and Controls

	ERAS patients (*n*=145)	Non-ERAS controls (*n*=144)	*P* value
Overall 30-day complication rate (%)	92 (64)	105 (73)	0.1
Low grade	67 (46)	81 (56)	0.2
High grade	26 (18)	25 (17)	0.2
30-day readmission rate (%)	30 (21)	20 (14)	0.15
30-day GI complication rate (%)	19 (13)	40 (27)	0.003
POI/PBO*	10 (7)	34 (23)	<0.001
Intractable nausea/vomiting	4 (3)	3 (2.1)	0.5
Intractable diarrhea	1 (<1)	1 (<1)	NA
Intractable constipation	1 (<1)	–	NA
C. difficile diarrhea	3 (2)	1 (<1)	0.3
Median time to first GI complication, days	4	5	0.7
30-day readmission due to GI complication (%)	2/19 (10)	2/40 (5)	0.1

Abbreviations: ERAS, enhanced recovery after surgery; GI, gastrointestinal; NGT, nasogastric tube; PBO, partial bowel obstruction; POI, postoperative ileus.

* POI/PBO is defined as nausea or vomiting together with abdominal distension that requires stopping oral intake, possible NGT placement and intravenous fluid therapy.[142]

Adapted from Bazargani ST, Djaladat H, Ahmadi H, et al. Gastrointestinal complications following radical cystectomy using enhanced recovery protocol. Eur Urol Focus. 2017. S2405-4569(17)30088-3. [Epub ahead of print]

Multiple other studies have also looked into GI recovery after application of ERAS or fast-track protocols.[161,182-184] The systematic review performed by Ramirez and colleagues demonstrated that incidence of 30-day POI were lower with enhanced recovery protocol than traditional pathways.[182] Recent evidence has also shown certain aspects of the ERAS protocol, effectively inhibiting release of inflammatory cytokines, which along with a reduction in stress level can result in a protective effect on the immune system that may contribute to reduced complications.[202,203] The 90-day mortality rate of RC with ERAS did not differ from standard care.[142,144,204,205] Thus, ERAS protocols are designed to reduce the risk of perioperative morbidity and, to date, have contributed to significant reductions in length of stay, although complication rates remain high.[168] The development of ERAS protocols for patients undergoing RC represents a significant evolution in perioperative care.

6.4.3.5 Mortality

Given the surgical complexity and high rates of surgical morbidity, it is not surprising that 30-day mortality from RC can be as high as 3.9% in larger series.[206] Furthermore, the rate of mortality climbs significantly for those patients with advanced age.[207,208] In a population-based assessment of perioperative mortality after RC, age, stage, and histological subtype represented statistically significant and independent predictors of 90-day mortality. The combined use of these three variables and of tumour grade resulted in the most accurate model (70.1%) for prediction of individual probability of 90-day mortality after cystectomy.[189] Comorbidity status is also predictive of perioperative death and 5-year all-cause mortality after RC and should, therefore, be incorporated into patient counselling and risk-stratification models.[209]

Nutritional deficiency, as measured by preoperative weight loss, BMI, and serum albumin, is strong predictor of 90-day mortality and poor OS.[210,211] Sarcopenia, defined as severe loss of skeletal muscle mass, has been in the spotlight in recent years, as it is associated with poor prognosis and markedly reduced survival in patients with various types of cancers.[190-192] Sarcopenia can be classified based on lumbar skeletal muscle index or muscle cross-sectional area, both of which are measured on CT. To date, only two reports have investigated the association between sarcopenia and survival after RC.[193,194] In a study by Psutka *et al.*, 68.8% of patients undergoing RC were sarcopenic. Patients with sarcopenia were older but were otherwise similar to patients without sarcopenia. Sarcopenic patients had significantly worse 5-year CSS and OS compared with patients without sarcopenia.[193] In another study, performed by Smith *et al.*, a clear association was noted between major complications and lower total cross-sectional area in women. Sarcopenia was not significantly associated with complications in men in this study. However, there was a nonsignificant trend of sarcopenia with a reduced 2-year survival.[194] Taken together, these studies demonstrate that objective measures of sarcopenia can be considered as biomarkers with an improved ability to prognosticate patients. Further research confirming sarcopenia as a useful predictor of complications would support the development of targeted interventions to mitigate the untoward effects of sarcopenia before cancer surgery.[212]

Perioperative mortality is fairly low post-RC and is largely caused by cardiovascular or septic complications. Careful patient selection and meticulous surgical technique may help decrease the incidence of perioperative mortality.[141] Lastly, multiple studies have found that hospital and surgeon volume have a significant impact on in-hospital mortality and length of stay after RC. Patients undergoing RC procedures at higher-volume centres experience overall better perioperative outcomes and lower mortality

rates compared with their counterparts undergoing RC at lower-volume institutions.[213-217] Therefore, factors such as age, comorbidity, nutritional status, sarcopenia, and hospital volume should be considered when stratifying risk for bladder cancer RC candidates.

6.4.3.6 Quality of care indicators

Bladder cancer treatment and management is complex and challenging. Health care providers are constantly looking to improve quality of care and better treatment options. Metrics are being developed to better assess the quality of care provided by physicians; this is a growing trend and will likely play an increasing role in health care delivery in the future. Cooperberg and colleagues defined candidate measures for quality of care in the treatment of bladder cancer and the relationship to surgical outcomes.[218] They note that time to cystectomy after diagnosis, hospital volume, surgeon volume, nodal yield, and utilization of orthotopic diversion are associated with improved health care outcomes in patients receiving RC. However, despite clear recommendations from multiple urological associations, compliance with treatment guidelines ranges from a dismal 3% for postoperative MMC to 20% for surveillance cystoscopy and cytology.[219] Improved adherence to recommended guidelines and recognition of important quality of care measures will likely reduce the substantial morbidity of RC and help shape the future care of bladder cancer patients.

6.4.3.7 Recommendations

- Surgical complications associated with RC and urinary diversion should be reported in a uniform grading system. Currently, the best adapted graded system for cystectomy is the Clavien grading system **[LOE 2; GOR B]**.
- Surgical complications associated with RC and urinary diversion should include the length of follow-up for the patient cohort and a minimum of 30-day, but preference for 90-day, reported outcome **[LOE 3; GOR C]**.
- ASA score, age, comorbidities, sarcopenic status, previous treatment for bladder cancer or other pelvic diseases, surgeon and hospital volume of RC, and type of urinary diversion influence surgical outcome **[LOE 2; GOR B]**.
- Reduction in blood loss and blood transfusion is afforded by meticulous technique, use of modern surgical devices, and improved understanding of pelvic anatomy **[LOE 3; GOR C]**.
- Reduction of urinary extravasation and leak can be achieved with careful closure of the anastomosis or pouch, stenting of the ureteroenteric anastomosis, and maintenance of appropriate drainage **[LOE 3; GOR C]**.
- Reduction of symptomatic lymphocele formation can be achieved with appropriate identification of lymphatic channels, careful surgical technique, and an open peritoneal window. Initial treatment should begin with percutaneous drainage **[LOE 3; GOR C]**.
- Reduction of anastomotic strictures requires meticulous surgical technique, minimal ureteral dissection, well-perfused segment, generous spatulation, and careful apical suture placement **[LOE 3; GOR C]**.
- Reduction of metabolic disorders after urinary diversion requires preservation of distal ileum, serial monitoring of electrolytes and vitamin B-12 levels, understanding of bowel segment physiology, and appropriate emptying of urinary diversion **[LOE 3; GOR C]**.
- Reduction of DVT and pulmonary embolus can be achieved with use of low molecular weight heparin, early ambulation, and sequential compression devices **[LOE 2; GOR B]**.

- There is increasing evidence that implementation of ERAS protocols can successfully reduce complication rates, length of stay in hospital, and the time taken to get back to normal activities following RC **[LOE 3; GOR C]**.
- ERAS protocols should be standardized and outcomes audited following implementation **[LOE 3; GOR C]**.

6.4.4 Oncological outcome of radical surgery

6.4.4.1 Survival and outcomes according to American Joint Committee on Cancer/TNM staging

6.4.4.1.1 Overview

Long-term oncological outcomes of RC have been investigated in a multitude of studies deriving largely from high-volume centres across Europe, North Africa, and North America.[66,220] The oncological outcomes reported in these series are based on the tumour-node-metastasis (TNM) staging system, which consists of three parameters deemed vital for survival. These parameters are local tumour invasiveness (T: tumour stage), presence of positive lymph nodes (N: nodal stage), and distant metastatic disease (M: metastasis). Based on this concept, the American Joint Committee on Cancer (AJCC) proposed to categorize patients according six prognostic groups (0a, 0is, I, II, III, IV). The TNM staging system can be used to determine the clinical and pathological stages of patients with invasive bladder cancer. **Table 6–8** and **Table 6–9** list the 2017 TNM staging system with the corresponding AJCC prognostic groups.[221]

TABLE 6–8 2017 TNM Staging[221]

Category	Status
T	
TX	No primary tumour assessment
T0	No primary tumour detectable
Ta	Noninvasive papillary carcinoma
Tis	CIS
T1	Invasion of the subepithelial connective tissue
T2	Invasion of the muscle layer
T2a	Invasion into the inner half of the muscle layer
T2b	Invasion into the outer half of the muscle layer
T3	Invasion of the perivesical fatty tissue
T3a	Microscopic perivesical invasion
T3b	Macroscopic perivesical invasion

Abbreviations: CIS, carcinoma in situ; TNM, tumour-node-metastasis.

continued on **page 580**

TABLE 6–8 2017 TNM Staging[221], *Cont'd*

Category	Status
T4	Invasion of organs or structures such as prostatic stroma, the seminal vesicles, uterus, vagina, pelvic wall, or abdominal wall.
T4a	Invasion of prostatic stroma, uterus, and/or vagina
T4b	Invasion of the pelvic or abdominal wall
N	
NX	Regional lymph nodes cannot be assessed due to lack of information
N0	No regional lymph node spread
N1	Single lymph node metastasis in the true pelvic region (perivesical, obturator, internal and external iliac, or sacral lymph node)
N2	Multiple lymph nodes in the true pelvic region
N3	Single or multiple lymph nodes in the common iliac region
M	
MX	Distant metastases cannot be assessed
M0	No signs of distant metastasis
M1	Distant metastasis present
M1a	Distant metastasis limited to lymph nodes beyond the common iliacs
M1b	Non-lymph-node distant metastasis

Abbreviations: CIS, carcinoma in situ; TNM, tumour-node-metastasis.

TABLE 6–9 AJCC Stages of Bladder Cancer[5,221]

Stage	TNM status
Stage 0a	Ta, N0, M0
Stage 0is	Tis, N0, M0
Stage I	T1, N0, M0
Stage II	T2a–T2b, N0, M0
Stage III	T3a–T4a, N0, M0
Stage IV	T4b, N0, M0, or any T, N1–N3, M0 or any T, any N, M1

Abbreviations: AJCC, American Joint Committee on Cancer; TNM, tumour-node-metastasis.

A large, retrospective, single-centre study reported on the long-term oncological outcomes of 1,054 patients treated with RC and pelvic lymphadenectomy.[66] RFS and OS rates at 5 years were 68% and 66%, respectively, and at 10 years they were 60% and 43%, respectively. In a study from Mansoura, Egypt, where the majority of cases were squamous cell carcinoma (59%), the overall 5-year survival outcome was 48%. Surgical factors that have been documented to influence outcome include soft

tissue margin and extent of LND. Patients with organ-confined, node-negative disease (<T2N0; AJCC stages <0a) have overall disease-specific survival rates of 60% to 85% over 5 and 10 years.[215,222] In contrast, the 5-year disease-specific survival rate for patients with node-negative extravesical disease (pT3a-4a, pN0; AJCC stage III) is in the 50% range, while patients with node-positive disease who have undergone a LND can expect a 30% chance of long-term RFS.[66] The outcomes for salvage cystectomy are generally worse than those of primary cystectomy in nonirradiated patients, although robust studies on this matter are not available. In select cases, surgery can offer a prolonged survival even in the presence of gross nodal disease.

In a series of 84 patients from MSKCC, a 10-year survival rate of 24% was noted in the group.[223] Additional single- and multicentre studies have further substantiated these findings and led to the current standard where RC is the mainstay treatment for patients with MIBC.[220,224]

In a larger cohort (2,039 patients) long-term study performed by the University of Southern California with a median follow-up of 12 years, 31% patients recurred and 61% died. The 5-year RFS and OS probabilities were 64% and 55%, respectively. Patients suffered from organ-confined (56%), extravesical (21%), and node-positive disease (23%). The 5-year RFS probabilities for these stages were 81%, 54%, and 32%, respectively. Corresponding 5-year OS probabilities were 72%, 40%, and 28%, respectively. Gender, stage, tumour upstaging, surgical margin status, lymphovascular invasion status, and NAC and AC were associated with RFS. A total of 4.5% of all patients with recurrences had pelvic recurrence without distant metastasis, and 24.1% of all recurrences appeared at distant sites with/without pelvic recurrence at the last follow-up. Only 4.8% of patients with pT2N0 disease experienced pelvic recurrence, as opposed to 7.4% of those with pT3N0 and 9% of node-positive patients. Patients with significant disease following neoadjuvant cisplatin-based chemotherapy have very poor prognosis. Significant factors influencing outcome include soft tissue margins and lymph node involvement.[95]

6.4.4.1.2 Outcomes of patients with stage pT0 or carcinoma *in situ*–only disease

The percentage of patients without evidence of the primary tumour (classified as pT0) at RC has been shown to range between 5% to 7%, with a risk of concomitant lymph node metastasis of approximately 3% to 7.5%.[225,226] In one study, the following clinical stages were reported at preoperative transurethral resection (TUR) in 56 patients with final pT0 disease: Tis (9%), Ta (4%), T1 (32%), T2 (52%), and T3 (4%).[226] The probability of remaining disease-free at 5 years in patients with invasive bladder cancer (pT1–T2) staged pT0 at RC is 89% to 90% and is significantly higher than in cases with residual pT1–T2 bladder cancer.[225,226] In a large international study with a total of 228 patients with pT0 bladder cancer at final pathological analysis, risk factors that independently correlated with decreased survival were the presence of lymph node metastasis and female gender.[225]

For patients with CIS only who had undergone RC due to refractory conservative treatment, the overall disease-free survival (DFS) and CSS was 74% and 85%, respectively. However, 36% of these patients experienced disease upstaging (≥pT1) at RC. Similar to patients with pT0 disease, risk factors for decreased CSS were found to be the presence of lymph node metastases, lymphovascular invasion, and female gender.[227]

A recent study looked into the survival of patients with MIBC who demonstrate complete clinical response (*c*T0) to NAC and then reject subsequent RC. *c*T0 was defined as negative cytology, cystoscopy with TURBT, and imaging. *c*T0 patients refusing RC were followed up with cytology, cystoscopy with biopsy, and cross-sectional imaging. Forty-eight patients were identified with MIBC that was *c*T0 after NAC. NAC regimens were 46% methotrexate or vinblastine or doxorubicin or cisplatin, 39% gemcitabine or cisplatin, and 15% other platinum-based therapies. Seven patients underwent immediate RC, whereas 41 elected for bladder preservation with close surveillance. In those 41 patients, 5-year CSS was 87%, DFS was 58%, and cystectomy-free survival was 79%. A total of 46% relapsed, with a 5.4-month median recurrence time. Thus, bladder preservation may be a reasonable option in a highly select subset of patients with MIBC who are complete clinical responders after NAC. Future studies should focus on identifying clinical and molecular factors associated with a durable pathologic complete response (pCR) after NAC.[46]

6.4.4.1.3 pT2 substaging

In 1997, the AJCC updated the TNM staging system and introduced new substaging categories for tumour stages T2 and T3,[228] with later versions published in 2002 and 2009.[229] The substratification was thought to provide improved risk assessment for follow-up strategies and enhance counselling of patients for adjuvant treatment options. However, recent retrospective studies in patients with node-negative, pT2a–T2b bladder cancer, classified as AJCC stage II,[230] have challenged the prognostic importance of substratifying pT2 tumours into those involving the inner (T2a) or outer half of the detrusor muscle (T2b) and suggested consolidating both substages into one.[231-233] Yet, a large retrospective study that included 311 patients with pT2 bladder cancer demonstrated that pT2b-classified patients had a higher risk of lymph node tumour involvement than pT2a-classified patients.[233] Nonetheless, this study had limitations: the extent of lymphadenectomy and number of retrieved lymph nodes were not precisely reported, which might have biased the final survival analysis.[233] Additionally, patients with nonurothelial cell carcinoma or those who underwent NAC were not excluded from the analysis.[231,232] Another multicentre series with 565 patients with pT2 urothelial carcinoma of the bladder attempted to overcome these limitations and reported significant differences in survival between the two substages in node-negative pT2 disease.[234] These findings were also confirmed in a mixed cohort of 1,737 patients with pT2 bladder cancer, where 54% of patients had squamous cell carcinomas.[235] In this study, the 5-year DFS was significantly higher for patients with pT2aN0 compared to those with pT2bN0 bladder cancer. In another series, this significant difference in RFS and CSS was further confirmed for patients with pT2 urothelial carcinoma of the bladder who were treated with extended pelvic lymphadenectomy.[236] Moreover, another recent multicentre study proposed a weighted prognostic model for patients with node-negative pT2 bladder cancer. Among different independent risk factors (presence of high-grade disease or lymphovascular invasion), pT2 substaging was the strongest predictor of RFS.[237] Taken together, these data support the prognostic importance of the current substratification in node-negative pT2 bladder cancer.

6.4.4.1.4 Organ-confined bladder cancer

One study assumed that microscopic extension of the tumour into the perivesical fat (pT3) does not confer a significantly higher risk for lymph-node tumour involvement and decreased survival compared to MIBC.[238] This study included 381 patients and found no significant differences in RFS between pT2 and pT3a; however, stages pT2 and pT3b showed significant differences. Moreover, a trend was reported for CSS between patients with pT2 and pT3a disease.[238] In contrast, a different

study examining 134 pT2b patients and 236 patients with pT3a–T3b bladder cancer found RFS and CSS to be significantly improved for pT2b compared to pT3a patients.[239] Moreover, a study analyzing the outcomes of 2,238 patients with pT2b–T3b bladder cancer found a significantly higher rate of node-positive disease and all-cause mortality in patients with node-negative pT3a versus pT2b bladder cancer.[240] With this in mind, it seems anatomically intuitive to define organ-confined MIBC as stages ≤pT2bpN0cM0.

In a retrospective analysis, the University of Southern California followed a cohort of 1,488 RC patients. The 10-year RFS was 78% to 80% in patients with organ-confined lymph node–negative disease, 53% to 60% in patients with extravesical lymph node–negative disease, and 30% in lymph node–positive disease. Systemic chemotherapy did not have a significant effect on survival in the entire cohort. This study revealed that, although outcomes have become fairly predictable, there have been no improvements in the survival of patients undergoing RC over the last three decades.[241] In a recent study by Dalbagni and colleagues, regression analysis of RC cohort of 1,488 patients revealed that patient age, pT stage, and NAC were significant factors for survival. Disease-specific survival was 67%, with a median survival of 94 months. In a multiple proportional hazards analysis, only pT stage and previous chemotherapy were significant predictors of disease-specific survival. Moreover, a significant difference was seen in the OS and disease-specific survival between patients with organ-confined and non–organ-confined tumours. No difference in survival rates among patients with pT4a to pT3 tumours was observed. No significant difference was found in patient outcomes among the different histological subtypes. Thus, in this study, organ-confined and non–organ-confined tumour grouping was better suited for evaluating adjuvant clinical trials.[222]

6.4.4.1.5 pT3 substaging

The prognostic significance of substaging patients with pT3 bladder cancer into those with microscopic (pT3a) and macroscopic (pT3b) perivesical fat invasion as implemented by the 2002 TNM staging system is also controversial. The majority of larger studies found that the risk of lymph-node metastases was significantly higher for patients classified with pT3b when compared to pT3a,[239,240,242] while some smaller studies did not.[243] A recent single analysis focused on outcomes in 75 patients with node-negative pT3 bladder cancer.[231] Actuarial 5-year RFS and CSS for patients with pT3apN0 relative to pT3bpN0 classified disease were not significantly different, with 68% versus 72% and 54% versus 42%, respectively. However, a larger multicentre study that evaluated 808 patients with pT3N0 bladder cancer (median follow-up: 43 months) treated with RC without any neoadjuvant modality reported significantly improved 5-year RFS (61% vs. 48%) and CSS (64% vs. 55%) rates for patients with pT3a versus pT3b disease.[22,242] These finding are further supported by another multicentre study in which a weighted prognostic model was constructed for 578 patients with node-negative pT3 bladder cancer. pT3 substaging was found to independently contribute to the relative risk of RFS.[237] In conclusion, the present data support the current concept of substaging in node-negative pT3 bladder cancer, whereas in node-positive pT3 disease a prognostic significance cannot be attributed to this substratification.[239,243]

6.4.4.1.6 Stage pT4 bladder cancer

Studies have demonstrated that RC is a feasible therapeutic option for patients with T4 bladder cancer.[244] Nevertheless, the oncological outcome of these patients when treated without neoadjuvant therapy is generally poor, although some patients may achieve long-term survival. Risk factors

that independently determine an adverse oncological outcome are the presence of positive soft tissue surgical margins, lymph node metastasis, lymphovascular invasion, tumours infiltrating the abdominal or pelvic wall (staged as pT4b), and female sex.[227] In this respect, several series have demonstrated that a positive soft tissue surgical margin *per se* is a strong independent risk factor for decreased RFS and CSS.[128,245] Female patients are at an especially increased risk for positive soft tissue surgical margins during RC.[127] However, since complete tumour removal cannot be achieved by surgery alone in case of abdominal or pelvic wall infiltration, cystectomy should be preserved in this stage for relief of symptoms such as recurrent macrohematuria, pain, fistula formation, and therapy-refractory urgency.[246]

The importance of AC for the treatment of pT3/T4 patients has been demonstrated by a recent study. In this series, patients with pT3/T4 and/or pN+ urothelial carcinoma of the bladder received either NAC and RC followed by AC (23.4%) or NAC and RC followed by observation (76.6%). Median OS was significantly longer for NAC and RC followed by AC compared to NAC and RC followed by observation. The 5-year adjusted OS rates were 36.8% for NAC and RC followed by AC compared to 24.7% for NAC and RC followed by observation. The OS benefit of NAC and RC followed by AC decreased significantly with age, whereas no significant interaction was observed with sex, Charlson Comorbidity Index, pT/N stage, and surgical margin status.[247] These findings support the addition of AC to the treatment regimens of pT3/T4 patients.

6.4.4.1.7 Prognostication in lymph node–positive bladder cancer

Oncological outcome in patients with node-positive bladder cancer at RC is generally poor, with a 5-year RFS ranging between 34% and 43%.[66,220,248] Nonetheless, long-term survival has been described, especially in patients with low-volume lymph node metastasis.[98,116,248] The following pathological and clinical parameters have been investigated as critical determinants for survival in node-positive disease: number and size of positive lymph nodes,[116] extracapsular extension of lymph node metastasis,[87] number of retrieved lymph nodes,[249] aggregate lymph node metastasis diameter,[250] and the anatomical extent of lymphadenectomy reflecting the surgical meticulousness of the lymph node removal.[98] The current pTNM staging system differentiates lymph node tumour involvement according to the number and size of positive lymph nodes (see **Table 6–8**), but does not sufficiently reflect the surgeon's capability of removing all the affected nodal tissue. In a study by Herr and Donat, a total of 84 patients with grossly node positive (N2–3) bladder cancer that was found at cystectomy and who underwent EPLND have been followed for up to 10 years. Twenty-four percent of patients were still alive, while 76% succumbed to disease. Median survival time was 19 months for all patients and 10 years for surviving patients. Of the entire cohort, 53 patients had clinical stage T2 (organ confined) tumours; of those, 32% survived versus 9.7% with stage T3 (extravesical) tumours. Therefore, a proportion of patients with grossly node-positive bladder cancer can be cured with RC and thorough PLND.[223]

With growing evidence for the prognostic role of the extent of lymphadenectomy in improving outcomes for invasive bladder cancer,[98,116] the concept of lymph node density has been proposed.[101,104] It is defined as the number of positive lymph nodes divided by the overall number of retrieved lymph nodes. In most series, a cut-off value of 20% has been reported to statistically optimally distinguish between different outcomes.[249,251,252] Various studies also found that lymph node density outperformed

pTNM-based predictions (pN1–N3) in terms of RFS and disease-specific survival.[101,104,252] Likewise, lymph node density was a superior prognosticator in node-positive patients after adjusting for the use of AC.[252,253] On the contrary, Fleischmann *et al* reported that lymph node density lost its independent prognostic value after accounting for the presence of extracapsular extension of lymph node metastasis.[87] Although the concept of lymph node density seems to be a promising alternative for improving the prognostication of patients with lymph node–positive bladder cancer, there are several unresolved issues that hinder its unquestionable adoption into clinical practice. In addition to the retrospective design of the studies in favour of the concept of lymph node density, the proposed cut-off values are based on statistical calculations and are highly dependent on the surgical extent and meticulousness of lymphadenectomy. Even when considering a defined anatomical template of extended pelvic lymphadenectomy, the number of retrieved lymph nodes can vary significantly from patient to patient,[29,98] and this might have implications for the proposed cut-off values. Furthermore, different pathological processing techniques and evaluation methods of lymph nodes have to be taken into account.[66] Moreover, in the neoadjuvant setting, lymph node density was not found to be of prognostic value.[254] In this respect, prospective trials are certainly needed to better address the role of lymph node density in patients with lymph node–positive bladder cancer.

6.4.4.1.8 Late recurrence

Soria and colleagues set out to characterize the outcomes and identify clinicopathological predictors of late recurrence and postrecurrence survival in patients with bladder cancer treated with RC. Late recurrence was defined as occurring more than 5 years after RC. In a cohort of 1,652 bladder cancer patients, 33% experienced disease recurrence. Of these, 12.2% experienced late recurrence, with a median time to recurrence of 86 months. Late recurrence was more likely to be located in the urothelium. Multivariable analysis identified younger age and non–organ-confined disease as predictors of late recurrence. Postrecurrence 5-year OS was worse in patients who experienced early recurrence compared with those with late recurrence and in those with nonurothelial recurrence compared to those with disease recurrence in the remaining urothelium. Older age, non–organ-confined disease at RC, and nonurothelial recurrence site were independently associated with postrecurrence OS. These findings reinforce the need for lifelong follow-up of bladder cancer patients after RC.[255]

6.4.4.2 Impact of pathological parameters on outcomes

6.4.4.2.1 Variant histology

NAC provides a significant survival benefit in pure urothelial bladder cancer. The effect of NAC on the probability of non–organ-confined disease and OS after RC was assessed in patients with histological variants. Variants were categorized as micropapillary or sarcomatoid differentiation, squamous cell carcinoma, adenocarcinoma, neuroendocrine tumours, and other histology. Patients with neuroendocrine tumours benefited from NAC, as evidenced by better OS and lower rates of non–organ-confined disease at the time of RC. For tumours with micropapillary differentiation, sarcomatoid differentiation, or adenocarcinoma, NAC decreased the frequency of non–organ-confined disease at the time of RC. However, this favourable effect did not translate into a statistically significant OS benefit for these patients, potentially due to the aggressive tumour biology.[256]

6.4.4.2.2 Lymphovascular invasion

Lymphovascular invasion in pathologically node-negative bladder cancer was found to independently predict poor CSS and OS after RC, whereas in node-positive disease its independent prognostic significance was not confirmed.[257,258] Therefore, in node-negative disease, its presence might indicate micrometastasis and, thus, help improve risk assessment and guide clinicians in counselling patients for adjuvant treatment options or clinical trials.[259] However, when assessing lymphovascular invasion on conventional histological sections, its accurate detection can be hampered due to retraction artifacts and difficulties in identifying small lymphatic or blood vessels[260] which, in turn, delimitates its prognostic value and stresses the importance of additional immunohistochemical studies.

6.4.4.2.3 Molecular markers

The predictive value of molecular markers for risk assessment in invasive bladder cancer has been evaluated in a subset of studies. These biomarkers play an important role in cell-cycle regulatory or angiogenetic mechanisms. The following markers have been investigated for their involvement in bladder cancer: E-cadherin, pRB, surviving, p53, p16, p21, p27, cyclin E, and Ki-67.[261-263] Expression levels of these markers can be assessed in cystectomy specimens by immunohistochemistry, and the combination of different markers has been shown to improve the predictive accuracy of clinical and pathological risk factors.[262] A study that incorporated preoperative C-reactive protein concentrations as a serological marker in a multivariate predictive model showed that increased levels were not only associated with decreased CSS but also increased the predictive ability of standard pathological risk factors, including tumour stage, lymph-node density, and resection margin status.[264] Nonetheless, thus far, no established molecular markers can be unequivocally recommended for risk assessment in invasive bladder cancer on a routine basis. However, this is likely to change as cancer diagnosis and treatment becomes more and more molecular.

6.4.4.3 Impact of clinical parameters on outcome

The validity of survival analyses in retrospective invasive bladder cancer studies with short or intermediate follow-up time intervals is constantly in question. Indeed, the median time for local or distant recurrence after RC in studies with a median follow-up of more than 10 years ranges between 7 and 18 months.[66] A study set up to investigate the validity of DFS rates at 2 or 3 years following RC found that DFS rates at these time points correlated well with and can be potential intermediate surrogates for 5-year OS.[265]

Another important parameter that might influence the outcomes of bladder cancer patients is surgical expertise. A meta-analysis addressed the ongoing debate on the relationship between high-volume centres and oncological outcome. A significant positive association on survival was not found for either hospital or surgeon volume.[266] Nonetheless, older patients might derive the highest benefit when treated in a high-volume centre.[215] In this respect, the largest series on cystectomy to date, derived from the SEER database, analyzed the outcomes of 13,796 bladder cancer patients and demonstrated that patients above 80 years of age had an increased risk for postoperative morbidity but not mortality. The ability of patients older than 80 years of age to undergo cystectomy had the highest impact on risk reduction of cancer-related and non–cancer-related mortality.[246]

However, elderly patients with MIBC can pose a therapeutic dilemma, given their multiple comorbidities that may preclude surgery. Treatment patterns and survival outcomes were evaluated in a registry-based analysis of this patient population. The NCDB was queried for muscle invasive (cT2-T4aN0M0) bladder cancer in patients 80 years old or older. Patients included in the study underwent either TURBT followed by RC, RC plus chemotherapy, radiation therapy alone, chemotherapy alone, chemoradiation, or no treatment. A total of 9,270 patients were identified, with a median follow-up of 12.8 months. Median OS in patients treated with RC alone was 23.2 months, which was superior to that of chemotherapy alone or radiation therapy alone. Those treated with chemoradiation had a median OS of 27.3 months, which did not statistically differ from that of RC alone. Surgery plus chemotherapy showed the longest median OS of 34.5 months Thus, the best OS was seen in patients treated with surgery plus chemotherapy, while no difference in OS was observed between chemoradiation and RC alone.[258]

Travelling distance to the treating institution may be another factor that plays a role in treatment outcome. Ryan and colleagues investigated the relationship between travelling distance to treatment and outcomes. A total of 34,729 patients with MIBC (cT2a-T4 N0 M0) were queried. Travelling farther for treatment was associated with a lower probability of overall mortality. This was significant for patients with cT2 disease and those treated at academic centres, but not for the 11,059 patients who underwent RC. This is likely due to longer travelling distance being associated with surgery at a high-volume institution and receipt of NAC. Thus, patients who travelled farther for bladder cancer treatment did not experience inferior survival outcomes, and travelling to academic institutions was associated with reduced mortality.[267]

Bladder-preservation therapy is an alternative treatment to RC and can be considered where appropriate. OS trends were examined in patients undergoing RC or bladder-preservation therapy for muscle-invasive urothelial carcinoma of the bladder. Receipt of bladder-preservation therapy was associated with decreased OS compared with RC in patients with stage II to III urothelial carcinoma. However, increasingly stringent definitions of bladder-preservation therapy and more rigorous statistical methods adjusting for selection biases attenuated the observed differences in survival.[268]

With the advancement of medical robotics and the increasing prevalence of robot involvement in surgical procedures, a study examining the outcomes of RARC compared to ORC was conducted for muscle-invasive urothelial bladder cancer. No differences in efficacy outcomes or ability to deliver AC were observed between RARC and ORC.[269]

Health-related quality of life (HRQoL) is an important aspect of treatment that is often overlooked. In a recent study, HRQoL was evaluated in patients with bladder cancer and compared to noncancer controls and patients with colorectal cancer using data from SEER Medicare Health Outcomes Survey (MHOS). Patients with bladder cancer who underwent RC experienced significant declines in multiple components of physical- and mental-health–related QoL compared with noncancer controls, which mirror those of patients with colorectal cancer.[270] Another study examined the long-term (>5 years) HRQoL outcomes following RC, comparing Indiana pouch (IP), neobladder (NB), and ileal conduit (IC). When adjusted for gender, age at surgery, surgeon, and time since surgery, IC and IP patients had significantly better urinary function than NB patients. Among men ≥65 years of age, IC patients had significantly better urinary function than NB patients. Among men <65 years of

age, IC and IP patients had significantly better urinary function than NB patients. Among women older than 65 years, bowel bother was significantly better for IC patients than IP patients. Prospective longitudinal studies using validated HRQoL tools will further help guide preoperative diversion choice decisions between patient and surgeon.[271]

Cost is an inevitable part of health care and parameters effecting treatment costs should be considered. A recent study attempted to assess surgeon- and hospital-level variations in costs and predictors of high- and low-cost RC. In this study, 23,173 patients who underwent RC for bladder cancer in 208 hospitals in the United States were evaluated. Postoperative morbidity, patient comorbidities, and year of surgery contributed most to observed variations in costs, while other hospital- and surgical-related characteristics such as volume, use of robot assistance, and type of urinary diversion contribute less to outlier costs.[272]

6.4.4.4 Nomograms to predict outcome

In 2006, bladder cancer nomograms were established by the Bladder Cancer Research Consortium (BCRC) and the International Bladder Cancer Research Consortium (IBCNC). Both the nomograms are freely available on the internet.[273,274] The BCRC nomogram is based on a total of 731 patients treated in three North American institutions. In this nomogram, the standard predictors of the AJCC-based prediction model, the pT and pN stage, were complemented by the following parameters: age, gender, tumour grade at cystectomy, presence of lymphovascular invasion, presence of CIS, NAC, and adjuvant chemotherapy and RT. The addition of these parameters increased the predictive accuracy of the BCRC nomogram by 3.2% compared to the AJCC-based predictions.[275]

In contrast, the IBCNC nomogram relies on more than 9,000 cystectomy patients who were treated at 12 centres worldwide. In this nomogram, the following parameters have been added to the pT and pN stage: age, gender, tumour grade, number of days from diagnosis to cystectomy, and final histology. This nomogram has been shown to improve the AJCC-based predictions by 7%.[273] The original data sets of both nomograms used 200-bootstrap resamples for reducing overfit bias and for interval validation. Currently, only a few smaller series have externally validated these data, but they have confirmed an approximately 4% improvement in the predictive accuracy for both nomograms.[276]

Nevertheless, some limitations must be taken into account when addressing each patient's individual risk of recurrence and death by the use of these nomograms. In the IBCNC nomogram, a considerable number of patients with squamous cell carcinomas were included but their primary clinical and pathological parameters were not published. This makes its general applicability difficult.[273] In this respect, the BCRC nomogram provides detailed patient data, but the number of included patients is relatively low. In addition, the independent prognostic relevance of some of the parameters included (i.e. adjuvant treatment modalities) remains controversial. Nonetheless, both nomograms can be regarded as important tools for estimation of outcomes in patients treated with radical surgery.

Recently, an additional nomogram assessing cancer and all-cause mortality following RC was developed by Williams and colleagues. A Cox proportional hazards model was used to develop the nomogram with the goal of predicting 3- and 5-year OS and CSS with external validation. Patients who underwent RC were mostly younger, male, married, non-Hispanic white, and had fewer comorbidities than those who did not undergo RC. Married patients, in comparison with their unmarried

counterparts, had both improved OS and CSS. The nomogram, developed using SEER-Medicare data, was able to predict 3- and 5-year OS and CSS rates, with concordance indices of 0.65 and 0.66. This validated generalizable instrument has been converted into an online tool (Radical Cystectomy Survival Calculator) to provide a benefit-risk assessment for patients considering RC.[277]

6.4.4.5 Recommendations

- The AJCC substratifications in node-negative pT2 and pT3 bladder cancer are of prognostic value **[LOE 2; GOR B]**.
- According to the TNM staging system, organ-confined bladder cancer has to be defined as ≤pT2bN0M0 **[LOE 2; GOR B]**.
- Nomograms provide improved prognostic information for oncological outcomes before and after radical surgery, as compared to predictions based on pathological TNM staging. However, their general applicability has not yet been sufficiently established by external validation **[LOE 3; GOR C]**.
- In patients older than 80 years, RC is associated with the highest risk reduction on cancer-related and non–cancer-related mortality **[LOE 3; GOR C]**.
- Based on the scarce data available, the routine use of molecular markers for risk assessment after RC in invasive bladder cannot be recommended **[LOE 3; GOR D]**.

6.4.5 Quality of life

6.4.5.1 Health-related quality of life after radical cystectomy

HRQoL outcomes after surgery remain critical for measuring impact of any surgical modality. Studies investigating HRQoL in patients undergoing RC and urinary diversion are currently lacking proper credence and power.[278-280] Many of the studies are retrospective or cross-sectional by design and do not use validated questionnaires, while lacking baseline or preoperative assessment.

Gilbert *et al.* evaluated HRQoL outcomes for patients with bladder cancer using Bladder Cancer Index (BCI) in 315 patients. Domains of the BCI included urinary, sexual, and bowel function, and bother domain.[281] Patients undergoing RC had lower sexual function scores than patients who kept their native bladder. The difference in urinary and bowel domains between cystectomy and noncystectomy patients differed by type of urinary diversion, IC or NB.

It is important to keep in mind that much of the long-term morbidity of RC is associated with urinary diversion, not extirpation. HRQoL relates to functional outcomes, and there is currently a limited amount of data on functional outcomes.[131] Functional outcomes are dependent on surgical choices, e.g. continent versus noncontinent diversion, with additional variables such as natural voiding versus required intermittent self-catheterization. Although continence rates after RC are directly related to the surgical approach, they are influenced by multiple factors, including patient age and mental status, an intact and innervated urethral sphincter, urethral length, low-pressure/large-capacity reservoir (>300 mL), absence of bacteriuria, and completeness of voiding.[282] The 2012 EAU International Consultation on Bladder Cancer reviewed the data published on urinary diversion between 1970 and 2012 and found that, in patients with ORC and orthotopic bladder substitution, daytime and nighttime continence is achieved in 85% to 90% and 60% to 80% of patients, respectively.[282] Continence after orthotopic bladder substitution continues to improve up to 12 months after surgery. It is therefore preferable to assess continence stratified by daytime versus nighttime continence and by gender.[282-284]

A recent meta-analysis of non RCTs on HRQoL after RC using validated questionnaires showed a significant advantage of ileal orthotopic neobladder (IONB) compared to IC in terms of HR-QoL,[279] whilst a retrospective cross-sectionals study with matched-pair analysis on IC versus IONB concluded that IONB and IC after RC were similar in terms of global health status. It showed that IONB provided better results in some aspects of HR-QoL related to bowel function, but a worsening of urinary and sexual functions.[285]

A prospective QoL outcomes study compared patients who underwent IC urinary diversion with those who underwent IONB reconstruction after RC.[286] The European Organization for Research and Treatment of Cancer QoL questionnaire C30 was used to analyse QoL before surgery and 6, 12, and 18 months after surgery and found that IONB is better than IC in terms of physical functioning, role functioning, social functioning, global health status/QoL, and financial expenditure. The study concluded that IONB reconstruction provides better QoL outcomes than does IC urinary diversion.[286]

6.4.5.2 Impact of minimally invasive approach on health-related quality of life

There remains a paucity of literature evaluating HRQoL in patients undergoing minimally invasive treatment modalities.[280] A meta-analysis comparing complication rates and HRQoL after RARC versus ORC that included four RCTs (239 patients overall) found that the evidence was of low to moderate quality and concluded no significant difference regarding HRQoL.[280]

Yuh *et al.* prospectively evaluated short-term HRQoL outcomes after RARC and IC diversion using the Functional Assessment of Cancer Therapy - Bladder Cancer (FACT-Bl) questionnaire.[287] The FACT-Bl questionnaire includes 5 domains: well-being, physical, social/family, emotional, and functional, as well as 12 additional bladder cancer–specific questions. Thirty-four patients were included in the study, with follow-up questionnaires at 1-, 3-, and 6-month postoperative time periods. As expected, scores decreased significantly at the initial period, with improvement to baseline at the 6-month period. Emotional domains improved almost immediately after surgery and exceeded baseline scores at 6 months.

If we consider robotic continent urinary diversion, in most published series a Studer IONB has been created[131] and, although current cohorts are small, reported functional outcomes are encouraging. Tyritzis *et al.*,[284] in a series of 70 patients, reported daytime continence of 88.2% in male patients who had undergone nerve-sparing surgery, whilst nighttime continence reached 73.5% at 12 months. Similar rates were achieved for males who had undergone non–nerve-sparing surgery at 12 months (83.3 and 88.9%, respectively). Of female patients, 66.7% were found to be continent during the day and 66.7% at night at 12 months. All continence rates showed significant improvement at 12 months compared with the 6-month follow-up. A total of 81.2% of male patients were potent with or without phosphodiesterase type 5 inhibitor medication at 12 months. In this series, all eight female patients received a nerve-sparing procedure by preserving the autonomic nerves on the anterior vaginal wall. Of the evaluated male nerve-sparing, male non–nerving-sparing and female patient groups, 84.4, 23.8, and 66.7% of patients, respectively, were sexually active postoperatively. In sexual functionality in females, important outcome measures after the reconstruction of the vagina include both the ability to have sexual intercourse and the absence of dyspareunia.

6.4.5.3 Recommendations

- There is evidence for improved HRQoL for orthotopic NB reconstruction compared to IC urinary diversion **[LOE 3; GOR C]**.
- Appropriate patient selection for urinary diversion type is critical to achieving improved HRQoL outcomes following RC **[LOE 3; GOR C]**.
- More high-quality RCTs are needed to confirm current findings regarding HRQoL **[LOE 3; GOR C]**.

6.5 Perioperative Systemic Therapy

6.5.1 Neoadjuvant chemotherapy

6.5.1.1 Introduction

The standard of care for muscle-invasive urothelial carcinoma of the bladder in the absence of metastatic disease at initial diagnosis remains RC with bilateral PLND. In spite of potentially curative surgery, approximately one-half of patients with muscle-invasive urothelial carcinoma (stages T2–4,) develop metastatic disease within 2 years.[288] At 5 years, the survival rate after cystectomy is, at best, 65%, with a typical range between 36% to 48% (level 3)[66,222,224] depending on the presence of extravesical extension (pT3) and lymph node metastases (N1–N3). Both factors are associated with an increased risk for recurrence following cystectomy. In contemporary series, 5-year OS rates up to 57% have been reported in patients with clinically unsuspected N1 disease, as compared to 0% to 27% for those with larger volume N2 to N3 disease.[66,103,223] This may be because patients already harbour clinically undetectable micrometastases at the time of surgery.

6.5.1.2 Advantages and disadvantages of neoadjuvant chemotherapy

NAC or AC has the potential of eradicating micrometastases and improving survival in patients with muscle-invasive urothelial carcinoma of the bladder. This seems to be particularly true for patients with pathological extravesical and lymph node–positive disease.[289]

Administration of chemotherapy prior to surgery (neoadjuvant) versus after (adjuvant) offers several potential advantages. Patients may be able to tolerate treatment better, and the response of the primary tumour to chemotherapy can be assessed,[290-294] providing prognostic significance. In a study of patients treated with neoadjuvant cisplatin-based therapy followed by definitive surgery, 91% of patients who responded to chemotherapy (defined as pathological stage ≤T1) were alive at a median follow-up of 25 months, in contrast to 37% of nonresponders.[292]

Downstaging of the tumour may provide an indication of the activity of NAC, especially in patients who have a pCR and in patients who are pT1 stage after therapy. Those patients with residual disease at RC should probably be offered clinical trials evaluating non–cross-resistant alternative agents. A CR after neoadjuvant therapy may also permit consideration of organ preservation in selected cases. The standard of care is that the majority of patients require and undergo cystectomy or radical RT.

An important potential disadvantage of NAC is the discordance between clinical and pathological staging. In a study reported by Scher *et al.*,[295] while 57% of patients achieved a clinical and cystoscopic complete response (CR) following neoadjuvant methotrexate, vinblastine, doxorubicin, and cisplatin chemotherapy (MVAC), only 30% had a pCR at subsequent cystectomy.

A potential disadvantage of NAC is that patients achieving a complete clinical response at the TURBT after chemotherapy may refuse cystectomy. Another theoretical disadvantage of the neoadjuvant approach is the possibility that some low-stage, low-risk patients may unnecessarily receive NAC. Conversely, delay of definitive local treatment could potentially be associated with disease progression.[296]

The primary disadvantage of the AC paradigm may be that it does not appear feasible in one-third of patients within 90 days after RC due to postoperative complications or slow recovery of functional status.[142,297] Also, approximately 40% of patients who would be candidates for NAC may not be candidates for postoperative cisplatin because of a perioperative decline in renal function.[298]

Besides all these pros and cons, both approaches are targeting microscopic disease and the question is what is the best option for an individual patient? No published trials have directly compared pure populations of NAC versus AC except for the MDA trial, whose endpoint was to show the feasibility and safety of neoadjuvant compared to adjuvant therapy.[299] In the absence of a specific randomized trial that has compared optimal NAC and AC regimens in association with cystectomy, it is not possible to make a definitive recommendation about the utility of AC as compared to neoadjuvant treatment. This will be further discussed in the subsequent section on AC.

Before reviewing the accumulating data from controlled, prospective trials on NAC for bladder cancer, it is beneficial to acknowledge a growing consensus among many investigators for at least selective use of NAC in subgroups of patients who are known to be at high risk for micrometastatic disease, including those with bulky primary tumours, hydronephrosis due to the primary tumour, mixed histology, and possibly lymphovascular invasion within the primary tumour.[300]

6.5.1.3 Randomized trials evaluating role of neoadjuvant chemotherapy for bladder cancer

NAC theoretically should provide benefit to patients, whether it is given before cystectomy or before RT. In the United States, RC is the preferred local therapy for patients who have a good performance status. In most of Europe, RC is also the preferred option, although some institutions consider local radical RT as an alternative.

Several randomized trials have explored whether NAC improves survival in bladder cancer. The results of these randomized trials are presented in **Table 6–10**.[6,301] Some studies suffered from small sample size, suboptimal chemotherapy, premature closure, or inadequate follow-up time.[302] Among these trials, single-agent regimens failed to show a survival benefit from neoadjuvant therapy.[303] However, well-designed multiagent chemotherapy trials utilizing effective chemotherapeutic regimens have helped to demonstrate an improvement in survival. These trials have shifted the treatment paradigm in muscle-invasive disease, favouring the use of NAC.[5,6]

TABLE 6–10 Randomized Phase 3 Trials of Neoadjuvant Chemotherapy for Bladder Cancer

Series	Study Population	Year	No. Of Patients	Chemotherapy	Follow-up* (Range)	OS† (%)	OS HR (95% CI)	Significant (Y/N)
Cortesi[332]	cT2-T4 N0M0	(Unpublished)	171	MVEC	-	52.4% vs. 57.7%	-	N
Wallace *et al.*[333]	cT2-T4 NxM0	1991	255‡	Cisplatin	-	71.1% vs. 65.8%	1.13 (0.80-1.57)	N
Cannobio[334]	cT2-T4N0	1995	104	Cisplatin 5-flurouracil		40% vs. 29%	-	N
Martinez-Pineiro *et al.*[303] (Spain)	cT2-T4a, Nx-N2, M0	1995	122	Cisplatin	78.2 mo (48 to 101)	35.5% vs. 37.3%	-	N
Bassi *et al.*[335] (GUONE)	cT2-4aN0	1996	206	MVAC	-	55% vs. 54%	-	N
Italian (GISTV)[336]	cT2-T4b, N0	1996	171	MVEC	-	52% vs. 57.6%	-	N
Coppin *et al.*[337] (NCI-CTC)	cT2-T4b	1996	102	Cisplatin	78 mo	16% vs. 13%; p=0.34	0.75 (90% CI, 0.50-1.12)	N
Abol-Enein *et al.*[338]	cT2-T4a Nx M0	1997	196	CMV	-	-	-	-
Shipley *et al.*[307]	cT2 to T4aNXM0	1998	123	CMV	60 mo	48% vs. 49%	-	N
Sengelov *et al.*[339] (Denmark DAVECA 8901 and 8902)	cT2-T4b, NX-3 M0	2002	153	CM	-	19% vs. 24%	-	N

Abbreviations: CI, confidence interval; CM, cisplatin and methotrexate; CMV: cisplatin, methotrexate, and vinblastine; EORTC, European Organisation for Research and Treatment of Cancer; GC, gemcitabine and cisplatin; GUONE, Gruppo Uro-Oncologico del Nord Est; HR, hazard ratio; MRC, Medical Research Council; MVAC: methotrexate, vinblastine, adriamycin, cisplatin; MVEC: methotrexate, vinblastine, epirubicin, cisplatin; NAC, neoadjuvant chemotherapy; NCI, National Cancer Institute; OS, overall survival; RT, radiotherapy; SCC, squamous cell carcinoma; TCC, transitional cell carcinoma.

* Mean or median follow-up time (in months) as reported by each study during time of publication. Types of range reported include minimum to maximum, inter-quartile range, and 95% CI.

† Based on number of events out of total number of patients in treatment (neoadjuvant) versus control arm (local treatment—radical cystectomy or RT).

‡ All 255 patients underwent NAC, but the control arm received local treatment in the form of RT in two different regimens (1) 159 patients received 45-50 Gy in 22 F and (2) 96 patients received 65 Gy in 22F + 10–15 Gy.

continued on **page 595**

TABLE 6–10 Randomized Phase 3 Trials of Neoadjuvant Chemotherapy for Bladder Cancer, *Cont'd*

Series	Study Population	Year	No. Of Patients	Chemotherapy	Follow-up* (Range)	OS† (%)	OS HR (95% CI)	Significant (Y/N)
Grossman *et al.*[5] (SWOG Intergroup)	cT2-T4a	2003	317	MVAC	104 mo	57% vs. 43%; p=0.06	1.33 (1.00-1.76)	Y
Sherif *et al.*[340] (Nordic I and II)	cT2-T4a, Nx, M0	2004	620	Cisplatin/ Adriamycin + RT (Nordic I) or cisplatin/ methotrexate (Nordic II)	56.4 mo	56% vs. 48%	0.80 (0.64-0.99)	Y
International Collaboration of Trialists[318] (MRC-EORTC/ BA06 30894)	cT2-4a, N0-x, M0	2011	976	CMV	120 mo	36% vs. 30%; p=0.037	0.84 (0.72-0.99)	Y
Kitamura *et al.*[315] (Japan JCOG0209)	cT2-T4N0	2014	130	MVAC	55 mo	72% vs. 62%	0.65 (0.19-2.18) *one-sided p=0.07	N
Osman *et al.*[314] (Egypt)	cT2-4N0M0	2014	60	GC	36 mo	60% vs. 50% (3-year OS)	-	N
Khaled *et al.*[316] (Egypt)	cT3-4, N0-2, M0 TCC + SCC	2014	114	GC	37.4 mo	51.9% vs. 51.2% (3-year OS)	-	N

Abbreviations: CI, confidence interval; CM, cisplatin and methotrexate; CMV: cisplatin, methotrexate, and vinblastine; EORTC, European Organisation for Research and Treatment of Cancer; GC, gemcitabine and cisplatin; GUONE, Gruppo Uro-Oncologico del Nord Est; HR, hazard ratio; MRC, Medical Research Council; MVAC: methotrexate, vinblastine, adriamycin, cisplatin; MVEC: methotrexate, vinblastine, epirubicin, cisplatin; NAC, neoadjuvant chemotherapy; NCI, National Cancer Institute; OS, overall survival; RT, radiotherapy; SCC, squamous cell carcinoma; TCC, transitional cell carcinoma.

* Mean or median follow-up time (in months) as reported by each study during time of publication. Types of range reported include minimum to maximum, inter-quartile range, and 95% CI.

† Based on number of events out of total number of patients in treatment (neoadjuvant) versus control arm (local treatment—radical cystectomy or RT).

‡ All 255 patients underwent NAC, but the control arm received local treatment in the form of RT in two different regimens (1) 159 patients received 45-50 Gy in 22 F and (2) 96 patients received 65 Gy in 22F + 10–15 Gy.

The SWOG Intergroup Trial randomized patients with T2–T4a transitional cell carcinoma (TCC) of the bladder to RC alone (154 patients) versus 3 cycles of MVAC followed by RC (153 patients).[5] The use of NAC was associated with a higher rate of complete pathological response (38% vs. 15%; p<0.001). At a median follow-up of 8.7 years, improvements in median survival (77 vs. 46 months; p=0.06) and 5-year survival (57% vs. 43%; p=0.06) favoured the neoadjuvant MVAC arm. Because of its size, this

trial had limited potential to discern a clinically meaningful difference. This trend toward improved survival favouring MVAC–treated patients with an estimated reduction in the risk of death by 25% (HR 1.33) provides some evidence of the benefit **[LOE 1]**.[5] There were no treatment-related deaths, and NAC did not adversely impact the ability to proceed with RC or increase adverse events related to surgery.

Several studies have been published based on retrospective analysis of this trial database. As an example, surgical factors were evaluated in 268 patients with MIBC who underwent RC in this SWOG Intergroup trial.[95] One hundred and six surgeons at 109 institutions performed these RCs. Half of the patients received neoadjuvant MVAC. The 5-year postcystectomy survival and local recurrence rates in all patients who underwent cystectomy were 54% and 15%, respectively. Surgical variables associated with longer post-cystectomy survival were negative margins (HR 0.37, p=0.0007) and removal of ≥10 nodes (HR 0.51, p=0.0001). These associations did not differ by treatment arm (p=0.21 for all tests of interactions between treatment and surgical variables). Predictors of local recurrence were positive margins (OR 11.2, p=0.0001) and removal of <10 nodes (OR 5.1, p=0.002). The quality of surgery was an independent prognostic factor for outcome after adjustments were made for pathological factors and NAC usage **[LOE 2]**.

Another recent analysis evaluated the impact of histology when neoadjuvant MVAC was given in this trial. There was evidence of a survival benefit from chemotherapy in patients with mixed tumours.[304] Presence of squamous or glandular differentiation in locally advanced urothelial carcinoma of the bladder does not seem to confer resistance to MVAC and, in fact, may be an indication for the use of NAC before RC.

The Medical Research Council (MRC)/European Organisation for Research and Treatment of Cancer (EORTC) performed a large trial in which 976 patients from 106 institutions were enrolled and randomized to neoadjuvant cisplatin, methotrexate, and vinblastine chemotherapy (CMV) (491 patients) or no NAC (485 patients) over a 5.5-year period. This trial was performed more or less during the same time as the SWOG trial. The results of this trial were updated at a median follow-up of approximately 7 years.[6] Management of the primary tumour involved cystectomy, radiation therapy, or both, and was left to the choice of investigators. An initial 8% improvement in time to progression and a 5.5% difference in absolute 3-year survival (HR, 0.85; 95% CI, 0.71-1.02) favouring the NAC arm was reported. When results were published in 1999, a nonsignificant trend toward improvement in survival was observed in patients in the CMV arm. In a 2002 update from American Society of Clinical Oncology (ASCO), with follow-up of 7.4 years, a statistically significant improvement in survival was observed for patients who received NAC (p=0.048; HR, 0.85; 95% CI, 0.72-1.0). This trial, well powered and with adequate follow-up, demonstrated both a survival benefit and improved locoregional control with neoadjuvant CMV chemotherapy; however, the predefined endpoint with an improvement in survival of 10% was, in fact, not reached. Survival at 5 years was 50% with CMV compared with 44% with RT; at 8 years, it was 43% with CMV and 37% with RT **[LOE 1]**.

A trial that was almost identical to the SWOG study was performed by the Gruppo Uro-Oncologico del Nord Est (GUONE) cooperative group in Italy.[305] Over a 6.5-year period, 206 patients were randomly assigned to neoadjuvant MVAC before cystectomy or to cystectomy alone. No clear differences in survival were demonstrated, as 3-year survival was 62% for the MVAC–treated patients and 68% for patients in the cystectomy-alone arm **[LOE 2]**.

The Nordic Cystectomy Trial I evaluated neoadjuvant doxorubicin, cisplatin, and preoperative RT before cystectomy versus preoperative RT and cystectomy alone. A 15% survival difference in favour of patients treated with chemoradiotherapy was seen in only a subset analysis of patients with T3 or T4 disease. Investigators were unable to confirm this survival advantage in the subsequent Nordic Cystectomy Trial II, in which 317 patients were randomly assigned to cystectomy or cystectomy preceded by methotrexate and cisplatin (without RT).[306] However, combining the two trials provided positive results in favour of NAC **[LOE 2]**.[307]

6.5.1.4 Meta-analysis of randomized trials

Because of the uncertainties of the definitive value of NAC in terms of survival, a meta-analysis of NAC trials was performed by the Advanced Bladder Cancer group from the Cochrane collaboration.[7] Data from 2,688 patients treated in 10 randomized trials evaluating NAC for invasive urothelial carcinoma were reviewed. Of note, this analysis did not include data from the SWOG Intergroup trial. Compared to local treatment alone, neoadjuvant platinum-based combination chemotherapy was associated with a significant benefit in OS (HR, 0.87; 95% CI, 0.78-0.98; p=0.016), translating to a 5% absolute survival benefit at 5 years (OS increased from 45% to 50%). When trials utilizing single-agent cisplatin were included, the survival benefit did not achieve statistical significance (HR, 0.91; 95% CI, 0.83-1.01; p=0.084) **[LOE 2]**. However, single-agent cisplatin did not show an improvement in survival (p=0.26) compared with no neoadjuvant therapy. As all platinum-based combination trials were analysed as a group, it is not possible to discern the best combination for use in neoadjuvant therapy. It is important that this first meta-analysis did not include the second largest RCT from SWOG Intergroup.

A subsequently reported meta-analysis that included individual patient data from 3005 individuals enrolled in 11 randomized trials, including the SWOG Intergroup data, extrapolated from the published report[5] confirmed the survival benefit for neoadjuvant cisplatin-based therapy compared to local therapy alone[7,308] (HR, 0.86; 95% CI, 0.77-0.95; p=0.003), with an absolute improvement of 5% in 5-year OS. Additionally, there was a significant DFS benefit with the use of platinum-based NAC (HR, 0.78; 95% CI, 0.71-0.86; p<0.001), with an absolute improvement of 9% in 5-year DFS.

In 2004, a very similar meta-analysis of neoadjuvant randomized controlled trials was conducted in Canada.[309] A total of 16 eligible trials including 3,315 patients were identified, and 2,605 patients provided data suitable for a meta-analysis of OS, for which the pooled HR was 0.90 (95% CI, 82%-99%; p=0.02). When restricted to the only eight trials using cisplatin-based combination chemotherapy, the pooled HR was 0.87 (95% CI, 78%-96%; p=0.006), consistent with an absolute OS benefit of 6.5% (from 50% to 56.5%) (95% CI, 2%-11%). A major pathological response was associated with improved OS in 4 trials. Neoadjuvant cisplatin– based chemotherapy improved OS in muscle-invasive urothelial carcinoma, but the size of the effect was modest **[LOE 2]**.

The use of perioperative chemotherapy has been limited until 2003 to 2005, when these meta-analyses were published. Among 7,161 analysable patients in the NCDB with stage III bladder cancer diagnosed between 1998 and 2003, perioperative chemotherapy was administered to 11.6% of patients, with 10.4% receiving AC and 1.2% receiving NAC.[310] After 2003, there has been a slight increase in its use. A more recent NCDB analysis on 40,388 patients aged 18 to 99 years diagnosed with muscle invasive (stages II to IV) bladder cancer found that the incidence of those who received chemotherapy increased from 27.0% in 2003 to 34.5% in 2007 due to an increase in NAC and chemotherapy without surgery.[311] Clinical Practice Guidelines (CPG) can help to increase the implementation of NAC. A Canadian study[312] has shown that neoadjuvant referral and treatment rates increased after publication of the CPG. However, overall referral and treatment rates with NAC prior to RC remained low. Based on these observations and despite level I evidence, neoadjuvant cisplatin-based chemotherapies continue to be underutilized in the management of bladder cancer, even at high-volume tertiary centres.[313]

Latest randomized trials and updated meta-analysis

Recently, three further RCTs were published, but they did not demonstrate any OS benefit.[314-316] For instance, the Japan Oncology Group analysed 130 patients and found no significant difference in OS between those who received NAC MVAC plus RC or RC alone (JCOG0209).[315] However, this study suffered from an early closure due to the slow accrual. Although there was no significant OS benefit demonstrated, the rate of complete pathological response was greater in the NAC versus RC-alone group (34% vs. 9%; $p<0.01$).

Accordingly, an updated meta-analysis of summary data was published in 2016, showing a persistent OS benefit with the use of NAC, even after including all these negative trials (HR, 0.87; 95% CI, 0.79-0.96). This benefit was even greater when only considering patients who received cisplatin-based regimens (HR, 0.84; 95% CI, 0.76-0.93).[317]

Long-term oncological outcomes

In 2011, the International Collaboration of Trialists evaluated long-term oncological outcomes after NAC, updating their previously historical RCT.[318] With a median follow-up of 8 years, a significant OS benefit was demonstrated, with a 16% reduction in the risk of death from any cause (HR, 0.84; 95% CI, 0.72-0.99; $p=0.037$). This translates to a 6% improvement in 10-year OS (from 30% to 36%). Additionally, all other oncological outcomes were in favour of the use of NAC, given that such treatment was associated with a 23% reduction in the risk of metastases (HR, 0.77; 95% CI, 0.66-0.90; $p=0.001$) and a 18% reduction in the risk of disease recurrence (HR, 0.82; 95% CI, 0.70-0.95; $p=0.008$). Only a nonsignificant benefit favouring the NAC group was observed for DFS (HR, 0.83; 95% CI, 0.68-1.00; $p=0.050$).

Real-world data and optimizing the selection of patients who may benefit from neoadjuvant chemotherapy

Outside the setting of clinical trials that tend to have strict inclusion/exclusion criteria, numerous investigators have evaluated the real-world use and benefit of NAC using retrospective cohort studies. A large series using the NCDB from 2003 to 2012 identified 1,739 patients with cTanyN1-3M0 bladder cancer and found that 5-year OS rates were the highest for those who received NAC followed

by RC (31%), followed by those who received cystectomy followed by AC (26%), followed by those who underwent cystectomy alone (19%).[319] Compared with cystectomy alone, NAC was significantly associated with improved OS (HR, 0.80; 95% CI, 0.66-0.97).

Several observational studies focused on identifying the best candidates for NAC prior to RC. Culp *et al* proposed a risk-stratified approach for the use of NAC,[300] defining high-risk patients as those with the clinical presence of hydroureteronephrosis, cT3b-T4a disease, and/or histological evidence of lymphovascular invasion, or micropapillary or neuroendocrine features on TUR. Culp *et al* found that high-risk patients exhibited poorer 5-year OS (47.0% vs. 64.8%) and decreased disease-specific survival (64.3% vs. 83.5%) and PFS (62.0% vs. 84.1%) probabilities compared to low-risk patients (p<0.001). These were subsequently externally validated by two other groups led by Moschini[320] and von Rundstedt[321] highlighting the interest of selecting individuals who may be more likely to experience tumour downstaging and ultimately benefit from NAC.

In fact, downstaging to ypT0 disease at surgery is of upmost importance, given that NAC may be only effective for these patients. Indeed, a recent report by Bhindi *et al* has evaluated the impact of residual disease at surgery after matching 180 patients who received NAC plus RC to 324 controls who received RC alone on the basis of pT and pN stages.[322] On multivariable analysis, the investigators found that NAC was associated with a DFS, CSS, and OS benefit only in patients who experienced ypT0 disease at RC, while such treatment was associated with adverse oncological outcomes in those with residual disease at RC.

6.5.1.5 Novel combinations as neoadjuvant therapy for bladder cancer

Only the MVAC regimen has been extensively evaluated in the neoadjuvant setting for bladder cancer, with most trials using this regimen. On this basis, MVAC has the strongest evidence-based data for neoadjuvant use.

Neoadjuvant dose-dense or accelerated MVAC

As a retrospective study evaluating the administration of dose-dense MVAC as NAC, in 2012 Blick *et al.* found reasonable grade 3/4 toxicity rates, favourable complete pathological response (43% of 60 surgical patients), and good objective radiologic local response (83% of 57 evaluable patients).[323] Subsequently, two prospective phase 2 trials published in 2014 demonstrated the efficacy and tolerability of dose-dense MVAC (DD-MVAC). The first, by Plimack *et al.*, recruited 44 patients with cT2-4a N0-1 disease, of which 15 out of 40 eligible patients (38%; 95% CI, 23%-53%) were pT0 at cystectomy, and another 6 patients (14%) were downstaged to nonmuscle invasive disease. The accelerated 6-week DD-MVAC regimen demonstrated comparable pT0 rates to the standard 12-week regimen.[324] A second trial, by Choueiri *et al.*, showed that, of the 39 recruited patients with cT2-4 N0-1 M0 disease, 49% (80% CI: 38-61) achieved pathological response of ≤pT1N0M0.[325]

Neoadjuvant paclitaxel, carboplatin, gemcitabine

Nevertheless, there are promising results from newer combinations such as gemcitabine, cisplatin/carboplatin with or without paclitaxel in patients with metastatic disease, which have led to the investigation of these regimens in the neoadjuvant and adjuvant settings. Although these newer regimens are promising, there are no data from randomized trials supporting their use in the neoadjuvant setting[326] and limited data from phase 2 trials.

