Molecular and histopathology directed therapy for advanced bladder cancer

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Key points

- Combining sequencing and transcriptomic technologies might improve the identification of clinically relevant bladder cancer subgroups.
- Molecular subtyping has helped us to identify bladder tumours that respond well to cytotoxic chemotherapy.
- Targeted therapies have had a limited role in bladder cancer management, but the next generation of specific targets are showing promise, as exemplified by fibroblast growth factor receptor 3 (FGFR3).
- Immune checkpoint inhibition leads to deep and durable responses in a small subgroup of patients.
- Composite molecular signatures are showing promise as predictors of treatment response and should be tested in prospective clinical trials.
- Real-time serial biopsies during the course of treatment will be required to help direct therapy in an evolving tumour landscape.

Abstract

Bladder cancer is a heterogeneous group of tumours with at least 40 histological subgroups that leads to ~165,000 deaths worldwide each year[1]. Patients with localized disease can be cured with surgical resection or radiotherapy, but such curative options are limited in the setting of recurrent disease or distant spread, in which case systemic therapy is used to control disease and palliate symptoms. Cytotoxic chemotherapy has been the mainstay of treatment for advanced bladder cancer, but high-quality evidence is lacking to inform the management of rare subgroups that are often excluded from studies. Advances in molecular pathology, the development of targeted therapies, and the resurgence of immunotherapy has led to the reclassification of bladder cancer subgroups and rigorous efforts to define predictive biomarkers for cancer therapies. In this Review, we present the current evidence for the management of conventional, variant, and divergent urothelial cancer subtypes, as well as nonurothelial bladder cancers, and discuss how the integration of genomic, transcriptomic and proteomic characterisation of bladder cancer could guide future therapies.

[H1] Introduction

For the first time in over 30 years, the treatment algorithm for advanced bladder cancer is rapidly evolving, with an expanded range of effective therapies beyond cytotoxic chemotherapy now approved (**FIG. 1**). With novel and still-emerging therapeutic advances in the fields of immunotherapy and molecularly targeted therapy, a renewed focus on bladder cancer subtypes has emerged in an attempt to personalize systemic therapy. The vast majority of bladder cancers are urothelial carcinomas, but up to 10% are nonurothelial carcinomas **[G]** [2]. Urothelial carcinomas (previously known as transitional cell carcinoma) can be subdivided according to their histopathological characteristics into conventional urothelial carcinoma **[G]** , urothelial carcinoma with divergent differentiation **[G]** , or variant urothelial carcinoma **[G]**[3].The importance of these categorisations for the correct diagnosis, treatment, and prognostication of bladder cancer is recognized in the 2016 WHO revised classification and pathology guidelines for urogenital cancers[3] (**TABLE 1**). However, most therapeutic clinical trials have historically not included patients with nonurothelial carcinoma and have not reported on the divergent or variant urothelial carcinoma subgroups. The management of these rare histologies, therefore, has been based on extrapolated data from other tumour types and limited clinical experience (for example, small case series) without a deep biological understanding.

In this Review, we outline how new molecular approaches, such as DNA sequencing and RNA profiling, have uncovered distinct biological subtypes of urothelial carcinoma that are prognostic, predictive of treatment responses, and, in many instances, align with the morphological phenotype. We discuss how this molecular taxonomy and histopathological evidence could support the deployment of chemotherapy, immune-checkpoint inhibitors (ICIs), and targeted agents for advanced bladder cancer. In addition, we discuss the pathology-driven management of variant urothelial carcinoma and nonurothelial carcinoma and comment on promising future therapies.

[H1] Molecular profiling and classification

Advances in sequencing technology over the past 15 years have enabled international collaborative efforts to catalogue the spectrum of somatic mutations in solid tumours, including urothelial bladder cancer[4]. Our understanding of the pattern of genomic aberrations and their influence on tumour biology and behaviour has changed substantially as a direct result of this work. Early work exploring phenotypes of bladder cancer postulated that bladder cancer develops along two distinct pathways, the

papillary and non-papillary pathways. In this model, 80% of tumours are papillary exophytic lesions arising from hyperplastic epithelium and are non-muscle-invasive bladder cancers (NMIBCs) that have a propensity for recurrence but are rarely life-threatening, whereas ~20% are non-papillary high-grade muscle-invasive bladder cancers (MIBCs) arising from carcinoma in situ that cause the most morbidity and have a propensity for metastatic spread[5]; these tumours rarely arise from papillary precursors.

Work over the past decade using gene expression and somatic mutational profiling in MIBC has defined the luminal **[G]** and basal **[G]** subgroups as commonly recurring subtypes, although discrepancies between the various classifications have beenreported (**FIG. 2**)[6-9], and subsequent efforts have been made to consolidate the major molecular taxonomies. Substantial work in this area has been undertaken by groups at Lund University, The Cancer Genome Atlas (TCGA), University of North Carolina at Charlotte (UNCC), and MD Anderson Cancer Centre (MDACC).

