Bladder cancer

Lars Dyrskjøt[1,2], Donna E. Hansel[3], Jason A. Efstathiou[4], Margaret A. Knowles[5], Matthew D. Galsky[6], Jeremy Teoh[7] and Dan Theodorescu[8,9,10,†]

[1] Department of Molecular Medicine, Aarhus University Hospital, Aarhus Denmark

[2] Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

[3] Division of Pathology and Laboratory Medicine, University of Texas MD Anderson Cancer Center, Houston, TX, USA

[4] Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA USA

[5] Division of Molecular Medicine, Leeds Institute of Medical Research at St James's,

St James's University Hospital, Leeds, UK.

[6] Division of Hematology and Medical Oncology, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY.

[7] S.H. Ho Urology Centre, Department of Surgery, The Chinese University of Hong Kong, Hong Kong, China.

[8] Department of Urology, Cedars-Sinai Medical Center, Los Angeles, CA, USA.

[9] Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA.

[10] Samuel Oschin Comprehensive Cancer Institute, Los Angeles, CA, USA.

[†]E-mail: dan.theodorescu@cshs.org

Abstract

Bladder cancer is a global health issue with sex differences in incidence and prognosis. Bladder cancer has distinct molecular subtypes with multiple pathogenic pathways depending on whether the disease is non-muscle-invasive or muscle-invasive . The mutational burden is higher in muscle-invasive than in non-muscle invasive disease. Commonly mutated genes include *TERT, FGFR3, TP53, PIK3CA, STAG2* and genes involved in chromatin modification. Subtyping of both forms of bladder cancer is likely to change considerably with the advent of single-cell analysis methods. Early detection signifies a better disease prognosis; thus, minimally invasive diagnostic options are needed to improve patient outcomes. Urine-based tests are available for disease diagnosis and surveillance, and analysis of blood-based cell-free DNA is a promising tool for detection of minimal residual disease and metastatic relapse. Transurethral resection is the cornerstone treatment for non-muscle-invasive bladder cancer and intravesical therapy can further improve oncological outcomes. For muscle-invasive bladder cancer, radical cystectomy with neoadjuvant chemotherapy is the standard of care with evidence supporting trimodality therapy. Immune checkpoint inhibitors have demonstrated benefit in non-muscle-invasive, muscle-invasive, and metastatic bladder cancer. Effective management requires a multi-disciplinary approach that considers patient characteristics and molecular disease characteristics.

[H1] Introduction

In 2020, 573,278 people were newly diagnosed with bladder cancer worldwide[1,2], and this number is expected to double by 2040 based on World Health Organization predictions [3]. If detected early before muscle invasion, this disease is often treatable and can be managed with minimal effects on survival. Muscle-invasive disease can metastasize, predominantly to lymph nodes, bones, lungs and liver[4], and is associated with a median survival of ~15 months[5].

The bladder wall consists of 5-7 epithelial cell layers with surface umbrella cells (urothelium) with underlying layers of fibroconnective tissue and vessels (lamina propria), thick muscular bundles (muscularis propria or detrusor muscle) and peri-vesical fat **(Figure 1)**. Urothelial cells are the primary cells of origin of bladder cancer and urothelial cancer is the most common form of bladder cancer, affecting ~95% of patients [6,7]. Tobacco use is the primary risk factor in ~50% of bladder cancer diagnoses[8,9], as the urothelium is exposed to carcinogenic tobacco metabolites eliminated via the urine[10]. Other urothelial-cell-derived bladder cancer types, occurring in <2% of patients, include small cell carcinoma, squamous cell carcinoma and adenocarcinoma [7].

At diagnosis, urothelial cancer is categorized as either non-muscle-invasive bladder cancer (NMIBC; stages Tis, Ta and T1) or muscle-invasive bladder cancer (MIBC; stages T2-T4) when the disease has grown into the muscularis propria. The overall categorisation of the disease into NMIBC and MIBC is used frequently as treatment modalities differ substantially between these entities; however, withing the NMIBC category, Ta tumors have a much more benign disease course than T1 and Tis tumors, and treatment of these subtypes is also markedly different [7]. The different tumour stages are associated with different genetic features, which can be used as markers for minimally invasive diagnostics and disease aggressiveness[11,12]. The importance of these markers in disease management will further increase as molecular pathology will become more predominant in diagnosis, treatment selection and follow-up planning. The most informative molecular markers to date are genetic variants of *TP53*, *ERCC1*, and *FGFR3* as markers of disease progression, chemotherapy sensitivity and small molecule therapeutic selection, respectively[11,12].

Of note, bladder cancer incidence and aggressiveness differ considerably between men and women[13]. For instance, bladder cancer is the 6th most common cancer in biologic males, but only the 17th most common cancer in biologic females [14]. However, women present clinically with more advanced disease and have a poorer prognosis[15,16] and, perhaps, a lower survival than men (possibly confined to the first 2 years after diagnosis)[17]. In the past few years,

efforts have also been made to understand the role of race in bladder cancer biology[18] and further advances in this field are expected in the future.

This Primer focuses on urothelial cancer, the most common form of bladder cancer. We summarize the epidemiology of the disease, with a focus on risk factors, discuss mechanisms of pathogenesis including genetic alterations, and provide an overview of current diagnostic methods. In addition, we review current treatment modalities employed at different disease stages, discuss the quality of life of patients with the disease, and discuss outstanding issues and research questions.

[H1] Epidemiology

[H2] Incidence and mortality

Bladder cancer incidence is highest in higher-income regions of the world, including Europe, North America, and Western Asia, and is also increased in regions affected by schistosoma parasites, such as Northern Africa[19]. By contrast, South America, Eastern Asia, the Caribbean, and Middle and Southern Africa have much lower rates of bladder cancer. The differences in bladder cancer incidence between these regions has been linked to the prevalence of tobacco use, occupational exposure to aromatic amines in industry, arsenic in drinking water and other causes[2,20] In 2020, nearly 600,000 people were diagnosed with bladder cancer globally, predominantly affecting individuals >55 years of age and men [1,2] (**Figure 2, Figure 3**). Bladder cancer is the 10th most common cause of cancer globally and the 13th most common cause of mortality from cancer [19]. Ongoing efforts to mitigate risk factors, improve timely diagnosis, better understand sex differences, and expand therapy seems to have resulted in decreasing global rates of bladder cancer diagnoses and deaths [21].

[H2] Risk factors

[H3] Cigarette smoking.

Cigarette smoking is the most prominent contributor to bladder cancer development in most countries, with ~50% of all cases linked to this risk factor[8,9]. A global decline in smoking prevalence might have contributed to improving rates of bladder cancer diagnoses and deaths; however, trends vary considerably by country [21]. More than 1 billion people are estimated to smoke tobacco globally but smoking prevalence has decreased since 1990 by ~27% in men and 38% in women [22,23]. The highest reductions seem to have occurred in

higher socioeconomic groups, which probably reflects higher health awareness and enhanced access to healthcare in this population[22,23].

[H3] Parasitic infection and chronic inflammation.

Infection with *Schistosoma haematobium*, a parasite in the blood fluke family, is a relatively unique risk factor for bladder cancer in Northern Africa [24]. Parasites infect individuals via the skin when swimming in water containing schistosome cercariae and, following maturation in the liver, can deposit eggs within the bladder and mesenteric plexus. Calcification of the eggs and resultant chronic inflammation of the bladder lining leads primarily to the development of squamous cell carcinoma[25]. Efforts to eradicate this parasite have resulted in a decrease in bladder cancer incidence[26]. In addition to parasitic infection, other conditions that can increase chronic inflammation may contribute to the development of bladder cancer, including presence of diverticula, alterations in gut or urinary tract microbiome, and dysfunction of the immune system [27].

[H3] Sex and age.

Sex and age are two key epidemiological features associated with the development of bladder cancer. Men are more commonly affected by the disease, with the male:female ratio remaining relatively steady at approximately 4:1 [21]. This discrepancy is reflected in the finding that bladder cancer is the 6th most common cancer in men worldwide and the 4th most common cancer in men in the USA[1,21]. Several explanations have been proposed, including differences in smoking rates and exposure to specific compounds in work environments, hormonal factors and the effects of sex chromosomes[13] . Bladder cancer more commonly affects older individuals, with an average age at diagnosis of 73 years and >90% of cases occurring in persons >55 years of age. The discrepancy between sexes exists irrespective of age at diagnosis[1,21].

[H3] Occupational exposure.

Occupational exposure to certain chemicals is another risk factor for bladder cancer. Exposure to aromatic amines, such as benzidine and beta-naphthylamine in the dye industry, exposure to hair dyes, paint products, and other occupational exposures to organic compounds may increase the risk of bladder cancer [28]. Processing of rubber and textiles, as well as exposure to diesel fumes, may also be associated with an increased risk of bladder cancer [29].

[H3] Genetic factors.

Risk factors in the development of bladder cancer include hereditary (germline) DNA alterations. For example, hereditary nonpolyposis colon cancer (Lynch syndrome), is indicated in the development of urothelial carcinoma, accounting for ~5% of upper tract urothelial carcinomas and probably also cases of bladder cancer, although studies are ongoing[30,31]. In this hereditary disease, mutations in mismatch repair genes *MLH1*, *MSH2*, *MSH6*, and *PMS2* result in microsatellite instability, with mutations in MSH2 and associated microsatellite instability posing a high risk for the development of urothelial carcinoma[30].

[H1] Mechanisms/pathophysiology

Overall, NMIBC (stages Tis, Ta and T1) and MIBC (stages T2-T4) have distinct molecular profiles with considerable molecular heterogeneity within each disease category. T1 tumors often share molecular characteristics with MIBC but these tumours usually differ substantially from low grade Ta tumors (**Figure 4**) [32–34]. There is no obligate pathway from NMIBC to MIBC and it seems that these tumour categories have largely non-overlapping pathogenesis pathways. Histopathological and molecular data indicate that the flat lesion carcinoma *in situ* is the major precursor of MIBC, whereas most papillary NMIBC arise from normal-appearing urothelium. Nevertheless, progression from initially non-invasive to invasive disease occurs in some NMIBC patients, particularly those with lamina-propria-invasive tumors.

[H2] The normal urothelium

The urothelium is composed of basal, intermediate and superficial cell layers, the latter specialized to form a tight barrier that prevents urine absorption. This barrier function relies on expression of uroplakins[35] and claudin family members in tight junctions[36]. Keratin 20 is restricted to the umbrella cells[37]. This normally quiescent epithelium can proliferate rapidly in response to damage. Whether a definitive stem cell exists is unclear but evidence suggests that human basal cells have regenerative capacity[38]. In mouse models, both basal and intermediate cells are implicated as tumour cells of origin [39]. PPARγ, a member of the nuclear receptor superfamily, is a regulator of urothelial differentiation, whose activation leads to expression of uroplakins, relevant keratins and claudins via transcription factors FOXA1, GATA2 and ELF3. In the absence of PPARγ activation, p63 maintains the undifferentiated (basal) phenotype[40].

[H2] Field cancerization

Field cancerization, the acquisition of pro-tumorigenic mutations and genomic alterations in normal cell lineages, has been associated with the development of bladder cancer[41]. The origin of transformed cells among normal appearing urothelial cells is unclear, with original speculation that cancer cells from tumors migrate in the urothelium or are shed from tumors and implanted between normal cells[42]. This is referred to as the 'tumor-first-field-later' theory. In the past decade, it has been suggested that field cancerization evolves from transformed stem cells in the urothelium that expand and drive tumor formation ('field-first-tumor-later' theory)[43,44]. Both theories may explain frequent recurrences of clonally related bladder tumors that develop years apart[45]. Whole-organ mapping studies demonstrated that genetic alterations can be divided into two categories: low-frequency mutations and high-frequency mutations increasing with disease progression. Based on this, it was estimated that bladder carcinogenesis spans 10-15 years, with a progressive phase of 1-2 years involving the high-frequency mutations[46]. In another study, patients with a high level of field cancerization had poor survival, and tumors from these patients harbored a high mutational burden, high neoantigen load, and high tumor-associated CD8 T-cell exhaustion[47]. Importantly, non-synonymous mutations in known bladder cancer driver genes, such as chromatin-remodeling genes and *TP53, STAG2* and *PIK3CA,* have been identified in non-diseased bladders[48], as well as in histologically tumor-free urothelium from patients with bladder cancer[49].

[H2] Common genetic alterations

Mutational signatures are similar regardless of tumour grade and stage despite largely non-overlapping pathogenesis pathways[34,50]. There is a major contribution from the activity of the APOBEC family of cytidine deaminases, accounting for more than 60% of all single nucleotide mutations [34,51,52] but only few known tobacco-use-related signatures despite the association of tobacco use with risk. Compared with NMIBC, the overall mutational burden is much higher in MIBC (>7 mutations per Mb), surpassed only by lung cancer and melanoma[53], and large structural alterations and aneuploidy are more common [54].

Deletions of chromosome 9 are found in ~50% of both NMIBC and MIBC. These deletions include the *CDKN2A* locus (9p21), encoding p16 and p14ARF, which are regulators of the RB and TP53 pathways, respectively. On 9q, loss of *TSC1* that regulates mTOR signaling has been found, and 9q loss is associated with upregulated expression of mTOR targets[55]. Interestingly, mTOR has been implicated as a regulator of telomerase reverse transcriptase (TERT) gene transcription. In addition to maintenance of telomere integrity, TERT has non-canonical functions including upregulation of oncogenic signaling pathways[56] ,is crucial in maintaining tumor immortality and contributes to tumor progression in bladder cancer[57–60].

Other copy number alterations in NMIBC (8-22%) include gains of 1q, 5p, 18q, 20p and 20q and losses on 8p, 11p, 17p and 18q, particularly in stage T1 tumors[32]. These regions are more commonly altered in MIBC in which amplifications of 3p25 (*PPARG*), 6p22 (*E2F3*), 7p11 (*EGFR*), 17q12 (*ERBB2*) and 19q12 (*CCNE1*) are also found[52]. High-level DNA amplification is uncommon in NMIBC[61].

Commonly mutated genes are shown in **Tables 1** and **2**. Extremely common in all tumor grades and stages (70-80%) are mutations in the promoter of the telomerase reverse transcriptase *TERT*[58,62,63], which are associated with upregulated expression. Apart from *TERT*, mutated genes and mutation frequencies differ considerably between NMIBC and MIBC. The mutational profile of lamina propria-invasive tumors (stage T1) is more closely related to that of MIBC compared with stage Ta NMIBC However, the mutational profile of stage T1 tumors does not indicate the presence of some tumors with MIBC-like features and some with Ta-like features but rather that individual T1 tumors often contain both Ta-like and MIBC-like features [34].

[H2] NMIBC

NMIBC is characterized by *FGFR3* point mutations (in ~60% of patients), which are associated with low tumor grade and stage[55]. The most common of these mutations (S249C) is predicted to result from APOBEC activity[64]. In cultured normal human urothelial cells, mutant FGFR3 drives cell overgrowth at confluence, suggesting a potential contribution to urothelial hyperplasia *in vivo*[65]. Mutation of RAS genes and *FGFR3* are mutually exclusive, with mutation of one or the other in 90% of stage Ta tumors[55]. APOBEC target mutations in *PIK3CA* hotspot codons are found in ~30% of NMIBC patients, often with mutations in *FGFR3* or RAS genes[34], indicating that most NMIBC have activation of both Ras-MAPK and PI3K signaling. Loss of 9q, including *TSC1* in 50% of patients, provides activation of the PI3K pathway downstream of mTOR. In stage T1, gain of function mutations in *ERBB2* and *ERBB3* that provide PI3K activation[53] are present in ~15% of tumors, and often co-occur[34].

Mutations of *STAG2* and other chromatin regulators (*KDM6A, KMT2D, KMD2C, CREBBP, EP300* and *ARID1A*) are common. Inactivation of one or more of these regulators is found in >65% of patients with NMIBC, with *KDM6A* mutations more common in stage Ta than stage T1 and *ARID1A* mutations more common in stage T1 tumours[34]. The exact roles of these genes in bladder cancer are not well understood and some mutations can be found in normal urothelium of cancer-bearing bladders. Compatible with this is the role for KDM6A in the regulation of normal urothelial differentiation[66,67] and its antagonistic effect on FGFR3 activation[66]. Mutation of *STAG2*, a subunit of the cohesin complex, is more common in

bladder cancer than in other cancers and is implicated in negative regulation of basal cell identity[68]. Inactivating mutations and loss of expression are present in ~30% of low-grade Ta tumours, often with *FGFR3, PIK3CA* and/or *KDM6A* mutations, but in fewer T1 tumors[34,69,70].

[H2] MIBC and metastatic disease

MIBC exhibits remarkable intra-tumour genetic heterogeneity[71]. Despite limited sampling, key players have been clearly identified[52] (**Tables 1 and 2**). Almost all MIBC have loss of cell cycle checkpoints via *TP53, RB1* and/or *ATM* mutations and/or alterations affecting their regulators, for example *E2F3* and *MDM2* amplification, mutation of *FBXW7* (8%) and deletion of *CDKN2A*. Response to DNA damage and DNA repair pathways (for example through loss of function of *ATM* or *ERCC2* mutation[72]) are also affected; *ERCC2* is also implicated in (24%) of T1 cases[34].

Overall involvement of chromatin modifiers in MIBC is similar to that in NMIBC except that the distribution of mutations differs. Activating point mutations in *FGFR3* and *PIK3CA* are less common than in NMIBC, though upregulated expression of FGFR3 is frequent. Activating translocations involving *FGFR3* are found in some tumors (2-5%)[73]. Upregulated expression and/or isoform switching of FGFR1, with potential effect on epithelial-mesenchymal transition (EMT)[74,75] are also found in some tumours. *FGFR3, PIK3CA, KDM6A* and *STAG2* mutations often co-occur and, in the tumors with this mutation profile and luminal phenotype, loss of 9p (p16 and p14ARF) may contribute to progression[76]. Activation of the Ras-MAPK/PI3K pathways is estimated to occur in ~70% of MIBC[52], commonly via mutation or upregulation of upstream regulators, including gain of function mutations of *ERBB2* and *ERBB3*, or amplification of *ERBB2* and *EGFR*[52]. Loss of *PTEN* and *TSC1* also contribute to AKT/mTOR activation[77]. Other pathways implicated in MIBC include upregulated MET signaling[78] and the NOTCH pathway[79].