In a phase 2 trial of 68 patients with adequate renal function and clinical T3 or T2 with hydronephrosis, N0, M0 bladder cancer received three cycles of neoadjuvant paclitaxel, carboplatin, and gemcitabine (PCaG) with a primary endpoint of pCR. Patients with T4 or node-positive patients received six cycles of PCaG with an endpoint of resectability.[327] The caveat is that this regimen was fairly toxic in a population with adequate baseline renal function and may often warrant prophylactic granulocyte growth factors in accordance with guidelines.

The SWOG conducted a phase 2 trial of three cycles of neoadjuvant PCaG followed by cystoscopic surveillance or immediate RC for patients with cT0 status after chemotherapy.[328] Patients with cT0 status could elect immediate RC or cystoscopic surveillance, and those who did not achieve cT0 status underwent immediate RC. There was an unacceptably high rate (60%) of persistent cancer at RC in patients presumed to have pT0 status, which suggests that RC is a critical component of therapy.

Neoadjuvant gemcitabine and cisplatin

While the GC doublet has not been validated in the perioperative setting, recent retrospective data from MSKCC shows that the GC regimen produces a pCR rate of 35%, similar to MVAC.[329] Multiple other reports on use of this doublet for metastatic disease show very similar response rates and survival to that obtained with MVAC, and with lower toxicity. In contrast to the above MSKCC study, data from the Cleveland Clinic showed that only 7% of patients achieved a pCR with mostly GC and other non-MVAC-based regimens, mainly administered in community oncology practices.[326] There are emerging data from large well-analyzed retrospective studies **[LOE 3]** showing the efficacy of GC compared to MVAC.

A large study comparing GC to MVAC conducted by the Retrospective International Study of Cancers of the Urothelial Tract (RISC) Investigators across 28 international centres included a total of 212 patients (146 patients in the GC cohort and 66 patients in the MVAC cohort). They found no significant difference in the pCR rate when adjusted for propensity scores between the two regimens (OR, 0.91; 95% CI, 0.48-1.72; p=0.77).[330] Another larger multicentre study across 19 centres included 935 patients with cT2-4N0M0 disease, of which the majority (64%) received GC, and 19.6% received MVAC. The investigators found that the rate of pT0N0 disease for patients receiving GC was 23.9%, compared with 24.5% for MVAC (p=0.2). On multivariable analysis, there was no significant difference between MVAC and GC in pT0N0 (OR, 0.89; 95% CI, 0.61-1.34; p=0.6).[331]

In summary, cystectomy is considered to be the gold standard of treatment for patients with localized MIBC. NAC was intended for patients with operable clinical stage T2 to T4a muscle-invasive disease. The rationale for giving chemotherapy before cystectomy or full-dose RT is based on the intent to treat micrometastatic disease present at diagnosis. A discrepancy between clinical and pathological staging can be expected. Toxicity and mortality associated with NAC are acceptable. Available data suggest that, for average-risk patients with cT2 cancer, the benefit of adding chemotherapy to local therapy is, at best, modest. Likewise, available studies suggest a much more substantial benefit for patients with high-risk disease, such as cT3b cancers. Presence of squamous or glandular differentiation in locally advanced urothelial carcinoma of the bladder does not seem to confer resistance

to MVAC. The quality of surgery is a confounding factor in these studies. Meta-analysis of cisplatin-containing combination NAC trials revealed a 5% difference in favour of NAC. Unfortunately, in this case, in which small differences in survival can be seen, it is regrettable that the data on QoL are inadequate.

6.5.1.6 Recommendations

- Cystectomy is considered the gold standard of treatment for localized MIBC **[LOE 2; GOR B]**.
- A discrepancy between clinical/cystoscopic and pathological staging can be anticipated after NAC and, therefore, cystectomy is not obviated by response **[LOE 2; GOR B]**.
- Toxicity and mortality associated with NAC are acceptable **[LOE 2; GOR B]**. However, few data on QoL are available.
- Meta-analysis of cisplatin-containing combination NAC trials revealed a modest benefit in favour of NAC **[LOE 1; GOR B]**.
- Cisplatin-based combination chemotherapy should be offered to all eligible patients with *c*T2-T4aN0M0 urothelial bladder cancer **[LOE 1; GOR A]**.
- We recommend using DD-MVAC as the NAC regimen for appropriately selected cases **[LOE 2; GOR B]**.
- Although other regimens, such as GC, have similar activity in patients with metastatic disease, there are no data from randomized trials in the neoadjuvant setting to support the use of regimens other than MVAC. Retrospective datasets in the NAC setting show comparable pCR rates between GC and MVAC **[LOE 2; GOR B]**.
- Available data suggest that, for average-risk cancer patients with *c*T2, the benefit of adding chemotherapy to local therapy is, at best, modest, but benefits still outweigh the risks. Likewise, all available studies suggest a much more substantial benefit for patients with high-risk disease, such as *c*T3b cancers or those thought to have lymph node involvement **[LOE 2; GOR B]**.
- Carboplatin-based regimens should not be used in the neoadjuvant setting **[LOE 2; GOR B]**.
- No predictive biomarker has an established role to exclude patients from neoadjuvant platinum-based therapy **[LOE 3; GOR D]**.
- The quality of the surgery is a confounding factor in interpreting these studies **[LOE 3; GOR C]**.
- Following cystectomy in patients who did not receive NAC, we suggest consideration of AC (see next section) with a cisplatin-based regimen for patients who have perivesical tumour extension (stage T3 or higher) or regional lymph node involvement **[LOE 2; GOR C]**.
- Presence of squamous or glandular differentiation in locally advanced urothelial carcinoma of the bladder does not seem to confer resistance to MVAC, and in fact may be an indication for the use of NAC before RC **[LOE 3; GOR C]**.

6.5.2 Adjuvant chemotherapy

6.5.2.1 Introduction

Despite the high rate of downstaging and response in the neoadjuvant and metastatic setting, cisplatin-based chemotherapy is underutilized in the treatment of urothelial cancer. As a consequence, more than 50% of patients with high-grade bladder cancer and muscle invasion ultimately die of disseminated disease. High-risk patients with pT3-pT4 pN0 have a 5-year OS of 47% after cystectomy; patients with lymph node metastases have an overall 5-year survival rate of up to 31% after RC.[66,341] In a recent contemporary analysis, pT3-pT4 pN0 high-risk patients undergoing RC without neoadjuvant or adjuvant therapy had a slightly better 5-year disease-specific survival of 65.8% to 46.1% and a poor 5-year disease-specific survival of 22.4% in node-positive disease (any pT).[342] In contrast, patients pT2pN0 had favourable 5-year disease-specific survival rates of 73.5%, pointing out that the risk patients are prone to is different.[342] Despite a high risk of relapse, translating the high response seen in advanced disease into long-term survival in the locally advanced setting has proven difficult.[343,344] The chemotherapy agents used in urothelial cancer have recently been reviewed and will not be discussed in detail here.[345]

AC for bladder cancer is controversial. This controversy is fuelled by suboptimal outcomes for locally advanced patients treated with RC alone, a small potential benefit of chemotherapy, and a sequence of trials that have been underpowered and/or closed early due to poor accrual, as well as the presence of more definitive evidence for NAC. Recently, this discussion was opened again due to the release of three randomized phase 3 trials and a meta-analysis that included two of these trials.[346-349] Neoadjuvant cisplatin-based combination chemotherapy is the standard of care for medically fit patients with high-grade stage T2 or greater bladder cancer based on level 2b evidence for improved OS, and is discussed in detail above. An alternative is the use of concurrent cisplatin and radiation therapy for which there is also level 2b evidence, albeit in a more highly selective cohort of patients. At this time, there is no proven value to NAC or AC for patients undergoing definitive chemoradiation (see discussion in the section on radiation therapy).

Despite definitive evidence for its use, relatively few patients are offered and receive NAC before surgery,[310,350] although this may be increasingly driven by clinical practice guidelines.[301,351-353] In tandem with commonly observed upstaging of bladder cancer patients at surgery,[354,355] slow adoption of neoadjuvant treatment has resulted in clinicians being confronted with patients who have not had the potential benefit of chemotherapy in combination with surgery, but who have pathological staging that portends a risk of relapse of up to 70%. This scenario begs the question as to whether patients would be better treated with immediate postoperative chemotherapy or observed for possible relapse and treated at that time. This question was the subject of some studies whose results presented recently.

Unfortunately, there have been methodological issues with many of the studies undertaken in the postoperative chemotherapy setting, and we are left with meta-analyses for our best evidence. In 2006, a pooled analysis supported AC, with a more extensive meta-analysis demonstrating a benefit to adjuvant cisplatin combination chemotherapy.[356,357] Since these analyses were undertaken, three major phase 3 studies were presented: Spanish Oncology Genitourinary Group (SOGUG) 99/01 (abstract),[348] Cancer and Leukemia Group B (CALGB) Italian Multicentric,[346] and EORTC 30994.[349]

These trials continue the pattern of premature closure for poor accrual seen in earlier studies, but contributed in composite to this field. Recently, a large cohort analysis assessing the effect of AC from several large centres has been published,[358] suggesting the greatest impact of AC is seen in patients with extravesical extension or N+ disease.

6.5.2.2 A short history of early clinical trials of adjuvant chemotherapy in bladder cancer

Multiple cisplatin-based combinations have been evaluated in the adjuvant setting (see **Table 6–11**).[359] Logothetis *et al.* administered cyclophosphamide, doxorubicin and cisplatin (CISCA) to a group of 71 post-cystectomy patients with resected nodal metastases, extravehicular extension, lymph vascular invasion, or pelvic visceral invasion.[360] These patients were compared in a nonrandomized fashion to 62 high-risk patients and 206 low-risk patients who did not receive AC. They concluded that adjuvant CISCA conferred a 2-year DFS advantage to patients with unfavourable pathological findings (70% vs. 30%; p=0.00012). The earliest RCT of combination chemotherapy administered to patients after RC was conducted at the University of Southern California.[361] Seventy patients with pT3, pT4, or node-positive TCC were offered to receive AC or observation. Chemotherapy consisted of cyclophosphamide or cisplatin at the beginning, and later of a combinatory regimen of CISCA with the randomisation to treatment or observation. The curves for DFS separated but were not significantly improved.[361] Consequently, the authors set up a prospective comparative trial.[362] Ninety-one patients with pT3/4, or node positive TCC were randomized to receive four cycles of CISCA (cisplatin, cyclophosphamide, doxorubicin). Chemotherapy resulted in a significant improvement in the risk of disease recurrence at 3 years (30% vs. 54%; p=0.011, unstratified Wilcoxon test), but only a trend to benefit in the overall risk of death (34% vs. 50%; p=0.099, unstratified Wilcoxon). The median survival of patients on chemotherapy was reported to be 4.25 years versus 2.41 years for patients in the observation group.[362] This study has been criticized for the fact that only 33 out of 44 patients assigned to the chemotherapy arm received one or more cycles of CAP (cyclophosphamide/doxorubicin/cisplatin), for the small sample size, and for deficiencies in statistical analysis such as the use of the Wilcoxon test emphasizing early differences. Nonetheless, the study was provocative in revealing the potential benefit of AC and in highlighting the difficulties involved in conducting such trials.

TABLE 6–11 Summary of Key Studies of Adjuvant Chemotherapy in Bladder Cancer

Centre	Regimen	Outcome	Comment	Reference
University of Mainz	MVEC/MVAC	Early stopping due to interim analysis favouring chemotherapy	Underpowered	Stockle *et al.*[366]; Lehmann *et al.*[367]
University of Southern California	Cisplatin-based	Modest benefit for chemotherapy	Methodological issues	Skinner *et al.*[362]
Stanford University	Cisplatin, methotrexate, vinblastine	Early stopping due to interim analysis favouring chemotherapy	Underpowered, delayed time to progression (p=0.01) effect on survival	Freiha *et al.*[368]
SOGUG 99/01	Cisplatin, gemcitabine, paclitaxel vs. observation	Early termination due to poor accrual	Major benefit to chemotherapy arm	Paz-Ares *et al.*[348]
CALGB-90104	Rapid sequence AG-ITP chemotherapy with G-CSF vs. cisplatin, gemcitabine	Early termination due to poor accrual	No results reported	Bajorin[370]
Italian Multicentric study	Cisplatin, gemcitabine vs. observation	Early termination due to poor accrual and futility	Nonsignificant, underpowered; trend to better outcome in nonchemotherapy arm	Cognetti *et al.*[346]
EORTC 30994	GC, MVAC or DD-MVAC vs. chemotherapy at relapse	Early termination due to poor accrual	Nonsignificant for OS, significant benefit for PFS	Sternberg *et al.*[349]

Abbreviations: AG-ITP, Sequential chemotherapy with doxorubicin-gemcitabine (AG) followed by fosfamide, paclitaxel and cisplatin (ITP); CALGB, Cancer and Leukemia Group B; CAP, cyclophosphamide/doxorubicin/cisplatin; DD-MVAC, dose-dense methotrexate, vinblastine, doxorubicin, and cisplatin chemotherapy; EORTC, European Organisation for Research and Treatment of Cancer; GC, gemcitabine and cisplatin; MVAC, methotrexate, vinblastine, doxorubicin, and cisplatin chemotherapy; MVEC, methotrexate, vinblastine, epirubicin, and cisplatin chemotherapy; OS, overall survival; PFS, progression-free survival; SOGUG, Spanish Oncology Genitourinary Group.

A subsequent trial of AC with three cycles of cisplatin alone did not result in any survival benefit in a randomized study of 77 patients.[363] Potential explanations for the lack of significant benefit include the usage of single-agent cisplatin, the small sample size, the inclusion of patients with lower T stage (pT1/2: 25 patients), and the high proportion of lymph node–negative disease (70/77). In addition, only 21 of the 37 patients (57%) received the planned three cycles of chemotherapy. Seven patients refused chemotherapy and nine patients had dose reductions but were included in the intention-to-treat analysis.[363]

Given the superiority of the MVAC combination over single-agent cisplatin in the metastatic setting,[364] it became important to evaluate the MVAC or MVEC (using epirubicin rather than adriamycin or doxorubicin) combinations in the adjuvant setting. Stockle *et al.* randomized patients with pT3, pT4, and/or pelvic lymph nodes to three cycles of MVAC or MVEC versus observation.[365,366] While planned to accrue 100 patients, the study was closed after an interim analysis of 49 randomized patients revealed a significant advantage in relapse-free survival with chemotherapy (p=0.0015). This trial has been interpreted with caution given its early closure and the fact that only 62% of patients randomized to chemotherapy completed the three cycles of treatment. Furthermore, patients in the observation arm were not offered chemotherapy at relapse. Two to three years later, the same

authors reported their longer experience with adjuvant MVAC/MVEC in 83 patients. Forty-nine of the patients had been enrolled in the prospective trial before it was closed, while the remaining 38 had received MVAC/MVEC as a routinely recommended therapy based on the interim results of the trial. Longer follow-up of the patients (38 to 78 months) who were on the trial confirmed significant improvement in PFS in the AC group (p=0.0005). The continued advantage in PFS with more mature data offered support to the beneficial role of chemotherapy. Subsequently, the authors provided complete long-term survival data with a PFS HR of 2.84 (95% CI, 1.46–5.54; p=0.002) for control versus AC and an OS HR of 1.75 (95% CI, 0.95–3.23; p=0.069) with 17.4% versus 26.9% of survival.[367]

The combination of cisplatin, vinblastine, and methotrexate was utilized as adjuvant therapy in a prospective randomized trial of four cycles of CMV versus observation following cystectomy at Stanford University.[368] Patients accrued to this trial had pT3b and pT4 TCC with or without lymph node involvement. Data were reported on 50 out of 55 enrolled patients. Twenty-two out of twenty-five patients randomized to adjuvant therapy received the total number of four planned cycles. With a median follow-up of 62 months, a significant difference in freedom from progression was noted between the chemotherapy and the observation group (median of 37 months vs. 12 months, respectively; p=0.01). No significant difference in OS was noted.

6.5.2.3 History of meta-analysis and composite analysis

In 2006, an analysis was published of both composite trials and a meta-analysis of individual trials of patients who were treated in AC trials compared to observation studies that were published before September 2004. The analysis, based on individual patient data, was limited in its ability to be definitive in most eyes, with only 491 patients from six RCTs included. The overall HR for survival of 0.75 (95% CI, 0.60-0.96; p=0.019) suggested a 25% relative reduction in the risk of death for chemotherapy compared to controls.[357] The authors commented on the small number of patients and relative poor quality of data going into the meta-analysis and highlighted the need for accrual to ongoing phase 3 trials examining adjuvant therapy.

Contemporaneously, Dr. Ruggeri and colleagues undertook a composite analysis from published data from all phase 3 studies of AC published.[356] While less stringent than the Cochrane review, the conclusions were similar, with a benefit to AC for OS (RR, 0.74; 95% CI, 0.62-0.88; p=0.001) and DFS (RR, 0.65; 95% CI, 0.54-0.78; p<0.001).

Concurrently, investigators have begun to compare different adjuvant regimens. Investigators at MD Anderson presented data comparing three cycles of preoperative chemotherapy with MVAC and three afterwards with the same chemotherapy given, only postoperatively[299] in a group of patients at high risk for extravesical extension or nodal involvement at surgery. This study demonstrated a high likelihood of extravesical extension or nodal involvement in nearly 80% of patients treated with initial surgery, and no difference in survival whether the chemotherapy was given in the neoadjuvant or adjuvant setting. While there was no difference between the two approaches in terms of outcome, it did demonstrate the feasibility of preoperative chemotherapy and, in particular, that such therapy did not result in deterioration or toxicity so that the patient had delayed surgery or missed it altogether. The German Urologic Oncology groups ran a phase 3 trial for patients with stage pT3a-4a and/or pathological node-positive transitional-cell carcinoma of the bladder after RC, randomizing

327 patients to either cisplatin and methotrexate (CM) or MVEC.[369] The 5-year PFS, tumour-specific survival, and OS rates were not significantly different between the two arms, although patients given MVEC had higher rates of grade 3 or 4 leukopenia (22%) than those given CM (7%, p=0.0001).

The meta-analysis and composite analysis represent a watershed in perioperative chemotherapy for bladder cancer, in part because they suggested benefit from chemotherapy but highlighted the relative poor quality of the trials undertaken in the area. The advent of phase 3 evidence for neoadjuvant MVAC at around this time also shaped thinking, with neoadjuvant therapy becoming a standard of care. Despite this, most patients were not being offered chemotherapy before surgery. When presented with the all-too-common scenario of upstaging and/or more definitive evidence of risk of relapse in the surgical pathology report, many clinicians and patients considered postoperative treatment despite deficiencies in evidence to support this approach.

6.5.2.4 Recent phase 3 trials

Three major phase 3 trials have finished accrual and were presented, while the results of the fourth trial, CALGB 90104, are still unpublished. They share a common theme: AC for transitional cell predominant urothelial cancer coupled with the acrimony of early closure for slow accrual.

The CALGB 90104 trial saw patients with TCC of the bladder treated with cystectomy and with a creatinine clearance >60 mL/min randomized to either a rapid-cycling regimen of chemotherapy with four cycles doxorubicin-gemcitabine given at 14-day intervals with granulocyte colony stimulating factor support followed by four cycles of paclitaxel-cisplatin given at 21-day intervals compared to adjuvant gemcitabine with cisplatin in a 4-week cycle.[370] Up to four cycles of 4 weeks each of each regimen were administered. Accrued patients were stratified based on pathological criteria according to primary tumour status (<T4 vs. T4), number of positive lymph nodes (0 or unknown vs. 1–5 versus >5), and number of dissected nodes (0–10 or unknown vs. >10). Patients commenced chemotherapy no earlier than 42 days and no more than 3 months after surgery. The accrual target was 800 patients; however, the study was halted in less than 2 years after opening, due to slow accrual, with fewer than 100 patients enrolled. Subsequent analysis of MSKCC data from studies used to develop the rapid-cycling regimen in more advanced disease, which also incorporated ifosfamide, suggested issues with toxicity in the accelerated therapy arm that was also an issue in this trial.[371] In retrospect, CALGB 90104 may have been overly ambitious in attempting to move a dose-dense regimen forward in the same step as the integration of newer agents. Published results from this trial are still awaited, while the study has been completed.[370]

The Italian multicentric trial saw 194 patients with pT2G3, pT3-4, N0-2 transitional cell bladder carcinoma treated with RC and then randomised to immediate chemotherapy (n=102) or a control (n=92) arm of observation and chemotherapy at relapse.[346] Patients were stratified by centre and lymph node metastases. Those patients given AC were randomized to two slightly different schedules of GC over a 4-week cycle given for four cycles. The primary endpoint was OS. Median follow-up was 35 months, and the 5-year OS of the whole series was 48.5% (SE 4.2%) and 53.7% for the control group versus 43.4% in the immediate chemotherapy arm (p=0.34). The 5-year DFS was 39.5% (SE 3.9%) for the whole cohort, 42.3% (control) and 37.2% (chemotherapy; p=0.70). There was no difference between the two GC regimens. The study recruited a relatively high proportion of patients with lymph node–negative disease (pN0: 49,4%).[346] The outcome data from this trial ran counter to prior

experience, where DFS has been increased on chemotherapy arms. The authors concluded that there was no role for AC after cystectomy for locally advanced bladder cancer, but that the trial has a high chance to be falsely negative as only one-third of the planned patients were accrued.[346]

The Spanish Urologic Oncology Group opened the 99/01 trial comparing four cycles of paclitaxel, cisplatin, and gemcitabine (PCG) to observation.[348] This regimen was used based on the results of a phase 1/2 trial in the advanced disease setting.[372] In a subsequent phase 3 trial in advanced urothelial cancer, the addition of paclitaxel increased the efficacy in terms of RR when compared to GC, with a benefit in OS seen only in patients having the bladder as primary origin of the tumour (*post hoc* analysis). In the intention-to-treat population, only a trend of prolonged OS was observed (p=0.075), with a 14% reduction in the risk of death.[373] The adjuvant 99/01 trial accrued patients with pT3-4 and/or lymph node–positive bladder cancer with a creatinine clearance >50 mL/min and mandated chemotherapy commencement within 8 weeks of cystectomy, whereas prior studies had allowed up to 12 weeks after surgery. The trial enrolled 142 patients between July 2000 and July 2007, when it was closed early due to poor accrual. Toxicity in the triple drug chemotherapy arm was acceptable, with a single treatment-related death due to sepsis. At a median follow-up of 30 months, OS was significantly prolonged in the PCG arm (n=68; median not reached; 5-year OS: 60%) compared to observation (n=74; median 26 months; 5-year OS: 31%) (p<0.0009). DFS (p<0.0001) and disease-specific survival (p<0.0002) were also superior in the PCG arm. The final results in full publication are still awaited. The results from this trial raise several questions:

- Does the addition of paclitaxel to PCG increase the efficacy in this setting compared to GC or MVAC, when data for the addition of paclitaxel to GC in the advanced setting only showed a benefit (post hoc) in patients with bladder as primary origin?[374]
- Did the stringency of time to commencement of chemotherapy (8 weeks postoperatively) in this trial contribute to the difference seen in OS?
- Given data from several centres suggesting diminished survival outcomes in patients with later commencement of chemotherapy compared to those starting earlier,[299] should clinical trials and clinical practice outside trials mandate commencement within 8 weeks of surgery?

The EORTC-30994 is a trial of immediate versus deferred chemotherapy. The trial accrued patients with pT3-4 and/or lymph node–positive transitional cell bladder cancer and with a creatinine clearance >60 mL/min to either four cycles of cisplatin-based combination of chemotherapy of physician preference (GC, MVAC, or DD-MVAC[375]) or chemotherapy with the same regimen at first relapse.[349] Both standard MVAC and GC were given on a 4-week cycle, whereas DD-MVAC was given every 2 weeks. Patients were stratified for institution, pT category, and lymph node status according to the number of nodes dissected. Those patients randomised to the chemotherapy arm were required to start treatment within 90 days of surgery. The study was unique in allowing a range of chemotherapy regimens, including a more intense delivery of cytotoxic drug with DD-MVAC. The accrual target was 660 patients, but this trial recruited from April 2002 to August 2008 and was also closed prematurely due to slow accrual after 284 patients. Median follow-up of the trial was 7.0 years. The primary endpoint of OS was not improved in the immediate treatment group compared to the deferred group (HR, 0.78; 95% CI, 0.56–1.08; p=0.13) with a median OS of 6.74 years (95% CI, 3.85–not reached) and 4.60 years (2.15–6.25), which corresponds to an OS rate of 53.6% (immediate) and 47.7% (deferred).

PFS was significantly prolonged on the immediate group compared to the deferred treatment (HR, 0.54; 95% CI, 0.4–0.73; $p<0.0001$), with a 5-year PFS of 47.6% (95% CI, 38.8–55.9) in the immediate treatment group and 31.8% (24.2–39.6) in the deferred treatment group.[349] In the subgroup analysis, lymph node involvement demonstrated a significant interaction with OS, suggesting that patients with node negative disease ($n=86$ of 284 patients, 30%) had the most prominent benefit from immediate chemotherapy, and not patients with lymph node–positive disease, in contradiction to the general belief. Here, 5-year OS in the pN0 group was 79.5% for immediate versus 59.0% in the deferred treatment group ($p=0.012$), while there was no significant interaction in patients with positive lymph node status. Sixty percent of patients (52 of 86) with pN0 had fewer than 15 lymph nodes dissected during cystectomy, suggesting that the chance of understaging due to missed lymph node metastasis was relatively high.

The EORTC-30994 publication incorporated these results in a literature-based meta-analysis, extending the results of the recent meta-analysis by Leow *et al.* and noting a benefit of immediate treatment on OS (HR, 0.77; 95% CI, 0.65–0.91; $p=0.002$). Nevertheless, data obtained are based on published HR rather than on individual patient data and suffer from a substantial heterogeneity[347,349] (**Figure 6–4 and Figure 6–5**).

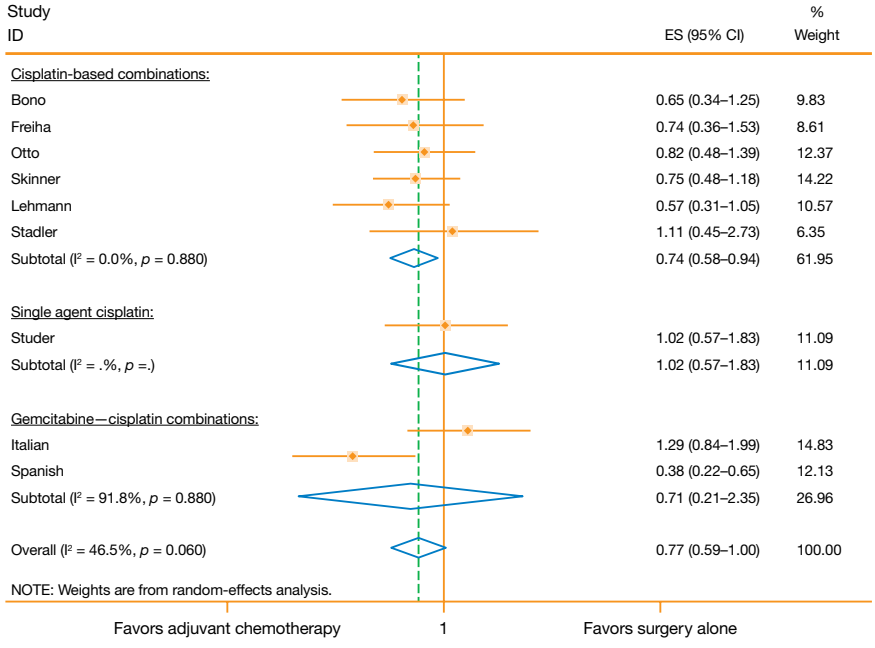


FIGURE 6–4
Hazard Ratios for Overall Survival in Bladder Patients Treated With Adjuvant Chemotherapy or Observation That Were Included in the 2014 Meta-Analysis[347]

Abbreviations: CI, confidence interval.

Leow JJ, Martin-Doyle W, Rajagopal PS, et al. *Adjuvant chemotherapy for invasive bladder cancer: a 2013 updated systematic review and meta-analysis of randomized trials.* Eur Urol. *2014;66(1):42–54.*

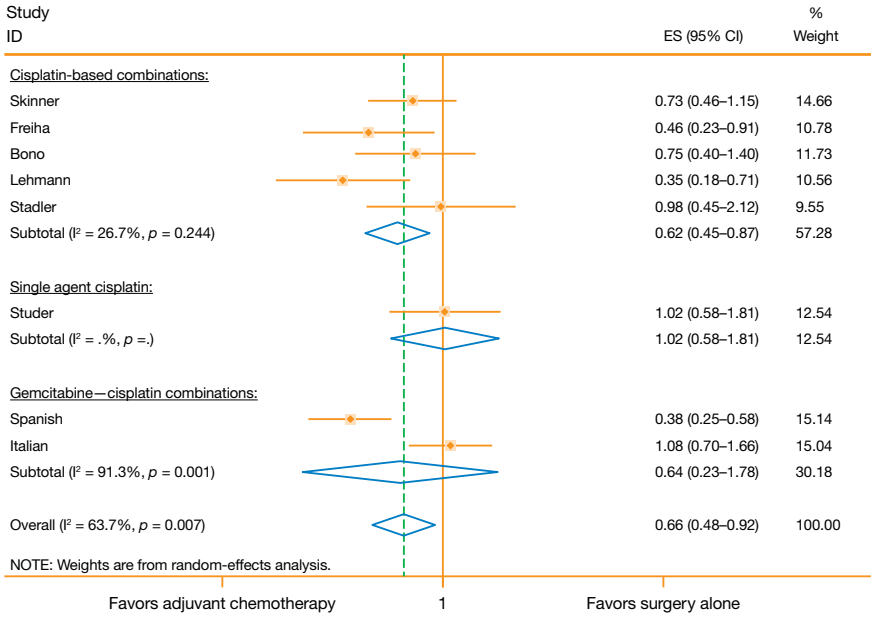


FIGURE 6–5
Hazard Ratios for Disease-Free Survival in Bladder Patients Treated With Adjuvant Chemotherapy or Observation That Were Included in the 2014 Meta-Analysis[347]

Abbreviations: CI, confidence interval.

Leow JJ, Martin-Doyle W, Rajagopal PS, et al. *Adjuvant chemotherapy for invasive bladder cancer: a 2013 updated systematic review and meta-analysis of randomized trials.* Eur Urol. *2014;66(1):42–54.*

Although the meta-analysis of Leow *et al.* did not incorporate the latest results of EORTC-30994, it provides data on 945 patients included in nine RCTs. The above-mentioned meta-analysis included three new trials and updated one compared to the recent Advanced Bladder Cancer Meta-analysis, Collaboration in 2006.[347,357] HR for OS in favour for AC was similar to that reported above, with a value of 0.77 (95% CI, 0.59–0.99; p=0.044), and DFS was improved by 34% (HR, 0.66; 95% CI, 0.48–0.92; p=0.014). In the sensitivity analysis, this meta-analysis demonstrated heterogeneity in outcome due to different ratios of pN1+ versus pN0 patients between the trials. After stratification of studies by nodal ratio, the observed heterogeneity was corrected. Interestingly, the HR for DFS was more favourable in studies with a higher nodal involvement (HR, 0.39; 95% CI, 0.28–0.54) compared to a HR of 0.89 (95% CI, 0.69–1.15) in studies with less nodal involvement. This is in contrast to the results of the EORTC-30994 trial, in which patients with pN0 had the most prominent effect from immediate chemotherapy. Therefore, the risk group of patients who will have the greatest benefit of AC still remains to be defined.

6.5.2.5 Data from larger cohort studies

A recent collaborative effort between 11 major centres has yielded an international cohort analysis of off-trial AC.[358] Patients were grouped into quintiles based on risk characteristics for relapse and death and chemotherapy impact assessed across the cohort as a whole, but also within each risk segment. The cohort consisted of 3,947 patients undergoing cystectomy and LND between 1979 and 2008, 932 (23.6%) of whom received AC, the largest analysis of an AC cohort to date. AC was independently associated with improved survival (HR, 0.83; 95% CI, 0.72%-0.97%; p=0.017). Significantly, risk group predicated the survival impact of chemotherapy on outcome. Increasing benefit from AC was seen across higher-risk subgroups (p<0.001), especially in those with extravesical extension or nodal involvement. There was a significant improvement in survival between the treated and nontreated patients in the highest-risk quintile (HR, 0.75; 95% CI, 0.62-0.90; p=0.002). This group

was characterized by an estimated 32.8% 5-year probability of CSS, with 86.6% of patients having both stage T3 or greater and nodal involvement. These data may be useful in stratifying and selecting patients for future studies.

Analysis from the same group of investigators suggests that 2- and 3-year DFS after cystectomy is a strong surrogate for 5-year OS.[265] This surrogacy needs to be tested in prospective cohorts treated with AC, but may be key in planning studies with an initial phase 2 accrual in the adjuvant setting before expanding to a larger phase 3 cohort if DFS and toxicity endpoints are met.

Galsky *et al.* used data from NCDB to investigate the benefit associated with AC in patients with pT3–4 and/or lymph node–positive bladder cancer. A total of 5,653 patients treated between 2003 and 2006 were identified, of whom 23% had received AC. The authors used propensity scores, and found an improvement in OS with the use of AC compared with no chemotherapy (HR, 0.70; 95% CI, 0.64–0.76).[376]

6.5.2.6 Role of adjuvant chemotherapy in patients who already had neoadjuvant chemotherapy

The studies mentioned earlier evaluated patients who underwent surgery with curative intent, followed by AC. None of these patients underwent NAC prior to surgery. Evaluating the role of AC, after receipt of NAC followed by surgery, is under-investigated at this current juncture, with no randomized trials conducted. A small observational study (n=161) attempted to evaluate this and found that the median RFS was 17.5 months in the AC compared to 13.7 months in the non-AC group (p=0.78).[377] After adjusting for pT, pN, and margin status, receipt of AC remained an insignificant predictor for RFS (HR, 0.89; 95% CI, 0.48-1.68). CSS was similar (23 vs. 22 months; p=0.65) and remained insignificant after adjusting for pathological confounders.

Recently, Seisen *et al.* used the NCDB to further evaluate this on a larger scale, identifying 788 patients with pT3/4 and/or pN+ disease, all of whom received NAC followed by RC.[247] Of these, only 23% received AC after RC. With a median follow-up of nearly 4 years, the authors found that those who also received AC had improved 5-year OS rates (36.8%) compared with those who did not (24.7%), with a significant OS benefit shown on propensity-weighted Cox proportional hazards regression (HR, 0.48; 95% CI, 0.61-0.99; p=0.046). This may represent sufficient preliminary evidence to garner support for a randomized trial to determine if patients, particularly those with adverse pathological features, may benefit from further AC after surgery.

6.5.2.7 Biomarkers and other indicators of potential adjuvant chemotherapy benefit

The literature is beset with multiple analyses of individual markers of outcome after cystectomy. p53 aberrance, cycle cell gene dysregulation, and presence of lymphovascular invasion identify patients with low-risk (<pT2) disease who are at heightened risk of relapse.[257,378,379] Recent attempts have been made to link these markers with systemic therapy interventions. In the p53 MVAC trial, patients were screened for p53 abrogation and randomized to either three cycles of MVAC or observation.[380] The trial was closed due to futility contingent upon slow accrual and low event rate. The final analysis did not demonstrate an advantage for MVAC chemotherapy in patients whose tumours contained abnormal p53; in fact, those patients had a nonsignificant trend to a higher relapse and death with

chemotherapy. This result proved disappointing and once again highlighted the difficulty of running trials at the adjuvant interface in bladder cancer. Current biomarker efforts led by the International Bladder Cancer Consortium have been directed at large-scale tissue microarray construction and analysis for putative markers of chemotherapy response such as ERCC1 (platinum drugs),[381-384] ribonucleotide reductase (gemcitabine),[385,386] topoisomerase II (doxorubicin, epirubicin),[387,388] and beta-tubulin (taxanes).[386,389,390] Hopefully these studies will delineate relationships between markers and therapies as well as defining magnitude of effect to help power those studies. Targeted monoclonal and small molecule agents remain of interest in urothelial cancer and a focus of studies that will attempt to treat patients that have the target present in their tumour and therefore are more likely to respond.

Immune checkpoint inhibitors demonstrated an OS advantage in second-line treatment of metastatic urothelial bladder cancer[391] and are currently being explored in the adjuvant setting in three large phase 3 trials with nivolumab versus placebo (CheckMate 274, ClinicalTrials.gov number: NCT02632409), atezolizumab versus observation (IMvigor010, ClinicalTrials.gov number: NCT02450331), and pembrolizumab versus observation (AMBASSADOR, ClinicalTrials.gov number: NCT03244384).

6.5.2.8 Summary

The body of evidence supports the use of perioperative chemotherapy. However, the best evidence is for neoadjuvant rather than adjuvant therapy. Several studies have suggested a 5% to 15% absolute advantage for chemotherapy in the postoperative setting, supported by the most updated meta-analysis by Leow *et al.*[347] Given the small incremental benefit to AC, the demonstration of a survival advantage may take a trial with several thousand patients, unless patients accrued can be stratified for risk to benefit by clinical or pathological parameters and/or biomarkers predictive of relapse risk and/or chemotherapy benefit. The optimal timing and intensity of chemotherapy in the adjuvant setting remains to be determined. Accrual to trials of adjuvant therapy in urothelial cancer represents a major challenge, but might be overcome by the currently ongoing phase 3 Immune checkpoint inhibitor trials, which are likely to be fully recruited.

6.5.2.9 Recommendations

- Adjuvant cisplatin-based chemotherapy is supported by a recent large cohort analysis **[LOE 2]**, several randomised clinical trials **[LOE 1]**, and the results of two meta-analysis and composite analysis of randomized trials **[LOE 1]**. However, the trials used in meta-analyses were flawed, mainly due to poor recruitment and early termination, and so make definitive conclusions difficult. On that basis, the group provides a **GOR B** for adjuvant cisplatin-based chemotherapy in the patient with pT3/4 and/or lymph node–positive cancer at cystectomy who has not had NAC and is medically fit.
- Adjuvant regimens not containing cisplatin (including those containing carboplatin) should not be routinely used outside of clinical trials because of a lack of evidence for their benefit in that setting [**LOE 2; GOR C**]. Patients who cannot tolerate cisplatin-based combination therapy should be included in the clinical trials or observed.
- Until the current equipoise is resolved for AC in this setting, clinical trial remains the best choice for patients with locally advanced bladder cancer [**LOE 3; GOR C**].

6.6 Prognostic and Predictive Biomarkers: Current and Future Applications

Recent advances in molecular biology and immunology have set the framework for the discovery and validation of putative biomarkers that may have prognostic and/or predictive value. This is even more relevant and urgent in the era of personalized/individualized medicine, in which we attempt to select the right treatment for the right patient at the right time. This concept of customized therapy selection using properly validated biomarkers may help improve outcomes. However, the development of clinically useful biomarkers is very difficult and is considered the Holy Grail of modern medicine. A biomarker needs to prove not only analytical and clinical validity, but also clinical utility (**Table 6–12**). An important distinction should be made between a *prognostic* biomarker (estimation of outcome regardless of selected therapy) versus a *predictive* biomarker (estimation of response/benefit to a particular therapy). The former can aid more in overall prognostication and the latter in specific treatment selection. Both can contribute to clinical decision-making, as well as clinical trial eligibility and stratification.

TABLE 6–12 Features of Biomarkers

Analytical validity	Can the used assay detect and accurately measure the biomarker of interest?
Clinical validity (biological relevance)	Is the biomarker associated with a disease or outcome or response to treatment? What is the clinical performance?
Clinical utility	Does the use of the biomarker improve diagnosis or outcomes?

Systemic administration of perioperative chemotherapy for MIBC may result in substantial overtreatment in a number of patients who could be cured by RC and PLND alone. In addition, it may lead to adverse outcomes in chemo-resistant individuals by delaying curative surgery and/or adding unnecessary toxicity. Based on these observations, several biomarkers attempting to predict response to perioperative chemotherapy have been explored, aiming to improve patient selection for such treatments. Biomarkers can be classified as clinical (e.g. age, stage, performance status, hydronephrosis, smoking status, etc.), pathological (e.g. grade, histological subtype, lymphovascular invasion, CIS, surgical margins, etc.), and molecular (e.g. genomic, transcriptomic/gene expression, epigenetic, proteomic, protein expression, etc.)

Several early candidate markers have been tested in cohorts of patients treated with NAC. These are summarized below:

- p53 aberrancy, as inferred by immunohistochemistry overexpression, confers a poor prognosis in muscle-invasive bladder urothelial carcinoma.[378,392] In one report of 90 patients undergoing neoadjuvant MVAC chemotherapy, patients with mutant p53 were three times more likely to die from their disease than those with wild-type p53.[392] The impact of p53 overexpression on survival was predominantly in T2 and T3a tumours. Conversely, a retrospective analysis of data suggested that AC enhanced survival in patients with p53 mutant tumours.[393] However, neither the prognostic nor the predictive role (in response to AC) of p53 protein was confirmed in a prospective phase 3 trial.[394]
- Ki67, p53, and angiogenesis (microvessel staining with CD34) were assessed by immunohistochemistry in 94 patients who accrued to the SWOG 8710 phase 3 neoadjuvant MVAC trial.5,395 There was a trend toward shorter DFS and OS in patients with higher Ki67 expression. Increased p53 nuclear expression was associated with a shorter OS, but this was not statistically significant. No association or trend between microvessel density and outcome parameters was noted. The study was very limited in power, given the small number of specimens available.
- BRCA1 mRNA expression was analyzed by quantitative PCR in tumour biopsies obtained by TUR from 51 patients with locally advanced bladder cancer receiving NAC. A close correlation was found between BRCA1 mRNA levels and pathological response. Low levels of BRCA1 were shown to predict response to neoadjuvant cisplatin-based chemotherapy and correlated with longer DFS.[396]
- *In vitro,* XAF1 expression enhances the apoptotic response of tumour cells to chemotherapeutic agents. In vivo, in a paired sample study from 14 bladder cancer patients treated with a combination of neoadjuvant GC, patients with high levels of XAF1 in their tumour had increased rates of response, PFS, and OS.[397]

Genomic approaches to predict response to NAC have been tested in various forms. Takata *et al.*,[398] in a retrospective study of patients with invasive bladder cancer who received neoadjuvant MVAC chemotherapy, found that 14 predictive genes separated the responder group (defined as no residual muscle invasive disease) from the nonresponder group. This system accurately predicted the drug responses of eight out of nine test cases. To further validate the clinical significance of the system, the investigators applied it to 22 additional cases of patients with bladder cancer and found that the scoring system correctly predicted clinical response for 19 of the 22 test cases.[399]

A novel gene expression strategy is commonly referred to as the COXEN model.[400,401] In this model, the National Cancer Institute (NCI)-60 panel of 60 cancer cell lines from 9 common tumour types was used to generate gene expression signatures, which were correlated to the half maximal inhibitory concentration (IC50) values of a large catalogue of approved drugs. To make the model more applicable to bladder cancer, a panel of bladder cancer cell lines were integrated into the model. In order to predict an individual patient's response to a specific combination chemotherapy, the gene expression of the patient tumour is compared to the cell line gene expression signatures that predict response to that combination chemotherapy. This model has been tested in several retrospective patient cohorts and is currently being evaluated prospectively in a cooperative group trial (S1314, "COXEN Trial," ClinicalTrials.gov number: NCT02177695), which completed accrual in December 2017. This trial

randomized patients to MVAC or GC NAC. It is not powered to determine a difference between the different regimens, but instead to test the ability of the COXEN model and other candidate predictive markers to predict response to the two different chemotherapy regimens.

Gene expression may also be important for prediction of response to NAC in the context of molecular subtypes. There has been a number of such RNA-based classifications with significant but not complete overlap.[402-405] The most recent comprehensive integrative molecular analysis of The Cancer Genome Atlas (TCGA)[406] reported five distinct molecular subtypes correlating with outcomes, and it suggested potential future treatment selection based on the molecular profile.

Choi *et al.* identified a *p53*-like molecular subtype, mostly within luminal tumours, that was associated with reduced sensitivity to cisplatin-based NAC.[405,407] In contrast, basal tumours with high-proliferative phenotype can respond better to cisplatin-based NAC. The data were corroborated by a recent study using transcriptome microarray analysis of tumour tissue obtained before NAC and subsequently developed a single-sample genomic subtyping classifier.[53] Patients with basal tumours had higher response to NAC, suggesting that the predictive role of gene expression profiling certainly merits further validation in this setting.

Comprehensive evaluation of genomic alterations may represent an additional strategy for optimal selection of patients for NAC. Elegant *in vitro* analyses showed that missense mutations of ERCC2, a nucleotide excision repair gene, based on exome sequencing, predicted response to cisplatin-based NAC.[408] Whole-exome sequencing on pretreatment tumour tissue and germline DNA from 50 patients with MIBC who got NAC prior to RC confirmed the hypothesis that somatic ERCC2 mutations correlate with CR to cisplatin-based chemotherapy (9).[409] In addition, mutations in ERBB2/human epidermal growth factor receptor 2 (HER2) were shown to correlate with favourable response to NAC.[409] Also, aberrations in DNA repair genes (ATM, RB1, or FANCC) were found to predict pathological response to cisplatin-based NAC and were associated with longer OS in those patients.[410]

Immunohistochemistry-based studies suggested that protein-expression biomarkers may predict response to NAC. For example, bladder expression of the transcription factor NrF2 was shown to correlate with resistance to cisplatin *in vitro* and shorter OS in patients who received NAC.[411] Similarly, bladder overexpression of an inhibitor of the apoptotic cascade (Bcl-2) correlated with lack of response to NAC.[412] Moreover, the expression of GDPD3 and SPRED1 was also shown to correlate with response rates to NAC.[413]

Another study reported higher intracellular platinum concentration in cystectomy biospecimens with pCR after NAC for MIBC compared to cases with residual tumour, suggesting that increased platinum accumulation may affect chemosensitivity.[414] The authors suggested that factors modulating intracellular platinum concentration, e.g. expression of transporters, warrant further assessment as putative predictive biomarkers of response to cisplatin-based NAC. Interestingly, another study did show a strong correlation between tumour expression of copper transporter receptor 1, which plays an important role in platinum uptake, and pathological outcome in platinum-treated MIBC.[415]

Overall, there is significant interest for ongoing assessment of molecular biomarkers that could be predictive of response to NAC. However, with a few exceptions, most studies include heterogeneous and relatively limited sample size populations, and none of the aforementioned molecular biomarkers are used in routine clinical practice. A number of currently designed clinical trials (either within or outside the cooperative research group setting) are using molecular biomarkers to prospectively allocate patients to specific therapies based on thorough molecular profiling, recapitulating the concept being tested in the NCI-MATCH (ClinicalTrials.gov number: NCT02465060) and other similar trials. Moreover, the putative predictive role of several biomarkers, e.g. tumour mutational load, gene expression profiling, protein expression, DNA repair gene mutations, homologous recombination deficiency, microsatellite instability, loss of heterozygosity, and others, in regard to response to immunotherapy-based approaches, is also being tested in the clinical setting. It is conceivable that a composite panel of relevant predictive biomarkers may be available upon validation to assist in the optimal patient selection for particular therapies in the future.

6.6.1 Recommendations

Due to the low levels of evidence **[LOE 4]**, biomarkers are currently not recommended for determining prognosis or predicting response to treatment in patients with bladder cancer **[GOR D]**.

6.7 Bladder-sparing Treatments for Localized Disease

6.7.1 Transurethral resection of the bladder and partial cystectomy with or without multimodal therapy

6.7.1.1 Level of evidence reviewed

To date, no randomized studies have been performed comparing TUR or partial cystectomy (PC) of invasive (TNM stages T2-T4 N0Mx) bladder cancer as monotherapy to other standard of care modalities such as RC or combined modality therapy. The literature on this subject consists of a few carefully performed clinical trials that are observational in nature; these studies are generally comparative, nonrandomized, and uncontrolled clinical experiences and are consistent, at best, with **LOE 2; GOR B**.

6.7.1.2 TUR monotherapy

6.7.1.2.1 Indications and patient selection

TUR monotherapy is appropriate for the treatment of patients with T2-T3 N0Mx bladder cancer in whom local endoscopic resection is likely to produce complete removal of the tumour exclusive of concomitant noninvasive disease (i.e., CIS). Patients most appropriate for this approach have tumours that: 1) are small, 2) are completely resectable, 3) have negative tumour bed and periphery biopsies, 4) are not associated with upper tract compromise (i.e. hydronephrosis), and 5) are not associated with radiographic evidence of locoregional extension of disease (T3b, N+) at the time of first treatment

6.7.1.2.2 Surgical technique

The essential components of a successful TUR of invasive bladder cancer is complete resection (R0) of all visible tumour, including extension into perivesical fat if necessary, and negative confirmatory biopsies of the base and periphery of the resection bed.[416] A continuous flow resectoscope should be used to ensure low intravesical pressure. Techniques to minimize obturator reflex include minimizing bladder distension, use of general anesthesia with neuromuscular blockade, and reducing the diathermy cutting current.[417] If tumour is involving the ureteral orifice, resection of the orifice may be performed with an acceptable complication rate (hydronephrosis 13%, iatrogenic stricture 4%).[418] Proper technique of resecting the ureteral orifice involves using a pure cutting current followed by selective pinpoint coagulation. A temporary ureteral stent can generally be avoided.

6.7.1.2.3 Outcomes

TUR monotherapy of invasive bladder cancers has been discussed in the urological literature since the 1940s.[419] Multiple groups evaluated the utility of this approach either in isolation or in combination with systemically administered polychemotherapy.[420-422] Comparing TUR to other standards of the day, including RC, radiation monotherapy, and combination therapy, Henry *et al* observed that the 5-year survival rate for patients with T2 tumours treated by TUR only was 63%.[423] In 1998, Solsona and coworkers reported the results of a comparative, nonrandomized study of 133 patients with T2-3 bladder cancer treated by TUR. The clinical course of these patients was compared to a concurrent group of patients treated by RC in the same centre.[424] These investigators reported that,

for patients with negative TUR bed biopsies following initial TUR, disease-specific survival was equivalent to that observed in the cystectomy group. Furthermore, patients in the TUR group with negative muscle biopsies but with CIS were found to respond favourably to intravesical therapy. Herr *et al* reported on a similar experience in 155 patients treated at a single institution with 10-year follow-up.[425] Investigators from MD Anderson Cancer Center reported that while TUR as monotherapy was effective in appropriately selected individuals with invasive bladder tumours, this approach was only applicable to approximately 11% of the patients at their centre presenting with invasive bladder cancers.[426] Recently, the Valencia group has updated its experience with 15-year follow-up data. This most recent report supports the authors' contention that, in their hands at least, TUR monotherapy produces high rates of disease-specific survival across all age groups treated.[427]

6.7.1.3 TUR in multimodal treatment

An R0 TUR is the essential first step of successful trimodality therapy. When compared to patients having an incomplete TUR upon starting trimodality therapy, an R0 TUR is associated with higher CR rate (84% vs. 58%; $p<0.001$), lower salvage cystectomy rate (24% vs. 43%; $p<0.001$), and better OS (57% vs. 43% 5-year OS; $p=0.003$) and DSS (68% vs. 56% 5-year DSS; $p=0.03$).[44,428] Consideration of performing a second TUR prior to initiating chemotherapy and RT is also recommended, since it is associated with better DSS (69% vs. 42% 5-year DSS, $p=0.046$).[429]

6.7.1.4 Partial cystectomy as monotherapy

6.7.1.4.1 Indications and patient selection

PC is an alternative form of therapy for muscle-invasive disease that may be used in a highly selected cohort of patients.[430] A very small subset of patients (<5%) presenting with muscle invasive urothelial cancer will be eligible for PC when applying strict criteria. The principal limiting factor is tumour location, which should be high on the dome or anterior wall, away from the bladder neck. Tumours on the posterior or lateral walls may also be treated with PC if anatomically accessible.[431,432] Tumours should be primary rather than recurrent, and there should be no CIS on bladder biopsies.

6.7.1.4.2 Surgical technique

A 2-cm circumferential margin of normal mucosa is recommended and tumours should be 3 cm or less in diameter. Partial cystectomy (PC) may also be used in patients with a tumour in a diverticulum in sites other than the dome and may require ureteral reimplantation in select patients.[433]

A few small case series also report the technical aspects and safety of laparoscopic/robotic PC for urothelial cancer and the use of cystoscopy for tumour localization and initial identification of resection margins [**LOE 4C; GOR C**].[434,435] Golombos *et al.* recently described the Weill Cornell Medical College technique for robotic-assisted PC.[436] After the peritoneum is insufflated and trocars are placed, the border of resection is marked on the exterior bladder surface using electrocautery, which is guided by light from a cystoscope. The bladder is then mobilized and subsequently incised away from the tumour site, taking care to avoid direct tumour manipulation. The tumour is then excised en bloc and placed in an Endo Catch™ bag. If the margin of resection involves the ureteral orifice and/or distal ureter, a ureteral reimplantation is performed. The bladder is then closed in two layers and a bilateral PLND is completed.

6.7.1.4.3 Outcomes

Three recent papers describe the contemporary experiences from MSKCC, MD Anderson Cancer Center, and Mayo Clinic.[431,433,437] The MSKCC series described 58 patients with primary nonurachal bladder cancer treated from 1995 to 2001.[433] This represented 6.2% of patients presenting for surgical therapy. All but five patients had a unilateral or bilateral pelvic lymphadenectomy, and nine patients had node metastases. A total of seven patients had tumour in a diverticulum. Of five patients with a final positive margin, three had negative intraoperative frozen sections of the margins, presumably due to sampling error. On univariate analysis, CIS and multifocality were associated with nonmuscle invasive recurrence and lymph node metastases and PSMs were associated with advanced recurrence. CIS and node metastases were independent predictors of advanced recurrence. Median follow-up was 31 months and 69% were alive and disease free, while 22% died of disease.

In the MD Anderson series, 37 patients underwent PC for curative intent between 1982 and 2003.[431] All patients had pT2 or pT3 disease and 14% had node metastases. Long-term cancer control was achieved in 65% with an intact bladder, with a median follow-up of 53 months. Nonmuscle invasive recurrences occurred in nine (24%) patients, and all were treated successfully. On multivariate analysis, only pathological tumour stage was associated with RFS.

The Mayo Clinic group performed a comparative survival analysis between patients undergoing PC and a matched RC cohort.[437] There was no difference in 10-year RFS (61% vs. 66%; p=0.63), 10-year OS (36% vs. 36%; p=0.39), or 10-year CSS (58% vs. 63%; p=0.67) between PC and RC groups, respectively. Sixteen out of eighty-six (19%) patients initially treated with PC eventually underwent salvage RC.

Smaldone *et al.* reported a single surgeon series of 25 patients operated on over a 10-year period.[432] Their protocol included 25 Gy of preoperative RT delivered to the abdominal wall in five fractionated doses, intra-operative intravesical thiotepa, and, postoperatively, 6 weeks of intravesical BCG. Preoperative RT and/or intra-operative intravesical chemotherapy have been reported in many series in an effort to minimize the risk of wound implantation, but there is no evidence to support their routine use **[LOE 4C; GOR C]**. Despite strict criteria of cT1 or cT2 disease, 36% were upstaged to pT3 and 12% had node metastases, though only two-thirds of patients underwent a pelvic lymphadenectomy. Five-year RFS and disease-specific survival probabilities were 62% and 84%, respectively, and tumour size was the only variable associated with recurrence. These data support a highly selective use of PC in patients with MIBC **[LOE 3; GOR B]**.

A recent series studied 39 patients treated in a variety of ways, including PC, for tumour in a diverticulum.[438] Thirteen patients demonstrated T2 or greater disease and had a 45% 5-year survival rate. Those patients with Ta and T1 disease had better long-term survival (83% and 72%, respectively).

Several recent studies suggest that PC is over-utilized particular, in nonacademic settings. In a population-based study in Quebec, 30% of patients with invasive bladder cancer underwent PC over a 22-year period.[439] Equally concerning is that only 23% of patients had a pelvic lymphadenectomy and 24% of patients required a salvage RC. Review of data from the Nationwide Inpatient Sample revealed that patients undergoing PC were older and had more comorbidities than those undergoing RC, and complications were more likely to occur at hospitals with lower surgical volume.[440,441]

Hollenbeck *et al.* queried the SEER and National Inpatient Sample databases from 1988 to 2000 and found that, in 2000, PC was still performed in 13% to 17% of patients, and more commonly in rural, nonteaching, low-volume hospitals.[442] More recently, Fedeli and colleagues reviewed the US NCDB from 2003 to 2007 and found a lower utilization that decreased over time from 10% to 7%.[311] Capitanio *et al* reviewed the SEER-9 database from 1988 to 2004 that included 7,243 patients with stages pT1-4N1-2M0 treated with PC (22%) or RC.[443] They performed a matched analysis utilizing pT and pN stage, grade, race, age, and year of surgery, and suggested that the use of PC did not undermine long-term cancer control. These data should be interpreted with caution, as within this same database 24% of patients did not appear to have any node dissection and an additional 18% had only 1 to 5 nodes removed, suggesting that surgical quality was less that optimal regardless of the use of PC or RC.

6.7.1.5 Partial cystectomy after neoadjuvant chemotherapy

PC has been incorporated into bladder-sparing protocols after initial NAC in highly selected patients with localized tumours. Sternberg *et al* reported on 13 patients among 104 treated with a bladder-sparing approach in mind who had PC.[47] The 5-year survival for this select cohort was 69%. In a separate report from MSKCC, 36 patients underwent PC after NAC and restaging TUR.[444] Interestingly, of the 21 patients who were cT0 after NAC, 7 (33%) had residual tumour in PC specimen. Five-year OS was 63%, which is comparable to contemporary RC series. There may also be a role for PC as an alternative to RC in the setting of post-chemoradiation therapy.[445]

6.7.1.6 Recommendations

- TUR monotherapy is an alternative to RC in appropriately selected (see Recommendation 2, below) and counselled patients with T2-T3a N0Mx bladder cancer **[LOE 3; GOR C]**.
- Patients most appropriate for this approach have tumours that **[LOE 3; GOR C]**:
  - Are small
  - Are completely resectable
  - Have negative tumour bed and periphery biopsies
  - Are not associated with upper tract compromise (i.e. hydronephrosis)
  - Are not associated with radiographic evidence of locoregional extension of disease (T3b, N+) at the time of first treatment
- TUR monotherapy should be discussed as part of the informed consent process of patients contemplating management options for invasive bladder tumours (TNM stages T2-3a N0Mx) **[LOE 3; GOR C]**.
- If technically feasible, an R0 resection should be attempted during TUR prior to multimodal therapy, since it is associated with higher CR, decreased need for salvage cystectomy, and more favourable survival **[LOE 2; GOR C]**.
- Highly selected patients with focal invasive cancers and cT0 or minimal residual disease after NAC may be candidates for bladder sparing with either TUR or PC **[LOE 3; GOR C]**.

6.7.2 Radiation-based trimodality treatment strategies

6.7.2.1 Introduction

The treatment options for muscularis propria-invasive bladder tumours can broadly be divided into those that involve removal of the bladder and those that spare it. There is a significant difference among countries in the path of care that is most often used to treat these patients. For instance, in the United States only a minority of the patients are offered radiation therapy. However, in the United Kingdom 60% of eligible patients receive radical radiation therapy, with surgery reserved for failures. The treatment of many other cancers in North America, Europe, and around the world include organ-preserving therapy as the, or one of the, current standards of care for malignancies of the breast, larynx, anus, head and neck, soft tissues (sarcoma), and prostate. In each case, radical surgical extirpation can often be avoided without compromising patient survival. Improved RT techniques combined with an enhanced understanding of the optimal chemotherapeutic regimens have promoted multimodality therapy in selected patients with muscle invading bladder cancer (MIBC) as a viable alternative to radical surgery alone. Cystectomy is effective in achieving local tumour control and patients are often cured by contemporary cystectomy, with major series reporting 5-year pelvic control rates of 80% to 90% and 5-year OS rates from 40% to 60%.[5,66,220,222,446] It is this standard that MIBC treatment with bladder-preserving strategies must meet.

Contemporary radiation-based bladder-sparing therapy algorithms consist of: (1) maximal TURBT, (2) induction EBRT with concurrent chemotherapy, (3) cystoscopic assessment of treatment response with prompt cystectomy for nonresponders, and (4) active cystoscopic surveillance with a cystectomy at the first sign of invasive recurrence (**Figure 6–6**). These algorithms were developed as a result of the lack of adequate local control of MIBC treated by TURBT, by chemotherapy, or by RT when used alone.

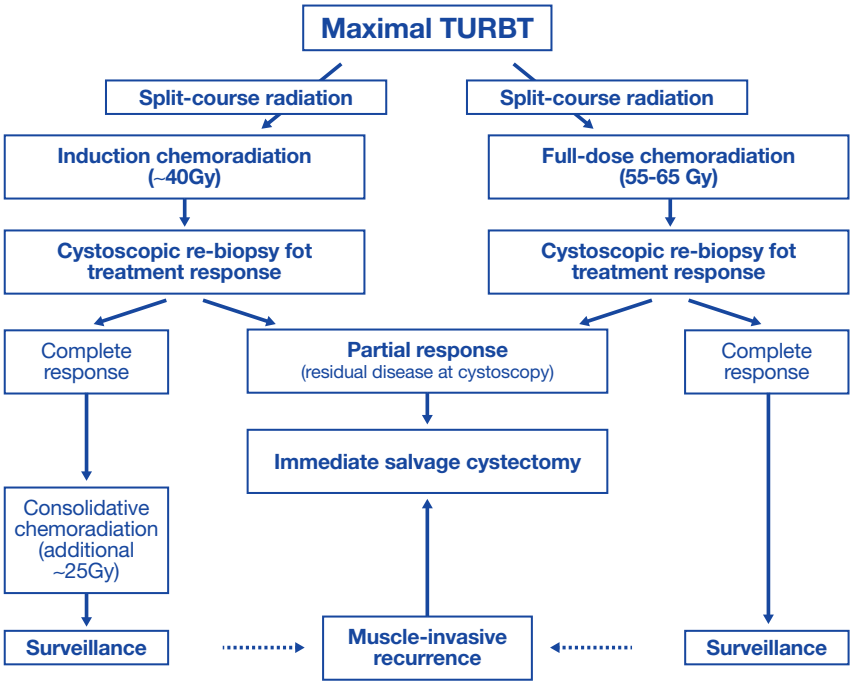


FIGURE 6–6
Current Schema for Trimodality Treatment of Muscle-invasive Bladder Cancer MIBC With Selective Bladder Preservation

Abbreviations: TURBT, transurethral resection of the bladder tumour.

"U" represents intervention by a urologist.

6.7.2.2 External beam radiation alone with salvage cystectomy reserved for tumour recurrence

From the 1960s through the 1980s, the most common type of bladder-sparing treatment was EBRT alone. Since the 1980s, in the United States radiation treatment has generally been reserved for patients judged too unfit for cystectomy on the basis of comorbid conditions or due to disease extent. These negative selection criteria may have contributed to the relatively poor results achieved with radiation therapy alone compared to cystectomy (see **Table 6–13**). Approximately 10% to 15% of patients are excluded from treatment by RC at the time of operation because previously unrecognized unresectable tumour spread is found. Thus in cystectomy series, but not radiation series, some of the patients with advanced local spread tumour are excluded, so this may be another selection bias favouring cystectomy.

TABLE 6–13 Results of Radical Radiation Therapy Alone (Monotherapy): Muscle-invading Bladder Cancer

Study		5-year survival rates		
	Number	T2	T3(+/-T4a)	All Stages
MD Anderson Cancer Center (Slack *et al.*[447])	32		22%	
National Bladder Cancer Group*	35			40%
Edinburgh (Duncan *et al.*[498])	889	40%	26%	36%
London Hospital (Jenkins *et al.*[499])	182	46%	35%	40%
Princess Margaret Hospital (Gospodarowicz *et al.*[500])	121	59%	39%	45%
Danish National Study (Sell *et al.*[449])	95		23%	
Norway (Fossa *et al.*[501])	308	38%	14%	24%
UK Cooperative Group (Horwich *et al.*[451])	91		28%	

* SD Cutler, National Cancer Institute, unpublished observations, 1983.

Modified from Zietman AL, Shipley WU, Kaufman DS. Organ-conserving approaches to muscle-invasive bladder cancer: future alternatives to radical cystectomy. Ann Med. *2000;32(1):34–42.*[69]

In the 1960s, the Cooperative Surgical Adjuvant Bladder Study group randomized 475 patients with stage T2-T4a bladder cancer to preoperative radiation therapy (45 Gy) followed by open surgery or surgery alone.[447] The study was large but compromised by incomplete data collection and follow-up. Of the 138 patients who completed preoperative radiation therapy and surgery on protocol, 34% had a complete pathological response of their bladder tumour (stage pT0). Furthermore, those with a complete pathological response at RC had a survival advantage of 55% versus 32% compared to those who had residual tumour at the time of cystectomy. This led, in the 1970s, to four randomized trials comparing EBRT alone (60 Gy) with RC reserved for local recurrence to the standard group receiving preoperative radiation therapy (40–50 Gy) with immediate RC (see **Table 6–14**).[448-450] Three of these trials showed equivalent OS with either approach. These studies provided Level 1b evidence that a bladder-preserving approach with radiation therapy alone and salvage RC for local recurrence was not significantly different in OS in this "pre-neobladder" era.