Luminal bladder cancers can be further separated into urothelial-like urothelial carcinomas (TCGA cluster I), which are enriched for *FGFR3* and *KDM6A* mutations, and genomically unstable urothelial carcinomas (TCGA cluster II), which are characterized by overexpression of peroxisome proliferator-activated receptor γ (PPAR-γ) and enrichment for *TP53* and *ERCC2* mutations[7], with some studies having demonstrated overexpression of the oestrogen receptor α (ERα)[10]. The characteristics of basal-like tumours have been variously described by different groups, including the UNCC[8] and MDACC[7,10], and these tumours correspond to the TCGA cluster III (and arguably the claudin-low TCGA cluster IV) and the Lund University squamous-like group[9]. Basal-like tumours are enriched for epithelial–mesenchymal transition (EMT) gene signatures[11], with loss of *RB1* and *NFE2L2* as well as dysregulation of nuclear factor-κB (NF-κB) and hypoxia-inducible factor 1-α (HIF1α) signalling. A 2016 consensus meeting[12] clarified the molecular taxonomy with respect to the existence of a basal-squamous-like (BASQ) subset, defined by overexpression of cytokeratin 5 (KRT5) and KRT14 and concurrent downregulation of urothelial markers forkhead box protein A1 (FOXA1) and trans-acting T-cell-specific transcription factor GATA3 (GATA3).

Further incremental gains in molecular taxonomy have been made using multiplatform approaches for molecular phenotyping, which have expanded and revised the molecular classifications. A 2017 TCGA study of 412 MIBCs integrated the use of whole-exome sequencing, copy number analysis, methylation analysis, and RNA-based and proteomic inputs[13]. This study revealed >50 recurrently mutated genes and that a high mutational burden in bladder cancer was related to apolipoprotein B mRNA editing

catalytic polypeptide-like (APOBEC)-mediated mutagenesis. Studies from other solid tumours indicate that APOBEC cytidine deaminases, which are operative during physiological RNA editing, can cause clusters of somatic mutations, and APOBEC3A specifically has been linked with the phenomenon of hypermutation[14]. Moreover, APOBEC mutational signatures been shown to be responsible for up to 68% of all mutations seen in a number of solid tumours[15]. Of relevance to MIBC prognosis, unsupervised clustering by mutational signature revealed that tumours with a very high mutational burden had an improved prognosis (with a 5-year survival of 75%)[13]. Furthermore, comprehensive RNA profiling, including long noncoding RNA (lncRNA), microRNA (miRNA), and messenger RNA (mRNA) analysis identified 5 distinct transcriptional and genomic signatures, each of which correlated with a distinct clinical outcome[13]. The 'luminal papillary' group (~35% of the cohort) had the best outcomes, whereas the novel 'neuronal' group (~5% of the cohort), which clinically behave similarly to neuroendocrine tumours but lack histological features of neuroendocrine or small cell differentiation, had the worst outcomes.

Molecular profiling and classification of tumour tissues has yielded insight into tumour biology and patient prognosis across populations. This approach has been explored further to inform the selection of molecularly targeted therapies for individual patients. Indeed, a panel gene sequencing study in 97 high-grade bladder cancer tumours demonstrated potentially druggable gene mutations in pathways such as the mitogen activated protein kinase (MAPK) pathway (for example, *BRAF* and *MEK1/2*) and the phosphoinositide 3-kinase (PI3K) pathway (for example, *PIK3CA*) in 61% of tumours[16]. In addition, international collaborative efforts applying massively parallel next-generation sequencing (NGS) to >100 metastatic urothelial cancers revealed activating mutations in the receptor tyrosine kinase (RTK)–RAS pathway (39% of tumours), including *FGFR3* (14% of tumours) and *ERBB3* (13% of tumours), and in the PI3K–RAC-alpha serine/threonine-protein kinase (AKT)–mechanistic target of rapamycin (mTOR) pathway (38% of tumours), including *PIK3CA* (16% of tumours) and *AKT3* (12% of tumours)[17]. Furthermore, the study was the first to identify activating fusion transcripts of the *FGFR3* gene (producing the FGFR3–TACC3 fusion protein) in three tumours. Many of these point mutations have previously been shown to have prognostic value; for example, *FGFR3* mutations are confer a good long-term cancer-specific survival in MIBC tumours[18].

Moving beyond annotating mutation frequencies in known cancer genes, genomic and transcriptomic data across multiple cancer types coupled with new statistical algorithms have identified rare, mutually exclusive, cancer-associated

mutations by virtue of the fact that they the gene products commonly cluster in the same protein complexes or signalling pathways. In bladder cancer, this approach identified rare somatic mutations in the switch/sucrose nonfermentable (SWI/SNF) chromatin remodelling complex and the ubiquitin carboxyl-terminal hydrolase BAP1 (BAP1) deubiquitinase complex[19]. The identification of gene mutations in components of these complexes, such as *ARID1A* and *BAP1*, could potentially be exploited therapeutically[20,21].