[H2] Tumor microenvironment

The tumor microenvironment (TME) comprises both malignant and non-malignant cells. Cancer-associated fibroblasts (CAFs) are the most common non-malignant cells in bladder cancer, forming distinct regions within the tumor[80], and these CAFs have been associated with tumor aggressiveness, chemoresistance and reduced response to immune checkpoint inhibitor therapy [80–82] . Tumor-associated macrophages (TAMs) are another important non-malignant population in bladder cancer[83]. TAMs are recruited to sites of inflammation and hypoxia within the TME but, like CAFs, they are co-opted by cancerous cells to promote an immune suppressive environment, drug resistance and metastasis[84–90]. Resistance to

inhibition of PD-1 or PD-L1 in urothelial cancer has also been linked to a proinflammatory cellular state of myeloid phagocytic cells detectable in tumor and blood [91]. Tumor-infiltrating lymphocytes (TILs) are immune cells clear cancerous cells. Mostly composed of CD8+ T cells, TILs develop and expand to recognize foreign antigens present on cancer cells or antigen-presenting cells. Of note, bladder cancer, and MIBC in particular, has a high level of mutational burden[92,93], providing neoantigens for the immune cells to recognize. However, the beneficial effect in bladder cancer is lower than expected because of low numbers of TILs in the tumor and/or inactivation of TILs that do reach malignant cells. In MIBC, the presence of TILs in or adjacent to the tumor is a predictor of patient response and survival to immune checkpoint inhibitors (ICI)[94]. The degree of stromal cell infiltration, most notably CAFs, into tumors also determines patient response to immune therapies. Patients with high numbers of TILs and low stromal gene tumor signatures have an improved survival and response to immune therapies[95]. The discoidin domain (DDR1 and DDR2) collagen receptors, which are commonly found on cancer cells and fibroblasts, have been implicated as biomarkers for ICI response in bladder cancer and other cancer types in both the experimental setting[89] and patients [96]. This important finding supports the link between collagen deposition, fibroblasts and resistance to ICI. Future clinical trials of targeted therapies, such as DDR1 and/or DDR2 inhibition combined with ICIs would be expected to enhance the effectiveness of ICI.

[H2] Biologic sex differences

Bladder cancer incidence and aggressiveness differ substantially between men and women[13] . Absence of X chromosome gene *KDM6A* leads to an increased incidence of bladder cancer in mouse models[97] but, notably, only in female animals. *KDM6A* is mutated in 24% of patients with bladder cancer and its experimental depletion in human bladder cancer cells enhanced *in vitro* cell proliferation, migration, and *in vivo* tumor growth; however, the limited number of cell lines investigated prevents a conclusion whether this effect is sex dependent[60].

In addition to sex-chromosome-mediated effects, androgen receptor (AR) signaling can lead to sexual dimorphism in bladder cancer incidence and therapeutic response. Two studies in 2022 demonstrated that T cell-intrinsic AR promotes $CD8^+$ T cell exhaustion in the tumor microenvironment (TME)[98,99]. Furthermore, AR can suppress expression of CD44[100], a well-known driver of tumor progression and metastasis in bladder cancer[101–103] and other cancer types[104]. In mouse studies, AR deletion reduces the incidence of bladder cancer induced by standard orally ingested chemical carcinogens that accumulate in urine and are analogs of those found in cigarette smoke[105]. However, the role of AR in humans is less clear[106,107]. Use

of the 5α-reductase inhibitor finasteride was found to reduce bladder cancer incidence in white and hispanic, but not black men[108]. Intriguingly, black men have higher free testosterone levels than white men[109], yet a lower incidence of bladder cancer[110]. By contrast, reduced AR expression in bladder cancer is associated with more advanced stage[100,111] and aggressive tumor subtypes[112]. Inhibition of AR signaling has shown promise in men with reduced recurrence of NMIBC[113–115].

In a systematic review of 18 studies, the incidence and clinical outcomes of bladder cancer were investigated in patients who received androgen suppression therapy[109]. 5α-reductase inhibitors or androgen deprivation therapy were not significantly associated with a reduced risk of bladder cancer incidence or cancer-specific, overall or progression-free survival. In subgroup analysis, only finasteride use was associated with reduced bladder cancer risk, and recurrence-free survival was improved in those receiving androgen suppression therapy compared with nonusers. Hence, finasteride use may represent a strategy for reducing bladder cancer incidence, and overall androgen suppression may reduce recurrence risk in patients with a history of bladder cancer. Only randomized trials with well characterized study populations can definitively prove these observations.

The Y chromosome is essential for male sex determination and spermatogenesis[116]. In aging men, loss of the Y chromosome (LOY) in hematopoietic cells has been associated with increased risk of several diseases, including cardiac fibrosis[117] and multiple cancer types [117–120]. In bladder cancer, LOY has been found in 10-40% of tumors [121–127]. This is unsurprising as bladder cancer is commonly caused by environmental exposures, such as tobacco and industrial chemicals that are known to result in DNA damage and LOY [128–130]. Recent studies have shown that LOY and the corresponding loss of Y genes *KDM5D* and *UTY*, which are chromatin modifiers, confer an aggressive phenotype to bladder cancer through acquiring ability to evade the adaptive immune system[18]. Fortunately, this also makes LOY tumors more vulnerable to ICI. This landmark study is the first to show LOY drives cancer biology and the host immune response to cancer [131].

[H1] Diagnosis and screening

[H2] Clinical Presentation

Around 75% of patients with bladder cancer present with painless, visible (gross) hematuria, which warrants early medical attention[132]. In a prospective observational study, 22.4% of patients presenting with visible hematuria were found to have bladder cancer, with the incidence increasing with age: only 4.7% of those <35 years of age compared with 35% of

those >75 years of age[133]. Rates of urologic referral of patients with hematuria is generally low [134] and, therefore, the reported rates of bladder cancer can differ in the literature. Patients may also present with microscopic or non-visible hematuria commonly detected upon health checkup, and bladder cancer was found in 3.3-5.2% of that population[133 135]. Presentation with microscopic hematuria seems to correspond to a low disease stage[136]. In a multi-center cohort study- in patients with microscopic hematuria, 68.8% had Ta/Tis disease, 19.6% had T1 disease and 11.6% had T2 disease, whereas in patients presenting with gross hematuria, 55.9% had Ta/Tis disease, 19.6% had T1 disease and 17.9% had T2 disease[136].

Bladder cancer is rare in children, with an incidence of only 0.1-0.4%[137,138]. In a systematic review including 243 pediatric patients with bladder cancer[139], gross hematuria was the commonest presentation (75.6%), followed by lower urinary tract symptoms (8.6%) and abdominal and/or flank pain (3.4%). Most of the patients presented with Ta (86.4%) and low-grade (93.4%) disease; T2 or above disease was uncommon (4.1%).

[H2] Diagnosis

Diagnostic evaluation of patients with hematuria should include a physical examination including rectal and vaginal bimanual palpation to assess for pelvic masses suggesting a locally advanced tumor [140], although the risk of both clinical under-staging and over-staging is well known [141,142]. Cystoscopy is considered the gold standard for diagnosing bladder cancer. White-light imaging cystoscopy is the conventional method to detect bladder cancer but may miss some lesions, such as carcinoma-in-situ (CIS). CIS usually presents as a velvet-like, reddish area that is difficult to detect and differentiate from inflammation[143], which has led to advanced cystoscopy technologies, such as narrow-band imaging, photodynamic diagnosis and Image 1S to enhance bladder cancer detection (**Supplementary Table 1**).

If a lesion is seen on cystoscopy, this is followed by examination under anesthesia at time of transurethral resection bladder tumor (TURBT), although the risk of both clinical under-staging and over-staging with this assessment is well known[142]. Pathological work up of patients includes the use of urine-based evaluation to detect malignant cells and/or analysis of biopsy or TURBT samples of visibly identifiable lesions.

[H3] Urine-based diagnosis of bladder cancer.

Urine cytology is the most cost-effective urine-based method to diagnose high-grade bladder cancer[144]. The sensitivity of this analysis is suboptimal but its specificity is high, especially for high-grade urothelial carcinoma; thus, urine cytology remains the gold standard in the diagnosis of bladder cancer compared with marker-based studies in urine[145,146]. Urine

cytology specimens are classified according to the Paris System for Reporting Urinary Cytology published in 2016, which subdivides specimens into nondiagnostic, negative for high-grade urothelial carcinoma (NHGUC), atypical urothelial cells (AUC), suspicious for high-grade urothelial carcinoma (SHGUC), high-grade urothelial carcinoma (HGUC), low-grade urothelial neoplasm (LGUN), and other malignancies[145]. The risk of cancer with a diagnosis of HGUC is >90% using this classification system[145,146]. Of note, any cytology classification approach to low-grade urothelial carcinomas yields lower sensitivity than those for high-grade carcinomas owing to the more cohesive nature of low-grade lesions and the much closer similarity of low-grade lesions to normal cellular morphology[147].

Over the past decades, extensive effort has gone into the development of protein- and molecular-based urine tests to diagnose bladder cancer. These efforts have resulted in numerous FDA-approved tests, including cell-free DNA tests[148–151]. Methodologies of these tests include, for example, detection of proteins elevated in dividing cells using antibody-based methods to detection of chromosome aneuploidy by fluorescence in situ hybridization[149,152]. Although many of these tests show higher sensitivity in detection of bladder cancer than urine cytology, they are often limited by lower specificity, false positive results, and better utility in high-grade lesions[148–151]. Efforts to identify new markers, including *TERT* and *FGFR3* alterations, are ongoing, but hurdles remain to determine whether these will outperform existing approaches to urine-based diagnosis [153].

[H3] Circulating tumour DNA analysis.

In addition to tumor markers in urine, cell-free DNA with tumor-specific alterations is released into the blood circulation (circulating tumor DNA; ctDNA) mainly by cell death[154]. ctDNA is cleared through nuclease digestion, renal clearance, and uptake by the liver and spleen[155–158]. The half-life of ctDNA is ~2 hours[159], which makes ctDNA useful for real-time tracking of tumor burden following surgery and during oncological treatment. Analysis of ctDNA in plasma has shown promising results for detection of minimal residual disease and metastatic relapse in multiple cancer types, including bladder cancer[160]. In one prospective study, ctDNA measurements detected clinical relapse on average 3 months earlier than CT scans and better predicted outcome following neoadjuvant chemotherapy compared than pathological response[160,161]. Furthermore, ctDNA levels have been shown to correlate to pathological complete response (pCR) and outcome following neoadjuvant immunotherapy[162]. Of note, another study used ctDNA measurements to document a survival benefit with adjuvant immunotherapy in patients positive for ctDNA[163,164]. These results are overall promising, especially for detection of minimal residual disease and for guiding adjuvant treatment, but further replication in large cohorts and development of

optimal laboratory procedures for clinical use are needed. Furthermore, additional knowledge of ctDNA assay sensitivity and specificity is needed to address false positive and false negative rates in specific settings. ctDNA guided clinical intervention trials are currently ongoing to determine the benefit of blood-based tests to guide adjuvant immunotherapy (for example, IMVIGOR011 and TOMBOLA) [165,166]. Importantly, ctDNA analysis can also identify genomic alterations associated with metastatic disease[167,168], potentially serving as actionable therapeutic targets.

[H3] Tissue-based diagnosis of bladder cancer.

Analysis of samples from biopsy or TURBT at the time of cystoscopy is the most common method of initial diagnosis. Pathological analysis confirms presence of cancer, histological type, and stage. Bladder carcinoma is subdivided by grade into low-grade and high-grade categories, with low-grade carcinomas showing frequent recurrence but limited progression[169]. High-grade carcinomas can be either NMIBC or MIBC, of which NMIBC commonly show recurrence and progression to MIBC, requiring more aggressive clinical management and follow-up.

More than 90% of all bladder carcinoma histological subtypes are of urothelial histology, with the remainder comprising squamous cell carcinoma, adenocarcinoma, and neuroendocrine carcinoma[170,169] (**Figure 5**). These broad categories describe 'pure' or non-mixed carcinomas representing a single histological type of carcinoma. Urothelial carcinoma itself can occur as a broad array of variants or subtypes, such as micropapillary, plasmacytoid, nested and lymphoepithelioma-like carcinomas. These categories are defined by the WHO Classification of Tumours of the Urinary System and Male Genital Organs[169]. Several subtypes have been associated with unique molecular and/or therapeutic considerations. Micropapillary urothelial carcinoma, which shows clusters of inversely-polarized nests of tumor cells within prominent retraction spaces, has a disproportionately higher rate of *ERBB2* amplification than conventional urothelial carcinoma[171–173]. This amplification has been identified in up to 40% of micropapillary urothelial carcinomas, resulting in efforts to selectively target this pathway[171]. Plasmacytoid urothelial carcinoma, which is defined by distinct *CDH1* mutations and a morphology that shows single, plasma-cell-like cells that are highly infiltrative, is another research focus[174]. Micropapillary and plasmacytoid urothelial carcinomas are biologically aggressive subtypes and optimizing the approach to these diagnostic categories has resulted in some institutions advocating early cystectomy regardless of stage [169]. Furthermore, micropapillary urothelial carcinoma is often variably mixed with conventional urothelial carcinoma, with higher proportions of micropapillary urothelial carcinoma portending more aggressive pathological behavior [175,176]. Despite their urothelial carcinoma

origin, these two examples of urothelial carcinoma subtypes highlight the dramatic differences of urothelial carcinoma evolution and differentiation, which complicates a unified approach to understanding and treating bladder cancer.

In addition to histological subtyping, pathological analysis determines depth of invasion of the carcinoma at biopsy or TURBT and also following cystectomy. Pathological (after cystectomy) staging is defined by the American Joint Committee on Cancer (AJCC), currently in its 8th edition[177]. NMIBC occurs as either papillary (pTa) or flat urothelial carcinoma in situ (pTis). Invasion of the lamina propria (pT1), invasion of the muscularis propria (pT2), perivesical fat (pT3), and involvement of adjacent organs (pT4) is associated with a progressive reduction in survival [177]. Determination of pathological stage on cystectomy specimens is straight-forward, but diagnosis and staging on TURBT samples is challenging owing to the extent of sampling, interpretation artifact due to cautery or crush phenomenon, and lack of objective markers to conclusively determine if muscularis propria is present.

Use of tissue to predict progression from lamina propria-invasive (T1) disease to muscle-invasive carcinoma has been a subject of interest for some time. A recommendation was made in the of the AJCC manual 8th edition to attempt substaging T1 disease based on numerous studies that showed that a larger amount of tumor in the lamina propria correlated with a higher rate of progression [177]. However, various approaches were used in the studies, including different cut-off criteria used for substaging, use of surface orientation in some approaches that was impossible to perform on a considerable subset of specimens, and diverse outcome endpoints. An additional confounder was the challenge of not knowing with certainty whether the lesion was fully resected. Comparison of these various approaches showed that an aggregate tumor measurement of ≥2.3 mm outperformed other histology-based approaches in predicting progression to muscle-invasive disease [178]. Since the endorsement of attempted substaging of T1 disease by the AJCC, numerous studies have evaluated additional approaches to predicting progression to MIBC, including histological, molecular and/or protein biomarkers [179,180]. Ultimately, these are challenging endeavors given uncertainty regarding presence of residual tumor, effects of precedent therapies on disease progression, and cellular heterogeneity associated with bladder cancer.

[H2] Staging

Diagnostic imaging is critical for both local and distant staging. During a workup of hematuria, abdominopelvic imaging including imaging of the upper urinary tract (renal pelvis and ureters) should be performed to assess for a bladder mass (ideally prior to TURBT)[181–183]. Imaging informs both location and extent of disease (including potential upper tract involvement, extravesical extension, hydronephrosis, nodal involvement or distant metastatic disease). CT urography (CTU) with and without intravenous contrast is preferred and has largely replaced intravenous pyelogram[184,185]. In patients with poor renal function or allergy to iodinated contrast, MR urogram with gadolinium-based contrast may be considered[186]. Renal ultrasonography or CT without contrast combined with a retrograde ureteropyelography is done in patients who cannot receive iodinated or gadolinium-based contrast[184,185].

In addition to CTU, MRI of the pelvis with and without intravenous contrast may be considered for further local staging, especially depth of bladder wall invasion[187]. The best evidence supporting use of MRI is in MIBC in the pre-TURBT setting to improve staging[188]. Multiparametric MRI has improved soft tissue resolution compared with CT, and the Vesical Imaging Reporting and Data System (VI-RADS) score has been developed to predict likelihood of muscle invasion[189]. MRI may also have potential to assess response after treatment, including TURBT, neoadjuvant chemotherapy and/or chemoradiation[190].

For patients with NMIBC, chest and other metastatic imaging is not necessary, whereas for patients with MIBC, chest CT is recommended [141]. Bone scan and brain MRI have limited value and are typically reserved for symptomatic or very high-risk (stage, tumor size, adverse pathology) patients[191]. 18F-fluorodeoxy glucose-PET (FDG PET)/CT is not as commonly used and does not have a clearly established role in patients with localized disease, although it may have more value in locally advanced disease and in when metastatic disease is suspected[192–195].

[H2] Prognostic and predictive biomarkers

In NMIBC, several prognostic biomarkers have been described; however, none have yet been implemented in clinical decision making. For example, in one study, patients with NMIBC at high risk for progression were subdivided into groups with good, moderate, and poor risk of progression based on mutations in *FGFR3* and methylation of *GATA2*[196]. In addition, studies using measurements of genome-wide copy number alterations (CNAs) through array-based comparative genomic hybridization[55] or SNP array analysis[32] separated

patients with Ta tumors or NMIBC, respectively, into different groups and found an association between a high level of CNAs and poor outcomes. Furthermore, tumor mutational burden (TMB) and APOBEC-associated mutations have been associated with increased NMIBC aggressiveness[32]. However, when analyzing T1 tumors only, a high TMB was associated with better survival[197]. Earlier studies of gene expression subtypes in NMIBC identified two major molecular subtypes associated with disease aggressiveness[198,199]. Five subtypes of bladder cancer were identified when considering the whole spectrum of bladder cancer stages, and urothelial-like, genomically unstable, and a group of infiltrated cases were specifically associated with NMIBC[200]. Three expression-based subtypes were reported by the UROMOL consortium, which showed different clinical outcomes and molecular characteristics[33]. The work from the UROMOL consortium was later expanded and four subtypes were identified: the UROMOL2021 classification system showed overlap with previously reported subtypes, but with increased granularity[32]. In another multi-omics approach, further molecular heterogeneity within disease stage categories was discovered, enabling further subclassification of Ta and T1 tumors[34].

In MIBC, several classification systems based on gene expression subtypes have been reported, ranging from two major subtypes (luminal and basal)[201], to six subtypes[202]. A consensus classification of six subtypes using previous classification systems has been reported[203]. The subtypes harbor different molecular alterations and immune cell characteristics and, overall, have been reported to be prognostic. In patients with MIBC, high TMB and neoantigen loads have been associated with particularly good survival, and high mutational contribution from APOBEC mutational processes was also associated with improved survival[52], similar to observations in T1 tumors[197].