TABLE 6–14 Five-Year Survival Data From Four Randomized Trials Comparing Preoperative Radiation Therapy (40–50 Gy) With Immediate Cystectomy to Radiation Therapy Alone (60 Gy) With Salvage Cystectomy for Recurrence

Study	No. of patients	5-year survival with preoperative RT and cystectomy (%)	5-year survival with RT and salvage cystectomy (%)	Statistical significance	Notes
Urologic Cooperative Group, UK (Leone *et al.*[9])	189	39	28	None	
Danish National Cancer Group (Hussain *et al.*[8])	183	29	23	None	
National Bladder Cancer Group*	72	27	49	None	
MD Anderson Cancer Center (Hussain *et al.*[8])	67	45	22	Significant	Large T3 tumours included

Abbreviation: RT, radiation therapy.

** SD Cutler, National Cancer Institute, unpublished observations, 1983.*

Alternative radiation therapy fractionation schemes were explored, including the use of twice-daily radiation with the potential advantage of improved biological effect to better control rapidly proliferating tumours. This also had the practical advantage of a more rapid completion of radiation therapy and thus a shorter interval to salvage cystectomy for the nonresponders. A randomized trial from the United Kingdom studied accelerated (twice-daily) radiation monotherapy in 229 patients with MIBC treated with radiation therapy alone.[451] There was no advantage with the twice-daily schedule over the conventional once-daily radiation schedule, and acute bowel and bladder toxicities were higher. Additionally, the recent results of Radiation Therapy Oncology Group (RTOG) 0712 further support this. Investigators looked at bladder preservation with twice-daily radiation plus 5-flurouracil or cisplatin versus daily radiation plus gemcitabine. Preliminary results have demonstrated comparable rates of distant metastases, CR, bladder preservation, and toxicities among the two treatment arms.[452] The biological rationale underlying accelerated fractionation radiation therapy in bladder cancer therefore remains unproven. Thus, off-protocol once–daily radiation treatments remain reasonable.

6.7.2.3 External beam radiation therapy combined with other modalities and with salvage cystectomy for recurrence

By the late 1980s, single institutions reported the combination of a visibly complete TURBT followed by radiation therapy led to improved local control.[453,454] By 1994, the group from the University of Erlangen-Nuremberg referred to the visibly complete TURBT, when followed by a tumour bed negative biopsy, as a R0 resection.[454] The importance of the visibly complete TURBT was also seen in many subsequent co-operative group trials using radiation concurrent with chemotherapy, such as in RTOG 89-03.[307]

Some groups in Holland, Belgium, and France combined EBRT with brachytherapy (for the delivery of precision partial bladder radiation therapy). Brachytherapy is done by an open cystotomy with an implant using iridium-192 as low dose—rate irradiation with doses of up to 40 Gy combined with external beam radiation doses of 30 Gy. The majority of the reported series include patients who first

underwent maximal tumour resection by either PC or an open TURBT. The approach is reserved for patients with solitary bladder tumours less then 5 cm in diameter. The 5-year survival rates reported are from 62% to 84%, with a disease-specific survival rate approaching 80%.[455-457]

6.7.2.3.1 External beam radiation combined with concurrent radiosensitizing chemotherapy

Encouraging results were obtained when cisplatin was first made available by the NCI to the National Bladder Cancer Group in the early 1980s for patients with MIBC who were unsuitable for RC. In a protocol combining the use of concurrent cisplatin with conventional doses of radiation carried out from 1981 to 1986 in 68 patients with MIBC, 64% of patients with clinical stage T2 tumours and 22% of those with clinical stage T3-T4a disease had long-term survival rates.[453] These promising findings of combining cisplatin with radiation led to a randomized trial of radiation therapy with or without concurrent cisplatin in 99 patients with clinical stage T3 MIBC conducted by the National Cancer Institute of Canada.[337] This trial showed a significant improvement in pelvic tumour local control at 5 years in patients treated with cisplatin and radiation therapy (68%) versus radiation therapy alone (47%). Similarly, early prospective studies at the MSKCC that combined chemotherapy and TURBT resulted in T0 tumour response rates nearly twice those of chemotherapy alone, but these rates were less than 50%.[68,422] The results of combining TURBT with chemotherapy but without radiation have not been as successful in organ-preservation as the combination of chemotherapy and radiation therapy combined with TURBT. A study of 104 patients treated with TURBT and MVAC showed a T0 tumour response rate of 49% and, in these 52 patients, the 5-year survival rate was 67%.[47] However, 66% of the 104 patients required RC, suggesting a relatively low local bladder tumour control with chemotherapy and TURBT alone. Modern trimodality therapy combining radiation and chemotherapy with TURBT has led to substantially higher T0 tumour response rates (64%—87%) and less need for salvage cystectomy. This has tempered further interest in the treatment with only chemotherapy and TURBT.

A second randomized trial comparing radiation alone to radiation plus concurrent chemotherapy with 5-FU and MMC was reported by a multicentre group led from Birmingham, England, involving 360 patients. The results showed no measurable differences in toxicities. There was a significant increase in pelvic disease-free rates at 2 years (67% free of recurrence compared to 54% with radiation alone, p=0.02) and at 5 years (62% and 51%, respectively).[12] The 5-year OS rate was increased from 35% to 48%. Seventy-five percent of the patients treated with 5-FU and MMC concurrent with radiation therapy reported no late side effects. Of the 25% who did report side effects, fewer then 5% reported them as serious. Patients on this protocol underwent urological evaluation of their bladder capacity before and after treatment. There was a median reduction in bladder capacity of 10%, which was the same in both groups.

6.7.2.3.2 Cooperative group and single institution trials with concurrent chemotherapy and radiation combined with TURBT

Over the last 25 years, single institutions in North America and Europe and multi-institutional cooperative groups, including RTOG and SWOG, have enrolled over 1,000 patients with MIBC in bladder-preserving protocols. Several variables have been tested, including evaluating more than cisplatin alone as the radiation-sensitizing chemotherapy and evaluating alternative radiation schemes.

The BA06 30894 was an international multicentre randomized trial of nearly 1,000 patients treated from 1989 to 1995 that looked at the addition of neoadjuvant CMV chemotherapy followed by definitive treatment with radiation or surgery.[318] While a survival benefit (HR, 0.84; 95% CI, 0.72-0.99; p=0.37) was demonstrated with NAC, when stratified by definitive surgery versus radiation, these finding did not persist in the radiation group, although this subgroup analysis may have been underpowered (HR, 0.80; 95% CI, 0.63-1.02; p=0.07). Importantly, this study was looking at NAC followed by radiation (or surgery) alone, not true trimodality therapy with concurrent chemotherapy and with complete TURBT.

Beginning in the late 1980s, some single institutions and the RTOG were studying NAC in addition to trimodality therapy for operable patients with MIBC (**Table 6–15**). Encouraging results led to the opening of RTOG 8903, a phase 3 trial comparing concurrent cisplatin and radiation with or without neoadjuvant CMV chemotherapy. This study was closed prematurely after accrual of 123 of the planned 174 patients because there was an unexpectedly high rate of leucopenia in the MCV arm.[307] With a median follow-up of 5 years, the OS rate was 48%, and 49% in patients who were randomized to the neoadjuvant MCV arm. Likewise, there was no statistically significant difference in the T0 tumour response rate, distant metastasis, nor the 5-year survival with an intact bladder.

TABLE 6–15 Results of Trimodality Treatment for Muscle-invasive Bladder Cancer for Selective Bladder Preservation

Multimodality therapy	No. of patients	5-year OS (%)	5-year survival with intact bladder (%)	Study location	Reference
TURBT, MCV, ERBT + cisplatin	91	62 (4-year)	44 (4 year)	RTOG 8802	Tester *et al.*[502,74]
TURBT, 5-FU, EBRT + cisplatin	120	63	NA	University of Paris	Housset *et al.*[503]
TURBT, +/-MCV, EBRT + cisplatin	123	49	38	RTOG 8903	Shipley *et al.*[307]
TURBT, EBRT + cisplatin, carbo, or 5-FU and cisplatin	415	50	42	University of Erlangen-Nuremberg	Rodel *et al.*[464]
TURBT, EBRT + TAX and cisplatin, adj. GEM and cisplatin	80	56	47	RTOG 9906	Kaufman *et al.*[504]
EBRT + 5-FU + MMC	182	48	NA	BC2001	James *et al.*[12]
TURBT, EBRT + TAX/5-FU + cisplatin	97	71–75	67-71	RTOG 0233	Mitin *et al.*[15]
TURBT, CMV, EBR + cisplatin/5-FU/ TAX/gem	468	57	NA	Pooled RTOG	Mak *et al.*[460]
TURBT, CMV, EBRT + cisplatin/5-FU/ TAX/gem	475	57	46	MGH	Giacalone *et al.*[460]

Abbreviations: 5-FU, 5-fluorouracil; carbo, carboplatin; EBRT, external beam radiation therapy; GEM, gemcitabine; CMV, cisplatin, methotrexate, vinblastine; MGH, Massachusetts General Hospital; MMC, mitomycin C; NA, not available; OS, overall survival; RTOG, Radiation Therapy Oncology Group; TAX, paclitaxel; TURBT, transurethral resection of the bladder tumour.

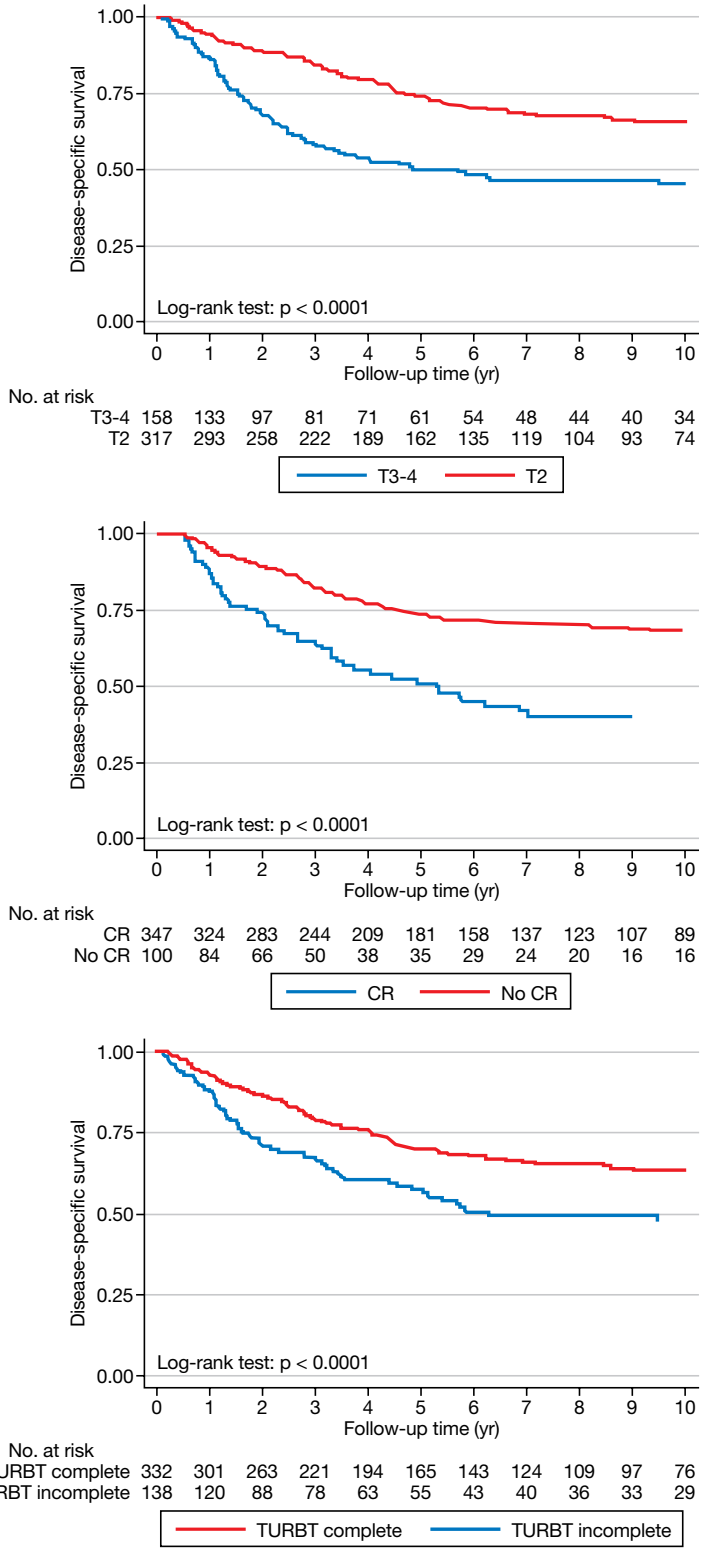
Two European phase 3 trials have studied the role of NAC before radiation alone. The Danish Group treated patients with MIBC with NAC before radiation and showed an insignificant 5% decrease in 5-year survival compared to patients treated with radiation alone (19% vs. 24%).[339] The other phase 3 trial, led in the United Kingdom by the MRC Group, studied neoadjuvant CMV before radical radiation or RC.[6,458] In the radical radiation sub-group of 415 patients, there was an insignificant trend to better survival in those treated with neoadjuvant CMV than in those treated with radiation alone. A meta-analysis of those two trials showed no significant difference in survival (30.4% vs. 28.1%, p=0.33) with the addition of the NAC.[459]

Two recent updates have published long-term outcomes in cooperative group and single institution trials. The first is a pooled analysis of RTOG protocols 8802, 8903, 9506, 9706, 9906, and 0233.[460] Reporting the results of 468 patients with a median follow-up of 4.3 years, they demonstrated 5-year and 10-year OS rates of 57% and 36%. The second is the Massachusetts General Hospital (MGH) experience, which included many patients treated on these protocols: 475 patients with MIBC treated at MGH who were entered on successive prospective trimodality protocols from 1986 to 2013.[44] Bladder-sparing trimodality therapy was reserved for those patients who had a complete clinical response at the mid-point of concurrent chemoradiation after radiation dosage of 40 Gy. These patients then received consolidation with additional chemotherapy and radiation, for the total dose of 64–65 Gy. Incomplete responders were advised to undergo an RC, as were patients whose invasive tumours recurred after the full 64–65 Gy treatment. All patients were treated with an aggressive TURBT, which was visibly complete in 70% of patients. All patients were treated with cisplatin concurrently with radiation therapy. With the median follow-up for all surviving patients of 7.21 years, the 5-year, 10-year, and 15-year actuarial OS rates were 57%, 39%, and 25%, respectively (stage T2, 65%, 46%, and 29%; stage T3-T4a, 42%, 26%, and 17%). The 5-year, 10-year, and 15-year disease-specific survivals were 66%, 59%, and 56% (stage T2, 74%, 66%, and 60%; stage T3-T4a, 50%, 45%, and 45%). The disease-specific survival rates stratified by clinical stage are shown in **Figure 6-7**, which demonstrates that there were very few late recurrences at least up to 10 years. These results are similar to those in contemporary cystectomy series. In this series, the 5-year, 10-year, and 15-year disease-specific survival for the 102 patients undergoing cystectomy were 55%, 44%, and 44%, respectively. This indicates the very important contribution of prompt cystectomy for disease control in patients whose tumours recur. An evaluation of patients undergoing salvage cystectomy at the MGH indicates quite acceptable surgical morbidity or mortality compared to major primary cystectomy series.[461] Interestingly, the outcomes and tolerability of trimodality therapy in the elderly from this series appears to be comparable to that of younger patients. This will be important in accrual for future trials, and this treatment option should not be excluded based on age alone.[462]

FIGURE 6–7
Kaplan-Meier Plot for Long-term Disease-specific Survival for All Patients With Selective Bladder Preservation Stratified by Clinical T Stage, Response to Therapy, and Completeness of TURBT From the Massachusetts General Hospital Experience[44,29]

Abbreviations: CR, complete response; TURBT, transurethral resection of the bladder tumour.

Shariat SF, Palapattu GS, Karakiewicz PI, et al. *Discrepancy between clinical and pathologic stage: impact on prognosis after radical cystectomy.* Eur Urol. *2007;51(1):137–149; discussion 149–151.Giacalone NJ, Shipley WU, Clayman RH,* et al. *Long-term outcomes after bladder-preserving tri-modality therapy for patients with muscle-invasive bladder cancer: an updated analysis of the Massachusetts General Hospital Experience.* Eur Urol. *2017;71(6):952–960.*



6.7.2.3.2 Comparison of survival outcomes following curative therapy in contemporary series by cystectomy or by bladder-preserving trimodality therapy with cystectomy reserved for recurrence

Comparing results of bladder-preserving therapy to those of contemporary RC series is confounded by the discordance between clinical staging (TURBT) and pathological (cystectomy) staging. Clinical staging is more likely to underestimate the extent of disease with regard to penetration into the muscularis propria or beyond than is pathological (cystectomy) staging.[463] Thus, if any favourable outcome bias exists among these selected staging options, it is in favour of the pathologically reported cystectomy series. For patients with MIBC, the OS outcomes following either contemporary RC at major single institutions or by trimodality therapy are shown in **Table 6–16**. The University of Southern California reported on 633 patients undergoing RC with pathological stages T2-T4a with an OS rate at 5-years of 48%, and at 10 years of 32%.[66] The MSKCC contemporary RC series showed that, in 184 patients with tumours pathological stage P2-P4, the 5-year OS rate was 36%.[222] The actuarial survival rate of all 269 patients with pathological stages ranging from P0 to P4 in this series was 45%. These results are similar to the MGH series,[44] as well as those from the University of Erlangen-Nuremberg[454,464] and RTOG. Interestingly, these results do not appear to be limited to MIBC histologies of pure urothelial carcinoma, as variant urothelial carcinomas (i.e. those with squamous or glandular differentiation) have recently been shown to have comparable rate of CR, OS, disease-specific survival, and salvage cystectomy.[465] The similarity in survival between cystectomy and bladder-preserving trimodality therapy is likely in part due to the prompt use of cystectomy when necessary for recurrence in the bladder-preservation series. The importance of life-long cystoscopic surveillance cannot be understated, as even in those who have had a CR to trimodality therapy, one in four will develop a nonmuscle invasive recurrence, with some occurring over a decade beyond initial therapy.[466]

TABLE 6–16 Muscle-invasive Bladder Cancer: Survival Outcomes Following Curative Therapy in Contemporary Series

Series	Stages	Number	OS	
			5-year	10-year
Cystectomy:				
University of Southern California 2001 (Stein *et al.*[66])	pT2-pT4a	633	48%	32%
MSKCC 2001 (Dalbagni *et al.*[222])	pT2-pT4a	181	36%	27%
SWOG/ECOG/CALGB*,§ 2002 (Grossman *et al.*[5])	cT2-cT4a	317	49%	34%
University of Southern California + University of Bern 2001† (Zehnder *et al.*[505])	pT2-pT3	959	50%	39%
U. Ulm 2012‡ (Hautmann *et al.*[342])	pT1-pT4a	1,100	58%	44%
Selective Bladder Preservation:				
U. Erlangen-Nuremberg* 2002 (Dunst *et al.*[454]; Rodel *et al.*[464])	cT2-cT4a	326	45%	29%
MGH* 2017 (Giacalone *et al.*[44])	cT2-cT4a	475	57%	39%
RTOG* 1998 (Shipley *et al.*[307])	cT2-cT4a	123	49%	–
BC2001* 2012 (James *et al.*[12])	cT2-4a	182	48%	–
RTOG pooled* 2014 (Mak *et al.*[460])	cT2-4a	468	57%	36%

Abbreviations: CALGB, Cancer and Leukemia Group B; ECOG, Eastern Cooperative Oncology Group; MGH, Massachusetts General Hospital; MSKCC, Memorial Sloan Kettering Cancer Centre; MVAC, methotrexate, vinblastine, doxorubicin, and cisplatin chemotherapy; OS, overall survival; RTOG, Radiation Therapy Oncology Group.

* These series included all patients by their intention-to-treat.
† Estimated survival statistics
‡ Included 26% pT1 and 18% pN+
§ 50% of patients were randomly assigned to receive 3 cycles of neoadjuvant MVAC.

These observational data demonstrating similar survival outcomes are further supported by a recent propensity score analysis that matched patients treated with cystectomy and patients treated with trimodality therapy in a multidisciplinary bladder cancer clinic. The 112 patients included after matching demonstrated a 5-year disease specific survival rate of 73.2% in the cystectomy group versus 76.6% in the trimodality group (p=0.49).[19] Furthermore, a recent meta-analysis and systematic review also found no difference in disease-specific survival (at 5 or 10 years), PFS (at 10 years), or OS (at 5 year or 10 years) between cystectomy versus trimodality therapy.[467]

6.7.2.4 Quality of life after definitive radiation for muscle-invasive bladder cancer

If the survival and cancer control outcomes appear similar in the absence of Level 1 evidence between cystectomy and trimodality therapy, then the different morbidity profiles and QoL considerations of the treatment modalities become paramount. The instruments to assess QoL have been well

established for prostate cancers and gynecologic cancers, but not for bladder cancer. The instruments that are currently used for bladder cancer patients are adaptations, and thus their validity is somewhat uncertain. These studies are also limited by incomplete sampling of all potential participants, which leaves unclear whether or not the nonparticipants are those who have had a worse outcome or who are the most satisfied. Despite these limitations, some general principles can be derived from this literature.[468]

Minimal late pelvic toxicity is certainly required for successful implementation of a selective bladder preserving protocol. Long-term bowel and bladder toxicity after chemoradiotherapy was investigated in patients enrolled in prospective sequential RTOG trials (8903, 9506, 9706, and 9906). One study reported on 157 patients who underwent combined modality therapy and who survived at least 2 years from the start of treatment with their bladders intact. The median follow-up was 5.4 years.[469] Seven percent of the patients experienced late grade 3 or 4 pelvic toxicity (5.7 % genitourinary and 1.9% GI). In only one of nine patients did a grade 3–4 genitourinary toxicity persist. This indicates that rates of late pelvic toxicity for patients who undergo selective bladder preservation and retain their native bladder are low.

Zeitman and colleagues reported a study on patients receiving TURBT, chemotherapy, and radiation in the treatment of the bladder cancers at the MGH.[470] Of 221 patients with clinical stage T2-T4a cancer of the bladder treated at the MGH from 1986 to 2000, 71 were alive with their native bladders and disease free in 2001. These patients were asked to undergo a urodynamic study and to complete a QoL questionnaire. Sixty-nine percent participated in some component of the study, with a median time from the trimodality therapy of 6.3 years. This long follow-up is sufficient to capture the majority of the late radiation side effects. Seventy-five percent of patients have normal functioning bladders by urodynamic study. Reduced bladder compliance, a recognized complication of radiation, was seen in 22% of patients. However, distressing bladder symptoms were seen only in one-third of these patients. Two women showed bladder hypersensitivity, involuntary contractions, and incontinence. The questionnaires showed that bladder symptoms were uncommon overall in both sexes. However, 19% of women reported problems with bladder control, and 11% of them wore pads. The distress from urinary symptoms was only half as common as symptom prevalence. Bowel symptoms occurred in 22% of patients, and caused distress in 14%. The majority of men retained sexual function with or without use of sildenafil. Global HRQoL was high. The majority of the patients treated by trimodality therapy therefore retained good bladder function. It was concluded that there is a small but detectable level of lasting bowel dysfunction and distress, and that this might be judged as the additional price that these patients have to pay to retain their bladders.

Two cross-sectional questionnaire studies, one from Sweden and one from Italy, have compared the outcome following radiation with the outcome following cystectomy.[471,472] The questionnaire results for urinary function following radiation are very similar to those recorded in the MGH study. Over 74% of the patients recorded good urinary function. Both studies compared bowel functions in irradiated patients with those seen in patients undergoing cystectomy. In both, the bowel symptoms were greater for those receiving radiation than for those receiving cystectomy (10% vs. 3% and 32% vs. 24%, respectively), but in neither was this statistically significant. In contrast to men who had been irradiated for prostate cancer, the majority of the male bladder-sparing patients reported adequate erectile function (full or sufficient for intercourse), and only 8% reported dissatisfaction with their

sexual lives. In the Swedish and Italian series, 38% and 25% of the men retained functional erections, as compared to 15% and 8% of cystectomy controls. Use of sildenafil by patients in the MGH series may have been the major reason for better-retained erectile function.

A third, more recent, cross-sectional bi-institutional questionnaire study attempted to compare the QoL of cystectomy versus trimodality therapy in 226 patients treated over 20 years.[473] With a response rate of 77%, a median follow-up period of over 5.5 years, using six different validated QoL instruments and propensity score matching, multivariable analysis demonstrated better general QoL in those who received trimodality therapy versus RC. Trimodality therapy was also associated with superior physical, social, emotional, and cognitive functioning as well as bowel and sexual function. Urinary symptom scores were similar. The availability of long-term outcomes and these QoL data permit comparative analyses beyond systematic reviews.[468] A recent comparative effectiveness modelling study using the primary endpoint of quality-adjusted life years showed a potential gain of over 1 quality-adjusted life year with bladder-preserving trimodality therapy relative to cystectomy.[474] The model results demonstrated their robustness by holding up to a myriad of sensitivity analyses. Results from these study designs remain hypothesis-generating and subsequent prospective investigations, which could provide higher levels of evidence, are warranted. This level of evidence may be a long time coming, as the difficulty in obtaining Level 1 evidence on this topic was illustrated by the SPARE (Selective bladder Preservation Against Radical Excision) randomized trial, which closed early due to low accrual.[475,476] This further highlights the importance of modelling studies in addressing this evidence gap.

6.7.2.5 Translational research: molecular tumour markers and genetic signatures as prognostic or predictive of response to radiation treatment

Tumour suppressor genes such as p53 and pRB have been studied in detail in bladder cancer, but both markers have led to contradictory data in the assessment of risk for disease progression and survival.[477] Cell-cycle regulatory proteins p27 and Ki-67 might predict recurrence and disease progression.[478-480] Additionally, in a *post hoc* analysis, the expression of hypoxia-inducible factor-1α has been shown to predict benefit from the addition of carbogen and nicotinamide (CON) to radiation therapy in the BCON phase 3 trial of radiation alone or with CON.[481,482] None of these strategies are yet ready for routine clinical use.

The bladder tumour's pre-treatment apoptotic index or altered expression of the RB1 or the BCL2 genes might alter tumour response to radiation therapy.[483-485] RTOG investigated the outcome of 73 patients treated in four RTOG bladder-preserving protocols and noted that, among patients treated with transurethral surgery and chemotherapy concurrent with radiation altered expression of p53, CDKN2A and pRB had no prognostic significance, but overexpression of HER2 (ERBB2) correlated significantly with a reduced CR rate (50% vs. 81%; p=0.03). The aim of targeted therapies is to interfere with molecular events related to tumour proliferation.[486] Examples of these therapies are cetuximab, an anti-epidermal growth factor receptor (EGFR) monoclonal antibody; gefitinib and erlotinib, EGFR-specific inhibitors; trastuzumab, an anti-HER2-related monoclonal antibody; and bevacizumab, a vascular endothelial growth factor (VEGF) monoclonal antibody.[487] Both EGFR and HER2 are targets identified on cancer cells, whereas VEGF is a target that acts on the tumour microenvironment. Studies have shown reduced CR rates when HER2 is overexpressed.[487-489] These results

have led to RTOG-0524, a phase 1/2 trial for patients with MIBC who are not fit for a cystectomy. This investigated paclitaxel and daily radiation therapy with trastuzumab given to patients whose tumours overexpress HER2. This study showed the addition of trastuzumab to patients with HER2-positive tumours resulted in comparable efficacy and toxicity.[490] This is one of the first examples of a molecular targeted therapy being added to treatment for patients with localized MIBC.

A group in Leeds and Oxford in the United Kingdom, in collaboration with the Ontario Cancer Institute in Toronto, has evaluated MRE11 expression in MIBC patients treated with radical radiation or with cystectomy.[491] MRE11 is one of a panel of DNA damage signalling proteins active in the process of DNA double-strand break repair.[492] DNA double-stand breaks are the most lethal form of injury produced by ionizing radiation and by some chemotherapy agents. MRE11 had been singled out as one possible predictor of radiation treatment outcome. The cohorts of patients treated with radical radiation and a separate cohort of patients also treated in Leeds by RC have documented that high MRE11 protein expression by the tumour predicts improved outcome with radiation therapy but not in the cystectomy cohorts. Comparing the high MRE11 patients by treatment showed that the patients treated with radiation have a significantly higher disease-specific survival than those treated with immediate cystectomy (p=0.02), while those with low MRE11 protein expression do insignificantly better with surgery than with radiation. These results tentatively identify MRE11 overexpression as a predictive molecular marker of improved cause-specific survival following radiation therapy for MIBC. A challenge in the implementation of MRE11 as a biomarker is standardization of assays and identification of appropriate metrics. A retrospective investigation done on 135 patients treated with chemoradiation on several pooled cooperative group trials demonstrated the potential of using of a quantitative immunohistochemistry assay for MRE11, coupled with using the nuclear-to-cytoplasm ratio to normalize measurements, as a predictive standardized biomarker.[493]

Much like in other cancers, efforts are ongoing to subtype MIBC via genomic information.[53,494] Coupling subtype with clinical response could help better selection of patients for the appropriate therapy. For example, using transcriptome-wide gene expression profiles of 189 MIBC TUBRT samples from patients undergoing trimodality therapy at MGH, tumours were classified into the basal, basal claudin-low, infiltrated luminal, or luminal subtypes, and were shown to demonstrate differential clinical outcomes.[495] These preliminary data are promising, but ultimately will need further validation on prospective clinical trials.

Finally, there has been an explosion in immunotherapy-related investigations in oncology, and bladder cancer is no exception.[496] The PD-L1 inhibitor atezolizumab has shown durable response rates, good tolerability, and encouraging survival in patients with locally advanced and metastatic bladder cancer who have progressed through chemotherapy.[497,498] This monoclonal antibody produced increased response in those with increased levels of PD-L1 expression. How these therapies can be utilized in the setting of definitive bladder-preserving therapy for MIBC is an area of ongoing investigation and will likely be a focus of future trial designs.

In conclusion, in select patients with MIBC, bladder-preserving therapy with cystectomy reserved for tumour recurrence represents a safe and effective alternative to an immediate RC. Cumulative published data of more than 1,000 patients in single institution and multi-institution cooperative group trials demonstrate that trimodality therapy results in excellent local control in 70% of patients

with MIBC, while preserving a native functional bladder without compromising long-term survival. The 10-year OS and disease-specific survival rates in the bladder-sparing protocols are comparable to the overall results reported with contemporary RC. Moreover, the 15-year results indicate a plateau in disease-specific survival, suggesting no evidence of increased rates of recurrence with longer follow-up times. Life-long bladder surveillance is essential. Prompt cystectomy for tumour recurrence is necessary to prevent tumour dissemination. Thus, bladder-preserving therapy is a bona fide option and valid alternative to RC in selected patients. This approach should be discussed along with all of the other treatment options during overall initial treatment planning. This approach contributes significantly to the QoL of the patients so treated and represents a unique opportunity for urological surgeons, radiation oncologists, and medical oncologists to work hand-in-hand in a joint effort to provide patients with the best treatment option for this disease.

6.7.2.6 Recommendations for radiation-based bladder preserving strategies for MIBC

- Radiation therapy followed by salvage cystectomy for tumour recurrence has comparable survival to preoperative radiation therapy and cystectomy [**LOE 1; GOR A**].
- Radiation therapy and chemotherapy result in a higher rate of pT0 status and locoregional DFS than does radiation therapy alone [**LOE 1; GOR A**].
- Combined radiation and chemotherapy allow good preservation of bladder function in the great majority of patients [**LOE 2; GOR B**].
- There is inadequate clinical trial evidence to indicate that NAC prior to chemoradiation therapy improves survival [**LOE 1; GOR A**].
- Complete TURBT, when possible, is associated with higher rates of local tumour control and higher cure rates than incomplete initial tumour resection for selected patients in trimodality radiation/chemotherapy trials [**LOE 2; GOR B**].
- Data suggest that high expression of the molecular marker MRE11 may be a putative predictor for cause-specific survival following radical radiation therapy for MIBC [**LOE 3; GOR B**].
- Trimodality therapy consisting of TURBT plus concurrent radiosensitizing chemotherapy and radiation is judged safely possible and, when combined with early salvage cystectomy for recurrence, this bladder-preserving treatment approach offers a chance for long-term cure and survival in selected patients comparable to RC, and affords a >70% chance of maintaining a well-functioning native bladder. QoL studies have demonstrated that the retained native bladder functions well and long-term toxicity of chemoradiation to pelvic organs is low. These reports support the acceptance of modern bladder-sparing trimodality therapy for selected patients as a proven alternative to cystectomy [**LOE 3; GOR C**].

See **Table 6–17** for a summary of these recommendations.

TABLE 6–17 Summary of Recommendations

Treatment/Comparison	Evidence	LOE	GOR
RT alone vs. 40 Gy + Cystectomy	3 of 4 RCTs report similar survival	1b	A
Chemoradiotherapy vs. RT alone	2 RCTs report significant improvement in bladder tumour eradication	1b	A
Neoadjuvant CT with RT or chemoradiotherapy	3 RCTs and 1 meta-analysis report no benefit	1a	A
Chemoradiotherapy preserves good bladder function	4 QoL studies and RTOG protocols report good tolerance	2a	B
Complete TURBT with chemoradiotherapy	3 reports (one phase 3, two phase 2) show benefit	2a	B
Predictive biomarkers of outcome after RT	MRE11 expression predicts improved CSS (three studies)	3	C
Trimodality therapy vs. immediate cystectomy	Comparison of several contemporary series and the results of one meta-analysis report similar 5- and 10-year survival	3	C

Abbreviations: CSS, cancer-specific survival; CT, computed tomography; GOR, grade of recommendation; LOE, level of evidence; QoL, quality of life; RCT, randomized control trial; RT, radiotherapy; RTOG, Radiation Therapy Oncology Group; TURBT, transurethral resection of the bladder tumour.

6.8 Follow-up After Radical Surgery

6.8.1 Evidence to determine optimal follow-up

Due to the lack of comparative literature regarding an optimal follow-up scheme, the early and late morbidity, incidence and location of recurrences, life expectancy, and uptake of various examination modalities must be taken into consideration to propose a follow-up scheme after RC and various forms of urinary diversion.

In a large single-centre series, 90-day complication rate were seen in 58% of patients.[204] Late morbidity was usually linked to the type of urinary diversion. Lower morbidity and (perioperative) mortality is associated with higher case load and therefore more experience.[216]

Risk of recurrence: A nomogram based on 728 patients who underwent cystectomy was presented. Standard predictors were pathological stage of the primary tumour (pTN) and nodal status (pN) **LOE 2b**. The prediction of recurrent disease increased by 3.2% when the nomogram included age, lymphovascular invasion, CIS, NAC, AC, and adjuvant RT.[275] This nomogram can be used to predict the individual risk of systemic relapse and to develop a risk-adapted follow-up protocol.

Patients have poor prognosis after pelvic recurrence. Even with treatment, the median survival ranges from 4 to 8 months following diagnosis. Definitive therapy can prolong survival, but mostly provides significant palliation of symptoms. Treatment comprises systemic chemotherapy, local surgery, or RT.[506]

Survival: According to a multi-institutional database of 888 consecutive patients undergoing RC for BC, the 5-year RFS was 58% and the CSS was 66%.[507]

Long-term oncological outcome of RC was analyzed in a series of 2,287 patients who underwent RC between 1998 and 2008.[508] The mean and median follow-up time was 35 and 29 months, respectively. The 5-year OS, RFS, and CSS was 57%, 48%, and 67%, respectively, with a distant recurrence and local recurrence rate of 37% and 6%, respectively.

Imaging studies: Although general recommendations are not advised based on high level of evidence, closer follow-up could be considered in patients with locally advanced disease or lymph node involvement. The suggested follow-up includes 4-monthly CT scans during the first year, 6-monthly until the third year, and annual imaging thereafter. Patients with multifocal disease, nonmuscle-invasive bladder cancer (NMIBC) with CIS, or positive ureteral margins are at higher risk of developing upper tract urothelial carcinoma, which can develop late (>3 years). In those cases, monitoring of the upper urinary tract is mandatory during follow-up. CT is to be used to assess the upper urinary tract.[509]

PET has not been shown to improve sensitivity in patients with metastatic urogenital cancer overstaging by doing CT scanning alone **[LOE 3]**. Bone scintigraphy, CT scans, ^{18}F–FDG PET/CT and whole-body MRI represent potential imaging studies to diagnose and to monitor skeletal metastases.[41]

Local recurrence: Contemporary cystectomy has a 5% to 15% probability of pelvic recurrence. Most recurrence manifests during the first 24 months, often within 6 to 18 months after surgery. However, late recurrence can occur up to 5 years after cystectomy. Pathological stage and lymph node status are predictive for pelvic recurrence, as well as positive margins, extent of LND, and perioperative chemotherapy.

The incidence of new urethral tumours after RC is 1.5% to 6.0% in men, with a mean recurrence-free interval of 13.5 to 39.0 months and median survival of 28 to 38 months, of which >50% die from systemic disease. Secondary urethral tumours are likely to occur at 1 to 3 years after surgery. Prophylactic urethrectomy at cystectomy is no longer justified in most patients. Independent predictors for urethral recurrence are: cystectomy for NMIBC, prostate involvement, and history of recurrent NMIBC.[506] In women, the main risk factor is bladder neck disease. Many studies have demonstrated that the risk of urethral recurrence after orthotopic diversion (0.9%–4.0%) is significantly less than after nonorthotopic diversion (6.4%–11.1%).[506]

There is a significant survival advantage in men with urethral recurrence diagnosed asymptomatically versus symptomatically, so follow-up of the male urethra by ureteroscopy every 2 years is indicated in patients at risk for urethral recurrence.[506] Treatment for urethral CIS by BCG instillations has success rates of 83%. For treatment of urethral recurrence following RC and ileal bladder substitution[510] in invasive disease, urethrectomy should be performed if the urethra is the only site of disease.

Distant recurrence: Distant recurrences are seen in up to 50% of patients treated with cystectomy. Most recurrences are seen in the first 24 months, although progression has been observed after more than 10.[511] The most likely sites for distant recurrence are extra-pelvic lymph nodes, lungs, liver, and bone.[512] Treatment of metastatic disease with cisplatin-based combination chemotherapy with either MVAC or cisplatin and gemcitabine result in a mean survival time of around 14 months. Consideration must also be given to the possibility of longer survival in patients with minimal metastatic disease undergoing multimodal treatment, including metastasectomy. There are reports of survival rates of 28% to 33% at 5 years in patients undergoing resection of metastases after objective response to chemotherapy.[513]

Upper urinary urothelial carcinomas occur in 1.8% to 6.0% of cases and represent the most common sites of late recurrence (3-year DFS following RC). Median OS is 10 to 55 months, and 60% to 67% of patients die of metastatic disease.[506] A meta-analysis found that 38% of upper tract urothelial carcinoma recurrence was diagnosed by follow-up investigation, whereas in the remaining 62%, diagnosis was based on symptoms. When urine cytology was used in surveillance, the rate of primary detection was 7% and 29.6% with upper urinary tract imaging.[509] This meta-analysis concluded that patients with noninvasive cancer are twice as likely to have upper tract urothelial carcinoma as patients with invasive disease. Multifocality increases the risk of recurrence by three-fold, while positive ureteral or urethral margins increase the risk by seven-fold. Radical nephroureterectomy can prolong survival.[506]

Follow-up of functional outcomes and complications: Apart from oncological surveillance, patients having undergone a urinary diversion deserve functional follow-up. Complications related to urinary diversion are detected in 45% of patients during the first 5 years of follow-up. This rate increases

over time, and exceeds 54% after 15 years of follow-up. Therefore, long-term follow-up of functional outcomes is desirable[506] **[LOE 3]**, and may stop after 15 years. The functional complications may include stenosis of uretero-intestinal anastomosis, stoma complications in patients with IC, NB continence problems, and emptying dysfunction.

6.8.2 Recommendations

- Postoperative follow-up after RC should be performed in a risk-adapted approach using currently available nomograms **[LOE 3; GOR C]**.
- Symptom-oriented follow-up might result in the same long-term outcome as standardized follow-up protocols, but at a lower cost **[LOE 3; GOR C]**.
- CT scan represents the imaging modality of choice to identify lung, lymph node, and liver metastasis **[LOE 2; GOR B]**.

6.9 Summary of Recommendations

6.4.1.6 Recommendations for removal of the tumour-bearing bladder and regional lymph nodes

- Preservation of the anterior and membranous urethra, including parts of the prostate and seminal vesicles for reasons of fertility, potency, and continence are technical variations to the nerve-sparing approach, which may improve patients' QoL but must be attentively judged against possible oncological risks **[LOE 3; GOR C]**.
- In female patients, standard anterior pelvic exenteration includes the entire urethra, adjacent vagina, uterus, distal ureters, and respective lymph nodes **[LOE 3; GOR C]**. The urethra-supplying autonomous nerves can be preserved in case of a planned orthotopic NB **[LOE 3; GOR C]**.
- RC in patients with MIBC should be performed within 3 months after initial diagnosis of stage T2 to T4 disease **[LOE 3; GOR B]**.
- The more lymph nodes are removed, the higher the probability of detecting at least one positive lymph node. However, there is no real threshold of the numbers of lymph nodes that need to be removed **[LOE 2; GOR B]**.
- Although there is some evidence from retrospective and prospective analyses that an extended pelvic lymphadenectomy might be associated with an improvement in 5-year PFS the most recent prospective randomized multicentre study did not show any evidence but did show an increased incidence of lymphoceles ($p<0.01$) **[LOE 2; GOR B]**.
- LND should include all lymphatic tissues around the common iliac, external iliac, and internal iliac groups, as well as the obturator group bilaterally, since up to one-third of all positive nodes are located around the common iliac artery **[LOE 3; GOR B]**.

6.4.2.8 Recommendations for minimally invasive approach: laparoscopic and robotic-assisted radical cystectomy (RARC)

- RARC is a surgical option for locally advanced bladder cancer with oncological outcomes similar to those of open series **[LOE 2; GOR B]**.
- High-volume centres with dedicated minimally invasive surgical teams have shown better results than smaller centres **[LOE 2; GOR C]**.
- Difficult cases should be avoided early in the surgeon's learning curve; see **Table 6–3** for relative contraindications **[LOE 2; GOR C]**.

6.4.3.7 Recommendations for surgical outcome: morbidity and mortality

- Surgical complications associated with RC and urinary diversion should be reported in a uniform grading system. Currently, the best adapted graded system for cystectomy is the Clavien grading system **[LOE 2; GOR B]**.
- Surgical complications associated with RC and urinary diversion should include the length of follow-up for the patient cohort and a minimum of 30-day, but preference for 90-day, reported outcome **[LOE 3; GOR C]**.
- ASA score, age, comorbidities, sarcopenic status, previous treatment for bladder cancer or other pelvic diseases, surgeon and hospital volume of RC, and type of urinary diversion influence surgical outcome **[LOE 2; GOR B]**.
- Reduction in blood loss and blood transfusion is afforded by meticulous technique, use of modern surgical devices, and improved understanding of pelvic anatomy **[LOE 3; GOR C]**.
- Reduction of urinary extravasation and leak can be achieved with careful closure of the anastomosis or pouch, stenting of the ureteroenteric anastomosis, and maintenance of appropriate drainage **[LOE 3; GOR C]**.
- Reduction of symptomatic lymphocele formation can be achieved with appropriate identification of lymphatic channels, careful surgical technique, and an open peritoneal window. Initial treatment should begin with percutaneous drainage **[LOE 3; GOR C]**.
- Reduction of anastomotic strictures requires meticulous surgical technique, minimal ureteral dissection, well-perfused segment, generous spatulation, and careful apical suture placement **[LOE 3; GOR C]**.
- Reduction of metabolic disorders after urinary diversion requires preservation of distal ileum, serial monitoring of electrolytes and vitamin B-12 levels, understanding of bowel segment physiology, and appropriate emptying of urinary diversion **[LOE 3; GOR C]**.
- Reduction of DVT and pulmonary embolus can be achieved with use of low molecular weight heparin, early ambulation, and sequential compression devices **[LOE 2; GOR B]**.
- There is increasing evidence that implementation of ERAS protocols can successfully reduce complication rates, length of stay in hospital, and the time taken to get back to normal activities following RC **[LOE 3; GOR C]**.
- ERAS protocols should be standardized and outcomes audited following implementation **[LOE 3; GOR C]**.

6.4.4.5 Recommendations for oncological outcome of radical surgery

- The AJCC substratifications in node-negative pT2 and pT3 bladder cancer are of prognostic value **[LOE 2; GOR B]**.
- According to the TNM staging system, organ-confined bladder cancer has to be defined as ≤pT2bN0M0 **[LOE 2; GOR B]**.
- Nomograms provide improved prognostic information for oncological outcomes before and after radical surgery, as compared to predictions based on pathological TNM staging. However, their general applicability has not yet been sufficiently established by external validation **[LOE 3; GOR C]**.
- In patients older than 80 years, RC is associated with the highest risk reduction on cancer-related and non–cancer-related mortality **[LOE 3; GOR C]**.
- Based on the scarce data available, the routine use of molecular markers for risk assessment after RC in invasive bladder cannot be recommended **[LOE 3; GOR D]**.

6.4.5.3 Recommendations for quality of life

- There is evidence for improved HRQoL for orthotopic NB reconstruction compared to IC urinary diversion **[LOE 3; GOR C]**.
- Appropriate patient selection for urinary diversion type is critical to achieving improved HRQoL outcomes following RC **[LOE 3; GOR C]**.
- More high-quality RCTs are needed to confirm current findings regarding HRQoL **[LOE 3; GOR C]**.

6.5.1.6 Recommendations for neoadjuvant chemotherapy

- Cystectomy is considered the gold standard of treatment for localized MIBC **[LOE 2; GOR B]**.
- A discrepancy between clinical/cystoscopic and pathological staging can be anticipated after NAC and, therefore, cystectomy is not obviated by response **[LOE 2; GOR B]**.
- Toxicity and mortality associated with NAC are acceptable **[LOE 2; GOR B]**. However, few data on QoL are available.
- Meta-analysis of cisplatin-containing combination NAC trials revealed a modest benefit in favour of NAC **[LOE 1; GOR B]**.
- Cisplatin-based combination chemotherapy should be offered to all eligible patients with *c*T2-T4aN0M0 urothelial bladder cancer **[LOE 1; GOR A]**.
- We recommend using (dose-dense) MVAC as the NAC regimen for appropriately selected cases **[LOE 2; GOR B]**.
- Although other regimens, such as GC, have similar activity in patients with metastatic disease, there are no data from randomized trials in the neoadjuvant setting to support the use of regimens other than MVAC. Retrospective datasets in the NAC setting show comparable pCR rates between GC and MVAC **[LOE 2; GOR B]**.
- Available data suggest that, for average-risk cancer patients with cT2, the benefit of adding chemotherapy to local therapy is, at best, modest, but benefits still outweigh the risks. Likewise, all available studies suggest a much more substantial benefit for patients with high-risk disease, such as cT3b cancers or those thought to have lymph node involvement **[LOE 2; GOR B]**.
- Carboplatin-based regimens should not be used in the neoadjuvant setting **[LOE 2; GOR B]**.
- No predictive biomarker has an established role to exclude patients from neoadjuvant platinum-based therapy **[LOE 3; GOR D]**.
- The quality of the surgery is a confounding factor in interpreting these studies **[LOE 3; GOR C]**.
- Following cystectomy in patients who did not receive NAC, we suggest consideration of AC with a cisplatin-based regimen for patients who have perivesical tumour extension (stage T3 or higher) or regional lymph node involvement **[LOE 2; GOR C]**.
- Presence of squamous or glandular differentiation in locally advanced urothelial carcinoma of the bladder does not seem to confer resistance to MVAC, and in fact may be an indication for the use of NAC before RC **[LOE 3; GOR C]**.

6.5.2.9 Recommendations for adjuvant chemotherapy

- Adjuvant cisplatin-based chemotherapy is supported by a recent large cohort analysis **[LOE 2]**, several randomised clinical trials **[LOE 1]**, and the results of two meta-analysis and composite analysis of randomized trials **[LOE 1]**. However, the trials used in meta-analyses were flawed, mainly due to poor recruitment and early termination, and so make definitive conclusions difficult. On that basis, the group provides a **Grade B recommendation** for adjuvant cisplatin-based chemotherapy in the patient with pT3/4 and/or lymph node–positive cancer at cystectomy who has not had NAC and is medically fit.
- Adjuvant regimens not containing cisplatin (including those containing carboplatin) should not be routinely used outside of clinical trials because of a lack of evidence for their benefit in that setting **[LOE 2; GOR C]**. Patients who cannot tolerate cisplatin-based combination therapy should be included in the clinical trials or observed.
- Until the current equipoise is resolved for AC in this setting, clinical trial remains the best choice for patients with locally advanced bladder cancer **[LOE 3; GOR C]**.

6.6.1 Recommendations for prognostic and predictive biomarkers: current and future applications

- Due to the low levels of evidence **[LOE 4]**, biomarkers are currently not recommended for determining prognosis or predicting response to treatment in patients with bladder cancer **[GOR D]**.

6.7.1.6 Recommendations for transurethral resection of the bladder and partial cystectomy with or without multimodal therapy

- TUR monotherapy is an alternative to RC in appropriately selected (see Recommendation 2, below) and counselled patients with T2-T3a N0Mx bladder cancer **[LOE 3; GOR C]**.
- Patients most appropriate for this approach have tumours that **[LOE 3; GOR C]**:
  - Are small
  - Are completely resectable
  - Have negative tumour bed and periphery biopsies
  - Are not associated with upper tract compromise (i.e. hydronephrosis)
  - Are not associated with radiographic evidence of locoregional extension of disease (T3b, N+) at the time of first treatment
- TUR monotherapy should be discussed as part of the informed consent process of patients contemplating management options for invasive bladder tumours (TNM stages T2-3a N0Mx) **[LOE 3; GOR C]**.
- If technically feasible, an R0 resection should be attempted during TUR prior to multimodal therapy, since it is associated with higher CR, decreased need for salvage cystectomy, and more favourable survival **[LOE 2; GOR C]**.
- Highly selected patients with focal invasive cancers and cT0 or minimal residual disease after NAC may be candidates for bladder sparing with either TUR or PC **[LOE 3; GOR C]**.

6.7.2.6 Recommendations for radiation-based trimodality treatment strategies

- Radiation therapy followed by salvage cystectomy for tumour recurrence has comparable survival to preoperative radiation therapy and cystectomy [**LOE 1; GOR A**].
- Radiation therapy and chemotherapy result in a higher rate of pT0 status and locoregional DFS than does radiation therapy alone [**LOE 1; GOR A**].
- Combined radiation and chemotherapy allow good preservation of bladder function in the great majority of patients [**LOE 2; GOR B**].
- There is inadequate clinical trial evidence to indicate that NAC prior to chemoradiation therapy improves survival [**LOE 1; GOR A**].
- Complete TURBT, when possible, is associated with higher rates of local tumour control and higher cure rates than incomplete initial tumour resection for selected patients in trimodality radiation/chemotherapy trials [**LOE 2; GOR B**].
- Data suggest that high expression of the molecular marker MRE11 may be a putative predictor for cause-specific survival following radical radiation therapy for MIBC [**LOE 3; GOR B**].
- Trimodality therapy consisting of TURBT plus concurrent radiosensitizing chemotherapy and radiation is judged safely possible and, when combined with early salvage cystectomy for recurrence, this bladder-preserving treatment approach offers a chance for long-term cure and survival in selected patients comparable to RC, and affords a >70% chance of maintaining a well-functioning native bladder. QoL studies have demonstrated that the retained native bladder functions well and long-term toxicity of chemoradiation to pelvic organs is low. These reports support the acceptance of modern bladder-sparing trimodality therapy for selected patients as a proven alternative to cystectomy [**LOE 3; GOR C**].

6.8.2 Recommendations for follow-up after radical surgery

- Postoperative follow-up after RC should be performed in a risk-adapted approach using currently available nomograms [**LOE 3; GOR C**].
- Symptom-oriented follow-up might result in the same long-term outcome as standardized follow-up protocols, but at a lower cost [**LOE 3; GOR C**].
- CT scan represents the imaging modality of choice to identify lung, lymph node, and liver metastasis [**LOE 2; GOR B**].

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C7

Urinary Diversion

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CONTENTS

Urinary Diversion

7.1 General Aspects

7.1.1 Metabolic consequences

The bowel is commonly used for reconstructive purposes when performing urinary diversion (UD). The subsequent consequences may vary depending on the bowel segments used. Loss of bowel length reduces the absorptive capacity of the bowel and its contact with urine, resulting in a shift of electrolytes and consequent metabolic changes. These are influenced by comorbidities such as impaired renal and hepatic function, and previous bowel resection, as well as by the patient's age. Moreover, the movement of water and electrolytes is impacted by the bowel segment used for diversion, the length of the bowel segment, the time that the urine is retained in the reservoir, the concentration of urinary solutes, urinary pH, and osmolality. In this chapter, we will discuss the risks and metabolic consequences associated with the bowel segment resected for UD.

7.1.2 General considerations regarding reabsorption of water and urinary solutes

The movement of water through the bowel wall mainly depends on the osmotic gradient and the efficiency of tight junctions. While the wall of the stomach shows the least water permeability, large water shifts are seen in the jejunum. Therefore, use of the jejunum as a segment for UD is not recommended. The ileal wall is more water-permeable than the colonic wall. However, when there is prolonged contact of the urine with the reservoir wall, iso-osmolarity will occur in all segments. This movement of water will result in loss or reabsorption of water, depending on the initial osmolality of the urine.[1] Urine osmolality depends on many factors, such as fluid intake and diet, and may be particularly altered in the presence of illness and dehydration. In addition, water movement depends on the length of the bowel segment used and the duration of contact of the urine with the bowel wall. While only a minority of patients suffer from metabolic consequences after conduit diversions, this risk increases to 50% in the case of continent diversions.[2] Impaired renal function reduces the capability to compensate the water movement through the bowel wall, and in these patients, a noncontinent diversion should be considered. Finally, the absorptive capacity, particularly of ileal reservoirs, decreases over time. Over the passage of time, only some patients will have significant problems and require treatment for acidosis.[3,4]

7.1.2.1 Stomach

Selecting the stomach for segmentation has the obvious disadvantage of its anatomic distance to the pelvic floor and is rarely used for orthotopic reconstruction in adults. The use of the body of the stomach may lead to hypergastrinemia due to the reduced negative feedback by acid that is mainly secreted in the reconstruction rather than the remaining stomach. A potential consequence is ulceration in the reservoir. The parietal cell in the stomach produces the intrinsic factor needed to form complexes with vitamin B_{12}. The formation is mandatory for vitamin B_{12} absorption in the terminal ileum. The disconnection of the stomach from the gastrointestinal tract may lead to decreases in the available intrinsic factor and subsequently to vitamin B_{12} deficiency. Due to the loss of hydrochloric

acid, sodium, and potassium in the urine, patients may become dehydrated and present with hyponatremic, hypochloremic, and hypokalemic alkalosis. This can be reversed by increased fluid intake, carbonic acid taken with meals, and intravenous saline replacement.[5]

7.1.2.2 Ileum

This segment is frequently used for several reasons. First, the length of the mesentery almost always allows a tension-free anastomosis with the pelvic floor. Second, only rare pathologies, such as diverticula or malignancies, prevent its use. Third, the loss of a significant length of the ileum in terms of its specific absorptive properties is tolerable. Moreover, in a functional terminal ileum, only few malabsorptive sequelae are to be expected.[6] In particular, if no more than 60 cm of ileum is used, the remaining ileum will compensate this loss by dilatation, elongation, and villous hypertrophy. Bile acid depletion is rarely seen with resections of less than 100 cm of ileum and the liver is normally able to compensate by increased production. However, there may be an increase in bile acids entering the colon, which can account for altered stool frequency, such as in chologenic diarrhea.[7] As mentioned above, these alterations depend on several factors, including the osmolality of the urine. In the case of dilute urine, loss of sodium and chloride into the reservoir may be seen. This happens in exchange for potassium and hydrogen ions, and results in a hypovolemic, hypochloremic, hyperkalemic acidosis. The subsequent aldosterone release leads to increased potassium and decreased sodium diuresis. This maintains the dilute urine and supports the abnormality through persistent sodium loss in exchange for hydrogen ions and increased potassium absorption by the ileal reservoir wall. Therefore, the patient is advised to increase salt intake to prevent dilute urine. Administration of alkalinizing agents such as sodium bicarbonate is also effective in restoring normal acid-base status. Because ileal segments absorb more potassium than colonic segments, treatment with potassium citrate is also possible, but is generally more appropriate for patients with colonic reservoirs.

7.1.2.2.1 Terminal ileum

Like the proximal ileum, the terminal ileum is often used for orthotopic UD and the length of the mesenterium usually allows a tension-free anastomosis with the pelvic floor. Preservation of 35 cm to 50 cm of distal ileum has been shown to prevent vitamin B_{12} and bile acid malabsorption.[8] When the terminal ileum is used, about a third of patients require vitamin B_{12} supplementation.[9] The efficiency of tight junctions, the permeability of the wall, and the resorptive properties are virtually the same as for ileal reservoirs. Therefore, hypovolemic, hypochloremic, hyperkalemic acidosis can be observed when dilute urine enters the reservoir. Treatment should be performed as described for ileal reservoirs.

7.1.2.2.2 Ileocecal valve

The ileocecal region serves as a valve from the colon to the distal ileum and regulates the passage of content from the small bowel into the colon. Moreover, it regulates the small-bowel movement and increases the transit time from 0.8 hours to 2.5 hours. Consequently, resection of this segment may result in diarrhea and its reconstruction has been advocated when an ileocecal pouch is formed.[10] Due to its function as a valve from the colon to the distal ileum, reflux from colonic organisms in the distal small bowel may occur in the event of resection. This can lead to cleavage of bile acids from conjugates and reduction of their reabsorption, which may cause fat malabsorption and steatorrhea. Moreover, these unabsorbed fatty acids bind calcium, resulting in an increase in oxalate absorption and hyperoxaluria, and the formation of urinary tract stones. The increased small-bowel transit

reinforces this consequence. Treatment with cholestyramine 4 g three times per day has been shown to effectively reduce stool frequency.[11] However, cholestyramine also reduces the absorption of fat-soluble vitamins A, D, E, and K; therefore, long-term use should be avoided.[12] Moreover, patients should be discouraged from restricting their fluid intake to reduce diarrhea. This mainly results in dehydration and water loss due to hyperosmolar urine. The ileocecal valve also regulates the passage of small-bowel content to the cecum. Although rapid infusion may cause diarrhea, the large absorptive capacity of the cecum is usually not exceeded.

7.1.2.3 Colon

The location of the sigmoid colon and its proximity to the pelvic floor support the use of this segment for reconstruction. However, the anatomic distance to the pelvis rarely prevents the formation of ileal reservoirs. In general, the colon does not suffer malabsorptive sequelae. The role of the right-sided colon in the storage and absorption of salt is important for water recovery, which is particularly important in cases of concomitant resection of the ileocecal valve. The use of the right-sided colon therefore carries a high risk of bile salt loss, diarrhea, and vitamin B_{12} malabsorption. Osmotic equilibrium is slower and there is less water loss in colonic segments due to the higher efficacy of the colonic tight junctions compared to the ileal tight junctions. Water recovery in the colon is achieved through active sodium and chloride reabsorption. This can lead to hyperosmolarity with a subsequent decrease in aldosterone and an increase in antidiuretic hormone release. The highly concentrated urine results in further sodium and chloride absorption, which translates into a higher risk of hyperchloremic, hyperkalemic acidosis.[13] Due to the reabsorption of water, these patients also may have a higher incidence of hypertension. Treatment is virtually the same as for ileal reservoirs. However, the risk of hypokalemic acidosis is higher compared to ileal reservoirs; maintenance of a normal acid-base status can be achieved by administration of potassium citrate.

7.1.3 Clinical symptoms and follow-up of metabolic consequences

Regular monitoring of acid-base balance should be performed. Normal serum pH and bicarbonate levels does not exclude a compensated metabolic acidosis. Therefore, regular venous blood gas analysis and body weight measurement are required. If the patient becomes unwell and complains of epigastric burning or vomiting, one should suspect acidosis and electrolyte disturbance. In patients with impaired renal function and in cases of colonic diversion, the risk of metabolic acidosis is even higher. In these cases, one should consider using the ileum instead or performing a non-continent form of diversion. Moreover, regular voiding and complete drainage of the reservoir are critical.

Symptoms of vitamin B_{12} deficiency include anemia, fatigue, pallor, weakness, and shortness of breath. Gastrointestinal symptoms may also be reported, such as alterations in bowel motility (mild diarrhea or constipation). Importantly, severe neurological consequences of vitamin B_{12} deficiency may be irreversible, resulting in peripheral neuropathy (sensory and motor, with absent reflexes), spinal-cord degeneration (altered reflexes such as the Babinski reflex), optic atrophy, seizures, or dementia. The body storage of vitamin B_{12} takes about 2 to 3 years to exhaust, and appearance of deficiency after surgery may be delayed. Therefore, regular and careful monitoring of serum vitamin B_{12} is recommended. Confirmed vitamin B_{12} deficiency requires lifelong supplementation. A summary of the metabolic consequences associated with different bowel segments is shown in **Table 7–1**.

TABLE 7–1 Summary of the Advantages, Disadvantages, and Metabolic Consequences After Urinary Diversion According to Bowel Segment

Bowel segment	Advantages	Disadvantages	Malabsorption and metabolic consequences	Treatment of metabolic consequences
Stomach	▪ Least water permeability	▪ Anatomic distance to the pelvic floor	▪ Hypergastrinemia ▪ Vitamin B_{12} deficiency ▪ Hyponatremic hypochloremic alkalosis	▪ PPIs or H_2 blockers ▪ Irrigation of the bladder with bicarbonate ▪ Vitamin B_{12} supplementation* ▪ Fluid intake, carbonic acid, intravenous saline
Jejunum		▪ Highest water permeability; not recommended for urinary diversions		
Ileum	▪ No malabsorptive sequelae ▪ Rare pathologies that prevent its use ▪ Loss of significant length can be tolerated	▪ Higher water permeability than the colonic wall	▪ Dilute urine: hypovolemic, hypochloremic, hyperkalemic acidosis	▪ Oral sodium bicarbonate, catheter drainage, Ringer lactate infusion
Terminal ileum	▪ Length of the mesentery	▪ Higher water permeability than the colonic wall ▪ Impact on small bowel transit time; increased stool frequency when resected	▪ Vitamin B_{12} deficiency ▪ Dilute urine: hypovolemic, hypochloremic, hyperkalemic acidosis	▪ Vitamin B_{12} supplementation* ▪ Oral sodium bicarbonate, catheter drainage, Ringer lactate infusion
Ileocecal valve		▪ Regulates the small bowel transit time	▪ Steatorrhea ▪ Vitamin B_{12} deficiency	▪ Cholestyramine 3×4 g per day ▪ Vitamin B_{12} supplementation*

Abbreviation: PPIs, proton pump inhibitors.

* Lifelong supplementation required.
† Mainly in cases of concomitant resection of the ileocecal valve and use of the right-sided colon.
‡ Or potassium citrate, in cases of hypokalemic acidosis.

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TABLE 7–1 Summary of the Advantages, Disadvantages, and Metabolic Consequences After Urinary Diversion According to Bowel Segment, *Cont'd*

Bowel segment	Advantages	Disadvantages	Malabsorption and metabolic consequences	Treatment of metabolic consequences
Colon	▪ Less water-permeable than the ileal wall ▪ Proximity of the sigmoid colon to the pelvic floor ▪ No malabsorptive sequelae	▪ Storage function of right-sided colon	▪ Bile salt loss† ▪ Diarrhea† ▪ Vitamin B_{12} deficiency† ▪ Higher concentrated urine: hyperchloremic, hyperkalemic acidosis	▪ Vitamin B_{12} supplementation* ▪ Oral sodium bicarbonate‡ ▪ Catheter drainage, Ringer lactate infusion

Abbreviation: PPIs, proton pump inhibitors.

*Lifelong supplementation required.
†Mainly in cases of concomitant resection of the ileocecal valve and use of the right-sided colon.
‡Or potassium citrate, in cases of hypokalemic acidosis.

7.1.4 Recommendations

- The terminal ileum is frequently used and recommended for UD. The length of the mesentery almost always allows a tension-free anastomosis with the pelvic floor. **Level of evidence (LOE) 3; GRADE C**
- In a functional terminal ileum, few malabsorptive sequelae are to be expected. **LOE 3; GRADE C**
- Use of the right-sided colon carries a high risk of hyperchloremic, hyperkalemic acidosis. **LOE 3; GRADE C**
- When the terminal ileum is used, about a third of patients require vitamin B_{12} supplementation. Confirmed vitamin B_{12} deficiency requires lifelong supplementation. **LOE 3; GRADE B**
- Regular venous blood gas analysis and body weight measurement are required. **LOE 3; GRADE B**

7.1.5 Secondary tumours

Since Hammer first reported an adenocarcinoma 10 years following vesicosigmoidostomy (VST) in 1929,[14] well over 200 such cases following ureterosigmoidostomy (UST) have been published, the latency period usually exceeding 20 years. Currently, it is accepted worldwide that increased colon tumour risk represents a complication following UST: the risk is estimated as 8- to 550-fold greater than the general population, depending on the patient's age at the time of operation.[15,16] In a literature review from 2004, Austen and Kälble reported about 81 secondary tumours in UDs via isolated intestinal segments, the majority of them in ileocystoplasties.[16] Whether this means there is a generally

increased tumour risk in all UDs or whether there are differences between different types cannot not be determined, due to the absence of long-term follow-up data on the total number of procedures performed.

