Bladder cancer

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Abstract

Bladder cancer is a highly prevalent disease associated with substantial morbidity, mortality and cost. Environmental or occupational exposures to carcinogens, and especially tobacco, are the main risk factors for bladder cancer. Most bladder cancers are diagnosed after patients complain of macroscopic haematuria, and cases are confirmed after transurethral resection of bladder tumour (TURBT), which also serves as the first stage of treatment. Bladder cancer develops via two distinct pathways, giving rise to non-muscle-invasive papillary tumours and non-papillary (solid) muscle-invasive tumours. Both subtypes have unique pathological features and different molecular characteristics. Indeed, The Cancer Genome Atlas project identified genetic drivers of muscle-invasive bladder cancer (MIBC) as well as subtypes of MIBC with unique characteristics and therapeutic responses. For non-muscle-invasive bladder cancer (NMIBC), intravesical therapies (primarily Bacillus Calmette–Guérin (BCG)) with

maintenance are the main treatments to prevent recurrence and progression after initial TURBT; additional therapies are needed for those who do not respond to BCG. For localized MIBC, optimizing care is important as is the goal to reduce morbidity of removing the bladder. In metastatic disease, advancements in genetic understanding and immunotherapy are being translated into novel therapies .

[H1] Introduction

Cancer of the urinary bladder is the 9th most common malignant disease and 13th most common cause of cancer death worldwide[1]. Indeed, 76,960 new cases of bladder cancer and 16,390 bladder cancer deaths were predicted to occur in 2016 in United States alone[2]. In 2012, 429,793 cases of bladder cancer were diagnosed and 165,084 deaths were recorded globally[3]. Men are more affected than women (3.2:0.9 ratio) and disease incidence increases with age[1]. The most common symptom of bladder cancer is either microscopic or macroscopic (visible) blood in the urine (haematuria), which occurs in 13.7% and 78.3% of patients, respectively[4]. Among patients with bladder cancer, macroscopic haematuria is associated with advanced pathological stage. However, many patients with microscopic haematuria are not adequately evaluated and there is no active screening for bladder cancer[5].

Bladder cancer generally originates from epithelium (urothelium) that covers the inner surface of the bladder and urothelial carcinomas represent the most common type of bladder cancer. Bladder cancers with variant histology (that is, with distinct histomorphological phenotypes) have also been described (10-25% of cases)[6] and include squamous cell carcinoma, small-cell carcinoma and adenocarcinoma. High-grade urothelial carcinomas can be of micropapillary, sarcomatoid, plasmacytoid, nested and microcystic variants and have the propensity for divergent differentiation into, for example, squamous and glandular histologies. Variant histology bladder cancers are associated with locally aggressive disease, metastasis and poor response to existing therapies; however, controversy persists over the true influence histology has on outcomes. In general, individualized management should be applied to these patients in the light of the existing limited literature.

Tumours that invade the detrusor muscle are considered muscle invasive bladder cancer (MIBC) and are more likely to metastasize to lymph nodes or other organs. Approximately 75% of newly diagnosed patients have non-muscle-invasive bladder cancer (NMIBC) and 25% have MIBC[7] or metastatic disease[8] (Figure 1). The stage at diagnosis of bladder cancer has not changed over the past 10 years based on data from the Surveillance, Epidemiology, and End Results (SEER) registry in the United States because

active screening is not available. Accordingly, mortality rates have not changed as there is no cure for metastatic disease.[9]

The Cancer Genome Atlas project (TCGA) has advanced our understanding of bladder cancer[10]. This genetic characterization study of MIBCs has provided information not only on genetic drivers that might serve as therapeutic targets, as well as providing information of subtypes or clusters of invasive disease. These clusters might be associated with prognostic factors and unique therapeutic approaches and eventually provide a step forward towards individualized patient management. In this Primer, we discuss urothelial (the main type) bladder cancer epidemiology, pathophysiology, diagnosis, screening and prevention as well as management and quality of life issues.

[H2]Epidemiology

[H2] Incidence and mortality

The incidence of bladder cancer differs considerably between geographical regions such that age-standardized incidence (ASI) is almost three times greater in more-developed areas (ASI of 9.5 per 100,000 population) than in less-developed countries (ASI of 3.3 per 100,000 population) (Table 1). The highest ASI is observed in European countries (namely, Spain, Italy, Denmark and Switzerland), North America, some northern African countries (for example, Egypt) and western Asia (Turkey and Israel), particularly in men[11]. By contrast, the lowest rates were noted in Central and South America, Sub-Saharan Africa and South-East Asia[11]. Urothelial cancers remain the most common type of bladder cancer in North America and Europe; however, in Egypt, 10-40% of bladder cancers are squamous cell cancers that are associated with the blood flukes *Schistosoma* infections[12]. Introduction of efficient anti-bilharzial drugs together with increased cigarette smoking resulted in significant decline of squamous cell cancer incidence and a shift towards more urothelial cancers in Egypt in recent years[11].

Although rates of bladder cancer are higher in white populations than in other ethnicities, survival is worse for black individuals[8,13], a finding shown in the United States and São Paulo, Brasil[14].

Unfortunately, information about incidence in black populations around the world are generally lacking. The low rates in Africa can be attributed to access to health care and competing risks of mortality[11]. Additionally, global variations of bladder cancer mortality are less conspicuous than of incidence. This reduced variability might result from less difference in the way patients with advanced-stage cancers are diagnosed and registered in health systems[15]. However, regional mortality differences reflect, at least in part, the disparate access to modern healthcare systems, sensitive diagnostic facilities and up-to-date treatment protocols. Fortunately, at the global level, age-standardized bladder cancer mortality has declined recently, especially in the most developed countries. Exceptions include Central and South America (for example, Brazil and Cuba), some central, southern and eastern European countries (for example, Bulgaria, Croatia, Hungary and Romania) as well as the Baltic countries (for example, Latvia) that have experienced rapid economic transition over the past 15 years. However, according to the WHO, the number of bladder cancer cases and deaths are expected to almost double in the near future[16]. This phenomenon is explained by the increase in life expectancy over time as the majority of bladder cancer is diagnosed >65 years of age. The average life expectancy has increased by 3% since 1950 and the life expectancy gap between more and less developed countries has diminished by 4.9 years since 2000 (Ref.[17]).

[H2] Risk factors

Cigarette smoking is the most common risk factor for bladder cancer, with estimates that tobacco is responsible for half of all cases[18], with 20-30 years lag time between the exposure and diagnosis[19]. Current bladder cancer incidence is highest, albeit not uniformly, in regions that had high smoking rates in the 1980s[11,20]. In particular, in Spain and in Italy, age-standardized smoking rates in 1980 amounted to 44.4% and 44.3%, respectively; the greatest bladder cancer incidence in men in these countries was 36.7 per 100,000 in 2003 and 33.2 per 100,000 in 2007. Since then, smoking prevalence has declined substantially in high-income countries, and incidence and mortality have tended to mirror this trend[11]. Thus, smoking patterns may at least partially explain geographical diversities in bladder cancer

epidemiology. Unfortunately, the WHO has reported increases in tobacco consumption in large areas of the less-developed world, including Africa, the Middle East, Eastern Europe, countries of the former Soviet Union and Asia, where governmental control over the cigarette market (including marketing) is less stringent and public debate on the detrimental effects of tobacco is lacking[21].

In addition to cigarette smoking, associations between a number of environmental factors and bladder cancer have been extensively investigated. Diets low in fruits and vegetables, and urban living are all linked — although not invariably — to increased bladder cancer risk[22,23]. Furthermore, some evidence suggests alcohol intake slightly increases the risk, but epidemiological data are confounded by other risk factors[24]. Metabolic syndrome in men was also reported to influence bladder cancer risk, but no direct or indirect association has been conclusively shown with either risk or prognosis[25]. Consumption of water or food polluted with arsenic might explain some regional bladder cancer data. For example, arsenic pollution was correlated with bladder cancer risk in Argentina, Chile and Bangladesh[8,26]. Ambient air pollution (AAP) is also suggested to influence bladder cancer risk in less-developed countries. Direct combustion chemicals (mainly diesel and gasoline engine exhausts, stationary power plants and indoor air pollution) are major sources of AAP[26].

Occupational exposures have long been associated with bladder cancer risk. According to the recent analysis, the greatest risk occurs in industrial areas processing paint, rubber, petroleum products and dye workers, whereas the greatest risk of bladder cancer-specific mortality occurs in electrical and chemical process workers[23,27]. Along with widespread urbanization, many manufacturing processes are being transferred from more developed to less developed countries, potentially posing increased occupational hazards to the local workers. Nevertheless, no more than 8% of bladder cancer cases are thought to be attributable to such exposures[28] and the global impact of occupational risks on bladder cancer incidence to explain geographic diversities remains to be fully elucidated. Additionally, unemployment, number of physically unhealthy days, number of days exposed to air pollution ozone, percent of houses with well-derived water, employment in the mining industry, urban living and ethnicity are all linked to bladder cancer mortality[23,24,26–28].