Several studies sought to develop predictive biomarkers in both NMIBC and MIBC. In relation to Bacillus Calmette–Guérin (BCG) treatment in NMIBC, high PD-L1 expression has been associated with BCG-unresponsiveness, linking immune inhibitory pathways to BCG failure[204]. In another study, T cell exhaustion in the tumor was associated with outcome following BCG instillations[205]. In one study, molecular profiling of high-risk BCG-naive NMIBC and recurrent tumours after BCG treatment found three distinct BCG response subtypes (BRS1–3)[206]. Patients with BRS3 tumors had reduced recurrence-free and progression-free survival compared with BRS1 and BRS2. BRS3 tumors expressed high EMT and basal markers and had an immunosuppressive profile. Tumors that recurred after BCG were enriched for BRS3. In a second cohort of BCG-naive patients with high-risk NMIBC, BRS molecular subtypes outperformed guideline-recommended risk stratification based on clinicopathological variables .

In MIBC, expression of and mutations in genes involved in DNA damage response (DDR) are associated with a particularly good outcome following chemotherapy and chemoradiation [207,208,209,210]. Some of these genomic alterations have been tested in a clinical trial evaluating bladder sparing approaches; however, the study did not reach the primary endpoint and further study refinements are needed[211]. In addition, a CD8+ T-effector cell phenotype, high TMB and high neoantigen load have been demonstrated to be predictors of immunotherapy response in MIBC, whereas lack of response was associated with a signature of transforming growth factor β (TGFβ) signaling in fibroblasts[212]. Other studies demonstrated that MIBC tumors of the luminal subtypes show an improved response to chemotherapy[213,214], but contradicting results have also been reported[215]. Further gene expression profiling studies have shown that increased immune cell infiltration in MIBC is associated with improved outcomes after chemoradiation, whereas increased stromal infiltration is associated with worse outcomes after neoadjuvant chemotherapy and cystectomy [216]. Several seminal studies have shown substantial intratumor heterogeneity using single-cell and spatial transcriptomic analysis, which is likely complicating the utility of current subtype classifications for clinical outcome prediction[80,217].

[H1] Management

The management of bladder cancer requires careful consideration of disease stage and tumour characteristics, as well as the patient's demographics, comorbidities and preferences. Optimal treatment involves a multidisciplinary approach that may include surgery, chemotherapy, radiation therapy, immunotherapy, and targeted therapy.

[H2] TURBT and en-bloc resection of bladder tumor

TURBT is a diagnostic, staging and, for NMIBC, therapeutic tool, making it a cornerstone in management. The procedure starts with a comprehensive inspection of the bladder, followed by resection of the exophytic part of the tumour, and separate resection of the underlying bladder wall and the edges of the resection area[143]. TURBT has two main goals: complete (possibly curative) resection in the case of NMIBC; and proper local staging and expediting subsequent definite treatment in the case of MIBC . To ensure complete tumour eradication in NMIBC, the quality of resection is extremely important, but the procedure is highly dependent on the operator’s skills and experience [218]. Although TURBT aims to resect NMIBC completely, this is not always possible due to its technical difficulty and fear of bladder perforation. A second TURBT, 2-6 weeks later, is indicated if the tumour was not completely resected in first TURBT, if the patient has T1 disease, or if detrusor muscle is

absent in the first TURBT specimen with the exception of Ta low-grade tumours and primary CIS[143]. Second TURBT may be associated with improved progression-free survival in patients with T1 NMIBC[219]. A meta-analysis of 81 studies found that the pooled rates of any residual tumours and upstaging on second TURBT were 31.4% and 2.8%, respectively [220], highlighting the limitations of the conventional TURBT procedure. In the case of MIBC, maximal TURBT is also important to optimise subsequent treatment, such as radical cystectomy and trimodality therapy (TMT) [221,222]. Maximal resection of all visible bladder tumors down to the detrusor muscle layer should be pursued even when MIBC is suspected endoscopically [221,222].

En-bloc resection of bladder tumor (ERBT), that is removal of bladder tumor in one piece, has been proposed as a potentially more favorable surgical approach than conventional TURBT[223,224]. Results from three randomized trials comparing ERBT and TURBT have been reported [225,226,227]. In one trial[225], the rate of detrusor muscle presence for ERBT was non-inferior to TURBT (94% vs 95%), and T1 substaging was more feasible in the ERBT group (100% vs 80%, p=0.02). In a second trial[226], the ERBT group had a higher rate of detrusor muscle presence (80.7% vs 71.1%, p=0.01) and a lower rate of bladder perforation (5.6% vs 12%, difference -6.4%, 95% CI -12.2 to −0.6%) than the TURBT group. In a third trial[227], ERBT resulted in a reduction in 1-year recurrence rate from 38.1% to 28.5% (p=0.007), and 30-day complications were similar between the two groups.

A single dose of intravesical chemotherapy (commonly mitomycin C or epirubicin) immediately after TURBT is associated with a decreased risk of recurrence[228]. A systematic review and individual patient data meta-analysis of a total of 2,278 patients found that a single dose of intravesical chemotherapy reduced the risk of recurrence by 35% ($p<0.001$)[228]. However, this benefit was not observed in patients with a prior recurrence rate of >1 recurrence per year, or in patients with an European Organization for Research and Treatment of Cancer (EORTC) recurrence score of ≥5 [228]. Single-dose intravesical chemotherapy should not be given when there is a concern for bladder perforation, as chemotherapy extravasation can result in severe consequences [229].

Although TURBT with or without single-dose intravesical chemotherapy is the standard of care in treating NMIBC, it is a major surgery requiring formal anaesthesia, which could be a burden for patients with recurring diseases. As the risk of disease progression for recurrent Ta low-grade bladder tumours is low, fulguration or laser vaporisation of small papillary recurrences on an outpatient basis has been proposed to reduce the therapeutic burden

[143,230,231]. In particular for patients at advanced age, watchful waiting with urine cytology and regular cystoscopy without resection can also be considered [232].

[H2] Intravesical therapy for NMIBC

Intravesical therapy with BCG vaccine was first proposed in 1976 as a type of immunotherapy to treat bladder cancer[233] and became a standard of care for NMIBC. A randomized study to investigate the optimal BCG schedule for intermediate-risk and high-risk NMIBC with a primary outcome of disease-free interval, concluded that 1 year and 3 years of full-dose BCG should be given to patients with intermediate-risk and high-risk NMIBC, respectively[234]. Adverse effects of BCG include inflammation and/or infection of the bladder, prostate, epididymis and testis, as well as general malaise, fever and BCG sepsis[143]. Since 2013, an intermittent BCG shortage has been a global problem and alternative treatment options are urgently needed[235,236]. Intravesical maintenance chemotherapy can be an alternative in intermediate-risk NMIBC, but its efficacy in high-risk NMIBC is limited[143]. New intravesical therapies, such as intravesical gene therapy with nadofaragene firadenovec[237], and systemic ICI with pembrolizumab[238] have been approved by the FDA for BCG-unresponsive NMIBC with CIS, with or without papillary tumors.

Intravesical maintenance chemotherapy, given repeatedly on a weekly or monthly basis [239,240], has been investigated as an alternative to intravesical BCG therapy. A meta-analysis compared TURBT plus intravesical maintenance chemotherapy with TURBT only and found that the use of intravesical maintenance chemotherapy was associated with a 44% reduction in 1-year recurrence ($p<0.001$) [241]. In an individual patient data meta-analysis comparing intravesical maintenance chemotherapy and intravesical BCG, the use of BCG was associated with a 32% reduction in the risk of recurrence ($p<0.001$) [240]. In patients with intermediate-risk NMIBC who cannot tolerate intravesical BCG, intravesical maintenance chemotherapy can be considered noting its inferiority in oncological efficacy.

[H2] Radical Cystectomy

Radical cystectomy is a standard of care in localized MIBC[183] and in patients with BCG-unresponsive NMIBC[183]. The surgery itself includes three major components: cystectomy, pelvic lymph node dissection (LND) and urinary diversion. In men, standard radical cystectomy includes removal of the bladder, prostate, seminal vesicles and distal ureters[183]. In women, standard radical cystectomy includes removal of the bladder, the entire urethra, anterior vaginal wall, uterus, and distal ureters[183]. Standard LND includes removal of bilateral

obturator, internal and external iliac lymph nodes. Two randomized trials investigated the role of extended LND (including the common iliac, presacral, and up to at least the aortic bifurcation), and found that extended LND was associated with more grade ≥3 complications [242,243] but no benefit in recurrence-free survival [242], cancer-specific survival [242], disease-free survival[243] and overall survival [242,243]. For urinary diversion, ileal conduit and orthotopic neobladder are commonly performed. The choice of urinary diversion depends on patient factors (for example, age, renal function, ability to perform self-catheterization and patient preference) and disease factor (for example, urethral involvement, locally advanced disease and need for adjuvant therapy)[244]. Patients should be carefully counselled about the advantages and disadvantages of each option, so that a shared decision can be made in the patient's best interest. Radical cystectomy can be performed in an open, laparoscopic or robot-assisted approach. In a meta-analysis comparing robot-assisted radical cystectomy (RARC) with open radical cystectomy (ORC) no difference in terms of recurrence-free survival (HR 0.99, 95% CI 0.75-1.31) and overall survival (HR 0.98, 95% CI 0.73-1.30) was found [245]. RARC had a lower transfusion rate (OR 0.42, 95% CI 0.30-0.59), but a longer operative time (mean difference 78.54 minutes, 95% CI 45.87-111.21 minutes) than ORC [245]. Overall complications, major complications, positive margin rates and length of hospital stay did not differ[245]. High-quality data comparing RARC with intracorporeal versus extracorporeal urinary diversion are lacking, although non-randomised studies favoured the intracorporeal approach showing benefits in blood loss and hospital stay [246,247]. High-quality data on laparoscopic radical cystectomy is limited [245].

Some patients with pT3/T4 pN0–2 bladder cancer (N0, no regional lymph node metastasis; N1, metastasis in a single regional lymph node; N2, metastasis in multiple regional lymph nodes) may be candidates for postoperative adjuvant pelvic radiotherapy to the pelvic lymph nodes with or without the cystectomy bed following radical cystectomy[248,249]. Addition of adjuvant radiotherapy to chemotherapy alone was associated with improved local relapse-free survival[250].

Partial cystectomy may be considered in highly selected patients, including those with solitary tumours at favourable locations, such as the bladder dome, without concomitant CIS [251]. Special caution must be taken to avoid urine and tumour spillage during the procedure. To date, there are no randomized trials comparing partial with radical cystectomy, but previous retrospective studies showed comparable results [251]. Patient selection is key should partial cystectomy be contemplated.

[H2] TMT

TMT is a bladder-preserving treatment of MIBC that includes a maximal, ideally visibly complete, TURBT followed by concurrent radiosensitizing chemotherapy and radiotherapy (chemoradiotherapy). TMT is an accepted alternative to radical cystectomy for selected patients with MIBC who have a desire to retain their native bladder or who are medically unfit for radical cystectomy[182,183,252] and may be most effective in patients with specific characteristics (**Box 1**). Randomized controlled trials comparing TMT to radical cystectomy closed due to lack of accrual[253], but best available data from prospective TMT trials (including from NRG/RTOG in the USA and from UK-based trials), meta-analyses and multi-institutional cohorts demonstrate comparable survival[254–258]. Chemoradiotherapy is considered standard in patients who can tolerate combined therapy, following a phase III randomized BC2001 trial that showed that concurrent chemoradiotherapy with 5-FU and mitomycin leads to improved locoregional disease control compared with external beam radiotherapy (EBRT) alone[257]. Other options for concurrent chemotherapy include cisplatin-based regimens or single-agent gemcitabine[259]. Ongoing randomized trials are investigating the addition of immunotherapy (for example, atezolizumab or pembrolizumab) to TMT [260,261] .

Life-long post-treatment bladder surveillance is essential for the detection of in-bladder recurrences (10-year rates: NMIBC 20-26%, MIBC 13-18%) or second primary tumours, and 10-15% of patients may require a salvage cystectomy, which is associated with a higher risk of overall and major late complications than primary cystectomy and most often requires an incontinent urinary diversion [262]. Patients with MIBC, who are appropriate candidates, should be offered the choice between radical cystectomy and TMT approaches. MIBC treatment, and in particular TMT, requires close multidisciplinary collaboration and environments that enable shared and informed decision-making[263]. A multi-institutional study in 722 patients (440 radical cystectomy, 282 TMT) used propensity score matching and logistic regression to show similar oncological outcomes between these two treatment modalities [258]. Although there are no conclusive randomized trials supporting the equivalence of TMT to radical cystectomy for selected patients in bladder cancer, the current evidence from other studies as summarized above supports that TMT, in the setting of multidisciplinary shared decision making, should be offered to all suitable candidates with MIBC and not only to patients with considerable comorbidities for whom surgery is not an option.

Bladder-preserving TMT has also been evaluated in a small phase II single-arm study in patients with recurrent high-grade NMIBC following intravesical therapy for whom the next step would be cystectomy, with chemoradiotherapy leading to favorable 88% cystectomy-free survival results at 3 years[264].

Radiotherapy of the primary tumour and possible sites of metastases may also have a role in oligometastatic bladder cancer. Studies suggest a possible survival benefit when adding local therapy to the bladder (including radiotherapy over chemotherapy alone) in metastatic disease[265,266] and when using metastasis-directed therapy[267,268]. However, data are limited in the adjuvant, recurrent NMIBC and oligometastatic settings, and further prospective research is needed.

[H2] Perioperative systemic therapy

For patients with MIBC, the risk of metastatic recurrence despite curative-intent local therapy (that is, radical cystectomy or TMT) is high and systemic therapy has been explored to further improve outcomes. The BA06 30894 trial compared neoadjuvant cisplatin, methotrexate plus vinblastine (CMV) followed by definitive local therapy versus definitive local therapy in patients with clinical stage T2-T4aN0M0 and is the largest neoadjuvant study reported to date[269]. This trial revealed that neoadjuvant CMV improved survival (HR 0.84; 95% CI, 0.72-0.99). The Southwest Oncology Group 8710 trial randomized patients with clinical stage T2-4aN0M0 to neoadjuvant methotrexate, vinblastine, doxorubicin plus cisplatin (MVAC) followed by cystectomy versus cystectomy alone[270]. This trial reported an improvement in overall survival with neoadjuvant MVAC (HR 0.75; 95% CI, 0.57-1.00). Importantly, these trials of neoadjuvant cisplatin-based chemotherapy have revealed an increased likelihood of achieving a pathological complete response at cystectomy with neoadjuvant chemotherapy followed by cystectomy versus cystectomy alone[270]. Meta-analyses of the neoadjuvant chemotherapy trials in MIBC have confirmed the survival benefit leading to this approach becoming standard care[271]. The optimal form of neoadjuvant chemotherapy, gemcitabine plus cisplatin or dose-dense MVAC remains controversial.[272,273,274]

Deferring decisions regarding the use of systemic therapy for MIBC to the post-operative setting is attractive given the ability to base treatment decisions on more precise pathological staging rather than clinical staging. Notwithstanding, clinical trials exploring adjuvant chemotherapy in patients with pT3-4 and/or pN+ urothelial cancer of the bladder have provided less robust evidence[275] despite observational analyses and meta-analyses suggesting a benefit[275,276].

There has historically been no standard perioperative systemic therapy to decrease the risk of recurrence after curative-intent surgery in cisplatin-ineligible patients with high-risk pathological features at cystectomy (pT3 and/or pN+) or patients who received prior neoadjuvant therapy with high-risk pathological features at cystectomy (pT3 and/or pN+).

Two phase 3 trials with a similar design sought to define the role of adjuvant PD-1 or PD-L1 blockade in this population by randomly allocating patients to 1 year of adjuvant PD-1 or PD-L1 blockade versus observation or placebo. Checkmate 274 demonstrated a significant improvement in disease-free survival in the overall population (HR 0.70; 95% CI 0.55–0.90) and in the subset of patients with tumors with increased PD-L1 expression (HR 0.55; 95% CI 0.35–0.85)[277], leading to regulatory approval of adjuvant nivolumab for bladder cancer in several parts of the world. IMvigor 010 did not demonstrate an improvement in the primary end point of disease free survival.[278] However, an exploratory analysis suggested a disease-free and overall survival benefit with adjuvant atezolizumab versus placebo in patients with detectable baseline ctDNA[163] paving the way for ctDNA-based studies of adjuvant therapy in bladder cancer.

[H2] Systemic therapy for metastatic bladder cancer

Cisplatin-based combination chemotherapy became a standard treatment for metastatic bladder cancer in the early 1990s after a randomized clinical trial demonstrated a survival benefit with MVAC versus cisplatin alone[279]. A series of subsequent randomized trials found that administration of MVAC in a dose-dense fashion and/or with granulocyte colony stimulating factor support was associated with less toxicity and possibly enhanced efficacy[280,281] and that the combination of gemcitabine plus cisplatin yielded similar efficacy but less toxicity than MVAC[282]. Although cisplatin-based chemotherapy became a standard of care for patients with metastatic urothelial cancer, many patients with bladder cancer are of advanced age and many are cisplatin ineligible[283]. For these patients, gemcitabine plus carboplatin is generally substituted.[284]

By 2015, PD-1 and PD-L1 ICIs had demonstrated durable responses in 20-25% of patients with metastatic urothelial cancer and received regulatory approval initially in patients progressing despite first-line platinum-based chemotherapy and, subsequently, as first-line treatment for cisplatin-ineligible patients[285–289]. Only the approval of pembrolizumab in patients with platinum-resistant metastatic urothelial cancer was based on a randomized phase 3 trial[287] with the remainder based on single-arm phase 2 studies. Potential adverse events with PD-1 and PD-L1 ICIs include, but are not limited to, immune-related adverse events, such as colitis, pneumonitis, dermatitis, hepatitis and endocrinopathies. Although requiring thorough validation in larger series, if the early data showing that LOY tumors are more vulnerable to ICIs holds, this would be a potentially valuable marker to stratify patients to this approach [131].

Several phase 3 trials were launched to optimize the use of these therapies. IMvigor 130[290] and Keynote 361[291] compared platinum-based chemotherapy versus PD-1 or PD-L1 blockade versus platinum-based chemotherapy plus PD-1 or PD-L1 blockade as first-line treatment for metastatic urothelial cancer. These trials failed to demonstrate a benefit of concurrent platinum-based chemotherapy plus PD-1 or PD-L1 blockade versus platinum-based chemotherapy alone. A randomized phase 2 and 3 trial compared switch maintenance PD-1 or PD-L1 blockade (pembrolizumab and atezolizumab, respectively) versus placebo or observation in patients with at least stable disease after initial platinum-based chemotherapy[292,293]. These trials met their primary endpoints, with the phase 3 JAVELIN-Bladder 100 demonstrating an overall survival benefit, resulting in switch maintenance ICI being adopted into standard treatment paradigms. After decades of investigation, platinum-based chemotherapy remains standard care for first-line treatment for most patients with metastatic urothelial cancer with switch maintenance ICI employed for patients with stable disease after ~4-6 cycles of chemotherapy. However, in some regions the combination of an antibody–drug conjugate (enfortumab vedotin) plus pembrolizumab has received regulatory approval as first-line treatment for cisplatin-ineligible patients, based on relatively high response rates and promising response durations [294]. Several new therapies with distinct mechanisms of action have subsequently been integrated into standard therapeutic strategies for metastatic bladder cancer **(Table 3)**.