In summary, the aforementioned molecular phenotyping approaches have enabled the sophisticated annotation of distinct groups of invasive bladder cancer. This work is relevant for patients as it has clear implications for the aetiology, biological drivers, and prognosis of these tumours.

[H2] Liquid biopsy

The evolution of molecular and genomic changes that occur during treatment in other tumour types have been well annotated and can inform rational therapies. However, the need for repeat tissue biopsies over time to track and understand the clonal evolution of cancer is challenging for patients, as these biopsies are invasive and their associated sequelae are often unacceptable and are associated with risk. The technical advances in DNA amplification and sequencing that have enabled the attainment of comparable molecular information from liquid biopsies **[G]** have rapidly gained traction. A 2017 study of 51 patients with aggressive or metastatic urothelial carcinoma demonstrated the feasibility of acquiring a snapshot of therapeutically relevant information from circulating tumour DNA (ctDNA)[22]. Potentially tractable amplifications in *ERBB2* were seen in 20% of patients and hotspot mutations in *PIK3CA* were seen in another 20%. This promising real-time molecular data could negate some of the issues associated with inadequate sampling as a result of intratumoral heterogeneity that is inherent to single tissue biopsies, although this issue is counterbalanced by a false negative rate in patients with a ctDNA tumour fraction that is below the level of detection of currently available assays. In addition to plasma, urine has also been shown to yield important molecular information in advanced bladder cancer. Urinary detection of cell-free DNA (cfDNA) has been associated with metastatic relapse in bladder cancer[23], and urine-derived lymphocytes — which reflect the bladder tumour immune microenvironment — exhibit dynamic changes in the expression of immune checkpoint molecules during cytotoxic treatment[24]. Liquid biopsies will become increasing important for the molecular profiling of bladder cancer and, consequently, for prognostication and allocation of therapy.

[H1] Management of urothelial bladder cancer

Conventional urothelial carcinoma is characterised by exophytic papillary-like structures covered by atypical urothelial cells (**TABLE 1**, **FIG. 3a**)[25]. Importantly, urothelial carcinoma has a propensity for divergent differentiation [26](**TABLE 1**, **FIG. 3b,c)**, and a number of urothelial carcinoma variants exist (**TABLE 1**, **FIG. 3d–m**). Currently, most clinicians treat advanced conventional urothelial carcinoma, urothelial carcinoma with divergent differentiation, and variant urothelial carcinoma in the same way, as data to support a stratified approach is lacking. Thus, the main treatment options are chemotherapy and immune checkpoint inhibitors (ICIs), as well as molecularly targeted therapies within clinical studies (**FIG. 1**, **TABLE. 2)**.

[H2] Role of cytotoxic chemotherapy

Combination chemotherapy has been the mainstay of treatment for advanced urothelial carcinoma, a disease that is responsive to chemotherapy in the short term. However, the durability of response is variable and the prognosis of patients with advanced disease remains poor[27]. The antitumour activity of cisplatin-based regimens has been established for decades[28]. Initially, the most frequently used platinum-based chemotherapy combination was methotrexate, vinblastine, doxorubicin and cisplatin (MVAC), on the basis of superior efficacy compared to cisplatin alone in a Phase III study[29]. However, MVAC has considerable toxicity with some studies identifying a 3–4% drug-related death rate[29,30]. The need for an alternative regimen with a similar survival benefit but without the inherent toxicity of MVAC was identified and the combination of gemcitabine and cisplatin (GC) was compared to MVAC in a large, international phase III study of 405 patients with advanced or metastatic bladder cancer[27]. At a median follow-up period of 19 months, the study showed similar overall survival (OS) benefit between the GC and MVAC groups (median OS 13.8 months versus 14.8 months; HR 1.04; *P*=0.75), and the GC group had the desired improved safety profile with lower rates of febrile neutropenia, neutropenic sepsis, and mucositis, as well as fewer drug-related deaths. A 5-year updated analysis confirmed that GC was noninferior to MVAC and, therefore, GC became the standard first-line regimen in many centres world-wide for advanced disease[31]. However, MVAC might still have a role, as a dose-dense regimen with growth factor support had improved efficacy and toxicity compared with standard MVAC; this regimen might be an option for select fit patients[32].

The taxanes have been shown to have some activity in advanced disease. A 2012 study investigated GC in a three-drug regimen with paclitaxel[33]. A higher response rate

was observed for the paclitaxel–cisplatin–gemcitabine regimen compared with GC, but the triplet did not reach the primary predefined end point of OS improvement, and toxicity was also increased in the triplet group. Other combinations in the first-line setting — cisplatin–paclitaxel, gemcitabine–paclitaxel, and docetaxel-based regimens — have shown modest activity, but current data are insufficient to recommend these alternative first-line regimens.