Some evidence supports a genetic predisposition to bladder cancer; genes involved in the metabolism of carcinogens such as N-acetyl transferase and *GSTM1*-null genotypes are associated with increased risk[29].

Specifically, cigarette-smoking women with the *GSTM1*-null genotype are more prone to bladder cancer diagnosis than their non-smoking counterparts[13]. Large genome-wide association studies (GWAS) found sequence variants that can increase the risk for bladder cancer; for example, alterations in the urea transporter encoded by *SLC14A* are associated with renal urine concentration and can influence the contact of carcinogens with urothelial surfaces[30–34]. Furthermore, according to a novel, bioinformatic approach towards measurements of gene–gene interactions in two GWAS, decarboxylase protein complexes were proposed as associated with bladder cancer susceptibility[35], which is a potential druggable target.

[H1]Mechanisms/pathophysiology

Pathological and clinical information from mouse models and human samples indicate that urothelial carcinoma develops via two distinct pathways, giving rise to papillary NMIBCs and non-papillary (solid) MIBCs (Figure 2). In mouse models, low-level expression of mutant *Hras* gives rise to flat or papillary urothelial hyperplastic lesions and high-level expression to NMIBCs[36]. Similarly, in humans, the predicted precursors of NMIBC are flat or papillary urothelial hyperplastic lesions. Two common alterations in NMIBC, deletion of chromosome 9 and point mutation of *FGFR3,* are also evident in these hyperplastic precursors[37–39], and where papillary NMIBC from the same patient has been studied, these alterations are shared[37–39], suggesting a clonal relationship. Urothelial papilloma, which is considered a benign tumour, also shows frequent *FGFR3* mutation[40].

By contrast, generation of (solid) MIBCs in mouse models requires inactivation of one or more of the tumour suppressor genes *Tp53, Rb1* and *Pten*[41–43] and tumours in these models are preceded by development of flat urothelial dysplasia and carcinoma *in situ* (CIS) lesions. Similarly in humans, high risk of development of MIBC in patients with dysplasia or CIS is well-documented[44] and these lesions share features with high-grade and invasive bladder cancers, including mutations in *TP53* (Ref.[45]), stabilised p53 expression[46], upregulated expression of cytokeratin 20 (CK20) and HER2/neu[47], and reduced expression of PTEN with concomitant upregulation of the phosphatidylinositol 3-kinase (PI3K) pathway[48] — features that facilitate cell proliferation and survival.

[H2] Clonality and cell of origin

Given that bladder cancer is typically a multifocal disease, questions have been raised concerning its clonality. Indeed, chronic exposure of the urothelium to carcinogens might lead to the development of multiple cancers from different cells of origin. Alternatively, multiple monoclonal lesions could arise from seeding of cells liberated during surgery, intraepithelial expansion or spread from a single tumour clone.

In women with bladder cancer, the random inactivation of one X chromosome during embryogenesis provides an ideal cell of origin marker; X-inactivation analysis has indicated that although some multifocal or metachronous tumours are oligoclonal[49], the majority are monoclonal[50]. However, other studies using multiple molecular markers or genome-wide features provide clear evidence for sub-clonal evolution of tumours that are predicted to have arisen from a single cell of origin[51,52].

Evidence from whole bladder mapping studies in MIBC shows that broad regions of the urothelium are replaced by the monoclonal expansion of cells that represent tumour precursors; sub-clonal molecular evolution within these populations enables construction of the temporal series of genomic events leading to tumour formation[53]. Indeed, large areas of the urothelium with normal or dysplastic morphology represent clonal 'fields' of altered cells within which loss of heterozygosity (LOH) on 3q22, 5q22-23, 9q21, 10p26, 13q14 and 17q13 have been identified[53,54], all of which are common alterations in bladder cancer. The most detailed analysis has been carried out on a region spanning the *RB1* locus on chromosome 13, which showed LOH in the absence of *RB1* mutation in urothelial fields that had minimal or no morphological change. Regional genes on chromosome 13 proposed to drive early clonal expansion included *ITM2B*, which was shown to be silenced by hypermethylation, with *RB1* mutation occurring as a secondary event during tumour progression[55,56].

The tumour-initiating cells in NMIBC are thought to be those in the intermediate (that is, non-basal) layers of the bladder wall[57], findings supported by lineage tracing studies in animals[58]. Lineage-tracing studies in mice have also identified sonic hedgehog (Shh)-expressing basal cells that can repopulate the normal urothelium following injury[59] and can give rise to MIBC in a carcinogen-induced mouse model[60]. Stem cells isolated from undifferentiated human bladder tumours also show a phenotype ($CD44^+$, $KRT14^+/KRT5^+$, $KRT20^-$) that is similar to cells that reside in the basal layer of the normal urothelium;

these cells have tumour-initiating ability as xenografts[57,61]. These different cells of origin for NMIBC and MIBC might determine or constrain the molecular events that subsequently occur during tumour development. The differentiation markers of these cells of origin are apparent in the expression signatures of NMIBC and MIBC (below).

[H2]Molecular landscape

Although bladder cancer is a smoking-related cancer, recent genome sequencing studies have not found a predominant mutational signature of tobacco smoke exposure. Instead, a major contribution of APOBEC cytidine deaminases to the mutation signature has been detected in both NMIBC and MIBC[10,62]. Despite similar mutational features, the mutational load and overall changes in genomic architecture in the two groups are distinct. Averages of 169–195 mutations per sample have been reported from exome sequencing of NMIBC[62,63] compared with 302 in MIBC[10]. NMIBC frequently have diploid or near diploid karyotypes and few copy number alterations[64], whereas MIBC are commonly aneuploid, with many numerical chromosomal alterations, re-arrangements and copy number changes[10,64]. These differences in mutational landscape are accompanied by major differences in the overall patterns of mutated genes in these two major groups of tumours (see below)[65].

[H3] NMIBC. Most low grade tumours are genomically stable and the most common copy number alteration is deletion of chromosome 9 (~50% of tumours)[66]. The *CDKN2A* locus (9p21) encodes p16 and $p14^{ARF}$, which are negative regulators of the retinoblastoma (RB) pathway and p53 pathway respectively. Chromosome 9 loss also implicates *TSC1*, a tumour suppressor that regulates mTOR signalling (see below). Genome-wide copy number analyses have also identified deletions of chromosome arms 10q, 11p, 11q, 17p, 18q, 19p and 19q in up to 20% of cases, most involving entire chromosome arms[64,67]. Gain of 20q has been reported, but high level DNA amplification is infrequent in this tumour group.

NMIBCs are characterized by activating point mutations in *FGFR3* in the majority of cases [68]. In cultured normal human urothelial cells, expression of mutant *FGFR3* leads to activation of the RAS-MAPK pathway and a phenotype of cell overgrowth at confluence *in vitro*, suggesting that *FGFR3* activation might contribute to early urothelial hyperplasia [69]. *FGFR3* is also activated in some cases by chromosomal translocation. The fusion proteins resulting from these translocations show loss of the

final exon of *FGFR3* and fusion in-frame to *TACC3* (encoding transforming acid coiled-coil containing protein 3), or less commonly to *BAIAP2L1* (BAI1-associated protein 2-like 1). These fusions proteins are potent transforming oncogenes [70,71]. As activating mutations of one of the *RAS* genes are also found in some NMIBCs, and these are mutually exclusive with *FGFR3* mutation[72], it is estimated that activation of the RAS-MAPK pathway may contribute to development of >80% of NMIBCs (Table 2). Activating mutations in phosphatidylinositol-3-kinase alpha (*PIK3CA)* are also common in NMIBC, and frequently found with *FGFR3* mutation[73–75].

Inactivated tumour suppressor genes include *TSC1* (9q34), which is mutated in ~15% of cases[73]. A few mutations in *TSC2* are also reported[76]. The TSC1:TSC2 complex regulates the mTOR branch of the PI3K pathway; loss of one copy and/or mutation of *TSC1* in many of these tumours suggests that upregulated mTOR signalling is a major feature of NMIBC. Frequent inactivating mutations in the cohesin complex tumour suppressor gene *STAG2* are also characteristic of NMIBC[77,78]. However the role of its loss in generating aneuploidy, as reported in other tumour types, is unlikely to be important in these genomically stable tumours[63,77]. Whole-exome sequencing has also revealed inactivating mutations in several chromatin-modifying proteins including *KDM6A*, *CREBBP*, *EP300* and *ARID1A.* These are present at significantly higher frequencies than in any other cancer type, including MIBC [10,62,63,79], indicating that epigenetic alterations are likely to play a major part in shaping the phenotype of these tumours (Table 3).

[H3]MIBC. The genome of MIBC typically shows complex copy number changes and re-arrangements and synchronous and metachronous MIBC are often genomically divergent, suggesting rapid sub-clonal evolution. Many regions of genomic amplification are reported, some containing genes with known oncogenic function, for example, *E2F3*, *MDM2* and *ERBB2* (Table 2). Regions of homozygous deletion include 9p21 (p16 and p14^{ARF}), 10q23(*PTEN*), 2q36, 4q35 and 13q14 (*RB1)*[10,64,67,80].