[H1] Quality of life

A cross-sectional survey investigated the health-related quality of life (HRQoL) of 1,796 bladder patients, of whom 868 (48%) had NMIBC, 893 (50%) received radical cystectomy or radiotherapy, and 35 (1.9%) had unknown treatment[295]. Most patients (69%) reported at least one problem in any EQ-5D dimension[295]. HRQoL outcomes adjusted for age and sex were similar across all stages and treatment groups. Sexual problems were common in male patients and increased with younger age and radical treatment[295]. A prospective study of 133 patients using the Short-Form 36-item survey (SF-36) found that patients' physical functioning, social functioning, and role-emotional worsened with first, second and third TURBT, and finally improved when TURBT was performed ≥4 times[296]. Patient's mental health was also impaired at first TURBT, but gradually returned to normal with repeated TURBT.

A study investigated the QoL of 103 patients with NMIBC who received intravesical BCG or mitomycin C, using the EORTC QLQ-C30 and QLQ-BLS24 questionnaires[297]. QoL seemed to drop after the induction course and returned to baseline at 3 months. QoL was more affected in patients >70 years, especially in those who received intravesical BCG therapy. In another study, QoL of 106 patients with NMIBC who underwent intravesical chemotherapy was evaluated using the EORTC QLQ-C30 and the Core Lower Urinary Tract Symptom Score (CLSS) questionnaire, finding that global health status and social functioning decreased, and that CLSS also worsened significantly[298].

A meta-analysis investigated the HRQoL following radical cystectomy and urinary diversion [299]. All included studies reported an initial deterioration in overall HRQoL, but general health, functional and emotional domains at 12 months after surgery were similar to or better than baseline. Overall, there was no significant difference in HRQoL between continent and incontinent urinary diversion. Subgroup analysis showed greater improvement in physical health for patients undergoing incontinent urinary diversion, but mental health and social health did not differ between diversion types [299]. Qualitative analysis showed that patients with neobladder had better emotional function and body image than those with cutaneous diversion [299].

A meta-analysis comparing RARC and ORC showed no significant difference in QoL (standard mean difference -0.02, 95% CI -0.17-0.13, p=0.78). In the RAZOR study comparing RARC plus extracorporeal urinary diversion and ORC, no significant difference in the Functional Assessment of Cancer (FACT)-Vanderbilt Cystectomy Index was found between the two groups at any time point. In the iROC study comparing RARC plus intracorporeal urinary diversion and ORC, patients undergoing ORC had worse QoL at 5 weeks and greater disability at 5 weeks and 12 weeks, but their QoL improved with time and QoL did not differ between RARC and ORC after 12 weeks[300].

TMT experiences have shown favorable toxicity profiles and good long-term QoL. Late pelvic (genitourinary or gastrointestinal) grade ≥3 toxicity rates from NRG/RTOG and BC2001 trials are acceptable and low (1-6%)[257,301]. Analysis of long-term survivors from four NRG/RTOG trials showed that TMT was associated with 5.7% genitourinary and 1.9% gastrointestinal late grade 3 toxic effects (that rarely persist), and no late grade 4 toxic effects or treatment-related deaths[301]. In TMT series, <1% of patients require cystectomy due to treatment-related toxicity. Other studies, from prospective trials and retrospective cohorts, using validated instruments, as well as urodynamic studies, in long-term survivors of TMT for

MIBC made three quality of life related findings. First, the BC2001 trial showed short-term declines in HRQoL during treatment and immediately following chemoradiation, as would be expected, but these improved to baseline levels after 6 months with no impairment from the addition of chemotherapy[302]. Second, most patients have normally functioning bladders following therapy [303]. Third, TMT resulted in QoL gains compared with radical cystectomy, including modestly better general HRQoL, markedly better sexual function and QoL, better informed decision-making, less concerns about appearance and less life interference from cancer or cancer treatment[304].

A Swedish bladder cancer database investigated the natural history of patients unable or unwilling to receive therapy with curative intent [305]. Among patients with T2-3 M0 disease, a median of 2.4 hospitalizations per patient occurred during the first 12 months of diagnosis, and half of these hospitalizations were due to cancer or genitourinary symptoms [305]. These patients experienced substantial disease-specific morbidity, which might have been avoided if they underwent treatment with curative intent [305].

Several large phase 3 trials have evaluated QoL of patients with bladder cancer receiving systemic therapy. There are limited available instruments that have been designed and validated to assess both general and bladder cancer-specific quality of life domains in these patients. The FACT-Bl (Functional Assessment of Cancer Therapy-Bladder) is a 39-item questionnaire that integrates questions regarding general quality of life domains (FACT-General), as well as a cancer site-specific bladder subscale, and has been assessed for validity in a cohort of patients with metastatic bladder cancer receiving ICI[306]. This tool, as well as the National Comprehensive Cancer Network/Functional Assessment of Cancer Therapy Bladder Symptom Index-18 (FBlSI-18), European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30), and EuroQol-4D (EQ-5D) have been most commonly employed in bladder cancer trials.

The effects of neoadjuvant cisplatin-based chemotherapy on QoL are not well studied. In a randomized trial comparing two cycles of neoadjuvant MVAC followed by cystectomy versus cystectomy alone in 99 patients, quality of life was assessed using the FACT-Bl instrument[307]. QoL after completion of chemotherapy was lower than baseline scores in domains including physical and functional well-being as well as total FACT-Bl scores; however, there was no difference in these domains between study arms on follow-up after radical cystectomy. In the Checkmate 274 trial comparing adjuvant nivolumab versus placebo in 709 patients, QoL was assessed using the EORTC QLQ-C30 and the EQ-5D-3L[308]: adjuvant nivolumab was noninferior to placebo on changes from baseline across all major domains. In the JAVELIN-Bladder 100 trial of switch maintenance avelumab versus

observation in 700 patients with at least stable disease after first-line platinum-based chemotherapy, the FBISI-18 and EQ-5D-5L instruments were explored[309]. Switch maintenance avelumab was demonstrated to have minimal effects on quality of life. QoL with ICI was also assessed in the Keynote-045 trial comparing pembrolizumab versus chemotherapy in 519 patients with platinum-resistant metastatic bladder cancer[310]. Pembrolizumab prolonged the time to deterioration in global QOL compared with chemotherapy (median, 3.5 months v 2.3 months; hazard ratio, 0.72; nominal one-sided P = .004). QoL with systemic therapy in patients with bladder cancer is complex to measure and interpret given the variability of instruments, timepoints, and heterogeneity in clinical disease states with differential effects of disease-related and treatment-related burden.

Because of unique biology, such as high recurrence rates, procedural requirements related to surveillance and expensive treatments, bladder cancer management contributes considerably to medical costs. In the USA, the overall annual costs of cancer were $183 billion in 2015 and are projected to increase to $246 billion by 2030[311]. Bladder cancer contributed $7.93 billion in 2015, with an anticipated increase of $11.6 billion by 2030 . Similarly, among European Union members, cancer costs totaled €152.8 billion in 2012, of which bladder cancer contributed €5.24 billion (adjusted to 2019 values) [312]. Multiple cost-effectiveness analyses and reviews have been published and provide perspectives on the cost, efficacy, and effects on quality of life of interventions in patients with bladder cancer [313–315].

[H1] Outlook

Bladder cancer is a considerable and growing global health issue and its prevalence is expected to increase by 2040. However, with advances in molecular biology and therapy culminating progressive advances over the past 100 years (**Figure 6**), there is hope for the development of more effective diagnostic and treatment options that can improve patient outcomes.

One promising area of research is the development of minimally invasive diagnostic tools, such as urine-based or blood-based tests that can detect disease recurrences and minimal residual disease. These tests could provide a less invasive and more convenient alternative to current diagnostic methods. Furthermore, the tests could ultimately lead to new ways for guiding oncological decisions and follow-up programs. Further research in this area should focus on validation of clinical applicability of the tests in clinical trials, to demonstrate clinical utility. Furthermore, development of robust multi-cancer early detection tests may ultimately

lead to better screening for bladder cancer and in this way detect the disease at earlier stages.

The development of precision medicine approaches is also critical for improving bladder cancer management. The distinct molecular profiles of NMIBC and MIBC suggest that personalized treatment approaches based on the specific genetic mutations of tumor could lead to more effective outcomes. Similarly, understanding the sex and race differences in bladder cancer incidence and prognosis can help tailor treatment approaches to individual patients and improve outcomes. In addition, there is an urgent need to delineate tumor heterogeneity using single-cell and spatial transcriptomic analysis, which is likely compromising the utility of current subtype classifications for clinical outcome prediction[80,217]. These approaches will likely provide much needed clues to clinically tractable approaches that can be used to determine the primary driver populations in each specific tumor and use that driver as a prognosticator, predictor or therapeutic target. Finally, it is essential to continue to prioritize research into the causes and risk factors for bladder cancer. With a better understanding of the disease's underlying biology, more effective prevention strategies can be developed to identify patients who are at increased risk for developing bladder cancer. This could include lifestyle interventions, such as smoking cessation and dietary changes, as well as targeted screening for high-risk populations.

Machine learning, a subdiscipline of artificial intelligence that focuses on data analytics, has played a prominent role in cancer research and care because of the complexity of the disease and the availability of big data from technologies such as genomics and imaging. Applications include predicting regulatory elements in DNA sequences, predicting disease risk in populations, and diagnosing cancer from pathology and radiology images, as well as modeling and prediction of physiologic and biologic behaviors or systems biology [316,317]. A seminal study demonstrated the “Molecular Twin” precision medicine platform (MT-POP) technology, which applied AI on muti-omic and digital pathology data obtained from patients with cancer to provide a novel parsimonious biomarker model. This model greatly improved prediction of recurrence compared to current markers used in patients undergoing surgery for pancreatic cancer (Osipov, NATURE Cancer, 2023, in press; to be added later) This study demonstrated how the power of AI technology coupled with multi-omics can provide clinically tractable and cost-effective marker panels, potentially providing avenues to democratize precision medicine worldwide across most cancer types.

In conclusion, the outlook for bladder cancer is promising, with multiple advances in the understanding of the biological context of bladder cancer, development of novel non-invasive test methods for potentially guiding treatment and, finally, the development of multiple novel

oncological treatments. A multi-disciplinary approach that considers sex and race differences, as well as the genetic and molecular characteristics of the disease, will be critical for improving patient outcomes and reducing the global burden of bladder cancer.

References

1. Sung, H. *et al.* Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **71**, 209–249 (2021).
2. Antoni, S. *et al.* Bladder Cancer Incidence and Mortality: A Global Overview and Recent Trends. *Eur. Urol.* **71**, 96–108 (2017).
3. Ferlay, J. *et al.* Global cancer observatory: Cancer tomorrow [online]. Lyon, France: International agency for research on cancer. Preprint at (2020).
4. Tran, L., Xiao, J.-F., Agarwal, N., Duex, J. E. & Theodorescu, D. Advances in bladder cancer biology and therapy. *Nat. Rev. Cancer* **21**, 104–121 (2021).
5. Facchini, G. *et al.* Advanced/metastatic bladder cancer: current status and future directions. *Eur. Rev. Med. Pharmacol. Sci.* **24**, 11536–11552 (2020).
6. Dancik, G. M., Owens, C. R., Iczkowski, K. A. & Theodorescu, D. A cell of origin gene signature indicates human bladder cancer has distinct cellular progenitors. *Stem Cells* **32**, 974–982 (2014).
7. Partin, A. W., Peters, C. A., Kavoussi, L. R., Dmochowski, R. R. & Wein, A. J. *Campbell-Walsh-Wein Urology Twelfth Edition Review*. (Elsevier Health Sciences, 2020).
8. Freedman, N. D., Silverman, D. T., Hollenbeck, A. R., Schatzkin, A. & Abnet, C. C. Association between smoking and risk of bladder cancer among men and women. *JAMA* **306**, 737–745 (2011).
9. Wilhelm-Benartzi, C. S. *et al.* Association of secondhand smoke exposures with DNA methylation in bladder carcinomas. *Cancer Causes Control* **22**, 1205–1213 (2011).
10. Bellamri, M. *et al.* DNA Damage and Oxidative Stress of Tobacco Smoke Condensate in Human Bladder Epithelial Cells. *Chem. Res. Toxicol.* **35**, 1863–1880 (2022).
11. Matuszczak, M. & Salagierski, M. Diagnostic and Prognostic Potential of Biomarkers CYFRA 21.1, ERCC1, p53, FGFR3 and TATI in Bladder Cancers. *Int. J. Mol. Sci.* **21**, (2020).
12. Mertens, L. S. *et al.* Prognostic markers in invasive bladder cancer: FGFR3 mutation status versus P53 and KI-67 expression: a multi-center, multi-laboratory analysis in 1058 radical cystectomy patients. *Urol. Oncol.* **40**, 110.e1–110.e9 (2022).
13. Theodorescu, D., Li, Z. & Li, X. Sex differences in bladder cancer: emerging data and call to action. *Nat. Rev. Urol.* **19**, 447–449 (2022).
14. Bladder cancer statistics. *WCRF International* https://www.wcrf.org/cancer-trends/bladder-cancer-statistics/ (2022).
15. Dobruch, J. *et al.* Gender and Bladder Cancer: A Collaborative Review of Etiology, Biology, and Outcomes. *Eur. Urol.* **69**, 300–310 (2016).
16. Hyldgaard, J. M. & Jensen, J. B. The Inequality of Females in Bladder Cancer. *APMIS* **129**, 694–699 (2021).
17. Radkiewicz, C. *et al.* Sex Differences in Urothelial Bladder Cancer Survival. *Clin. Genitourin. Cancer* **18**, 26–34.e6 (2020).
18. You, S. *et al.* Characterizing molecular subtypes of high-risk non-muscle-invasive bladder cancer in African American patients. *Urol. Oncol.* **40**, 410.e19–410.e27 (2022).
19. Saginala, K. *et al.* Epidemiology of Bladder Cancer. *Med Sci (Basel)* **8**, (2020).
20. Richters, A., Aben, K. K. H. & Kiemeney, L. A. L. M. The global burden of urinary bladder cancer: an update. *World J. Urol.* **38**, 1895–1904 (2020).
21. Safiri, S., Kolahi, A.-A., Naghavi, M. & Global Burden of Disease Bladder Cancer Collaborators. Global, regional and national burden of bladder cancer and its attributable risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease study 2019. *BMJ Glob Health* **6**, (2021).
22. Dai, X., Gakidou, E. & Lopez, A. D. Evolution of the global smoking epidemic over the past half century: strengthening the evidence base for policy action. *Tob. Control* **31**, 129–137 (2022).
23. Flor, L. S., Reitsma, M. B., Gupta, V., Ng, M. & Gakidou, E. The effects of tobacco control policies on global smoking prevalence. *Nat. Med.* **27**, 239–243 (2021).

24. Ishida, K. & Hsieh, M. H. Understanding Urogenital Schistosomiasis-Related Bladder Cancer: An Update. *Front. Med.* **5**, 223 (2018).
25. Zaghloul, M. S., Zaghloul, T. M., Bishr, M. K. & Baumann, B. C. Urinary schistosomiasis and the associated bladder cancer: update. *J. Egypt. Natl. Canc. Inst.* **32**, 44 (2020).
26. Salem, S., Mitchell, R. E., El-Alim El-Dorey, A., Smith, J. A. & Barocas, D. A. Successful control of schistosomiasis and the changing epidemiology of bladder cancer in Egypt. *BJU Int.* **107**, 206–211 (2011).
27. Martin, A., Woolbright, B. L., Umar, S., Ingersoll, M. A. & Taylor, J. A., 3rd. Bladder cancer, inflammageing and microbiomes. *Nat. Rev. Urol.* **19**, 495–509 (2022).
28. Lobo, N. *et al.* Epidemiology, Screening, and Prevention of Bladder Cancer. *Eur Urol Oncol* **5**, 628–639 (2022).
29. Letašiová, S. *et al.* Bladder cancer, a review of the environmental risk factors. *Environ. Health* **11 Suppl 1**, S11 (2012).
30. van der Post, R. S. *et al.* Risk of urothelial bladder cancer in Lynch syndrome is increased, in particular among MSH2 mutation carriers. *J. Med. Genet.* **47**, 464–470 (2010).
31. Lindner, A. K. *et al.* Lynch Syndrome: Its Impact on Urothelial Carcinoma. *Int. J. Mol. Sci.* **22**, (2021).
32. Lindskrog, S. V. *et al.* An integrated multi-omics analysis identifies prognostic molecular subtypes of non-muscle-invasive bladder cancer. *Nat. Commun.* **12**, 2301 (2021).
An update to the 2016 UROMOL consortium study, representing the largest multi-omics analysis to characterize the molecular landscape in early-stage bladder cancer.
33. Hedegaard, J. *et al.* Comprehensive Transcriptional Analysis of Early-Stage Urothelial Carcinoma. *Cancer Cell* **30**, 27–42 (2016).
34. Hurst, C. D. *et al.* Stage-stratified molecular profiling of non-muscle-invasive bladder cancer enhances biological, clinical, and therapeutic insight. *Cell Rep Med* **2**, 100472 (2021).
35. Sun, T. T., Zhao, H., Provet, J., Aebi, U. & Wu, X. R. Formation of asymmetric unit membrane during urothelial differentiation. *Mol. Biol. Rep.* **23**, 3–11 (1996).
36. Varley, C. L. *et al.* PPARgamma-regulated tight junction development during human urothelial cytodifferentiation. *J. Cell. Physiol.* **208**, 407–417 (2006).
37. Southgate, J., Harnden, P. & Trejdosiewicz, L. K. Cytokeratin expression patterns in normal and malignant urothelium: a review of the biological and diagnostic implications. *Histol. Histopathol.* **14**, 657–664 (1999).
38. Wezel, F., Pearson, J. & Southgate, J. Plasticity of in vitro-generated urothelial cells for functional tissue formation. *Tissue Eng. Part A* **20**, 1358–1368 (2014).
39. Wiessner, G. B., Plumber, S. A., Xiang, T. & Mendelsohn, C. L. Development, regeneration and tumorigenesis of the urothelium. *Development* **149**, (2022).
40. Fishwick, C. *et al.* Heterarchy of transcription factors driving basal and luminal cell phenotypes in human urothelium. *Cell Death Differ.* **24**, 809–818 (2017).
41. Curtius, K., Wright, N. A. & Graham, T. A. An evolutionary perspective on field cancerization. *Nat. Rev. Cancer* **18**, 19–32 (2018).
42. Sidransky, D. *et al.* Clonal origin of bladder cancer. *N. Engl. J. Med.* **326**, 737–740 (1992).
43. Höglund, M. On the origin of syn- and metachronous urothelial carcinomas. *Eur. Urol.* **51**, 1185–93; discussion 1193 (2007).
44. Höglund, M. Bladder cancer, a two phased disease? *Semin. Cancer Biol.* **17**, 225–232 (2007).
45. Lamy, P. *et al.* Paired Exome Analysis Reveals Clonal Evolution and Potential Therapeutic Targets in Urothelial Carcinoma. *Cancer Res.* **76**, 5894–5906 (2016).
46. Bondaruk, J. *et al.* The origin of bladder cancer from mucosal field effects. *iScience* **25**, 104551 (2022).
47. Strandgaard, T. *et al.* Field Cancerization Is Associated with Tumor Development, T-cell Exhaustion, and Clinical Outcomes in Bladder Cancer. *Eur. Urol.* (2023) doi:10.1016/j.eururo.2023.07.014.