In the Crissey and Gittes rat model,[17] there was no significant difference in tumour growth following VST, with or without separation of urine and feces by a proximal colostomy. It was also revealed that urine alone can induce secondary tumours. The histological findings, however, were different in the 2 groups. In the colostomy group, both urothelial carcinomas and adenocarcinomas arose directly at the vesicosigmoidal anastomosis, whereas in the group without colostomy, only adenocarcinomas did so. In a second experiment, there was a significant decrease in the number of adenocarcinomas in rats with VST, from 40% to 5%, when a segment of small intestine was interposed between the bladder and the sigmoid colon. This finding suggests a diminished tumour risk in UDs using the ileum.[18] However, as always in animal experiments, the question is how these results can be translated to the human situation.

Tumour prevalence in the different forms of UD, the latency period of the tumours, and mean time from operation to data collection are shown in **Table 7–2**.

TABLE 7–2 Tumour Prevalence (secondary tumours per number of urinary diversions) in Different Forms of Urinary Diversions[19,52]

Type of urinary diversion	Secondary tumours/total tumours (%)	Tumour latency period, years Median (range)	Time from date of urinary diversion to data collection, years Mean (range)
Ileal neobladder	2/4,190 (0.005)	3.0	6.3 (1–23)
Ileocecal bladder	3/239 (1.26)	4.0 (2–10)	13.6 (1–21)
Colonic neobladder	1/70 (1.43)	6.0	9.9 (3–13)
Ileocecal pouch	3/2,181 (0.14)	12.0 (2–19)	8.8 (1–24)
Ileocystoplasty	4/233 (1.71)	21.5 (5–31)	10.5 (1–24)
Colocystoplasty	0/20	–	4.9 (2–13)
Ileal conduit	2/8,637 (0.002)	14.0 (2–20)	8.9 (1–37)
Colon conduit	1/430 (0.23)	40.0	24.4 (1–37)
Ureterocutaneostomy	0/1,138	–	10.3 (1–37)
Ureterosigmoidostomy	16/620 (2.58)	26.0 (4–38)	18.9 (1–37)

A German multicentre study[19] of 44 high-volume clinics analyzed 17,758 procedures, including all existing types of diversion techniques. In these 44 institutions, 32 secondary tumours were reported. Specifically, the tumour risk was 22-fold higher in USTs and 13-fold higher in cystoplasties than in the other continent diversions such as neobladders (NBs).

The tumour risk in ileal conduits and NBs is minimal.[19] The latency period of the secondary tumours (**Tables 7-3** and **7-4**) is shorter if the operation is performed because of malignant disease. Because of the latter finding, it can be speculated that an adenoma–adenocarcinoma sequence exists for secondary tumours, similar to that observed in normal colon carcinogenesis. As in the animal experiments, histological findings and tumour locations differed among the types of UD. Of the 16 secondary tumours in UST, 15 (94%) were adenomas and adenocarcinomas located directly at the urointestinal anastomosis, whereas 44% of the tumours in isolated intestinal segments developed in the intestinal part of the UD, and not all were adenomas and adenocarcinomas.[19]

Due to the theoretical selection bias because of missing follow-up data for some of the 17,758 patients, one should be careful when drawing conclusions from the study, especially concerning the low-volume diversions such as ileocecal or colon NBs. The significantly higher risk in ileocecal or colon NBs compared to ileal neobladders (INBs) could therefore be a result of either the increased colon cancer risk compared to the ileum or the UD itself. Nevertheless, tumour prevalence, histology, and location in high-volume diversions are in accordance with animal experiments,[17,18] case reports,[15,16] and epidemiological data,[15] suggesting a significantly increased tumour risk following UST and ileocystoplasty compared to the general population. By contrast, it appears that NBs, especially ileal conduits (ICs), bear a minimal to nonexistent specific tumour risk.

TABLE 7–3 Latency Period of Secondary Tumours Relative to Primary Indication for Urinary Diversion[19]

Primary disease	Ureterosigmoidostomy, years Median (range)	Cystoplasty, years Median (range)	Isolated intestinal segments, years Median (range)
Benign	28.5 (4–38)	28.0 (5–31)	33.0 (19–40)
Malignant	7.0 (6–22)	0	6.0 (2–20)

TABLE 7–4 Latency Period of Secondary Tumours Relative to Histologic Findings[19]

Primary disease	Ureterosigmoidostomy, years Median (range)	Cystoplasty, years Median (range)	Isolated intestinal segments, years Median (range)
Benign	22.5 (17–28)	18.0 (5–31)	6.0 (2–6)
Malignant	29.0 (4–38)	21.5 (14–29)	12.0 (2–40)

It may be concluded from these results that regular endoscopic controls in UST and cystoplasties are mandatory from the fifth postoperative year onward, whereas in the case of NBs, conduits, and pouches, it seems sufficient to perform endoscopy in the presence of symptoms such as hematuria or new-onset hydronephrosis. The cancer risk following UST is emphasized by the study of Petterson *et al.*[20] With 25 patients who had undergone UST for benign disease (mainly bladder exstrophy), a telephone interview was performed 49 to 66 years postoperatively. Only 2 patients were still alive with intact UST; 20 had been converted; 9 patients had developed secondary tumours and 7 of them had died. The authors concluded that when UST is performed in childhood, it should be routinely converted after puberty.[20]

Taking into account the fact that young adults with a well-functioning UST since childhood usually refuse to be converted as a matter of routine, the conclusion is that at least annual or biannual colonoscopies should be required in these patients. In addition, UST should be avoided in adults with a life expectancy of at least 20 years. Due to the increased cancer incidence in the colon compared to the ileum—independent of the specific risk—INBs should be preferred over colon or ileocecal NBs.

7.1.6 Recommendations

- For patients with a UST, regular endoscopic controls and cystoplasties are mandatory from the fifth postoperative year onward due to the higher risk of developing tumour (adenocarcinoma), whereas in the case of NBs, conduits, and pouches, it seems sufficient to perform endoscopy in the presence of symptoms such as hematuria or new-onset hydronephrosis.
  LOE 3; GRADE B

7.1.7 Pelvic radiation and urinary diversion

Radiation therapy plays an important role in managing cancers of the gastrointestinal, genitourinary, and gynecologic systems. Performing radical cystectomy (RC) and UD following a course of pelvic or abdominal radiation therapy, or both, can pose unique challenges, leading to a higher rate of perioperative and long-term complications. Pelvic radiation may produce damaging exposure of the cecum, appendix, and terminal ileum. Additional segments of small bowel located in the pelvis as a result of adhesions from prior treatment or disease may also be affected as a result of radiation scatter. Furthermore, pelvic tissues may be affected as a result of prior radiation exposure, leading to fibrosis that eliminates normal tissue planes and detrimentally affects the ability of the surgeon to preserve critical structures involved in continence.

Radiation effects leading to small-vessel damage may also affect the distal ureteral segments in the pelvis, leading to suboptimal healing and increasing the risk of developing ureteroenteric stricture. Intraoperative inspection of the small and large intestine plays an important role in the selection of appropriate bowel segments for the desired reconstruction. Bowel that shows thickening or fibrotic changes from prior radiation exposure should be avoided, as use of such segments may lead to poor healing and urinary leaks. Additionally, fibrotic bowel will not expand well, leading to higher internal pressures and poor overall functional outcomes. Great care should be taken to use non-irradiated segments for the bowel anastomosis when re-establishing continuity of the gastrointestinal tract to minimize the risk of leaks, abscess, and possible fistula formation.

7.1.7.1 Conduit urinary diversion

Initial recommendations for UD in patients who have received pelvic radiation included the preferred use of the transverse colon as a conduit.[21–23] The presumption was that avoiding terminal ileal segments of the small bowel would minimize complications such as perioperative leaks and long-term risk of strictures.[24]

Although use of the transverse colon minimized the potential for using a radiated segment of intestine, a series of transverse colon conduits in irradiated patients demonstrated that the long-term risk of stricture development at the ureteral–colonic connection was not eliminated.[25] Others reported that ileum could be used safely with a complication risk similar to that observed with colonic conduits

in the setting of prior pelvic radiation treatment. In a series of 62 patients who failed definitive radiation therapy for their bladder cancer, IC diversion was performed at the time of salvage cystectomy. In this series of patients, treated between 1969 and 1979, 2 patients developed a perioperative urinary leak. A total of 4 ureteroileal strictures were reported during longer-term follow-up.[26] A series reporting UD complications in 212 gynecologic patients have also documented morbidity associated with conduit formation. These patients, who had a wide range of gynecologic primaries and of whom 93% of whom had received prior pelvic radiation therapy, underwent surgery that included the formation of a conduit diversion using the ileum, sigmoid colon, transverse colon, or jejunum. Overall complications included a 3% rate of urinary leak or fistula; 17% of patients lost a renal unit; and 6% required surgical revision of the conduit stoma.[27]

7.1.7.2 Continent cutaneous urinary diversion

Although experience with the conduit continued in the 1970s and 1980s, evidence emerged that patient quality of life (QoL) may be improved by using alternative diversion options, such as continent cutaneous urinary reservoirs.[28] Unlike the conduit, which required lifelong use of an external appliance, continent cutaneous reservoirs provided the advantage of internal storage of urine. The elimination of the external bag was thought to improve QoL, particularly body image.

A variety of techniques have been developed to fashion the body of the reservoir, the catheterizable channel, and the continence mechanism. As the use of continent cutaneous UD became more popular, reports emerged on outcomes in patients with prior radiation therapy.[29–33] Comparison of complications following RC and UD remains a challenge. Variations in methodology for collecting and reporting complications have made it difficult to achieve valid cross-comparison between series, including comparisons between series of irradiated and non-irradiated patients. A perioperative urinary leak was observed in 20% of the irradiated patients, a rate not significantly different from that reported in a similar contemporary group of non-irradiated patients who also received a Kock urinary reservoir. Diarrhea requiring hospitalization or medical therapy was more common in the radiated group: 18% versus 2%.[33] Others reported similar outcomes using the Kock pouch, with complications comparable with those in non-irradiated patients.[34]

Right colon- or ileocecal-based reservoirs have also been used in previously irradiated patients. The potential advantage of using the ascending colon is that the fixed segment of right colon may be less likely to have sustained damage from radiation directed at the pelvic region. Initial reports using the ileocecal pouch designs noted reasonable early complication rates.[30,35,36] However, longer-term follow-up revealed that complications involving the stoma and ureterocolonic strictures were more likely to develop in the post-radiation setting. A patient series in which 130 modified Indiana pouches were performed demonstrated a 5-fold increase in ureteral strictures in irradiated patients compared to those who had not been irradiated prior to surgery.[37] A similar 3-fold increase in the risk of ureteral strictures in irradiated patients was reported in a series in which Mainz 1 reservoirs were used.[29] In a series of 62 irradiated women, Penalver *et al.* reported that one-third of patients experienced ureteral complications.[38] Based on the high reported ureterointestinal complication rates, technical alterations were recommended, such as shortening the length of the ureteral tunnel to lower the obstruction rate.[37]

Others have used designs that include an afferent ileal limb to the reservoir, allowing the use of more proximal ureteral segments to avoid damage to distal ureteral segments.[39,40] Use of an afferent limb provided a means to discard the segments of irradiated distal ureter, permitting the ureterointestinal connection to be made with healthy, better vascularized proximal ureter.

Bochner *et al.* demonstrated the use of an afferent limb as a ureteral substitution segment in a ureteroileocecal appendicostomy design led to a reduction in the long-term ureterointestinal stricture rate to 11%.[32] Differences in stomal complications for continent cutaneous reservoirs in the irradiated setting have also been evaluated.[29,31,38,39,41,42] Ileocecal value-based, intussuscepted ileal nipple systems or appendiceal flap valve mechanisms for continence have been described.

Given the proximity of the cecum, ileocecal valve, and appendix to the pelvis, knowing whether these tissues perform similarly in patients with prior pelvic radiation is critical to understanding the long-term complication risk. Wammack *et al.* reported outcomes of a series of 36 irradiated patients compared to 385 non-irradiated patients. The continence mechanism was preferably the appendix; however, an invaginated ileal nipple valve was used in those patients in whom the appendix was unsuitable. After a median follow-up of 57 months of patients who had received a Mainz 1 reservoir, a 38.9% versus 10.6% stomal stenosis rate and a 25% versus 5.7% stomal continence failure rate were noted in irradiated versus non-irradiated patients, respectively.[29] Using the appendix mechanism as the catheterizable channel has proven to be a reliable valve to prevent leakage; however, stomal stenosis does occur at a relatively high rate. In the non-irradiated setting, only a minor local procedure is required to resolve appendiceal stomal stenosis, but following radiation therapy, open or more extensive revisions are more frequently required.[29] Bochner *et al.* found that stomal stenosis occurred in 23% of irradiated patients who received an appendiceal stoma as part of a ureteroileocecal appendicostomy reservoir, but no cases of significant stomal incontinence were observed.[32]

7.1.7.3 Orthotopic urinary diversion

Historically, it was thought that patients who have received prior pelvic radiation therapy would not be appropriate candidates for an orthotopic reconstruction. As highlighted above, damage to the lower intestinal system and ureters was thought to result in higher rates of diversion-related complications. Additionally, the radiation-related pelvic fibrosis and subsequent scarring was thought to potentially lead to poorer functional outcomes, either as a direct result of damage to the sphincteric mechanism, surrounding innervation, or vascular supply or secondarily, as a consequence of more difficult surgical dissection at the time of salvage cystectomy.

Several decades of surgical experience with orthotopic reconstruction have clearly demonstrated that the ability to spare important control muscles, neural innovation, and vascular supply to the remnant urethra optimizes functional recovery following RC and NB reconstruction. As early experience with orthotopic reconstruction grew, some centres began to selectively offer NB reconstruction to patients who had received pelvic radiation.[43,44]

Bochner *et al.* reported one of the earliest experiences of orthotopic reconstruction in the post-radiation setting by means of a hemi-Kock pouch (bilateral ureteroileal urethrostomy).[44] A total of 18 patients with a median of 28 months' follow-up were studied. Most underwent RC or anterior extenteration, except for 3 patients who underwent total pelvic exenteration. A total of 6 complications

were reported (33%), including an afferent limb stenosis, prolonged urinary leak in 2 cases, and an enterocutaneous fistula. Daytime complete control was reported in 67% with nighttime complete control in 56%. Hautmann *et al.* reported a 22-year experience with orthotopic reconstruction after RC or anterior exenteration.[45] Of 1,570 patients treated during this period, 25 (1.6%) were selected for salvage surgery after radiation NB reconstruction. Using a comprehensive complications database, they noted that 66% of the 25 salvage RC and NB patients experienced a complication of any grade within 90 days after surgery, an overall complication rate comparable to that seen in other larger, non-irradiated RC series using similar methodology for collection of data on complications.[46] Major complications (grade 3–5) were noted in 28% of these patients; continence was described as seriously compromised in 24%. Eisenberg *et al.* reported on 148 irradiated patients, including 48 who received NBs after RC.[47] Using similar standardized complication-reporting techniques, a 77% overall complication rate was reported. The postoperative complication rates were not significantly different when comparing continent (NB, cutaneous reservoir) diversion to the IC patients.[47] It would seem from the cumulative evidence published to date that in properly selected patients, orthotopic reconstruction can be performed with reasonable functional outcomes. Although complications can be expected in the majority of patients undergoing RC and UD, the overall complication rates in irradiated patients are similar to those in non-irradiated patients undergoing similar extensive surgery and reconstruction.

7.1.7.4 Postdiversion pelvic radiation therapy

One additional area of concern regarding radiation and UD arises in patients who have already undergone reconstruction but are exposed to postoperative radiation therapy. This is primarily important in patients who have undergone orthotopic reconstruction and require pelvic radiation after surgery. This situation may arise in urothelial cancer patients who experience a pelvic recurrence following surgery; however, colorectal cancers may develop in the postoperative period in patients who have undergone NB reconstruction and require pelvic radiation therapy.

Only a limited amount of literature directly addresses this issue, but a recent multi-institutional evaluation reported on 25 NB patients with a median follow-up of 10 months.[48] Most patients received adjuvant radiation therapy and the dose ranged from 39.6 Gy to 65 Gy. The reported maximal dose of radiation to the NB was available for 12 patients and ranged from 42.6 Gy to 58 Gy. Sixteen patients had a gastrointestinal toxicity of grade 2 or less and 12 reported genitourinary system toxicity. Only one of those experiencing genitourinary toxicity had a grade 3 issue (hematuria requiring transfusion), while of the remaining 11, three had grade 2 and eight had grade 1 toxicity. Although the follow-up was limited in this series, it would appear that the NB can tolerate moderate levels of radiation, although gastrointestinal toxicity remains a concern.

7.1.8 Recommendations

- Radiation damage to the cecum, appendix, and small bowel must be considered and evaluated when determining the most appropriate form of UD. **LOE 3; GRADE B**

- Patient selection and adherence to meticulous surgical technique can provide acceptable outcomes in irradiated patients who require diversion. **LOE 3; GRADE B**

7.1.9 Pregnancy and sexual dysfunction after radical cystectomy and urinary diversion

7.1.9.1 Introduction

While QoL and functional issues related to sexuality and sexual health are fundamental problems that affect most men and women who undergo cystectomy and UD, they often do not receive the warranted attention relative to other issues and concerns that these patients may face. This gap likely exists for several reasons, ranging from the older age distribution associated with cystectomy and UD, to more immediate and pressing concerns and consequences that patients struggle with after surgery. Still, cystectomy and UD can have a tremendous impact on sexual health that is long-lasting, if not permanent, in most cases.[49]

Although some men and women may place concerns about their sexual health on hold while they focus on more immediate challenges, such as adjusting to their new life with a UD, others may want to be more proactive about regaining sexual function and returning to a more normal sex life. Because impacts on sexual health and function resulting from cystectomy and UD are lower priority issues, however, patients may be ill-informed regarding their frequency and causes, and physicians may underappreciate them or be ill-equipped to manage them. Although it is clear that cystectomy and UD contribute to sexual dysfunction,[50,51] the interconnection of the contributing and causal factors can be confounding. For example, factors that contribute to sexual dysfunction after cystectomy and UD include physical and functional changes, changes in self-perception and body image, emotional issues related to recovery from major surgery and adjustment to a new normal, and social strains in a relationship that may negatively affect intimacy and partner interest.[52]

Bundling these different factors within a taxonomy is not necessarily straightforward, in part because of their overlap, but in general, a few broad categories (that is, physical and iatrogenic, psychological, partner, and life course–related) should be considered. Besides briefly reviewing each of these contributing or causal groups, this review will consider the incidence of and risk factors for male and female sexual dysfunction occurring after cystectomy and UD, as well as management strategies. In addition, existing data regarding reproductive health and pregnancy after UD performed with cystectomy or as a stand-alone reconstructive surgery will also be reviewed.

7.1.9.2 Taxonomy of sexual dysfunction

7.1.9.2.1 Organic and iatrogenic

Age-related sexual and erectile dysfunction is common in the general population, so the contribution of underlying sexual dysfunction (such as erectile dysfunction in men) should be considered. The prevalence of erectile dysfunction among American men older than 50 years of age who also have hypertension or diabetes, for example, is approximately 50%.[53] The incidence of erectile dysfunction rises to over 70% in men aged 70 years and older, which is near the average age of bladder cancer patients who are managed with cystectomy.[54] Beyond age-related functional decline, iatrogenic injury associated with extirpative pelvic surgery is one of the main determinants of sexual dysfunction after cystectomy and UD.[52] In men, this means loss of erections associated with injury to the cavernosal nerves. In women, collateral dissection or resection of vaginal tissue can result in iatrogenic changes in vaginal size, capacity, compliance, or function. Other iatrogenic consequences should also be noted, including orgasmic dysfunction and changes in penile length that can occur after cystoprostatectomy, regardless of return of erectile function.[55]

7.1.9.2.2 Psychological

Psychological stressors are not uncommon after major surgeries, particular ones that result in an altered body.[56] Though not necessarily directly linked to sexual dysfunction through physical or physiological pathways, the emotional reaction to or preoccupation associated with a urostomy, or poor urine control and nighttime accidents after NB, can effectively dampen intimacy and interest in sex for patients with UDs, resulting in sexual dysfunction. Although NB diversions may mitigate some of the social interference associated with UD, it is not fully clear whether self-perception or body image differs significantly between patients after NB or IC diversion, so it is possible that psychological factors associated with body image issues could contribute to psychogenic sexual dysfunction in both groups.[57,58] However, depression and anxiety do appear to be relatively low in most men and women after cystectomy and UD, and the exact way and extent to which emotional factors or distress cause sexual dysfunction in this patient population is unclear.[59]

7.1.9.2.3 Life course and partner response

Patients may also experience fundamental changes in both their relationships and their life priorities. Månsson *et al.* studied postoperative adjustments in patients after cystectomy, and found that although relationships with friends did not change, intimate relationships with spouses or partners were strained by sexual problems.[60] In another study, Somani and colleagues interviewed 32 cystectomy patients before and after surgery and found that social relationships were a major determinant of QoL.[61] These data highlight the importance of relationships and other social factors on patients' perceptions and health outcomes. Recognizing the relationship between social and psychological stresses related to UD and sexual dysfunction is critical. Altered body image after undergoing either a conduit or continent UD, and the anxiety associated with potential urinary incontinence can further negatively impact sexual function. In addition to the patient's perceived psychological distress, partners experience stress related to UD. The presence of a stoma, external urostomy appliance, or catheterizable channel may contribute to sexual dysfunction or a lack of sexual interest among couples. While the effect of repulsion to sexual intimacy in UD patients has not been well studied, its marked effect has been demonstrated among colon ostomates.[62]

7.1.9.3 Male sexual dysfunction

Changes in sexual function and health may be underappreciated after cystectomy and UD. For example, erectile dysfunction has been reported in up to 80% of men.[63] In one study, Månsson and colleagues reported significant decreases in average erectile function scores after cystectomy and UD, which dropped from 2.3 prior to surgery to less than 0.2 afterward on a 5-point scale of the FACT-BL questionnaire.[64] In another study by the same group, almost every man surveyed (total *n*=65) reported that their sexual potency had been negatively affected by surgery, and only 17% (11/65) were able to achieve erections after cystectomy and UD.[59]

Other studies also report both low interest in sexual activity and inability to maintain erections after cystectomy and UD, although that finding is not universal.[63,65] In contrast, for example, Hekal and colleagues reported that most men treated with cystectomy and UD could achieve adequate erections when nerve-sparing surgery was performed.[66] In that case series, 12 of 21 patients had complete and spontaneous tumescence after nerve-sparing cystectomy, while 5 of 21 required phosphodiesterase-5 (PDE-5) inhibitors and 4 required intracorporeal injections (ICIs) of prostaglandin E1 (PGE1). All patients in the non–nerve-sparing cystectomy group required injection therapy.[66] Similarly, when Zippe and colleagues compared return of erections in 49 men treated with nerve-sparing cystectomy or standard non–nerve-sparing cystectomy, they found a 50% rate (8/16) of return of erectile function in men treated with nerve sparing compared to only 14% (9/49) in men treated with cystectomy and UD that did not include nerve sparing.[67] Other studies suggest that prostate-sparing cystectomy and UD may preserve sexual function postoperatively as well.[68,69]

Management of male erectile dysfunction after cystectomy and UD typically begins with oral medications and then moves to more direct therapies (e.g., injections) before surgery is considered. First-line therapy typically consists of PDE-5 inhibitors, followed by ICI or transurethral suppositories. In cases in which medical therapy fails, surgical management with penile prosthesis placement is an alternative effective, albeit invasive approach. PDE-5 inhibitors have a long track record in managing organic and iatrogenic erectile dysfunction. In the context of prostatectomy, the effectiveness of PDE-5 inhibitors appears to be directly related to the degree of preservation of neurovascular bundles at the time of surgery. For example, Zippe *et al.* showed that 71.7% of patients with neurovascular bundle preservation responded to PDE-5 inhibitor therapy, while only 15.4% those with neuromuscular bundle sacrifice responded to medical therapy.[70] This class of medication has been studied for both on-demand use and for penile rehabilitation. Studies involving men treated with prostatectomy have shown a statistically significant benefit of PDE-5 inhibitors over placebo with daily use for rehabilitation.[71–74]

In patients who do not responded to PDE-5 inhibitors, ICIs and transurethral suppositories represent effective treatment options. Injection therapy typically consists of a combination of alprostadil, a PGE1 derivative, papaverine, and phentolamine. Alprostadil stimulates adenylate cyclase to increase conversion of AMP to cAMP, while papaverine inhibits phosphodiesterases, and phetolamine inhibits α-1 receptors to prevent detumescence. Injection therapies have also been studied in the rehabilitative setting and for on-demand use after prostatectomy. ICI has shown to be effective post-prostatectomy, even in those in whom PDE-5 inhibitors has failed.[75–78]

Similarly, transurethral suppository therapy has been used in post-prostatectomy patients and is an effective tool in the treatment of erectile dysfunction after surgery.[79] The barriers to these treatments include burning, pain, and discomfort with self-injections or urethral administration.

Sexual counselling is an important additional measure to address these potential barriers to adherence. For example, counselling has been shown to improve the efficacy of ICI treatment by decreasing dropout rates and even increasing the number of patients who respond to first-line oral therapy.[77,80] These strategies appear to be effective among cystectomy patients as well. For example, response rates to a rehabilitative program of initial PDE-5 inhibitor therapy, with escalation to ICI if necessary, resulted in 58% of patients achieving erections with PDE-5 inhibitors alone after nerve-sparing cystectomy and UD.[66] Another 21% reported partial erections with oral medications, while a slightly smaller percentage needed to transition to injection therapy.[66]

Timing of rehabilitation also appears to make a difference. In one study involving bladder cancer patients managed with nerve-sparing cystectomy and UD, men who started PDE-5 inhibitor therapy earlier (2 months after surgery) achieved better erectile function compared to those who started at 6 months postoperatively.[81] Despite the evidence supporting penile rehabilitation, use of erectile aids appears to be fairly limited after cystectomy and UD. In a population-level study examining the use of erectile dysfunction treatments, overall use varied from 8% to 15% after cystectomy and UD, with PDE-5 inhibitors representing the most common type of therapy prescribed or used for erectile dysfunction.[82] These results seem staggeringly low relative to the high rates of erectile and sexual dysfunction reported among men who undergo cystectomy and UD.

7.1.9.4 Female sexual dysfunction

In females, sexual dysfunction after UD is primarily related to nerve damage affecting sensation, changes to vaginal anatomy that affect compliance, capacity, or both, and decreased lubrication.[83] In one study, 80% of women who had been treated with vaginal-sparing cystectomy and UD remained sexually active.[84] In contrast, others have reported more disappointing results. Zippe and colleagues found that fewer than half of women were sexually active after cystectomy; the most commonly reported complaints were inability to achieve orgasm (45%), decreased lubrication (41%), decreased sexual desire (37%), and dyspareunia (22%).[52]

Another recent study reported that more than 65% of women were sexually active after vaginal-sparing cystectomy.[85] Booth and colleagues recently noted that sexual activity decreased from a baseline of 78% prior to cystectomy to 37% after surgery in 71 women surveyed with the Female Sexual Function Index (FSFI) 1 year after cystectomy and UD. Problems with lubrication, orgasm, and pain were the most distressing issues for these women.[86]

Perhaps more concerning, a significant number of women may experience or perceive a deleterious change in the intimate relationship with their partner. For example, one study found that 39% (29/73) of women reported worsening relationships with their husbands, and that 26% of previously sexually active women became inactive after cystectomy and UD.[87] As in other studies, relatively poor sexual functional outcomes and satisfaction were related to lost libido (89%), dyspareunia (43%), loss of orgasms (63%), and urinary problems related to sexual activity (63%).[87,88] Volkmer and colleagues reported that younger age (younger than 60 years), cystectomy performed for non-cancer indications,

and partnership are important factors that influence sexuality and sexual function after cystectomy and UD. In this recent review of pooled results of 11 studies covering 361 women, loss of sexual desire, and orgasm disorders were found to be the most frequent problems that contribute to female sexual dysfunction after cystectomy and UD. Dyspareunia was reported by a quarter of women, while vaginal lubrication disorders were reported by nearly 10%. Organ-sparing cystectomy markedly decreased the incidence of sexual dysfunction from approximately 60% to only 10%.[89] Rubenwolf and colleagues reported on sexual function and fertility among women who were managed with continent UD for classic bladder exstrophy.[90] Twenty-nine women managed between 1969 and 2014 reported a mean FSFI score of 28.4 out of a total score of 36. According to responses to the FSFI, 31% of the women met criteria for sexual dysfunction.[90]

Management of female sexual dysfunction is based on its principal cause. Currently, female sexual dysfunction is defined as a disorder of sexual desire, arousal, orgasm, and sexual pain that results in distress.[83] Because sexual dysfunction after RC is typically iatrogenic in nature and can be caused by permanent consequences associated with cystectomy, such as nerve injury or altered vaginal anatomy, hormonal therapy, a mainstay of female sexual dysfunction management, may or may not be effective. Psychogenic causes should be evaluated and ruled out; referrals for sexual counselling may be useful in such cases. Medical therapy may consist of estrogens, androgens, dopaminergic agonists, nitric oxide donors, prostaglandins, or α-melanocyte–stimulating hormones.[83] Women may also benefit from pelvic floor rehabilitation and massage after healing from their surgery, in order to soften tissue planes and improve the compliance of vaginal tissue. The most effective treatment for female sexual dysfunction associated with cystectomy and UD is preservation and prevention. Limiting collateral tissue injury or removal using more limited dissection, such as with vaginal and urethral-sparing cystectomy, is associated with a lower risk of female sexual dysfunction and higher rates of sexual satisfaction after surgery.[91]

7.1.9.5 Pregnancy after urinary diversion

Pregnancy following fertility-sparing cystectomy and UD is possible, but rare, so limited information has been published about it. Not unexpectedly, the information that is available comes from small case series consisting of younger women treated with cystectomy, diversion, or both, for mostly non-cancer indications.[92]

Although data regarding pregnancy outcomes in women previously treated with cystectomy and UD are sparse, a comprehensive review of 252 pregnancies among 188 women after UD was reported by Hautmann and colleagues, and provides perhaps the most reliable data on this topic.[93] Of those cases, the majority were not contemporaneous, surgeries were typically performed for non-cancer indications, and diversions ranged across a fairly broad spectrum: 57 were in women with conduit UDs, 47 in women with enterocystoplasty, 64 in women with UST, 19 in women with a colon pouch, and 1 in a woman with an NB.[93]

Several problems appear to be relatively frequent across different types of UD. Antenatal hydronephrosis, symptomatic or clinically significant urinary tract infections, and changes in urine elimination (e.g., leakage, catheterization) are the most common. Pregnancy may interfere with normal

diversion emptying during the middle and late stages of the pregnancy, and recurrent urinary tract infections are also a concern. Although vaginal delivery is possible after UD, most deliveries have been performed by caesarean section.[93]

Early case reports regarding successful pregnancy after UD appeared in the medical literature starting in the late 1960s.[94–96] Schumacher and colleagues reported on their experience of 6 women (18–33 years old) with Mainz pouches for congenital urogenital conditions. The women later became pregnant and were delivered via caesarean section. Three of 7 pregnancies were uncomplicated, while the other 4 were notable for hydronephrosis, pyelonephritis, or both. All pregnancies resulted in healthy newborns.[95]

Pregnancy after UST may be characterized by hydronephrosis, urinary tract infections, and exacerbated metabolic acidosis, which can be managed with sodium potassium hydrogen citrate.[97,98] A survey of 54 women with urostomies who became pregnant revealed fertility problems in 15% of survey respondents and a high rate of stomal problems (68.5%) in the late term of the pregnancy (second or third trimester). It is noteworthy that stomal issues typically were successfully managed conservatively without surgery.[99]

Most reports of pregnancy following continent UD have been in the setting of catheterizable colon pouches, and characterized by near-universal hydronephrosis, bacteriuria, and urinary tract infections that can be successfully treated with culture-directed antibiotic therapy, prophylactic therapy, or both, with occasional need for percutaneous nephrostomy.[100]

Other studies of women with either a UST or IC reported no clinically significant upper-tract obstruction and safe vaginal delivery, with caesarean delivery reserved for obstetric indications.[101,102] In the case of orthotropic diversions, the lower segment of the uterus—the typical site of entry into the uterine cavity—cannot be easily or safely accessed, and requires an upper segment caesarean section.[91,103] A case series of 11 women treated in childhood or adolescence with UD who then became pregnant reported the experience and outcomes of 12 pregnancies delivered between 1989 and 2003.[104] Problems with emptying requiring clean intermittent catheterization were universal and most women (10/12) experienced a urinary tract infection during their pregnancy. Eight pregnancies were delivered by caesarean section, 2 by vaginal delivery, and 1 by combined delivery. All cases returned to baseline urinary function and emptying after delivery.[104]

7.1.10 Recommendations

- The etiology of sexual dysfunction after UD is multifactorial, and although effective management options are available, broad awareness of the importance of these long-term consequences of cystectomy and UD have gained a firm footing only over the last several years. **LOE 2; GRADE B**

- Nerve-sparing and organ-preserving approaches to surgery are the most effective strategies to avoid the constellation of complications that negatively affect sexual function and pregnancy. **LOE 2; GRADE B**

7.1.11 Enhanced recovery after surgery

There has been an increasing focus on improving perioperative outcomes for patients undergoing cystectomy with UD. In many high-volume cystectomy centres across the globe, the previous orthodoxy regarding perioperative care after cystectomy has been replaced with new approaches commonly referred to as enhanced recovery after surgery pathways, which purportedly expedite postoperative convalescence, decrease costs, and improve quality.[105] Originally described in the late 1990s,[106] ERAS pathways are standardized multidisciplinary protocols that aim to improve surgical outcomes by reducing variation in perioperative and postoperative best practices.[107] Despite the interest in ERAS pathways among urologic surgeons, however, the evidence supporting their use in cystectomy patients is not always rooted in robust experimental data. In this chapter, we performed a nonsystematic narrative review of the literature with 4 pedagogic objectives in mind: 1) to describe the key elements of ERAS protocols for cystectomy patients; 2) to describe the theoretical reasons behind some of these elements as well as ERAS pathways in general; 3) to review the available data supporting the use of ERAS pathways in cystectomy patients, with an emphasis on the methodological limitations that temper strong causal inferences; and 4) to highlight areas of research that should be prioritized by future investigators in this space.

7.1.11.1 Key elements of ERAS pathways for cystectomy patients

While there is no single best ERAS pathway, several common themes have emerged across many different published pathways for cystectomy patients. What follows is a brief synopsis of these key elements, categorized according to timing in relation to surgery (preoperative, intraoperative, and postoperative). Recognizing that many of these elements may vary according to institutional preference as well as type of surgery (open vs. robot), these key features seem to have the most universal acceptance.

7.1.11.1.1 Preoperative

Preoperative verbal, written, and videographic information for the patient and caregiver is an important part of the ERAS pathway. This includes details about the operation, hospitalization, postoperative care, and expected functional trajectory. Providing the patient with comprehensive information ensures compliance with protocols, sets expectations, and equips the patient with the resources necessary to answer the myriad questions that arise during the preoperative evaluation. Malnourished patients are at increased risk of complications and should receive perioperative enteral nutrition support if needed.[108–110] Current smokers also have a higher than expected postoperative complication rate, making smoking cessation an integral part of the ERAS pathway as well.[111] It is unknown whether physical activity prior to cystectomy is beneficial, but for the deconditioned patient, a regimented exercise program may offer some benefit.[112] While there is some evidence to support the omission of mechanical bowel preparation among cystectomy patients,[113,114] there is no strong evidence supporting a preoperative fasting period. In fact, some evidence links preoperative carbohydrate loading with improved muscle strength, reduced hospitalization stay, and return of gut function.[115]

7.1.11.1.2 Intraoperative

Venous thromboembolism (VTE) is a major complication after cystectomy with an incidence of at least 3%.[116] Compression stockings and periprocedural prophylactic heparin should be administered to reduce the risk of VTE; some groups even advocate prolonged use (up to 4 weeks postoperatively).[117] Use of a thoracic epidural is controversial. Epidurals can result in peripheral vasodilatation and postural hypotension, which may hamper early ambulation.[117] Nevertheless, some have advocated their use in the open literature,[118] while others have advocated their avoidance.[119] Whether and for how long a drain should be left near the resection site or diversion site is also controversial. Because the drain can potentially impair bowel recovery, some have suggested that it should be omitted.[120] Yet, few surgeons have ever regretted leaving a drain after surgery, as long as it is removed in a timely fashion, if not otherwise needed for the management of a leak or infection.

With respect to anesthesia, the key goals are to prevent hypothermia and hypoxemia, and to avoid hypovolemia, overhydration, and the use of opioid-based analgesics.[121] While a high level of evidence is lacking in this regard, careful monitoring and the maintenance of open lines of communication between the surgical and anesthesia teams is critical to reduce surgical and anesthetic-related complications.

7.1.11.1.3 Postoperative

Several studies have found that early removal of the nasogastric tube can reduce postoperative complications.[122–124] The practice at the Arizona Mayo Clinic is to remove it immediately after extubation. With respect to the ureteral stents and Foley catheters (for orthotropic diversion patients), no study has yet defined the optimal timing for removal. Perhaps the most common complication after cystectomy and UD is postoperative ileus. To a large extent, ERAS protocols have been developed in an effort to mitigate this complication. Promotility drugs, such as metoclopramide, erythromycin, serotonin receptor antagonists, and naloxone, have demonstrated less satisfactory results.[117] However, alvimopan has demonstrated promising results, showing earlier return of bowel function, reducing costs and hospitalization lengths.[125,126] Chewing gum, avoidance of narcotics, early mobilization, early oral feeding, and robotic approaches may offer additional benefits in this regard.[127–129]

7.1.11.2 The theory behind the beneficial effect of enhanced recovery after surgery

There are several theoretical reasons why ERAS protocols would improve outcomes for cystectomy patients. First, many of the principles of ERAS are rooted in human physiology. For example, preoperative carbohydrate loading leads to better insulin sensitivity, and helps preserve lean body mass and muscle strength[130]; judicious fluid management reduces the risk of postoperative ileus by maintaining splanchnic perfusion[131]; and early ambulation and prompt oral feeding promote homeostasis.[118] These and other aspects of ERAS protocols make use of well-known principles in human physiology to optimize the recovery of patients who are undergoing an extraordinarily complex operation with well-documented risks.

Second, ERAS protocols use a multidisciplinary approach which is adaptive to local care needs that have been identified at the organizational level. Unlike national guidelines, ERAS pathways are designed by local experts to ensure that current best practices are delivered to the population directly under their care while respecting prevailing cultural norms. This adaptive approach allows more flexibility for the inclusion of practices that are uniquely needed at that institution. Take, for example, the study by Karl *et al.*, which is the only randomized trial in this space.[132] The authors argue that, in Germany, the classic endpoints of ERAS studies, such as length of stay, are not really of interest to German patients and investigators, primarily because the German healthcare system covers inpatient stays until all drains and stents are removed. Furthermore, German patients, in general, are not "used to, nor appreciate, being discharged home earlier after surgery." Therefore, the ERAS protocols in this study centred on QoL and pain control, as opposed to length of stay and time to bowel function, which underscores how the implementation of ERAS pathways can promote effective management strategies without compromising cultural expectations.

Lastly, ERAS pathways have the potential advantage of reducing variation in care processes, even if the protocols differ. ERAS protocols are cohesive management paradigms that set targets for perioperative and postoperative outcomes and, importantly, provide a conceptual framework for the strategic sequence and timing of the steps required to reach these targets with optimum efficiency. Emerging evidence suggests that the implementation of standardized protocols, regardless of the specific items in the protocol to a certain extent, can not only mitigate noncompliance with recommended processes of care, but also lead to better patient outcomes.[133] Institutions that use electronic medical records for computerized entry of physician orders may achieve even greater gains in the improvements in compliance, quality, and efficiencies of care. Taken together, ERAS pathways improve outcomes primarily through the promotion of effective strategies based on local care needs and through the reductions in variations in perioperative best practices.

7.1.11.3 The evidence supporting enhanced recovery after surgery in cystectomy patients

Although several studies have been published supporting the use of ERAS for cystectomy patients, there is striking variation in both the magnitude and the direction of the effect of ERAS protocols on perioperative outcomes.[119,134–144] (**Table 7–5**) For example, some studies show that ERAS pathways can reduce the length of hospitalization,[119,134,136,140,143,145] whereas others do not[137,139,141,145]; some studies show that ERAS pathways can expedite the recovery time for bowel activity,[137,139,141] yet others do not[136,140]; some studies show that ERAS protocols can improve the rates of readmission,[136,141,142] but yet again, others do not.[139,140,143] In light of this wide variability in study results, as well as the relative dearth of experimental data from randomized controlled trials (RCTs), a clear rationale existed for a systematic review of the literature and a meta-analysis to evaluate the comparative effectiveness of ERAS pathways versus standard of care on various perioperative outcomes of interest.[146] In this study, applying ERAS protocols reduced the length of the index hospitalization, lowered the rate of low-grade complications, and improved the time to bowel function. However, no difference in overall readmission rates was noted.

TABLE 7–5 Enhanced Recovery After Surgery Protocol Details for Selected Studies

Study	Preoperative/intraoperative					Postoperative				Comments
	MBP	CL	EDA	FM	NGT,[a] hours	Prokinetics	EOF/EM	Opioid-sparing	Drain	
Pruthi *et al.*[134]	Y	NR	NR	NR	24 h	Y	Y/Y	Y	NR	
Maffezzini *et al.*[135]	Y	NR	Y	Y	8 h	Y	Y/Y	Y	NR	
Arumainayagam *et al.*[136]	N	NR	Y	NR	N	Y	Y/Y	Y	24 h[b]	Stent removed on discharge
Mukhtar *et al.*[137]	N	Y	Y	Y	N	NR	Y/Y	Y	N	
Saar *et al.*[138]	Y	Y	N	NR	N	NR	Y/Y	Y	N	
Cerruto *et al.*[139]	Y	Y	Y	Y	N	Y	Y/Y	Y	24 h[b]	
Daneshmand *et al.*[119]	N	Y	N	Y	N	Y	Y/Y	Y	NR	Alvimopan/neostigmine
Guan *et al.*[145]	N	N	N	Y	N	Y	Y/Y	Y	NR	
Smith *et al.*[140]	N	Y	Y	Y	N	Y	Y/Y	Y	Y	
Perrson *et al.*[141]	N	Y	Y	Y	N	NR	Y/Y	Y	Y	
Koupparis *et al.*[142]	NR	Y	Y	Y	N	Y	Y/Y	Y	NR	
Collins *et al.*[143]	N	Y	N	Y	N	Y	Y/Y	Y	48 h[b]	Targiniq
Xu *et al.*[144]	N	Y	N	Y	N	Y	Y/Y	Y	NR	

Abbreviations: CL, carbohydrate loading; EDA, epidural anesthesia; EM, early mobilization; EOF, early oral feeding; FM, goal-directed fluid management; MBP, mechanical bowel preparation; N, no; NGT, nasogastric tube; NR, not reported; Y, yes.

[a] If the NGT was removed at the end of the surgery, the study was classified as not leaving an NGT postoperatively.
[b] If the drainage output was less than 5 mL, the drain was removed after 24 or 48 hours, as specified.

While these findings are compelling and seem to support the implementation of ERAS for cystectomy patients, they must be contextualized within the obvious limitation that the 13 studies synthesized in this review constituted mostly of low-level evidence. These studies were mostly retrospective observational studies, some of which used historical controls, which clearly biased the effect estimates in favour of ERAS. This highlights the need for experimental data from which more robust estimates of the effect of ERAS could be derived.

As mentioned earlier, there has been one randomized trial evaluating the effect of ERAS protocols on cystectomy outcomes. But, as previously mentioned, the endpoints from this study differ slightly from the endpoints used in other studies, due to nuances in the German healthcare system and fundamentally different cultural expectations from German patients regarding their hospitalization. As a result, the investigators focused their investigation on QoL endpoints, finding that pain control, wound complications, and time spent in the intensive care unit were all significantly better with ERAS pathways compared to standard-of-care pathways.

Each study uses a perioperative pathway that is distinct in some way from all pathways used in the other studies (see **Table 7–5** for protocols from selected studies). It may be argued that the aim of studying ERAS for cystectomy patients is not to determine which pathway is best or which elements should be universally adopted. Rather, the purpose of research in this space is to determine whether these pathways have an effect on the intended targets set by the vested local stakeholders. The differences in the pathways notwithstanding, the current body of evidence suggests that merely adopting a standardized, multimodal, interdisciplinary protocol for the perioperative management of cystectomy patients may be as important to improving perioperative outcomes as any individual element by itself. This spirit of relativity is captured by the recent European Association of Urology scientific working group on ERAS after robotic cystectomy,[117] as well as by the Department of Health Enhanced Recovery Partnership Programme across 4 different surgical specialties in the United Kingdom.[147]

7.1.11.4 Future research need

There is no question that significant work remains to be done in this arena by future investigators caring for cystectomy patients. While an appropriately conducted randomized study could offer unbiased estimates of the effect of ERAS on cystectomy outcomes of interest, randomized studies are often unfeasible due to expense and lack of clinical equipoise. Therefore, a natural experiment employing a difference-in-differences approach may be the next logical step, as it would similarly offer relatively unbiased effect estimates while mitigating the confounding effect of contemporaneous changes to clinical care (robotics, alvimopan, etc). Either approach would represent significant improvements over the current battery of studies, which use a pre-post case series approach. Yet, it is important to recognize that, despite all the limitations, these pioneering studies have promulgated new ways of thinking about caring for cystectomy patients, for which legions of cystectomy patients are undoubtedly grateful.

7.1.12 Recommendations

- ERAS pathways for patients undergoing cystectomy and UD may improve perioperative outcomes. **LOE 2; GRADE B**

- Usually, implementation of the ERAS protocol has resulted in significantly reduced length of hospital stay and decreased cost, but with comparable rates of complications and readmission. **LOE 2; GRADE B**

7.1.13 Quality of life following urinary diversion

7.1.13.1 Introduction

Several factors may affect the choice of UD after RC, including patient, physician, and general factors.[148] The term health-related quality of life (HR-QoL) relates to a subjective sense of well-being encompassing physical, psychological, social, and spiritual dimensions.[148–151] There is a lack of good data evaluating HR-QoL in patients with an orthotopic bladder substitution (OBS) versus other UDs.[152] Although the HR-QoL of patients with a well-functioning OBS seems to be higher than that seen in patients with other forms of UD, RCTs using validated HR-QoL outcome instruments are warranted to render definitive conclusions on this matter.[153,154]

7.1.13.2 Systematic reviews and meta-analyses of nonrandomized clinical trials (LOE 2b–3)

Two meta-analyses have been published in order to update data from all relevant published studies comparing different UDs, without any definitive conclusions.[155,156] Pooled effect sizes from the meta-analysis by Cerruto *et al.* showed a slightly, nonsignificantly higher HR-QoL in patients with OBS compared to those with IC, reaching a significant advantage in the ileal OBS subgroups.[155] The meta-analysis by Yang *et al.* showed no difference in overall QoL comparing continent and incontinent diversions.[156] Their subgroup analysis demonstrated greater improvement in physical health for patients with incontinent diversions compared to those with continent diversions. Their qualitative analysis showed patients with NB had superior emotional function and body image compared to those with cutaneous diversions.[156] While patient choice is key to selection of reconstruction method, IC surgery is associated with adverse patient selection.[157] Thus, although systematic reviews suggested a slightly better QoL in the OBS group, this preoperative patient selection bias may inhibit any reasonable comparison of outcomes between different UDs.[157]

7.1.13.3 Validated instruments used to evaluate health-related quality of life following urinary diversion

Table 7–6 summarizes the most commonly validated HR-QoL instruments used to compare different UDs.[51,155,158–160,161,162] Both generic and cancer-specific questionnaires are useful when comparing patients with different UDs, but are less effective when only one type of UD has to be evaluated. To address this problem, an HR-QoL questionnaire specific to ileal OBS has been developed.[163] The Ileal Orthotopic Neobladder-Patient Reported Outcome (IONB-PRO) questionnaire revealed that at a follow-up of >36 months, absence of urinary incontinence was an independent predictor of better functioning in terms of fatigue, and relational and emotional life.[164] To obtain a clear description of the evolution of the needs and expectations of the patients with IC over time, Cerruto *et al.*[165] used a "narrative-based" approach, identifying 2 major profiles, positive and negative. A positive profile was statistically more prevalent in older patients, with a longer follow-up and lower complication rates.

TABLE 7–6 Different Validated Instruments Mostly Used in the Literature to Evaluate Health-related Quality of Life Following Urinary Diversion[155]

Instrument	Generic	Cancer–specific	Bladder cancer –specific	Brief description
BCI (Bladder Cancer Index)			X	36-item questionnaire for patients with bladder cancer, urinary diversion, or both evaluating 3 domains (urinary function, bowel habits, and sexual function)
BDI (Beck Depression Inventory)	X			21-question, multiple-choice inventory for individuals aged 13 and over, with the aim of measuring the severity of depression, with items relating to hopelessness, irritability, fatigue, and weight loss
EORTC QLQC30 (European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire)		X		30-item questionnaire with 5 functional scales (physical, role, cognitive, emotional, and social), 3-symptom scale (fatigue, pain, and nausea or vomiting), and a global health and quality-of-life scale
EORTC QLQ BLM30 (European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-Bladder Cancer Module)			X	30-item questionnaire for patients with muscle-invasive bladder cancer with additional items concerning urostomy problems, body image, and use of catheters
FACT-G (Functional Assessment of Cancer Therapy-General)	X	X		27-item questionnaire evaluating 4 domains (physical, social/family, emotional, and functional well-being)
FACT-BL (Functional Assessment of Cancer Therapy-Bladder Cancer)			X	27+12–item questionnaire specific for patients with bladder cancer
FACT-VCI (Functional Assessment of Cancer Therapy-Vanderbilt Cystectomy Index)			X	27+12–item questionnaire specific for patients with bladder cancer who underwent cystectomy and various urinary diversions
HADS (Hospital Anxiety and Depression Scale)	X			14-item scale with ordinal data to determine anxiety (7 items) and depression (7 items) experienced by patients
MTC (Meta-Contrast Technique)	X			Projective test measuring personality factors, especially defensive strategies
POMS (Profile of Mood Status)	X			65-item psychological rating scale used to evaluate mood states
QWB (Quality of Well-Being Scale)	X			71-item questionnaire (20 minutes to complete) evaluating overall status and well-being with 4 domains (physical activities, social activities, mobility, and symptom/problem complexes)
SEIQoL-DW (Schedule for Evaluation of Individual Quality of Life-Direct Weighting)	X			Semistructured interview-based instrument with 5 domains (cues) elicited by the interviewer; the patient evaluates the relative importance of each QoL with a disk
SF-36 (36-Item Short Form Survey)	X			36-item survey evaluating 2 major domains: physical health (physical functioning, physical role functioning, bodily pain, and general health perceptions) and mental health (vitality, social role functioning, emotional role functioning, and mental health)

7.1.13.4 Gender

Although male patients reported a better HR-QoL in an OBS subgroup,[155] there is little information regarding the impact of gender on HR-QoL after RC. In a series of long-term, disease-free female survivors after RC, no difference between IC and ileal OBS subgroups was found[166]; in contrast, women with cutaneous ureterostomy showed a worse HR-QoL compared with those with other UDs, mostly due to the worse physical and emotional perception of their body image.

7.1.13.5 Body image

Kikuchi *et al.* found significantly worse QoL scores regarding body image in the IC group.[65] However, in preoperatively well-counselled patients, body image did not appear to be an important consideration.[61] At a maximum follow-up of 96 months, Hedgepeth *et al.*[57] did not find differences in body image scores between IC and OBS patients, with older patients having slightly better scores.

7.1.13.6 Age

The few studies that have addressed the relationship between age and QoL after UD[57,167,171] have yielded inconclusive results. Some did not document significant differences among UD subgroups in any QoL aspects in elderly patients[169,170]; others found significantly lower scores for role-physical functioning and role-emotional functioning in patients aged ≥65 years. In contrast, Hedgepeth *et al.*[57] recorded slightly better scores in older patients. Metcalfe *et al.*[171] reported that younger age was independently associated with increased QoL. D'Agostino *et al.* observed that increased age and the resulting poor management of OBS may have a negative impact on socioemotional aspects of QoL.[167]

7.1.13.7 Sexual function

Data on this topic are very sparse in the literature. In long-term follow-up, factors such as age and comorbidities may negatively impact on sexual functioning; simultaneous presence of these factors and a UD may explain the lack of interest in sexual life.[167]

7.1.13.8 Follow-up

Time represents a key point in patient satisfaction with IC because a long coexistence with a UD may change the patient attitude towards it. The UD may, in effect, become a part of the patient and the patient will have longer practice in management of the UD, with impacts on the degree of adaptation to the presence of the UD.[155] Time seems to improve function and other scores for both IC and OBS patients.[57] Analyzing the HR-QoL in patients after OBS, socioemotional factors as well as social life tend to decrease significantly in the long-term follow-up.[167] Currently, an OBS requires correct management by the patients, mainly during the nighttime. This reveals that sleep disorders are more frequent in the long-term follow-up, while in the intermediate follow-up, insomnia seems less relevant.[167] At 3, 12, and 18 months postoperatively, physical, role, social, and Global Health Status/Quality of Life (GHS/QoL) scores were significantly better in patients with OBS compared to those with IC,[172,173] as was the impact of the financial burden related to UD.[172] **[LOE 2b]**. Peri- and postoperative complications may affect HR-QoL subdomains without a significant impact on GHS/QoL.[173] OBS represented an independent predictor for better overall HR-QoL at 3 months postoperatively, but not at 12 months.[173] **[LOE 2b]**.

7.1.14 Recommendations

- OBS seems to provide better quality-of-life outcomes than other UDs mainly in the short and intermediate term.
  LOE 2; GRADE B

7.2 Types of Urinary Diversion

7.2.1 Ileal conduit

7.2.1.1 Introduction

In 1887, Bardenheuer performed the first cystectomy in Cologne. Because of massive hemorrhage, he decided not to proceed in accordance with the original intention of implanting the ureters into the rectum; instead, the ureters were left lying free in the pelvis, with urine draining into the cystectomy cavity, and the patient died within a few days.[175] Over time, diversion with ICs and other forms of incontinent diversion were performed more frequently than rectal diversions, becoming the mainstay of diversion for many years, and nowadays, they are performed frequently as other forms, such as ureterocutaneostomy in the palliative setting.[176–178] The main reasons for the switch away from rectal diversions were the associated metabolic problems, the increased risk of intestinal carcinoma, and the occurrence of chronic recurrent upper urinary tract infections.[179]

The IC technique first reported by Seifert was taken up and improved by Bricker[180,181] (**Figure 7–1**). The surgical principle was the implantation of both ureters into the proximal end of a distal ileal segment.[182] Other forms of diversion using colonic or gastric segments have since been abandoned.[183–185]

Use of the jejunum was abandoned due to characteristic metabolic changes in 40% to 65% of patients, with hypovolemia, decreased estimated glomerular filtration rate (eGFR), and aldosterone-induced sodium resorption in the distal tubule resulting in hyponatremia, hyperkalemia, hypochloridemia, azotemia, and acidosis with renal insufficiency as the main risk factors (Fontaine *et al.*, 1997).

The use of the colon has been propagated by several groups.[186–188] Three parts of the colon are used: the transverse colon; the sigmoid; and the ileocecal valve. The transverse colon is used in patients after radiotherapy to the pelvis[189] and in those with very short ureters.

The sigmoid may be used in cases of pelvic exenteration with a need for a colostomy.[190] The ileocecal part of the colon can be used when long ureteral segments need to be replaced.[191] Contraindications are chronic inflammatory diseases of the bowel such as Crohn's disease or ulcerative colitis, chronic diarrhea, and diverticulitis.

7.2.1.2 Indications and contraindications

Urinary conduit using the ileum is the most commonly performed conduit procedure.[192] The consequences of UD for the patient, including stress, can be severe. There may be functional complications, such as recurrent infections of the upper urinary tract, deterioration of kidney function, problems with management of the stoma, and impaired QoL and body image.[193] In general, an IC is indicated in patients who do not qualify for a continent diversion or who do not want to comply with the inconveniences of a continent diversion. In this context, the patient's physical and mental status, age, body habitus (obesity, malformations), extent of disease, prognosis, urethral involvement, kidney and liver function, and expectations and preferences, as well as the surgeon's experience and preferences, often play an important role in the decision. Other factors, such as previous surgery, pelvic irradiation, or

both, may also affect the decision regarding which form of UD to perform. Important contraindications are short bowel syndrome, inflammatory intestinal diseases (Crohn's disease and ulcerative colitis), and high anesthesiological and operative risk.

7.2.1.3 Preoperative preparation

Preoperatively, a stoma plate with the bag filled with water should be worn by the patient in everyday situations for test purposes, in order to define the optimal position of the stoma. The optimal position should be marked preoperatively. Otherwise, ERAS principles should be applied.[132,194]

7.2.1.4 Description of surgical technique

From the distal ileum (approximately 25 cm proximal to the ileocecal valve), a segment of 10 cm to 20 cm is isolated by diaphanoscopy, with consideration of the blood supply by the ileocolic artery (**Figure 7–2**). An ileal segment that is too long will show a tendency toward kinking, with consequent problems regarding urinary transport. Continuity is restored by tension-free anastomosis, be it by suture of the ileal segment or by stapling.[195] Ureters are then implanted refluxively into the proximal end of the ileal segment.

A variety of different techniques have been described, such as the individual end-to-side technique according to Nesbit or the end-to-end anastomosis forming a plate with 2 two ureter stumps before implantation.[196–198] Antirefluxive measures and implantation techniques have been abandoned due to poor results.[199–201] In any case, it is important to preserve as much periureteral tissue as possible to avoid ischemic strictures of the ureteral anastomosis. The aboral end of the IC is then pulled tension-free through a circular skin excision (approximately 3 cm in diameter), and a crosswise incision of the anterior and dorsal fascial sheaths of the rectus abdominis muscle is made. Muscle fibres should be dissected, not transected, in the direction of the fibres. It is important to have muscle on all sides of the IC to avoid parastomal hernias. Fixation of the IC to the 4 corners of the fascial crosswise incision, with avoidance of sutures to the mesentery of the conduit, prevents gliding of the conduit and hernia formation. Further support may be offered by sutures between the conduit and the dorsal fascia, again avoiding sutures to the mesentery. The aboral end of the stoma should protrude about 3 cm above the skin level to obtain a stoma that protrudes 5 mm to 10 mm after everting sutures from the skin to the distal end of the conduit (**Figure 7–2**).

7.2.1.5 Functional aspects

A direct comparison of functional results and HR-QoL after UD does not exist. HR-QoL studies seem to indicate that results are comparable after all interventions, but that there are significant differences in specific domains, such as perception of body image, urinary incontinence, and impaired sexuality.[202,203] From the literature, it can be concluded that these problems are mainly related to conduit derivations. In terms of body image, in a retrospective Korean study, patients who underwent orthotopic INB had a superior body image compared to those who underwent an IC.[204] This could not be confirmed in another study, where no difference in body image scores between IC and NB patients was found after surgery.[57] A systematic review demonstrated that family, relationships, health, and finance were the most important determinants of QoL. Only 2 studies reported a better QoL in favour of NBs, while 2 other studies suggested a better body image perception in patients with NB. The authors of this systematic review concluded that QoL is good, irrespective of the type of UD.[61]

Recent studies have demonstrated no significant difference in QoL between patients with incontinent diversion and patients with continent diversion. Although Philip *et al.*[205] found that patients with an OBS report better physical functioning, this did not translate into a better QoL as compared to patients with an IC. This indicates that, over time, patients adapt to the new situation and learn to live with the impairments. Sixty-three per cent of patients with an IC felt "less complete," 43% were ashamed because of the stoma, and 58% were worried about a poorly sealed stoma. Similar results were obtained by Sogni *et al.*[169] These authors used the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQC30) and the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-Bladder Cancer Module (EORTC QLQ BLM30) in their analysis to evaluate HR-QoL data in 34 patients undergoing cystectomy. Patients received either an IC or an OBS. Although the global health score was higher in the group undergoing an OBS, there was no statistical difference in the final analysis. Autorino *et al.*[206] obtained comparable HR-QoL scores for both patient groups. In their study, Pahernik *et al.* demonstrated that a conversion from an incontinent conduit to a continent diversion (Mainz pouch I) is a safe and feasible option for patients who wish to change from an incontinent to a continent diversion, with acceptable complication rates, stable renal function, and improved patient satisfaction postoperatively. The conversion was performed in 39 patients after a mean of 11 years.[207]

7.2.1.6 Complications

A short-term complication highly relevant to IC is urine leak at the ureteroileal anastomosis, which occurs in 2% to 5.5% of patients.[61] In an early IC series, there was an astonishingly high rate of urine leak.[197,208] This problem can be obviated by proper surgical technique and placement of ureteral stents.

Two studies showed that stenting reduced the risk of early upper-tract dilatation and was associated with improved bowel function.[209,210] Stenting was not associated with an increased risk of ureteroileal stricture or upper-tract infection. In the series reported by Jin *et al.* with a 20-year follow-up, 60% of patients showed a deterioration of renal function and up to 7% of patients required dialysis due to renal insufficiency.[211] Madersbacher *et al.* analyzed all conduit-associated complications in 131 patients occurring later than 3 months after surgery and recorded that 66% of patients had conduit-associated complications.[212] The most frequent complications were renal insufficiency (27%), problems with the stoma (24%), intestinal problems (24%), symptomatic upper urinary tract infections (23%), conduit and ureter anastomotic stenosis and strictures (14%), and urolithiasis (9%). In the first 5 years of their analysis, 45% of patients developed complications. This percentage increased over time to 50%, 54%, and 94% among patients who survived 10, 15, and longer than 15 years, respectively. In the last of these groups, changes in the upper urinary tract were seen in 50% of patients and urolithiasis was observed in 38%.

Samuel *et al.* analyzed data from 178 patients who had a minimum follow-up of 4 years[213] and found a deterioration of renal function in 29%. As predisposing factors in patients with nondilated renal units, hypertension, recurrent urosepticemia, and an eGFR <50 mL/min/1.73 m^2 were identified. In a recently published large series of 1,057 patients undergoing cystectomy at the Mayo Clinic, 1,045 patients received an IC and 12 a colon conduit.[193] Mean survival was 4.1 years. The mean follow-up in the 213 surviving patients was 15.5 years. Overall complications were observed in 61% of patients. The foremost complications were associated with the bowel (20.3%). Further complications

were renal (20%), infectious (16.5%), stoma associated (15.4%), and urolithiasis (15.3%). Metabolic complications had the lowest incidence (12.8%). The authors concluded that derivation with an IC leads to a high complication rate, but with a low reoperation rate.

In terms of deterioration of renal function, the Bern group showed the importance of long-term follow-up data.[211] This retrospective study included 50 patients with an IC and 111 patients with an INB with a follow-up of 10 or more years. The median eGFR of patients with an IC decreased from 65.5 mL/min/1.73 m^2 preoperatively to 57 mL/min/1.73 m^2 after 10 years. In the patients with an INB, a decrease from 68 mL/min/1.73 m^2 to 66 mL/min/1.73 m^2 was observed. Overall, 18 patients (36%) with an IC and 23 patients (21%) with an INB demonstrated a deterioration of kidney function. Seven of 12 patients (58%) with an IC who showed obstruction (ureteroileal stricture, stoma stenosis, parastomal hernia) and 17 of 46 patients with an INB (37%) with obstruction (ureteroileal or nipple stricture, bladder outlet obstruction) developed a deterioration of renal function. Logistic regression analysis confirmed that obstruction was the main factor responsible for worsening of kidney function (p=0.045 for patients with IC and p=0.002 for patients with NB). These studies demonstrate the need for long-term follow-up in these patients, as a large proportion of complications occur later and only a follow-up of more than 10 years allows identification of patients with deterioration of kidney function.

7.2.2 Recommendations

- IC diversion remains the most commonly used method for reconstruction of the urinary tract in conjunction with RC. **LOE 2; GRADE B**

- Several studies confirm a high incidence of upper-tract complications, probably increasing with length of follow-up. It is difficult to draw definitive comparisons with other diversion techniques. **LOE 2; GRADE B**

7.2.3 Orthotopic neobladder in men

7.2.3.1 Introduction

Ten commandments have been developed for achieving good results with OBS[214]:

- The procedure should be performed by a high-volume surgeon
- Do not overextend the indication
- The surgeon should have experience with nerve-sparing radical prostatectomy and bowel surgery
- Use the ileum whenever possible
- Maximum detubularization is a must
- Use a stented, freely refluxive ileoureterostomy
- The low-pressure, compliant, freely refluxive reservoir is standard
- Be aware of the myriad of potential complications
- The full armamentarium of diversion techniques must be available
- Meticulous follow-up must be guaranteed

7.2.3.2 Indications and contraindications

The indication for cystectomy is almost always bladder cancer. The extent of pelvic disease has little bearing on the appropriateness of OBS. If pelvic recurrence does develop, it does not usually have a significant impact on the function of OBS, and patients who have positive pelvic nodes can achieve good functional results with OBS.