In patients who are cisplatin-ineligible (owing to impaired renal function, poor performance status or comorbidities), carboplatin can used as an alternative to cisplatin. The doublet gemcitabine and carboplatin and the triplet methotrexate, carboplatin, and vinblastine (M-CAVI) are active in patients who are unfit for cisplatin with response rates in the order of 30–40%, with less toxicity seen with the gemcitabine and carboplatin doublet[34]. However, patients with both poor performance status and impaired renal function derived limited benefit, and new strategies are needed.

Following first-line treatment, responses to second-line chemotherapy are highly variable, and progression-free survival (PFS) is short with both single-agent chemotherapy and combination treatments in the second-line setting[35]. Vinflunine, a third-generation vinca alkaloid, showed modest activity compared with best supportive care (ORR 8.6% versus 0%; *P*=0.006), which was sufficient to gain approval in Europe in the second-line setting[36]. Single-agent paclitaxel is well tolerated with some activity[37], but ICIs are now the preferred option in this setting.

Patients relapsing with advanced urothelial carcinoma might have previously received chemotherapy in the neoadjuvant setting. MVAC followed by radical cystectomy was found to improve survival compared with radical cystectomy alone[38]. Similar to the advanced setting, GC has since become a more favoured regimen over MVAC, owing to its more acceptable toxicity profile. Although no randomised trials have compared GC and MVAC in the neoadjuvant setting, as GC was noninferior to MVAC in the metastatic setting, GC has been adopted as the most commonly used regimen in the neoadjuvant setting[28]. Choice of first-line treatment for advanced disease might be influenced, in part, by the time to first progression with metastatic disease, with some rationale for re-challenge with platinum-based chemotherapy [39,40].

In summary, cisplatin-based regimens remain the mainstay of first-line cytotoxic treatment for advanced urothelial malignancy, with only modest activity seen with other drug combinations in both the first-line and second-line setting.

[H3] Potential predictive biomarkers for chemotherapy response

In the future, predictive biomarkers for chemotherapy response will help to identify patients who are most likely to benefit from chemotherapy and, therefore, avoid unnecessary and perhaps ineffective treatment of those individuals who are unlikely to respond, in addition to avoiding unnecessary toxicity. Using the molecular taxonomy and extrapolating from data on neoadjuvant platinum-based chemotherapy in muscle-invasive, localized urothelial cancer, basal tumours and the comparable classifications of TCGA clusterIII, urobasal B, and Lund squamous cell carcinoma (SCC)-like seem to be more likely to benefit from chemotherapy than nonbasal groups[41]. This group of basal tumours are not perfectly aligned between the different classifications, as exemplified by the lack of chemotherapy benefit in the seemingly overlapping claudin-low and TCGA cluster IV groups[42]. Tumours with impaired DNA repair capacity might be expected to be more sensitive to DNA-damaging agents, as would p53 mutant tumours that are genomically unstable with high mutational burdens. For example, DNA repair pathway mutations in genes such as *ERCC2*, *FANCC*, *ATM*, *RB1*, among others, can predict responses to neoadjuvant platinum-based chemotherapies and to targeted therapies on the basis of mutational status[43 44]. Conversely, MIBC tumours expressing a wild-type p53 gene expression signature have been shown to be resistant to neoadjuvant chemotherapy[10]. Thus, it might be possible in the future to select patients who are most likely to respond to specific cytotoxic agents.

[H2] Emerging role of immunotherapy

Immunotherapy has been used to treat bladder cancer for >40 years following the seminal work by Morales et al.[45] in 1976 demonstrating tumour responses using Bacillus Calmette–Guérin (BCG), a tuberculosis vaccine. However, the recent identification of immune checkpoint molecules that can be targeted using monoclonal antibodies has led to a paradigm shift in cancer therapy, with trials thus far suggesting that bladder cancer is one of the most immunotherapy-responsive solid tumours. The most thoroughly investigated immune checkpoint ligand and receptor pair to date is programmed cell death 1 ligand 1 (PD-L1) and programmed cell death 1 (PD-1), respectively. PD1 is an inhibitory T-cell surface receptor that promotes self-tolerance by suppressing T-cell activation [46]. Upon binding of its ligand, PDL1 (or PDL2), PD1 recruits a phosphatase, tyrosine-protein phosphatase non-receptor type 11 (SHP2), which abrogates SHP2 translocation to lipid rafts and, consequently, causes downregulation of SRC family kinase-dependent T-cell receptor (TCR) signalling[47]. PDL1 is often overexpressed in bladder cancer cells and in tumour-associated stromal compartments, and its expression is correlated with tumour aggressiveness[48]. Moreover, high PD1

expression in general is associated with T-cell exhaustion[49] **[G]**. Together, these data supported a rationale for testing anti-PD1 and anti-PDL1 agents in bladder cancer.

[H3] PD-1 antibodies. Nivolumab and pembrolizumab were the first two PD1 monoclonal antibodies (mAbs) to receive FDA approval in the USA. Both are IgG4 humanised mAbs that function predominantly by steric interference of PD1–PDL1 interaction and have been shown to have very similar molecular and preclinical characteristics[50].