MIBCs share many molecular features with other solid cancers, particularly loss of function of key tumour suppressors (Table 3), leading to escape from cell cycle checkpoints and dysregulation of major signalling pathways. *TP53* and *RB1* are frequently mutated and regulators of their pathways are also altered[10] (for example, amplification of *MDM2* and *E2F3* and homozygous deletion of *CDKN2A*).

Hemizygous deletion, homozygous deletion and/or reduced expression of *PTEN* is found in many cases[73,81]. Other mutations in the PI3K pathway include *TSC1* mutation, mutations of *PIK3CA* (at lower frequency than in NMIBC) and a few *AKT1* mutations[82]. The upstream pathway activator *ERBB2* shows amplification, mutation or over-expression in a subset of cases[10]. In the micropapillary variant in particular, high frequency of *ERBB2* amplification and mutation has been shown[83].

Although *FGFR3* mutations are less frequent in MIBC than in NMIBC, up to 40% of MIBCs show upregulated expression[84]. Isoform switching (generated by alternative splicing of specific exons) of FGFR3 and FGFR1 are prevalent in MIBC[85,86], with predicted effects on ligand binding and potential for increased autocrine or paracrine signalling. Activation of FGFR1 has been shown to induce epithelial–mesenchymal transition (EMT, whereby cells acquire migratory and invasive properties) in preclinical models[87], suggesting a potential role in MIBC metastasis. In addition to FGFR signalling in these tumours, *RAS* mutations and mutational inactivation of NOTCH pathway genes[88] also contribute to MAPK pathway activation. Mutations in *APC*, nuclear accumulation of β-catenin (*CTNNB1*) and loss of expression of the WNT antagonists secreted frizzled receptor proteins (SFRPs) and WNT inhibitory factor 1 (WIF1) [89,90] also implicate the WNT signalling pathway in some MIBCs[91,92].

As in NMIBC, a major contribution of epigenetic changes in MIBC is clear[10]; genome-wide analysis indicates the importance of both DNA methylation and histone methylation in gene silencing[93]. Extensive analysis of chromatin marks and relationships to expression and mutational status has not yet been carried out. However, a role for histone modification in regions of copy number-independent gene silencing identified in aggressive MIBC with a CIS-associated expression signature has been demonstrated[94]. Distinct differences in DNA methylation exist between NMIBC and MIBC, with common hypomethylation in non-CpG islands in NMIBC and widespread promoter hypermethylation in MIBC[95,96]. Many specific DNA methylation changes have clinical and pathological associations[97].

Finally, some molecular features in MIBC have been related to response to chemotherapy. For example, tumours with *ERBB2* mutations are reported to show good response to neoadjuvant chemotherapy[98]. In addition, response to cisplatin-based chemotherapy has been related to the presence of mutations in *ERCC2* (Ref.[99]). *ERCC2* encodes a DNA helicase with a key role in nucleotide excision repair. *ERCC2*-

297 mutant tumours have a higher mutational load than other MIBC[10], which has been associated with the
298 presence of a distinct genomic signature prevalent in MIBC from smokers[100].

299

300 **[H2]Molecular subtypes**

301 Heterogeneity in clinical outcomes of patients suggest that biologically relevant subtypes might exist
302 within and between NMBIC and MIBC.

303

304 Transcriptional profiles currently provide the most well-defined subtypes. The subtypes described, their
305 relationships and biological implications have been compared analytically[101,102] and discussed in detail in
306 recent reviews[103,104]. The initial Lund study of tumours of all grades and stages defined five subtypes:
307 termed urobasal A, genomically unstable, (immune-cell) infiltrated, squamous cell carcinoma-like and
308 urobasal B [105]. These subtype assignments did not absolutely correlate with tumour grade and stage,
309 which might have highly relevant prognostic implications[105]. Subsequently, three major transcriptional
310 profiling studies focussed on MIBC[10,106,107] and one on NMIBC[108]. To date, these classifications have used
311 different nomenclatures (Figure 3). Whilst it has been possible using bioinformatics approaches to align
312 these to reveal overlaps, it will be essential to assess which signatures provide the most clinically useful
313 information and to develop a unified nomenclature system to describe these.

314

315 Subtypes based on DNA copy number alteration and on DNA methylation profiles have also been
316 described, some with prognostic associations[64,109]. Three subtypes based on mutations and regions of
317 DNA amplification and deletion were reported in The Cancer Genome Atlas (TCGA) study of MIBC[10]. It is
318 not yet clear how well these align with the transcriptional subtypes but it can be anticipated that
319 integration and unified description of epigenetic, copy number and transcriptional data will ultimately
320 deliver data with improved clinical relevance.

321

322 **[H3] NMIBC.** In the Lund study[105], low-grade Ta tumours were predominantly classified as urobasal A,
323 characterized by high levels of markers of urothelial differentiation, cell adhesion genes, an *FGFR3*-
324 related signature and early cell cycle genes such as cyclin D1. Genomically unstable tumours have higher
325 expression of late cell cycle genes such as cyclins B and E, but retain markers of urothelial differentiation

including uroplakins. Infiltrated tumours show high levels of expression of immune cell and stromal markers compatible with a high proportion of non-tumour cells in the sample. Stage T1 and high-grade tumours contained fewer rrobasal A tumours, more genomically unstable and infiltrated tumours (subtypes that show overlap with the broad luminal subtype of MIBC, see below), but some were classified as urobasal B and squamous cell carcinoma-like (both basal-like groups).

The UROMOL study (using samples from 460 patients with low-grade and high-grade Ta and T1 tumours and CIS) described three expression classes[108] (Figure 3). Class 1 contained many Ta tumours and showed best prognosis; these lesions were similar to Lund urobasal A group. Class 2 contained more T1 and high-grade tumours and those from patients with high European Organisation for Research and Treatment of Cancer (EORTC) risk score for recurrence and progression[110], and this class included the majority of patients who progressed to MIBC. Indeed, the majority of MIBC samples analysed in parallel were assigned to this class[108]. Class 2 tumours were also characterized by expression of late cell cycle, EMT-related, stem cell-related and CIS signature genes[111] but retained expression of uroplakins, implying that these may represent tumours of origin for luminal MIBC that retain markers of urothelial differentiation. Indeed, *FGFR3*-activated MIBCs frequently have deletions of *CDKN2A*[10,101] and homozygous deletion of *CDKN2A* has been shown previously to identify a subset of *FGFR3*-mutant NMIBC with high risk of progression[112]. Class 3 tumours share features with the urobasal A subtype (including *FGFR3* mutation), but also had features of the basal signature defined in MIBC[106] (that is, a phenotype of $KRT5^{+}$, $KRT14^{+}$, $CD44^{+}$, $KRT20^{-}$, $PPARG^{-}$). The upregulation of many long non-coding RNAs, some of which have been implicated in oncogene-induced senescence, in class 3 tumours supports that these may represent a 'dormant' luminal tumour state[108].

[H3] MIBC. MIBC analyses have identified two major groups of tumours that have been termed 'luminal' and 'basal' (the 'UNC' subtyping), which show strong similarities to subtypes defined in breast cancer[113]. Further subdivision into three[107] ('MDA' subtyping) or four[10] ('TCGA' subtyping) categories provides further biological insight and relationships with disease outcome (Figure 3).

MIBC luminal tumours commonly show papillary histology and express markers of urothelial differentiation (such as the uroplakins and *KRT20*), E-cadherin (*CDH1*), *FGFR3* and early cell cycles genes

(for example, *CCND1*). Also expressed are the transcription factors peroxisome proliferator-activated receptor γ (PPARG), oestrogen receptor (ER) and tumour protein p63 and their targets. Basal tumours express markers of the basal layer of the urothelium (such as CD44, KRT5, KRT6B and KRT14). Many show squamous differentiation, and some show low claudin gene expression and markers characteristic of EMT.

Outcome data indicates significantly worse prognosis for patients with tumours defined as basal (TCGA II, TCGA IV, urobasal B or squamous cell carcinoma-like) rather than luminal (TCGA I or urobasal A)[105,107]. Women with MIBC show poorer outcomes than men[114] and interestingly seem to have increased prevalence of basal-type tumours[106]. Data also indicates that the MDA *TP53*-like subtype, which contains tumours with overlap with both luminal and basal types, is associated with a higher frequency of resistance to neoadjuvant chemotherapy[107].

Several actionable therapeutic targets segregate with specific expression subtypes. These targets include FGFR3 and ERBB2 in luminal tumours, EGFR in tumours with squamous differentiation and immune checkpoint inhibitors in basal tumours with evidence of a high immune infiltration. The potential role for EGFR inhibition in basal subtype tumours has been examined in depth, and preclinical evaluation (human cell line and mouse model) provides support for this approach[115]. A specific signature for claudin-low basal tumours has recently been defined that is related to low PPARG and high nuclear factor (NF)-kB activity; these tumours are enriched for a tumour-initiating cell expression signature[61], have an EMT phenotype and high immune infiltration. Accordingly, these tumours might respond to immune checkpoint inhibition[116]. Given this potential prognostic and therapeutic relevance, it will be important to develop methods that can rapidly assign subtype membership using routine clinical samples. Recent efforts to identify these subtypes using conventional histopathology and immunohistochemistry[117,118] could facilitate this.