The risk of urethral recurrence after OBS is 5% to 10% and it usually occurs within the first 2 years. Risk factors include multifocal disease, carcinoma *in situ*, prior intravesical chemotherapy, ureteral disease, and urothelial cancer in the distal prostatic urethra. Paraffin-embedded biopsies are more reliable than an intraoperative frozen section. An intraoperative frozen section is considered sufficient by many centres. Biopsies carried out before cystectomy enable discussion of the result with the patient, who then has greater confidence in the OBS.[152]

7.2.3.2.1 Age and motivation

Although there is no age cutoff for OBS, in practice, many patients over the age of 80 will opt for a conduit, as the postoperative course is less arduous and urinary incontinence is less likely. The motivation of the patient is the most important factor when considering the suitability for an OBS, although it is difficult to assess this objectively.[152,153,215]

7.2.3.2.2 Sphincter function

Urinary continence after OBS depends on adequate urethral sphincter function and reservoir behaviour.[4,216] During patient selection, it is essential for realistic expectations to be discussed by the patient and the surgeon. Contraindications to an OBS[152] are:

- Urinary stress incontinence
- Damaged rhabdosphincter or incompetent urethra
- Tumour infiltration of the distal prostatic urethra
- Impaired renal function (serum creatinine >150 mmol/L)
- Severely impaired liver function
- Severe intestinal disease (e.g., Crohn's disease)
- Inadequate intellectual capacity, dexterity, or mobility
- Patient incompliance regarding active postoperative re-education and regular follow-up

7.2.3.3 Preoperative considerations

7.2.3.3.1 Reservoir configuration

A spherical reservoir has 4 times the capacity and a quarter of the pressure compared to a cylinder made from the same length of bowel. Larger reservoirs have lower end-filling pressures and better continence, particularly in the early postoperative period. Popular techniques include an ileal afferent limb OBS using 55 cm of distal ileum, preserving the 25 cm of terminal ileum, and the W-shaped OBS.[4,216,217]

7.2.3.3.2 Use the ileum whenever possible

A comparison of gastric, ileal, ileocolic, right colic, and sigmoid segments has shown an advantage for the ileum over any other segment regarding function and urodynamics, transformation after exposure to urine, change in absorptive capacity, adaptation of mucosa from an absorptive to a storage

function, incidence of metabolic disorders, later-volume increase, capacity at first and at maximum contraction, involuntary contractions, motor activity, distensibility, and suitability for patients with decreased kidney function.[217]

7.2.3.3.3 Maximum detubularization and crossfolding

The peristaltic contractions of the tubularized segment generate high pressure peaks that lead to leakage when the bladder pressure exceeds the urethral pressure. In a detubularized reservoir, the bladder end-filling pressure is <30 cm of water, which is less than the urethral pressure. By detubularizing and folding, a new tubular segment is obtained with double the width, half the length, and double the volume. Moreover, the body of the reservoir counteracts pressure peaks, and thus, a large volume is obtained at low pressure. It is also possible to preserve a tubular segment for ureteral implantation, such as the chimneys for an INB or a longer tubular segment for the Studer technique. The peak pressures of these tubular segments are lost in the low bladder pressure.[216]

7.2.3.3.4 Stented, freely refluxive ileoureterostomy

Conventional wisdom suggests the need for an antireflux mechanism. Reflux prevention in a low-pressure OBS, however, may not be as beneficial as anticipated. First, with detubularized bowel segments and the absence of coordinated contractions, no appreciable pressure is generated. Second, the increase in intra-abdominal pressure results in identical pressure rises in both NB and ureters, allowing no reflux.[4] The pressure in the reservoir cannot be higher than the peristaltic force of the ureters. With a major pressure peak within the reservoir that exceeds the urethral closure pressure, the external sphincter generally acts as a safety valve, allowing urinary leakage.

7.2.3.3.5 Low-pressure, compliant, freely refluxive reservoir is standard

It is mandatory to avoid gut segments that are too long. No more than 60 cm of ileum seems safe to avoid metabolic disorders. An optimal volume of 500 mL for the OBS has been advocated. The pressure in the lower ureter is 20 cm to 30 cm of water. The OBS end-filling pressure is 20 cm for an optimal cystometric capacity of 500 cm. This pressure difference is a safety margin. During the filling phase of the OBS, the absence of coordinated contractions guarantees a low-pressure reservoir. During voiding, the Valsalva manoeuvre increases the pressure in the OBS, abdomen, and renal pelvis simultaneously. Thus, a direct ureteral OBS anastomosis can be performed.[216] Even if there is reflux during voiding, it is only transient and grade 1, without renal damage. But in the case of overdistention of the OBS, reflux will occur.

7.2.3.4 Description of surgical technique

There are different techniques using ileal segments of OBS.[4,216,217] According to Hautmann, key operative steps of the INB with chimneys (3 cm each) are as follows[218]:

- Isolate a 60- to 70-cm segment of ileum, 20 cm to 25 cm proximal to the ileocecal valve.
- Measurement of the segment should be done in 10-cm steps, with epidural anesthesia ceased 1 hour prior to measurement of bowel length.
- Make a long distal and a short proximal mesoileal incision.
- Restore bowel continuity. Use staplers.
- Closure of the ileal trap.

- Construction of the reservoir. Four lengths of ileum are arranged in the shape of a W with 3-cm to 5-cm-long chimneys on each side of the W, using 5 to 6 Babcock clamps.
- Other than the 2 chimneys, the bowel is opened on the antimesenteric border, except for a 5- to 7-cm section centred around the marking suture (OBS outlet), which is detubularized close to the mesenteric border to create a U-shaped flap.
- Two to 3 cm from the tip of that flap, a buttonhole is excised (**Figure 7–3**).
- An ileal plate is formed by sewing together the cut edges of the antimesenteric borders.
- Six (UR6 5/8 needle) 2-0 polyglycolic acid sutures, incorporating only 2 mm to 3 mm of the urethral sphincter and exiting at the mucosal edges, are placed (**Figure 7–3**).
- A 22-F catheter is placed through the buttonhole.
- The inner sutures are passed through the NB outlet without grasping the ileum. The corresponding outer sutures grasp the entire ileal wall 5 mm to 8 mm away from the NB outlet (**Figure 7–3**).
- Applying gentle traction, the catheter and ileal plate are manipulated down to the urethral remnant. The knots are tied inside the reservoir (**Figure 7–4**).
- The lower third of the anterior wall of the NB is closed (**Figure 7–5**).
- In 10% of patients, the ileourethral anastomosis may cause difficulties. Some of the following techniques are helpful: loosening the retractor; straightening the operating table; removing the sacral cushion; neutralizing the extended position of the patient; bringing up the perineum with a sponge stick; freeing the cecum and descending colon as in retroperitoneal lymph node dissection; moving up the NB outlet to the tip of the U-shaped flap; or performing an end-to-end anastomosis after tubularization of the U-shaped flap. Any incisions of the mesentery of the NB should be avoided. The NB mesentery should not be pulled roughly to the pelvic floor.
- Refluxing ileoureteral anastomosis (**Figure 7–6**): The ureters are trimmed as appropriate for their chimney. The anastomosis is done extraperitoneally above the common iliac vessels using a Wallace-type technique, without competing with the bowel mesentery. This part of the procedure is facilitated when the distance between the chimneys is 7 cm to 10 cm. This chimney modification simplifies the flexibility of the procedure. Major advantages include: extra length for the NB to reach the ureter; simplified abdominal or flank access to the ureterointestinal anastomosis, in case re-operation is required; and technically easier ureterointestinal anastomosis.
- Ureteral stents are brought through the anterior NB suture line. The remaining anterior NB wall is closed in a T shape with running 2-0 simple absorbable sutures. No cystostomy tube is placed. Two 20-F silicone drains are placed into the small pelvis.
- Extraperitonealization of the entire NB including the ileoureteral anastomoses (**Figure 7–7**).

7.2.3.5 Postoperative management

Excessive mucus production of the OBS may occasionally cause a problem. Therefore, the OBS is rinsed with 50 mL of saline twice a day, starting on the second postoperative day. Routinely, the ureteral stents are removed between the seventh and the ninth postoperative day. As soon as the urine is in contact with the OBS mucosa, reabsorption of electrolytes may occur. Therefore, the base excess is checked at weekly intervals for the first 4 weeks and monthly thereafter. Approximately 50%

of all patients need temporary alkalinizing therapy. The urethral catheter is removed after 14 days, after a cystogram has demonstrated complete healing of the anastomosis. If there is still leakage from the anastomosis, it is treated by prolonged catheter drainage.[152,218]

7.2.3.6 Functional aspects

7.2.3.6.1 Voiding

The mechanism of voiding for patients with an OBS is well described[4,216] in the case of an ileal reservoir (not a cecal reservoir). A passive pressure rise occurs in the reservoir during filling, and this action stretches the wall, activating intestinal stretch receptors and resulting in a sensation of filling. The patient learns this new sensation during postoperative voiding rehabilitation. For an ileal reservoir, voiding occurs by reducing outlet pressure, and pelvic floor and sphincter relaxation, combined with slight straining. If straining occurs, the upper tracts and the reservoir are equally subjected to the resulting abdominal pressure rise, and so no pressure gradient is created from the reservoir toward the upper tracts. In the early postoperative period, men should sit to void, to help them to learn how to empty the reservoir. Late voiding difficulty occurs in 20% of men.[152]

7.2.3.6.2 Continence

Continence requires careful preservation of the urethral sphincter and a low-pressure reservoir by doubly crossfolding detubularized ileum (see above) to achieve the desired reservoir volume of 500 mL. In the first postoperative weeks, the patient has to work actively to stretch the reservoir. Stretching the reservoir is done by delaying voiding when the patient feels the urge to void is irresistible and that leakage may occur. A rapid increase in reservoir capacity following surgery allows daytime continence to be achieved. Nighttime continence is established less quickly. During sleep, a detrusor–sphincter reflex normally increases outlet pressure as the bladder wall stretches during filling: this reflex is lost after cystectomy. Consequently, as the reservoir fills at night, additional outlet contraction is not recruited, and when the rise in reservoir pressure exceeds outlet pressure, leakage occurs.[152] The intestinal wall of the OBS will secrete water into the reservoir and render its contents iso-osmolar, and so overnight urine output is greater after OBS than before cystectomy.[216,217] Men achieve continence by day and by night in 92% and 76% of cases, respectively.[4,216,217] Attempted nerve sparing may improve daytime continence while increasing age worsens it. Men with type 2 diabetes gain daytime continence more slowly than controls and are less likely to achieve nighttime continence.[216,217,219]

7.2.3.7 Upper-tract preservation

Long-term upper-tract outcomes are excellent: as few as 2.7% of patients develop ureteroileal stricture if a direct end-to-side ureteroileal anastomosis is used. Stents after OBS substitution have been shown to improve outcomes.[192] These data are consistent with the outcome of a randomized surgical trial carried out to determine the effect of different afferent mechanisms with an OBS. Results showed clearly that the use of an antireflux procedure is associated with a worse outcome than a freely refluxive procedure. This was confirmed in further randomized studies.[220–222]

7.2.3.8 Complications

Even in the most experienced hands, OBS is a morbid procedure, with contemporary single-institution series reporting postoperative complications in the range of 25% to 57%, in-hospital mortality of ≤3%, and re-operation rates in the range of 2.3% to 17%.[46,223] The disparity in the quality of surgical complication reporting in urologic oncology makes it impossible to compare the morbidity of surgical techniques and outcomes.[220] Between 1986 and 2008, the Department of Urology at Ulm, Germany, performed 1,013 RCs with OBS. All complications within 90 days of surgery were defined, categorized into 11 categories, and classified with an established 5-grade modification of the original Clavien-Dindo classification system, as used at the Memorial Sloan Kettering Cancer Center. The results showed that only 42.4% of the patients had no early complications. Overall, 11.1% of complications were grade 1, 25.3% were grade 2, 16.7% were grade 3, 2.3% were grade 4, and 2.3% were grade 5.[221,222]

7.2.3.9 Follow-up

Critical components for good long-term results include not only surgical finesse, but also patient compliance and meticulous postoperative care. Immediate postoperative management should include the following steps:

- Subcutaneous heparin prophylaxis into the arm instead of the thigh to prevent lymphoceles
- Bowel stimulation with parasympathicomimetics from day 2 or 3 onward
- Withdrawal of ureteral stents on day 5 to 7 after bowel activity resumption
- Removal of the suprapubic tube (if any) on day 8 to 10 (cystogram)
- Withdrawal of the urethral catheter on day 10 to 12

Following catheter withdrawal, patients are carefully instructed on how to void. Initially, they should empty the NB in a sitting position by relaxing the pelvic floor and increasing the abdominal pressure. The following points must be observed:

- Voiding without residual urine
- Sterile urine
- Alarm clock at night
- Venous blood gas analysis every second day
- Supplement of bicarbonate (2 g–6 g) and salt

Fluid intake is gradually increased and body weight is checked. Reservoir capacity is increased by adhering to regular voiding intervals: 2 hours at first, thereafter 3 hours, and later 4 hours. The aim is a capacity of 500 mL. Meticulous long-term follow-up is essential regarding metabolism (vitamin B_{12}, electrolytes, base excess), continence, volume of voided urine (400 mL–500 mL), sterile urine, residual urine (if yes, check regular voiding intervals), and bladder neck obstruction (if yes, perform incision or resection).[152,224]

7.2.4 Recommendations

- Continence and voiding function following OBS are determined primarily by characteristics of the reservoir and by a preserved, innervated outlet mechanism. **LOE 2; GRADE B**

- Ileum seems to be superior to sigmoid or stomach, which can be used when necessary, but entails higher incontinence rates. **LOE 2; GRADE B**

- Reflux prevention is not a major concern and does not justify the use of an antireflux mechanism with a high complication rate. **LOE 3; GRADE B**

7.2.5 Orthotopic neobladder in women

7.2.5.1 Indications and contraindications

7.2.5.1.1 Patient-related factors

Patient selection has a significant impact on both oncological and functional outcome, and decision-making should be the result of a detailed discussion between the physician and the patient. Most contraindications for continent UD apply to both men and women, but there are some factors specific to women. Preexisting stress urinary incontinence will not improve after surgery and is a relative contraindication to an OBS. Some women, however, will accept the need for further incontinence surgery and the ensuing need for intermittent self-catheterization, although in these cases, a continent catheterizable reservoir may be a better option. Other forms of incontinence involving the bladder should improve after surgery. Age alone is not a criterion for refraining from offering continent diversion.[225,226] Recent publications have demonstrated comparable functional and oncological outcomes in well-selected patients. In females, the impact of age on functional outcome is not well studied and may play a larger role than in men. There is general agreement that in women over 75 years of age, there is an increased risk of incontinence, but in appropriately selected women, excellent functional outcome can be achieved in >75-year-olds. On the other hand, nerve sparing and preservation of the uterus (avoidance of hysterectomy) have been documented to have a significant impact on functional outcome.[227]

7.2.5.1.2 Oncologic factors

Maintenance of function must be placed second to oncological outcome. As preservation of the urethra was initially considered dangerous, based on de Paepe finding 36% urethral involvement in female cystectomy specimens, orthotopic diversion was avoided in females.[228] However, Coloby and colleagues evaluated 47 consecutive cystectomy specimens with step sectioning through the urethra and found that only 7% of cases displayed urethral involvement, all of which also showed bladder neck involvement.[229] Stenzl obtained similar findings in a review of a large number of specimens with localized invasive cancer, as did Stein and Chen.[230–232]

Stein and colleagues found that 50% of women with bladder neck tumours had no tumour in the urethra. In most of these studies, tumour involvement of the trigone, the presence of carcinoma *in situ*, and multifocal primary tumours were not predictors of urethral recurrence. It is now standard to require a negative urethral margin prior to proceeding with NB construction in women.[4,152,233,234]

There are increasing reports of improved functional outcome with comparable oncological outcome in patients with reproductive organ-sparing cystectomy. In these patients, the lack of a trigonal/bladder floor tumour, a palpable posterior mass, and clinical lymphadenopathy were associated with the lowest risk of pelvic organ involvement.[235]

7.2.5.1.3 Prerequisites for urinary diversion

Absolute:

- Adequate renal function (eGFR >35 mL/min/1.73 m²)
- Adequate hepatic function
- Adequate available bowel
- Negative urethral margin
- Intact urethral function

Relative:

- Adequate cognitive function
- The ability to adhere to regular follow-up
- Patient preference and social situation

7.2.5.1.4 Specific contraindications to orthotopic diversion in women

Absolute:

- Cancer invading the anterior vagina
- Positive bladder neck biopsies

Relative:

- Prior pelvic radiation
- Locally extensive disease at surgery (stage T4)
- History of stress incontinence

It is reasonable to advise against NB reconstruction for a woman with invasive bladder neck involvement, or suspected invasion of the vaginal wall or cervix. However, patients may be considered for NB diversion if the bladder neck/urethral margin is negative. It appears that overall, 60% to 70% of women undergoing cystectomy might be reasonable candidates for continent diversion.[236]

7.2.5.2 Description of surgical technique

In the past, OBS was rarely performed in women, based on the belief that the bladder neck was the primary continence mechanism in women.[237] However, it was ultimately recognized that the urethra alone could provide continence if the sphincter mechanism was carefully preserved. Colleselli and colleagues carried out elegant cadaver studies showing that the primary rhabdosphincter in adult women is an "omega"-shaped structure under the pubic symphysis deep to the endopelvic fascia surrounding the distal third of the urethra.[238] The main nerves innervating the sphincter and maintaining sensory input to the urethra are the somatic pudendal nerves, which run under the endopelvic fascia, while the pelvic plexus supplies the smooth muscle of the bladder neck and urethra.

In animal studies, pudendal nerve stimulation led to a pressure increase in the distal urethra, while stimulation of the pelvic plexus elicited a response in the proximal urethra.[239] Bilateral denervation resulted in marked denervation of the smooth muscle cells.[240] The pelvic plexus is located medial to the internal iliac arteries. and extends from the lateral aspect of the vagina (allocated to the urogenital tract) and rectum (allocated to the rectum) to the bladder neck.[241]

There is consensus that preservation of rhabdosphincter function is critical to maintenance of continence in women undergoing OBS,[152,227,233,242–244] but pudendal innervation of the rhabdosphincter should not be affected by cystectomy, as the pudendal nerve courses below the pelvic floor.[227,245] However, some authors suggest that preservation of the pelvic plexus in a "nerve-sparing" approach is crucial, as the autonomic nerves control urethral closing pressure at rest and are essential to maintain good urinary continence, the ability to empty the bladder completely, normal sexual function, and coordinated rectal and anal function.[152,233,244–246] To that end, dissection along the vagina should be performed very ventrally (1 o'clock and 11 o'clock positions) for nerve sparing, whereas on the tumour-bearing side, dissection is along the lateral vaginal wall.

Another factor reported to affect continence is preservation of the uterus. Gross *et al.* showed that patients with a preserved uterus were more likely to be continent, had a longer functional urethral length, and had higher closing pressures at rest, compared to patients with hysterectomy.[226] In line with this observation, in well-selected patients, reproductive organ-sparing cystectomy has been postulated to improve functional outcome, which supports the relevance of nerve sparing, as this allows a very ventral resection.[247]

7.2.5.3 Functional aspects

7.2.5.3.1 Continence

Comparison of continence results reported in different series is difficult, due to a lack of consensus on definitions, variable follow-up periods, and different mechanisms of data collection. Some of the larger series reporting continence results with NBs have not separated out patients by gender[224,233,248] (**Table 7–7**); however, some smaller studies have evaluated outcome specifically in women.[234,249–255] Few studies have used the gold standard of a validated, anonymous questionnaire to evaluate continence. In addition, urinary retention may develop as a late event, so length of follow-up is an important variable in these reports.

In one of the largest series, Ali-el-Dein and associates reported on a total of 192 women who underwent an RC and OBS, with 177 patients evaluable at a mean follow-up of 54 months. Overall, 89% of the women were continent during the day, 70% were continent at night, 5% were completely incontinent, and 16% were in chronic retention.[249] Stein and colleagues completed a mailed questionnaire study using the validated Bladder Cancer Index. Among the 56 women who returned the questionnaire (64% of the 87 surviving women), significant daytime incontinence was reported in 23% and nighttime incontinence in 34%. Somewhat surprisingly, 61% reported that they catheterized at least once per day and 39% always voided by self-catheterization. Only 18% of the women who catheterized reported that it was a moderate bother and 56% reported it was no bother at all.[251]

TABLE 7–7 Functional Outcomes in Series Limited to Females

Author	Patients, n	Follow-up, months	Continence, %		ISC, %
			Day	Night	
Hautmann *et al.*[259]	116	60	83	83	50 (5 years)
Granberg *et al.*[254]	49	29	90	57	35
Ali-el-Dein *et al.*[249]	177	54	92	72	16
Stenzl *et al.*[252]	83	24	82	72	11
Stein *et al.*[251]	56	82	77	66	39
Anderson *et al.*[250]	49	37	57	45	31
Burkhard F, Studer U (unpublished)	107	12	86	61	13
Jentzmik *et al.*[255]	50	73	82	77	58
Lee *et al.*[253]	53	24	87	85	21

Abbreviation: ISC, intermittent self-catheterization.

7.2.5.3.2 Sexual function and quality of life

Few studies have examined the postoperative sexual function of women undergoing cystectomy and UD.[97,256,257] Results suggest that sexual dysfunction is common (59%), and may be potentially improved (10%) by leaving the uterus intact when possible and preserving the autonomic nerves lateral to the vagina.[97] Comparisons of most aspects of QoL in women between types of UD are limited, but do not show any convincing differences.[190,258] Continence status seems to be a deciding factor concerning QoL. In a series of only long-term, disease-free female survivors after RC, Gacci *et al.* did not find a significant difference between an IC and an OBS; however, women with cutaneous ureterostomy showed a worse HR-QoL compared to those with an IC or OBS. In this study, physical well-being and emotional perception of body image were the prominent factors.[166]

7.2.5.4 Complications

Most of the early and late complications in women undergoing RC and NB are identical to those in men and are managed in a similar fashion.[4,249,259] Two complications, however, are different in female patients.

7.2.5.4.1 Pouch–vaginal fistula

This complication occurs in 1% to 5% of patients, even in experienced hands.[4,260,261] Awareness of this potential complication is important at the time of surgery and the best means of prevention is appropriate surgical technique.[230] When possible, the anterior vaginal wall should be left intact, taking a strip of vagina only in cases with close approximation of the tumour. Any vaginal incision should be carefully closed in a watertight manner with an absorbable continuous suture and eversion of the mucosa. If possible, the level of resection should differ slightly between vagina and urethra to avoid overlying sutures. An omental flap may be transposed to the pelvis and tacked to the pelvic floor on either side of the urethral anastomosis.[234,244] These fistulas rarely heal spontaneously, except in the first few weeks after surgery, and a prolonged trial of catheter drainage or more proximal diversion is not warranted.

Vaginal estrogen supplementation may help healing prior to fistula repair. Repair can be attempted vaginally if the fistula is low and small.[262,263] Transabdominal repair should be considered for high and large fistulas. When attempts to repair a vaginal fistula have failed, the patient may be better served by conversion to a cutaneous diversion.

7.2.5.4.2 Urinary retention

Urinary retention is clearly more common in women than men undergoing orthotopic diversion (**Table 7–7**). Retention can occur in the early postoperative period, but often appears after an initial phase of good NB function and emptying.[227] In the Ulm series of 116 women, the rate of retention increased steadily over time to approximately 50% by 5 years.[234] The etiology remains intensely debated. One proposed mechanism is a mechanical kink in the urethra–pouch anastomosis due to downward migration of the bladder substitute during the Valsalva manoeuvre, failure to preserve periurethral supporting structures, and cystocele (pouchocele) formation.[244,250,264] This can be documented on a lateral straining cystogram. However, not all patients with retention have this finding. Other suggested etiologies include autonomic denervation of the proximal urethra and disordered reinnervation resulting in inability to relax the sphincter.[227,243,265]

Serial urethral pressure profiles have shown an increase in urethral length, in maximal urethral closing pressure at rest, and in the continence product in patients developing emptying problems 3 to 6 months after surgery. This may be explained by recovery from neuropraxia occurring in the early postoperative period. Since the first description of this potentially undesirable late complication of OBS in women in 1996,[266] a number of authors have suggested modifications in surgical technique to try to prevent the problem and have presented data to suggest improved outcomes.[243,244,267,268] However, all are consecutive series and because the complication may appear late, such reports may be biased by shorter follow-up in the "new" group.

Nevertheless, some attempt to fill the posterior pelvis, and re-establish anterior and superior fixation of the new bladder seems to be warranted. At the University of Southern California, a sacrocolpopexy, with mesh and omental transposition laid between the bladder and vagina, has been routinely performed.[242] Ali-el-Dein described anchoring the vaginal apex to the preserved round ligaments and also used an omental flap.[244]

However, others believe these manoeuvres are unnecessary,[89] and recent results suggest that they have not prevented retention (see below). The Bern group has suggested that the location of the urethral opening in the pouch is an important variable.[4] A study from the group in Ulm suggested that patients in whom the bladder neck itself is preserved (for example, those with nonurothelial tumours) have a higher risk of retention than those in whom the urethra is divided just below the bladder neck.[269] Increasing interest has focused on the role of nerve-sparing techniques in women to preserve both voiding and sexual function.[246] Preservation of pelvic organs, when possible, may be an effective way to achieve nerve sparing and thus help prevent retention, though again, this question has not been subjected to a randomized trial and such a trial may be difficult.[247]

Treatment of retention is by intermittent catheterization. Alpha-blockers are not effective.[244] Transurethral resection of a urethral fold and open reduction of the pouch size with anterior fixation to the abdominal wall have also been described. It is clear that every woman undergoing NB

reconstruction should be advised that intermittent catheterization may be required for adequate emptying, and must be willing and able to learn how to perform this. Many women who are dry, but require self-catheterization, seem content with the diversion in spite of this setback.[251]

7.2.6 Recommendations

- Orthotopic NB reconstruction is an attractive option for selected women undergoing RC for bladder cancer. Oncologic outcomes appear to be excellent with appropriate selection criteria. **LOE 3; GRADE B**

- Careful attention to patient selection, surgical technique, and follow-up are important to optimize functional results. **LOE 3; GRADE B**

7.2.7 Continent cutaneous diversion

7.2.7.1 Indications and contraindications

Continent cutaneous diversion (CCD) became popular in the 1980s, prior to the widespread acceptance of NB reconstruction. Skinner and colleagues adopted the cutaneous Kock pouch CCD with an intussuscepted nipple valve continence mechanism and reported on a large series in 1987.[270] Simultaneously, Rowland described a pouch using right colon with a reinforced ileocecal valve and tapered distal ileal catheterizable segment.[271] Early enthusiasm was tempered by the high late complication rate related to the efferent continence mechanism. With the wide adoption of the simpler NB reconstruction, this form of diversion has become much less popular. Recent reports show that in centres with extensive experience with continent diversion, CCD was performed in 0% to 30% of patients, with many surgeons doing few or none.[272,273]

CCD is indicated in patients who prefer continent diversion, but require urethrectomy, have a compromised urethral sphincter, or otherwise prefer this form of diversion. Acceptable renal function and available bowel are required, along with adequate manual dexterity and a commitment to adhere to a schedule of regular self-catheterization to empty. Some groups have suggested that CCD may be preferable for women compared to orthotopic diversion, even if the urethra is usable.[274]

The advantages of CCD over NB include immediate continence and, once the reservoir has expanded, ability to void less frequently and often sleep through the night. However, the disadvantages of CCD are a longer, more complex surgical procedure, absolute dependence on catheterization for emptying, and higher rates of late complications related to the efferent continence mechanism that often require surgery to resolve.

7.2.7.2 Preoperative preparation

The components of the ERAS perioperative management can generally be applied to patients undergoing CCD. Many surgeons are hesitant to omit a mechanical bowel prep in these patients if they are planning to use the right colon, which must be opened widely and reconfigured, resulting in stool spill. An alternative is to wash out the isolated colon segment prior to opening the bowel through a small cecostomy catheter. No randomized trial has directly addressed the safety of omitting the bowel prep in CCD patients.

Maffezini reported on 68 patients who received only a "minimal mechanical prep" with acceptable results.[275] In general, antibiotics should be limited to 24 hours unless there is evidence of infection. A large-bore catheter is placed percutaneously into the reservoir to drain it during the early post-operative period and to manage mucus, and left until the reservoir has healed (typically 2–3 weeks). A temporary pelvic drain should be placed in case of urinary leak. Early feeding and ambulation are encouraged.

7.2.7.3 Description of surgical technique

A variety of techniques for construction of a CCD have been described. All use detubularized segments of bowel, with either the right colon, ileum, or a combination of the two, folded to form a spherical shape. The use of approximately 60 cm of ileum, or a combination of ileum and colon, is associated with somewhat lower pressures than pure colonic reservoirs, but a minimum of 26 cm to 30 cm of colon results in an acceptable low-pressure volume for most patients.[270] The primary variability between different constructs is the formation of the efferent catheterizable limb, which has been the source of most late complications.[276]

The most commonly used options are tapered distal ileum with a reinforced ileocecal valve as the continence mechanism (Indiana pouch and variations),[270,271,277] an intussuscepted ileal valve (Kock and Mainz pouch I, Lundiana),[278–281] and the *in situ* appendix, or tunnelled ileum, using the Mitrofanoff principle (right colon pouch with appendix stoma, Monti, Charleston, T-pouch).[40,281–283] There are no randomized studies comparing these different constructs, and in individual series, none is clearly superior to the others. However, constructs using surgical staples have high rates of stone formation and those using the appendix have the highest rates of stomal stenosis.[278,284]

There is controversy regarding the need to prevent reflux in patients undergoing CCD. Although these reservoirs are always colonized with bacteria, the low-pressure nature of the reservoir may protect the upper tracts from infection. There are convincing data that tunnelled ureteral reimplants directly into the colon, as in the classic Indiana pouch, has a higher rate of ureteral stenosis than a refluxing anastomosis.[277] Direct anastomosis to the distal ileum with the native ileocecal valve as the antireflux mechanism, as described in the right colon pouch with appendix or Monti efferent limb, accomplishes both goals.[40] However, others have advocated a direct implantation into the colon as an acceptable alternative.[277,285]

CCD has recently been applied to patients undergoing robot-assisted radical cystectomy (RARC). Most RARC series contain very few patents with CCD. The City of Hope group has one of the largest experiences with extracorporeal construction. They describe mobilizing the right colon and performing the bowel anastomosis laparoscopically through the robotic ports, and then doing the reconstruction extracorporeally.[285] Goh and then the USC group recently reported on very early experience with complete intracorporeal construction of an Indiana-type CCD.[286,287]

7.2.7.4 Complications

Most authors report continence rates of 85% or better with various forms of CCD construction.[32,40,277,278,281,286,288–290] Continence is usually immediate, though pouch volume will increase and the pressure will decrease during the initial few months.

Because of the routine catheterization, bacterial colonization of the reservoir is the norm and any attempt to sterilize the urine is fruitless. Many patients receive unnecessary antibiotics from primary care providers. However, febrile infections and even urosepsis do occur, especially in the early post-operative period. Reported rates of early and late febrile infections range from 20% to 40%,[288,291] which is probably higher than with INB or conduit. New late onset of symptomatic infections should institute a search for potential aggravating causes, such as kidney or pouch stones, or hydronephrosis. The late re-operation rate with CCD is high, up to 50% or more with long-term follow-up.[278,285]

Stomal problems are common and often require surgery to resolve. They include primarily difficulty passing the catheter and incontinence. The former may be due to stenosis at the skin level, kinking or outpouching of the catheterizable channel, or acute overdistention of the pouch. This is an emergency and most emergency room physicians (and many urologists) do not know how to troubleshoot the problem. If a catheter cannot be passed, a percutaneous cystostomy under ultrasound guidance is the safest acute management.[292]

Stenosis at the skin level can be managed with office dilation, Y-V plasty, or formal revision of the stoma.[276] Obstruction deeper along the passageway that cannot consistently be negotiated by the patient requires surgical revision. This can be prevented by careful attention to detail during construction of the efferent limb. Incontinence that is persistent and bothersome also usually requires surgical revision or even replacement of the continence mechanism to resolve.

Pouch rupture is a rare complication of CCD. The incidence is unknown, but likely well under 1%.[293] It can rarely be caused by acute overdistention of the pouch, traumatic catheterization, or blunt abdominal trauma with a full reservoir. Prior radiation may increase the risk. Patients present with abdominal pain and diagnosis is made by conventional or computed-tomography cystogram. Management is usually surgical, especially because of the bacterial colonization of the urine in the reservoir.[294]

Stones in the kidneys and pouch are common in these patients. Pouch stones are more likely when surgical staples are used anywhere in pouch construction, but can occur in all pouches, and the incidence increases with time. Rates of 5% to 20% or higher have been reported. Pouch stones can be managed with laser lithotripsy using a flexible cystoscope inserted through the stoma, or percutaneously with a rigid scope, taking care not to go through overlying bowel. Large stones are probably best managed with a small incision and open extraction. Renal and ureteral stones usually require an antegrade approach because of difficulty accessing the ureterocolonic anastomosis.[295]

Bowel complications also appear to be higher in patients undergoing CCD using an ileocecal segment. Frees and colleagues compared age- and gender-matched patients who underwent either CCD or IC, and found that the former complained of increased stool frequency and diarrhea.[296] This is perhaps not surprising, but has not been confirmed by other groups or compared, for example, to patients receiving a continent reservoir made entirely of ileum.

7.2.8 Recommendations

- CCD is an acceptable option for UD following cystectomy. Advantages of this diversion include excellent immediate continence and less frequent voiding. **LOE 3; GRADE C**

- Significant disadvantages include longer operative time, dependence on catheterization to empty, and increase in infections, bowel symptoms, and late complications requiring surgical revision. **LOE 3; GRADE C**

7.2.9 Anal diversion

7.2.9.1 Indications and contraindications

A prerequisite to a successful continent anal diversion is a competent anal sphincter to control continence and allow spontaneous evacuation. This excludes most patients with neuropathy of the pelvic floor innervation (e.g., secondary to myelomeningocele or spinal cord trauma) if the anal sphincter control is compromised. Moreover, patients with other forms of reduced anal sphincter control (e.g., secondary to surgical interventions for treatment of hemorrhoids or anal fistulas) may not be good candidates for this procedure. In any case, competence of the anal sphincter and confidence of the patient to accommodate sufficient amounts of liquids in the rectum must be tested preoperatively. This is easily accomplished by instilling 200 mL to 350 mL of warm saline into the rectum and observing the patient's response during normal physical activities. If the patient is comfortable with this situation and is able to hold the saline for 3 or more hours, she or he may be a good candidate for the procedure. Anal profilometry is another option for preoperative anal sphincter assessment, but is only required in equivocal cases. In anal profilometry, the resting closure pressure should be greater than 60 cm H_2O and the closing pressure under stress greater than 100 cm H_2O.

Contraindications to continent anal UD are a reduced renal function (eGFR <50% of age-adjusted normal limit, serum creatinine >1.5 mg/dL); grade III or higher hydroureteronephrosis or a history of recurrent pyelonephritis; benign or malignant rectosigmoid pathology, such as ulcerative colitis, diverticulitis, polyposis, previous or present adenocarcinoma; previous or planned adjuvant radiotherapy of the pelvis; and lack of anal sphincter control.

7.2.9.2 Preoperative preparation

Preoperatively, coexistent large-bowel pathology must be excluded by colonoscopy, computed tomography colonography, or conventional colonography with double contrast. Twenty-four hours before surgery, patients are placed on a clear liquid diet. The afternoon before surgery, mechanical bowel cleansing is mandatory for this type of surgery. Preferably, this is achieved in an antegrade fashion by administration of 3 L of a hyperosmotic solution (such as polyethylene glycol), either by drinking or through a nasogastric tube. Retrograde bowel cleansing by administration of one or several enemas may be performed in addition or as an alternative.

On the operating table, before draping the patient, a rectal tube must be placed, into which the ureteral stents are later inserted for their intraoperative extraction through the anus. The patient is placed supine in a slight anti-Trendelenburg position. Before skin incision, broad-spectrum antibiotic therapy is given, consisting of either a broad-spectrum penicillin (such as piperacillin–tazobactam) or a fourth-generation cephalosporin plus metronidazole and an aminoglycoside.

7.2.9.3 Description of surgical technique

The Mainz pouch II, as described by Fisch and Hohenfellner in 1991,[29] is a modified UST through the addition of rectosigmoid pouch formation. Briefly, the rectosigmoid colon is detubularized and reconfigured into a spherical shape to reduce the complications of pyelonephritis and anal incontinence. Detubularization of these bowel segments interrupts circular bowel contractions and decreases storage pressures, and spherical reconstruction increases capacity, so that both urinary continence and upper-tract protection are improved. Both ureters are mobilized up into Gerota's fascia.

Care must be taken to preserve the ureteral adventitia with its longitudinal blood supply. Vascular connections between the gonadal vessels and the ureter should be preserved. The left ureter is pulled with a curved clamp through the mesentery of the descending colon or sigmoid colon, at a site where compression from the inferior mesenteric artery or another major vessel of the mesentery is unlikely, into a position in front of the promontory, so that a straight course without kinking is achieved. Stay sutures are placed into the rectosigmoid at a position where they reach without tension to the promontory, to which the pouch will be sutured later on (**Figure 7–8A**). The bowel segments are opened along the anterior tenia (dashed line in **Figure 7–8B**) over a distance of about 20 cm. The rectosigmoid is mechanically cleaned by several wet swabs with gentamicin. If the sigmoid colon is short and the intended side-to-side anastomosis would be under some tension, the descending colon must be mobilized up to the left colonic flexure with division of the phrenocolic ligament. The posterior wall of the pouch is established by a double-layer, side-to-side anastomosis. The seromuscular layer is sutured with either interrupted or running absorbable 4-0 monofilament sutures (such as polydioxanone sutures [PDS]), and the mucosa is sutured with a running absorbable 5-0 monofilament suture (such as gluconate).

To prepare a submucosal tunnel, 4 stay sutures are placed over a distance of 4 cm through the intestinal mucosa and muscularis (**Figure 7–9**). At the proximal end of the submucosal tunnel, a small segment of colon mucosa is excised and the underlying muscular layer of the posterior wall of the pouch is incised crosswise to allow an unobstructed pull-through of the ureter into the intestine. A curved clamp is inserted through the incision and the ureter is pulled through into the intestinal lumen, avoiding kinking or angulation. The ureter is ventrally spatulated over 2 mm to 3 mm. For tunnel preparation, submucosal injection of a small amount (1 mL to 2 mL) of saline facilitates separation of mucosa from muscularis. With a curved clamp, the ureter is pulled through the tunnel and anchored at the most distal aspect of its neo-orifice at the 6 o'clock position, with 2 absorbable 5-0 monofilament sutures (such as gluconate), through the mucosa and muscularis of the intestine (**Figure 7–10**A). The neo-orifice of the spatulated ureter is completed by several ureteromucosal absorbable 6-0 monofilament sutures (such as gluconate). On the left side, preparation of the submucosal tunnel and pull-through of the ureter is performed in the same way as on the right side (**Figure 7–10B**).

Kinking of the ureter, compression of the ureter by a narrow entry through the muscular layer, or a tight submucosal tunnel must be avoided. The back wall of the pouch is fixed with one or two nonabsorbable 4-0 sutures (such as polypropylene) through its seromuscular layer to the periosteum of the promontory on the right side of the mesentery of the sigmoid. When tying the sutures, care must be taken that the ureters are not compressed and continue to run in a straight direction into the pouch without kinking or angulation. Size 6 French ureteral stents are inserted into each ureter and secured to the intestinal mucosa by rapidly absorbable 4-0 monofilament sutures (such as gluconate). Both ureteral stents are inserted into the side holes of the rectal tube, which is pulled back to bring out the stents anally. However, the rectal tube is reinserted in parallel to the stents, in order to serve as a rectal drainage of urine, which may pass alongside the stents. The anterior aspect of the pouch is closed in 2 layers: the mucosa is closed with a running absorbable 5-0 monofilament suture (such as gluconate) and the seromuscularis is closed with either interrupted or running absorbable 4-0 monofilament sutures (such as PDS). The mesenteric windows are closed and the pouch is covered with greater omentum. At the end of the procedure, both ureteral stents and the rectal tube are secured to the perianal skin by separate stitches.

7.2.9.4 Postoperative management

Antibiotics (piperacillin–tazobactam and metronidazole) are continued after surgery until the ureteral stents have been removed. For postoperative drainage of the stomach, we prefer intraoperative insertion of a 12 French balloon gastrostomy catheter rather than a nasogastric tube for patient comfort. Patients are allowed to start drinking on the day of surgery and are mobilized as early as the first day after surgery.

Jackson-Pratt drains are placed behind the pouch and into the small pelvis, if cystectomy has been performed. The gravity drains are removed as soon as the drainage is less than 50 mL/24 hours. Diet is advanced as bowel function returns. Bowel movements mostly start on the fifth postoperative day and the rectal tube should be removed at this time. The skin fixation of the ureteral stents can be removed on postoperative day 9.

The patient will lose the stents as soon as the rapidly dissolving mucosal fixation sutures break. Before discharge, upper-tract drainage should be checked by intravenous pyelography or renal ultrasonography. Blood gas analysis should be used to check for metabolic acidosis. With a base excess lower than -2.5 mmol/L, alkali substitution using Na+ bicarbonate, Ca2+/Na+ citrate, or K+/Na+ citrate should be instituted and initially checked at 2-week intervals. Renal ultrasonography should be repeated after 6 weeks to assure normal upper-tract urinary drainage. Owing to an increased risk of adenoma and subsequent adenocarcinoma formation, annual colonoscopy should be instituted from the fifth postoperative year. However, caution must be taken not to biopsy the ureteral orifices, which may be mistaken as an adenoma by an inexperienced endoscopist.

7.2.9.5 Follow-up

According to published series, daytime urinary continence is achieved in 98% (weighted mean; range, 88%–100%) and nighttime continence in 90% (weighted mean; range, 73%–100%).[298] Early postoperative complications, such as urinary leakage and ileus, are reported in 3.3% to 29.9% of cases; late complications, such as pyelonephritis, ureteral implantation stenosis, and metabolic acidosis in 3.7% to 28%; ureter stenosis dilatation, reimplantation, or both in 3.2% to 11%; and administration of

alkalizing agents for treatment or prevention of metabolic acidosis in 33% to 69%.[299–307] Metabolic acidosis is a frequent concern, but can be controlled if alkali substitution is initiated with preventive rather than therapeutic intent. Consequently, blood gas analysis rather than pH and blood serum chloride should be checked at regular intervals. With a base excess below 2.5 mmol/L in blood gas analysis, alkali substitution should be instituted to prevent clinically symptomatic metabolic acidosis.

After Mainz pouch II UD, QoL is very good in the majority of patients.[308,309] Nevertheless, after UST, there is an increased risk of benign and malignant tumour formation. After UST, the risk of secondary adenoma formation is increased after a mean of 10 years, if the diversion has been performed for a malignant disease, and after a mean of 20 years, if the diversion has been performed for a benign condition.

Again after UST, the risk of secondary adenocarcinoma formation is increased after a mean of 13 years, if the diversion has been performed for a malignant disease and after a mean of 26 years, if the diversion has been performed for a benign condition. In a multicentre study of more than 17,000 patients, UST had the highest risk of formation of secondary benign and malignant tumours at 2.58% among all types of UD.[19] The adenoma–adenocarcinoma sequence has a mean latency of about 3 years before malignancy develops, so that within this time period, cure may be possible without radical resection of the sigmoid colon and undiversion. Even if tumour formation has not been reported to date in a Mainz pouch II, annual colonoscopy should also be instituted from the fifth postoperative year after Mainz pouch II UD, with the aim of diagnosing (and treating) a possible tumour at the stage of an adenoma before an adenocarcinoma develops.

Over the years, indications for both UST and Mainz pouch II have been restricted to older patients (for example, after cystectomy for bladder cancer) in whom the latency period until development of a colorectal tumour would exceed their life expectancy, and to younger patients who will not accept a stoma for cultural, socioeconomic, or cosmetic reasons, and would otherwise have no alternative to a UD. These indications frequently apply in patients in developing countries, such as females with incurable vesicovaginal fistulas,[310–315] children and adolescents with bladder exstrophy,[316–324] and patients with other rare conditions and diseases.[325,326] In 2002, Türk reported the first series of 11 patients with RC and Mainz pouch II UD, performed entirely laparoscopically with intracorporeal UD and delivery of the pathological specimen through the anus.[327] Since then, several other laparoscopic series have been reported.[328–330]

7.2.10 Recommendations

- A prerequisite for a successful continent anal diversion is a competent anal sphincter to control continence and allow spontaneous evacuation. **LOE 3; Grade B**

- A regular endoscopic control in UST and cystoplasties are mandatory from the fifth postoperative year onward, due to the higher risk of tumour development (adenocarcinoma). **LOE 3; Grade B**

7.2.11 Extracorporeal diversion (robotic)

7.2.11.1 Indications and contraindications

Robot-assisted radical cystectomy (RARC), described for the first time by Menon *et al.*,[331] is currently accepted as a standard treatment for muscle-invasive bladder cancer.[332] However, consensus on the way in which the reconstruction of the urinary apparatus should be performed is a matter of debate within the urological community. Even if the evolution of robotic surgery has made intracorporeal diversion easier, extracorporeal urinary diversion (ECUD) still represents the preferred surgical approach due to the complexity of intracorporeal reconstruction.[333] The potential advantages of a RARC are lower complication rates and faster recovery without compromising oncological outcomes. Nevertheless, a recently published randomized clinical trial failed to find any real benefit of robot-assisted techniques over standard open surgery for patients undergoing RARC and ECUD.[334] Consequently, it is still not clear whether the extracorporeal approach to performance of the diversion undermines the benefits of RARC.

7.2.11.2 Preoperative preparation

Information about RARC with ECUD and type of UD is available for 1,577 patients (**Table 7–8**). According to these studies, the most common type of diversion is the IC (51.3%), followed by NB (34.2%). Mean operative time, including cystectomy and UD reconstruction, was 399.4 minutes (range, 230–554 minutes), and mean blood loss was 357.6 mL (range 167–573 mL). The mean duration of hospital stay was 10.9 days (range, 4.8–20.7). RARC with ECUD seems to be a safe procedure, with an intraoperative complication rate ranging from 0% to 3%. The available literature reports have not identified any patient characteristics affecting surgical outcomes. Of particular note, one study analyzed the relationship between body mass index (BMI) and perioperative outcomes in 49 patients undergoing RARC and ECUD, and found no significant difference in patients with BMI <25, 25–29, and >30.[335]

TABLE 7–8 Available Series of Robot-assisted Radical Cystectomy with Extracorporeal Urinary Diversion and Information About the Type of Urinary Diversion

Study	No. of patients	Study design	Type of diversion, n	Mean/median operative time, minutes	Median/mean blood loss, mL	LHS, days
Menon *et al.*[331]	17	Retrospective	IC, 3 NB, 14	300	–	–
Menon *et al.*[386]	3	Retrospective	NB, 3	323	167	–
Guru *et al.*[387]	20	Prospective	IC, 17 NB, 3	442	555	10
Mottrie *et al.*[388]	27	Retrospective	IC, 19 NB, 8	340	301	–

Abbreviations: CCD, continent cutaneous conduit; IC, ileal conduit; LHS, length of hospital stay; NB, neobladder.

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TABLE 7–8 Available Series of Robot-assisted Radical Cystectomy with Extracorporeal Urinary Diversion and Information About the Type of Urinary Diversion, *Cont'd*

Study	No. of patients	Study design	Type of diversion, n	Mean/median operative time, minutes	Median/mean blood loss, mL	LHS, days
Murphy *et al.*[389]	23	Retrospective	IC, 19 NB, 4	397	278	11.6
Lowentritt *et al.*[390]	20	Retrospective	IC, 20	375	338	5
Pruthi *et al.*[391]	12	Retrospective	IC, 9 NB, 3	276	221	4.8
Gamboa *et al.*[392]	41	Retrospective	IC, 24 Continent diversion, 17	497	254	8
Yuh *et al.*[393]	73	Retrospective	IC, 67 NB, 6	378	573	10
Josephson *et al.*[394]	58	Retrospective	NB, 58	480	450	10
Kang *et al.*[395]	104	Retrospective	IC, 60 NB, 44	554	526	18.4
Kauffman *et al.*[396]	79	Retrospective	IC, 46 NB, 25 CCD, 8	360	400	5
Kwon *et al.*[397]	17	Prospective	IC, 13 NB, 4	379	210	20.7
Manoharan *et al.*[398]	14	Retrospective	NB, 14	360	310	8.5
Khan *et al.*[399]	50	Retrospective	IC, 45 NB, 5	361	340	10
Yuh *et al.*[400]	196	Retrospective	IC, 62 NB, 86 CCD, 48	432	–	9
Lau *et al.*[401]	23	Retrospective	IC, 17 Continent diversion, 6	384	300	13
Treiyer *et al.*[402]	91	Retrospective	IC, 68 NB, 23	412	294	18.8
Torrey *et al.*[285]	34	Retrospective	Indiana pouch, 34	510	504	12.9

Abbreviations: CCD, continent cutaneous conduit; IC, ileal conduit; LHS, length of hospital stay; NB, neobladder.

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TABLE 7–8 Available Series of Robot-assisted Radical Cystectomy with Extracorporeal Urinary Diversion and Information About the Type of Urinary Diversion, *Cont'd*

Study	No. of patients	Study design	Type of diversion, n	Mean/median operative time, minutes	Median/mean blood loss, mL	LHS, days
Mmeje *et al.*[403]	50	Prospective	IC, 42 NB, 8	–	–	–
Khan *et al.*[404]	14	Retrospective	IC, 12 NB, 2	384	317	12.6
Pham *et al.*[405]	11	Retrospective	NB, 11	496	315	–
Xylinas *et al.*[341]	175	Retrospective	IC, 109 NB, 40 CCD, 26	360	400	7
Nazmy *et al.*[291]	196	Retrospective	IC, 62 NB, 86 Indiana pouch, 48	–	–	–
Lin *et al.*[406]	5	Retrospective	IC, 5	230	310	–
Yuh *et al.*[407]	162	Retrospective	IC, 48 NB, 72 Indiana pouch, 42	438	400	–
Overall	1,577		IC, 809 (51.3%) NB, 539 (34.2%) Other, 229 (14.5%)	395.12 (range, 230–554)	355 (range, 167–573)	10.9 (range, 4.8–20.7)

Abbreviations: CCD, continent cutaneous conduit; IC, ileal conduit; LHS, length of hospital stay; NB, neobladder.

A number of studies have compared RARC followed by ECUD with open radical cystectomy (ORC) (**Table 7–9**). The most important advantages deriving from RARC were a reduction of blood loss and a shorter hospital stay, while operative time was shorter after ORC.[336] Despite these data, the few available randomized clinical trials comparing RARC and ORC failed to find any significant differences in length of hospital stay or duration of the surgical procedure. In terms of complication rates, at 1 month after surgery, RARC and ORC were found to be comparable, while 90 days after surgery, RARC reduced both the number of complications of any grade and the number of grade 3 complications ($p<0.001$). Despite a significant reduction in 90-day complication rates, no differences in terms of mortality were found between RARC with ECUD and ORC.

TABLE 7–9 Randomized Clinical Trials Comparing Robot-assisted Radical Cystectomy and Extracorporeal Urinary Diversion with Open Radical Cystectomy

Study	No. of patients, ORC/ RARC	IC, ORC/ RARC	NB, ORC/ RARC	Median blood loss, mL (mean)	Operative time, hours	Overall complication rate, %	Median LHS,* days
Nix *et al.*[128]	20/21	14/14	6/7	ORC 575 RARC 258	ORC 3.52 (SD 3.4) RARC 4.20 (SD 4.2)	ORC 50% RARC 33%	
Parekh *et al.*[408]	20/20	NA	NA	ORC 800 (400–1,125) RARC 400 (300–762)	ORC 5 (IQR 4–5.3) RARC 4.7 (IQR 4–6)	N/A	ORC 6 RARC 6
Bochner *et al.*[334]	58/60	23/27	35/33	ORC 676±338 RARC 516±427	ORC 5.5 (SD 1.3) RARC 7.6 (SD 1.3)	N/A	ORC 8 RARC 8
Khan *et al.*[409]	20/20	17/18	3/2	ORC 808 (329) RARC 585 (618)	ORC 4.9 (SD 1.1) RARC 6.5 (SD 1.6)	ORC 71% RARC 42%	ORC 14.4 RARC 11.9

Abbreviations: IC, ileal conduit; LHS, length of hospital stay; NB, neobladder; ORC, open radical cystectomy; RARC, robot-assisted radical cystectomy.

7.2.11.3 Description of surgical technique

In order to perform a RARC with ECUD, the robot is undocked after completing the extirpative portion of the procedure. Surgical principles for UD reconstruction are the same as those described for open surgery. Similarly, preoperative preparation and postoperative management do not differ from open surgery, and are mainly based on the type of diversion. The ECUD is usually made through the incision used for the extraction of the specimen. Several sites have been proposed: periumbilical midline, infraumbilical midline, Pfannenstiel, and McBurney. The infraumbilical midline incision is the most frequently adopted one, since it provides the best access to the ureters and the afferent limb of the diversion, regardless of body habitus. The degree of ureteral mobilization necessary to perform the ureteroenteric anastomosis at the level of the skin incision still represents an open debate. Regardless of the level of the ureteral dissection, the most important aspects are minimization of the tension and performance of the ureteral anastomosis as proximally to the ureter as possible.[337] The ureteroenteric anastomosis can be performed in an open or robotic approach. In the case of robotic anastomosis, at the end of the reconstruction of the UD, the incision is closed and the robot is redocked to perform the ureteral anastomosis.

A similar procedure is adopted in the case of NB: the bowel anastomosis and pouch construction are performed extracorporeally, while the urethral–enteric anastomosis is performed robotically using the van Velthoven technique with absorbable suture. According to the European Association of Urology guidelines, indications for RARC and ORC overlap.

7.2.11.4 Functional aspects

Data about long-term functional and oncological outcomes are still lacking. Moreover, the current knowledge derives from selected centres early in their learning curves, in which patients have potentially been selected for the robotic technique, thus avoiding more advanced-stage or technically difficult cases. This limitation is particularly evident in the reporting of functional outcomes, since the

quality of nerve sparing, and its role in potency recovery and continence are still poorly understood. Yuh *et al.* reviewed the available literature about functional outcomes after RARC.[338] The main issues that complicate the interpretation of these data are the lack of a standardized method in reporting outcomes and the widespread differences in patient selection. Globally, data on continence after RARC with continent urinary diversion are available for fewer than 200 patients. Moreover, this information is affected by significant differences in outcomes assessment and a short follow-up.

According to the available literature, 6-month daytime continence rates range from 48% to 100%, while after 12 months of follow-up, continence recovery varies from 83% to 100%. Torrey *et al.* published a series of 34 RARC with extracorporeal reconstruction of an Indiana pouch.[285] They defined continence as absence of urinary leakage after surgery. After a mean follow-up of 20 months, 97% of the patients were continent, while one developed stomal incontinence after 1 year of continence and regained continence after revision. Currently, information on potency recovery after RARC is available in 7 studies. This outcome is strictly related to neurovascular bundle preservation. Nerve-sparing procedures have been performed in 20% to 100% of patients, depending on the series. Most of the data about sexual function are based on use of the International Index of Erectile Function and the use of PDE-5 inhibitors is quite common. According to the available studies, potency rates range from 41% to 75% when a potency recovery definition is clearly reported (3 of 7 studies).

7.2.12 Intracorporeal diversion (robotic)

7.2.12.1 Indications and contraindications

In general, the indications for RARC with intracorporeal urinary diversion (ICUD) are identical to ORC. However, care should be taken in patient selection. The extent of tumour, patient comorbidities such as impaired cardiac, pulmonary and renal function, as well as cognitive function should be considered when deciding on the appropriate type of UD. Patients with decreased pulmonary compliance who cannot tolerate prolonged Trendelenburg positioning are not candidates for the robotic-assisted technique. Furthermore, if the patient has a history of previous extensive abdominal surgery, RARC may be contraindicated. Patients with bulky disease should be avoided early in the operative learning curve (**Table 7–10**). The patient should be informed of the possibility of conversion to open surgery.

TABLE 7–10 Challenging Cases Recommended Only for Surgeons Experienced in Robot-assisted Radical Cystectomy

Patients with high body mass index
Salvage cystectomy following chemotherapy and radiation treatment
Patients with clinically advanced disease (T3/T4)
Patients with clinical lymphadenopathy
Previous history of surgery, e.g., previous prostatectomy, abdominoperineal resection, or low anterior resection surgery
Patient with large bulky tumour
Patients with multiple previous lower abdominal surgeries
Patients with previous history of pelvic radiation for malignancy, e.g., prostate or rectal cancer

7.2.12.2 Preoperative preparation

ERAS guidelines should be followed. The following procedures are particularly relevant to cystectomy patients.[339]

- No preoperative bowel preparation is necessary.
- European and American guidelines recommend no solid food 6 hours before surgery, but allow liquids up to 2 hours before surgery.
- Carbohydrate fluid intake 2 hours before surgery may be beneficial.
- Preoperative intravenous broad-spectrum antibiotics are recommended in most centres 24 to 48 hours after surgery.
- Low-molecular-weight heparin (4,000 units) for up to 4 weeks after surgery is also recommended in ERAS guidelines.

7.2.12.3 **Surgical technique description**

The surgical principles for urinary reconstruction are identical, whether conducted with open laparoscopic or robot-assisted techniques. Three options for urinary diversion are mainly used after cystectomy: incontinent cutaneous diversion; continent orthotopic diversion; and CCD. All 3 diversions can be performed with intracorporeal robot-assisted technique.

7.2.12.3.1 Patient position

After induction of general anaesthesia, the patient is placed in lithotomy position, with arms adducted and padded. The lower limb calves are placed and secured within stirrups where they can be abducted and slightly lowered on spreader bars. The table is placed in the 25° Trendelenburg position during the radical cystectomy and extended pelvic lymph node dissection (ePLND).

7.2.12.3.2 Equipment

The technique is challenging, requiring conventional laparoscopic infrastructure as well as an assistant skilled in conventional laparoscopy.

7.2.12.3.3 Trocar configuration

Port placement is critical for successful robotic surgery. A 6-port technique is most often used, with the camera port placed 5 cm above the umbilicus in the midline. The camera port is placed by a small mini-laparotomy as described by Hasson[340] and the other ports are placed in view of the camera. Two robotic ports are placed symmetrically and level with the umbilicus on the left and right side, lateral to the rectus sheath. A third robotic instrument port is placed just above and medial to the left anterior superior iliac spine through a 15-mm port, thereby enabling laparoscopic stapling by the assistant when the third robotic port is temporarily disconnected. Two assistant ports are placed on either side of the right robotic instrument port. The pneumoperitoneum can be set to 10 mmHg to 12 mmHg.

7.2.12.3.4 Orthotopic bladder substitution; intracorporeal technique

Several different techniques for ICUD have been described.[341–343] In the present overview, we concentrate on the technique described from Karolinska University Hospital Stockholm. After completing the radical cystectomy and the ePLND, the UD is performed. For the UD, the Trendelenburg position may be decreased to 10° to 15°, so as to facilitate the bowel dropping into the pelvis.

Step 1: Anastomosis between the ileum and the urethra. The 0° lens is used for this initial step. The ileum is sufficiently mobilized to reach down to the urethra without tension. A posterior reconstruction is performed where the Denonvilliers fascia on the rectum may be sutured down to the rectourethralis muscle. The reconstructed plane is then sutured to the ileum 1 cm dorsocephalad to where the urethral anastomosis will be made. This enables the anastomosis between the NB and urethra to be performed without tension, and this manoeuvre also ensures the NB will be placed correctly in the small pelvis during the entire procedure. A 20F opening is made on the antimesenteric side of ileum using cold robotic scissors. The anastomosis is sutured according to the van Velthoven technique with 2 times 16 cm 2-0 Quill™ suture, allowing for 10 to 12 suture passes.

Several surgical tricks have been described to reduce tension in the anastomosis[344]:

- Vessel loops through the bowel mesentery
- Decrease Trendelenburg position
- Scarring of the peritoneum on the bowel mesentery

Step 2: NB formation. The orthotopic NB is fashioned from a 55-cm segment of terminal ileum. The intestine is isolated using laparoscopic Endo GIA™ with a 60-mm intestinal stapler. The stapler is inserted by the assistant surgeon using the hybrid 15-mm port. The ileum is stapled 10 cm distal and 45 cm proximal to the urethroileal anastomosis. The continuity of the small bowel is restored by using an Endo-GIA (60 mm intestinal stapler), positioning the distal and proximal end of the ileum side-to-side with the antimesenteric sides facing each other; a subsequent 45-mm stapler may be used to elongate the anastomosis. An additional transverse firing of the Endo-GIA 60-mm staple is then used to close the open ends of the ileal limbs. The distal 40 cm of the isolated ileal segment is detubularized along its antimesenteric border, preserving a 15-cm intact proximal isoperistaltic afferent limb for the entero-ureteric anastomoses. After detubularization, the posterior part of the reservoir is closed using a multiple running suture (15 cm 3-0 V-Loc™) in a seromuscular fashion. After the posterior part is sutured, the NB is folded and the distal half of the anterior part of the reservoir is sutured. The proximal half of the anterior part of the reservoir is left open to allow placement of the ureteric stents and is closed in the last part of the procedure.

Step 3: Ureteric NB anastomosis. The anastomosis between the ureters and the afferent limb is performed using the Wallace technique. Using the fourth arm, the ureters are aligned. The ureters are then incised and spatulated for 2 cm to 3 cm. The posterior walls of the ureters are sutured side-to-side, using a 15-cm running 5-0 PDS. Before the anastomosis between the ureters and the intestinal loop is made, 2 single-J 40-cm ureteric stents are introduced with the Seldinger technique through 2 separate 4-mm incisions at the lower part of abdominal wall. The stents are pulled through the afferent limb and pushed up into the ureters on each side. The ureters are then sutured to the afferent limb of the pouch, using 2 times 16 cm 3-0 Quill suture. After the entero-ureteric anastomosis is completed, the stents are sutured and fixed to the skin.

Step 4: Closure of the NB. The remaining part of the NB is then closed with a running 3-0 V-Loc suture. The balloon of the indwelling catheter is filled with 10 mL of sterile water. The NB is then filled with 100 mL of saline to check for leakage. Extra suturing to secure a watertight reservoir and anastomosis is fundamental to decreasing postoperative complications. A 21F passive drain is introduced and placed in the small pelvis. The urethral catheter is removed after 21 days.

7.2.12.3.5 Ileal conduit, intracorporeal technique

Twenty centimetres of intestine are isolated from the terminal ileum, using an Endo-GIA with 60-mm intestinal staples. The continuity of the small bowel is restored as described above. The distal end of the conduit is fashioned as a stoma by the surgical assistant at the previously marked site on the abdominal wall. The left ureter is tunnelled under the sigmoid mesentery to the right side. The ureters are then incised and spatulated 2 cm to 3 cm. The Wallace technique is used here as described above. Single-J ureteric stents are then introduced through the isolated ileal segment (IC). The stents are then pushed up into the ureters on each side and the ureteroenteric anastomosis is completed, using a 2 times 16-cm 4-0 Quill suture.

7.2.12.3.6 Functional aspects: continence and erectile function

Functional outcomes are important quality indicators, especially in the NB diversion. Promising functional outcomes for RARC have been reported, but limited amount of information is available (**Table 7–11**). Collins *et al.* have extracted data for daytime and nighttime continence and potency at 12 months. Daytime continence ranged between 64% and 100%, nocturnal continence between 17% and 72%, and potency at 81% in the nerve-sparing patients. **Table 7–2** shows the available RARC studies of NBs with published functional outcomes.[343,345–351]

TABLE 7–11 Functional Outcomes of Robot-assisted Radical Cystectomy and Intracorporeal Urinary Diversion

Author	Patients, n	Daytime continence, %	Nighttime continence, %	Potency, %
Akbulut *et al.*[346]	7	86	71	55
Goh *et al.*[343]	8	75	–	–
Canda *et al.*[347]	17	65	18	9
Tyritzis *et al.*[345]	70	90	75	81
Simone *et al.*[348]	45	74	55	NA
Tan *et al.*[349]	20	95	65	NA
Asimakopoulos *et al.*[350]	40	100	72	72
Satkunasivam *et al.*[351]	28	42*	38*	NA

*Definition of continence=almost dry to slightly wet.

There is one study to date which included urodynamic data when comparing RARC with intracorporeal NB and ORC with NB by Satkunasivam *et al.*[351] The authors stated that the RARC NB had similar urodynamic characteristics to the ORC neobladder, but the RARC daytime continence was inferior to ORC. Patient urinary bother was similar between the 2 procedures. The study had limitations: it was retrospective, with a small number of patients (28 RARC, 79 ORC), and a very short follow-up for RARC (9.4 months compared to 62.1 months for ORC).

7.2.12.3.7 Upper-tract preservation and renal function

There is no evidence to suggest that the management of the upper tract should be different after RARC compared to ORC.

7.2.12.4 Postoperative management

ERAS guidelines should be followed (see separate chapter). The ERAS pathway has been known for many years and is beneficial for the patient's postoperative course. It was introduced by the open colorectal surgeons[106] and recently gained significant interest because of RARC. Minimally invasive surgery has been added as one of the 22 elements of ERAS.[118] ERP has shown that it decreases length of stay, postoperative ileus, complications, and risk for readmission at 30 days.[146]

The following procedures are particularly relevant to cystectomy patients[339]:

- Routine postoperative intensive care unit monitoring not necessary
- Monitoring of metabolic abnormalities
- Frequent irrigation of urinary catheters to clear the NB from mucus
- Minimum ureteral stenting for 5 days
- No long-term use of nasogastric tubes routinely
- No parenteral nutrition routinely
- Early oral feeding
- Early ambulation
- Multimodal postoperative analgesia to minimize opioids
- Deep-breathing exercises to minimize post-operative respiratory complications

Postoperatively, patients were followed at 6 weeks; at 6, 12, and 24 months; and once a year thereafter. Postoperative functional outcome regarding continence and potency rates were assessed. Radiological examination includes computed tomography of the thorax and abdomen every 6 months for first 2 years, after which computed tomography is performed yearly.