Based on the promising activity seen in advanced urothelial cancer in the early phase KEYNOTE-012 study, which reported an overall response rate (ORR) of 26% (**TABLE 3**), the phase III KEYNOTE-045 trial was conducted in 542 patients previously treated with platinum-based chemotherapy randomized to either pembrolizumab or physicians choice of chemotherapy. In KEYNOTE-045, pembrolizumab treatment led to a 2.9 month increase in OS and was associated with reduced toxicity[51] (**TABLE 3**). Pembrolizumab was also tested in 374 patients with bladder cancer who were ineligible to receive cisplatin-based chemotherapy in the phase II KEYNOTE-052 study. PD-L1 expression of tumour biopsies at study entry was centrally reviewed, and the study reported an ORR of 24% at data cut-off and a staggering durable response rate of 89% (percentage of responders), although the median follow-up duration was short at 5 months[52] (**TABLE 3**).

Nivolumab was tested in 86 patients with advanced urothelial cancer who had progressed on previous platinum-based chemotherapy in the phase I/II CheckMate 032 trial. Nivolumab monotherapy led to an ORR of 24.4% and grade 3–4 toxicity occurred in 22% of patients[53] (**TABLE 3**). Given the good tolerability and the substantial number and durability of responses, these findings led to the larger phase II Checkmate 275 study in the same patient population[54]. The median ORR was 19.6% (with a range of 16.1–28.4%) and positively correlated with PD-L1 immunostaining on tumour cells; grade 3–4 adverse events occurred in 18% of patients.

[H3] PD-L1 antibodies. Atezolizumab, durvalumab and avelumab are fully humanised IgG1 MAbs targeting PDL1 that have demonstrated efficacy in bladder cancer. Crystallographic studies of durvalumab and avelumab show that they predominantly interact with the front β-sheet face of PD-L1, albeit from distinct orientations, and that the relative contribution of the heavy and light chain of the antibody differs between the antibodies[55]. In a binding assay in which the PDL1 protein was immobilised on a chip and the single chain variable fragments of durvalumab, atezolizumab, and avelumab

were flowed over the chip, avelumab had the highest PDL1 binding efficacy, with durvalumab and atezolizumab demonstrating similar binding kinetics[55].

Atezolizumab received FDA approval in May 2016 in the USA for patients who had progressed after either first-line platinum-based chemotherapy or within 1 year of neoadjuvant chemotherapy on the basis of results from the phase I PDC4989g study, showing safety and an ORR of 26.2%, and the phase II IMvigor 210 study, which showed appreciable response rates and notable durability of response[56,57]. In the IMvigor 210 study, which evaluated both first-line atezolizumab in cisplatin-ineligible patients (Cohort 1) and second-line atezolizumab (Cohort 2), the ORRs were 23% and 15%, respectively, and across the two studies ranged between 8–28%, depending on PD-L1 expression on infiltrating immune cells (ICs)[56](**TABLE 3**). To confirm the efficacy of atezolizumab in the second-line setting, the phase III IMvigor 211 trial was conducted and randomised between atezolizumab and physicians choice of chemotherapy[58]. The primary end point was OS stratified by level of PDL1 positivity on infiltrating ICs and, in contrast to the KEYNOTE-045 study, no difference in survival was observed (**TABLE 3**). In general, although the ORRs and the OS benefit were promising, this surprisingly negative trial outcome might be attributable to the unexpectedly high response rates of vinflunine in the control group. Furthermore, in the exploratory subgroup analysis of IMvigor 211, tumours with high tumour mutational burden and high PDL1 expression had a marked survival improvement in the atezolizumab arm, further stressing the importance of identifying robust predictive biomarkers in this disease.

In order to test the safety and activity of durvalumab in metastatic urothelial bladder cancer, a phase I/II study was conducted in 61 patients. Enrollement of the first 20 patients was independent of PDL1 expression; however, following review, the protocol was amended to only recruit patients with a minimum PD-L1 expression of 5% on tumour cells. To assess the contribution of PD-L1 expression to response, patients were subsequently analysed and dichotomized according to PDL1-high (≥25% PDL1 expression level on either tumour cells or ICs) or PDL1-low (<25% on both tumour cells and ICs) status. In these 61 patients, 42 were evaluable for response at the time of the initial report and demonstrated an ORR of 31% which was increased to 46.4% in the PDL1-high group[59] (**TABLE 3**). This study continued to recruit and an updated report, including patients with advanced urothelial cancer who were platinum-ineligible as well as patients previously treated with platinum, demonstrated a more modest ORR of 17.8%, ranging between 5.1–27.6% depending on PDL1 status[60] (**TABLE 3**).