[H1]Diagnosis, screening and prevention

[H2] Signs and symptoms

Most patients are diagnosed because of (painless) haematuria; the incidence of bladder cancer is 10-

20% in patients with macroscopic haematuria and 2-5% in referred populations with microscopic haematuria[119,120]. Usually, microscopic painless haematuria is incidental in urine tests performed for general health assessment.

Bladder cancer can also be suspected if the patient presents with non-specific symptoms of the lower urinary tract associated with impaired storage of urine, namely increased urinary urge, frequency and dysuria. These symptoms are more frequent in patients with CIS than with papillary (pathological Ta) tumours and should prompt urological assessment[4,121,122]. Urine analysis, either by dip-stick, microscopy or both, should demonstrate presence of haematuria and rule out urinary tract infection, which can mimic and/or co-occur with bladder cancer. Accordingly, following treatment of urinary tract infection, absence of these symptoms and haematuria must be confirmed[123].

[H2] Evaluation and diagnosis

Evaluation of patients suspected of having bladder cancer is performed using cystoscopy (Figure 4), which is an outpatient endoscopic procedure performed with a flexible scope and with local anesthesia[124]. Any abnormal finding such as reddish flat, papillary or solid lesions requires histological evaluation because benign conditions, such as inflammatory diseases, can mimic the bladder cancer. Histology can be obtained by transurethral biopsy or resection of the entire area (see Management). Often, inspection of cells in the urine (cytology) is performed as an adjunct measure to detect missed cancer; cells with malignant appearance are highly suspicious of presence of cancerous lesions in the bladder and again warrant cystoscopic and histological investigation. Importantly, no current urinary-based tumours markers have demonstrated sufficient sensitivity and specificity to replace cystoscopy in detection of bladder cancer[121,125,126].

However, CIS is an entity that is challenging to diagnose cystoscopically because these lesions can be hardly discernible from normal bladder tissue. Instead, microscopic urinary analysis is required to identify atypical cells[127] and diagnosis is confirmed with histological assessment of bladder tissue samples (Figure 4). Cystoscopic detection of CIS may be enhanced by fluorescence cystoscopy[128] or narrow band imaging[129]. These technologies improve the differentiation of tumorous lesions from

normal tissue by taking advantage of increased metabolic activity (blue-light) and vessel architecture (narrow band) in cancer and have higher specificity for bladder cancers than traditional cystoscopy.

Imaging of the upper urinary tract, that is, the renal collecting system and the ureter, is an important component of evaluation of patients with haematuria but plays a minor part in diagnosis of bladder cancer. Although CT and MRI urography have been suggested to improve accuracy of diagnosis, ultrasound imaging has been suggested to suffice[121,130]. In patients with confirmed MIBC, CT imaging is mandated to stage and assess potential distant spread[121].

[H2] Staging and prognosis

Bladder cancer prognosis (and management) depends on bladder cancer histopathology (NMIBC or MIBC)[121,131]. Indeed, histology is the only reliable determining factor of tumour biology to inform management. However, although prognostication cannot be exact, the depth of tumour infiltration into the bladder wall can provide a simple stratification of risk. For example, tumours confined to the inner lining of the bladder (the mucosa) that do not invade the lamina propria are classified as stage Ta according to the Tumour, Node, Metastasis classification system (Figure 1)[131]. Tumours invading the lamina propria are classified as stage T1; these tumours are characterized by an adverse tumour biology similar to muscle-invasive tumours[132]. Tumours penetrating the bladder detrusor muscle and beyond are highly aggressive[131,133].

Additionally, grading (the extent to which the cells are differentiated) is important for the assessment of Ta tumours because well-differentiated (lower grade tumours) are less aggressive than high-grade lesions[134,135]. In 2016, a third edition of the WHO classification was issued, accentuating the changes proposed by the 2004 over the 1973 classification (Figure 1). While benefit over the 1973 classification has not been evaluated systematically yet, the current (2016) version provides uniform terminologies and clearer definitions, especially in increasingly recognized divergent differentiation in MIBC.

Overall, the importance of histopathology in bladder cancer diagnosis, prognostication and treatment is clear. However, interobserver and intraobserver variability in staging and grading is a limitation of the technique. For example, significant interobserver variability in the classification of stage T1 versus Ta

tumours and tumour grading using both the 1997 and 2004 WHO classifications have been reported[121,132,136].

[H2] Screening and prevention

Screening for early detection of bladder cancer is not available owing to the low incidence rates compared with common cancers like prostate and breast cancer. However, several non-randomized trials have demonstrated the ability to detect bladder cancer early using blood detection in the urine or urine-based tumour markers, such as cytology or nuclear matrix protein number 22 (NMP22), which reflects mitotic activity[137]. Although the value of, for example, urinary cytology, is clear in the follow-up care of patients with NMIBC (see below, Management), the low detection rates limit current application in a screening setting[138]. Additionally, screening has been proposed for individuals at high risk of developing bladder cancer — those with substantial exposure to risk factors such as smokers. In contrast to other common malignancies, genetic predisposition has little utility in predicting who will develop bladder cancer, but genetics do exert an influence via regulating susceptibility to environmental risk factors[139].

Prevention of bladder cancer focuses on avoiding tobacco exposure as well as environmental and occupational carcinogens. Tobacco cessation is challenging and many patients have difficulty in stopping smoking[140]. Physicians have an obligation to inform patients about their risks and encouraging tobacco cessation.

[H1]Management

Initially, all newly diagnosed bladder tumours require an endoscopic resection under general or spinal anaesthesia, which is a transurethral resection of bladder tumour (TURBT), to enable thorough visualization of the bladder and appropriate resection with an attempt to include muscle for accurate staging. Various guidelines are available on managing bladder cancer, including from the European Association of Urology, American Urological Association, Society of Urologic Oncology, and the US National Comprehensive Cancer Network[121,141–144]. Although generally concordant, these

recommendations do have important differences owing to varying levels of evidentiary support[143]. In general, NMIBCs are frequently managed with endoscopic resection and risk-based intravesical therapy (that is, bladder instillation) whereas MIBCs are managed with more-aggressive treatments such as cystectomy (bladder removal) with or without chemotherapy.

[H2]TURBT

TURBT not only has a diagnostic role, but also a therapeutic one and can be a sufficient and potentially curative therapy depending on the pathological features of the tumour. TURBT is performed by passing an endoscopic instrument (a resectoscope) through the urethra. Small tumours can be resected *en bloc* with the electrified wire loop of the resectoscope whereas larger tumours are resected in multiple fractions. Patients with NMIBC can be stratified into three risk groups according to the number of tumours, tumour size, recurrence rate, tumour stage, presence of CIS and tumour grade to further guide therapy after initial TURBT[144] (Table 4). Patients with low-risk disease are often treated with the initial TURBT if all disease was visibly resected, but remain under close surveillance owing to risk of recurrences. Although many surveillance protocols have been recommended, at a minimum, cystoscopy should be performed 3 months postoperatively and at decreasing frequencies for up to 5 years. Future low-grade recurrences can be treated with TURBT or in-office fulguration (heat ablation).

Under-staging the true pathology at the initial TURBT in patients with high-risk disease is an important risk; there is an up to a 20% chance of upstaging a patient with T1 disease to T2 even if muscle was present in the resected tissue initially, and up to a 40% chance of upstaging if muscle was not present[121]. Furthermore, even if the tumour is accurately staged at initial TURBT, there is a 50% chance of an incomplete resection resulting from factors such as multiplicity, size and location of the tumour[145]. True staging is of utmost important in bladder cancer to determine the appropriate treatment after initial TURBT. Thus, a repeat TURBT is recommended within 2-6 weeks in patients with known incompletely resected tumour, or tumours invading the lamina propria (T1; Figure 1), and should be considered in high-grade non-invasive disease (except CIS alone) to improve staging accuracy and increase recurrence-free survival[145,146]. A randomized controlled study has shown that repeat TURBT after newly diagnosed T1 bladder cancer improves recurrence-free survival and progression-free survival by 25% and 14% at 5 years, respectively[147].

Advances in the TURBT technique are being slowly adopted and evaluated. For example, blue-light fluorescent cystoscopy uses hexaminolevulinate hydrochloride (a photo-sensitizing haeme precursor instilled 1 hour prior to cystoscopy) to detect the pathological accumulation of fluorescent porphyrin products in bladder cancers. The technique has demonstrated the ability to improve diagnosis and actually decrease short and long-term recurrence[148]. However, some issues with a high false-positive rate that decreases with operator experience have been reported, and the proprietary equipment is costly. Nonetheless, this technology is very promising with respect to improved diagnosis and treatment of NMIBC[149].

Bipolar electrocautery is another new technology that uses less energy and voltage than the standard monopolar cautery of the TURBT loop as the circuit does not pass through the patient. Additionally, isotonic fluids can be used which have minimal effects on the serum of the patient compared with monopolar resection (although this is less a concern during bladder resections than, for example, when the technique is used to ablate prostate tissue)[150]. Other potential advantages are being explored to improve TURBT, including minimizing the obturator muscle reflex in the pelvis, decreasing bladder perforation and reducing the effects of cautery artefacts on pathology; however, more trials are needed prior to conclusions being drawn[151].