7.2.12.4.1 Complications

Even in the most experienced hands, the rates of overall complications after cystectomy are high, reaching up to 64%, while the rates of Clavien-Dindo ≥3 complications can be as high as 41%.[152] In systematic analyses, meta-analyses, and large national healthcare registries, it seems that there is a consistent pattern in complications and outcomes comparing RARC to ORC.[336,338,339,349,352–362] RARC shows decreased blood loss, lower transfusion rates, shorter hospitalizations, and fewer overall complications, which may be expected from a minimally invasive procedure (**Tables 7–12** and **7–13**). On the other hand, the operative times are longer for RARC compared to ORC.

Blood loss and transfusion rates are classified as grade 2 complications in the Clavien-Dindo classification system, and may be downplayed in complication reporting. However, blood loss leading to transfusion is a major predictor of worse oncological prognosis.[363–365] A recent meta-analysis of patients suggested that transfusion in patients who underwent RC was associated with increased overall mortality, cancer-specific mortality, and cancer recurrence.[363] The same result was recorded by Siemens *et al.*, who apart from worse survival, also recorded an association of transfusion with poorer early outcomes, such as longer hospitalization and higher readmission rates.[366] Four RCTs comparing RARC and ORC have published their early outcomes, and the recent meta-analysis by Tan *et al.* of those RCTs concluded that RARC is better in blood loss and wound complications and worse in operation time.[349]

TABLE 7–12 Operative Data on Robot-assisted Radical Cystectomy and Intracorporeal Urinary Diversion

Author	Year	Patients, n	RARC	ORC	RARC	ORC	RARC	ORC
			Operation time, hours		Blood loss, cc		LOS, days	
Wang *et al.*[363]	2015	54	6.5	6	400	750	5	8
Nix *et al.*[128]	2010	41	4.2	3.5	278	575	5	6
Parekh *et al.*[408]	2013	47	5	4.8	400	800	6	6
Khan *et al.*[409]	2012	100	6.4	5.3	337	1,352	10	19
Richards *et al.*[369]	2012	70	7.7	6.1	275	600	7	14
Knox *et al.*[368]	2013	142	7.8	6.6	276	1,522	6	11
Bochner *et al.*[334]	2015	118	7.6	5.5	516	676	8	8
Khan *et al.*[409]	2016	40	6.5	4.8	585	808	12	14
Hu *et al.*[359]	2016	1,317	NR	NR	NR	NR	8	8

Abbreviations: ICUD, intracorporeal urinary diversion; LOS, length of stay; NR, not reported; ORC, open radical cystectomy; RARC, robot-assisted radical cystectomy.

TABLE 7–13 Complication Rates and Oncological Outcomes After Robot-assisted Radical Cystectomy and Intracorporeal Urinary Diversion

Author	Year	Patients, n	Follow-up, months	Complication rates, %	Mortality, %
Wang *et al.*[363]	2015	54	NR	21 vs. 24	0 vs. 0
Nix *et al.*[128]*	2010	41	NR	33 vs. 50	0 vs. 5
Parekh *et al.*[408]*	2013	47	NR	25 vs. 25 (Clavien-Dindo >2)	NR
Khan *et al.*[409]	2012	100	38	42 vs. 71	0 vs. 2
Richards *et al.*[369]	2012	70	NR	10 vs. 35	0 vs. 5
Knox *et al.*[368]	2013	142	NR	43 vs. 64	1 vs. 2
Bochner *et al.*[334]	2015	118	3	62 vs. 66	0 vs. 2
Khan *et al.*[409]	2016	40	12	55 vs. 70	0 vs. 0
Tan *et al.*[349]	2015	184	34	NR	NR
Hu *et al.*[359]	2016	1,317	44	8.0 vs. 10 (Clavien-Dindo >2)	NR

Abbreviation: NR, not reported.

Radical cystectomy by any approach has associated significant perioperative complication and mortality rates.[367] Recent publications reported perioperative complication rates from open radical cystectomy ranging from 49% to 64%; high-grade (Clavien-Dindo ≥3) complication rates ranged

from 13% to 40%; and 90-day mortality ranged from 0% to 4.5% (38-40). RARC is also associated with a high rate of complications.[338,352] However, a robotic approach may be better tolerated in elderly patients,[368,369] suggesting that RARC may be indicated in this susceptible patient group. Significant hesitancy was seen globally in adopting the totally intracorporeal technique for bowel handling and reconstruction of the UD.

However, in light of data published by the International Radical Cystectomy Consortium (IRCC), it seems that shifting to the intracorporeal technique might be justified. In this multicentre, retrospective review of 167 patients undergoing RARC with intracorporeal diversion (IC, 106; NB, 61), and 768 patients undergoing RARC with ECUD (IC, 570; NB, 198), the intracorporeal patients were at lower risk of experiencing a postoperative complication at 90 days (32%).[43] In addition, the authors reported a statistically significant lower risk for gastrointestinal and infectious complications in favour of the intracorporeal approach, which are the most common major complications at 90 days.[46] The totally intracorporeal technique provides some theoretical advantages: the bowel stays inside the abdomen; there is no hypothermia or loss of fluids via osmosis; there is less bleeding; there is less need for ureteral dissection; and there is less traction to the bowel and ureters.

7.2.13 Recommendations (robotic cystectomy)

- The most important advantages deriving from RARC were a reduction of blood loss and a shorter hospital stay, while operative time was shorter after ORC. **LOE 2; GRADE B**

- Data on long-term functional and oncological outcomes are still lacking. Moreover, the current knowledge derives from selected centres early in their learning curves, in which patients have potentially been selected for the robotic technique, thus avoiding more advanced-stage or technically difficult cases. **LOE 2; GRADE B**

- While we are still waiting for stronger scientific evidence, RARC with intracorporeal urinary diversion appears to be a viable alternative to an open operation, offering patients the advantages of a minimally invasive approach. **LOE 3; GRADE C**

7.2.14 Palliative diversion

7.2.14.1 Indications and contraindications

The vast majority of patients with malignant ureteral obstruction present with an advanced stage of the disease, with other sites of metastases being commonly documented at the time of presentation.[370–372] Extrinsic ureteral obstruction secondary to malignancy is commonly a late manifestation of metastatic disease, while primary prostate and the urinary bladder cancers are the most common causes.[370–372] Currently, there is no consensus on optimal management of malignant ureteral obstruction. In the absence of randomized trials or matched-pair comparisons, any conclusions are based on cohort series with a low LOE.

The issue of palliative UD is largely neglected in major guidelines. Herein, the term "palliative urinary diversion" is used to refer to the insertion of a double-J ureteral catheter, a percutaneous nephrostomy (PCN), or subcutaneous drainage in the presence of a malignant upper urinary tract obstruction. Other forms of UD occasionally used in the palliative setting, such as IC or ureterocutaneostomy, are not considered here. Ethical concerns arise in the management of ureteral obstruction in patients with incurable malignancies because decompression procedures may merely prolong patient suffering.[370–372] Neither double-J ureteral catheter placement nor PCN is exempt from complications. Aside from the impact of clinical and surgical complications (detailed below), postinterventional QoL is usually limited owing to impairment arising from urinary symptoms, pain, and a poor performance status already present to prior palliative UD.

The overall survival rate of patients undergoing palliative UD remains poor. In the most recent large-scale, 2-centre study, Cordeiro *et al.* prospectively enrolled 208 patients with a median survival of 144 days.[372] Twenty-one per cent of patients died during hospitalization.[372] Overall survival did not differ according to mode of palliative diversion.[372] Shekarriz *et al.* evaluated 103 patients and observed a median survival of 112 days following palliative diversion, and in Ishioka *et al.*'s series of 140 patients, the median overall survival was 96 days.[370,373]

Several groups have attempted to identify prognosticators for these patients that may aid in the decision-making process. Cordeiro *et al.* identified the number of events related to malignancy (>4) and Eastern Cooperative Oncology Group (ECOG) index >2 as risk factors for a shorter survival.[372] The median 6-month survival was 57.3% for patients with no risk factor, compared to 36.3% in those with 1 risk factor and 14.3% in those with 2 risk factors.[372] Ishioka *et al.* identified a serum albumin level of <0.7 mmol/L, a low degree of hydronephrosis, and 3 or more events related to disseminated malignancy as predictors of poor outcome.[373] The authors created a risk stratification model with favourable, intermediate, and unfavourable groups, and recorded 6-month survival rates of 69%, 24%, and 2%, respectively.[373] In a study of 49 patients, Lienert *et al.* identified serum levels of albumin <0.17 nmol/L and sodium <135 mmol/L and 3 or more events related to dissemination as risk factors.[374] Patients in the favourable-risk group (no risk factor) had a mean survival of 278 days versus 173 days for the intermediate-risk group (1 risk factor) and 63 days for those in the high-risk group (2 or more risk factors).[374]

In summary, the survival of patient undergoing palliative UD is in the range of 100 days and several prognosticators have been established. The decision to perform palliative UD should be approached with great caution in patients with a poor performance status, low serum albumin levels, and 3 or more events related to malignancy. In this cohort, the 6-month survival rate is less than 15%, and in some series, below 5%, questioning the indication for palliative UD in this setting.

7.2.14.2 Description of surgical technique

There is no consensus on whether the initial attempt at a palliative UD should be made via ureteral stenting or PCN. Insertion of a ureteral double-J stent is not always feasible because of extensive pelvic disease, anatomic deformities, bleeding, or ureteral compression. In a series of 186 patients, insertion of a ureteral double-J catheter failed in 21% of patients.[371] Ganatra *et al.* reported on 157 patients who

all underwent an initial attempt to insert a ureteral double-J catheter. A total of 24 (15.3%) patients required immediate PCN placement and a further 32 (20.3%) patients experienced late failure of the ureteral stent, and also received a PCN.[375]

Observation of direct tumour invasion during cystoscopy was a significant risk factor for progression to PCN.[375] As an alternative to conventional forms of palliative UD, a subcutaneous pyelovesical bypass was developed more than a decade ago. Desgrandchamps *et al.* reported on a series of 19 patients who received 27 subcutaneous tubes as a palliative UD.[376] All patients had a PCN as the initial form of diversion. The mean operating time was 73 minutes for unilateral diversion and 105 minutes for bilateral diversion, with no relevant intra- or perioperative complications.[376] The mean follow-up was 7.8 months and 6.6 months for the 15 patients (79%) who died during follow-up. The authors observed an improvement of the function scale (EORTC QLC-30) as a result of the elimination of the PCN and a parallel worsening of the symptom scale secondary to disease progression.[376] Patient ratings of the global QoL and satisfaction with the UD were improved because of the absence of the PCN. The authors concluded that the subcutaneous pyelovesical bypass provides a better QoL than a standard PCN in terminally ill patients by making them external-tube free.[376] Although this technique was developed more than a decade ago, it did not gain widespread acceptance and recent publications are scant.

7.2.14.3 Functional aspects

Apart from clinical and surgical complications, postinterventional QoL can be impaired because of lower urinary tract symptoms, pain, and poor functional performance status. Only a few studies have systematically addressed functional and QoL aspects following palliative UD.

Shekarriz *et al.* analyzed performance on the Karnofsky Performance Scale (KPS) following palliative diversion (ureteral stenting of PCN) according to the following grading[370]: 0, hospitalized until death; 1, bedridden at home, severe pain despite analgesia; 2, moderate disability, moderate pain despite analgesia; 3, mild disability, pain free with medication; 4, normal. After UD, the KPS score in all patients averaged 2.[370] In this study of 103 patients, 86% of patients had persistent cancer-related symptoms and poor functional status after diversion.[370] Only 13 patients (14%) were free of pain with normal functional status after the procedure.[370] Fourteen patients (15%) never left the hospital after the intervention.[370]

Monsky *et al.* assessed various QoL parameters after palliative UD.[377] Forty-six patients received either a PCN (n=16), a double-J stent (n=15), or an internal/external nephroureteral stent.[377] QoL surveys were administered at 7, 30, and 90 days after the intervention, and covered symptoms and physical, social, functional, and emotional well-being aspects.[377] Responses to QoL surveys did not differ between patients receiving PCN, double-J ureteral stent, or the internal/external nephroureteral stent at 7, 30, or 90 days.[377] Patients with double-J stents experienced more urinary symptoms and pain (p<0.05) compared to those with nephrostomies. PCNs were associated with more frequent minor complications requiring additional interventions.[377]

7.2.14.4 Upper urinary tract preservation

As indicated above, the issue of long-term renal function preservation is not of paramount clinical relevance, given the limited life expectancy in the range of 100 days following palliative UD. As expected, renal function improves following palliative diversion. Ishioka *et al.* reported on the immediate effect of palliative PCN insertion on renal function in 140 patients.[373] Serum creatinine levels declined from 4.3 mg/dL preoperatively to 1.4 mg/dL following PCN placement.[373] In Shekarriz's series, the serum creatinine declined from 6.8 mg/dL preoperatively to 3.3 mg/dL ($p<0.0001$).[370] The mid-term effect of UD on renal function is not well established.

7.2.14.5 Complications

Neither ureteral stent placement nor PCN is free of complications. In Cordeiro's series, complications related to PCN were pyelonephritis in 34 (22.7%), hospital readmission in 26 (17.3%), dislodgment of the nephrostomy catheter requiring replacement in 14 (9.3%), hematuria in 6 (4%), and blood transfusion in 2 (1.3%).[372] Complications related to ureteral stenting were hospital readmission in 11 (19%), pyelonephritis in 3 (5.2%), and stent obstruction in 10 (17%).[372] Shekarriz *et al.* rated the complications in their series as either minor (hematuria, catheter blockade, urinary tract infection) or major (significant bleeding, bladder tamponade requiring surgical intervention).[370] Overall, 68.4% developed procedural-related complications during the postoperative course: 63% had minor complications and 5.4% had major complications.[370] In 30% of patients, dislodgment of the percutaneous nephrostomy occurred and required replacement.[370] Given the limited life expectancy of these patients, no differentiation was made between early and late complications.

7.2.14.6 Follow-up

No recommendations regarding follow-up are available. Follow-up should be highly individualized based on various factors, such as underlying malignancy, metastatic burden, QoL, pain, and family support. Shekarriz *et al.* found that following palliative UD, patients spent approximately 50% of their remaining life span in hospital care.[370] Harrington *et al.* followed up 42 patients with malignant ureteral obstruction and observed that they spent 23% of their remaining survival time in hospital care.[378] Hence, control visits should be set as cautiously as justifiable from the medical standpoint.

7.2.15 Recommendations

- The indication for palliative diversion in poor-risk patients is highly questionable. An attempt at ureteral stenting is warranted, yet the mid-term progression rate to PCN is as high as 50%. **LOE 3; GRADE C**

- Each type of UD has its pros and cons, and careful selection is necessary to balance benefits against risks in an effort to offer the best individual option to the patient. This is particularly true for older and frail patients. **LOE 3; GRADE C**

7.3 Conclusions and Recommendations

Diversion procedures are the most difficult open, laparoscopic, and robotic procedures to perform, more so, if the diversion is performed intracorporeally. The risk associated with RC and UD derives not only from the technical challenges of the procedure, but also from the nature of the patients who require it.[152] RC and UD are two steps of the same operation. Published studies uniformly report the complications of RC, but ignore that the majority of complications are related to the diversion. This might seem semantic, but it is not: complications can be caused by the RC, the underlying disease, the excluded gut segment, or the diversion itself. The incidence of early complications (defined as occurring either during the hospital stay or within 90 days of surgery) has been reported retrospectively to be in the range of 20% to 57%.[380] The International Consultation on Urological Diseases has looked at the published evidence and produced recommendations at various levels. For proper assignment to levels of evidence, one has to consider study design (prospective or retrospective), number of patients enrolled, whether or not the study cohort consists of all available patients, type of assessment tool and its psychometric properties (validity reliability), and response rate. Unfortunately, only 4 RCTs exist within the field of UD. All address ileoureterostomy (see section on OBS in men). Consequently, almost all studies used in this report are of level 3 evidence; that is to say, good-quality retrospective studies or case series, – or level 4 evidence, including expert opinion based on "first principles" research. Therefore, the grades of recommendations given are of grade C only. A grade C recommendation is given when expert opinion is delivered without a formal analytical process.

7.3.1 Secondary tumours

Patients who have undergone IC, CCD, or OBS do not seem to be at increased risk of secondary malignancy. By comparison, the risk is slightly higher after cystoplasty, albeit not increased sufficiently to support endoscopic surveillance. However, the present knowledge regarding gastric cystoplasty is insufficient; hence patients should be followed up after such surgery. Furthermore, yearly colonoscopy is recommended in cases involving UST, beginning 10 years after the procedure.

7.3.2 Prior radiation

Surgeons must be mindful that the effects of prior radiation exposure may significantly alter options for UD, complications, and long-term outcomes. Diversion-related complications are significant in patients who have undergone prior pelvic radiation therapy. Radiation damage to the cecum, appendix, and small bowel must be considered and evaluated when determining the most appropriate form of UD. Experience has shown, however, that thoughtful patient selection and adherence to meticulous surgical technique can provide acceptable outcomes in irradiated patients who require diversion, including continent reconstruction to the abdominal wall or urethra.

7.3.3 Enhanced recovery after surgery

Although there is accumulating evidence supporting the use of ERAS pathways in cystectomy patients, most studies are retrospective or underpowered. Thus, high-quality, prospective multicentre studies are needed to assess the different elements of ERAS protocols, such as optimal perioperative nutritional support, as well as the type and duration of pelvic and urinary catheterization, and the need to tailor ERAS elements in open versus minimally invasive surgery.[381] Usually, implementation of the ERAS protocol has resulted in significantly reduced length of hospital stay and decreased cost, though regrettably, with comparable rates of complications and readmission.

7.3.4 Renal function over time

Urinary tract obstruction remains the main factor responsible for eGFR deterioration after UD. Few literature reports are available on methods of evaluating eGFR, definitions of eGFR outcome, and factors predictive of outcome, and the current evidence is too weak to draw solid conclusions. Further well-designed studies and consensus reports on methods of assessment and definitions of eGFR are warranted.[382]

7.3.5 Quality of life

It appears that OBS provides better QoL outcomes than other forms of UD, especially in the short and intermediate term. However, comparison between different types of UD is difficult because of several factors—lack of RCTs, short follow-up, limitations in research methodology, models used, heterogeneity of clinical characteristics—leading to reduced utility of HR-QoL assessments in the clinical trial setting.

7.3.6 Hospital and surgical volume

Increasing facility RC volume is associated with increased rates of receipt of continent UD in both open and robotic RC. The committee strongly recommends that this type of surgery be performed only at high-volume hospitals. The committee continues to consider a minimum annual hospital case load of 25 surgeries, carried out by no more than 2 surgeons, to be the definition of a high-volume centre. Fifty per cent of RCs in the United States are done by surgeons doing no more than 2 to 5 cases annually, resulting in a nationwide OBS rate of fewer than 10%. In Germany, the nationwide median hospital RC number is 20. This results in a 36% annual OBS rate.[383]

7.3.7 Use of regenerative tissue for urinary diversion

There is much interest in developing tissue-engineered urinary diversions (TEUDs) in order to reduce the significant morbidity that results from using the alimentary tract in the urinary system. Thus far, all preclinical and clinical experiences in regenerating the lower urinary tract have shown histological evidence of complete urinary tissue recapitulation. This represents a major advance in the field of regenerative medicine; however, functional outcomes, in particular urinary storage,

contractile capacity, and neuronal innervation, have not been demonstrated to date in human clinical trials. Therefore, all research efforts must focus on this aspect of TEUDs before patients with benign pathology or bladder cancer can be expected to benefit from this form of regenerative medicine.[384]

7.3.8 Ileal conduit

IC diversion remains the most commonly used method for reconstructing the urinary tract in conjunction with RC. Associated complications, early as well as late, are legion. Several studies confirm a high incidence of upper tract complications, probably increasing with length of follow-up. It is difficult to draw definitive comparisons with other diversion techniques.

7.3.9 Orthotopic bladder substitution in the male

Worldwide, the use of OBS is declining. The reasons for this trend are the imperfect continence, the robot, costs, and the increasing age and frailty of patients. Population-based data from the United States show an OBS rate of 8%. This is in sharp contrast to the 60% to 80% OBS rate at pioneering institutions and dedicated high-volume RC centres. Technical reasons for imperfect continence are the use of too small, not crossfolded, and imperfectly detubularized reservoirs. This is particularly true for (intracorporeal) robotic reservoirs.

7.3.10 Orthotopic bladder substitution in the female

OBS in the female is greatly underused. OBS reconstruction is an attractive option for selected women undergoing RC for bladder cancer. Oncologic outcomes appear to be excellent with appropriate selection criteria. Careful attention to patient selection, surgical technique, and follow-up are important to optimize functional results. Additional studies are necessary to allow surgeons to minimize incontinence and urinary retention in these patients.

7.3.11 Continent cutaneous diversion

CCD is an acceptable option for UD following RC. Advantages of this diversion include excellent immediate continence and less frequent voiding. Significant disadvantages include longer operative time, dependence on catheterization to empty, an increase in infections, bowel symptoms, and late complications requiring surgical revision.

7.3.12 Cutaneous ureterostomy

The emergence of an increasingly aging and frail population undergoing RC and UD has rekindled interest in UD with a lower risk of perioperative complications, such as cutaneous ureterostomy. Contemporary results show that cutaneous ureterostomy with a single stoma can represent a valid alternative to IC in elderly patients with relevant comorbidities, reducing perioperative complications without a significant impairment of QoL.[385]

7.3.13 Palliative diversions

The prognosis for patients with malignant ureteral obstruction undergoing palliative diversion is poor, with a median survival in the range of 100 days and patients spending 20% to 50% of their remaining life span in hospital care. Several prognostic models have been developed and in poor-risk patients, the survival is in the range of 2 months. The indication for palliative diversion in poor-risk patients is highly questionable. An attempt at ureteral stenting is warranted, yet the mid-term progression rate to PCN is as high as 50%. Each type of UD has its pros and cons, and careful selection is necessary to balance benefits against risks in an effort to offer the best individual option to the patient. This is particularly true for older and frail patients.

FIGURE 7–1
Bricker Ileal Conduit

Adapted from Bricker EM. Surg Clin North Am. 1950;30:1511–1521.

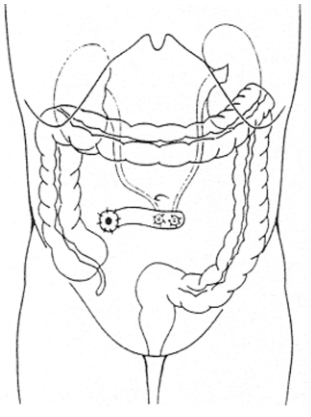


FIGURE 7–2
A: Crosswise incision of the ventral fascia of the rectus abdominis muscle.
B: The aboral end of the ileal conduit is pulled through the tunnel and is fixated to the skin with everting locking sutures.
C: The final result.

Adapted from Jr HF. Atlas of Urologic Surgery. *Elsevier: 2007.*

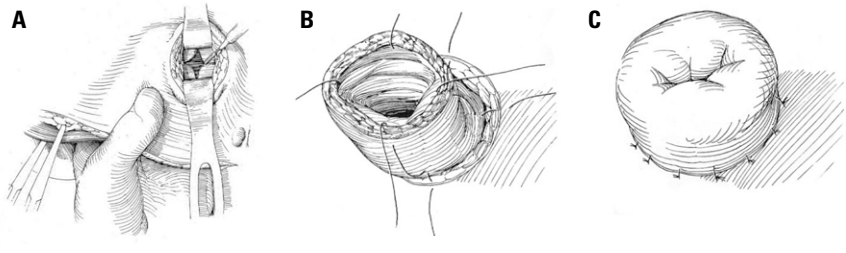


FIGURE 7–3
A buttonhole is excised 2 cm to 3 cm from the tip of the U-shaped flap

Source: Hautmann RE. Surgery illustrated - surgical atlas ileal neobladder. BJU Int. 2010;105:1024–1035.

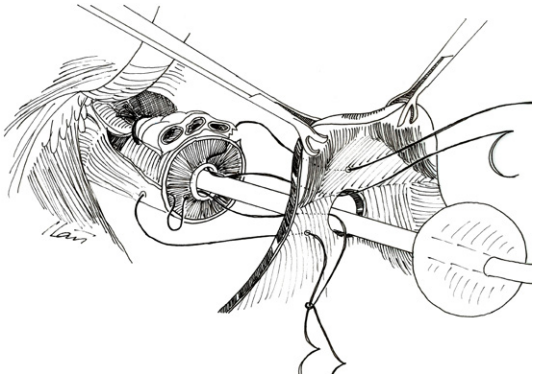


FIGURE 7–4
Under gentle traction, the catheter and ileal plate are manipulated down to the urethral remnant.

Source: Hautmann RE. Surgery illustrated - surgical atlas ileal neobladder. BJU Int. 2010;105:1024–1035.

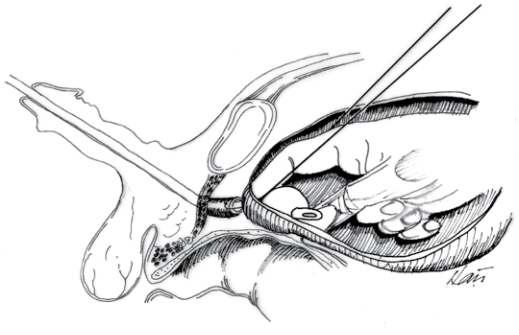


FIGURE 7–5
The lower third of the anterior wall of the neobladder is closed.

Source: Hautmann RE. Surgery illustrated - surgical atlas ileal neobladder. BJU Int. 2010;105:1024–1035.

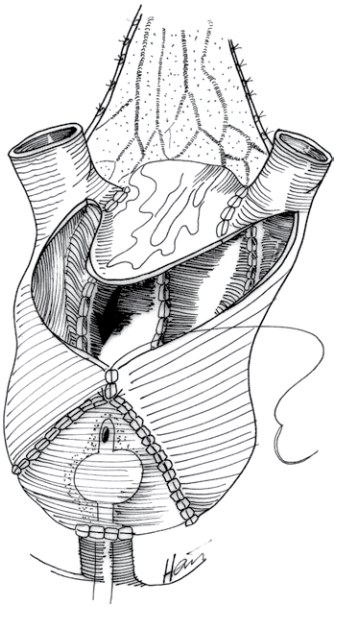


FIGURE 7–6
Refluxing Ileoureteral Anastomosis

Source: Hautmann RE. Surgery illustrated - surgical atlas ileal neobladder. BJU Int. 2010;105:1024–1035.

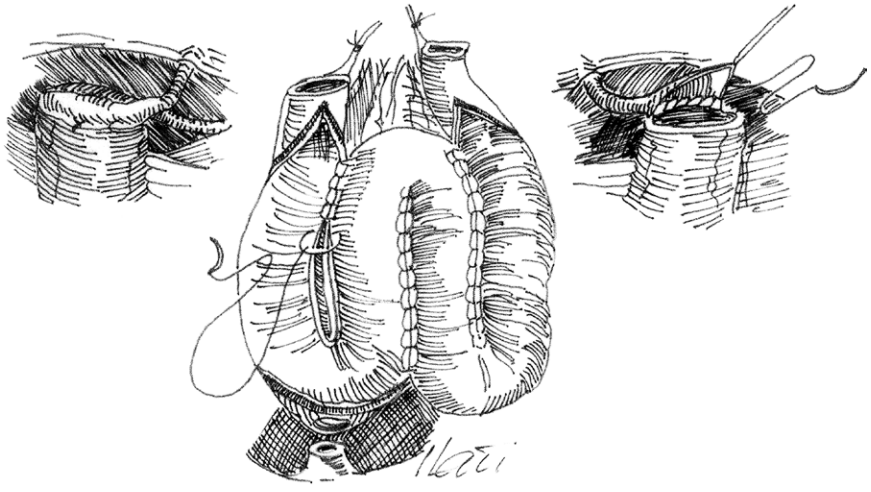


FIGURE 7–7
Extraperitonealization of the Entire Neobladder, Including the Ileoureteral Anastomoses

Source: Hautmann RE. Surgery illustrated - surgical atlas ileal neobladder. BJU Int. 2010;105:1024–1035.

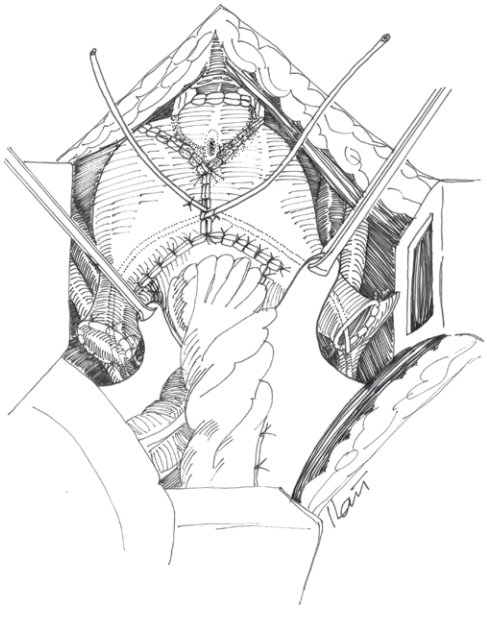


FIGURE 7–8

A: Stay sutures are placed into the rectosigmoid colon, where it reaches without tension to the promontory, to which it will be sutured later on.

B: The bowel segments are opened along the dashed line in the anterior tenia over a distance of about 20 cm.

Source: Hautmann RE. Surgery illustrated - surgical atlas ileal neobladder. BJU Int. 2010;105:1024–1035.

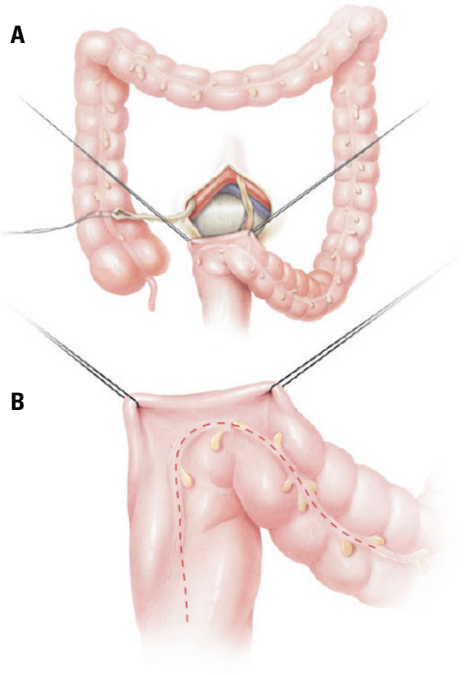


FIGURE 7–9

For preparation of the right submucosal tunnel, 4 stay sutures are placed over a distance of 4 cm through the intestinal mucosa and muscularis.

Source: Hautmann RE. Surgery illustrated - surgical atlas ileal neobladder. BJU Int. 2010;105:1024–1035.

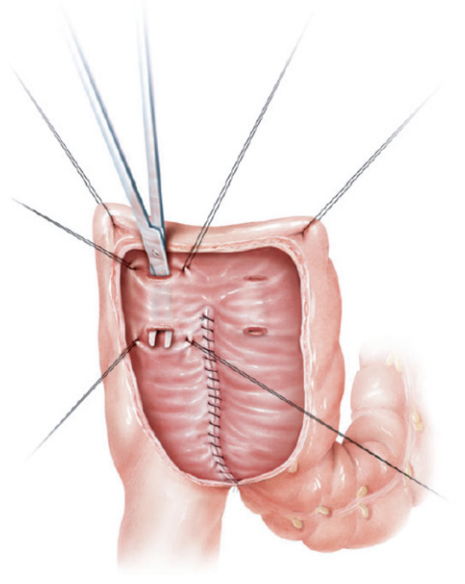
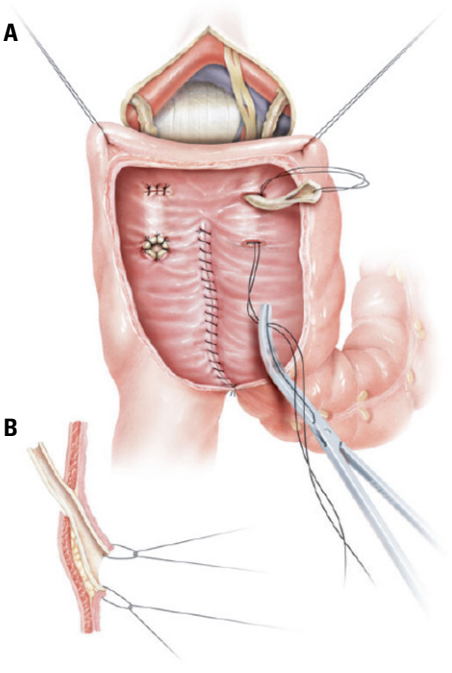


FIGURE 7–10

A: The left ureter is pulled on a stay suture through the submucosal tunnel by a curved clamp.

B: The neo-orifice is anchored at its most distal aspect at the 6 o'clock position with 2 absorbable 5-0 monofilament sutures through mucosa and muscularis of the intestine, and completed by several uretero-mucosal absorbable 6-0 monofilament sutures.

Source: Hautmann RE. Surgery illustrated - surgical atlas ileal neobladder. BJU Int. 2010;105:1024–1035.



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C8

Systemic Therapy for Metastatic Bladder Cancer

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Committee 8

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Systemic Therapy for Metastatic Bladder Cancer

8.1 First-line Treatment for Metastatic Urothelial Cancer: Cisplatin-eligible Patients

8.1.1 Introduction

Cisplatin-based chemotherapy was adopted as the standard of care front-line treatment for metastatic urothelial cancer (mUC) in the 1980s, when the combination of methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) showed objective response rates (ORRs) as high as 72% in patients with metastatic incurable urothelial cancers (UCs).[1] Subsequent trials easily proved that combination therapy was better than single-agent cisplatin.[2] Several decades of research into combination chemotherapy ensued; however, it soon became clear that we had reached a therapeutic plateau in clinical outcomes with combination chemotherapy that has not been surpassed in the intervening years.

8.1.2 Doublet chemotherapy

Early in the 21st century, the doublet of gemcitabine-cisplatin (GC) was accepted as a new standard of care for the treatment of patients with incurable UCs. Although the clinical trial did not meet the designed endpoint of an improvement in survival compared with traditional MVAC chemotherapy, the lower rates of neutropenia and mucositis resulted in a decreased frequency of neutropenic fever, providing a less toxic option than traditional MVAC chemotherapy.[3] However, it should be noted that even this new combination was toxic, with only 60% of patients completing treatment on a scheduled 4-week cycle and higher rates of thrombocytopenia compared with MVAC. Consequently, many patients have dropped the day-15 dose of gemcitabine, resulting in a more tolerable 3-week combination.

8.1.3 Dose-dense therapy

The use of dose-dense chemotherapy regimens has had a more significant impact on the toxicity of combination therapy, with only modest improvements in clinical activity. A small randomized trial of dose-dense MVAC (DDMVAC) was compared with the traditional 4-week cycle of MVAC, resulting in an improved complete response rate (21% vs. 9%; p=0.009) and twice the 2-year survival rate (24.7% vs. 11.6%; p=0.037).[4] The improved toxicity profile with decreased rates of mucositis, neutropenia, and thrombocytopenia has resulted in the use of DDMVAC in place of traditional MVAC, both in the metastatic and neoadjuvant settings, with several small studies in the latter suggesting similar response rates,[5-7] and long-term survival[5] as has been observed with traditional MVAC.

8.1.4 Triplet chemotherapy

One might argue that the clinical trial of gemcitabine, paclitaxel, and cisplatin (GTP) was also a study of dose density. This triplet combination, given on a 3-week schedule, was compared with the 4-week schedule of the doublet combination of GC.[8] Although this clinical trial did not meet the

designed endpoint of improvement in survival, there was an improvement in toxicity, with decreased frequency of thrombocytopenia and bleeding (11.4% vs. 6.8%; p=0.0031), resulting in more patients completing the triplet combination compared with the doublet combination.

Other combinations have not yielded an appreciable benefit compared with the current standards of GC or DDMVAC. Both the response rate and survival with MVAC were superior to a combination of cisplatin, cyclophosphamide, and doxorubicin (CISCA) (ORR, 65% vs. 46%; p<0.05; median overall survival [OS], 80 vs. 40 weeks; p=0.0003).[9] In an early attempt to modulate the immune response, alpha-interferon was combined with fluorouracil (5-FU), and cisplatin (FAP) and compared with traditional MVAC, suggesting similar survival (median OS, 12.5 months, both groups), but increased toxicity with higher rates of mucositis with FAP.[10] More recently, a trial combining bevacizumab and cisplatin with gemcitabine produced a promising OS of 20 months compared with historical controls. This combination has since been tested against cisplatin and gemcitabine on a 21-day schedule in the phase 3 NCI Cooperative group CALGB 90601 trial, which has completed accrual and is expected to report soon (see https://clinicaltrials.gov/show/NCT00942331). Other triplet combinations, including ifosfamide, paclitaxel, with cisplatin (ITP),[11] and ifosfamide with doxorubicin and gemcitabine (IAGem),[12] have suggested evidence of clinical activity in the treatment of UC. However, the difficulties in treating these often frail and elderly patients suggest that these more aggressive regimens, which require fluid hydration and sodium 2-mercaptoethanesulfonate (MESNA), are unlikely to be easily adopted by the general community. In patients who are not eligible for cisplatin due to comorbidities such as renal impairment, neuropathy, or heart failure or with performance status (PS), gemcitabine and carboplatin can be used. This combination was found to have a more favourable cancer control and toxicity profile compared with M-CAVI (i.e., the MVAC regimen modified to incorporate carboplatin) by the European Organisation for Research and Treatment of Cancer (EORTC).[13] Patients with these comorbidities and/or who are treated with carboplatin-based regimens tend to have poorer outcomes than patients receiving cisplatin, and so alternative novel therapies are very much needed for this population.

8.1.5 Front-line immunotherapy

There are several clinical trials currently ongoing studying immune checkpoint inhibitors in the front-line treatment of cisplatin-eligible patients.[14] Based on phase 2 data, multiple programmed cell death-1 (PD-1) or programmed cell death-ligand 1 (PD-L1) inhibitors show activity based on response and response durability in the first-line treatment of cisplatin-ineligible UC patients (see **Section 8.2** of this chapter). A phase 2 trial of the cytotoxic T lymphocyte–associated protein 4 (CTLA-4) inhibitor ipilimumab with cisplatin and gemcitabine was completed, with a response rate of 69% and a 1-year survival rate of 61%; while these outcome measures are similar to historical controls treated with cisplatin and gemcitabine, the addition of ipilimumab in the 3rd cycle was associated with circulating CD4-cell expansion that correlated positively with overall survival.[15] Several trials have focused on combination therapy of cisplatin- or carboplatin-based regimens with a PD-1– or PD-L1–directed immune checkpoint inhibitor either concomitantly or as a maintenance strategy following cisplatin-based chemotherapy. Additional trials are studying the impact of the combination of immune checkpoint inhibition with CTLA-4 and PD-1 or PD-L1 inhibition. Investigators in the field are eagerly anticipating the results of these trials within the next few years in the hopes that we may one day overcome the therapeutic plateau originally realized with cisplatin-based combination therapy for mUC.

8.2 First-line Treatment for Metastatic Urothelial Cancer: Cisplatin-ineligible Patients

8.2.1 Introduction

It is estimated that approximately 50% of patients with mUC are ineligible for cisplatin-based chemotherapy.[16] Poor kidney function from either comorbid medical conditions or obstruction of the ureters is a frequently cited reason in addition to poor hearing and peripheral neuropathy. Carboplatin-based doublets have played a frequent role in the treatment of these patients. However, the Food and Drug Administration (FDA) recently granted accelerated approval for pembrolizumab and atezolizumab for the front-line treatment of cisplatin-ineligible patients.

8.2.2 Carboplatin-based combinations

In an attempt to improve the toxicity profile of systemic chemotherapy, investigators have substituted carboplatin for cisplatin in several bladder regimens. An EORTC phase 2/3 trial compared two carboplatin-based regimens, gemcitabine and carboplatin (GCa) with M-CAVI, in 238 patients who were ineligible for cisplatin-based chemotherapy.[17] In addition to poor kidney function, this trial also allowed enrolment of patients with a PS of 2. There was no difference in response or survival when comparing GCa with M-CAVI (ORR, 36.1% vs. 21.0%; p=0.08; median OS, 9.3 vs. 8.1 months; p=0.64). However, M-CAVI had a higher rate of severe acute toxicity, including death, grade 4 thrombocytopenia with bleeding, grade 3 or 4 renal toxicity, neutropenic fever, or mucositis (21.2% M-CAVI vs. 9.3% GCa). In patients with both poor kidney function and poor PS, the ORR dropped, and severe toxicity rates increased for both GCa and M-CAVI (ORR, 25% vs. 27%; severe toxicity, 12.5% vs. 27.3%).

8.2.3 Triplet combination therapy

Triplet chemotherapy regimens have also been explored in patients with poor kidney function. A combination of gemcitabine with paclitaxel and carboplatin (GTCa) was explored in 60 patients with no or one prior chemotherapy regimen. This trial enrolled patients with both good kidney function, or poor kidney function requiring a serum creatinine ≤2.5 mg/dL.[18] The ORR was 43% with a median OS of 11 months. However, this regimen was considered more toxic than was typically observed with doublet-based chemotherapy, with grade 3 or 4 neutropenia occurring in 72% of patients. Another triplet combination of gemcitabine with paclitaxel and doxorubicin (GTA) enrolled 40 patients with previously untreated metastatic disease and a glomerular filtration rate (GFR) <60 mL/min.[19] Half of the patients enrolled had a GFR <40 mL/min. The ORR was 56.4%, with a median OS of 14.4 months. Only 33% of patients experienced grade 3 or 4 neutropenia on this trial, which did include same-day growth-factor support on treatment.

8.2.4 Front-line immunotherapy

Immunotherapy with immune checkpoint blockers such as avelumab, atezolizumab, durvalumab, pembrolizumab, and nivolumab has been found to be safe in patients with renal impairment. According to pharmacokinetics studies, renal function does not affect drug clearance. Therefore, package inserts for these drugs do not recommend dose adjustments for chronic kidney disease.[20–24] Two large trials of the anti-PD-1 and anti-PD-L1 agents pembrolizumab and atezolizumab, respectively, and for the first-line treatment of cisplatin-ineligible mUC patients found these agents to be safe in this patient population. There have also been several case reports of checkpoint blockers being safely administered to patients with end-stage renal disease (ESRD) on dialysis.[25] A major consideration in treating dialysis patients is the potential of anticancer drug ultrafiltration.[26] As these MAbs have large molecular weights, they are likely not dialyzable and may possibly be given without regard to the timing of dialysis. However, prospective trials are needed to understand the safety profile of anti-PD-1 and anti-PD-L1 therapies in patients with significant renal impairment and ESRD.

The anti–PD-1 antibody pembrolizumab phase 2 trial, KEYNOTE-052, recruited 370 previously untreated patients who were ineligible for cisplatin-based chemotherapy.[27] The ORR was 29%, including 7% of patients achieving a complete response (CR), with 82% of responders maintaining their response for more than 6 months. This regimen was well tolerated, with grade 3 or 4 events occurring in 19% of patients, making pembrolizumab an attractive option for cisplatin-ineligible patients.

The anti–PD-L1 antibody atezolizumab was approved following a phase 2 trial (IMVigor210) conducted in 119 untreated patients with mUC who were ineligible for cisplatin due to poor kidney function, hearing impairment, or peripheral neuropathy. The ORR was 23%, including 9% of patients who experienced a complete response; 70% of responders continued to respond at a median follow-up of 1.5 years. With longer follow-up, this cohort also had a median OS of 15.9 months, and treatment-related grade 3 or 4 events occurred in only 16% of patients.[28]

8.3 Targeted Agents and Biomarker-driven Strategies

8.3.1 Introduction

Historically, cisplatin-based combination chemotherapy has yielded improved survival in patients with mUC, and second-line chemotherapy with vinflunine or taxanes exhibited modest activity.[29–32] In fact, the median survival of patients receiving first-line cisplatin-based chemotherapy is only 12 to 15 months, while second-line taxanes or vinflunine yields a median survival of 6 to 8 months. Moreover, the median survival of cisplatin-ineligible patients receiving first-line carboplatin-based combination chemotherapy is only 8 to 9 months.[13]

Since 2016, the therapeutic landscape has witnessed exciting increments in survival outcomes provided by PD-1 and PD-L1 inhibitors.[33–37] In particular, pembrolizumab has extended overall survival as salvage systemic therapy compared with taxane or vinflunine chemotherapy (10.9 vs. 7.4 months).[33] Additionally, both pembrolizumab and atezolizumab gained accelerated approval by the FDA for the first-line therapy of cisplatin-ineligible patients with advanced UC based on nonrandomized phase 2 trials.[28] Phase 3 trials are now evaluating the role of PD-1 and PD-L1 inhibitors alone or in combination with platinum-based chemotherapy or CTLA-4 inhibitors as first-line therapy regardless of cisplatin eligibility.

However, most of the benefit from PD-1 and PD-L1 inhibitors appears confined to the minority of the 15% to 25% of patients who respond. Thus, the majority of patients garner either a modest or no benefit. Hence, there remains a major role for the development of biologic and targeted agents. Indeed, UC is a remarkably heterogeneous malignancy at the molecular level, suggesting that targeted therapy may play a major role in appropriately selected patients. Here, the emerging role of targeted agents and biomarker-driven strategies for mUC is reviewed. (Immunotherapy is reviewed in **Section 8.5** of this chapter.)

8.3.2 Biological rationale for targeted agents

The Cancer Genome Atlas (TCGA) project has highlighted the molecular heterogeneity of muscle-invasive bladder cancer (MIBC).[38] The majority (76%) of tumours harboured an inactivating mutation of chromatin regulatory genes. Additionally, recurrent mutations were noted in genes involved in cell-cycle regulation, chromatin regulation, and kinase signalling. Alterations of kinase genes including fibroblast growth factor receptor 3 (FGFR3), phosphatidylinositol 3 kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR), and mitogen-activated protein kinase (MAPK) pathways were frequently noted by the TCGA and other reports of tumour tissue alterations.[39]

Gene expression profiling led to multiple groups proposing major clusters of tumours including luminal and basal subtypes, which may have clinical implications.[40,41] Basal tumours are characterized by squamous and sarcomatoid histologic features, which express markers of stemness and epithelial-to-mesenchymal transition (EMT). Luminal subtypes are characterized by papillary features and

molecular aberrations observed in nonmuscle-invasive bladder cancer (NMIBC). The luminal TCGA "cluster II" or the "p53-like" subtype appears to be resistant to cisplatin-based chemotherapy, while the basal subtype appears chemosensitive as well as sensitive to immune checkpoint blockade.[42] Other data suggests that the luminal cluster-II subtype may also be sensitive to PD-1 and PD-L1 inhibitors. Basal tumours are also enriched for epidermal growth factor receptor (EGFR) and hypoxia-inducible factor, which may translate to sensitivity to EGFR and vascular endothelial growth factor (VEGF) inhibitors.[43] Luminal tumours frequently harbour activating FGFR3, ErbB2 receptor tyrosine kinase 2 (ERBB2), and ErbB2 receptor tyrosine kinase 3 (ERBB3) mutations. Thus, an improved understanding of tumour biology may inform the development of precision medicine. In an updated comprehensive analysis of 412 muscle-invasive bladder cancers characterized by multiple TCGA analytical platforms, 58 genes were significantly mutated, and the overall mutational load was associated with APOBEC-signature mutagenesis. In addition, the updated analyses have now identified 5 expression subtypes that may be helpful in the future to stratify response to different types of treatments.[44]

8.3.3 The activity of targeted agents

Data suggest a potential role for agents targeting specific molecules, which may be potential molecular drivers of subsets of patients with the disease.

8.3.3.1 Fibroblast growth factor receptor 3

FGFR inhibitors have demonstrated activity in a subset of tumours with FGFR3 alterations. Dovitinib, a multitargeted tyrosine kinase inhibitor (TKI) that inhibits VEGF receptors (VEGFRs) and FGFRs, was evaluated in a second-line mUC phase 2 trial.[45] The study was terminated early due to no responses in FGFR3-mutated tumours and only a 3.2% response rate in wild-type FGFR3 tumours. BGJ398, a more selective and potent FGFR3 inhibitor, yielded promising activity in patients with somatic FGFR3 alterations in a phase 1 trial.[46] The ORR in an expansion cohort of 25 evaluable pretreated patients receiving BGJ398 was 36%, including one unconfirmed CR.[47] Toxicities were manageable and included hyperphosphatemia, constipation, fatigue, and elevated serum creatinine. The pan-FGFR inhibitors BAY-1163877 (rogartinib) and JNJ-42756493 (erdafitinib) have also yielded responses in phase 1 trials enrolling mUC patients with FGFR tumour aberrations.[48,49] Early results from the phase 2 trial of erdafitinib suggest an ORR of around 42% using a continuous daily dose, with up-titration of the dose to achieve target phosphate levels.[166] Further investigation of these and other novel and potent FGFR inhibitors is planned or ongoing (**Table 8–1**). B-701, a monoclonal antibody (MAb) targeting FGFR3, is being evaluated in combination with docetaxel-based second-line therapy in a randomized phase 2 trial. This trial is enrolling a selected population harbouring tumour FGFR3 mutations or fusions and may offer a path to accelerated approval. In addition, a potentially underappreciated immunomodulatory role of the FGFR pathway has further enhanced the relevance of FGFR3 as a therapeutic target. In an analysis of TCGA UC samples, FGFR3 appeared to be the most frequent somatic mutation present in non-T-cell–inflamed tumours, suggesting an immunoinhibitory effect of FGFR3 on the tumour microenvironment.[50] Retrospective analysis of physician-reported outcomes suggests a low rate of response to prior immunotherapy (ORR in 1 of 22) in patients with FGFR3 mutations enrolled on treatment with erdafitinib.[166] The response rate to the optimal dose of erdafitinib was 73% in patients treated with a prior immuno-oncology (IO)

agent, suggesting a potential benefit from the sequence or combination. These observations form the basis for an ongoing phase 1 investigation of the FGFR inhibitor AZD4547 as monotherapy and in combination with the PD-L1 inhibitor durvalumab in mUC patients with FGFR tumour alterations.

8.3.3.2 HER family

Given that EGFR and HER2 are overexpressed on the majority of UC cells and appear to correlate with stage and outcomes, agents inhibiting these potential therapeutic targets have been investigated.[51–53] Unfortunately, agents targeting EGFR have demonstrated poor activity in clinical trials. Cetuximab did not improve outcomes when combined with first-line GC in a randomized phase 2 trial.[54] In another phase 2 trial, poor activity was observed with second-line cetuximab alone, while combination cetuximab plus paclitaxel yielded an ORR of 25%.[55] Gefitinib demonstrated poor activity in combination with GC in the first-line setting and as a single agent in the salvage setting.[56,57] None of the aforementioned EGFR targeting trials was restricted to an EGFR-altered population, and further study within biomarker-enriched patient subsets may be warranted.

In contrast, the targeting of HER2/HER3 has demonstrated encouraging results in selected patients. Trastuzumab demonstrated promising activity when evaluated in combination with first-line gemcitabine, carboplatin, and paclitaxel for mUC with overexpression of HER2 by immunohistochemistry (IHC), fluorescence *in situ* hybridization (FISH), and/or elevated serum HER2 levels.[58] However, in a separate randomized phase 2 trial, the combination of trastuzumab with GC did not demonstrate an increment in outcomes in patients with HER2-overexpressing (HER2 expression 3+ by IHC or 2+ by IHC plus FISH+) mUC.[59] Lapatinib demonstrated poor activity as salvage therapy and second-line switch maintenance, even in those with the highest HER1 or HER2 protein expression by IHC.[60–62] In contrast, afatinib, a more potent and irreversible inhibitor of HER1, HER2, and HER4, demonstrated activity in patients with advanced UC harbouring HER2 aberrations.[63] The median time to progression or discontinuation of afatinib was 6.6 months in patients with HER2 and/or ErbB3 alterations versus 1.4 months in those without alterations. Further evaluation of afatinib in selected patients is ongoing (**Table 8–1**). In addition, evaluation of the HER2-targeting antibody-drug conjugate (ADC) trastuzumab emtansine is ongoing in mUC patients with HER2 IHC+ tumours (**Table 8–1**). HER2 has also been targeted by employing the autologous antigen-presenting cell (APC) platform. APCs pulsed with the HER2 protein conjugated with granulocyte-macrophage colony-stimulating factor (GM-CSF) were evaluated in a randomized phase 2 adjuvant therapy trial for those with tumour HER2 ≥1+ by IHC. Although the results demonstrated safety, biologic activity, and immune memory generation, no extension of recurrence-free or overall survival was observed.[64,65]

8.3.3.3 PI3K/AKT/mTOR pathway

Activating aberrations of the PI3K/mTOR pathway may represent therapeutic targets in a subset of patients.[66–69] Despite the poor overall activity of everolimus, a subset of patients with tumours harbouring deletions in the TSC1 (tuberous sclerosis complex-1) gene or activating mTOR mutations exhibited durable disease control with the use of everolimus.[68] Furthermore, another phase 2 trial demonstrated partial responses (PRs) in 3 of 45 evaluable patients (6.9%) receiving temsirolimus as second-line therapy.[70] Thereafter, a retrospective analysis of this study suggested that single nucleotide polymorphisms (SNPs) of NR1I2 and its target genes CYP3A5 and ABCB1 may be associated with temsirolimus pharmacokinetics and toxicities.[71]

Everolimus, in combination with gemcitabine plus weekly fractionated cisplatin, is being studied in patients with creatinine clearance ≥40 mL/min in the first-line metastatic setting (**Table 8–1**). Another nonrandomized study is recruiting cisplatin-ineligible patients to assess everolimus alone or with paclitaxel (**Table 8–1**). Additionally, other oral PI3K inhibitors, buparlisib and sapanisertib, are being evaluated in phase 2 trials (**Table 8–1**).

8.3.3.4 Anti-angiogenic agents

Data generally supports targeting multiple angiogenesis-promoting pathways and specifically the VEGF pathway in UC.[72–75] Modest single-agent activity has been observed with sunitinib, pazopanib, and sorafenib in the first-line or salvage setting, which has been accompanied by toxicities typical for these multitargeted agents.[76–82] A randomized phase 2 trial could not demonstrate improved outcomes for pazopanib versus paclitaxel as second-line therapy.[83] Cabozantinib, a TKI targeting VEGFR and the receptor kinase MET, has demonstrated activity in a subset of patients for salvage therapy as a single agent and appears promising in combination with nivolumab or nivolumab plus ipilimumab.[84–86] Further development of this combination is planned.

Aflibercept displayed poor activity as a salvage single-agent therapy.[87] TRC105, an antibody targeting CD105, a transforming growth factor (TGF)-β coreceptor expressed on the endothelium, also did not appear to exhibit activity in the salvage setting.[88] Trebananib, a MAb that targets angiopoietin (Ang)-1 and Ang-2, displayed promise in combination with chemotherapy in a phase 1 trial.[89] While further development of trebananib in UC is not ongoing, a phase 2 trial is investigating regorafenib, a TKI that targets VEGFRs, TEK receptor tyrosine kinase (Tie2), platelet-derived growth factor receptor (PDGFR), and FGFR1 (**Table 8–1**).

Barring few exceptions, the combination of VEGF receptor TKIs with various chemotherapeutic agents appears toxic or did not confer increments of outcomes in most trials.[90–95] MAbs targeting VEGF or VEGFR2, in contrast, have been combined with chemotherapeutic regimens and may hold some promise. Bevacizumab in combination with GC first-line chemotherapy exhibited an ORR of 72%, including CR in 19% and median progression-free survival (PFS) and OS of 8.2 and 19.1 months, respectively.[96] Thromboembolic events were mitigated by amending to allow a lower dose of gemcitabine (1,000 mg/m^2). Bevacizumab has also been studied in a phase 2 trial in combination with GCa in cisplatin-ineligible or incurable patients, with activity suggesting an increment.[97] The phase 3 US Intergroup trial (**Table 8–1**) comparing GC with either placebo or bevacizumab has completed accrual and will provide definitive data.

8.3.3.5 Antibody-drug conjugates

Enfortumab vedotin (ASG-22ME) is an ADC that delivers monomethyl auristatin E to tumours expressing the surface adhesion protein Nectin-4, which is overexpressed in most UC tumours. A phase 1 trial enrolling 71 patients demonstrated antitumour activity with an ORR of 41%, including responses in 44% of patients with prior PD-1/PD-L1 inhibitor exposure and 47% with liver metastases.[98] Nineteen patients (28%) experienced a treatment-related adverse event of grade ≥3. Based on these data, a registration phase 2 trial for those with progressive disease following checkpoint inhibitor therapy is planned. Additionally, a trial evaluating enfortumab vedotin in combination with checkpoint inhibitors is also planned.

8.3.3.6 Other biologic targets

Apatorsen, an antisense oligonucleotide directed at heat shock protein (HSP)-27, has been shown to sensitize UC to several cytotoxic agents in a preclinical system.[99] Apatorsen was investigated in randomized phase 2 trials in combination with first-line GC and second-line docetaxel. In the first-line trial, while the overall outcomes were not statistically superior for adding apatorsen, the subgroup with poor-risk clinical features appeared to derive a benefit.[100] The second-line trial demonstrated a significant extension of overall survival (pre-specified criteria of one-sided $p<0.1$), the primary endpoint, with the addition of apatorsen (hazard ration [HR], 0.80; one-sided $p=0.08$).[101] Further evaluation of apatorsen may be warranted in selected patients. Novel agents targeting Aurora kinase A (AURKA) and cyclin-dependent kinase (CDK)-4/6 are ongoing (**Table 8–1**).

8.3.4 Clinical trial strategies to develop targeted therapy

Given the rapid evolution of systemic therapy for advanced UC, new strategies are necessary to make further advances. PD-1 and PD-L1 inhibitors (pembrolizumab and atezolizumab) are already available as first-line therapy for cisplatin-ineligible patients and are likely going to be available as first-line therapy for all patients if ongoing phase 3 trials validate their role in this setting. Nevertheless, there will remain patients who progress following definitive local therapy with or without perioperative chemotherapy, who will be candidates for PD-1 or PD-L1 inhibitors.

In this context, the following strategies for further development of targeted agents may hold relevance: 1) to identify subsets of patients progressing post–PD-1/PD-L1 inhibitors who may benefit from specific targeted agents; 2) to investigate the role of targeted agents alone or in combination with PD-1/PD-L1 inhibitors in those with both PD-1/PD-L1–naïve and post–PD1/PD-L1 inhibitor progressive disease; and 3) to identify the role of combining different targeted agents with each other and with chemotherapy.

Innovative clinical trial designs will need to address the molecular profile of the tumour instead of enrolling an unselected population. Moreover, the impact of validated baseline clinical prognostic factors should be accounted for when conducting nonrandomized trials.[102,103] Given the large number of potential therapeutic targets, the evaluation of multiple novel agents targeting different molecular aberrations in an accelerated fashion is necessary. The approach should probably use a staged adaptive design, which combines an umbrella trial and subsequent graduation of prime candidates with expanded targeted therapy trials. Activity may be analyzed using a Bayesian model, with more patients adaptively assigned to treatment arms based on efficacy. This concept has been employed in other malignancies, e.g., BATTLE (Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination), I-SPY-2 (Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging and Molecular Analysis-2), and LUNG-MAP (Lung Cancer Master Protocol) trials.

In this context, the BISCAY trial is a biomarker-directed, multi-arm, randomized phase 1b umbrella study in patients with progressive UC following prior chemotherapy. The primary objective is safety of new combinations of targeted agents and durvalumab, with secondary objectives of ORR and markers of response. The study will also explore whether combining targeted agents with durvalumab may trigger neoantigen release and accentuate response. The arms currently include durvalumab plus olaparib in those with somatic DNA repair gene alterations, durvalumab plus an FGFR3 inhibitor

in those with FGFR3 mutations or fusions, durvalumab plus a WEE1 inhibitor in those with WEE1 alterations, and a mutation-agnostic targeted therapy combination in patients with none of these somatic alterations. Tumour-agnostic trials enrolling patients selected for somatic molecular alterations targeted by biologic agents are another rational strategy when alterations are infrequent. For example, the TAPUR and NCI-MATCH basket trials are enrolling patients regardless of malignancy into cohorts based on molecular alterations. Another phase 2 trial is enrolling patients with advanced solid malignancies for everolimus in the presence of somatic TSC1- or TSC2-inactivating alterations or activating mTOR mutations (NCT02201212).

Preclinical and translational studies should continue to play a major role in discovering and prioritizing the most critical therapeutic targets. UC is a remarkably heterogeneous tumour, and large increments in outcomes are likely to require combinations of agents as well as biomarker-directed therapy. Moreover, molecular evolution of the malignancy could be evaluated by repeating biopsies or capitalizing on noninvasive profiling using circulating tumour (ct)-DNA profiling.[104,105] Investigators should also capitalize on different phases of the disease to expedite rational drug development, e.g., NMIBC, the neoadjuvant space for muscle-invasive resectable bladder cancer, and the second-line switch maintenance space following first-line chemotherapy for metastatic disease. The caveat is that biologic and even antitumour activity in these stages of disease may not always translate into benefit in the advanced disease setting.

8.3.5 Conclusions

Targeted agents will continue to be vigorously developed even in the era of immunotherapy. However, at this time, there is no clear role for employing targeted agents of any class for the routine off-protocol management of patients with mUC, and these agents should generally be offered on trials. Based on the data, Level of Evidence 2 and Grade of Recommendation B [**LOE 2; GOR B**] may be proposed for the use of the following as second- or later-line therapy: sunitinib; ramucirumab in combination with docetaxel; afatinib for patients harbouring somatic alterations of HER2/HER3; enfortumab vedotin; multiple FGFR3 inhibitors (BGJ398, BAY 1163877, and erdafitinib [JNJ-42756493]) for patients harbouring somatic FGFR3-activating mutations; and everolimus for patients harbouring somatic deletions or inactivating mutations of TSC1/2 or mTOR complex 1 (mTORC1)-activating mutations.

Despite the much-needed advances engendered by PD-1/PD-L1 inhibitors, the majority of patients derive modest or no benefit. A major challenge is the prioritization of various potential therapeutic targets and potential combinations. Thus, translational studies should continue to inform rational drug development. Finally, given that most aforementioned molecular alterations occur in minorities of patients, large international collaborative efforts are necessary to make rapid advances.

TABLE 8–1 Selected Ongoing Trials of Targeted Agents for Metastatic Urothelial Carcinoma

Phase	Therapeutic target	Line of therapy	Biomarker-based selection	Control arm	Experimental arm	Trial ID
3	VEGF	First-line, cisplatin-eligible	No	GC + placebo	GC + bevacizumab	NCT00942331
2	VEGF	First-line, cisplatin-ineligible	No	Atezolizumab	Bevacizumab + Atezolizumab	NCT03133390
3	VEGFR2	Second	No	Docetaxel + placebo	Docetaxel + ramucirumab	NCT02426125
1	MET/ VEGFRs	Salvage or first-line cisplatin-ineligible	No		Cabozantinib + atezolizumab	NCT03170960
2	VEGFRs, Tie2, FGFR1	Salvage	No		Regorafenib	NCT02459119
2	EGFR	First-line, cisplatin-eligible	No H-Ras or K-Ras mutation	Intense MVAC	Panitumumab + intense MVAC	NCT02818725
2	HER family	Salvage	ErbB2/ErbB3 mutations, ErbB2 amplifications, EGFR amplification		Afatinib	NCT02780687
2	HER2	Salvage	HER2+ (IHC)		Trastuzumab emtansine	NCT02999672
2	Ephrin-B2	Salvage	No		sEphB4-HSA + pembrolizumab	NCT02717156
3	FGFR1-4	Salvage	FGFR alteration	Chemotherapy	BAY-1163877	Pending
1/2	FGFR1-4	Salvage	FGFR alteration		BAY-1163877 + atezolizumab	Pending
2	FGFR3	Second	FGFR3 alteration (IHC→mutation)	Docetaxel	Docetaxel + B-701	NCT02401542
2	FGFR1-3	Second	FGFR alterations		Erdafitinib (JNJ-42756493)	NCT02365597
2	FGFR1-3	Salvage or first-line cisplatin-ineligible	FGF/FGFR alteration		INCB054828	NCT02872714
1	FGFR1-4	Second	FGFR alterations		PRN1371	NCT02608125
1	FGFR1-3	Second	FGFR3 alteration		AZD4547	NCT02546661

Abbreviations: CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor; ERBB, ErbB2 receptor tyrosine kinase; FGF, fibroblast growth factor; FGFR, FGF receptor; GC, gemcitabine and cisplatin; IHC, immunohistochemistry; mTOC1/2, mammalian target of rapamycin complex 1/2; mTOR, mammalian target of rapamycin; MVAC, methotrexate/vinblastine/doxorubicin/cisplatin; PI3K, phosphatidylinositol 3 kinase; TSC, tuberous sclerosis complex; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

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TABLE 8–1 Selected Ongoing Trials of Targeted Agents for Metastatic Urothelial Carcinoma, *Cont'd*

Phase	Therapeutic target	Line of therapy	Biomarker-based selection	Control arm	Experimental arm	Trial ID
2	ATR	First	No	GC	GC + VX970	NCT02567409
2	Aurora kinase A	Salvage	No		Alisertib	NCT02109328
2	CDK4/6	Salvage	Rb/CDKN2A+ →Rb/CCND1+ (IHC)		Palbociclib	NCT02334527
1	mTORC1	First-line	No		Everolimus + weekly cisplatin + gemcitabine	NCT01182168
2	mTORC1	First-line, cisplatin-ineligible	No		Everolimus alone or with paclitaxel	NCT01215136
2	PI3K	Salvage	Activating alterations in PI3K/Akt/mTOR pathway		Buparlisib	NCT01551030
2	mTORC1 and mTORC2	Salvage	TSC1 and/or TSC2 mutations		Sapanisertib	NCT03047213

Abbreviations: CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor; ERBB, ErbB2 receptor tyrosine kinase; FGF, fibroblast growth factor; FGFR, FGF receptor; GC, gemcitabine and cisplatin; IHC, immunohistochemistry; mTOC1/2, mammalian target of rapamycin complex 1/2; mTOR, mammalian target of rapamycin; MVAC, methotrexate/vinblastine/doxorubicin/cisplatin; PI3K, phosphatidylinositol 3 kinase; TSC, tuberous sclerosis complex; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

8.4 Second-line and Salvage Chemotherapy

8.4.1 Introduction

The landscape for the treatment of patients with mUC who have progressed after platinum-based chemotherapy has changed dramatically since the SIU-ICUD Bladder Cancer 2nd edition with the recent approval of five immune checkpoint inhibitors targeting PD-1 and PD-L1. Unfortunately, these agents only benefit a minority of patients and therefore continued efforts toward the evaluation and development of effective therapies in patients with advanced UC are needed. Chemotherapy has limited activity in patients who progress after platinum-based chemotherapy and additional studies will need to occur in order to understand the activity of chemotherapy in patients who progress after immune checkpoint inhibitors. Thus, this section does not address the use of chemotherapy after immune checkpoint blockade, a newly defined clinical state in patients with mUC.