48. Lawson, A. R. J. *et al.* Extensive heterogeneity in somatic mutation and selection in the human bladder. *Science* vol. 370 75–82 Preprint at https://doi.org/10.1126/science.aba8347 (2020).
49. Strandgaard, T. *et al.* Field cancerization impacts tumor development, T-cell exhaustion and clinical outcomes in bladder cancer. *bioRxiv* 2023.02.20.528920 (2023) doi:10.1101/2023.02.20.528920.
50. Alexandrov, L. B. *et al.* The repertoire of mutational signatures in human cancer. *Nature* **578**, 94–101 (2020).
51. Nordentoft, I. *et al.* Mutational context and diverse clonal development in early and late bladder cancer. *Cell Rep.* **7**, 1649–1663 (2014).
52. Robertson, A. G. *et al.* Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer. *Cell* **174**, 1033 (2018).
53. Alexandrov, L. B. *et al.* Signatures of mutational processes in human cancer. *Nature* **500**, 415–421 (2013).
54. Sandberg, A. A. Chromosome changes in bladder cancer: clinical and other correlations. *Cancer Genet. Cytogenet.* **19**, 163–175 (1986).
55. Hurst, C. D. *et al.* Genomic Subtypes of Non-invasive Bladder Cancer with Distinct Metabolic Profile and Female Gender Bias in KDM6A Mutation Frequency. *Cancer Cell* **32**, 701–715.e7 (2017).
The authors defined two major genomic subtypes of primary stage Ta tumors and found more mutations in the histone lysine demethylase KDM6A were present in non-invasive tumors from women than men, supporting the hypothesis that male and female bladder cancers have both common and different biological drivers.
56. Ségal-Bendirdjian, E. & Geli, V. Non-canonical Roles of Telomerase: Unraveling the Imbroglio. *Front Cell Dev Biol* **7**, 332 (2019).
57. Agarwal, N. *et al.* TRIM28 is a transcriptional activator of the mutant TERT promoter in human bladder cancer. *Proc. Natl. Acad. Sci. U. S. A.* **118**, (2021).
58. Borah, S. *et al.* Cancer. TERT promoter mutations and telomerase reactivation in urothelial cancer. *Science* **347**, 1006–1010 (2015).
59. Nickerson, M. L. *et al.* Molecular analysis of urothelial cancer cell lines for modeling tumor biology and drug response. *Oncogene* **36**, 35–46 (2017).
60. Nickerson, M. L. *et al.* Concurrent alterations in TERT, KDM6A, and the BRCA pathway in bladder cancer. *Clin. Cancer Res.* **20**, 4935–4948 (2014).
61. Hurst, C. D., Platt, F. M., Taylor, C. F. & Knowles, M. A. Novel tumor subgroups of urothelial carcinoma of the bladder defined by integrated genomic analysis. *Clin. Cancer Res.* **18**, 5865–5877 (2012).
62. Allory, Y. *et al.* Telomerase reverse transcriptase promoter mutations in bladder cancer: high frequency across stages, detection in urine, and lack of association with outcome. *Eur. Urol.* **65**, 360–366 (2014).
63. Stern, J. L., Theodorescu, D., Vogelstein, B., Papadopoulos, N. & Cech, T. R. Mutation of the TERT promoter, switch to active chromatin, and monoallelic TERT expression in multiple cancers. *Genes Dev.* **29**, 2219–2224 (2015).
64. Shi, M.-J. *et al.* APOBEC-mediated Mutagenesis as a Likely Cause of FGFR3 S249C Mutation Over-representation in Bladder Cancer. *Eur. Urol.* **76**, 9–13 (2019).
65. di Martino, E., L'Hôte, C. G., Kennedy, W., Tomlinson, D. C. & Knowles, M. A. Mutant fibroblast growth factor receptor 3 induces intracellular signaling and cellular transformation in a cell type- and mutation-specific manner. *Oncogene* **28**, 4306–4316 (2009).
66. Barrows, D., Feng, L., Carroll, T. S. & Allis, C. D. Loss of UTX/KDM6A and the activation of FGFR3 converge to regulate differentiation gene-expression programs in bladder cancer. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 25732–25741 (2020).
67. Qiu, H. *et al.* KDM6A loss triggers an epigenetic switch that disrupts urothelial differentiation and drives cell proliferation in bladder cancer. *Cancer Res.* (2023) doi:10.1158/0008-5472.CAN-

22-1444.
68. Richart, L. *et al.* STAG2 loss-of-function affects short-range genomic contacts and modulates the basal-luminal transcriptional program of bladder cancer cells. *Nucleic Acids Res.* **49**, 11005–11021 (2021).
69. Taylor, C. F., Platt, F. M., Hurst, C. D., Thygesen, H. H. & Knowles, M. A. Frequent inactivating mutations of STAG2 in bladder cancer are associated with low tumour grade and stage and inversely related to chromosomal copy number changes. *Hum. Mol. Genet.* **23**, 1964–1974 (2014).
70. Gordon, N. S. *et al.* STAG2 Protein Expression in Non-muscle-invasive Bladder Cancer: Associations with Sex, Genomic and Transcriptomic Changes, and Clinical Outcomes. *Eur Urol Open Sci* **38**, 88–95 (2022).
71. Meeks, J. J. *et al.* Genomic heterogeneity in bladder cancer: challenges and possible solutions to improve outcomes. *Nat. Rev. Urol.* **17**, 259–270 (2020).
72. Li, Q. *et al.* ERCC2 Helicase Domain Mutations Confer Nucleotide Excision Repair Deficiency and Drive Cisplatin Sensitivity in Muscle-Invasive Bladder Cancer. *Clin. Cancer Res.* **25**, 977–988 (2019).
73. Williams, S. V., Hurst, C. D. & Knowles, M. A. Oncogenic FGFR3 gene fusions in bladder cancer. *Hum. Mol. Genet.* **22**, 795–803 (2013).
74. Tomlinson, D. C., Baxter, E. W., Loadman, P. M., Hull, M. A. & Knowles, M. A. FGFR1-induced epithelial to mesenchymal transition through MAPK/PLCγ/COX-2-mediated mechanisms. *PLoS One* **7**, e38972 (2012).
75. Tomlinson, D. C. & Knowles, M. A. Altered splicing of FGFR1 is associated with high tumor grade and stage and leads to increased sensitivity to FGF1 in bladder cancer. *Am. J. Pathol.* **177**, 2379–2386 (2010).
76. Rebouissou, S. *et al.* CDKN2A homozygous deletion is associated with muscle invasion in FGFR3-mutated urothelial bladder carcinoma. *J. Pathol.* **227**, 315–324 (2012).
77. Huan, J., Grivas, P., Birch, J. & Hansel, D. E. Emerging Roles for Mammalian Target of Rapamycin (mTOR) Complexes in Bladder Cancer Progression and Therapy. *Cancers* **14**, (2022).
78. Miyata, Y., Sagara, Y., Kanda, S., Hayashi, T. & Kanetake, H. Phosphorylated hepatocyte growth factor receptor/c-Met is associated with tumor growth and prognosis in patients with bladder cancer: correlation with matrix metalloproteinase-2 and -7 and E-cadherin. *Hum. Pathol.* **40**, 496–504 (2009).
79. Goriki, A. *et al.* Unravelling disparate roles of NOTCH in bladder cancer. *Nat. Rev. Urol.* **15**, 345–357 (2018).
80. Gouin, K. H., 3rd *et al.* An N-Cadherin 2 expressing epithelial cell subpopulation predicts response to surgery, chemotherapy and immunotherapy in bladder cancer. *Nat. Commun.* **12**, 4906 (2021).
This is the first publication evaluating by single-cell and spatial transcriptomics and proteomics, tumor heterogeneity in muscle-invasive bladder cancer and defines a new subtype architecture and specific tumor cell populations whose presence predict clinical outcomes after surgery and immunotherapy.
81. Su, S. *et al.* CD10+GPR77+ Cancer-Associated Fibroblasts Promote Cancer Formation and Chemoresistance by Sustaining Cancer Stemness. *Cell* **172**, 841–856.e16 (2018).
82. Lee, Y.-C. *et al.* The dynamic roles of the bladder tumour microenvironment. *Nat. Rev. Urol.* **19**, 515–533 (2022).
83. Qiu, S. *et al.* Tumor-associated macrophages promote bladder tumor growth through PI3K/AKT signal induced by collagen. *Cancer Sci.* **110**, 2110–2118 (2019).
84. Mezheyeuski, A. *et al.* Fibroblasts in urothelial bladder cancer define stroma phenotypes that are associated with clinical outcome. *Sci. Rep.* **10**, 281 (2020).
85. Long, X. *et al.* Cancer-associated fibroblasts promote cisplatin resistance in bladder cancer cells by increasing IGF-1/ERβ/Bcl-2 signalling. *Cell Death Dis.* **10**, 375 (2019).

86. Tran, L. & Theodorescu, D. Determinants of Resistance to Checkpoint Inhibitors. *Int. J. Mol. Sci.* **21**, (2020).
87. Chen, Y. *et al.* Tumor-associated macrophages: an accomplice in solid tumor progression. *J. Biomed. Sci.* **26**, 78 (2019).
88. Tu, M. M. *et al.* Inhibition of the CCL2 receptor, CCR2, enhances tumor response to immune checkpoint therapy. *Commun Biol* **3**, 720 (2020).
89. Tu, M. M. *et al.* Targeting DDR2 enhances tumor response to anti-PD-1 immunotherapy. *Sci Adv* **5**, eaav2437 (2019).
90. Said, N., Sanchez-Carbayo, M., Smith, S. C. & Theodorescu, D. RhoGDI2 suppresses lung metastasis in mice by reducing tumor versican expression and macrophage infiltration. *J. Clin. Invest.* **122**, 1503–1518 (2012).
91. Wang, L. *et al.* Myeloid Cell-associated Resistance to PD-1/PD-L1 Blockade in Urothelial Cancer Revealed Through Bulk and Single-cell RNA Sequencing. *Clin. Cancer Res.* **27**, 4287–4300 (2021).
92. Samstein, R. M. *et al.* Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat. Genet.* **51**, 202–206 (2019).
93. Cristescu, R. *et al.* Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science* **362**, (2018).
94. Pfannstiel, C. *et al.* The Tumor Immune Microenvironment Drives a Prognostic Relevance That Correlates with Bladder Cancer Subtypes. *Cancer Immunol Res* **7**, 923–938 (2019).
95. Wang, L. *et al.* EMT- and stroma-related gene expression and resistance to PD-1 blockade in urothelial cancer. *Nat. Commun.* **9**, 3503 (2018).
96. You, S. *et al.* Discoidin Domain Receptor-Driven Gene Signatures as Markers of Patient Response to Anti-PD-L1 Immune Checkpoint Therapy. *J. Natl. Cancer Inst.* **114**, 1380–1391 (2022).
97. Kaneko, S. & Li, X. X chromosome protects against bladder cancer in females via a KDM6A-dependent epigenetic mechanism. *Sci Adv* **4**, eaar5598 (2018).
98. Li, Z., Azar, J. H. & Rubinstein, M. P. Converting Tumoral PD-L1 into a 4-1BB Agonist for Safer and More Effective Cancer Immunotherapy. *Cancer discovery* vol. 12 1184–1186 (2022).
99. Kwon, H. *et al.* Androgen conspires with the CD8+ T cell exhaustion program and contributes to sex bias in cancer. *Sci Immunol* **7**, eabq2630 (2022).
100. Sottnik, J. L. *et al.* Androgen Receptor Regulates CD44 Expression in Bladder Cancer. *Cancer Res.* **81**, 2833–2846 (2021).
101. Calvete, J. *et al.* The coexpression of fibroblast activation protein (FAP) and basal-type markers (CK 5/6 and CD44) predicts prognosis in high-grade invasive urothelial carcinoma of the bladder. *Hum. Pathol.* **91**, 61–68 (2019).
102. Bellmunt, J. Stem-Like Signature Predicting Disease Progression in Early Stage Bladder Cancer. The Role of E2F3 and SOX4. *Biomedicines* **6**, (2018).
103. Sottnik, J. L. & Theodorescu, D. CD44: A metastasis driver and therapeutic target. *Oncoscience* **3**, 320–321 (2016).
104. Senbanjo, L. T. & Chellaiah, M. A. CD44: A Multifunctional Cell Surface Adhesion Receptor Is a Regulator of Progression and Metastasis of Cancer Cells. *Front Cell Dev Biol* **5**, 18 (2017).
105. Miyamoto, H. *et al.* Promotion of bladder cancer development and progression by androgen receptor signals. *J. Natl. Cancer Inst.* **99**, 558–568 (2007).
106. Morales, E. E. *et al.* Finasteride Reduces Risk of Bladder Cancer in a Large Prospective Screening Study. *Eur. Urol.* **69**, 407–410 (2016).
107. Sathianathen, N. J., Fan, Y., Jarosek, S. L., Lawrentschuk, N. L. & Konety, B. R. Finasteride does not prevent bladder cancer: A secondary analysis of the Medical Therapy for Prostatic Symptoms Study. *Urol. Oncol.* **36**, 338.e13–338.e17 (2018).
108. Zhu, D. *et al.* Finasteride Use and Risk of Bladder Cancer in a Multiethnic Population. *J. Urol.* **206**, 15–21 (2021).

109. Richard, A. *et al.* Racial variation in sex steroid hormone concentration in black and white men: a meta-analysis. *Andrology* **2**, 428–435 (2014).
110. Giaquinto, A. N. *et al.* Cancer statistics for African American/Black People 2022. *CA Cancer J. Clin.* **72**, 202–229 (2022).
111. Miyamoto, H. *et al.* Expression of androgen and oestrogen receptors and its prognostic significance in urothelial neoplasm of the urinary bladder. *BJU Int.* **109**, 1716–1726 (2012).
112. Tripathi, A. & Gupta, S. Androgen receptor in bladder cancer: A promising therapeutic target. *Asian J Urol* **7**, 284–290 (2020).
113. Xiang, P. *et al.* Impact of Androgen Suppression Therapy on the Risk and Prognosis of Bladder Cancer: A Systematic Review and Meta-Analysis. *Front. Oncol.* **11**, 784627 (2021).
114. Creta, M. *et al.* Inhibition of Androgen Signalling Improves the Outcomes of Therapies for Bladder Cancer: Results from a Systematic Review of Preclinical and Clinical Evidence and Meta-Analysis of Clinical Studies. *Diagnostics (Basel)* **11**, (2021).
115. Wu, S.-C. *et al.* Androgen Suppression Therapy Is Associated with Lower Recurrence of Non-muscle-invasive Bladder Cancer. *Eur Urol Focus* **7**, 142–147 (2021).
116. Maan, A. A. *et al.* The Y chromosome: a blueprint for men's health? *Eur. J. Hum. Genet.* **25**, 1181–1188 (2017).
117. Sano, S. *et al.* Hematopoietic loss of Y chromosome leads to cardiac fibrosis and heart failure mortality. *Science* **377**, 292–297 (2022).
118. Forsberg, L. A. *et al.* Mosaic loss of chromosome Y in peripheral blood is associated with shorter survival and higher risk of cancer. *Nat. Genet.* **46**, 624–628 (2014).
119. Kido, T. & Lau, Y.-F. C. Roles of the Y chromosome genes in human cancers. *Asian J. Androl.* **17**, 373–380 (2015).
120. Brown, D. W. & Machiela, M. J. Why Y? Downregulation of Chromosome Y Genes Potentially Contributes to Elevated Cancer Risk. *Journal of the National Cancer Institute* vol. 112 871–872 (2020).
121. Panani, A. D. & Roussos, C. Sex chromosome abnormalities in bladder cancer: Y polysomies are linked to PT1-grade III transitional cell carcinoma. *Anticancer Res.* **26**, 319–323 (2006).
122. Fadl-Elmula, I. *et al.* Karyotypic characterization of urinary bladder transitional cell carcinomas. *Genes Chromosomes Cancer* **29**, 256–265 (2000).
123. Sauter, G., Moch, H., Mihatsch, M. J. & Gasser, T. C. Molecular cytogenetics of bladder cancer progression. *Eur. Urol.* **33 Suppl 4**, 9–10 (1998).
124. Smeets, W., Pauwels, R., Laarakkers, L., Debruyne, F. & Geraedts, J. Chromosomal analysis of bladder cancer. III. Nonrandom alterations. *Cancer Genet. Cytogenet.* **29**, 29–41 (1987).
125. Sauter, G. *et al.* Y chromosome loss detected by FISH in bladder cancer. *Cancer Genet. Cytogenet.* **82**, 163–169 (1995).
126. Neuhaus, M. *et al.* Polysomies but not Y chromosome losses have prognostic significance in pTa/pT1 urinary bladder cancer. *Hum. Pathol.* **30**, 81–86 (1999).
127. Powell, I., Tyrkus, M. & Kleer, E. Apparent correlation of sex chromosome loss and disease course in urothelial cancer. *Cancer Genet. Cytogenet.* **50**, 97–101 (1990).
128. Siegel, R. L., Miller, K. D., Fuchs, H. E. & Jemal, A. Cancer statistics, 2022. *CA Cancer J. Clin.* **72**, 7–33 (2022).
129. Johansson, S. L. & Cohen, S. M. Epidemiology and etiology of bladder cancer. *Semin. Surg. Oncol.* **13**, 291–298 (1997).
130. Dumanski, J. P. *et al.* Mutagenesis. Smoking is associated with mosaic loss of chromosome Y. *Science* **347**, 81–83 (2015).
131. Abdel-Hafiz, H. A. *et al.* Y chromosome loss in cancer drives growth by evasion of adaptive immunity. *Nature* (2023) doi:10.1038/s41586-023-06234-x.
This is the first publication that mechanistically links cancer aggressiveness with loss of