[H2]Adjuvant intravesical therapy

In patients with intermediate and especially high-risk disease (Table 4) the use of adjuvant intravesical therapy is advised. Bacillus Calmette–Guérin (BCG), a vaccine primarily used against tuberculosis, has demonstrated both a decrease in recurrence and in progression of bladder cancer and has been shown to be superior to chemotherapy in multiple randomized controlled trials (RCTs) and meta-analyses[152,153]. Adjuvant therapy must include maintenance therapy for 1 year in intermediate-risk disease and for up to 3 years (if tolerable) for high-risk disease to achieve maximal efficacy; variety of protocols are available. BCG maintenance has been shown to lower the risk of progression by 37% compared with no BCG maintenance[154,155]. Patients who have persistent or worsening disease after an appropriate treatment course with BCG, those who experience disease relapse while on maintenance therapy or those who experience recurrence with an inadequate treatment course due to inability to tolerate BCG owing to adverse effects are deemed BCG failures[156]. For these patients, the most oncologically effective

treatment is radical cystectomy, although consideration can be given to bladder preservation strategies including intravesical chemotherapy, device-assisted intravesical therapy, and clinical trials[157].

Following TURBT, a single dose of intravesical therapy with chemotherapeutic agents (mitomycin or doxorubicin in the United States, as well as epirubicin or pirarubican in Europe) within 24 hours has been shown to decrease recurrence by 40% at 1 year and 15% at 5 years[158,159]. Intravesical chemotherapy should be given to patients immediately following TURBT with papillary lesions if there is no clinical concern for MIBC or bladder perforation during TURBT[121]. If there is suspicion of bladder perforation, immediate intravesical therapy should not be given due to the increased risk of complications[121]. The oncological benefit of adjuvant intravesical therapy is particularly useful in the patients with low-risk tumours as those with intermediate and high-risk disease are at greater risk of recurrence and likely require further adjuvant therapy.

[H2]Radical cystectomy

Radical cystectomy is the gold standard therapy for patients with MIBC as well as in those with NMIBC who fail intravesical treatment as defined above[141]. Furthermore, certain patients with T1 NMIBC (invasion of lamina propria) and high-risk features on TURBT — lymphovascular invasion, concomitant CIS, variant histology (especially micropapillary disease), large (>3cm) and multifocal tumours, and deep lamina propria invasion — can be considered for 'early' cystectomy[131–133]. Radical cystectomy typically includes prostatectomy in men and hysterectomy and partial resection of the vagina and urethra in women.

Although radical cystectomy is traditionally performed with an open technique, interest is increasing in minimally invasive approaches using the da Vinci® (Intuitive Surgical, Sunnyvale, California, United States) robotic system. The potential benefits of this approach include reduced blood loss and other benefits inherent to minimally invasive surgery, with multiple studies demonstrating equivalent oncologic and functional outcomes[160,161]. One RCT compared robotic to open cystectomy with equivocal findings; however, only the cystectomy portion of the operation was performed robotically and the urinary diversion portion (see below) was performed through a standard open incision — potentially mitigating the initial benefits of the robotic approach[162]. Another RCT comparing open and radical cystectomy, the RAZOR trial, is currently accruing; however, again the diversion can be performed

open[163]. Further studies are needed to compare total robotic radical cystectomy with intra-corporeal diversion to open radical cystectomy before further conclusions can be drawn[164].

[H3] Lymphadenectomy. The role of lymphadenectomy (lymph node dissection) in managing bladder cancer is crucial, and although thorough lymphadenectomy has a demonstrated survival advantage, debate remains regarding the specific template that should be used or number of lymph nodes that should be removed[165]. The standard dissection includes removal of lymph nodes along the external iliac vessels from the circumflex iliac vein caudally up to common iliac bifurcation cephalad and the genitofemoral nerve laterally to the ureter medially (Figure 5). In the extended template, the dissection is carried up along the common iliac vessel to the aortic bifurcation and presacral region; in the super-extended template, the dissection reaches the aorta to inferior mesenteric artery. Given that most agree that at a minimum a standard lymph node dissection should be performed, a randomized trial (SWOG S1011) is evaluating the standard versus extended lymph node dissection templates and we await the results (NCT01224665).

[H3] Urinary diversion. Following radical cystectomy and lymph node dissection, urinary diversion is required for rerouting urine flow from its normal pathway[166]. All urinary diversions use a segment of intestine to which the ureters are anastomosed and the other end is used to expel urine. Urinary diversions can be either continent such as continent cutaneous diversions and orthotopic neobladders or non-continent such as the ileal conduit. Ileal conduit diversions use a segment of ileum that is brought to the skin as a stoma; urine is collected in a urostomy bag. Continent cutaneous diversions vary in technique but essentially a 'pouch' is created from various segments of intestine and a channel is brought out flush with the skin with some form of continent mechanism requiring catheterizing to empty. In an orthotopic neobladder, a pouch is created and anastomosed to the native urethra with patients voiding through their native orifice. This type of diversion requires adequate renal function and an absence of cancer at the urethral margin.

[H3] Recovery. Great strides have been made in regards to recovery after radical cystectomy resulting from the implementation of specific Enhanced Recovery after Surgery (ERAS) protocols[167]. Preoperatively, these pathways encourage fluid hydration and use of medications such as alvimopan, which is a μ-opioid antagonist that aids recovery after bowel surgery. These medications mitigate the negative bowel adverse effects associated with opioid drugs and discourage routine bowel preparation

that can lead to dehydration and the subsequent need for intravenous fluids that can lead to bowel oedema and ileus (obstruction). Intraoperatively, ERAS protocols minimize fluid resuscitation and encourage removal of any nasogastric tubes prior to extubation. Postoperatively, the protocols encourage early ambulation, early feeding (typically with a regular diet by the second postoperative day) and the substitution and minimization of opioids pain medication for alternatives such as ketorolac, acetaminophen and tramadol. Such ERAS protocols have resulted in decreasing hospital stay and faster convalescence[168].

[H3] Survival. Following radical cystectomy, survival outcomes largely depend on final pathological staging. 10-year recurrence-free survival for patients with negative lymph nodes is 86% for pathologically confirmed T0 tumours, 76% for T1-pT3a, 61% for T3b, and 45% for T4, but drops to 34% regardless of stage when lymph nodes are positive[169]. Bladder cancer remains a lethal disease and despite radical cystectomy with seemingly good oncological outcomes, even in patients with apparent organ-confined disease and negative margins and lymph nodes many patients still experience recurrence thereby prompting consideration of adjuvant therapies.

[H2]Neoadjuvant chemotherapy

The role of neoadjuvant chemotherapy prior to radical cystectomy to help improve survival has been investigated in several RCTs and meta-analyses. One meta-analysis of 11 trials demonstrated a 5% survival advantage at 5 years[170].These studies used platinum-based chemotherapy; there is insufficient data to support non-cisplatin based therapy in the neoadjuvant setting. Some adjuvant studies have shown similar efficacy with the less-toxic gemcitabine-cisplatin combination, which has been extrapolated to the neoadjuvant setting[171].Thus, in patients with MIBC (and particularly in those with clinical $\geq$T3 disease), neoadjuvant chemotherapy should be given if they are able to tolerate a platinum-based regimen. If platinum-based chemotherapy cannot be tolerated, patients should proceed directly to radical cystectomy.

[H2]Adjuvant therapy

The role of adjuvant therapy following radical cystectomy remains unclear because individual studies hitherto have been underpowered. A recent RCT demonstrated no clear benefit in deferred versus immediate chemotherapy in patients with pathological $\geq$T3 disease or disease with lymph node involvement[172]. However, a meta-analysis including a total of 945 patients from nine RCTs showed a 23%

relative decrease in the risk of death with adjuvant gemcitabine-cisplatin compared with surgery alone. Similarly, a 34% reduction in the risk of disease recurrence was apparent from seven trials reporting this end point; the reduction was highest for those with positive nodal involvement[173]. Early evidence suggests that adjuvant radiotherapy might have a role in patients with adverse pathologic features (pathological ≥T3 disease, lymph node involvement or positive margins) on radical cystectomy and is being further explored in the NRG-GU001 trial (NCT02316548)[174].

[H2]Bladder preservation protocols for MIBC

Although radical cystectomy remains the gold standard for oncological efficacy in patients with MIBC, the surgery carries substantial quality of life implications; furthermore, some patients are not fit for surgery. Thus, various bladder-sparing options have been explored that could benefit very carefully selected patients. Radical TURBT, whereby an aggressive endoscopic resection is performed to resect all visible disease (while taking care not to cause a perforation) and cautery (either monopolar or bipolar) is used to ablate tissue as deep and wide as possible in an attempt to destroy as much tumour as safely possible, as a standalone treatment approach can have a durable effect on survival but should only be considered for patients who are not eligible for radical cystectomy or chemoradiation[175]. Survival data of 133 patients have revealed a satisfactory cancer-specific survival and progression-free survival with bladder preservation as 76.7% and 57.8% at 15 years, respectively. However, complete tumour resection (confirmed by negative biopsy of the tumour bed) is crucial for this type of management[175].