The phase I JAVELIN study of avelumab in patients with advanced solid tumours initially included 44 patients with metastatic urothelial carcinoma that have previously

received platinum-based chemotherapy and were unselected for PD-L1 expression. At a median follow up period of 11 months, the ORR was 18.2% (**TABLE 3**) [61]. Using a cut-off threshold for positive PD-L1 expression on tumour cells of ≥5%, the response rate was markedly higher (50.0% versus 4.3%) and PFS at 24 weeks was prolonged (58.3% versus 16.6%) in the PDL1^{+} group compared with PDL1^{-} group. For the expansion phase of the JAVELIN study, a protocol amendment was made to include patients who were platinium-naïve. This updated pooled cohort of 249 patients (including 161 evaluable patients that had previously received platinum-based chemotherapy for advanced disease) demonstrated a very similar ORR of 16%, although the influence of PDL1 positivity was less pronounced (24% versus 13%) between PDL1^{+} and PDL1^{-} groups[62].

[H3] Potential predictive biomarkers for ICI response. Responses, particularly durable responses, with ICIs are exciting, but the majority of patients do not respond and, therefore, predictive biomarkers are clearly needed[63]. The rate of immune-targeted drug development has been so rapid that a scientific rationale for their use in treatment has often lagged behind their implementation into the clinical arena. Early phase studies investigating ICIs in bladder cancer are now being analysed, which has shed some light on baseline predictive biomarkers.

Although PD1 and PDL1 monoclonal antibody therapies function by interrupting PD1–PDL1 inhibitory signalling at the T-cell immunological synapse **[G]** , PDL1 protein expression (as determined by immunohistochemistry) in metastatic urothelial cancer does not reproducibly correlate with treatment response. For example, although a trend towards high response rates was observed in PDL1-high groups of the second-line pembrolizumab (KEYNOTE-012), second-line durvalumab, second-line avelumab (JAVELIN), and first-line pembrolizumab (KEYNOTE-052) studies, no such relationship was noted in the studies of second-line nivolumab (CheckMate 275) or second-line pembrolizumab (KEYNOTE-045) (**TABLE 3**). Furthermore, even in the reports in which PDL1 expression correlated with response, the negative predictive value of this test was generally poor, meaning that it was unable to clinically discriminate between responders and nonresponders[52,60,64]. The interpretation of PD1 or PDL1 immunohistochemistry as a biomarker has been challenging due to nonconformity between different studies, which used different antibody clones, positivity thresholds, and cell types (tumour cells, ICs or a combination) (**TABLE 3**). Furthermore, the biomarker results are probably confounded by the complex biology of the immunological synapse and the plasticity and redundancy of a wide array of immune checkpoint molecules. These issues have led to a lack of harmonization of PD1 or PDL1

immunohistochemistry as a predictive biomarker in advanced bladder cancer.

Metastatic urothelial cancer is characterized by a high somatic mutation rate[65], particularly in the Lund Genomically Unstable (LGU) group and the TCGA cluster II[9,17]. Importantly, high tumour mutational burden (TMB) has been correlated with response to ICIs in metastatic urothelial cancer[66]. Using a computational approach combining somatic nucleotide variation datasets from individual patients with the patient-specific human leukocyte antigen (HLA) genotype, predictions of neoantigens that might strongly bind to patient-specific major histocompatibility complex (MHC) class I molecules can be made, which have been shown to correlate with response to ICIs in several cancer types[67-69]. Using this approach, a 2018 analysis of the IMvigor 210 study confirmed that high levels of predicted neoantigens were positively associated with response to atezolizumab[66] and seemed to be a stronger predictor of response than TMB alone.

Gene mutations causing defective DNA mismatch repair (dMMR) and defective DNA damage repair (DDRD) have been shown to be predictive biomarkers for response to ICIs, possibly because they cause increases in TMB[70,71]. Such mutations are being increasingly recognised in urothelial carcinoma. For example, germline mutations in DNA mismatch repair genes, such as occurs in Lynch syndrome **[G]** , leads to a 4.2-fold increased lifetime risk of urothelial carcinoma in men (from 1.8% to 7.5%)[72]. In 60 patients with metastatic urothelial carcinoma treated with either nivolumab or atezolizumab in clinical trials, detection of DDRD, particularly deleterious mutations in DDR genes, was strongly associated with response to anti-PD1 and anti-PDL1 agents and survival outcomes, and was superior to TMB[71]. Conversely, in the analysis of the larger IMvigor210 dataset, TMB seemed to have a stronger association with ICI response than DDRD[66]; however, whether this relationship would have still been observed if only deleterious mutations were used in this analysis (which could account for differences in these two reports) is unclear.

Transforming growth factor β (TGFβ) is a pleiotropic protein that is thought to be protumorigenic in advanced cancers through its role in stromal activation, angiogenesis, and EMT[73]. In a gene-set analysis of patients in the IMvigor210 study, genes related to TGFβ signalling within fibroblasts were enriched in nonresponders to ICIs, and expression of the two top-scoring TGFβ pathway genes, *TGFB1* and *TGFBR2*, were associated with reduced OS[66]. The functional importance of TGFβ was confirmed in two separate syngeneic mouse models (EMT6 and MC38) of immune-excluded tumours, in which physical exclusion of T-cells from the tumour parenchyma by the stromal barrier occurs. Using these models, co-treatment with monoclonal antibodies against

both PDL1 and TGFβ, but not either antibody alone, led to increases in a $CD8^+$ T-effector (T_{eff}) gene expression signature and tumour regression[66].