8.4.2 Monotherapy

Vinflunine, a microtubule-inhibiting vinca alkaloid, is one of the most thoroughly investigated agents in the second-line setting. A phase 2 trial recruited 51 patients, most of whom had progressed within 12 months, and demonstrated an 18% response rate (RR) and a median duration of response of 9.1 months.[106] A second phase 2 trial accrued 175 patients with disease progressing within 12 months of platinum-based chemotherapy and demonstrated an RR of 15% and median duration of response of 6 months.[107] Subsequently, a randomized phase 3 trial accrued 370 patients and compared vinflunine plus best supportive care (BSC) with BSC alone as second-line therapy.[108] This trial included patients progressing after front-line platinum-containing chemotherapy for metastatic disease, and excluded those who had received prior perioperative chemotherapy only. More than 80% of patients had progressed within 6 months after prior chemotherapy and more than 70% of patients had visceral metastatic disease. An extension of survival, the primary endpoint, was not demonstrated by an intention-to-treat (ITT) analysis (6.9 vs. 4.6 months; p=0.287), but there was a statistical improvement in RR (8.6% vs. 0%) and median PFS (3.0 vs. 1.5 months). Approximately 30% of patients in both arms received subsequent systemic therapy, which may have confounded the survival analysis. Multivariate Cox analysis adjusting for prognostic factors demonstrated a statistically significant extension of survival with vinflunine (p=0.036), reducing the risk for death by 23%. In another analysis of the eligible patient population (n=357), the median survival was significantly longer for vinflunine plus BSC compared with BSC alone (6.9 vs. 4.3 months; p=0.04). Based on this study, vinflunine was approved by the European Medicines Agency (EMA). The main grade 3 or 4 toxicities for vinflunine were neutropenia (50%), febrile neutropenia (6%), anemia (19%), fatigue (19%), and constipation (16%). A retrospective analysis of patients who received second-line vinflunine identified Eastern Cooperative Oncology Group (ECOG) PS greater than 0, hemoglobin <10 g/dL, and liver metastasis as poor prognostic factors.[109] Vinflunine has also been investigated as a maintenance strategy after first-line therapy in patients with advanced UC.[110] A multicentre, open-label, randomized, phase 2 trial of maintenance therapy with vinflunine plus BSC versus BSC alone

in a total of 88 patients demonstrated an improvement in PFS with vinflunine plus BSC (median PFS was 6.5 months [95% confidence interval {CI}, 2.0–11.1] in the vinflunine group and 4.2 months [95% CI, 2.1–6.3] in the BSC group [HR, 0.59; 95% CI, 0.37–0.96; p=0.031]).

Overall, vinflunine has limited activity in patients with mUC. An OS benefit was seen in the randomized phase 3 trial compared with BSC in the eligible patient population but not by ITT analysis [**LOE 2; GOR B**]. With only a single phase 2 study in 88 patients, there is insufficient evidence to make a formal recommendation for the use of vinflunine as a maintenance strategy.

Numerous other chemotherapeutic and VEGF-targeted agents have been evaluated as monotherapy in nonrandomized phase 2 trials, and modest or marginal activity has been demonstrated for some agents (**Table 8–2**). Eligibility criteria for these reported phase 2 trials have been highly variable and heterogeneous, enrolling patients treated with perioperative chemotherapy followed by front-line therapy for metastatic disease or enrolling those who had received perioperative chemotherapy alone and with no requirement for a defined treatment-free interval after front-line therapy. In addition, prior treatment may not have been clearly defined. This renders comparison of outcomes across trials extremely difficult. In general, these trials report RRs of 5% to 25%, median PFS of 2 to 3 months, and median survivals of 6 to 9 months (**Table 8–2**).

Taxanes (paclitaxel, docetaxel, nanoparticle-albumin-bound paclitaxel) have been evaluated following first-line GC, while gemcitabine and the taxanes, alone or in combination, have been employed following MVAC. Both docetaxel and paclitaxel have demonstrated modest RRs (10%–15%) and poor survival outcomes (6–9 months).[31,111] In spite of poor patient outcomes, in the absence of alternative therapies, taxanes had been a mainstay treatment for patients who had progressed after platinum-based chemotherapy until the recent approval of immune checkpoint inhibitors in the second-line setting. Somewhat ironically, the control arms from the recently reported randomized phase 3 trials comparing immune checkpoint blockade with chemotherapy in patients with platinum-resistant mUC provide the largest prospective datasets of second-line chemotherapy to date.[112,113] KEYNOTE-045 is an open-label, international, phase 3 trial that demonstrated an improvement in survival in patients with advanced UC with the anti–PD-1 antibody pembrolizumab compared with the investigator's choice of chemotherapy (paclitaxel, docetaxel, or vinflunine).[112] In the control arm (n=272), 168 patients received taxanes (84 docetaxel, 84 paclitaxel) and 87 received vinflunine. The median OS in the chemotherapy group was 7.4 months (95% CI, 6.1–8.3) with an estimated OS rate at 12 months of 30.7% (95% CI, 25.0–36.7). The RR in the chemotherapy group was 11.4% (95% CI, 7.9–15.8). Treatment-related adverse events occurred in 90.2% of the patients receiving chemotherapy, with grade 3, 4, or 5 events occurring in 49.4% of patients. IMvigor211 is a larger phase 3 trial that enrolled 931 patients and randomized patients 1:1 to atezolizumab versus the investigator's choice chemotherapy (paclitaxel, docetaxel, or vinflunine). This study did not demonstrate a survival improvement for atezolizumab versus chemotherapy although a biomarker-based hierarchical statistical analysis plan was used, which may have influenced these results. Notably, among patients randomized to chemotherapy, the median OS was 10.6 months (95% CI, 8.4–12.2) and the estimated OS rate at 12 months was 41% (95% CI, 32–50); the better-than-expected outcomes in the chemotherapy arm of this study were particularly driven by patients receiving vinflunine. These

studies provide further evidence for the modest, yet variable, activity of chemotherapy in the second-line setting and argue strongly for continued investigation of novel therapies in those patients who do not respond or who progress after immune checkpoint blockade.

Overall, taxanes appear to have limited activity in patients with mUC as second-line therapy, and with the more recent FDA approval of five immune checkpoint inhibitors in platinum-pretreated patients, taxane use has an even more limited role in the salvage setting [**LOE 2; GOR B**].

Second-line pemetrexed, a multitargeted antifolate, has been investigated in previously treated patients. One phase 2 study demonstrated modest activity, with an ORR of 8% (1 of 12 patients), thereby not meeting the criteria for expansion to the second stage of the optimal 2-stage Simon design and the trial was concluded.[114] Conversely, another phase 2 trial in previously treated patients with locally advanced or mUC demonstrated an RR of 27.7% and a median OS of 9.6 months (95% CI, 5.1–14.6 months).[115] A more recently reported large retrospective analysis of pemetrexed use at Memorial Sloan-Kettering Cancer Center identified 129 patients with platinum-resistant advanced UC treated with pemetrexed.[116] The ORR was 5% (95% CI, 1–9), median PFS was 2.4 months, and the 6-month PFS rate was 14%.

Pemetrexed has very limited activity in the management of patients with advanced UC who have progressed after platinum-based therapy, and with the approval of immune checkpoint inhibitors in this setting, the use of pemetrexed as salvage therapy is not recommended [**LOE 2; GOR B**].

More precise delivery has the potential to improve the therapeutic index of cytotoxic chemotherapy in mUC. Several large phase 2 trials are exploring antibody-drug conjugates directed at antigens highly expressed in UC such as nectin and Trop-2.[117,118] Preliminary results from these studies have reported promising response rates in the 30% to 40% range in patients who have progressed despite prior platinum-based chemotherapy and even immune checkpoint blockade, and trials seeking regulatory approval for these agents have been initiated.

Variable activity has been seen with single-agent VEGF-targeted therapy in patients with progressive UC. In particular, two single-arm phase 2 trials of pazopanib have demonstrated conflicting results.[79,119] A randomized phase 2 study investigating pazopanib versus weekly paclitaxel in patients with relapsed or progressive UC was terminated early due to futility.[120] Median OS was 8.0 months for paclitaxel (80% CI, 6.9–9.7 months) and 4.7 months for pazopanib (80% CI, 4.2–6.4 months) with an HR adjusted for baseline stratification factors of 1.28 (80% CI, 0.99–1.67; one-sided p=0.89). Median PFS was 4.1 months for paclitaxel (80% CI, 3.0–5.6 months) and 3.1 months for pazopanib (80% CI, 2.7–4.6 months; HR, 1.09; 80% CI, 0.85–1.40; one-sided p=0.67). In summary, VEGF receptor TKIs have limited activity in patients with advanced UC.

There is no clear role for single-agent VEGF receptor TKIs in the management of patients with mUC [**LOE 2; GOR B**].

8.4.3 Combination therapy

Combination regimens have also been evaluated as second-line therapy in phase 2 trials (**Table 8–2**). A German, randomized, phase 3 trial of 102 patients compared the strategy of administering 6 cycles of second-line gemcitabine-paclitaxel with continuation beyond 6 cycles until progression.[121] None of the patients had received previous paclitaxel, and approximately half had received previous gemcitabine. The median OS was 7 to 8 months and the median PFS was approximately 3 to 4 months in both groups. The strategy of a fixed number of cycles versus continuation until disease progression could not be evaluated, as a mean of only 4 cycles was delivered in both groups due to rapid tumour progression and toxicity.

Another trial evaluated carboplatin-paclitaxel following prior cisplatin-based chemotherapy not including paclitaxel, and reported an RR of 16%, median PFS of 4 months, and median survival of 6 months.[122] A phase 1/2 trial evaluated a combination of salvage weekly cisplatin, gemcitabine, and ifosfamide in a heterogeneous group of patients who had received previous platinum-based chemotherapy.[123] The RR was 40.8%, but hematologic toxicities appeared prohibitive. Similarly, the combination of pemetrexed and gemcitabine has demonstrated moderate activity coupled with substantial myelosuppression.[124,125] Scant data support re-administration of second-line MVAC following prior first-line MVAC in those patients with an excellent previous quality of response and relatively prolonged time to progression.[126] Limited retrospective data suggests that MVAC may have activity after GC and that GC may have activity after MVAC.[127,128]

Pooled data from salvage systemic therapy phase 2 trials have suggested a benefit for combination chemotherapy compared with single-agent therapy. An analysis of individual patient-level data from eight phase 2 trials of single-agent taxane versus taxane-containing combination chemotherapy in 370 patients demonstrated that combination chemotherapy was independently and significantly associated with improved OS (HR, 0.60; 95% CI, 0.45–0.82; p=0.001).[129] In contrast to this finding, a systematic review and meta-analysis evaluated single-agent or doublet chemotherapy in the second-line setting after platinum-based chemotherapy that included 46 arms of trials including 1,910 patients: 22 arms with single agent (n=1,202) and 24 arms with doublets (n=708).[130] Despite significant improvements in ORR and PFS, doublet regimens did not extend OS compared with single agents for the second-line chemotherapy of UC.

There is no clear benefit for the use of combination chemotherapy over single-agent chemotherapy in the salvage treatment of patients with mUC [**LOE 2; GOR B**].

The use of VEGF receptor TKIs such as sunitinib in combination with chemotherapy has proved challenging due to poor tolerability.[131] A recently reported open-label, three-arm, randomized, phase 2 trial in the second-line treatment of locally advanced or mUC compared docetaxel monotherapy with docetaxel combined with ramucirumab, a VEGF receptor 2 antibody, and docetaxel combined with icrucumab, a VEGF receptor 1 antibody.[132] The addition of ramucirumab to docetaxel resulted in an improvement in PFS compared with docetaxel monotherapy (median, 5.4 months; 95% CI, 3.1–6.9 months vs. 2.8 months; 95% CI, 1.9–3.6 months; stratified HR, 0.389; 95% CI, 0.235–0.643; p=0.0002). There was no benefit associated with the addition of icrucumab. The phase 3 RANGE trial randomized 530 patients with locally advanced, unresectable, or mUC whose disease had progressed on or after

platinum-based chemotherapy to docetaxel plus either ramucirumab (n=263) or placebo (n=267) with a primary endpoint of PFS.[133] PFS was prolonged significantly in patients allocated ramucirumab plus docetaxel versus placebo plus docetaxel (median 4.07 months [95% CI, 2.96–4.47] vs. 2.76 months [95% CI, 2.60–2.96]; HR, 0.757; 95% CI, 0.607–0.943; p=0.0118). Ramucirumab plus docetaxel is the first regimen in a phase 3 study to show superior progression-free survival over chemotherapy in patients with platinum-refractory advanced UC. OS data are expected. No formal recommendation for the use of ramucirumab in combination with docetaxel can be made at this time.

8.4.4 Levels of evidence and grades of recommendation for second-line and salvage chemotherapy

With the recent approval of five immune checkpoint inhibitors in the treatment of patients with mUC who have progressed after platinum-based chemotherapy, in the great majority of patients, the use of chemotherapy should be considered only after a trial of immune checkpoint blockade. Single-agent chemotherapy including vinflunine, paclitaxel, and docetaxel has very limited activity in the salvage setting [**LOE 2; GOR B**]. There is no clear role for the use of combination chemotherapy over single-agent chemotherapy in the salvage setting [**LOE 2; GOR B**]. Although promising data exists for the combination of ramucirumab and docetaxel, no formal recommendation for the combination can be made at this time.

TABLE 8–2 Selected Phase 2 Trials of Second-line Chemotherapy and VEGF-targeted Therapy for Metastatic Urothelial Carcinoma

Drug	N	RR %	PFS (mo)	OS (mo)
Ifosfamide[134]	56	20	2.4	5.5
Gemcitabine[135]	30	11	4.9	8.7
Gemcitabine[136]	35	22.5	NA	5.0
Weekly paclitaxel[111]	31	10	2.2	7.2
Docetaxel[31]	30	13	NA	9.0
Nab-paclitaxel[137]	35	44	NA	NA
Paclitaxel-gemcitabine[138]	41	60	NA	14.4
Ifosfamide-gemcitabine[139]	34	21	4.0	9.0
Carboplatin-paclitaxel[122]	44	16	4.0	6.0
Pemetrexed[115]	47	27.7	2.9	9.6

Abbreviations: NA, not available or stipulated in publication; OS, overall survival; PFS, progression-free survival; mo, months; RR, response rate; VEGF, vascular endothelial growth factor.

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TABLE 8–2 Selected Phase 2 Trials of Second-line Chemotherapy and VEGF-targeted Therapy for Metastatic Urothelial Carcinoma, *Cont'd*

Drug	N	RR %	PFS (mo)	OS (mo)
Pemetrexed[114]	12	8	NA	NA
Ixabepilone[140]	42	11.9	2.7	8.0
Oxaliplatin[141]	18	6	1.5	7.0
Vinflunine[107]	175	15	2.8	8.2
Vinflunine[106]	51	18	3.0	6.6
Irinotecan[142]	40	5	2.1	5.4
Topotecan[143]	44	9.1	1.5	6.3
Sorafenib[82]	27	0	NA	6.8
Sunitinib[77]	45	7	2.4	6.9
Pazopanib[79]	41	17.1	2.6	4.7
Pazopanib[119]	19	0	1.9	NA
Pazopanib[120]	66	4.5	3.1	4.7
Docetaxel-ramucirumab[132]	46	24	5.4	10.4

Abbreviations: NA, not available or stipulated in publication; OS, overall survival; PFS, progression-free survival; mo, months; RR, response rate; VEGF, vascular endothelial growth factor.

8.5 Second-line Immunotherapy of Bladder Cancer After Platinum-based Therapy

8.5.1 Immunotherapy for bladder cancer

Immune checkpoint blockade with MAbs directed against CTLA-4, PD-1, and PD-L1 are revolutionizing treatment paradigms across multiple cancer types. These therapies have shown striking antitumour activity in an increasing number of solid tumours and hematologic malignancies, including tumours previously not considered immune responsive. Bladder cancer, however, has long been known to be immune-responsive.[144] Intravesical instillation of bacillus Calmette-Guérin (BCG) induces infiltration of cytotoxic T lymphocytes (CTLs) and mediates cell-mediated cytotoxicity against bladder tumours in patients with NMIBC. The emergence of BCG-refractory disease in a subset of patients suggests that BCG resistance may be mediated by a complex mechanism of immune escape.[62] An effective antitumour immune response involves a series of events: (a) cancer cells release cancer antigens; (b) dendritic cells and APCs present these antigens; (c) APCs and T cells are primed and activated; (d) CTLs traffic to and infiltrate tumours; and (e) CTLs recognize and kill cancer cells.[145] This process provides a framework for understanding the mechanisms of response and resistance to cancer therapy. Divergence from any of these steps facilitates immune escape, while optimizing each of these steps provides new therapeutic opportunities. For instance, while intravesical and systemic chemotherapies work by direct cytotoxic effects and release of cancer-cell antigens, intravesical BCG works by causing T cells to infiltrate tumours. While therapeutic cancer vaccines and anti–CTLA-4 antibodies work by priming, activating, and expanding T cells, immune checkpoint blockers such as anti–PD-1 and anti–PD-L1 antibodies restore effector T-cell function against cancer cells at the tumour site.[146,147] Hence, the PD-1/PD-L1 pathway is a powerful target for therapeutic intervention in oncology.

8.5.2 Immune checkpoint inhibitors in platinum-refractory urothelial cancer

Patients with advanced or mUC who have progressive disease after platinum-based chemotherapy have a median survival of less than a year,[144] presenting a significant need for better treatment options. Second-line chemotherapy trials have not shown a survival advantage compared with best supportive care.[144] Even targeted therapies with proven activity in many types of cancer have not shown survival benefits in UC.[62]

In Europe and the United States, treatment for mUC has changed a great deal recently, mainly involving a switch from chemotherapy to immune checkpoint blockers. This is particularly true in platinum-refractory disease, where supportive randomized data exist.[146,147] Five checkpoint blockers have been approved in this setting by the US FDA: avelumab, atezolizumab, durvalumab, nivolumab, and pembrolizumab.[34,146–150] Nivolumab, pembrolizumab, and atezolizumab have been approved in Europe. These approvals are not all based on randomized phase 3 trials. Indeed, 2 approvals were based

on large phase 1 trials in the United States. This unusual occurrence reflects the current enthusiasm for treating patients with these agents in the clinical setting and is driven by the modest proportion of patients who achieve long-term, well-tolerated durable benefit.[151] One of the complicating features of these studies is the selection of patients for treatment, which has at times been based on the PD-L1 biomarker.[147] The results and implications of these trials are discussed later in this chapter.

8.5.3 Randomized phase 3 data on platinum-refractory urothelial cancer

The KEYNOTE-045 and IMvigor211 trials studied pembrolizumab and atezolizumab, respectively, in patients who had progressed after 1 or 2 lines of chemotherapy.[146,147] (**Table 8–3**). The study drugs were compared with chemotherapy, for which investigators were given a choice between taxanes and vinflunine due to the lack of a global standard of care. The major difference between the two trials was their primary endpoints: for pembrolizumab, OS in the ITT population; for atezolizumab, OS in the PD-L1–positive population (SP142 antibody >5% of immune cells staining positive).

In KEYNOTE-045, pembrolizumab achieved its primary endpoint. The OS HR was 0.73 (95% CI, 0.59–0.91; $p<0.01$). Median OS was 10.3 months and 7.4 months for pembrolizumab and chemotherapy, respectively. Landmark analysis showed that >44% of patients were alive at 12 months. Response rates (21%) were significantly higher with pembrolizumab. Moreover, duration of response was longer with immunotherapy. PFS was similar in both arms. Data on toxicity and quality of life supported pembrolizumab. These data, the most robust for any of the checkpoint blockers in this setting, are practice-changing.

The PD-L1 biomarker results (22C3 antibody with combined immune and tumour-component staining) were more controversial. Although results showed enrichment with pembrolizumab (HR, 0.59; 95% CI, 0.37–0.88), they did not meet predefined statistical endpoints. Sensitivity and specificity of this biomarker are not high enough to recommend this therapy in unselected patients. This is particularly important, as the benefits of therapy do not appear to be confined to the PD-L1–positive population.

IMvigor211 explored atezolizumab in PD-L1–positive patients, which included 25% of the 931 patients enrolled on trial. No OS benefit was seen in this population (HR, 0.87; 95% CI, 0.62–1.21). The median OS was 11.1 months (95% CI, 8.6–15.5) for atezolizumab and 10.6 months (95% CI, 8.4–12.2) for chemotherapy. The biomarker predicted responses to both chemotherapy and atezolizumab, with response rates of 23% in both arms in the PD-L1–positive population (vs. 13% in both arms in the ITT population). Together, these results showed that the biomarker selected responders to both immunotherapy and chemotherapy. The biomarker endpoint was chosen because of impressive results in the phase 1 and 2 trials. However, these single-arm studies were not able to distinguish between the prognostic and predictive factors of the biomarker. IMvigor211 highlights the risks of biomarker-driven approaches. Had the primary endpoint been the ITT population, as was the case in the pembrolizumab trial, the study would have had a positive result.

Statistical significance cannot be drawn from the ITT population due to the study design. Subsequent analysis is therefore exploratory. Analysis of these data has two goals: to get a better understanding of why the trial failed, and to quantify drug activity in the context with the previous phase 1 and 2 studies. Median OS was 8.6 months (95% CI, 7.8–9.8) and 8.0 months (95% CI, 7.2–8.6) for atezolizumab and chemotherapy, respectively. Response rates were 13% in both arms. The HR of OS in the ITT population was 0.85 (95% CI, 0.71–0.99). Duration of response was better with immunotherapy, and a tail to the Kaplan Meier curve occurred, with impressive landmark analysis. Adverse events were less frequent with atezolizumab, and quality-of-life data also favoured atezolizumab. Forest plot analysis showed inconsistent results with different chemotherapy agents and different anatomical origin of the UC. This was particularly true for vinflunine and upper-tract tumours. Atezolizumab appears to be an attractive alternative to chemotherapy, based on data from the phase 1 and 2 trials that also showed well-tolerated durable remissions. It is likely that these factors were important in the FDA and EMA giving a positive recommendation for atezolizumab in this setting.

TABLE 8–3 Summary of Randomized Phase 3 Trials in Platinum-refractory Urothelial Carcinoma

Study drug	KEYNOTE-045 pembrolizumab	IMvigor211 atezolizumab
Number of patients receiving study drug	270	467
PS 2	1%	0
Bladder primary	86%	69%
Patients with ≥2 risk factors	41%	23%
Visceral metastasis	89%	77%
Liver metastasis	34%	30%
≥2 previous lines of therapy	20%	19%
Vinflunine in control arm	34%	54%
PD-L1–positive patients	40%	25%
RR in ITT	21%	13%
OS in PD-L1–positive patients	HR, 0.59 (95% CI, 0.37–0.88)	HR, 0.87 (95% CI, 0.62–1.21)
RR in PD-L1–positive patients	22%	23%
OS in all patients	HR, 0.73 (95% CI, 0.59–0.91)	HR, 0.85 (95% CI, 0.71–0.99)

Abbreviations: CI, confidence interval; HR, hazard ratio; ITT; intent-to-treat; OS, overall survival; PD-L1, programmed cell death-ligand 1; PS, performance status; RR, response rate.

8.5.4 Phase 1 and 2 trials of durvalumab, avelumab, and nivolumab

There are no randomized data on durvalumab, avelumab, or nivolumab in platinum-refractory disease. All three agents have been given FDA approval based on phase 1 or 2 data.[148–150] The majority of phase 2 data for atezolizumab came from IMvigor210. This drug was the first to show high response rates, impressive landmark survival, and biomarker enrichment for response.[34] The randomized phase 3 data described above have superseded this study.[147]

It is noteworthy that each of the five checkpoint blockers employ a unique method of biomarker analysis. Also, some of the trials described below had enrichment phases, where only biomarker-positive patients were enrolled. Therefore, any form of cross-trial comparison is futile.

An overview of the agents with no randomized data in this setting underlines the consistency of the results. Nivolumab has both FDA and EMA approval. It was tested in a phase 2 study with 270 patients.[148] Response rates were 19.6% (15.0%–24.9%), and median OS was 8.7 months (6.1–NA). PD-L1 positivity was defined as >1% expression on tumour cells. Inconsistencies occurred with this biomarker. Durvalumab was tested in 191 patients. Response rates were 18%, and OS was 18.2 months (95% CI, 8.1–NA), although the analysis was performed with a median follow-up of 5.8 months.[150] Biomarker-positive patients (SP263-positive in immune and tumour cells) had better outcomes. A degree of patient selection occurred in this study due to biomarker enrichment. Avelumab was tested in 44 patients.[149] Response rates were 18.2%, and median OS was 13.7 months (95% CI, 8.5–NA). Conclusions to be drawn from these three studies include: a) a proportion of patients achieve long-term durable benefit with each of the drugs, usually between one-fifth and one-third of patients, depending on biomarker enrichment; b) the agents appear to be well tolerated, with similar adverse-event profiles; c) inconsistent results have been seen with the biomarker across the board. This has hampered development of the drugs and none of the agents has biomarker-driven regulatory approval; and d) a majority of patients gets no significant long-term benefit from these agents. Median PFS is always short, and disease progression is most common as a best response to therapy in this setting. This is particularly true for patients with liver metastasis.

These results elicit key questions concerning the next steps in this area of high unmet need. Can efficacy be improved? New combinations are needed to increase response rates and outcomes. These may or may not be immunotherapies or immune combinations, as chemotherapy and targeted therapy combinations hold promise as well. There are phase 2 data with ipilimumab/nivolumab and pembrolizumab/epacadostat in UC, with some promising results.[152,153] Randomized studies are planned with both combinations. Combinations with targeted therapy and durvalumab in selected patients are also ongoing (BISCAY NCT09432452). The next question is what can be done to better identify patients who have clear benefit. There is a need to find alternative biomarkers to PD-L1. Tumour mutational burden and immune gene signatures have been investigated with some success.[34] Finally, should we be testing these drugs earlier in the disease setting? A plethora of front-line trials and studies in earlier disease states are ongoing.

8.5.5 Mutational load and comparison with The Cancer Genome Atlas data

Predictive biomarkers of response to anti–PD-1 and anti–PD-L1 therapy are required to facilitate appropriate patient selection for treatment. PD-L1 staining by immunohistochemistry cannot reliably predict outcomes in UC, and the field is characterized by conflicting data.[28,34,36,146,148,154] Several new biomarkers have been studied for their ability to predict objective responses, although associations with OS have not yet been reported in randomized trials.

Bladder cancer has the third-highest mutational load of any solid tumour.[155] The TCGA project in bladder cancer revealed that the mean and median mutation rates for MIBC were 7.7 and 5.5 mutations/Mb within coding regions, leading to 302 protein-coding mutations per cancer.[156] These mutations are due to multiple processes, most commonly APOBEC-mediated mutagenesis (misregulated endogenous proteins involved in innate antiviral responses) and DNA repair defects (e.g., ERCC2 somatic mutation).[157,158] Nonsynonymous mutational load has a proven association with outcomes in immune checkpoint blockade for multiple solid tumours.[159,160]

In IMvigor210, the single-arm phase 2 study of atezolizumab with 2 cohorts (cohort 1, cisplatin-ineligible without prior chemotherapy for metastatic disease; cohort 2, progression after prior platinum-containing therapy), pretreatment tumour tissue was obtained for a subset of patients and DNA was extracted for mutational analysis using the FoundationOne assay. To determine mutational load, all coding short variant alterations (base substitutions and indels), including synonymous alterations, were counted and divided by the coding region size. In 150 cohort-2 patients with available tissue, median mutational load was significantly higher in atezolizumab responders compared with nonresponders (12.4/Mb vs. 6.4/Mb; $p<0.0001$). Interestingly, smoking status did not correlate with either response to atezolizumab or mutational load. In addition, patients whose tumour mutational load was in the highest quartile had improved survival compared with those in the lower 3 quartiles.[161] These findings were replicated in cohort 1 of the study, where a similarly pronounced benefit in OS was noted.[28] However, these data were derived from a single-arm study; therefore, whether this is truly a predictive versus prognostic biomarker is unclear.

Recent data have demonstrated that bladder cancer may be subdivided into multiple RNA-expression subtypes with distinct biology and clinical behaviour.[5,40,156,162–164] Multiple schemas have been proposed based on different genomic features. TCGA bladder cohort proposed categorizing as clusters I, II, III, and IV.[156] Clusters I and II are similar to luminal breast tumours, while clusters III and IV are similar to basal breast tumours. In IMvigor210, RNAseq was performed on 195 tumours, and RNA expression clusters were assigned based on TCGA schema. PD-L1 expression was high in the basal clusters III and IV, while CD8 T-effector gene expression was high in clusters II, III, and IV, and not in luminal cluster I. Intriguingly, objective response rates to atezolizumab were highest in cluster II (34%), compared with 10% in cluster I, 16% in cluster III, and 20% in cluster IV. In Checkmate 275, the single-arm phase 2 study of nivolumab, gene expression profiling was performed on 177 tumours classified by TCGA schema.[148] This dataset showed that the basal cluster III had the highest proportion of responders (30%), closely followed by the luminal cluster II (27%).[165] Whether the differences in subtype responses observed between these 2 studies is due to differences in targeting PD-L1 (atezolizumab) versus PD-1 (nivolumab), or to the relatively small groups of patients in each subtype is unclear.

Further evaluation of existing tumour classification schemes may yield deeper insights into the biology of immunotherapy response, and perhaps provide a better method of identifying potential responding tumours. Mutational load data suggest that while neoantigens associated with high numbers of mutations may predispose to a higher chance of antitumour response with immune checkpoint blockade, a high mutational load neither guarantees nor precludes response. Further work is needed to integrate these new biomarkers into the treatment of UC.

8.5.6 Summary

Immunotherapy has superseded chemotherapy for UC, largely driven by the urgent need for new treatments. Few patients are benefiting from anti–PD-1 or anti–PD-L1 therapy, a situation that calls for further intensive study. Although phase 2 data shows similarities across the board for these agents, both positive and negative randomized data exist, highlighting the need to identify better combination treatments and biomarkers.

8.6 Summary and Recommendations

8.6.1 Front-line treatment—cisplatin-eligible patients

- Front-line treatment for patients with unresectable or mUC of the bladder should consist of combination cisplatin-based chemotherapy. The cisplatin-based regimen can be MVAC, ddMVAC, or GC **[LOE 1; GOR A]**.
- Bajorin risk stratification can be employed in cisplatin-eligible patients with mUC **[LOE 2; GOR B]**.

8.6.2 Front-line treatment—cisplatin-ineligible patients

- In patients with renal impairment, advanced age, or poor PS, carboplatin and gemcitabine is recommended for front-line therapy **[LOE 2; GOR B]**.
- The addition of paclitaxel or other agents to gemcitabine plus cisplatin or to gemcitabine plus carboplatin in cisplatin-ineligible patients is not recommended **[LOE 2; GOR C]**.
- Immunotherapy with pembrolizumab or atezolizumab can be considered in the front-line setting in cisplatin-ineligible patients based on single-arm, phase 2 trials **[LOE 2; GOR C]**.

8.6.3 Second-line chemotherapy

- Risk stratification of patients in the second-line setting can be based on ECOG performance status (PS >1), hemoglobin level (<0 g/dL), presence of liver metastases, and time from previous chemotherapy **[LOE 2; GOR B]**.
- The administration of chemotherapy in the second-line setting will depend on the patient's PS, comorbidities, and age. The decision to treat will also depend on the patient's willingness to receive chemotherapy **[LOE 2; GOR B]**.
- Only marginal benefit is expected from standard chemotherapy in patients with poor PS (>1). Therefore, best supportive care should be considered in these patients **[LOE 3; GOR C]**.
- If renal function is adequate, progression occurs >6 months after first-line therapy, and patients present with PS of 0 or 1, re-exposure to first-line cisplatin-based treatment can be considered **[LOE 3; GOR B]**.
- Vinflunine is approved for second-line therapy after platinum-based therapy in Europe, but not in North America. Where available, it should be considered for second-line chemotherapy after prior platinum-based therapy **[LOE 2; GOR B]**.
- Monotherapy or combination chemotherapy especially with paclitaxel, docetaxel, pemetrexed, gemcitabine, and carboplatin may be considered in the second line **[LOE 3; GOR B]**.

8.6.4 Second-line immunotherapy

- PD-1–directed or PD-L1–directed checkpoint blockade demonstrates improved objective response rates and overall survival with less toxicity compared with second-line single-agent chemotherapy after prior platinum-based therapy. The level of evidence for superiority of checkpoint blockade over chemotherapy is highest for pembrolizumab. These agents should therefore be preferred over chemotherapy in this setting **[LOE 2; GOR B]**.
- PD-L1 expression by immunohistochemistry is inadequate to predict response to PD-1–directed and PD-L1–directed checkpoint inhibitors **[LOE 2; GOR D]**.
- There is insufficient evidence to support the use of total mutational burden to predict response to PD-1–directed and PD-L1–directed checkpoint inhibitors **[LOE 2; GOR D]**.

8.6.5 Targeted therapy

- Novel targeted therapies for UC are urgently needed **[GOR C]**.

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C9

Non-Urothelial Cancer of the Urinary Bladder

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CONTENTS

Non-Urothelial Cancer of the Urinary Bladder

9.1 Introduction

Pure non-urothelial bladder cancers comprise a small minority of about 5% of all bladder cancers. These are distinct from urothelial tumours, with a variant histologic component. They comprise several histologic subtypes. Squamous cell carcinoma (SCC) is perhaps the most prevalent of these subtypes, with a distinct etiology, pathogenesis, and phenotype. It is also the one subtype that is most closely linked to an infectious etiology and can be caused by infestation with the waterborne parasite *Schistosoma haematobium*. Other causes such as long-term irritation and trauma also bear an etiologic link to SCC. Adenocarcinoma of the urinary bladder is also well described and has a measurable prevalence. This subtype often arises from the glandular elements within the urachus, but non-urachal types are also prevalent. There appears to be a link between topographic location in the bladder and prognosis. The literature is replete with descriptions of other subtypes such as neuroendocrine, and sarcomatoid carcinoma. Some tumours where a variant histology such as micropapillary or nested forms dominate take on a biology different from that of standard urothelial tumours.

Non-urothelial tumours are generally thought to have a worse prognosis compared with urothelial tumours. However, this may not always be the case. Some of this may be due to stage-agnostic assessment of survival. Once corrected for stage and other patient-related factors, a significant proportion of non-urothelial tumours may have prognosis similar to that of urothelial tumours. Diagnosis, evaluation, and staging of non-urothelial bladder cancers use approaches that are generic to all bladder cancers. In some subtypes, unique features that would entail special diagnostic techniques may include identification of Schistosoma eggs in the bladder wall, serum or urinary catecholamine analysis in bladder pheochromocytoma. Special radiologic studies and immunostaining may also be necessary to demonstrate the tumour subtypes.

Management of non-urothelial cancers is largely based on experience from retrospective case series and some prospective data. There are very limited well-conducted clinical trials in this tumour subtype. This hampers the ability to make strong recommendations, which are generally based on high-level evidence. The rarity of these tumours will make it difficult to conduct randomized trials to assess ideal therapeutic strategies. The mainstay of therapy has been surgical resection followed by chemo or radiation therapy in some cases. Neuroendocrine tumours and lymphomas tend to be managed with primary chemotherapy, as is the case with similar tumours in other organs, recognizing the fact that they are primarily a systemic and chemoresponsive disease. Radiation therapy is often used as an adjunct either in a neoadjuvant or an adjuvant fashion. In this chapter, with the help of a broad collection of experts from urology and pathology, we have analyzed the available data and developed a set of consensus recommendations to help provide guidance in the management of the complicated collection of disease entities that represent non-urothelial bladder tumours. Most of the consensus statements are based on lower levels of evidence due to the data quality, and hence rely to a significant extent on the opinion of the expert panel.

9.2 Squamous Cell Carcinoma (SCC)

Squamous cell carcinoma may occur *de novo*, or in individuals who have been infected with the parasite *Schistosoma haematobium*. It is important to recognize the distinction between these two populations of patients, because the epidemiology, natural history, and treatment recommendations are different. Each will be discussed separately in the sections below.

9.2.1 SCC not associated with schistosomiasis

9.2.1.1 Epidemiology

Often referred to as non-bilharzial SCC, this subtype represents the most common non-urothelial bladder malignancy, accounting for 2% to 5% of cases in most contemporary cystectomy series.[1–5] These tumours are most often diagnosed during the seventh decade of life.

The incidence of SCC demonstrates less of a male predominance than does urothelial carcinoma. Compiling data from 915 patients in 10 series of patients with SCC, Johansson and Cohen[6] reported that the ratio of men to women was 1.4:1. Similar to urothelial carcinoma, however, women are more likely than men to present with advanced disease.[7] Data from the Netherlands Cancer Registry[8,9] corroborates this higher incidence of SCC noted in women relative to men (1:1.1) and T3/T4 tumours (21.7% vs. 14.5% in T3, and 14.5% vs. 8.4% in T4).

The incidence of SCC of the bladder with respect to time assessed using the Surveillance, Epidemiology, and End Results (SEER) United States population dataset showed a decrease during the period from 1973 to 2013 ($p<0.05$).[10] Data from the SEER program conducted between 1973 and 1997[11] shows that there is a large racial disparity in the incidence of SCC. With an annual incidence of 1.2 per 100,000 person-years, African Americans were twice as likely to develop these tumours as Caucasians, who had an annual incidence of 0.6 per 100,000 person-years.

9.2.1.2 Etiology

9.2.1.2.1 Pathogenesis

Chronic bladder irritation and inflammation caused by various conditions such as urinary retention, recurrent infection, urolithiasis, indwelling catheters, foreign body, and bladder exstrophy are known risk factors for the development of SCC. Keratinizing squamous metaplasia, which clinically presents as leukoplakia, often develops as a result of chronic irritation and has a documented association with squamous carcinoma.[12,13] The reported risk of developing SCC is estimated to be 21% to 42% in these patients, with a latent period of 4 to 28 years.[13] In a study of 34 patients with keratinizing squamous metaplasia, Khan *et al.* found 4 patients who presented with synchronous squamous carcinoma. In addition, 55% of 14 patients with extensive and 12.5% of 16 patients with limited keratinizing metaplasia developed subsequent SCC.[14] On the other hand, non-keratinizing squamous metaplasia, commonly seen in the trigone of women, is considered a normal variant.[15] However, Lagwinski and colleagues in their detailed analysis of 45 cases of SCC of the bladder reported that 44% of patients, the majority of whom were male, demonstrated non-keratinizing metaplasia as an associated superficial pathology. These findings suggest keratinization may not be a prerequisite for carcinogenesis.[16] The association between multifocal and/or extensive squamous metaplasia and

SCC has been demonstrated in several studies, and these patients require close follow-up. Although squamous metaplasia is a risk factor for SCC, currently available evidence is insufficient to support squamous metaplasia as a preneoplastic lesion in the bladder.

9.2.1.2.2 Spinal cord injury (SCI)

In the United States, patients with SCI represent the largest cohort of patients affected by SCC. Squamous cell carcinoma in this condition is believed to arise from chronic urinary tract inflammation[17] due to neurogenic bladder and the need for catheterization. Historically, the incidence of bladder cancer in patients with SCI was believed to be as high as 2.3% to 10%, with most cases representing SCC.[18–20] Patients with SCI with indwelling catheters were found to have the highest risk for the development of SCC; up to 10% of these individuals developed SCC at 10 years.[18] Interestingly, studies determined that patients performing clean intermittent self-catheterization were significantly less likely to develop SCC than those SCI patients with indwelling catheters.[21,22] Recently, these concepts have been scrutinized by data emerging from several contemporary series. A United States Department of Veterans Affairs (VA) review of admission data from 33,560 patients with SCI identified only 130 patients with bladder cancer, for an overall incidence of 0.39%.[23] Some 42 patient records were available for review, including 23 (55%) with urothelial carcinoma, 14 (33%) with SCC, and 4 (10%) with adenocarcinoma. It is noteworthy that in 26 patients with indwelling catheters, the incidence of SCC and urothelial carcinoma was equal, implicating chronic inflammation and tobacco abuse as competing risk factors. In another study of 2,900 SCI patients from several centres in the Southwest United States, bladder cancer was detected in only 8 (0.32%) patients, none of whom had an indwelling catheter.[24] Another study evaluated 15 SCI patients with SCC, who were followed over a 24-year period from a VA Center in Northern California.[25] The majority of these patients had never had an indwelling catheter. Finally, in the largest study to date, Pannek[26] evaluated 43,561 SCI patients from multiple urologic centres in Eastern Europe. In all, 48 patients with bladder cancer were identified, for an overall incidence of 0.11%. Only 7% of the patients in this series had indwelling catheters, and the prevalence of SCC was only 0.02%. Based upon these epidemiologic data, guidelines regarding the necessity, frequency, and the diagnostic testing needed for bladder cancer screening in the SCI patient population cannot be determined. Initial reports, in which a high percentage of patients with spinal cord injury developed SCC are flawed, primarily related to the retrospective manner in which the data were obtained, the small patient cohorts, and the less rigorous statistical evaluation than would be expected for a more contemporary evaluation. Although the incidence of SCC in a contemporary SCI population appears to be less than 1%, it is recommended that these patients should be monitored, particularly if they have indwelling catheters or have a history of tobacco abuse. Any history of hematuria should be evaluated.

9.2.1.2.3 Smoking

The relationship between SCC and cigarette smoking is not clear; Johansson and Cohen[6] found a higher incidence of SCC in smokers. SEER data support a direct correlation between quantity of cigarettes smoked and relative risk of developing SCC.[27] Indirect evidence from the Swedish Cancer Registry,[28] however, does not support an association between SCC and smoking. A review of this data, which plotted trends in bladder cancer in Sweden between 1960 and 1993, revealed that, despite a rising incidence of urothelial carcinoma in Swedish women (which correlated with an increased prevalence of smoking), the incidence of SCC remained relatively constant.

9.2.1.2.4 Other associations

Most evidence in the literature suggests a very limited or no role of human papilloma virus (HPV) infection in the development of bladder SCC. However, rare exceptions have been described in patients with neurogenic bladder undergoing chronic catheterization in whom HPV was detected in SCC presenting with basaloid features.[29] Squamous cell carcinoma has also been reported following radiation and cyclophosphamide therapy.[30,31] Other studies have revealed possible genetic and chromosomal changes that may be associated with SCC. Similar to urothelial carcinoma, abnormalities of chromosomes including monosomy 9, trisomy 7, and rearrangements of chromosomes 3, 8, 10, 13, and 17 have been detected in SCC.[32] Studies of uroplakin II gene expression found a significant difference in expression between urothelial carcinoma and SCC, with expression being greater in SCC. Uroplakins are the major differentiation products of the urothelium that also control the various pathways of urothelial differentiation.[5] Mutations of the p53 gene were of similar frequency but differed in type compared with urothelial carcinoma.[33] However, in addition to unique alterations common to squamous carcinoma in other locations, gene profiling studies show that bladder SCC shares a significant number of dysregulated genes with conventional urothelial carcinoma, suggesting a close evolution between the two cancers.[34] With the advent of molecular subtyping of urothelial carcinoma, there has been increased focus on predictive correlates of these subtypes. Although not pertaining directly to pure squamous carcinoma, the clinically aggressive and potentially chemotherapy-sensitive "basal" subtype is significantly enriched for the presence of squamous differentiation in urothelial carcinoma.[35]

9.2.1.3 Clinical features

The presenting symptoms in patients with non-bilharzial SCC are not distinguishable from those of urothelial carcinoma. Hematuria is the main clinical feature in 63% to 100% of patients. Irritative bladder symptoms are reported in two-thirds of patients. Weight loss, back or pelvic pain, and frank obstructive symptoms are less common and suggest advanced disease.[3] A urinary tract infection is present in 30% to 93% of patients at the time of diagnosis,[3,36,37] and symptoms have often been present for a prolonged period of time before the diagnosis is made.[3] Relatively few series of patients from Europe and the Americas have addressed pure SCC. Most of these are retrospective observational series that are more than 10 years old, and they use older staging and grading systems. Despite this, we know that the majority of patients present with a bulky, solitary tumour that extensively involves the bladder wall. These tumours are sessile lesions, often with ulceration and areas of squamous metaplasia adjacent to the primary tumour. A predilection for the trigone has been noted, but SCC can arise anywhere within the bladder. Tumours may occupy a bladder diverticulum and have been described in association with bladder calculi.[38] Squamous cell carcinoma is often locally advanced at the time of diagnosis. Debbagh *et al.*[39] reported that 10 (71%) of 14 patients in their series had a palpable tumour on rectal examination, and 11 (79%) had upper urinary tract obstruction. Pretreatment imaging studies may demonstrate hydronephrosis in 33% to 59% of cases.[3,5] Of 114 patients with SCC of the bladder in one series, 92% had T2 to T4 disease at the time of diagnosis, and most tumours were high grade.[2] As with urothelial carcinoma, clinical understaging is seen in as many as 73% of patients.

9.2.1.4 Treatment

Pure SCC of the bladder has a poor prognosis, with most patients succumbing within 1 to 3 years of diagnosis. Failure to provide loco-regional control is the hallmark of the difficulty in managing these patients, and little, if any, research has been directed toward improving the outcome of patients diagnosed with this disease. In a series of 120 patients from the Royal Marsden Hospital, the overall 5-year survival rate was 16%, with only 8% of patients developing metastatic disease and the rest developing local disease progression.[36]

9.2.1.4.1 Radiation

Irrespective of whether radiation is used as neoadjuvant or primary treatment, results have been uniformly poor and derived from older studies.[3,40] In one of the larger series reporting on patients treated before 1986 by Quilty and Duncan,[37] 51 patients were treated with radical radiotherapy, delivered with a 3-field beam-directed technique, covering the entire bladder, to a prescribed dose of 55 Gy over 4 weeks. Patients were treated prone, immediately after emptying their bladders. Only 4 patients in this series had T2 cancer, with a median survival of 14.3 months, and a 3-year survival rate of 26.8%. More recently, radiotherapy-only regimens are rarely used, except for palliation in patients unfit for surgery or in combinations with chemotherapy, because of side effects and advances in chemotherapy, transforming the role of radiation therapy to be a part of multimodality treatment that has shown some success in recent small series.[41,42]

9.2.1.4.2 Chemotherapy

Squamous cell carcinoma has been described as a chemotherapy-refractory disease, and there are no clear recommendations on whether or when to use neoadjuvant and adjuvant chemotherapy due to the rarity of the disease and the small number of patients included in retrospective studies. Nevertheless, complete remissions in pure SCC were reported following the administration of platinum-based regimens, such as methotrexate/vinblastine/epirubicin/cisplatin (MVEC), carboplatin/5-fluorouracil/leucovorin, cisplatin and taxanes, or cisplatin/gemcitabine/ifosfamide.[43–45]

9.2.1.4.3 Radical surgery

In contrast to urothelial cancer, effective intravesical therapy does not exist for non-muscle–invasive SCC, and therefore radical cystectomy (RC) is considered at presentation. While most of the surgical series are subject to selection bias, radical cystectomy seems to offer some advantages in patients with SCC. Serretta *et al.*[5] reported on 19 patients with pure SCC of the bladder undergoing radical cystectomy. With a mean follow-up of 52 months, 63% had died of local recurrence, with only one patient developing distant metastases. Rausch *et al.*[43] performed a retrospective single-centre analysis of 42 patients with SCC treated with surgery between 1989 and 2004. Stage pT3 was present in 55% of the patients, and nodal and distant metastases was identified in 26%. The overall 5-year survival rate was 26% (tumour specific 46%), with a median survival of 10.5 months. Three of four patients with pT2N0 bladder carcinoma were cured by cystectomy.[43] In another recent series of 45 patients,[16] in which 67% presented with T3 tumours, 37% of patients were alive without disease and 29% had died of disease at a median follow-up of 15 months following cystectomy. Similarly, Kassouf *et al.*[44] reported a 50% recurrence in 10 of 20 patients who had undergone cystectomy at only 5 months. The majority of patients who died of SCC had isolated pelvic recurrence in the absence of disseminated metastases, emphasizing the importance of local surgical control.

9.2.1.4.4 Impact of urinary diversion

The impact of the type of urinary diversion in patients with SCC is the subject of some discussion in the literature. In a series of 19 patients undergoing radical cystectomy and urinary diversion, all 3 patients with an orthotopic ileal neobladder developed recurrence at the anastomosis between the neobladder and the urethra.[46] In another series reported by Stenzl *et al.*,[47] intraoperative frozen-section biopsies were obtained from the bladder neck before orthotopic reconstruction. No local recurrences occurred in the 5 female patients in this series.

9.2.1.4.5 Prognostic comparison with urothelial carcinoma

Several large contemporary cystectomy series in the literature have compared outcomes in patients with urothelial carcinoma with those in patients with SCC. In a large series from Japan,[48] evaluating 1,042 patients treated with urothelial carcinoma and 89 patients with SCC, there was no significant difference observed in the 5-year post-cystectomy survival rate (68.0% urothelial carcinoma vs. 60.8% SCC). In a review of SEER data between 1988 and 2003,[49] patients with SCC had worse outcomes than those with urothelial carcinoma except for those patients with localized cancers who were treated by cystectomy.

9.2.1.4.6 Prevention and early detection

Several screening protocols have been advocated in an attempt to diagnose these tumours earlier, thereby improving outcomes. Broecker *et al.*[50] recommended annual cystoscopy and urine cytology in patients with SCI. Others have suggested routine random bladder biopsies every 1 to 2 years. Navon *et al.*[51] advocated urine cytology or random bladder biopsies in patients with spinal cord injuries for more than 10 years or in those with recurrent or chronic urinary tract infection. No validated biomarkers have been so far identified to help in the early diagnosis of SCC, and chemoprevention of this disease is nonexistent.

Recommendations

In summary, non-bilharzial SCC is an uncommon form of bladder cancer that usually presents at an advanced stage, generally recurs loco-regionally, and has an extremely poor prognosis. Death is most often related to loco-regional failure, and not to disseminated metastasis. Radiation alone should be probably reserved for palliative treatment, and the role of chemotherapy in this disease has not been well defined. The current literature supports cystectomy as the treatment of choice, when possible, after proper counselling of patients on the relatively low overall survival rate.

Consensus Statement	Level of Evidence (LOE)	Grade of Recommendation (GOR)
Patients with long-term indwelling catheters and chronic irritative symptoms or hematuria should undergo evaluation for possible development of SCC.	2	B
Patients with localized non-bilharzial SCC should be offered radical cystectomy with wide resection and regional lymphadenectomy as primary treatment.	2	B
Radiation therapy for non-bilharzial SCC should be reserved for palliation.	3	C
Chemotherapy can be offered in metastatic disease.	4	C

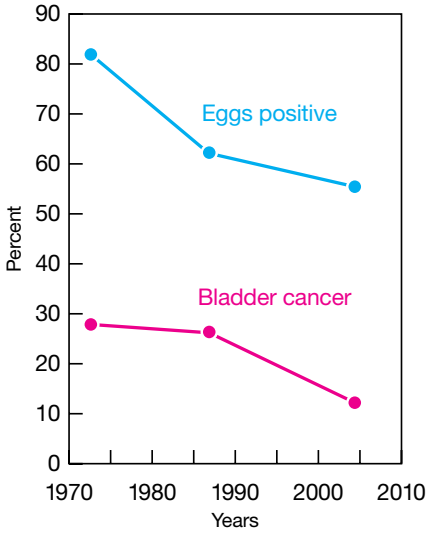
9.2.2 SCC associated with schistosomiasis

9.2.2.1 Epidemiology

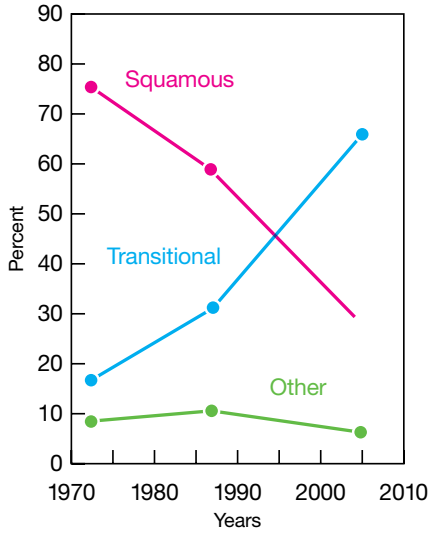
Squamous cell carcinoma is also prevalent where urinary *Schistosoma haematobium* is endemic. It is often referred to as schistosoma-related SCC or bilharzial SCC (B-SCC). The highest incidence of SCC of the bilharzial bladder occurs in Egypt.[52] In a report by Ghoneim *et al.*,[53] SCC accounted for 59% of 1,026 cystectomy specimens. A high incidence of SCC is also found in Iraq, the Gizan region in Southern Saudi Arabia, Yemen, and Sudan. In other parts of Africa, the disease has been reported in the Gold Coast region and in South Africa. However, the incidence in these countries is lower because Schistosoma haematobium is less endemic and less severe.[54] With a mean age at presentation of 46 years, the mean age of patients with B-SCC is 10 to 20 years younger than that seen with non-bilharzial SCC.[55] In areas in which schistosomiasis is endemic, some 80% of cancer specimens have shown histologic evidence of bilharzial infestation.[54] A lag period of approximately 30 years has been reported between infection with the parasite and subsequent development of the disease. The male to female ratio is 5:1.[56] This male predominance is attributed to sustained contact with infected water supplies that laborers often endure while working in various outdoor environments. Contemporary epidemiological studies have demonstrated a decline in the incidence of B-SCC, as public health efforts have been successful in eradicating schistosomiasis in many areas of the Nile Delta and in rural Egypt.[57] Interestingly, despite the decline in SCC, the incidence of bladder cancer remains high. This is believed to be a result of the high prevalence of tobacco use that has now created a significant rise in the incidence of urothelial cancer (**Figure 9–1**).[58]

FIGURE 9–1
Successful control of schistosomiasis and the changing epidemiology of bladder cancer in Egypt.

(Reproduced with permission from Salem S, et al. BJU Int. 2011;107(2):206–211.)[57]



The decline in the relative frequency of both bladder carcinoma and its bilharzial association during 37 year (1970-2007)



The change in the relative frequency of histological types of bladder carcinoma during 37 years (1970-2007)

9.2.2.2 Biology

Schistosoma haematobium is a blood trematode that most commonly inhabits the venous plexus of the urinary bladder. The lifespan of an adult worm ranges from 3 to 5 years, and a typical patient harbours hundreds of worms. The female parasite produces hundreds of eggs per day. These progressively move toward the bladder or ureters and are eliminated in the urine. However, about half are trapped in the tissue during the process of migration through the bladder wall and provoke an inflammatory response.[59] Bladder infection by *S. haematobium* usually results in various secondary changes such as ulceration, reactive urothelial proliferation, fibrosis, and squamous metaplasia.[60] These changes are often compounded by secondary bacterial infections.[61] Given the parasitic load and lifespan, the inflammatory response and related changes that result in the development of SCC occur repeatedly over a prolonged period of time and at an accelerated rate compared with patients with chronic bladder irritation due to other causes linked with non-bilharzial SCC. The few available studies in the literature do not show significant differences in cytogenetic and molecular abnormalities between bilharzial and non-bilharzial SCC.[62] However, comparative genomic hybridization data has shown more frequent losses of chromosomes 17p and 18p in squamous compared with transitional Schistosoma–associated bladder carcinoma.[63]

9.2.2.3 Clinical features

The clinical presentation of B-SCC is similar to conventional SCC in most respects, with dysuria, hematuria, and necroturia being the main symptoms. Imaging studies may frequently demonstrate calcifications in the bladder and distal ureters.[53] Grossly, tumours are generally of the nodular, fungating type, and are located in the dome or posterior or lateral walls of the bladder.[60] Most patients present for treatment at an advanced stage, and 25% of cases are inoperable when first seen.[55] This is because of the nonspecific symptoms of B-SCC, which often mimic simple bilharzial cystitis. Similar to urothelial bladder cancer, clinical understaging occurs in a high percentage of cases,[56] and B-SCC is often locally advanced at the time of diagnosis. In a study of 608 patients with B-SCC, pT1 disease was found in 2.6%, pT2 in 10.5%, pT3 in 80.0%, and pT4 in 6.9%.[53] Lymph node metastases were present in only 18.7% of cystectomy specimens. Interestingly, the vast majority of tumours were low grade, a factor that may account for the low incidence of lymph node positivity.[64]

9.2.2.4 Treatment

9.2.2.4.1 Radiation

Although growth characteristics of carcinoma of the bilharzial bladder identified by older studies were suggestive of favourable response to radiation therapy,[65] the experiences with external beam radiation therapy for definitive control of these tumours were disappointing. Factors that interfered with the efficiency of radiation treatment in these cases included coexisting Schistosoma-related urologic lesions, which interfere with local tissue tolerance, and considerable tumour bulk, which reduces local tumour control. Furthermore, the presence of radioresistant hypoxic tumour cells is suspected in light of the capillary vascular pattern of this cancer.[66]

While radiation alone has not been shown to be very effective, neoadjuvant radiation before surgery in B-SCC is associated with some therapeutic efficacy. Prospective randomized studies in this disease have suggested that treatment with preoperative radiotherapy improves disease-free survival over

RC alone.[67] Also, just as in the case of non-bilharzial SCC, the treating physician could consider multimodality therapy including radiation with concomitant chemotherapy as a useful alternative for unresectable bladder tumours or in cases where bladder preservation is desired.[68]

9.2.2.4.2 Radical cystectomy

Radical cystectomy and urinary diversion provides a logical treatment approach for patients with resectable tumours.[65,69] In an early series of 138 cases, Ghoneim *et al.*[69] reported a high perioperative mortality rate of 13.7%. This was primarily due to peritonitis, intestinal obstruction, and liver failure. Cardiopulmonary complications were uncommon among this relatively young group of patients. In this older series, the overall 5-year survival rate was 32.6%. Patients with pT1 and pT2 disease had a 43% overall 5-year survival rate, which compared favourably with 30% in patients with pT3 and pT4 tumours. Low-grade tumours were associated with a 46% overall 5-year survival rate, and high-grade disease was associated with a 5-year survival rate of 21%. Lymph node metastases were associated with a poor outcome, and they reduced the 5-year survival rate to 20%.[65] A more contemporary report evaluating 1,026 cystectomy patients from an area in which schistosomiasis is endemic[53] found that 59% of tumours were SCC. Bilharzial ova were identifiable in 88% of specimens. Interestingly, extravesical extension was not significantly different between patients with B-SCC and those with urothelial carcinoma (13.5% and 14.9%, respectively). Overall, the 5-year survival rate with B-SCC was 50.3%. Factors identified to have significant effect on survival after cystectomy included tumour stage and grade, lymph node involvement, and lymphovascular invasion. In fact, lymphovascular invasion has more prognostic significance with B-SCC than with urothelial carcinoma of the bladder.[70]

9.2.2.4.3 Chemotherapy

Several agents have been evaluated by Gad-el-Mawla *et al.*[71] in order to treat patients who are initially not believed to be surgically resectable. All trials were phase 2 studies in which a single agent was used, and the optimal results were obtained with epirubicin. Neoadjuvant and adjuvant epirubicin chemotherapy were used in a prospective, randomized study involving 71 patients with invasive B-SCC. Disease-free survival rates were 73.5% and 37.9%, favouring the chemotherapy group.[72] Additional long-term follow-up results have not been published. Studies investigating gemcitabine with cisplatin in the neoadjuvant setting showed conflicting results, precluding our ability to make any conclusions on the role of this neoadjuvant combination in treating B-SCC.[73]

Prevention and early detection

Bilharzial SCC is a preventable malignant disease. Primary prevention entails control of bilharziasis through snail control (the intermediate host of the parasite) and mass treatment of the rural population with oral antibilharzial drugs such as praziquantel. Secondary prevention includes early detection with urine cytology and selective screening of the population at risk. The yield of a single screening study done in a rural area in Egypt was 2 per 1,000 individuals. Chemoprevention by the administration of retinoids to revert to normal, precancerous atypical squamous metaplastic lesions was previously discussed as a feasible approach;[74] however, no recent studies were published on the effectiveness of this preventive strategy.

Recommendations

In summary, bilharzial SCC is the most common form of bladder cancer in endemic areas. It most often presents at an advanced stage but with low-grade cells. Cystectomy is the standard treatment, but long-term survival remains disappointing (**grade B**). Limited evidence (**grade B**) supports a potential role of neoadjuvant chemotherapy (NAC) and radiation therapy, but it is not yet sufficient to facilitate a recommendation.

Consensus Recommendation	LOE	GOR
Radical cystectomy should be offered as the primary therapy for patients with bilharzial SCC.	2	B
Neoadjuvant radiation therapy with or without chemotherapy could improve survival following radical cystectomy.	3	B

Immunotherapy in SCC of the bladder

Immunotherapy represents a promising adjuvant treatment after radical cystectomy that may improve outcome in patients with SCC of the bladder. Recent studies in transitional cell carcinoma have explored targeting the programmed cell death 1 (PD-1) pathway to increase T-cell response to malignant tissue.[75] A new programmed cell death-ligand 1 (PD-L1) agent atezolizumab has shown good activity in platinum-refractory metastatic transitional cell carcinoma and is now FDA approved in that setting.[76] While no current data support the use of immunotherapy in SCC of the bladder, it appears that the clinical benefit from drugs active in the PD-1 pathway is independent of histology, and may play a role in future treatment of all types of bladder carcinoma.

9.3 Urachal and Non-Urachal Adenocarcinoma

9.3.1 Definition

Adenocarcinomas are malignant tumours with glandular features. Adenocarcinomas of the urinary bladder are broadly divided into primary vesical tumours that originate *de novo* within the urinary bladder, tumours arising from the urachal remnant, and metastatic/local extension tumours arising in other organs.[77,78] Primary adenocarcinoma of the bladder arises in the urothelium and is characterized by a pure glandular phenotype; urachal adenocarcinoma originates from the urachal remnants, and metastatic adenocarcinoma represents the bladder involvement from pelvic and extra-pelvic adenocarcinomas. The presentation and management of adenocarcinomas may vary depending on the type of tumour.

9.3.2 Epidemiology

Primary adenocarcinoma of the urinary bladder constitutes 0.5% to 2% of all bladder malignancies. Urachal adenocarcinoma is less common than non-urachal bladder adenocarcinoma, and it represents the majority of malignant tumours that arise from the urachal remnants. Both urachal and not-urachal adenocarcinomas show highest incidence in the fifth or sixth decade of life, with a male-to-female ratio of 2:1 to 3:1.

In one of the largest reviews of patients undergoing surgery for bladder adenocarcinomas, Wright *et al.* identified 1,525 subjects between 1972 and 2003.[78] More than 90% of the subjects had primary vesical adenocarcinoma, suggesting that this is the more common entity. The urinary bladder is not a frequent site of secondary tumours, but it is the most common metastatic site among urinary tract organs: secondary carcinomas from colon, prostate, rectum, and uterine cervix may involve the bladder with direct extension and, less frequently, metastatic adenocarcinomas from the stomach and lung may also involve the bladder. Direct extension of a pelvic malignancy into the bladder may be synchronous or metachronous. A clinical suspicion of metastatic disease requires a confirmation biopsy. The differential diagnosis is frequently challenging for the pathologist.

Clinical data are important for the differential diagnosis and even the knowledge of the site of the biopsy is helpful for the diagnosis—colon and cervical adenocarcinomas involve the posterior wall of the bladder; prostate adenocarcinoma invades the bladder neck or trigone. However, the histologic features of the tumour may be misleading for the diagnosis—bladder adenocarcinoma may mimic a secondary tumour, and half of secondary tumours are adenocarcinomas.[79–81] In addition, high-grade urothelial carcinomas can show high variability in histologic features and may show glandular differentiation. In men, the most common secondary adenocarcinoma is prostatic in origin. Histological features alone may be not enough to distinguish between primary or secondary tumours, and the use of immunohistochemical staining is an important tool. Prostate-specific antigen (PSA), prostate-specific acid phosphatase (PSAP), and racemase stains support a prostatic origin, while GATA3, p63, cytokeratin 7 (CK7), and high molecular weight cytokeratin 34 E12 (34βE12)-positive stains suggest a urothelial differentiation.[82] In addition, both urachal and non-urachal adenocarcinomas do not stain with PSA, PSAP, or p63. The selection of a proper panel of immunohistochemical markers according to the clinical and histological findings may help in the diagnosis. **Table 9–1** itemizes the positive stains in different tumours.