Partial cystectomy can be cautiously considered for patients with a small (<3 cm), solitary tumour at the bladder dome with no associated CIS or tumours in a diverticulum[176]. One study compared 86 patients who underwent partial cystectomy with patients having radical cystectomy in a matched-pair fashion (1:2 ratio), but found no statistically significant difference in 10-year cancer-specific survival (58% compared with 63%, *P*=0.63) or overall survival (36% versus 39%, *P*=0.67).

Trimodal therapy combines radical TURBT with concomitant radio-sensitizing chemotherapy and external beam radiotherapy[177]. This strategy can result in 5-year cancer-specific survival rates of 50-82%; however, 25-30% of patients require salvage cystectomy for failure to respond[178]. Importantly, trimodal therapy severely limits the likelihood of future orthotopic neobladder diversion should salvage radical cystectomy be required, a limitation that must be discussed when counselling patients.

[H2]Metastatic disease

Patients diagnosed with metastatic bladder cancer during their initial workup or after radical cystectomy are treated with systemic chemotherapy. The standard first-line regimens are cisplatin-based such as methotrexate, vinblastine, doxorubicin and cisplatin (M-VAC); cisplatin, methotrexate and vinblastine (CMV); gemcitabine-cisplatin; or gemcitabine-cisplatin plus paclitaxel. Patients not eligible for cisplatin-based therapy can be treated with carboplatin or with taxanes, which might be inferior[179]. In patients who progress on platinum-based chemotherapy, some second-line options are available, albeit with mixed results[180].

The role of systemic immunotherapy in patients with metastatic bladder cancer has yielded some promising initial results with checkpoint inhibitors (see below, Outlook) and one such drug, atezolizumab (a programmed cell death (PD1) inhibitor) has been approved for second-line treatment and is now being investigated for first line treatment[181]. Meanwhile, studies are ongoing of pembrolizumab (NCT02560636, NCT02662062 and NCT02500121), nivolumab (NCT01928394 and NCT02387396) and ipilimumab (NCT02553642 and NCT01524991) in patients with metastatic bladder cancer.

[H1]Quality of life

Patients with bladder cancer face numerous potential challenges to quality of life owing to their disease and available treatments. Overall function and well-being as well as the specific domains of sexual, urinary and bowel function can be affected. Furthermore, high rate of recurrence and progression imposes one of the highest costs per person of all cancer types[182]. Patients with NMIBC contend with a high recurrence rate, low but variable rates of progression and frequent — often lifelong — invasive monitoring and intravesical treatments. Patients with MIBC face the potentially life-altering options of radical cystectomy with urinary diversion.

A growing literature describes the quality of life of these patients. The preponderance of data exist in the MIBC patient population, specifically around questions of the impact of urinary diversion type. For example, one systematic review suggested more favourable results for continent orthotopic diversion than for ileal conduit in studies published since 2012 (Ref.[183]). The existing literature is limited by heterogeneity in study design, with different questionnaires, frequently retrospective or cross-sectional

data collection and little baseline data. Currently, five bladder cancer-specific patient-reported outcome (PRO) instruments have been described (three of which have been validated) to further the goal of capturing patients' experience with these disease and treatment processes, with questionnaires focused on the experience of those with NMIBC [184,185], MIBC[186] and either NMIBC or MIBC[187]. A recent review details the methodological and contextual strengths and limitations of these instruments[188].

Recently, ERAS protocols focusing on the perioperative care with the components of reduced bowel preparation and standardized feeding schedule and analgesic regimen came into practice[189,190]. In the only RCT to date, the ERAS protocol reduced postoperative morbidity in terms of wound healing disorders, fever and thrombosis compared with a conservative regimen[191]. Time spent in the intermediate care unit was significantly lower and quality of life determined using the EORTC QLQ-30 questionnaire was significantly better postoperatively for those on the ERAS protocol. Larger trials are needed to validate these findings; improved perioperative management will probably mitigate of some of the morbidity associated with diversion procedures.

A growing body of experience supports the value of collecting PROs in routine care, ideally integrated into the electronic medical record (ePROs)[192]. Multiple prospective studies have demonstrated improved patient satisfaction, symptom management, quality of life and patient-clinician communication with the integration of ePROs into routine cancer care[193–196]. As these efforts gain traction and PRO data become routinely collected, bladder cancer care might improve in multiple dimensions[197]. Routine ePRO data collection could also be aggregated over time across diverse patient populations to inform our understanding of the comparative effectiveness of different bladder cancer treatment options. Ultimately, further development in this area could lead to the establishment of PRO-based quality performance measures for bladder cancer, based on standards established by the US National Quality Forum.

[H1]Outlook

While tobacco use is decreasing in some parts of the world, the impact of tobacco exposure can last for decades such that bladder cancer incidence is fairly stable and might increase as the population ages. Improving survival for bladder cancer will require earlier detection, more effective local control or novel therapies for metastatic disease.

[H2] Diagnosis and biomarkers

Two techniques are emerging to detect bladder cancer earlier. The first option is to screen high-risk patients, which has proven challenging owing to low incidence and lack of genetic markers for susceptibility. Indeed, the cost of designing an RCT to demonstrate survival benefit of screening is extremely high. The second option is to improve detection by improving compliance with current guidelines for evaluating haematuria. Current guidelines are frequently ignored[198] but it is possible that incorporation of risk stratification tools using clinical information or urine-based tumour markers could enhance early detection[199,200].

Emerging classes of urinary markers using DNA methylation and microRNAs (miRNAs) are currently being studied. For example, a recent review reported that urine markers of methylation revealed sensitivity in the range 65-100% and specificity in the range 77-100% in the detection setting[201]. Similarly, sensitivity of assessing miRNAs exfoliated from bladder cancer cells was 71-94%, specificity 51-100%[201]. Another model assessing expression of *IGFBP5*, *HOXA13*, *MDK*, *CDK1* and *CXCR2* in a voided urine sample (genotypic data) and age, sex, frequency of macroscopic haematuria and smoking history (phenotypic data) was able to correctly stratify 80% of patients with microscopic haematuria who did not have bladder cancer so they could avoid full urological work-up[202]. Incorporating markers into detection of bladder cancer will require validation to reduce the risk of false-negative results. On the other hand, identifying patients at high risk of cancer will improve detection if that patient was not going to be evaluated in the first place.

The role of sex steroids and their specific receptors is an emerging research area in the progression of bladder cancer[203]. This idea originates from the epidemiological studies showing that women are diagnosed with higher stage disease and have poorer outcomes after treatment than men[204]. Furthermore, early age at menopause (≤45 years versus ≥50 years) is associated with an increased risk of bladder cancer[205] whereas parous women, women who reported late menarche (≥15 years of age), and women who have used oestrogen and progestin therapy have lower risk of bladder cancer than women who have not been treated for menopause[206]. Data from basic science studies have shown that one of the most possible mechanisms for tumour progression involves UDP glucuronosyltransferase 1 (UGT1A)[207], an enzyme vital for the detoxification of major carcinogens such as aromatic amines. UGT1A

is differentially regulated by oestrogens and has protective role in normal urothelium but decreased levels are associated with recurrence and cancer progression in the neoplastic urothelium[207]. UGT1A has been identified as being regulated by the androgen receptor transcription factor (at least in prostate tissue[208]) and androgen-mediated signals promote bladder carcinogenesis by downregulating the expression of UGTs[209]. This finding was also supported by a clinical study evaluating the relationship between bladder cancer and androgen deprivation therapy[210]. Of 162 patients having both prostate cancer and NMIBC, 22% who received androgen deprivation therapy experienced bladder cancer recurrence compared with 50% in the control arm after a median follow-up of 62 months and 5-year actuarial recurrence-free survival was 76% versus 40%, respectively. Consequently, the potential role of sex steroids or their specific receptors might open new doors for novel targeted therapies for bladder cancer.

[H2] Surgery

Technological advancements in visualization of tumours using fluorescence cystoscopy and narrow-band imaging will likely improve detection and reduce recurrences due to inadequate initial resections. Furthermore, radical surgery and extent of lymphadenectomy for MIBC is still evolving. Recent studies have shown that the extent of lymphadenectomy is more important than lymph node count; for example, two recent meta-analyses revealed that extended lymphadenectomy might contribute to 5 year recurrence-free survival despite patients having worse preoperative factors in extended lymphadenectomy group than in the standard lymphadenectomy group[165,211] without increasing the overall complications rates. There are two randomized controlled studies (SWOG 1011 (NCT01224665) and Association of Urogenital Oncology and German Urological Association (NCT01215071) that will help guide optimal surgical anatomical boundaries.