The Cancer Genome Atlas (TCGA) molecular subtypes taxonomy of urothelial cancer defined in localized MIBC[74], has been applied to advanced disease and these subtypes have shown differential responses to atezolizumab, suggesting that the immune biology at least partially maps to the TCGA subtypes. In particular, in a post-hoc analysis of IMvigor 210, in which baseline tumour samples (primary 61%, metastatic 39%) were subtyped using the TCGA nomenclature, responses were slightly more frequent in the TCGA cluster II subtype compared to other subtypes such as the TCGA cluster IV (29.7% versus 14.3%), although statistical comparison was limited by small patient numbers in each group[75]. In an attempt to improve the taxonomy of immune-responsive tumours, the Powles group integrated the Lund classification with the TCGA classification to form three new subgroups — TCGA Luminal II only (TLII), Lund genomically unstable (LGU) only, or both TLII and LGU[66]. LGU tumours had a low $CD8^+$ T_{eff} gene signature expression and low TMB, but, surprisingly, up to 50% of patients responded to ICIs. TLII tumours had high $CD8^+T_{eff}$ gene signature expression and an intermediate level of TMB, and patients responded poorly to ICIs; moreover, these tumours were associated with a TGFβ gene signature in fibroblasts. The LGU–TLII group had high $CD8^+T_{eff}$ gene signature expression, high TMB, and low TGFβ gene signature expression in fibroblasts, which is the optimal triad of variables leading to almost 20% of patients attaining a complete response. The relative importance of these competing factors for immunotherapy response needs to be tested in prospective clinical studies.

[H3] Clinical predictors of response to ICIs. Clinical biomarkers have previously been proposed for patients with metastatic urothelial carcinoma who are treated with second-line chemotherapy[76], and a similar preliminary prognostic model for second-line treatment with atezolizumab has been developed[77]. Patients receiving atezolizumab in the IMvigor210 trial (n=310) and PCD4989g trial (n=68) were used as the training and validation cohorts, respectively. The factors included in the optimal prognostic model for OS (as they remained statistically significant in the multivariable analysis) were: Eastern Cooperative Oncology Group–Performance Status (ECOG-PS) 1 versus 0, liver metastasis, platelet count, neutrophil:lymphocyte ratio, lactate dehydrogenase (LDH) levels, and anaemia. Determining the general applicability of these results to other ICIs will require testing in other prospective trials and different patient cohorts, but these findings emphasize the potential importance of clinical 'low-tech' markers in

prognostication.

[H2] Role of molecularly targeted therapies

Studies in a variety of solid tumours investigating novel targeted agents have demonstrated how predictive biomarkers can alter treatment paradigms and enable patient selection when developing new therapeutic strategies. Urothelial carcinomas are known to harbour a large proportion of recurrently mutated genes in key protumorigenic signalling pathways, and studies have investigated a number of potential molecular targets that have shown promise in the preclinical setting (**TABLE 4**, **FIG. 4**).

The ubiquitous serine-threonine kinase mTOR is a downstream component of the PI3K–phosphatase and tensin homologue (PTEN)–AKT signalling pathway that has a key role in the regulation of cell growth, proliferation, protein synthesis, survival, and angiogenesis[78]. Two agents that inhibit mTOR, temsirolimus and everolimus, are approved for the treatment of patients with advanced kidney cancer[79]. Markers of mTOR activation include eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and the 70 kDa ribosomal protein S6 kinase 1 (p70S6K1), and expression of both 4E-BP1 and p70S6K1 is seen in urothelial carcinoma[80], suggesting this pathway is active and that mTOR is a potential therapeutic target. Everolimus is known to inhibit the growth of bladder cancer cell lines in vitro and also has activity in nude mouse xenograft models in vivo[81]. A single-arm phase II study of everolimus in 45 patients with metastatic urothelial carcinoma, although not reaching its primary end point of PFS, showed one partial response, one near-complete response, and twelve minor regressions (**TABLE 4**) [82]. Thus, in a subset of patients with urothelial carcinoma, mTOR inhibitors might have clinically significant antitumour activity.

In 2009, second-line lapatinib was shown to be well tolerated with anti-tumour activity in a single-arm phase II study in locally advanced or metastatic bladder carcinoma[83]. A UK-based phase III placebo-controlled study published in 2016 evaluated lapatinib, an epidermal growth factor receptor (EGFR; also known as HER1) and human epidermal growth factor receptor 2 (HER2; also known as ERBB2) tyrosine kinase inhibitor (TKI), in patients with $EGFR^+$ or $HER2^+$ advanced and/or metastatic urothelial carcinoma[84] (**TABLE 4**). Patients had completed first-line chemotherapy for metastatic disease and were randomized to placebo or lapatinib, which was used with the primary aim of delaying progression and maintaining the response to chemotherapy. The results did not show any clinical benefit with maintenance lapatinib, even in tumours with the highest EGFR and HER2 protein expression (+3 immunohistochemistry score); the best

response rate for lapatinib and placebo were 14% versus 8% (*P*=0.14) and no statistically significant difference was observed in OS or PFS compared with placebo.