TABLE 9–1 Panel of immunohistochemical markers useful in the differential diagnosis between primary and secondary adenocarcinoma of the bladder

	CK7	CK20	CDX2	β-catenin	PSA	racemace	PAX8	WT1	ER	PR	GATA3	p63	34βE12
Primary bladder adenocarcinoma	+/-	+/-	+/-	Membranous stain	-	-	-	-	-	-	Usually -	-	+
Urothelial carcinoma with glandular differentiation	+	+/-	Usually -	Membranous stain	-	-	-	-	-	-	Usually -	in the glandular component	+
Prostatic adenocarcinoma	-	-	-	-	+	+	-	-	-	-	-	-	-
Colonic adenocarcinoma	-/+	+	+	Nuclear stain	-	-	-	-	-	-	-	-	-
Clear cell renal cell carcinoma	-	-	-	-	-	-	+	-			-	-	-
Papillary renal cell carcinoma	+/-	-	-	-	-	+	+	-			-	-	-
Müllerian clear cell adenocarcinoma of the bladder	+	-	-		-	+/-	+	-	-	-			
High-grade serous carcinoma of the ovary	+	-	-					+	+	+	-	-	-
Clear cell adenocarcinoma of the female genital tract	+	-	-				+	-	-	-			

34βE12, high molecular weight cytokeratin 34 E12; CK7, cytokeratin 7; CK20, cytokeratin 20; PSA, prostate-specific antigen; ER, estrogen receptor; PR, progesterone receptor; +, usually positive; +/-, variable staining; -, usually negative.

9.3.3 Urachal and non-urachal adenocarcinoma—biologic differences

9.3.3.1 Histopathology

There are two main categories of adenocarcinomas of the urinary bladder: primary adenocarcinoma arising in the bladder, and adenocarcinoma arising in the urachal remnants. Among the tumours originating in the bladder itself, the following histologic subtypes are recognized: adenocarcinoma not otherwise specified (NOS), enteric, mucinous, signet ring cell, mixed, and tumours of Müllerian type (clear cell and endometrioid adenocarcinomas) (**Table 9–2**).[83]

Intestinal metaplasia may be the precursor for adenocarcinoma of the bladder. It has also been associated with chronic irritation.[84,85]

Grossly, adenocarcinoma of the bladder shows common features of a urothelial carcinoma, and it may present with mucus when it is histologically characterized by extensive mucinous and colloid features (**Figure 9–2**). In addition to the mucinous type, it may be enteric (**Figure 9–3**), signet ring cell, mixed types, or adenocarcinoma NOS. The enteric type looks like colonic adenocarcinoma; the mucinous (colloid) type shows tumour cells in mucin; the signet cell type may be pure, composed of only signet ring cells or mixed with other features. Adenocarcinoma NOS is characterized by a nonspecific glandular pattern. These tumours are classified into 3 grades: well, moderately, and poorly differentiated, based on the degree of glandular differentiation and nuclear pleiomorphism.

TABLE 9–2 Adenocarcinoma arising in the bladder

▪ Adenocarcinoma, not otherwise specified (NOS)
▪ Adenocarcinoma enteric type
▪ Adenocarcinoma mucinous type
▪ Adenocarcinoma signet-ring cell type
▪ Adenocarcinoma mixed type
▪ Clear-cell adenocarcinoma of Müllerian type
▪ Endometrioid adenocarcinoma of Müllerian type

Adenocarcinomas should be differentiated from urothelial carcinomas with glandular differentiation and from metastasis from colonic adenocarcinomas. Careful morphological evaluation with the recognition of urothelial features or histologic features that suggest secondary bladder involvement (such as necrosis, invasion of the bladder wall from outside)[86] can help make the right diagnosis.

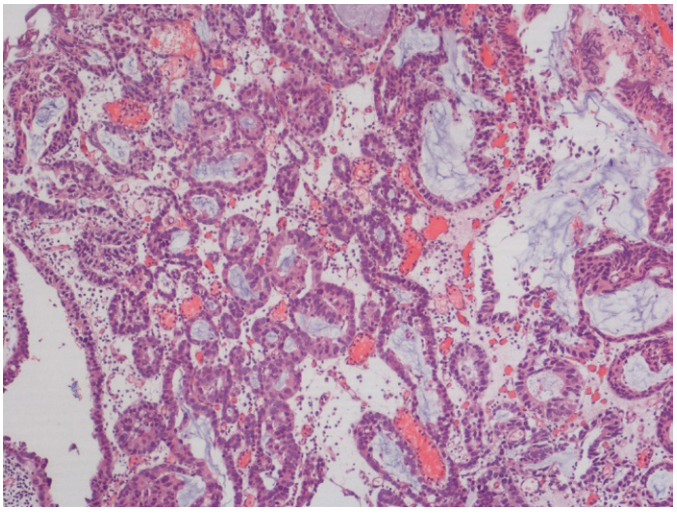


FIGURE 9–2
Adenocarcinoma of the bladder with focal mucinous component, hematoxylin and eosin (H&E) 10X.

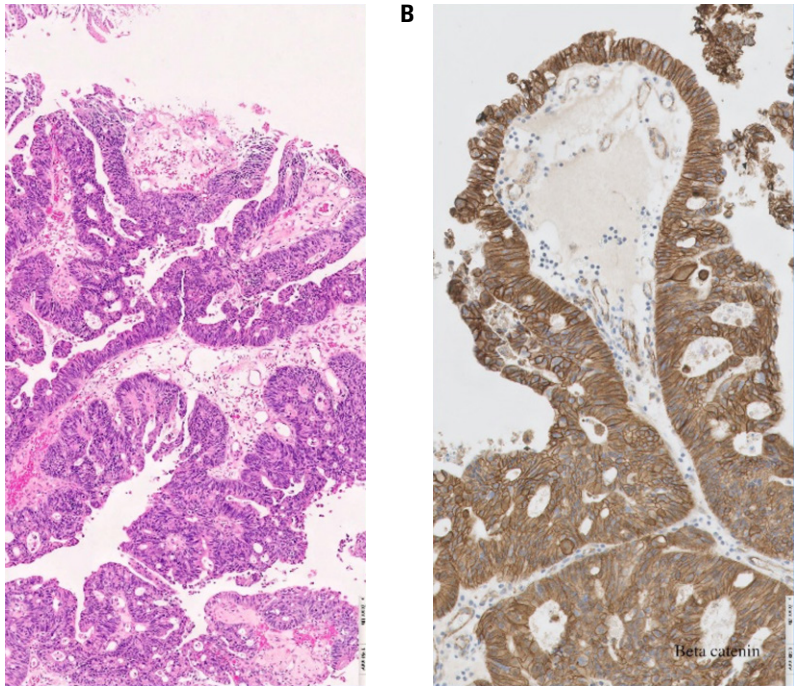
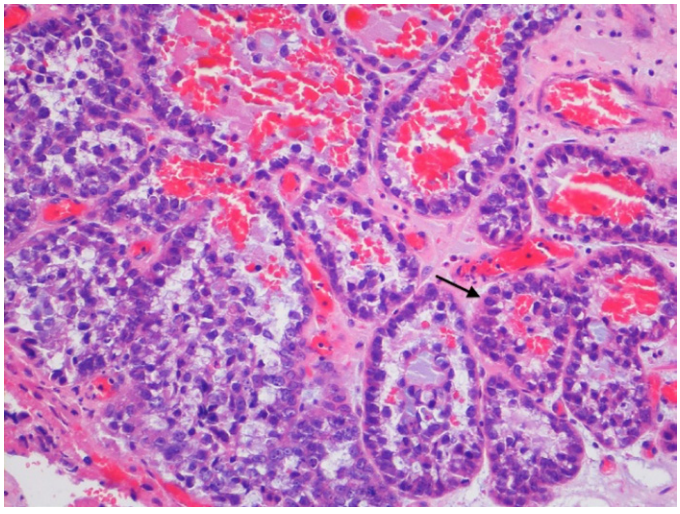


FIGURE 9–3
Bladder adenocarcinoma, enteric type: H&E 20X (A), β-catenin membranous–positive stain in the tumour cell (B). Courtesy of Antonio Lopez-Beltran.

Immunohistochemical staining may be an important tool for evaluation.[87] The immunohistochemical profile of bladder adenocarcinoma resembles that of colonic adenocarcinoma: CK7 and CK20 may be variably positive, and CDX2 and villin may show positive stain in both tumours. CDX2 has also been reported positive in a low percentage of urothelial carcinomas, and it stains positive with intestinal metaplasia of the bladder. Villin shows a negative stain in urothelial carcinoma with glandular differentiation, Beta-catenin shows a membrane positive stain in bladder adenocarcinoma such as in urothelial carcinoma, while in the majority of the cases, it shows a nuclear stain in colonic adenocarcinoma. GATA3 and p63 stains are negative in both colonic and bladder adenocarcinomas. However, both GATA3 and p63 may be useful to recognize the transitional component in urothelial carcinoma with glandular differentiation.

Müllerian type tumours of the bladder are very uncommon as are clear cell and endometrioid adenocarcinomas. Clear cell adenocarcinoma (**Figure 9–4**) resembles clear cell tumours of the female genital tract. It occurs more frequently in women (female-to-male ratio of 2:1), and in middle-aged and elderly patients. It has been more commonly described in the bladder neck, in the trigone, in a diverticulum, or in a Müllerian duct cyst.[88]

FIGURE 9–4
Clear cell adenocarcinoma of the urinary bladder, H&E 20X. The arrow points to the oxyphilic cytoplasm.



Clear cell carcinoma of the bladder of true Müllerian origin has been shown to have an association with concomitant endometriosis or müllerianosis.[89] Clear cell carcinomas of the urinary bladder may arise either in the vicinity of, or directly associated with, endometriosis and also within Müllerian duct cysts or remnants and endosalpingiosis.[90] The uncommon clear cell and endometrioid adenocarcinomas arise from endometriosis or müllerianosis of the bladder. The gross hallmark of the clear cell adenocarcinoma of the bladder is a unique polypoid mass.

Urothelial carcinoma may have glandular differentiation with presence of clear cells; however, in such tumours, the presence of both urothelial and clear cell features can be demonstrated. Conversely, like clear cell carcinoma of the female genital tract, clear cell adenocarcinoma of the urinary bladder is characterized by different patterns, and tubulo-cystic, papillary, and predominantly solid/diffuse are the most common patterns. The tumour cells may be clear cells containing glycogen, similar to those of clear cell renal cell carcinoma; hobnail cells with large nuclei that stick out in the lumen; and less frequently flat, cuboidal cells with oxyphilic cytoplasm.[91] A moderate-to-severe degree of cytologic atypia is observed, often with brisk mitotic activity. Endometrioid adenocarcinoma may present with different degrees of differentiation.

Immunohistochemically, there are many similarities between clear cell adenocarcinomas of the urinary tract and gynecological tumours.[92] Both lesions tend to stain positively for CAM 5.2, CK 7, epithelial membrane antigen (EMA), CA-125, Leu-M1, and hepatocyte nuclear factor 1 (HNF-1). Both lesions stain variably for CA-125, PAX8, and PAX2. However, the identification of associated Müllerian elements within sampled sections in the absence of associated urothelial neoplasia or areas of the tumour resembling nephrogenic crests may prove helpful in accurate diagnosis, in addition to immunohistochemistry.

Clear cell carcinoma of the urinary tract mimics a nephrogenic adenoma (**Table 9–3**). Differential diagnosis may be difficult on a biopsy specimen, and immunohistochemical markers have been proposed to aid diagnosis.[93] However, both lesions stain with CK7 and racemase; only p53 and Ki67 markers are useful in the distinction, with strong p53 staining and high Ki67 counting in the malignant tumours, though the diagnosis of atypical nephrogenic metaplasia may be difficult.

TABLE 9–3 Differentiating nephrogenic adenoma from clear cell adenocarcinoma

	Nephrogenic adenoma	Clear cell adenocarcinoma
PSA	-	-
34βE12	+	+/-
Cytokeratin 7	+	+
Cytokeratin 20	-	-/+
EMA	+	+
racemase	+	+
PAX2	+	+
PAX8	+	+/-
Ki67	< 5%	> 15%
p53	Focal +	+

Urachal epithelial tumours are uncommon tumours arising from the urachal vestiges. They are less common than non-urachal adenocarcinomas. They are more common in men, with a male-to-female ratio of 2:1 to 3:1. Urachal adenocarcinomas have been reported in patients from 20 to 90 years of age, including patients of younger age than bladder urothelial carcinoma and non-urachal adenocarcinoma. The etiology is unknown, but the tumour arises from metaplasia of urachal epithelium. Most patients present with hematuria. Other symptoms include umbilical or pelvic pain, mass, and weight loss. Patients may be asymptomatic.

The diagnosis of urachal adenocarcinoma, because of the histologic similarities with other adenocarcinomas and the proximity to the bowel, requires rigorous criteria such as the location of the tumour in the bladder dome and/or anterior wall, the epicentre of the carcinoma in the bladder wall, the absence of widespread cystitis cystica and/or cystitis glandularis beyond the dome or the anterior wall, and the absence of a known primary elsewhere (**Table 9–4**). The presence of urachal remnants in association with the tumour is supportive of the diagnosis, but their absence does not rule the diagnosis of urachal origin.[94]

TABLE 9–4 Criteria for the diagnosis of urachal adenocarcinoma

A- Mandatory criteria
Location of the tumour in the bladder dome and/or anterior wall
Epicentre of the carcinoma in the bladder wall
Absence of widespread cystitis cystica and/or cystitis glandularis beyond the dome or the anterior wall
Absence of a known primary elsewhere
B- Optional criteria
Presence of urachal remnants in association with the tumour

Grossly, the tumours are located in the dome and/or anterior wall, in the muscularis propria. They may be calcified on imaging and/or associated with cysts and urachal remnants. Histologically, urachal adenocarcinomas are classified as cystic or non-cystic.[95] Non-cystic adenocarcinomas represent about 80% of the urachal adenocarcinomas (**Table 9–5**). They exhibit solid and infiltrative growth. The subtypes include enteric (intestinal), mucinous (colloid), signet ring cell, adenocarcinoma NOS, and mixed types. The enteric (intestinal) type is characterized by stratified columnar epithelium similar to colorectal adenocarcinoma. The mucinous type shows predominance of pools of mucin, with clusters of malignant epithelium associated with them. These tumours are called colloid when the amount of mucin is large. The signet ring cell type shows predominant signet ring cells. Mixed type tumours have more than one of the previous features present. Adenocarcinoma NOS is not readily classifiable with enteric, mucinous, or signet ring cell features. Most adenocarcinomas are mucinous (50%), followed by enteric (24%), mixed (10%), NOS (9%), and signet ring cell (7%). Urachal adenocarcinomas may also be admixed with some non-glandular carcinoma components.

Patients with mucinous cystic tumours of urachal origin have an age incidence that is similar to patients with urachal non-cystic adenocarcinoma; however, they show a slight predominance among women (male-to-female ratio of 1:1.7). More mucinous cystic tumours of urachal origin are diagnosed incidentally in contrast to the non-cystic adenocarcinoma, which usually are symptomatic tumours.

TABLE 9–5 Histological classification of urachal adenocarcinomas

Non-cystic adenocarcinomas (80% of the urachal adenocarcinomas)
enteric (intestinal)
mucinous (colloid)
Signet ring cell
adenocarcinoma not otherwise specified (NOS)
mixed types
mixed with a minor non-glandular component
Cystic adenocarcinoma
mucinous cystic adenocarcinoma

Grossly, mucinous unilocular or multilocular cystic tumours of urachal origin measure from 1 to 8 cm in diameter. Mucinous cystadenocarcinoma is characterized by cellular atypia, architectural complexities of the lining, and presence of stromal invasion. Microscopic invasion is diagnosed when infiltration is less than 2 mm from the cyst wall and it is less than 5% of the tumour volume. Intraepithelial mucinous cystic carcinoma is distinguished from mucinous cystic tumour of low malignant potential by the presence of high-grade atypia of the tumour cells.

The immunohistochemical profile of urachal mucinous cystic tumours is similar to urachal non-cystic adenocarcinomas. These tumours stain positive for CDX2 and CK20 and variable for CK7, while the majority of the cases stain negative for β-catenin. Nuclear localization of β-catenin occurs in some cases.[96] Diffuse nuclear β-catenin and CK7 may help in the differential diagnosis of urachal adenocarcinoma of the enteric subtype; in fact, urachal adenocarcinoma is negative for nuclear β-catenin with focal or negative CK7, while colorectal adenocarcinoma shows diffuse positive nuclear β-catenin and negative CK7 stains.

9.3.3.2 Prognosis

Prognosis for bladder adenocarcinoma is poor, and it is predicted by pathological stage. Clear cell adenocarcinoma is associated with an aggressive clinical course and poor prognosis, similar to that observed in conventional urothelial carcinomas. However, low-stage exophytic tumours may have good outcomes.

Among the urachal adenocarcinomas, cystic tumours (both mucinous cystic tumour of low malignant potential and mucinous cystadenocarcinoma) are distinguished from the non-cystic tumours by their favourable behaviour. The prognosis for urachal cystic mucinous tumours is better than for urachal non-cystic mucinous tumours, as the group of non-invasive mucinous cystic urachal tumours includes mucinous cystadenoma, mucinous cystic tumour of low malignant potential, mucinous cystic tumour of low malignant potential with intraepithelial carcinoma, and microscopically or frankly invasive mucinous cystadenocarcinoma, with 65% of cystic tumours classified as mucinous cystic tumour of low malignant potential (**Table 9–6**).[97,98]

Overall, the prognosis for non-cystic urachal carcinomas is poor, and most large contemporary studies show a 5-year survival rate of around 45% to 50%.[83] The involvement of bladder fat, adjacent organs, abdominal wall and metastasis, and the presence of residual disease are associated with poor prognosis.

TABLE 9–6 Urachal mucinous cystic tumours

▪ mucinous cystadenoma
▪ mucinous cystic tumour of low malignant potential (65% of the urachal mucinous cystic tumours)
▪ mucinous cystic tumour of low malignant potential with intraepithelial carcinoma
▪ microscopically invasive mucinous cystadenocarcinoma
▪ invasive mucinous adenocarcinoma

9.3.3.3 Staging

The 8th edition of the tumour-node-metastasis (TNM) staging system of the American Joint Committee on Cancer (AJCC)[99] has been created for bladder urothelial carcinoma and is also used for other epithelial tumours of the bladder. Several staging systems have been proposed for urachal carcinomas. The Sheldon system[100] is most commonly used (**Table 9–7**). Stage I tumours are confined to the urachal mucosa and they are equivalent to pT1 bladder carcinoma. Stage II tumours are confined to the urachus similar to pT2 bladder carcinoma. Stage III urachal carcinoma is a tumour with extension: IIIA in case of extension to the bladder, IIIB in case of extension to the abdominal wall, IIIC in case of extension to the peritoneum, and IIID in case of extension to other viscera. Stage IVA tumours have metastases to lymph nodes, and IVB have metastases involving distant sites.

TABLE 9–7 Staging system of urachal adenocarcinoma by Sheldon (1984)[100]

Stage I	Carcinoma confined to the urachal mucosa
Stage II	Carcinoma invasion confined to the urachus
Stage III	Local carcinoma extension
IIIA	Extension to the bladder
IIIB	Extension to the abdominal wall
IIIC	Extension to the peritoneum
IIID	Extension to other viscera
Stage IV	Metastasis
IVA	Metastasis to lymph nodes
IVB	Metastasis to distant sites

9.3.4 Primary bladder adenocarcinomas

9.3.4.1 Presentation

Primary adenocarcinomas present with symptoms similar to other urothelial tumours, including hematuria and lower urinary tract symptoms such as dysuria and frequency of urination, while some patients may also complain of passing mucus in urine.[101] Cystoscopic appearance of the tumour may resemble primary urothelial cancers but most of these tumours are sessile, solid with possible ulceration of the overlying mucosa.[102] Primary vesical adenocarcinomas may be multiple and may arise from any part of the bladder, while urachal carcinomas typically arise at the dome.[53,103] The signet-cell variety of adenocarcinomas may not demonstrate a visible, exophytic lesion within the bladder and may, instead, grow beneath the epithelium in an infiltrative fashion.[104]

9.3.4.2 Diagnosis

Initial diagnosis of these tumours requires a standard work-up as for other bladder masses, including history, physical and laboratory examinations, and radiological investigations. Considering the rarity of primary bladder adenocarcinomas, it is essential to rule out secondary tumours by evaluating the common sites such as the bowel, lung, breast, and prostate for a primary lesion. However, one-third of adenocarcinomas of the bladder arise in urachal remnants, and evaluation of other primary sites in these cases is generally not recommended.[105,106] Therefore, an attempt should be made to identify urachal tumours, and all tumours at the dome should be treated as urachal cancers unless proven otherwise.[104]

The majority of bladder adenocarcinomas present with locally advanced or metastatic disease, with the SEER database review suggesting only 32% to be localized at the time of presentation.[78] Consistent with this report, a more recent review of the SEER database from 2004 to 2013 reported that only 35% patients had organ-confined disease and only 24% was low grade.[107] The risk for non-organ-confined disease was 2.24 times higher in adenocarcinoma patients compared with urothelial cancers.

9.3.4.3 Primary treatment

The standard treatment for all bladder adenocarcinomas is radical cystectomy with pelvic lymph node dissection.[108] Radical cystectomy confers a higher survival than TURBT alone, which is associated with low 5-year survival rates.[78,109] Primary radiotherapy and systemic therapies for primary bladder adenocarcinomas have limited effectiveness, and the lack of large series has resulted in the absence of well-established protocols for their management.[110] Galsky *et al.* reported measurable response in 11 patients of unresectable locally advanced or metastatic urachal adenocarcinoma using paclitaxel, cisplatin, and ifosfamide in a prospective study, with a median survival time of 24.8 months (10.2–32.3 months).[111] Yu *et al.*[112] treated 6 patients of locally advanced urachal or non-urachal (3 each) adenocarcinomas with gemcitabine, cisplatin, and S-1 for 3 cycles prior to surgery, and reported a complete and partial response in 2 patients each, and a stable and progressive disease in 1 patient each. However, the data is insufficient to support neoadjuvant therapies for adenocarcinoma of the bladder. Intravesical therapies have no role in the management of adenocarcinomas of the bladder and novel therapies are under investigation.[113]

9.3.4.4 Adjuvant treatment

The use of adjuvant radiation therapy may improve survival in patients with adenocarcinoma. Zaghloul *et al.*[114] reported their data on 216 patients of adenocarcinoma bladder, 82 of whom received radiation therapy in addition to radical cystectomy and pelvic lymphadenectomy while 134 did not. The 5-year disease-free survival rate in patients receiving radiation was significantly higher at 58% versus 33% for those who did not receive radiation. Adjuvant therapy on the lines of adenocarcinomas of the bowel using oxaliplatin, leucovorin, 5-fluorouracil (FOLFOX), gemcitabin, cisplatin combinations or iphosphamide, paclitaxel, cisplatin combinations may be used, preferably in a clinical trial setting.[106]

9.3.4.5 Prognosis

While there is a large discordance in overall 5-year survival rates between 11% and 55%, the prognosis is believed to be poorer than for urothelial cancers, and the 5-year survival rate averages 35% with a median survival time of 30 months.[78,103,108,114] One of the largest series on bladder adenocarcinomas from a schistosomiasis endemic zone included 185 patients and found a 55% 5-year disease free survival rate, with radical cystectomy being the most effective treatment, and survival depending on tumour stage and lymph node status.[108] Survival data from non-endemic zones suggests lower 5-year survival rates that may be related to the higher low-stage tumours found in the former report.[78,103] Contemporary data suggests that the 5-year survival rate may not be as poor as previously believed for patients with organ-confined, non-signet ring cell tumours.[107,115]

Age at diagnosis, grade, and stage of tumour have been the most consistent predictors of survival in patients with bladder adenocarcinoma.[78,107,115,116] Signet ring cell morphology of the tumour portends a poorer prognosis.[78,101,107] The poorer overall survival in these patients is possibly related to higher stage at presentation and the presence of such adverse pathological features.

9.3.5 Adenocarcinoma in exstrophy patients

Patients with exstrophy of the urinary bladder are at a high risk of developing adenocarcinomas, and the risk may be 27 times higher than the general population.[117] While the majority of these obvious anomalies are corrected in the neonatal period, delayed presentation due to adverse socioeconomic conditions is not uncommon.[118] Patients who undergo early repair or urinary diversion may also not be immune from the development of an adenocarcinoma.[119,120] Malignancies most often occur in the fourth and fifth decades of life, and surveillance cystoscopy with biopsy has been recommended.[121] However, the role of routine surveillance cystoscopy is not universally accepted, and a high index of suspicion with early investigation of symptomatic patients may be an alternative approach.[122]

9.3.6 Urachal adenocarcinomas

9.3.6.1 Presentation and diagnosis

Urachal adenocarcinomas most often arise at the dome of the bladder, the site of origin of the urachal remnant (**Figure 9–5**). Differentiating them from primary bladder cancer, in the absence of additional sites of tumour within the bladder, is difficult but supported by the presence of an intact overlying urothelium or minimal ulceration.[104,106] In addition to the clinical features of primary bladder adenocarcinomas, these patients may present with discharge from the umbilicus.[102] The disease is

rarely confined to the urachus at presentation, and a Mayo Clinic series found that 27 of 32 patients without metastasis had locally advanced disease at diagnosis.[106] The SEER database review noted that 30% patients with urachal tumours presented with distant disease even though they were younger than patients with primary vesical adenocarcinomas.[78]

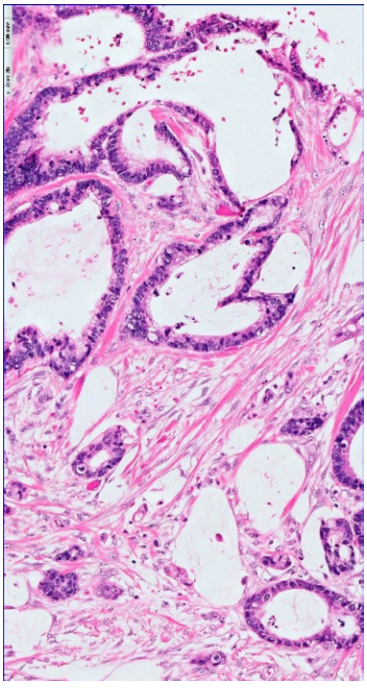


FIGURE 9–5
Urachal adenocarcinoma, H&E 20X. Courtesy of Antonio Lopez-Beltran.

9.3.6.2 Treatment

Although radical cystectomy with umbilectomy is the recommended primary treatment for bladder adenocarcinomas, conservative resections with partial cystectomy have shown similar, or better, survival outcomes as radical surgery when performed for urachal adenocarcinoma.[103,123] Henly *et al.*[123] compared survival in 30 patients who underwent partial cystectomy with 4 who underwent radical cystectomy, and they found similar overall survival at 5 years. The authors recommended that partial resection must include the entire urachal ligament and the umbilicus. Data from the Mayo Clinic cohort is similar.[106] Partial cystectomy is the favoured approach, with two-thirds of all patients receiving this option in one of the largest series reviewed.[78] The role of pelvic lymphadenectomy, along with partial or radical cystectomy, for urachal adenocarcinoma remains controversial.

9.3.6.3 Adjuvant therapy

Systemic therapy is reserved for patients who develop local or distant recurrence or have unresectable primary disease, and it consists of fluorouracil and cisplatin–based regimens. Chemotherapy has also been used in the adjuvant setting after radical surgery for patients with positive lymph nodes or surgical margins.[106]

9.3.6.4 Prognosis

Survival after partial or radical cystectomy for bladder adenocarcinoma may depend on tumour size at presentation, with tumours less than 4 cm having a better prognosis. However, local recurrence rates as high as 50% have been reported.[124] In comparison with primary adenocarcinomas of the bladder, urachal carcinomas may have a slight survival advantage. The 5-year overall survival rate for these patients is below 50% and the medial survival time is under 5 years.[78] The median overall survival of these patients is around 46 months from diagnosis (11–55% 5-year survival rate) and is associated with lymph nodal status and status of surgical margins but not the type of surgery (radical or partial).[79,80]

9.3.7 Secondary bladder adenocarcinomas

Secondary adenocarcinomas of the bladder include tumours that are metastatic to the bladder or involve the bladder through direct extension of tumours of adjacent organs. Primary sites of such tumours include the colon, rectum, prostate, lung, and breast. Secondary adenocarcinomas, though uncommon, may be more frequent than primary adenocarcinomas of the urinary bladder.[125]

9.3.8 Metastatic adenocarcinoma to the bladder

Adenocarcinoma metastasizing to the bladder has been reported from many organs that harbour primary adenocarcinomas.[126,127] This includes primary clear cell renal cancers where these tumours may occur both synchronously and after treatment of the primary lesion.[128,129] Some of the uncommon forms include mucin-secreting tumours from the gallbladder where the primary lesion may be occult or minimally symptomatic.[130] These tumours present with hematuria and both obstructive and irritative voiding symptoms. The majority of metastatic tumours will have evidence of additional site metastasis, either at the time of presentation or in follow-up. The management of bladder metastasis depends on the primary pathology and presence of additional metastasis. Most such tumours are identified through imaging and cystoscopy, and transurethral resection (TUR) is the primary treatment for diagnosis and symptom control. Systemic chemotherapy based on the primary tumour histology may be considered as additional therapy. However, radical resection for metastasis has not been used.

9.3.9 Locally advanced cancer of other organs

Adenocarcinomas arising in the prostate, rectum, and sigmoid may invade the urinary bladder through direct spread. These patients may have symptoms related to bladder involvement in the form of frequency, dysuria, hematuria, or gas in the urine in the case of fistulae. Kobayashi *et al.* reviewed 580 patients of bowel cancer operated over a 5-year period.[131] Among these, bladder involvement was suspected intra-operatively in 17 cases (2.9%). However, pathological involvement was documented in only 4 of 14 patients where a partial or total cystectomy was simultaneously performed. Preoperative imaging (computed tomography [CT] or magnetic resonance imaging [MRI]) could not predict the pathology, but preoperative cystoscopy identified a fistula in 2 and a visible tumour in 1 of these 4 patients. Edema was seen on cystoscopy in the 4th patient, but this alone seems to have a low predictive value, as it was also seen in 4 other patients who did not have pathological involvement.

The inability of preoperative imaging and cystoscopy to fully identify the population at risk has been previously documented.[132] Patients with locally advanced disease have a higher risk for bladder involvement, and a review of specimens of 46 patients who underwent pelvic exenteration for clinically advanced disease showed bladder invasion in 58%.[132] However, suspected locally advanced disease should be an indication for preoperative assessment for bladder involvement, as this may impact management decisions.

Partial or total cystectomy, en bloc with the primary tumour, is the most appropriate management for these locally invading tumours.[131–133] Ureteric reimplantation may be required if the involvement is in the region of the bladder trigone. Bladder-sparing approaches including partial cystectomy have gained validity through demonstration of long-term survival and local recurrence rates similar to pelvic exenteration.[132] However, dissection between the tumour and bladder must be done with caution, as it may violate the tumour and result in recurrence. While complication rates increase if a cystectomy is simultaneously performed, survival after such resections depends on the primary disease pathology rather than the involvement of the urinary bladder.[134,135]

Consensus Recommendation	LOE	GOR
Radical cystectomy is the primary treatment for non-urachal adenocarcinomas.	3	B
Adjuvant radiation or chemotherapy may be considered for locally advanced disease.	4	C
Limited resection with partial cystectomy and umbilectomy with lymph node dissection may be sufficient treatment for urachal tumours.	2/3	C
Exstrophy patients who have not undergone a cystectomy are at a higher risk for bladder adenocarcinoma and should be carefully followed.	3	B

9.4 Neuroendocrine Tumours of the Urinary Bladder

9.4.1 Small cell carcinoma

9.4.1.1 Definition

Small cell carcinoma of the bladder (SCCB) is a malignant neuroendocrine neoplasm of the urothelium that histologically mimics its pulmonary counterpart. It often coexists with conventional urothelial carcinoma, adenocarcinoma, or squamous cell carcinoma.[136]

9.4.1.2 Pathogenesis

The original theories of the cells of origin in SCCB included a tumour arising from a Kulchitsky-type cell or a tumour arising from a cell that is not normally present in the urinary bladder mucosa.[137] Cheng *et al.*[138] showed molecular genetic evidence of common clonal origin of coexisting SCCB of the urinary bladder, suggesting that the cell of origin was a multipotential, undifferentiated cell or stem cell.

9.4.1.3 Incidence

SCCB is a rare disease, accounting for 0.5% to 0.7% of all bladder tumours. It most commonly presents in the seventh decade of life, with a mean age at presentation of 66 years and a male-to-female ratio of 2:1 to 5:1.[139] Occasionally, patients have paraneoplastic syndromes with hypercalcemia, Cushing syndrome,[140] hypophosphatemia,[141] or a neurologic disorder.[142]

An analysis from the SEER registry of SCCB indicated a significant rise in the incidence of this tumour in the United States from 0.05 to 0.14 cases per 100,000 population between 1991 and 2005. This is likely to increase as the US population ages, but it may also be due to increased identification by pathologists.[143] Approximately 500 cases of these tumours have been reported in the literature.[59,139]

9.4.1.4 Gross

Macroscopically, a large, solid, polypoid, and sometimes necrotic tumour mass is found, but tumours may also appear sessile and ulcerated and extensively infiltrate the bladder.[136,139]

At cystoscopy, SCCB cannot be distinguished from bladder urothelial carcinoma by its gross appearance.[136,137,139]

Tumour sizes range from 1.5 cm to 13 cm. Most of the tumours are located on the lateral wall of the bladder and less commonly in the fundus, trigone, anterior wall, or dome of the bladder.[139]

9.4.1.5 Microscopic features

On microscopic examination, the tumour consists of sheets or nests of small or intermediate cells with molding, scant cytoplasm, inconspicuous nucleoli, and evenly dispersed "salt-and-pepper chromatin" (**Figure 9–6A**). Mitotic activity is usually brisk, and frequently crush artifact is found. Necrosis and vascular invasion are commonly present.[136,144,145]

9.4.1.6 Molecular pathology

At the molecular level, SCCB demonstrates chromosomal aberrations that are also present in small cell carcinoma of the lung.[144]

Loss of 4q, 5q, 10q, and 13q may be seen in SCCB. Allelic loss at 3p25-26, 9p21, 9q 32-33, and 17p13 and non-random inactivation of the X-chromosome have also been reported.[59,145]

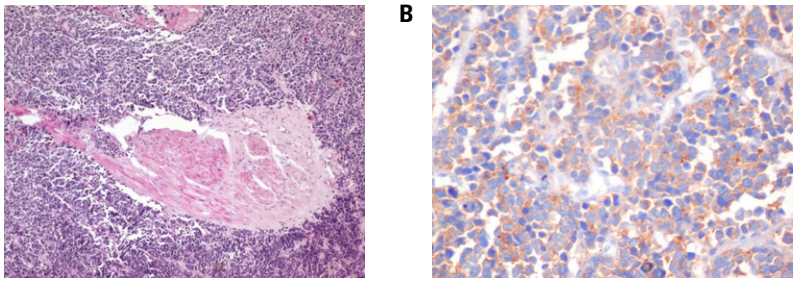
9.4.1.7 Differential diagnosis

Lymphoma, poorly differentiated urothelial carcinoma, poorly differentiated squamous cell carcinoma, and metastatic small cell neuroendocrine carcinoma from another primary should be considered. It is also important to distinguish SCCB from small cell carcinoma originating in the prostate. The identification of a urothelial component, including urothelial carcinoma *in situ*, would strongly support a primary bladder origin.[46,146] The distinction between prostate and SCCB origin can be difficult, especially in smaller biopsy specimens. PSA staining is usually negative and p501S has a low staining rate (20%). The prostate-specific TMPRss2-ERG fusion can rule in prostate origin, but negative staining does not rule it out.[147] Another diagnostic challenge can be alveolar rhabdomyosarcoma, although immunohistochemistry for muscular differentiation is helpful in such cases.[148]

9.4.1.8 Immunohistochemistry

Small cell carcinoma of the urinary bladder typically exhibits both epithelial and neuroendocrine differentiation. Immunohistochemical stains show high positivity for chromogranin (**Figure 9–6B**), synaptophysin, CD57 (Leu7), CD56, TTF1 (thyroid transcription factor 1), neuron-specific enolase, CAM 5.2, keratin 7, and epithelial membrane antigen.[149] Additionally, GATA 3 has been found in 32% of tumours.[146–149]

FIGURE 9–6
A Small cell carcinoma with involvement through the muscularis propria. Note the sheet-like growth pattern.
B Chromogranin A expression



On the other hand, immunohistochemical staining with CK20 is negative in SCCB but positive in 40% to 70% of urothelial carcinomas.[59,140,144]

9.4.1.9 Prognosis

SCCB is an aggressive disease. Similar to pulmonary small cell carcinoma, SCCB is often detected at advanced stage and has a dismal prognosis. A publication from the University of Southern California reported that the median survival time of SCCB patients was 13 months, and the 5-year survival rate was only 10%.[150] In the Mayo Clinic experience, median overall survival time of SCCB patients was 20 months, and 5-year survival rates for patients with stage II, III, and IV disease were 63.3%, 15.4%, and 10.5%, respectively.[151]

9.4.1.10 Treatment

After initial diagnosis on transurethral resection of bladder tumour (TURBT), thorough staging including chest CT should be performed to rule out a primary lung small cell carcinoma.

For primary SCCB, the preferred treatment for localized disease is cisplatin-based NAC with cisplatin and etoposide prior to radical cystectomy. In the literature, a number of different treatment strategies exist with their combinations, including initial chemotherapy followed by local control with radical or partial cystectomy or sometimes radiotherapy.[147] Because patients with SCCB may frequently have micrometastatic disease at diagnosis that is not detectable on imaging studies, the treatment paradigm emphasized initial systemic chemotherapy. TURBT alone should be used cautiously or not at all given the aggressiveness of SCCB.

In contrast to urothelial carcinoma, chemotherapy for SCCB is typically cisplatin (or carboplatin) and etoposide. Neoadjuvant chemotherapy was associated with improved survival outcome in a study at the MD Anderson Cancer Center.[152] The 5-year survival rate for patients who underwent NAC was 78%, which was significantly higher than the rate of 36% for patients who had initial cystectomy without NAC (p=0.26). Several reports presented cases of SCCB that were well controlled with local radiation treatment. However, most of the patients in those reports also received chemotherapy, and the effect of radiation alone could not be isolated.[153] An observational study from the National Cancer Database of 856 patients with small cell carcinoma reported that treatment with either radical cystectomy plus chemotherapy or chemoradiation therapy was associated with better overall survival than treatment with monotherapy.[154] Definitive data for the management of non-muscle-invasive (T1) small cell carcinoma is not available. Due to the potential for understaging with non-muscle-invasive small cell carcinoma and the known aggressiveness of the histology, multimodal therapy including chemotherapy plus cystectomy or radiation therapy should be considered.

9.4.2 Large cell neuroendocrine carcinoma

9.4.2.1 Definition

Large cell neuroendocrine carcinoma (LCNEC) of the urinary bladder has a phenotype composed of sheets or isolated undifferentiated cells that do not fit into urothelial, squamous, adenocarcinoma, or any other recognized category of bladder carcinoma.[155,156] LCNEC is described as a pure form or with other variants of urothelial carcinoma, including lymphoepithelioma-like carcinoma, squamous cell carcinoma, adenocarcinoma, and sarcomatoid carcinoma.[157,158]

9.4.2.2 Incidence

LCNEC is a tumour of rare incidence that occurs mostly in men. The age of diagnosis ranges from 61 years to 87 years (mean, 74 years).[156]

To date, approximately 20 cases of bladder LCNEC of pure and mixed histology have been reported in the literature,[159,160] but it seems possible that this type of neoplasm was previously underdiagnosed.

9.4.2.3 Pathogenesis

There are a number of theories for the cell of origin for LCNEC. The first is a similar hypothesis common to other neuroendocrine tumours of the urinary bladder including multipotent stem cells, originating from submucosa neuroendocrine cells or urinary tract epithelial metaplasia.[161] Another hypothesis is that the large neuroendocrine cells originate from the urachal epithelium.[162]

9.4.2.4 Gross

Most tumours appear as a nodular mass with a polypoid solid appearance and are difficult to distinguish from other types of bladder cancers.[163]

9.4.2.5 Microscopic features

Routine hematoxylin and eosin–stained sections show a high-grade malignant epithelial neoplasm composed of large cells with abundant cytoplasm, large nuclei with coarse chromatin and variable prominent nucleoli, and a mitotic count greater than 50 mitotic figures per 10 high-power fields. Scattered bizarre giant cells can be present, and the tumour also shows abundant necrosis.[164]

9.4.2.6 Differential Diagnosis

The differential diagnosis of primary urinary bladder LCNEC includes metastatic LCNEC, most frequently from lungs or intestines, local extension of poorly differentiated prostatic carcinomas, high-grade urothelial carcinoma, small cell neuroendocrine carcinoma, and some types of lymphoma.[160,162,164]

9.4.2.7 Immunohistochemistry

The diagnosis of LCNEC of the urinary bladder is established on morphologic criteria and, additionally immunohistochemical or ultrastructural evidence of neuroendocrine differentiation is required. The tumour cells show immunoreactivity to chromogranin A, CD56, neuron-specific enolase, and synaptophysin. In addition to neuroendocrine markers, the tumour cells typically show positive immunostaining for CAM 5.2, AE1/AE3, and EMA.[157,162]

9.4.2.8 Molecular pathology

There have been no studies of the genomic landscape of LCNEC tumours of the urinary bladder due to their rarity. The difference in mutational genes between pulmonary small cell and large cell neuroendocrine carcinoma has been explored. Distinct differentiated mutations between the two were found in Janus kinase 3 (JAK 3), NRAS, retinoblastoma 1 (RB1), and von Hippel-Lindau (VHL), with all absent in large cell but present in small cell carcinomas. Other mutational differences include isocitrate dehydrogenase (IDH), fibroblast growth factor receptor 1 and 2 (FGFR1 and FGFR2), kinase insert domain receptor (KDR), Kirsten rat sarcoma viral oncogene homologue (KRAS), and MET.[165]

9.4.2.9 Prognosis

Most patients with LCNEC present with high-stage disease and have a poor prognosis. To date, the only reported series of LCNEC of the bladder (n=5) compared with small cell carcinomas (n=20) reported no significant differences in survival.[165,166]

9.4.2.10 Treatment

There is no standard evidence-based chemotherapy for LCNEC. Akamatsu *et al.*[166] reported on a subject who underwent chemotherapy with carboplatin and etoposide with radical cystectomy for muscle-invasive LCNEC and had no recurrence for 16 months.

9.4.3 Well-differentiated neuroendocrine tumour ("carcinoid tumour")

According to the 2016 World Health Organization classification scheme, well-differentiated neuroendocrine carcinoma (WDNET or carcinoid tumour) of the bladder is recognized as a distinct entity of neuroendocrine neoplasms that is potentially malignant with histologic features similar to carcinoids found at other anatomic locations. Some patients can have metastases to regional lymph nodes or distant metastases.[156]

9.4.3.1 Incidence

WDNET of the urinary bladder is a very rare neoplasm, with fewer than 50 cases of pure form described in the literature. Other reports described "carcinoid tumours" with coexisting carcinomatous component such as adenocarcinoma or small cell carcinoma.[167] WDNET occurs predominantly in elderly patients (range, 30–75 years of age) with a slight male predominance.[168]

9.4.3.2 Gross

WDNETs of the bladder are usually small, with the largest reported tumour measuring 3 cm.[167] They often appeared as polypoid or smooth-surfaced submucosal nodules and sometimes associated with changes suggesting an inflammatory lesion by cystoscopic examination. These tumours have a predilection for the trigone and bladder neck regions.[168,169]

9.4.3.3 Microscopic examination

WDNETs at any sites usually have common structural features of a trabecular, ribbon-like, or rosette pattern. A unique feature seen in most WDNETs of the bladder is that the cells are arranged in a pseudoglandular pattern and are associated with cystitis cystica and cystitis glandularis.[167]

The cells are uniform with round to oval nuclei containing stippled chromatin, and generally with inconspicuous nucleoli and eosinophilic granules in the cytoplasm.[169] A case composed by oncocytic cells has also been described.[170]

9.4.3.4 Immunohistochemistry

Tumour cells show immunoreactivity for neuron-specific enolase, chromogranin, serotonin, and synaptophysin. In addition, bladder WDNETs can also express PSAP, but do not express other prostatic markers.[163]

9.4.3.5 Differential diagnosis

Differential diagnostic considerations include paraganglioma, nested variant of urothelial carcinoma, and metastatic prostate carcinoma.[169]

9.4.3.6 Prognosis

The prognosis of WDNET limited to the lamina propria is excellent. Moreover, WDNET involving the muscularis propria also appears to have favourable outcomes, albeit with conclusions based on short clinical follow-up.[171]

9.4.3.7 Treatment

The treatment for localized disease is similar to the treatment of carcinoids at other body sites and primarily involves surgical resection with clinical follow-up.[172]

Consensus Recommendations	LOE	GOR
Patients with locoregional pure small cell carcinoma of the bladder should initially be offered multimodal therapy with cisplatin-based neoadjuvant chemotherapy prior to radical cystectomy or chemoradiation.	2/3	B
Recommended chemotherapy for small cell carcinoma of the bladder is cisplatin and etoposide.	2	B
Due to potential for understaging of non-muscle-invasive (T1) small cell carcinoma, multimodal therapy including chemotherapy plus cystectomy or radiation therapy should be considered.	4	C
Chemotherapy followed by radiation therapy should be offered as an option for patients with locoregional disease.	3	C

9.5 Other Bladder Tumours

9.5.1 Micropapillary

Micropapillary bladder cancer (MPBC) is a variant of invasive urothelial carcinoma characterized by tight clusters of high-grade tumour cells that lack a fibrovascular core and are surrounded by retraction spaces.[155,173,174] The cell nuclei are typically polarized toward the periphery, and the cells may exhibit intracytoplasmic vacuolations and ring formations. MPBC is usually associated with conventional urothelial carcinoma (UC), but it may occur with SCC,[175] adenocarcinoma,[174] small cell carcinoma,[176] and sarcomatoid carcinoma.[177] The cutoff for diagnosis varies, but most studies require at least 10% of tumour with micropapillary features.[155,178] Although rare "non-invasive" forms have been described,[179] for management and prognostic purposes, the term micropapillary carcinoma is reserved by most experts only for the invasive forms, which are associated with worse outcome.

The diagnosis of MPBC is not straightforward and is often under-recognized and under-reported in non-academic centres. Even in centres of excellence, expert genitourinary pathologists exhibit significant interobserver variability.[180] With "classic" cases of MPBC there was 93% agreement; however, overall the reproducibility was only moderate (kappa, 0.54). This significant variation in histopathological reporting has the potential to bias clinical outcomes reported for individuals diagnosed with MPBC. Telomerase reverse transcriptase (TERT) mutation, which is the most common gene alteration in UC, is also frequently found in MPBC.[181] Gene expression profiling shows that MPBCs are almost exclusively luminal type and are enriched in PAPRγ. Gene expression profiling shows that micropapillary cancer,[182] a subset of MPBC, exhibits amplification of ERBB2 (HER2) that has been associated with poorer outcome.[183,184]

Muscle-invasive disease: The majority of studies report that MPBC with muscle invasion results in higher rates of locally advanced disease, distant disease, and poor survival compared with conventional UC.[174,176,177,185,186] A study by Ghoneim *et al.* found that 35 of 37 patients with MPBC had either non-organ-confined disease or lymph node metastases.[185] Samaratunga *et al.* have also identified >pT3 disease in 13 of 20 patients treated with radical cystectomy without NAC.[178] Other studies have also identified lymph node metastases rates of 34% and 62% in patients with MPBC not treated with NAC,[155,187] and this collective data clearly demonstrates the predilection for early local invasion and lymph node metastasis.

Micropapillary bladder cancer clearly has worse survival compared with conventional UC; however, interestingly when matched stage for stage, there does not appear to be any difference in survival in patients with MPBC compared with conventional UC.[188]

How the percentage of micropapillary affects outcomes is currently controversial. In the Mayo Clinic series, 75% of bladder cancers were completely (100%) composed of micropapillary architecture. They compared those with <10%, 10% to 50%, and >50%, and found no correlation of percentage micropapillary to cancer-specific survival (CSS).[188] Contrary to this, Compérat *et al.* studied 72 patients with MPBC and concluded that even a small percentage of MPBC was associated with disease-specific survival (DSS) on univariate analysis but not multivariable analysis.[236] Alvarado-Cabrero *et*

al. reported on 38 MPBC cases and found that those with >50% micropapillary architecture had an increased risk for mortality compared with conventional UC.[176] Samaratunga *et al.* also reported that the percentage of micropapillary architecture correlated with tumour stage.[178]

Response to neoadjuvant chemotherapy is also controversial. In a large study from the MD Anderson Cancer Center, 100 consecutive patients with MPBC were evaluated.[187] Of these, 23 had NAC followed by cystectomy and 55 had initial cystectomy. Both groups were equivalent with regards to stage; however, the percentage alive at 5 years was only 32% for those with NAC and 71% for those without, suggesting no significant benefit from NAC. This is supported by a study of 869 patients with MPBC from the National Cancer Database, where NAC also did not show a survival benefit compared with RC alone.[189] Others have argued the case for NAC, as the majority of patients have locally advanced or lymph node metastatic disease, although they have not shown an improvement in outcomes with NAC.[185] A study by Meeks *et al.* has shown that pT0 rates are higher after NAC compared with no NAC (45% vs. 13%) and survival in those with pT0 is substantially higher than in those without pT0 (92% vs. 25%), suggesting that there may be a place for NAC in the management of ≥T2 MPBC.[235] Another study from MD Anderson by Fernández *et al.* studied 103 patients and used risk stratification to determine those who would benefit most from NAC.[190] Those authors concluded that those with muscle-invasive disease and no hydronephrosis benefit most from NAC. Those with hydronephrosis did not respond to NAC and had a poor prognosis, regardless of treatment plan.

Non-muscle-invasive (NMI) MPBC has also been shown to be more aggressive than conventional bladder cancer. In a study of 72 patients with cT1 MPBC from the MD Anderson Cancer Center, 40 received primary intravesical bacillus Calmette-Guérin (BCG) and 26 had upfront cystectomy.[191] The 5-year DSS for BCG versus cystectomy was 60% versus 100%. Those that initially had BCG experienced a 75% disease recurrence rate, a 45% progression rate, and a 35% lymph node metastasis rate. Those having salvage cystectomy experienced a 24% 5-year survival rate. This study demonstrated that micropapillary bladder cancer is relatively resistant to intravesical BCG, and all patients should be considered for upfront cystectomy. A study from Memorial Sloan Kettering Cancer Center describes 36 patients with N0M1 MPBC.[237] In this small series, 15 underwent early cystectomy and 21 underwent BCG. Five-year cumulative mortality and metastases rates were 17% versus 25% (p=0.8) and 21% versus 34% (p=0.9) for the cystectomy and BCG groups, respectively. The authors concluded there was no difference between early cystectomy and BCG; however, the study was clearly underpowered. Although there are no major prospective studies confirming the importance of early upfront cystectomy in patients with non-muscle-invasive MPBC, a survey of 118 members of the Society of Urologic Oncology (SUO) demonstrated that 81% of members would offer upfront cystectomy to patients with N0MI MPBC.[193]

Consensus Recommendations	LOE	GOR
In patients found to have micropapillary features, quantification of the proportion of tumour that demonstrates micropapillary features is recommended.	3	C
Patients with high-risk micropapillary NMI bladder cancer (HG T1) should be offered early cystectomy, as response to BCG is poor.	4	C
Patients with ≥T2N0M0 micropapillary tumours should be considered for neoadjuvant chemotherapy.	4	C

9.5.2 Nested variant

Nested variant of bladder cancer (NVBC) is characterized by infiltrative small solid nests consisting of deceptively benign-appearing neoplastic cells.[194,195] This unusual histological appearance mimics florid von Brunn nests,[196] and pathologic diagnosis can be challenging, particularly in superficial samples without muscularis propria to demonstrate invasion. Presence of TERT promoter mutation in NVBC has been suggested in distinguishing from the cancer's benign mimics.[197] There is very little data regarding the clinical behaviour of NVBC, with most information derived from three studies of 30,[192] 52,[198] and 54[194] patients. Nested variant of bladder cancer is characterized by high rates of muscle invasion on TUR (70% vs. 31), extravesical disease (83% vs. 33%), and metastases (67% vs. 19%) compared with high-grade conventional UC.[192,198] Stage for stage however, NVBC does not appear to be more aggressive than conventional UC, with no significant difference in 10-year local recurrence-free survival (RFS) rates (83% vs. 80%; p=0.46) and 10-year CSS rates (41% vs. 46%; p=0.75).[198] This has also been replicated in another study of 54 patients with NVBC, where no difference was identified between NVBC and conventional UC and also between pure nested compared with mixed nested.[194]

There is very little literature on the outcomes of NMI NVBC. A study from Gofrit *et al.* reported on 100 patients with cTa/1 variant bladder cancer, of which 7 had nested variants.[199] Tumours were fully resected and treated with intravesical BCG. The 5-year DSS rate was 75%, and the progression-free survival (PFS) rate was 50%. Disease-specific, progression-free, and recurrence-free survival of NVBC was substantially better than micropapillary and more similar to UC with squamous or glandular differentiation.

There is no evidence to support a treatment algorithm different to that of standard UC; however, multimodal approaches are recommended, as this disease often presents late.

Consensus Recommendations	LOE	GOR
Treatment of nested variant of urothelial carcinoma can be similar to that of conventional urothelial carcinoma including neoadjuvant chemotherapy and radical cystectomy in appropriate cases with ≥T2N0M0 disease.	4	C

9.5.3 Plasmacytoid

Plasmacytoid UC (PUC) is a rare disease characterized by infiltrative discohesive neoplastic cells with abundant cytoplasm and eccentrically situated nuclei that resemble plasma cells.[145,166,200,201] This disease is characterized by presentation with locally advanced disease, similar to the other variants.

PUC however is unusual in that local invasion occurs typically along the peritoneum, and this site also remains a major site for recurrence. This disposition for peritoneal metastasis has made the tumour markers CA 125 and CA19-9 useful in managing the disease.[202] Truncating somatic alterations in the CDH1 gene occur in ~85% of PUC, which lead to loss of e-cadherin expression and may explain the cancer's unique pattern of infiltration and proclivity for peritoneal spread.[203] A subset of PUC exhibits HER2 protein expression and gene amplification.[204] Other clinically actionable alterations in genes such as phosphoinositide-3-kinase (PI3K) and tuberous sclerosis-1 (TSC1) may also be present.[203] The frequency of aneuploidy and complexity of genomic changes per tumour are greater in NVBC than UC.[205]

Very few studies report the outcomes of this rare variant; however, a series of 31 patients with plasmacytoid from MD Anderson have demonstrated a median overall survival of 17.7 months.[202] In this study, despite 80% pathological downstaging with NAC, relapses were common, and no survival difference was identified in those having NAC compared with those who did not.

Nigwekar *et al.* reported the outcomes of 17 patients with PUC, with no patient surviving longer than 1 year.[206] Lopez-Beltran *et al.* have also reported on 11 patients with PUC, with a median survival of 6.2 months.[145]

Because of the high rates of locally invasive disease and peritoneal metastasis, aggressive therapy incorporating radical cystectomy is important.

9.5.4 Sarcomatoid carcinoma

In the 2016 World Health Organization definition, sarcomatoid carcinoma of the bladder includes what was previously called carcinosarcoma and sarcomatoid carcinoma, merged because of the similarly poor outcome. Sarcomatoid carcinoma is characterized by high-grade spindle-cell dedifferentiation, whereas carcinosarcoma is defined by the presence of malignant heterologous mesenchymal elements (e.g., rhabdo-, osteo-, chondro-, lipo- or angosarcoma).[144,207,208]

In a series of 41 patients from 1998, Lopez-Beltran *et al.* reported that both carcinosarcoma and sarcomatoid tumours had similar presentation.[209] The most common epithelial component was urothelial in both types. Most patients had locally advanced tumours at the time of diagnosis (96% ≥T3). Despite aggressive surgical management, the outcome is poor, with a mean survival of 9.8 months. Treatment failure occurs within 1 to 2 years following treatment.[209]

A contemporary study from Canada reports 37 cases of sarcomatoid carcinoma, of whom more than 50% died of disease within a year of presentation.[210] In a population analysis by Wright *et al.*, 135 cases of sarcomatoid carcinoma of the bladder were described.[211] Median overall survival was 16 months and radical or partial cystectomy was performed in 47% of patients.[211] A study from the National Cancer Database by Sui *et al.* in 2016, described 489 patients with sarcomatoid bladder cancer.[189] At presentation, 41% were T2 and 15% T3 or above. In this study, multivariable analysis revealed that radical cystectomy alone and radical cystectomy with multimodal therapy resulted in a 44% and 41% reduction in mortality, respectively, compared with bladder preservation. The use of

neoadjuvant chemotherapy appears superior to radical cystectomy alone but did not reach statistical significance. In another report, metastatic disease has showed a favourable response to cisplatin and gemcitabine.[212]

It is clear that sarcomatoid carcinoma of the bladder presents late, but even when compared stage for stage with conventional UC, it has a worse survival. Radical cystectomy is the most common treatment, the results of which may be improved with neoadjuvant chemotherapy.

Consensus Recommendations	LOE	GOR
Benefits of neoadjuvant chemotherapy are minimal for plasmacytoid type of bladder cancer.	4	D
Radical cystectomy can be used as primary treatment for sarcomatoid carcinoma of the bladder.	3	C
Neoadjuvant chemotherapy can be used in select cases, as survival benefit is unclear.	4	D

9.5.5 Bladder sarcoma

Malignant soft tissue tumours represent the most common histologic type of the nonepithelial bladder tumours. Half of bladder sarcomas are leiomyosarcoma, 20% are rhabdomyosarcoma, and the remainder are angio-, osteo-, and carcinosarcoma.[213]

The histological pattern of leiomyosarcoma is characterized by vague fascicles of spindle-shaped cells and must be differentiated from sarcomatoid spindle-cell carcinoma, leiomyoma, postoperative spindle-cell nodule, and inflammatory myoblastic tumour. The incidence is higher in patients with previous local radiation treatment or systemic chemotherapy.[214] Most patients present with hematuria, and the diagnosis is made early. The majority of the tumours are high grade and may attain very large size before recognition. Tumour grade is established on the basis of mitotic rate and proliferation indices rather than nuclear atypia.[214] The preferred treatment for localized disease is radical cystectomy with negative margin resection. In the largest series to date, encompassing 24 patients, high-grade sarcomas experienced 50% disease-related mortality versus 0% of those with low grade.[215] Metastatic sarcomas are treated with multimodality protocols. Doxorubicin and ifosfamide are the most active single agents available.[216]

Rhabdomyosarcoma in the adult is rare and characterized by primitive round blue-cell neoplasm often with alveolar or unclassified histology and associated with anaplasia, and most likely different from pediatric variety, which typically shows the botryoid embryonal histology.[148] Due to the rarity of this disease, treatment options are not standardized. Treatment generally involves the use of neoadjuvant chemotherapy followed by complete resection, which is often radical cystectomy. Only one patient is reported in the literature to have long survival following radiotherapy;[217] however, radiotherapy is very useful for achieving local control after surgery and chemotherapy.[218]

Angiosarcomas are seen in association with radiotherapy (38%) and hemangioma (15%). Very few cases have been reported, so no consensus on treatment has been achieved. The cancer is however aggressive, and radical cystectomy followed by chemotherapy and radiotherapy is recommended.[219,220]

Complete tumour resection and negative margins are important, although long-term survival in uncommon, with the longest documented survival of 6 years. Chemotherapy includes ifosfamide and epirubicin.[221]

9.5.6 Paraganglioma and pheochromocytoma

These are extra-adrenal neoplasms derived from neural crest cells. Tumours arising within the adrenal medulla are termed pheochromocytoma, and those outside the adrenal, paraganglioma. Bladder paraganglioma accounts for 0.05% of bladder tumours and occurs in young adults (mean age, 43 years). It may be derived from embryonic rests of chromaffin cells in the detrusor sympathetic plexus. It accounts for 10% of extra-adrenal pheochromocytoma. Malignancy was demonstrated in 10% and characterized by local invasion, regional lymph node metastases, or distant spread.

Bladder paraganglioma may be hormonally active and presents with attacks of paroxysmal hypertension, headaches, palpitations, blurred vision, and sweating associated with the act of micturition.[222] If the disease is suspected, cystoscopy should be performed under adrenergic blockade in the operating room. The gross appearance is often a solitary, submucosal, or intramural nodule. Biopsy should be avoided. The diagnosis depends on CT scan or MRI for anatomical location and the extent of the lesion. Isotopic scanning using ^{131}Iodine metaiodinebenzylguinidine (MIBG) is the study of choice for localizing small pheochromocytomas with more than 90% specificity.[223] Positron emission tomography was recently used with high sensitivity as well.[222]

In a systematic review of bladder paraganglioma including 80 articles by Beilan *et al.*, 20% of patients were treated by TURBT alone, 70% with partial cystectomy, and 10% with radical cystectomy. After a median follow-up of 35 months, 14% suffered recurrence.[224] The surgery is employed under the same precautions as in adrenal pheochromocytoma with controlled adrenergic blockade.

It may be difficult to distinguish between benign and malignant lesions if the disease is localized. Lifelong follow-up is important, as the malignant pheochromocytoma may show local recurrence with or without metachronous metastases.

Consensus Recommendations	LOE	GOR
Suspected paragangliomas of the bladder should undergo examination with adrenergic blockade and cystoscopy.	3	C
MIBG scanning can be used as confirmatory radiographic evaluation for paraganglioma of the bladder.	3	C
Transurethral resection alone can be offered as an option for small (≤3 cm) tumours that are deemed completely resectable.	4	C
Partial or radical cystectomy should be used as primary curative therapy for paraganglioma of the bladder.	3	C

9.5.7 Bladder pseudotumours

Bladder pseudotumours (also known as inflammatory pseudotumour or pseudosarcomatous myofibroblastic proliferation) are rare and may resemble malignancy.[225,226] The etiology and histogenesis remain unclear. Some of these lesions present as "postoperative spindle-cell tumours." It may be

difficult to distinguish them from leiomyosarcoma on a histopathologic basis.[227] Pseudotumours may infiltrate deep into the muscularis propria but this does not indicate tumour aggressiveness.[226] Absence of significant nuclear atypia, less than 3 mitotic figures per high-power field, and presence of spindle cells with myxoid degeneration and eosinophilic cytoplasm favour pseudotumour. Up to about two-thirds of cases may exhibit anaplastic lymphoma kinase (ALK-1) protein expression and gene rearrangement that can be helpful in the diagnosis.[225,226,228,229] Local recurrence or distant metastases are rare following complete tumour excision. If the diagnosis is clear, transurethral resection or partial cystectomy is sufficient. Radical cystectomy may be required if the diagnosis is difficult to distinguish from bladder sarcoma.

9.5.8 Melanoma

Primary bladder melanoma is very rare, with only 30 cases reported.[230] It affects the urethra more than the bladder. The age of patients with primary bladder melanoma ranges from 34 to 84 years without sex preference. Macroscopic hematuria is the usual presenting symptom. The disease generally has a poor prognosis, with two-thirds of patients dying within 3 years.[231] Treatment is surgery, usually in the form of radical or partial cystectomy.

Secondary melanoma of the bladder is found in patients with widespread metastatic melanoma of the skin. The patient's history and careful examination of the skin are essential to confirm the primary nature of the tumour. The histologic picture of bladder melanoma is similar to other melanomas. It is composed of large pleomorphic cells arranged in nests with variable amounts of pigments. As this tumour is rare in the urinary tract, confirmation with expression of melanoma-associated markers such as S100, HMB45, or Melan-A should be made. The cell origin of bladder melanoma is undefined.

9.5.8 Lymphoma

Bladder lymphoma is usually a part of metastatic spread of systemic disease.[232–234] Primary extranodal lymphoma of the bladder is very rare. Microscopic analysis shows diffuse infiltration of lymphoid cells into the normal structures of the bladder.[233] Most bladder lymphomas are the extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) origin.[233,234] Primary lymphoma is more common in women.[234] It is mostly localized and of low grade with good prognosis.[233] Other high-grade lymphomas such as diffuse large B-cell lymphoma may occur.[232,234] Local irradiation is the recommended treatment with a high recurrence-free survival.[233,234]

Consensus Recommendations	LOE	GOR
Primary or secondary bladder lymphoma should be treated primarily with local radiation and/or chemotherapy.	3	C

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BLADDER CANCER: A JOINT SIU-ICUD INTERNATIONAL CONSULTATION

Advances in the field of bladder cancer have led to our expanded understanding of the molecular biology of bladder cancer, as well as breakthroughs in treatment.

The 2017 Société Internationale d'Urologie–International Consultation on Urological Diseases (SIU-ICUD) Joint Consultation on Bladder Cancer represents an update of the 2012 Consultation on the same topic. This book represents a huge effort from many of the world's thought leaders in bladder cancer and rising stars, as part of the SIU-ICUD Joint Consultation on Bladder Cancer, held in Lisbon, Portugal, and chaired by Peter Black and Paolo Gontero.

This ICUD publication details the consensus statements on some potentially contentious issues and addressed all the recent advances in the field. Composed of nine chapters, this book tackles the following topics: epidemiology, prevention, screening, diagnosis and evaluation; pathology; basic science; molecular markers; management of nonmuscle-invasive bladder cancer; urolothelial carcinoma; localized muscle-invasive bladder cancer; urinary diversion; systematic therapy for metastatic and non-urothelial cancer of the urinary bladder. Important additions to this update of the 2012 Consultation include the addition of immunotherapy for patients with metastatic bladder cancer, and a new chapter on the basic science of bladder cancer reflecting the recent progress in our understanding of the molecular biology of bladder cancer.

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Peter Black and Paolo Gontero
Chairs, SIU-ICUD Joint International Consultation on Bladder Cancer

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