Since the introduction of da Vinci surgical robot two decades ago, evidence has accumulated regarding the outcomes of robot-assisted radical cystectomy compared with conventional open surgery. Three RCTs, albeit with limited numbers of patients, showed that only intraoperative blood loss is better in robot-assisted radical cystectomy compared with open radical cystectomy[162,212,213]. Moreover, the study by Bochner et al.[162] was closed early after intention to treat analysis failed to demonstrate a 20% decrease in perioperative complications according the hypothesis. Despite the lack of superiority of

robot-assisted surgery in these early experiences, the outcomes of the multi-institutional, randomized, prospective, non-inferiority phase III RAZOR trial are awaited before making the precise judgement regarding the future of robot-assisted surgery in terms of perioperative and oncological outcomes in the management of MIBC[163].

[H2] Intravesical therapies

Tumours frequently recur despite BCG induction and maintenance therapy and the absence of effective treatments in this setting result in cystectomy being recommended in many patients prior to development of muscle-invasive disease. Accordingly, new treatments are being evaluated for use in patients with BCG-unresponsive disease, some of which involve use of viruses for therapeutic purposes. Oncolytic viruses that target cancer cells are being explored for bladder cancer[214]. Additionally, modified adenovirus that produces interferon α2b (IFN-α2b) to stimulate the host immune system is also being explored in bladder cancer[215]. In a phase I study, adenovirus mediated IFN-α2b gene therapy was administered by bladder instillation in 17 BCG-unresponsive patients; of the 14 patients treated with adequate dosage, six (43%) experienced complete response with an average duration of 31 months and two were disease free at the last follow-up[216]. In another study, five of seven patients treated with an augmented dose of the gene therapy were disease-free after a minimum follow-up of 23.9 months[217].

[H2] Systemic therapies

Whether the new molecular subtypes (Figure 3) have an effect on response to chemotherapy is being examined. One study demonstrated that a significant number of patients with high-grade disease (67%) with MDA luminal cell type were sensitive to neaoadjuvant MVAC chemotherapy whereas those with the *TP53*-like subtype were resistant to neoadjuvant chemotherapy [107]. Another group reported that somatic mutations in *ERCC2* (which encodes a nucleotide excision repair protein) correlate with response to cisplatin-based therapy in MIBC[99]. Accordingly, prediction of response to neoadjuvant chemotherapy using the genetic background of the resected tumour is a new fruitful area for cancer research. Indeed, a clinical trial of neoadjuvant treatment (SWOG 1314) has been initiated to compare gemcitabine-cisplatin versus MVAC and the ability of a gene expression profiling-based algorithm (CoXEN) to predict complete pathological response.

In the TCGA analysis, 69% of bladder tumours were found to harbour potentially ‘druggable’ mutations, including 42% with alterations in the PI3K/AKT/mTOR pathway and 45% with alterations in the

RTK/MAPK pathway (including *ERBB2*). Patients with specific targetable mutations can have long-term responses to targeted therapies, which are ineffective when given to patients without the mutations[218]. Future trials such as NCI-match (NCT 02465060) will hopefully shed light on effective strategies to treat patients in a personalized fashion and to identify agents that are effective in selected patients.

As briefly mentioned, checkpoint inhibitors are being explored as potential therapies in bladder cancer, building on advances in other cancer types. The major targets are cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed death 1 (PD-1) and programmed death-1 protein ligand (PD-L1), which function to dampen inflammatory immune responses to prevent an unregulated destructive inflammation (Figure 6)[219]. Another promising area of research is the combination of radiotherapy with immunotherapy, owing to the fact that tumour cell death by radiotherapy triggers immune response (so-called cytokine storm) that might be augmented by immunotherapeutic agents such as checkpoint inhibitors[219]. Studies with pembrolizumab in high-risk and recurrent NMIBC are ongoing (NCT02324582, NCT02808141, NCT02625962) but no clinical data are available in conjunction with radiotherapy.

Atezolizumab is a monoclonal antibody against PD1 that was approved by FDA in May 2016 for patients with metastatic bladder cancer who are unresponsive to platinum-based chemotherapy. In a phase II study (*n*=310 patients), four categories of immune infiltration were defined: IC3 (whereby ≥10% of the tumour area is infiltrated), IC2 (≥5% and <10%), IC1 (≥1% and <5%) and IC0 (<1%). Treatment with atezolizumab resulted in a significantly improved RECIST v1.1 objective response rate for each prespecified group (IC2-3: 27% (95% CI 19-37%, *P* <0·0001) and IC1-3: 18% (95% CI 13-24%, *P*=0·0004)) and in all patients (15% (95% CI 11-20%, *P*=0·0058)) compared with a historical control overall response rate of 10%[220]. Tumour-infiltrating immune cells expressing high level of the ligand to PD1 (programmed death-ligand 1 (PD-L1)), were shown to be associated with higher response rates to atezolizumab. Moreover, 84% of the patients responding to therapy were found to have ongoing responses after a median follow-up of 11.7 months. Despite these findings, some reservations abound for the use of PD-L1 as a predictive biomarker for the use of PD-1/PDL1 inhibitors owing to the multitude of PD-L1 antibodies, assays, scoring systems and thresholds for positivity. Eventually, these classes of drugs along with targeted agents may provide more avenues for treatment of patients with advanced-stage disease[221].

Overall, this is an exciting time for bladder cancer care, with advances in understanding and management of bladder cancer promising to be translated to improved survival and quality of life.

Competing interests

M.B. has received speaker honoraria from and consulted for Ipsen, Astellas, Janssen, Bayer and Takeda, and has received study grants from Ipsen and Janssen. Y.L. has served as a consultant for Photocure, Pacific Edge and MDxHealth and has conducted research studies with Abbott, Photocure, Cepheid, Pacific Edge and MDxHealth. All other authors declare no competing interests.

Author contributions

Introduction (O.S. and Y.L.); Epidemiology (J.D.); Mechanisms/pathophysiology (M.A.K.); Diagnosis, screening and prevention (M.B.); Management (M.A.); Quality of life (M.N.); Outlook (Y.L.); overview of the Primer (O.S. and Y.L.).

Figure 1. Types and stages of bladder cancer.

Staging of bladder cancer according to the Tumour–Node–Metastasis (TNM) system is shown. Bladder cancer generally originates from epithelium (urothelium) of bladder and is referred to as urothelial carcinoma. Papillary tumours that are confined to mucosa and invaded to lamina propria (submucosa) are classified as Ta and T1, respectively. Carcinoma *in situ* (Tis in the TNM system) is a flat, poorly differentiated tumour confined to mucosa. Stage 2 tumours either invade the muscle layer superficially (T2a) or deeply (T2b). T3 tumours invade beyond muscularis propria into perivesical fat (T3a invasion is microscopic, T3b is macroscopic). T4a tumours invade the prostate, uterus, vagina and/or bowel whereas T4b tumours invade the pelvic or abdominal walls. The prefix ‘c’ can be applied to specify the grade is based on clinical data; ‘p’ can be applied to the stage if confirmed by pathological assessment.
In 2004, the WHO and International Society of Urological Pathology (ISUP) published a novel histological classification of bladder cancer providing a different patient stratification between individual categories compared to the older 1973 WHO classification[134]. The most important grade differentiation is between low-grade and high-grade tumours (that is, the greater propensity for invasion). Notably, although CIS is confined to the mucosa (that is, non-invasive), it has a pronounced propensity to progress to invasive stages and is characterized by an aggressive tumour biology[135]. PUNLMP, papillary urothelial malignancy of low malignant potential.

Figure 2. Pathogenesis pathways. Two potential pathways of pathogenesis of papillary non-muscle-invasive bladder cancer (NMIBC) and solid muscle-invasive bladder cancer (MIBC) are shown. Low-grade papillary tumours can arise via simple hyperplasia and minimal dysplasia, and are characterized at the molecular level by loss of heterozygosity (LOH) of chromosome 9 and activating mutations of fibroblast growth factor receptor 3 (*FGFR3*), telomerase reverse transcriptase (*TERT*), phosphatidylinositol 3-kinase (namely, *PIK3CA*) and inactivating mutations of cohesin subunit SA-2 (*STAG2*), which have roles in cell proliferation, division and growth. MIBC is thought to arise via flat dysplasia and carcinoma *in situ* (CIS), which commonly show *TP53* mutation in addition to LOH at chromosome 9, but fewer *FGFR3* mutations. Low-grade papillary NMIBCs might progress to MIBC as a result of *CDKN2A* loss. Numerous potential differences in the molecular pathways to the major tumour types and their subtypes are known. Solid arrows indicate pathways for which there is histopathological and/or molecular evidence; uncertainty is indicated by dashed arrows. Adapted from Ref.[65]

Figure 3. Molecular subtypes of bladder cancer.