the Y chromosome and shows that this is due to the cancer cell evading T cell mediated immunity, opening up possibilities for biomarker and therapeutic development in cancer.
132. Cummings, K. B., Barone, J. G. & Ward, W. S. Diagnosis and staging of bladder cancer. *Urol. Clin. North Am.* **19**, 455–465 (1992).
133. Khadhouri, S. *et al.* The IDENTIFY study: the investigation and detection of urological neoplasia in patients referred with suspected urinary tract cancer - a multicentre observational study. *BJU Int.* **128**, 440–450 (2021).
134. Ghandour, R., Freifeld, Y., Singla, N. & Lotan, Y. Evaluation of Hematuria in a Large Public Health Care System. *Bladder Cancer* **5**, 119–129 (2019).
135. Rai, B. P. *et al.* Systematic Review of the Incidence of and Risk Factors for Urothelial Cancers and Renal Cell Carcinoma Among Patients with Haematuria. *Eur. Urol.* **82**, 182–192 (2022).
136. Ramirez, D. *et al.* Microscopic haematuria at time of diagnosis is associated with lower disease stage in patients with newly diagnosed bladder cancer. *BJU Int.* **117**, 783–786 (2016).
137. Alanee, S. & Shukla, A. R. Bladder malignancies in children aged <18 years: results from the Surveillance, Epidemiology and End Results database. *BJU Int.* **106**, 557–560 (2010).
138. Kutarski, P. W. & Padwell, A. Transitional cell carcinoma of the bladder in young adults. *Br. J. Urol.* **72**, 749–755 (1993).
139. Rezaee, M. E., Dunaway, C. M., Baker, M. L., Penna, F. J. & Chavez, D. R. Urothelial cell carcinoma of the bladder in pediatric patients: a systematic review and data analysis of the world literature. *J. Pediatr. Urol.* **15**, 309–314 (2019).
140. Czech, A. K. *et al.* Diagnostic accuracy of bimanual palpation in bladder cancer patients undergoing cystectomy: A prospective study. *Urol. Oncol.* **41**, 390.e27–390.e33 (2023).
141. Flaig, T. W. *et al.* Bladder Cancer, Version 3.2020, NCCN Clinical Practice Guidelines in Oncology. *J. Natl. Compr. Canc. Netw.* **18**, 329–354 (2020).
142. Ploeg, M. *et al.* Discrepancy between clinical staging through bimanual palpation and pathological staging after cystectomy. *Urol. Oncol.* **30**, 247–251 (2012).
143. Babjuk, M. *et al.* European Association of Urology Guidelines on Non-muscle-invasive Bladder Cancer (Ta, T1, and Carcinoma in Situ). *Eur. Urol.* **81**, 75–94 (2022).
144. Xing, J. & Reynolds, J. P. Diagnostic Advances in Urine Cytology. *Surg. Pathol. Clin.* **11**, 601–610 (2018).
145. Barkan, G. A. *et al.* The Paris System for Reporting Urinary Cytology: The Quest to Develop a Standardized Terminology. *Adv. Anat. Pathol.* **23**, 193–201 (2016).
146. Nikas, I. P. *et al.* The Paris System for Reporting Urinary Cytology: A Meta-Analysis. *J Pers Med* **12**, (2022).
147. Saprykina, E. V. & Sal'nik, B. I. [The role of lipid metabolism disorders in the mechanism of the hepatotoxic effects of rubomycin]. *Antibiot. Khimioter.* **33**, 452–455 (1988).
148. Guo, A. *et al.* Bladder tumour antigen (BTA stat) test compared to the urine cytology in the diagnosis of bladder cancer: A meta-analysis. *Can. Urol. Assoc. J.* **8**, E347–52 (2014).
149. Dimashkieh, H. *et al.* Evaluation of urovysion and cytology for bladder cancer detection: a study of 1835 paired urine samples with clinical and histologic correlation. *Cancer Cytopathol.* **121**, 591–597 (2013).
150. Zippe, C., Pandrangi, L. & Agarwal, A. NMP22 is a sensitive, cost-effective test in patients at risk for bladder cancer. *J. Urol.* **161**, 62–65 (1999).
151. He, H., Han, C., Hao, L. & Zang, G. ImmunoCyt test compared to cytology in the diagnosis of bladder cancer: A meta-analysis. *Oncol. Lett.* **12**, 83–88 (2016).
152. Wang, Z. *et al.* Evaluation of the NMP22 BladderChek test for detecting bladder cancer: a systematic review and meta-analysis. *Oncotarget* **8**, 100648–100656 (2017).
153. Jeong, S.-H. & Ku, J. H. Urinary Markers for Bladder Cancer Diagnosis and Monitoring. *Front Cell Dev Biol* **10**, 892067 (2022).
154. Heitzer, E., Auinger, L. & Speicher, M. R. Cell-Free DNA and Apoptosis: How Dead

Cells Inform About the Living. *Trends Mol. Med.* **26**, 519–528 (2020).
155. Cherepanova, A. V., Tamkovich, S. N., Bryzgunova, O. E., Vlassov, V. V. & Laktionov, P. P. Deoxyribonuclease activity and circulating DNA concentration in blood plasma of patients with prostate tumors. *Ann. N. Y. Acad. Sci.* **1137**, 218–221 (2008).
156. Tamkovich, S. N. *et al.* Circulating DNA and DNase activity in human blood. *Ann. N. Y. Acad. Sci.* **1075**, 191–196 (2006).
157. Yu, S. C. Y. *et al.* High-resolution profiling of fetal DNA clearance from maternal plasma by massively parallel sequencing. *Clin. Chem.* **59**, 1228–1237 (2013).
158. Khier, S. & Gahan, P. B. Hepatic Clearance of Cell-Free DNA: Possible Impact on Early Metastasis Diagnosis. *Mol. Diagn. Ther.* **25**, 677–682 (2021).
159. Khier, S. & Lohan, L. Kinetics of circulating cell-free DNA for biomedical applications: critical appraisal of the literature. *Future Sci OA* **4**, FSO295 (2018).
160. Christensen, E. *et al.* Early Detection of Metastatic Relapse and Monitoring of Therapeutic Efficacy by Ultra-Deep Sequencing of Plasma Cell-Free DNA in Patients With Urothelial Bladder Carcinoma. *J. Clin. Oncol.* JCO1802052 (2019).
This is the first larger prospective study showing that ctDNA measurements during chemotherapy and after cystectomy may be a very powerful biomarker for guiding treatment.
161. Christensen, E. *et al.* Cell-free urine- and plasma DNA mutational analysis predicts neoadjuvant chemotherapy response and outcome in patients with muscle invasive bladder cancer. *Clin. Cancer Res.* (2023) doi:10.1158/1078-0432.CCR-22-3250.
162. van Dorp, J. *et al.* High- or low-dose preoperative ipilimumab plus nivolumab in stage III urothelial cancer: the phase 1B NABUCCO trial. *Nat. Med.* (2023) doi:10.1038/s41591-022-02199-y.
163. Powles, T. *et al.* ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. *Nature* **595**, 432–437 (2021).
164. Powles, T. *et al.* Updated Overall Survival by Circulating Tumor DNA Status from the Phase 3 IMvigor010 Trial: Adjuvant Atezolizumab Versus Observation in Muscle-invasive Urothelial Carcinoma. *Eur. Urol.* (2023) doi:10.1016/j.eururo.2023.06.007.
165. US National Library of Medicine. ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT04660344 (2023).
166. US National Library of Medicine. ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT04138628 (2022)
167. Vandekerkhove, G. *et al.* Plasma ctDNA is a tumor tissue surrogate and enables clinical-genomic stratification of metastatic bladder cancer. *Nat. Commun.* **12**, 184 (2021).
168. Vandekerkhove, G. *et al.* Circulating Tumor DNA Reveals Clinically Actionable Somatic Genome of Metastatic Bladder Cancer. *Clin. Cancer Res.* **23**, 6487–6497 (2017).
169. Moch, H. *Urinary and Male Genital Tumours : WHO Classification of Tumours, 5th Edition, Volume 8*. vol. 8 576 (IARC Publications, 2022).
170. Pasin, E., Josephson, D. Y., Mitra, A. P., Cote, R. J. & Stein, J. P. Superficial bladder cancer: an update on etiology, molecular development, classification, and natural history. *Rev. Urol.* **10**, 31–43 (2008).
171. Ching, C. B. *et al.* HER2 gene amplification occurs frequently in the micropapillary variant of urothelial carcinoma: analysis by dual-color in situ hybridization. *Mod. Pathol.* **24**, 1111–1119 (2011).
172. Willis, D. L. *et al.* Micropapillary bladder cancer: current treatment patterns and review of the literature. *Urol. Oncol.* **32**, 826–832 (2014).
173. Isharwal, S. *et al.* Intratumoral heterogeneity of ERBB2 amplification and HER2 expression in micropapillary urothelial carcinoma. *Hum. Pathol.* **77**, 63–69 (2018).
174. Teo, M. Y. *et al.* Natural history, response to systemic therapy, and genomic landscape of plasmacytoid urothelial carcinoma. *Br. J. Cancer* **124**, 1214–1221 (2021).
175. Edgerton, N., Sirintrapun, S. J., Munoz, M., Chen, Z. & Osunkoya, A. O.

Micropapillary urothelial carcinoma of the urinary bladder: a clinicopathological analysis of 24 cases. *Int. J. Urol.* **18**, 49–54 (2011).
176. Fernández, M. I. *et al.* Clinical risk stratification in patients with surgically resectable micropapillary bladder cancer. *BJU Int.* **119**, 684–691 (2017).
177. *AJCC Cancer Staging Manual*. (Springer International Publishing).
178. Leivo, M. Z. *et al.* Analysis of T1 Bladder Cancer on Biopsy and Transurethral Resection Specimens: Comparison and Ranking of T1 Quantification Approaches to Predict Progression to Muscularis Propria Invasion. *Am. J. Surg. Pathol.* **42**, e1–e10 (2018).
179. Soria, F., Dutto, D. & Gontero, P. Clinical and biological markers for risk-stratification of T1 high-grade non-muscle invasive bladder cancer. *Curr. Opin. Urol.* **32**, 517–522 (2022).
180. Castaneda, P. R., Theodorescu, D., Rosser, C. J. & Ahdoot, M. Identifying novel biomarkers associated with bladder cancer treatment outcomes. *Front. Oncol.* **13**, 1114203 (2023).
181. Chang, S. S. *et al.* Treatment of Non-Metastatic Muscle-Invasive Bladder Cancer: AUA/ASCO/ASTRO/SUO Guideline. *J. Urol.* **198**, 552–559 (2017).
182. Flaig, T. W. *et al.* NCCN Guidelines® Insights: Bladder Cancer, Version 2.2022. *J. Natl. Compr. Canc. Netw.* **20**, 866–878 (2022).
183. Witjes, J. A. *et al.* European Association of Urology Guidelines on Muscle-invasive and Metastatic Bladder Cancer: Summary of the 2020 Guidelines. *Eur. Urol.* **79**, 82–104 (2021).
184. Hensley, P. J. *et al.* Contemporary Staging for Muscle-Invasive Bladder Cancer: Accuracy and Limitations. *Eur Urol Oncol* **5**, 403–411 (2022).
185. Mirmomen, S. M., Shinagare, A. B., Williams, K. E., Silverman, S. G. & Malayeri, A. A. Preoperative imaging for locoregional staging of bladder cancer. *Abdom Radiol (NY)* **44**, 3843–3857 (2019).
186. Tekes, A. *et al.* Dynamic MRI of bladder cancer: evaluation of staging accuracy. *AJR Am. J. Roentgenol.* **184**, 121–127 (2005).
187. Cornelissen, S. W. E., Veenboer, P. W., Wessels, F. J. & Meijer, R. P. Diagnostic Accuracy of Multiparametric MRI for Local Staging of Bladder Cancer: A Systematic Review and Meta-Analysis. *Urology* **145**, 22–29 (2020).
188. Gurram, S., Muthigi, A., Egan, J. & Stamatakis, L. Imaging in Localized Bladder Cancer: Can Current Diagnostic Modalities Provide Accurate Local Tumor Staging? *Curr. Urol. Rep.* **20**, 82 (2019).
189. Panebianco, V. *et al.* Multiparametric Magnetic Resonance Imaging for Bladder Cancer: Development of VI-RADS (Vesical Imaging-Reporting And Data System). *Eur. Urol.* **74**, 294–306 (2018).
190. Bandini, M. *et al.* The Value of Multiparametric Magnetic Resonance Imaging Sequences to Assist in the Decision Making of Muscle-invasive Bladder Cancer. *Eur Urol Oncol* **4**, 829–833 (2021).
191. Furrer, M. A. *et al.* Routine Preoperative Bone Scintigraphy Has Limited Impact on the Management of Patients with Invasive Bladder Cancer. *Eur Urol Focus* **7**, 1052–1060 (2021).
192. Ha, H. K., Koo, P. J. & Kim, S.-J. Diagnostic Accuracy of F-18 FDG PET/CT for Preoperative Lymph Node Staging in Newly Diagnosed Bladder Cancer Patients: A Systematic Review and Meta-Analysis. *Oncology* **95**, 31–38 (2018).
193. Mertens, L. S., Meijer, R. P. & Alfred Witjes, J. Positron Emission Tomography/Computed Tomography for Staging of Bladder Cancer: A Continuing Clinical Controversy. *Eur. Urol.* **83**, 95–96 (2023).
194. Apolo, A. B. *et al.* Clinical value of fluorine-18 2-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography in bladder cancer. *J. Clin. Oncol.* **28**, 3973–3978 (2010).
195. Kibel, A. S. *et al.* Prospective study of [18F]fluorodeoxyglucose positron emission tomography/computed tomography for staging of muscle-invasive bladder carcinoma. *J. Clin. Oncol.* **27**, 4314–4320 (2009).

196. van Kessel, K. E. M. *et al.* Molecular Markers Increase Precision of the European Association of Urology Non-Muscle-Invasive Bladder Cancer Progression Risk Groups. *Clin. Cancer Res.* **24**, 1586–1593 (2018).
197. Bellmunt, J. *et al.* Genomic Predictors of Good Outcome, Recurrence, or Progression in High-Grade T1 Non–Muscle-Invasive Bladder Cancer. *Cancer Research* vol. 80 4476–4486 Preprint at https://doi.org/10.1158/0008-5472.can-20-0977 (2020).
198. Dyrskjøt, L. *et al.* A molecular signature in superficial bladder carcinoma predicts clinical outcome. *Clin. Cancer Res.* **11**, 4029–4036 (2005).
199. Dyrskjøt, L. *et al.* Prognostic Impact of a 12-gene Progression Score in Non-muscle-invasive Bladder Cancer: A Prospective Multicentre Validation Study. *Eur. Urol.* **72**, 461–469 (2017).
200. Sjödahl, G. *et al.* A molecular taxonomy for urothelial carcinoma. *Clin. Cancer Res.* **18**, 3377–3386 (2012).
201. Damrauer, J. S. *et al.* Intrinsic subtypes of high-grade bladder cancer reflect the hallmarks of breast cancer biology. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 3110–3115 (2014).
202. Sjödahl, G., Eriksson, P., Liedberg, F. & Höglund, M. Molecular classification of urothelial carcinoma: global mRNA classification versus tumour-cell phenotype classification. *J. Pathol.* **242**, 113–125 (2017).
203. Kamoun, A. *et al.* A Consensus Molecular Classification of Muscle-invasive Bladder Cancer. *Eur. Urol.* (2019) doi:10.1016/j.eururo.2019.09.006.
A consensus classification system for MIBC that includes six subtype classes, demonstrating differences in underlying oncogenic mechanisms, infiltration by immune and stromal cells, and histological and clinical characteristics, including outcomes.
204. Kates, M. *et al.* Adaptive Immune Resistance to Intravesical BCG in Non–Muscle Invasive Bladder Cancer: Implications for Prospective BCG-Unresponsive Trials. *Clinical Cancer Research* vol. 26 882–891 Preprint at https://doi.org/10.1158/1078-0432.ccr-19-1920 (2020).
205. Strandgaard, T. *et al.* Elevated T-cell Exhaustion and Urinary Tumor DNA Levels Are Associated with Bacillus Calmette-Guérin Failure in Patients with Non–muscle-invasive Bladder Cancer. *Eur. Urol.* **82**, 646–656 (2022).
206. de Jong, F. C. *et al.* Non-muscle-invasive bladder cancer molecular subtypes predict differential response to intravesical Bacillus Calmette-Guérin. *Sci. Transl. Med.* **15**, eabn4118 (2023).
207. Van Allen, E. M. *et al.* Somatic ERCC2 mutations correlate with cisplatin sensitivity in muscle-invasive urothelial carcinoma. *Cancer Discov.* **4**, 1140–1153 (2014).
208. Liu, D. *et al.* Clinical Validation of Chemotherapy Response Biomarker ERCC2 in Muscle-Invasive Urothelial Bladder Carcinoma. *JAMA Oncol* **2**, 1094–1096 (2016).
209. Magliocco, A. M., Moughan, J. & Miyamoto, D. T. Analysis of MRE11 and Mortality Among Adults With Muscle-Invasive Bladder Cancer Managed With Trimodality Therapy. *JAMA Network* (2022).
210. Miyamoto, D. T., Mouw, K. W., Feng, F. Y., Shipley, W. U. & Efstathiou, J. A. Molecular biomarkers in bladder preservation therapy for muscle-invasive bladder cancer. *Lancet Oncol.* **19**, e683–e695 (2018).
211. Geynisman, D. M. *et al.* A phase II trial of risk-enabled therapy after initiating neoadjuvant chemotherapy for bladder cancer (RETAIN). *J. Clin. Orthod.* **41**, 438–438 (2023).
212. Mariathasan, S. *et al.* TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature* **554**, 544 (2018).
213. Taber, A. *et al.* Molecular correlates of cisplatin-based chemotherapy response in muscle invasive bladder cancer by integrated multi-omics analysis. *Nature Communications* vol. 11 Preprint at https://doi.org/10.1038/s41467-020-18640-0 (2020).
214. Sjödahl, G. *et al.* Different Responses to Neoadjuvant Chemotherapy in Urothelial Carcinoma Molecular Subtypes. *Eur. Urol.* **81**, 523–532 (2022).
215. Seiler, R. *et al.* Impact of Molecular Subtypes in Muscle-invasive Bladder Cancer on