Several subtypes of non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC) have been defined based on transcriptional features.

a | Data from UROMOL study was used to subgroup NMIBCs into three classes that showed differences in expression of cell cycle genes and markers of differentiation[108]. Class 1 tumours were characterized by high expression of early cell cycle genese and of uroplakins, which are markers of urothelial differentiation. Class 2 tumours expressed late cell cycle genes and retained expression of uroplakins.Class 3 tumours showed high *KRT5* and *KRT15* expression, characteristic of undifferentiated (basal) cells and high levels on long non-coding RNAs (lncRNAs).

b | Nomenclature and overlap of bladder cancer expression subtypes defined by the University of North Carolina (UNC)[106], MD Anderson Cancer Center (MDA)[107], The Cancer Genome Atlas Network (TCGA)[10] and Lund University (Lund)[105] projects. UNC, MDA and TCGA studies included MIBC alone; Lund included both MIBC and NMIBC. The approximate alignment of the subgroups is shown according to data from Ref.[103]. The UNC classification defines two major subtypes, one with features of urothelial differentiation that are found in the intermediate and superficial cells of the normal urothelium and the second with features of basal cells. The other systems further subdivide these two groups. Key markers and

actionable targets related to the Lund subtypes are shown. Note: overlap of UROMOL classifications in part **a** with those shown in part **b** is not yet clear. UroA, urobasal A subtype; UroB, urobasal B subtype; SCC, squamous cell carcinoma.

Figure 4. Diagnosing bladder cancer.

a | Normal bladder appearance by cystoscopy. **b** | Papillary bladder cancer by cystoscopy with **c** | confirming histology slide (haematoxylin eosin staining) of low-grade (pTa) non-muscle invasive bladder cancer. Magnification ×100. **d** | Muscle-invasive bladder cancer by cystoscopy with **e** | confirming histology slide (haematoxylin eosin staining) of high-grade (pT2) cancer. Magnification ×200. **f** | Cystoscopy image of the characteristic appearances of carcinoma *in situ* (CIS) as 'velvety' red patches at the base of the bladder. **g** | Blue-light cystoscopy image of CIS visible at the anterosuperior portion of the bladder with **h** | confirming histology slide (haematoxylin eosin staining). Scale bar=100 μm.

Figure 5. Anatomical extent of standard lymphadenectomy in bladder cancer. a | The standard lymph node dissection includes removal of lymph nodes along the external iliac blood vessels, from the node of Cloquet (superior-most node located under the inguinal ligament in the upper inner thigh) caudally up to the common iliac bifurcation cephalad and the genitofemoral nerve laterally to the ureter medially. The obturator nodes are also removed. b | The pelvic anatomy after the lymph nodes are removed.

Figure 6. Emerging immunotherapies for bladder cancer.

Various ligand–receptor interactions between T cells and antigen-presenting or cancer cells regulate the T cell response to antigen, providing numerous targets to enhance T cell responses and promote immune killing of cancer cells. For example, blockade of the T cell-inhibitory signals from the PDL1/PD1, PDL2/PD1, CD80/CTLA4, CD86/CTLA4 and CD276 (also known as B7-H3, the receptor for which is unknown) axes has shown promise in other cancer types and are actively being explored in bladder cancer. By contrast, promoting T cell-stimulatory pathways (for example, the CD137L/CD137 and CD40/CD40L axes) is also an option. Finally, engineering T cells to display chimeric antigen T cell receptors (such as CART-19) that have enhanced persistence is also being explored.

Table 1. Bladder cancer epidemiological data

Region		***n*** (×100,000)		**Age-standardized rate** (per 100,000 population)		**Cumulative risk** (%)	
		Incidence	**Mortality**	**Incidence**	**Mortality**	**Incidence**	**Mortality**
World	Men	3,303	1,230	9	3.2	1	0.3
	Women	994	420	2.2	0.9	0.2	0.1
More developed regions	Men	1,960	589	16.9	3.7	2.0	0.4
	Women	577	210	3.7	1.1	0.4	0.1
Less developed regions	Men	1,343	641	5.3	2.6	0.6	0.2
	Women	416	210	1.5	0.7	0.2	0.1

More developed regions are all regions of Europe plus Northern America, Australia, New Zealand and Japan. Less developed regions are all regions of Africa, Asia (excluding Japan), Latin America and the Caribbean, Melanesia, Micronesia and Polynesia. Data from www.globocan.iarc.fr.

Table 2. Oncogenes activated by mutation in bladder cancer*

Gene	Chromosome	Frequency (%)	Alteration	Refs
Low-grade stage Ta tumours				
TERT	5p15	73-83	Point mutation	[222,223]
FGFR3	4p16	60-70 80‡	Point mutation Upregulated expression	[68,84]
PIK3CA	3q26	16-25	Point mutation	[73,75]
HRAS	11p15	~10	Point mutation	[62,72]
KRAS	12p12	5	Point mutation	[72]
MDM2	12q14-q15	3	Amplification	[224]
AKT1	14q32	1-3	Point mutation	[82]
Muscle-invasive tumours				
E2F3	6p22	20	Gain/Amplification	[10]
PIK3CA	3q26	9-20	Point mutation	[73,75]
FGFR3	4p16	5-20 40‡	Point mutation Upregulated expression	[68,84]
MDM2	12q14-q15	5-15	Amplification	[64,224]
HRAS	11p15	5-12	Point mutation	[10,225]
ERBB3	12q13	11	Point mutation	[10]
CCND1	11q13	10	Amplification	[10]
RXRA	9q34	9	Mutation	[10]
ERBB2	17q12	7 4.5 42§	Gain/Amplification Mutation Amplification	[10,226]
EGFR	7p12	6	Gain/Amplification	[10]
FGFR1	8p12	6	Amplification	[227]
KRAS	12p12	6	Point mutation	[225]
AKT1	14q32	1-3	Point mutation	[82]

*Altered expression is listed for selected genes. ‡Change measured in the corresponding protein. §In the micropapillary variant.

946 **Table 3. Tumour suppressor genes inactivated by mutation in bladder cancer**
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Gene	Chromosome	Frequency (%)	Alteration	Refs
Low-grade stage Ta tumours*				
CDKN2A	9p21	50-60 18 15	Loss of heterozygosity Hemizygous deletion Homozygous deletion	[112,228]
KDM6A	Xp11	12-60	Inactivating mutation	[62,63]
STAG2	Xq25	32-36	Inactivating mutation	[77,229]
ELF3	1q32	20-25	Inactivating mutation	[62,63]
ARID1A	1p35	12-35	Inactivating mutation	[62,63]
EP300	22q13	12-25	Inactivating mutation	[62,63]
KMT2D	12q13	15-24	Inactivating mutation	[62,63]
TP53	17p13	6-20	Inactivating mutation	[76,230]
RBM10	Xp11	20	Inactivating mutation	[62]
CREBBP	16p13	16-20	Inactivating mutation	[62,225]
ERCC2	19q13	20	Inactivating mutation	[62,225]
ATM	11q22-23	5-15	Inactivating mutation	[62,63]
TSC1	9q34	~12	Inactivating mutation	[73,76]
RB1	13q14	~5	Inactivating mutation	[62,63]
Muscle-invasive tumours				
ARID1A	1p35	25	Inactivating mutation	[10]
TXNIP	1q21	7	Inactivating mutation	[10]
ELF3	1q32	8	Inactivating mutation	[10]
NFE2L2	2q31	8	Inactivating mutation	[10]
FBXW7	4q31	10	Inactivating mutation	[10]
APC	5q21-q22	6-16	Inactivating mutation	[76,91]
CDKN1A	6p21	14	Inactivating mutation	[10]
EP300	22q13	15	Inactivating mutation	[10]
CDKN2A	9p21	50-60 28 22	Loss of heterozygosity Hemizygous deletion Homozygous deletion	[112,228]
TSC1	9q34	8-12	Inactivating mutation	[10,73,76]
PTEN	10q23	13-58 17 1-6	Loss of heterozygosity or hemizygous deletion Mutation Homozygous deletion	[10,73,81,231]
ATM	11q22-23	14	Inactivating mutation	[10]
KMT2D	12q13	27	Inactivating mutation	[10]
MDM2	12q14-q15	4-9	Gain or amplification	[10,232]
RB1	13q14	13	Inactivating mutation	[10]
KLF5	13q22	8	Inactivating mutation	[10]
TSC2	16p13	2	Inactivating mutation	[76]

948 *Frequencies of inactivating mutations identified by exome sequencing in Ta tumours are derived from studies of much smaller numbers of
949 tumours than those of MIBC. For Ta tumours, only mutations reported in more than one study are listed.
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Table 4. Risk stratification, surveillance and treatment of non-muscle-invasive bladder cancer

Risk group[144]	**Pathological characteristics**	**Treatment after initial TURBT**	**Adjuvant intravesical treatment**
Low Risk	Solitary, low grade (Ta) tumours <3cm in size	Surveillance for ≥5 years	None
Intermediate Risk	• Solitary low grade Ta tumour >3cm in size • Multifocal low grade Ta tumours • Recurrence of low grade Ta tumour within 1 year of initial TURBT • High-grade Ta tumours <3cm in size	Life-long surveillance	Adjuvant immunotherapy with 1 year maintenance therapy
High Risk	• High-grade Ta tumours >3cm in size or multifocal • T1 tumour • Multifocal, recurrent and large (>3cm in size) low-grade tumours • Any CIS • BCG failure • Lymphoovascular invasion • Variant histology • Prostatic urethral involvement	Repeat TURBT	Adjuvant immunotherapy with 3 years maintenance therapy

TURBT, transurethral resection of bladder tumour.

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