Predicting Response and Survival after Neoadjuvant Chemotherapy. *Eur. Urol.* **72**, 544–554 (2017).
216. Efstathiou, J. A. *et al.* Impact of Immune and Stromal Infiltration on Outcomes Following Bladder-Sparing Trimodality Therapy for Muscle-Invasive Bladder Cancer. *Eur. Urol.* **76**, 59–68 (2019).
217. Lindskrog, S. V. *et al.* Single-nucleus and Spatially Resolved Intratumor Subtype Heterogeneity in Bladder Cancer. *Eur Urol Open Sci* **51**, 78–88 (2023).
218. Poletajew, S. *et al.* The Learning Curve for Transurethral Resection of Bladder Tumour: How Many is Enough to be Independent, Safe and Effective Surgeon? *J. Surg. Educ.* **77**, 978–985 (2020).
219. Divrik, R. T., Sahin, A. F., Yildirim, U., Altok, M. & Zorlu, F. Impact of routine second transurethral resection on the long-term outcome of patients with newly diagnosed pT1 urothelial carcinoma with respect to recurrence, progression rate, and disease-specific survival: a prospective randomised clinical trial. *Eur. Urol.* **58**, 185–190 (2010).
220. Yanagisawa, T. *et al.* Repeat Transurethral Resection for Non-muscle-invasive Bladder Cancer: An Updated Systematic Review and Meta-analysis in the Contemporary Era. *Eur Urol Focus* (2023) doi:10.1016/j.euf.2023.07.002.
221. Kirk, P. S. *et al.* Impact of Maximal Transurethral Resection on Pathological Outcomes at Cystectomy in a Large, Multi-institutional Cohort. *J. Urol.* **209**, 882–889 (2023).
222. Giacalone, N. J. *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.* **71**, 952–960 (2017).
223. Kitamura, K., Kataoka, K., Fujioka, H. & Kashiwai, K. Transurethral resection of a bladder tumor by the use of a polypectomy snare. *J. Urol.* **124**, 808–809 (1980).
224. Teoh, J. Y.-C. *et al.* En-bloc resection of bladder tumour as primary treatment for patients with non-muscle-invasive bladder cancer: routine implementation in a multi-centre setting. *World J. Urol.* **39**, 3353–3358 (2021).
225. Gallioli, A. *et al.* En Bloc Versus Conventional Transurethral Resection of Bladder Tumors: A Single-center Prospective Randomized Noninferiority Trial. *Eur Urol Oncol* **5**, 440–448 (2022).
226. D'Andrea, D. *et al.* En Bloc Versus Conventional Resection of Primary Bladder Tumor (eBLOC): A Prospective, Multicenter, Open-label, Phase 3 Randomized Controlled Trial. *Eur Urol Oncol* (2023) doi:10.1016/j.euo.2023.07.010.
227. Teoh, Y. C. J. *et al.* A0707 - Transurethral en bloc resection versus standard resection of bladder tumour: A multi-center randomized trial (EB-StaR Study). *Eur. Urol.* **83**, S997–S998 (2023).
228. Sylvester, R. J. *et al.* Systematic Review and Individual Patient Data Meta-analysis of Randomized Trials Comparing a Single Immediate Instillation of Chemotherapy After Transurethral Resection with Transurethral Resection Alone in Patients with Stage pTa-pT1 Urothelial Carcinoma of the Bladder: Which Patients Benefit from the Instillation? *Eur. Urol.* **69**, 231–244 (2016).
229. Mertens, L. S., Meinhardt, W., Rier, W. B., Nooter, R. I. & Horenblas, S. Extravasation of intravesical chemotherapy for non-muscle-invasive bladder cancer. *Urol. Int.* **89**, 332–336 (2012).
230. Xu, Y. *et al.* Comparing the treatment outcomes of potassium-titanyl-phosphate laser vaporization and transurethral electroresection for primary nonmuscle-invasive bladder cancer: A prospective, randomized study. *Lasers Surg. Med.* **47**, 306–311 (2015).
231. Planelles Gómez, J., Olmos Sánchez, L., Cardosa Benet, J. J., Martínez López, E. & Vidal Moreno, J. F. Holmium YAG Photocoagulation: Safe and Economical Alternative to Transurethral Resection in Small Nonmuscle-Invasive Bladder Tumors. *J. Endourol.* **31**, 674–678 (2017).
232. Gofrit, O. N., Pode, D., Lazar, A., Katz, R. & Shapiro, A. Watchful waiting policy in

recurrent Ta G1 bladder tumors. *Eur. Urol.* **49**, 303–6; discussion 306–7 (2006).
233. Morales, A., Eidinger, D. & Bruce, A. W. Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. *J. Urol.* **116**, 180–183 (1976).
234. Oddens, J. *et al.* Final results of an EORTC-GU cancers group randomized study of maintenance bacillus Calmette-Guérin in intermediate- and high-risk Ta, T1 papillary carcinoma of the urinary bladder: one-third dose versus full dose and 1 year versus 3 years of maintenance. *Eur. Urol.* **63**, 462–472 (2013).
235. Balasubramanian, A. *et al.* Adjuvant therapies for non-muscle-invasive bladder cancer: advances during BCG shortage. *World J. Urol.* **40**, 1111–1124 (2022).
236. Ourfali, S. *et al.* Recurrence Rate and Cost Consequence of the Shortage of Bacillus Calmette-Guérin Connaught Strain for Bladder Cancer Patients. *Eur Urol Focus* **7**, 111–116 (2021).
237. Boorjian, S. A. *et al.* Intravesical nadofaragene firadenovec gene therapy for BCG-unresponsive non-muscle-invasive bladder cancer: a single-arm, open-label, repeat-dose clinical trial. *Lancet Oncol.* **22**, 107–117 (2021).
238. Balar, A. V. *et al.* Pembrolizumab monotherapy for the treatment of high-risk non-muscle-invasive bladder cancer unresponsive to BCG (KEYNOTE-057): an open-label, single-arm, multicentre, phase 2 study. *Lancet Oncol.* **22**, 919–930 (2021).
239. Shang, P. F. *et al.* Intravesical Bacillus Calmette-Guérin versus epirubicin for Ta and T1 bladder cancer. *Cochrane Database Syst. Rev.* CD006885 (2011).
240. Malmström, P.-U. *et al.* An individual patient data meta-analysis of the long-term outcome of randomised studies comparing intravesical mitomycin C versus bacillus Calmette-Guérin for non-muscle-invasive bladder cancer. *Eur. Urol.* **56**, 247–256 (2009).
241. Huncharek, M., Geschwind, J. F., Witherspoon, B., McGarry, R. & Adcock, D. Intravesical chemotherapy prophylaxis in primary superficial bladder cancer: a meta-analysis of 3703 patients from 11 randomized trials. *J. Clin. Epidemiol.* **53**, 676–680 (2000).
242. Gschwend, J. E. *et al.* Extended Versus Limited Lymph Node Dissection in Bladder Cancer Patients Undergoing Radical Cystectomy: Survival Results from a Prospective, Randomized Trial. *Eur. Urol.* **75**, 604–611 (2019).
243. Lerner, S. P. *et al.* SWOG S1011: A phase III surgical trial to evaluate the benefit of a standard versus an extended lymphadenectomy performed at time of radical cystectomy for muscle invasive urothelial cancer. *J. Clin. Orthod.* **41**, 4508–4508 (2023).
244. Lee, R. K. *et al.* Urinary diversion after radical cystectomy for bladder cancer: options, patient selection, and outcomes. *BJU Int.* **113**, 11–23 (2014).
245. Kowalewski, K.-F. *et al.* Robotic-assisted Versus Laparoscopic Versus Open Radical Cystectomy-A Systematic Review and Network Meta-analysis of Randomized Controlled Trials. *Eur Urol Focus* **9**, 480–490 (2023).
246. Zhang, J. H. *et al.* Large Single Institution Comparison of Perioperative Outcomes and Complications of Open Radical Cystectomy, Intracorporeal Robot-Assisted Radical Cystectomy and Robotic Extracorporeal Approach. *J. Urol.* **203**, 512–521 (2020).
247. Teoh, J. Y.-C. *et al.* Perioperative Outcomes of Robot-Assisted Radical Cystectomy with Intracorporeal Versus Extracorporeal Urinary Diversion. *Ann. Surg. Oncol.* **28**, 9209–9215 (2021).
248. Baumann, B. C. *et al.* Validating a Local Failure Risk Stratification for Use in Prospective Studies of Adjuvant Radiation Therapy for Bladder Cancer. *Int. J. Radiat. Oncol. Biol. Phys.* **95**, 703–706 (2016).
249. Baumann, B. C. *et al.* Development and Validation of Consensus Contouring Guidelines for Adjuvant Radiation Therapy for Bladder Cancer After Radical Cystectomy. *Int. J. Radiat. Oncol. Biol. Phys.* **96**, 78–86 (2016).
250. Zaghloul, M. S. *et al.* Adjuvant Sandwich Chemotherapy Plus Radiotherapy vs Adjuvant Chemotherapy Alone for Locally Advanced Bladder Cancer After Radical Cystectomy: A Randomized Phase 2 Trial. *JAMA Surg.* **153**, e174591 (2018).

251. Peak, T. C. & Hemal, A. Partial cystectomy for muscle-invasive bladder cancer: a review of the literature. *Transl. Androl. Urol.* **9**, 2938–2945 (2020).
252. Compérat, E. *et al.* Current best practice for bladder cancer: a narrative review of diagnostics and treatments. *Lancet* **400**, 1712–1721 (2022).
253. Huddart, R. A., Hall, E., Lewis, R., Birtle, A. & SPARE Trial Management Group. Life and death of spare (selective bladder preservation against radical excision): reflections on why the spare trial closed. *BJU Int.* **106**, 753–755 (2010).
254. Vashistha, V. *et al.* Radical Cystectomy Compared to Combined Modality Treatment for Muscle-Invasive Bladder Cancer: A Systematic Review and Meta-Analysis. *Int. J. Radiat. Oncol. Biol. Phys.* **97**, 1002–1020 (2017).
255. Mak, R. H. *et al.* Long-term outcomes in patients with muscle-invasive bladder cancer after selective bladder-preserving combined-modality therapy: a pooled analysis of Radiation Therapy Oncology Group protocols 8802, 8903, 9506, 9706, 9906, and 0233. *J. Clin. Oncol.* **32**, 3801–3809 (2014).
256. Kamran, S. C. & Efstathiou, J. A. The Legacy of RTOG/NRG Protocols in Shaping Current Bladder Preservation Therapy in North America. *Semin. Radiat. Oncol.* **33**, 26–34 (2023).
257. James, N. D. *et al.* Radiotherapy with or without chemotherapy in muscle-invasive bladder cancer. *N. Engl. J. Med.* **366**, 1477–1488 (2012).
258. Zlotta, A. R. *et al.* Radical cystectomy versus trimodality therapy for muscle-invasive bladder cancer: a multi-institutional propensity score matched and weighted analysis. *Lancet Oncol.* (2023) doi:10.1016/S1470-2045(23)00170-5.
In the absence of randomized trials, this is the most definitive work suggesting that trimodality therapy for muscle-invasive bladder cancer is of value and should be considered in patient management.
259. Coen, J. J. *et al.* Bladder Preservation With Twice-a-Day Radiation Plus Fluorouracil/Cisplatin or Once Daily Radiation Plus Gemcitabine for Muscle-Invasive Bladder Cancer: NRG/RTOG 0712-A Randomized Phase II Trial. *J. Clin. Oncol.* **37**, 44–51 (2019).
260. US National Library of Medicine. ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT03775265 (2023).
261. US National Library of Medicine. ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT04241185 (2023)
262. Pieretti, A. *et al.* Complications and Outcomes of Salvage Cystectomy after Trimodality Therapy. *J. Urol.* **206**, 29–36 (2021).
263. Yerramilli, D., Moghanaki, D. M. & Efstathiou, J. A. Safeguarding Autonomy of Patients With Bladder Cancer. *Int. J. Radiat. Oncol. Biol. Phys.* **103**, 81–83 (2019).
264. Dahl, D. M. *et al.* NRG Oncology/RTOG 0926: Phase II Protocol for Patients With Stage T1 Bladder Cancer to Evaluate Selective Bladder Preserving Treatment by Radiation Therapy Concurrent With Radiosensitizing Chemotherapy Following a Thorough Transurethral Surgical Re-Staging. *Int. J. Radiat. Oncol. Biol. Phys.* **111**, S133–S134 (2021).
265. Seisen, T. *et al.* Efficacy of High-Intensity Local Treatment for Metastatic Urothelial Carcinoma of the Bladder: A Propensity Score-Weighted Analysis From the National Cancer Data Base. *J. Clin. Oncol.* **34**, 3529–3536 (2016).
266. Fischer-Valuck, B. W. *et al.* Association Between Local Radiation Therapy to the Primary Bladder Tumor and Overall Survival for Patients with Metastatic Urothelial Cancer Receiving Systemic Chemotherapy. *Eur Urol Oncol* **5**, 246–250 (2022).
267. Lehmann, J. *et al.* Surgery for metastatic urothelial carcinoma with curative intent: the German experience (AUO AB 30/05). *Eur. Urol.* **55**, 1293–1299 (2009).
268. Palma, D. A. *et al.* Stereotactic Ablative Radiotherapy for the Comprehensive Treatment of Oligometastatic Cancers: Long-Term Results of the SABR-COMET Phase II Randomized Trial. *J. Clin. Oncol.* **38**, 2830–2838 (2020).
269. International Collaboration of Trialists *et al.* International phase III trial assessing neoadjuvant cisplatin, methotrexate, and vinblastine chemotherapy for muscle-invasive bladder

cancer: long-term results of the BA06 30894 trial. *J. Clin. Oncol.* **29**, 2171–2177 (2011).
270. Grossman, H. B. *et al.* Neoadjuvant chemotherapy plus cystectomy compared with cystectomy alone for locally advanced bladder cancer. *N. Engl. J. Med.* **349**, 859–866 (2003).
271. Advanced Bladder Cancer (ABC) Meta-analysis Collaboration. Neoadjuvant chemotherapy in invasive bladder cancer: update of a systematic review and meta-analysis of individual patient data advanced bladder cancer (ABC) meta-analysis collaboration. *Eur. Urol.* **48**, 202–5; discussion 205–6 (2005).
272. Galsky, M. D. *et al.* Comparative effectiveness of gemcitabine plus cisplatin versus methotrexate, vinblastine, doxorubicin, plus cisplatin as neoadjuvant therapy for muscle-invasive bladder cancer. *Cancer* **121**, 2586–2593 (2015).
273. Flaig, T. W. *et al.* A Randomized Phase II Study of Coexpression Extrapolation (COXEN) with Neoadjuvant Chemotherapy for Bladder Cancer (SWOG S1314; NCT02177695). *Clin. Cancer Res.* **27**, 2435–2441 (2021).
274. Pfister, C. *et al.* Dose-Dense Methotrexate, Vinblastine, Doxorubicin, and Cisplatin or Gemcitabine and Cisplatin as Perioperative Chemotherapy for Patients With Nonmetastatic Muscle-Invasive Bladder Cancer: Results of the GETUG-AFU V05 VESPER Trial. *J. Clin. Oncol.* **40**, 2013–2022 (2022).
275. Sternberg, C. N. *et al.* Immediate versus deferred chemotherapy after radical cystectomy in patients with pT3-pT4 or N+ M0 urothelial carcinoma of the bladder (EORTC 30994): an intergroup, open-label, randomised phase 3 trial. *Lancet Oncol.* **16**, 76–86 (2015).
276. Galsky, M. D. *et al.* Effectiveness of Adjuvant Chemotherapy for Locally Advanced Bladder Cancer. *J. Clin. Oncol.* **34**, 825–832 (2016).
277. Bajorin, D. F. *et al.* Adjuvant Nivolumab versus Placebo in Muscle-Invasive Urothelial Carcinoma. *N. Engl. J. Med.* **384**, 2102–2114 (2021).
This trial demonstrated an improvement in disease-free survival with adjuvant PD-1 inhibition versus placebo as adjuvant therapy for patients with high-risk muscle-invasive urothelial cancer after radical resection.
278. Bellmunt, J. *et al.* Adjuvant atezolizumab versus observation in muscle-invasive urothelial carcinoma (IMvigor010): a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol.* **22**, 525–537 (2021).
279. Loehrer, P. J., Sr *et al.* A randomized comparison of cisplatin alone or in combination with methotrexate, vinblastine, and doxorubicin in patients with metastatic urothelial carcinoma: a cooperative group study. *J. Clin. Oncol.* **10**, 1066–1073 (1992).
280. Gabrilove, J. L. *et al.* Effect of granulocyte colony-stimulating factor on neutropenia and associated morbidity due to chemotherapy for transitional-cell carcinoma of the urothelium. *N. Engl. J. Med.* **318**, 1414–1422 (1988).
281. Sternberg, C. N. *et al.* Seven year update of an EORTC phase III trial of high-dose intensity M-VAC chemotherapy and G-CSF versus classic M-VAC in advanced urothelial tract tumours. *Eur. J. Cancer* **42**, 50–54 (2006).
282. von der Maase, H. *et al.* Long-term survival results of a randomized trial comparing gemcitabine plus cisplatin, with methotrexate, vinblastine, doxorubicin, plus cisplatin in patients with bladder cancer. *J. Clin. Oncol.* **23**, 4602–4608 (2005).
283. Galsky, M. D. *et al.* A consensus definition of patients with metastatic urothelial carcinoma who are unfit for cisplatin-based chemotherapy. *Lancet Oncol.* **12**, 211–214 (2011).
284. De Santis, M. *et al.* Randomized phase II/III trial assessing gemcitabine/carboplatin and methotrexate/carboplatin/vinblastine in patients with advanced urothelial cancer who are unfit for cisplatin-based chemotherapy: EORTC study 30986. *J. Clin. Oncol.* **30**, 191–199 (2012).
285. Sharma, P. *et al.* Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): a multicentre, single-arm, phase 2 trial. *Lancet Oncol.* **18**, 312–322 (2017).
286. Patel, M. R. *et al.* Avelumab in metastatic urothelial carcinoma after platinum failure (JAVELIN Solid Tumor): pooled results from two expansion cohorts of an open-label, phase 1 trial. *Lancet Oncol.* **19**, 51–64 (2018).

287. Bellmunt, J. *et al.* Pembrolizumab as Second-Line Therapy for Advanced Urothelial Carcinoma. *N. Engl. J. Med.* **376**, 1015–1026 (2017).
This trial demonstrated an improvement in survival with PD-1 inhibition versus chemotherapy in patients with metastatic urothelial cancer progressing despite prior platinum-based chemotherapy.
288. Balar, A. V. *et al.* First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. *Lancet Oncol.* **18**, 1483–1492 (2017).
289. Balar, A. V. *et al.* Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. *Lancet* **389**, 67–76 (2017).
290. Galsky, M. D. *et al.* Atezolizumab with or without chemotherapy in metastatic urothelial cancer (IMvigor130): a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet* **395**, 1547–1557 (2020).
291. Powles, T. *et al.* Pembrolizumab alone or combined with chemotherapy versus chemotherapy as first-line therapy for advanced urothelial carcinoma (KEYNOTE-361): a randomised, open-label, phase 3 trial. *Lancet Oncol.* **22**, 931–945 (2021).
292. Galsky, M. D. *et al.* Randomized Double-Blind Phase II Study of Maintenance Pembrolizumab Versus Placebo After First-Line Chemotherapy in Patients With Metastatic Urothelial Cancer. *J. Clin. Oncol.* **38**, 1797–1806 (2020).
293. Powles, T. *et al.* Avelumab Maintenance Therapy for Advanced or Metastatic Urothelial Carcinoma. *N. Engl. J. Med.* **383**, 1218–1230 (2020).
This trial demonstrated an improvement in survival with switch maintenance PD-L1 inhibition versus surveillance after first-line chemotherapy in patients with metastatic urothelial cancer.
294. Rugo, H. S. *et al.* LBA76 Overall survival (OS) results from the phase III TROPiCS-02 study of sacituzumab govitecan (SG) vs treatment of physician's choice (TPC) in patients (pts) with HR+/HER2-metastatic breast cancer (mBC). *Ann. Oncol.* **33**, S1386 (2022).
295. Catto, J. W. F. *et al.* Quality of Life After Bladder Cancer: A Cross-sectional Survey of Patient-reported Outcomes. *Eur. Urol.* **79**, 621–632 (2021).
296. Yoshimura, K. *et al.* Impact of superficial bladder cancer and transurethral resection on general health-related quality of life: an SF-36 survey. *Urology* **65**, 290–294 (2005).
297. Siracusano, S. *et al.* Health-related quality of life after BCG or MMC induction for non-muscle invasive bladder cancer. *Can. J. Urol.* **25**, 9480–9485 (2018).
298. Wei, L., Li, Q., Liang, H. & Jianbo, L. The quality of life in patients during intravesical treatment and correlation with local symptoms. *J. Chemother.* **26**, 165–168 (2014).
299. Yang, L. S. *et al.* A systematic review and meta-analysis of quality of life outcomes after radical cystectomy for bladder cancer. *Surg. Oncol.* **25**, 281–297 (2016).
300. Catto, J. W. F. *et al.* Effect of Robot-Assisted Radical Cystectomy With Intracorporeal Urinary Diversion vs Open Radical Cystectomy on 90-Day Morbidity and Mortality Among Patients With Bladder Cancer: A Randomized Clinical Trial. *JAMA* **327**, 2092–2103 (2022).
301. Efstathiou, J. A. *et al.* Late pelvic toxicity after bladder-sparing therapy in patients with invasive bladder cancer: RTOG 89-03, 95-06, 97-06, 99-06. *J. Clin. Oncol.* **27**, 4055–4061 (2009).
302. Huddart, R. A. *et al.* Patient-reported Quality of Life Outcomes in Patients Treated for Muscle-invasive Bladder Cancer with Radiotherapy ± Chemotherapy in the BC2001 Phase III Randomised Controlled Trial. *Eur. Urol.* **77**, 260–268 (2020).
303. Zietman, A. L. *et al.* Organ conservation in invasive bladder cancer by transurethral resection, chemotherapy and radiation: results of a urodynamic and quality of life study on long-term survivors. *J. Urol.* **170**, 1772–1776 (2003).
304. Mak, K. S. *et al.* Quality of Life in Long-term Survivors of Muscle-Invasive Bladder Cancer. *Int. J. Radiat. Oncol. Biol. Phys.* **96**, 1028–1036 (2016).

305. Westergren, D.-O., Gårdmark, T., Lindhagen, L., Chau, A. & Malmström, P.-U. A Nationwide, Population Based Analysis of Patients with Organ Confined, Muscle Invasive Bladder Cancer Not Receiving Curative Intent Therapy in Sweden from 1997 to 2014. *J. Urol.* **202**, 905–912 (2019).
306. Degboe, A., Ivanescu, C., Rohay, J. M., Turner, R. R. & Cella, D. Validity and performance of the Functional Assessment of Cancer Therapy-Bladder (FACT-Bl) among advanced urothelial cancer patients. *Support. Care Cancer* **27**, 4189–4198 (2019).
307. Kitamura, H. *et al.* Effect of neoadjuvant chemotherapy on health-related quality of life in patients with muscle-invasive bladder cancer: results from JCOG0209, a randomized phase III study. *Jpn. J. Clin. Oncol.* **50**, 1464–1469 (2020).
308. Witjes, J. A. *et al.* Health-related Quality of Life with Adjuvant Nivolumab After Radical Resection for High-risk Muscle-invasive Urothelial Carcinoma: Results from the Phase 3 CheckMate 274 Trial. *Eur Urol Oncol* **5**, 553–563 (2022).
309. Grivas, P. *et al.* Patient-reported Outcomes from JAVELIN Bladder 100: Avelumab First-line Maintenance Plus Best Supportive Care Versus Best Supportive Care Alone for Advanced Urothelial Carcinoma. *Eur. Urol.* **83**, 320–328 (2023).
310. Vaughn, D. J. *et al.* Health-Related Quality-of-Life Analysis From KEYNOTE-045: A Phase III Study of Pembrolizumab Versus Chemotherapy for Previously Treated Advanced Urothelial Cancer. *J. Clin. Oncol.* **36**, 1579–1587 (2018).
311. Mariotto, A. B., Enewold, L., Zhao, J., Zeruto, C. A. & Yabroff, K. R. Medical Care Costs Associated with Cancer Survivorship in the United States. *Cancer Epidemiol. Biomarkers Prev.* **29**, 1304–1312 (2020).
312. Leal, J., Luengo-Fernandez, R., Sullivan, R. & Witjes, J. A. Economic Burden of Bladder Cancer Across the European Union. *Eur. Urol.* **69**, 438–447 (2016).
313. Botteman, M. F., Pashos, C. L., Redaelli, A., Laskin, B. & Hauser, R. The health economics of bladder cancer: a comprehensive review of the published literature. *Pharmacoeconomics* **21**, 1315–1330 (2003).
314. Yeung, C., Dinh, T. & Lee, J. The health economics of bladder cancer: an updated review of the published literature. *Pharmacoeconomics* **32**, 1093–1104 (2014).
315. Joyce, D. D., Sharma, V. & Williams, S. B. Cost-Effectiveness and Economic Impact of Bladder Cancer Management: An Updated Review of the Literature. *Pharmacoeconomics* **41**, 751–769 (2023).
316. Kandoi, G., Acencio, M. L. & Lemke, N. Prediction of Druggable Proteins Using Machine Learning and Systems Biology: A Mini-Review. *Front. Physiol.* **6**, 366 (2015).
317. Koprowski, R. & Foster, K. R. Machine learning and medicine: book review and commentary. *Biomed. Eng. Online* **17**, 17 (2018).
318. Sanli, O. *et al.* Bladder cancer. *Nat Rev Dis Primers* **3**, 17022 (2017).
319. Loriot, Y. *et al.* Erdafitinib in Locally Advanced or Metastatic Urothelial Carcinoma. *N. Engl. J. Med.* **381**, 338–348 (2019).
320. Powles, T. *et al.* Enfortumab Vedotin in Previously Treated Advanced Urothelial Carcinoma. *N. Engl. J. Med.* **384**, 1125–1135 (2021).
321. Hoimes, C. J. *et al.* Enfortumab Vedotin Plus Pembrolizumab in Previously Untreated Advanced Urothelial Cancer. *J. Clin. Oncol.* **41**, 22–31 (2023).
322. Tagawa, S. T. *et al.* TROPHY-U-01: A Phase II Open-Label Study of Sacituzumab Govitecan in Patients With Metastatic Urothelial Carcinoma Progressing After Platinum-Based Chemotherapy and Checkpoint Inhibitors. *J. Clin. Oncol.* **39**, 2474–2485 (2021).

Author contributions

Introduction (L.D.; D.T.); Epidemiology (D.E.H); Mechanisms/pathophysiology (L.D.; M.A.K.; D.T.); Diagnosis, screening and prevention (L.D.; D.E.H.; J.T. J.A.E); Management (J.T.; J. A. E.; M.D.G.); Quality of life (J.T.; J.A.E; M.D.G.); Outlook (L.D.; D.T.); Overview of Primer (L.D.; D.T.).

Competing interests

[Au: The below queries relating to the COI of DEH, MDG and JAE have not been answered yet. Please check and answer my queries, and include the updated COI statement in the competing interest form https://www.nature.com/documents/nr-competing-interests.pdf]

L.D. has sponsored research agreements with Natera, C2i Genomics, AstraZeneca, Photocure, and Ferring, has an advisory/consulting role at Ferring, MSD and UroGen, and has received speaker honoraria from AstraZeneca, Pfizer and Roche, and is board member in BioXpedia.

D.E.H. AstraZeneca**[Au: Please specify the type of COI for AstraZeneca.]**

M.D.G. receives or has received**[Au: OK?]** research funding from Bristol Myers Squibb, Novartis, Dendreon, Astra Zeneca, Merck, and Genentech. M.D.G. is or was Advisory Board Member/Consultant**[Au: OK?]** for Bristol Myers Squibb, Merck, Genentech, AstraZeneca, Pfizer, EMD Serono, SeaGen, Janssen, Numab, Dragonfly, GlaxoSmithKline, Basilea, UroGen, Rappta Therapeutics, Alligator, Silverback, Fujifilm, Curis, Gilead, Bicycle, Asieris, Abbvie, Analog Devices.

J.A.E. is or was Consulting/Advisory Board Member and receives or has received honoraria**[Au: OK?]** from Blue Earth Diagnostics, Boston Scientific, AstraZeneca, Lantheus, IBA, Astellas, Pfizer, Merck, Roivant Pharma, Myovant Sciences, Janssen, Bayer Healthcare, Progenics Pharmaceuticals, Genentech, Gilead, Angiodynamics, UptoDate.

The other authors declare no competing interests.

FIGURES

Figure 1: Bladder cancer categories

Bladder cancer can be categorized into grades, which is the cytological appearance of the urothelium, and stages, which is determined by the spread and depth of bladder wall invasion of the tumour. Non-invasive papillary carcinomas are classified as pTa disease, whereas urothelial carcinoma in situ is classified as pTis disease. All invasive urothelial cancers arise from either high-grade papillary carcinoma or urothelial carcinoma situ.

Figure 2: Global incidence of bladder cancer

Global estimated incidence of bladder cancer in 2020 in men and women of all ages. Data are expressed as age-standardized rates (ASR; adjusted to World Standard Population) to account for differing age profiles among regions. Data was obtained from GLOBOCAN 2020. Map was produced by the World Health Organization (WHO) / International Agency for Research (IARC) (https://gco.iarc.fr/today).

Figure 3: Global mortality of bladder cancer

Global estimated mortality due to bladder cancer in 2020 in men and women of all ages. Data are expressed as age-standardized rates (ASR; adjusted to World Standard Population) to account for differing age profiles among regions. Data was obtained from GLOBOCAN 2020. Map was produced by the World Health Organization (WHO) / International Agency for Research (IARC) (https://gco.iarc.fr/today).

Figure 4. Pathogenesis pathways.

Potential pathogenesis pathways to papillary NMIBC and solid invasive MIBC, including key genomic events are shown (**Table 1, Table 2**). Solid arrows indicate pathways for which there is histopathologic and/or molecular evidence. Dashed arrows indicate pathways for which there is uncertainty. Estimated time for tumour development is shown on right.

Figure 5. Histopathology of bladder cancer

Normal urothelium (A) is defined by cellular polarization towards the luminal surface with individual cells relatively monotonous in appearance and containing open chromatin. Low-grade papillary urothelial carcinoma (B) shows papillary cores, in this image cut in cross-section, lined by urothelium that remains relatively monotonous and polarized but with hyperchromasia of some nuclei. Non-invasive high-grade neoplasia in the bladder may be papillary (C) or flat (D) and demonstrates disorganization, nuclear enlargement, nuclear pleomorphism, and hyperchromasia. High-grade lesions have the potential to invade beyond the basement membrane and into the underlying bladder wall.

Figure 6: Landmarks in understanding, diagnosis and treatment of bladder cancer

[Au: Please add a brief legend for this timeline, along the lines of "This timeline shows …" and ideally not re-iterating just the title but providing just a bit more general detail.

This timeline shows the seminal development in the bladder cancer highlighting both clinical, scientific and technical advances that have or will change clinical practice or scientific thinking in the field

In addition, I have now collated references for the timeline in the timeline ppt that I have sent to you. I will add these to the Figure legend once you sent the legend back to me. You do NOT need to add them to the reference list yourself. However, please check that all the references I have selected are correct. For this, I have provided links for each of the references so it's easy for you to quickly go through them. Thank you!]-you picked very well! The really old stuff, you can quote the textbook…I don't know the references and I don't have the book anymore. I just sent you the revised ppt

TABLES

Table 1. Oncogenes activated in bladder cancer

Gene	Chromosome	Frequency (%)			Alteration	Functions affected
		Ta	T1	T2+		
TERT	5p15	70-80	70-80	70-80	Point mutation	Senescence and other functions
FGFR3	4p16	80	30	10-15	Point mutation	Ras-MAPK signaling
		70-80	50-60	40	Upregulated expression	
PIK3CA	3q26	40	20	20	Point mutation	PI3K signaling
HRAS/KRAS	11p15/12p12	10-15	10-15	10-15	Point mutation	Ras-MAPK/PI3K signaling
ERBB2	17q12	≤2	10-15	10-15	Mutation or amplification	Ras-MAPK/PI3K signaling
ERBB3	12q13	≤2	10-15	10-15	Mutation	Ras-MAPK/PI3K signaling
EGFR	7p12	≤2	≥2	11	Amplification	Ras-MAPK/PI3K signaling
PPARG	3p25	≤2	10	15	Amplification	PPARG signaling
		≤2	9	3	Mutation	
RXRA	9q34	2	5	6	Mutation	PPARG signaling
E2F3	6p22	≤2	5-10	10-15	Amplification	Cell cycle regulation
MDM2	12q15	0	5-15	5-15	Amplification	Cell cycle regulation
CCND1	11q13	≤2	10	10	Amplification	Cell cycle regulation
CCNE1	19q12	≤2	≤2	10	Amplification	Cell cycle regulation

Genes with activating mutation or high-level DNA amplification in >10% of at least one bladder cancer stage are shown. If very low frequencies have been found in stage Ta tumors but samples were too few for accurate estimation, ≤2% is shown. Adapted from [318].

Table 2. Genes commonly inactivated by mutation in bladder cancer.

Gene	Chromosome	Frequency (%)			Alteration	Functions affected
		Ta	T1	T2+		
CDKN2A	9p21	30	60	60	Loss of heterozygosity, deletion	Cell cycle
		≤2	12	22	Homozygous deletion	
		1	7	7	Mutation	
RB1	13q14	0	14	17	Inactivating mutation	Cell cycle
ATM	11q22	12	16	14	Inactivating mutation	Cell cycle
CDKN1A	6p21	11	11	9	Inactivating mutation	Cell cycle
TP53	17p13	4	24	48	Inactivating mutation	Transcription
ELF3	1q32	8	22	12	Inactivating mutation	Transcription
ZFP36L1	14q24	12	11	6	Inactivating mutation	Transcription
KDM6A	Xp11	40	40	26	Inactivating mutation	Chromatin regulation
KMT2D	12q13	35	27	28	Inactivating mutation	Chromatin regulation
CREBBP	16p13	23	20	12	Inactivating mutation	Chromatin regulation
KMT2C	7q36	23	14	18	Inactivating mutation	Chromatin regulation
STAG2	Xq25	30	9	14	Inactivating mutation	Chromatin regulation
ARID1A	1p36	11	27	25	Inactivating mutation	Chromatin regulation
KMT2A	11q23	11	15	11	Inactivating mutation	Chromatin regulation
EP300	22q13	15	11	15	Inactivating mutation	Chromatin regulation
ASH1L	1q22	10	12	7	Inactivating mutation	Chromatin regulation
ARID2	12q12	7	11	8	Inactivating mutation	Chromatin regulation
ERCC2	19q13	4	24	18	Inactivating mutation	DNA repair
BRCA2	13q13	10	10	9	Inactivating mutation	DNA repair
PTEN	10q23	7-12	20-30	50	Loss of heterozygosity, deletion, mutation	Regulator of AKT signaling
TSC1	9q34	12	15	8	Inactivating mutation	Regulator of mTOR signaling
RBM10	Xp11	7	13	5	Inactivating mutation	RNA splicing

Genes affected in >10% of at least one bladder cancer stage are shown. Large genes not formally identified as significantly mutated or with unknown function are not listed.

Table 3. New systemic therapies for metastatic bladder cancer*

Drug	Mechanism of action	Evidence	Select adverse events
Erdafitinib	Small molecule inhibitor of fibroblast growth factor receptor 3	In a phase 2 study of patients with FGFR3-mutated metastatic urothelial cancer progressing despite prior platinum-based chemotherapy, erdafitinib demonstrated an objective response rate of 42%[319]	Hyperphosphatemia, stomatitis, hand-foot syndrome, as well as ocular disorders such as central serous retinopathy
Enfotumab vedotin	Antibody-drug conjugate comprised of a monoclonal antibody directed against Nectin-4 linked to a monomethyl auristatin E payload	The phase 3 EV-301 trial[320] randomized patients with metastatic urothelial cancer progressing despite prior platinum-based chemotherapy and PD-1 or PD-L1 blockade to treatment with enfortumab vedotin versus standard chemotherapy (docetaxel, paclitaxel, or vinflunine). The trial demonstrated an improvement in overall survival with enfortumab vedotin versus chemotherapy (HR 0.70; 95% CI 0.56–0.89; P = 0.001). The combination of enfortumab vedotin plus pembrolizumab has been explored as first-line treatment in cisplatin-ineligible patients with metastatic urothelial cancer[321], yielding a 73% response rate.	Peripheral neuropathy, hyperglycemia, rash
Sacituzumab govitecan	Antibody drug conjugate comprising a monoclonal antibody directed against Trop-2 linked to the topoisomerase I inhibitor SN-38 payload	A large phase 2 trial demonstrated an objective response rate of 27% with sacituzumab govitecan in patients with metastatic urothelial cancer progressing despite prior platinum based chemotherapy and PD-1 or PD-L1 immune checkpoint inhibition.[322].	Diarrhea, neutropenia

*New systemic therapies that have received regulatory in at least one region of the world are shown.

BOXES

Box 1. Optimal patient characteristics for trimodality bladder-sparing treatment for muscle-invasive bladder cancer

- Predominant urothelial cancer histology
- Unifocal tumor <7 cm in size
- Visibly complete TURBT
- Clinical stage T2-T3a
- Lack of extensive carcinoma in situ
- Absence of hydronephrosis
- Good bladder function