Studies have also investigated the fibroblast growth factor receptor (FGFR) family tyrosine kinases, which have known regulatory roles in tumour survival and growth[85]. The FGFR family consists of four isoforms and multiple ligands, which have roles in cell proliferation, differentiation, migration, and survival[86]. FGFR genetic aberrations are frequent in patients with urothelial carcinoma; 10–15% have *FGFR3* mutations, 6% have *FGFR3* translocations, 3% have *FGFR3* amplifications, and a further ~10% have similar aberrations in *FGFR1*, *FGFR2* and *FGFR4*[87,88].

A phase II study of dovitinib, a multi-targeted TKI with activity against FGFR3 demonstrated limited efficacy in previously-treated, unselected patients with advanced urothelial carcinoma[89] However, a phase I study of BGJ398, a pan-FGFR TKI, included a cohort of patient with urothelial carcinoma previously-treated with chemotherapy and reported an ORR of 38% in patients with *FGFR3* mutations[90] (**TABLE 4)**. Erdafitinib, another FGFR TKI, has been tested in a phase II study in patients with metastatic urothelial carcinoma who had specific *FGFR2* and/or *FGFR3* mutations or translocations and were randomized to either continuous or intermittent dosing schedules[91] (**TABLE 4**). The response rate in the continuous dosing arm was 35% compared to 24% in the intermittent arm. The response rates to FGFR TKIs in both of these trials, along with their manageable tolerability, is extremely promising given that *FGFR* aberrations are enriched in the TCGA luminal 1 subgroup, a group with limited successful treatment options as they seem to derive the least benefit from chemotherapy or ICIs[41].

As an alternative to targeting the intrinsic tumour growth pathways, targeted therapies may be used modulate the tumour vasculature with a view to improving the tumour uptake of drugs such as chemotherapy. This strategy was tested in the RANGE study where 530 patients with platinum refractory metastatic urothelial carcinoma were treated with docetaxel chemotherapy with or without ramucirumab, a vascular endothelial growth factor receptor 2 (VEGFR2) antibody (**TABLE 4**). PFS was prolonged significantly in patients allocated ramucirumab plus docetaxel versus placebo plus docetaxel (median PFS: 4.07 months versus 2.76 months; HR 0.76, *P*=0.0118)[92]. Furthermore, exploratory analysis has revealed a ramucirumab exposure–activity relationship[93], suggesting that dose optimisation could potentially enhance the benefit of this combination. This study was the first to show superior PFS for a cytotoxic and targeted therapy combination over chemotherapy alone in urothelial cancer, and has placed VEGFR2 inhibition firmly in the spotlight. A number of biomarker-directed

combination studies are now underway, which, if positive, could markedly influence the management of advanced bladder cancer in the future.

[H1] Management of variant urothelial cancer

Variant urothelial bladder cancer describes a subset of rare cancers with different clinical and biological phenotypes. The defining characteristic of these rare cancers is their high morbidity relative to their incidence, and the limited prospective clinical trial data to guide clinicians in selecting the optimal therapy in metastatic disease. In the developed world, the most common of these unusual variants are micropapillary urothelial cancer (MPUC), sarcomatoid urothelial carcinoma (SUC), and plasmacytoid urothelial carcinoma (PUC).

[H2] Micropapillary

Micropapillary urothelial cancer (MPUC) is a rare variant that has been estimated to represent 0.01–2.2% of urothelial tumours[94]. Histologically, the MPUC tumour is comprised of infiltrating tight clusters of micropapillary aggregates that are typically associated with vascular and lymphatic invasion (**FIG. 3E**). Clinically, this variant is associated with high rates of metastasis and poor outcomes[26]. Given its rarity, the evidence regarding the influence of chemotherapy in MPUC is limited to a number of conflicting institutional reports of neoadjuvant therapy in the localized setting. A review of 869 patients with MPUC from the US National Cancer Database showed no benefit of neoadjuvant chemotherapy and radical cystectomy compared with radical cystectomy alone[95]. A cohort of 72 patients with MPUC from a Parisien series has also been reported[96]; although informative, these reports are confounded by their nonrandomised and retrospective nature. Similarly, a group at the Memorial Sloan Kettering Cancer Center did not report a survival advantage for neoadjuvant chemotherapy in 82 patients with MPUC[97]. However, compared to immediate cystectomy, treatment with neoadjuvant chemotherapy was associated with a significant increase in the rate of pathological downstaging to pT0 (13% versus 45%; P=0.049)[97], which has been a reliable surrogate end point for OS benefit[98]. Given this downstaging effect of neoadjuvant chemotherapy in this MPUC cohort, we believe that the use of chemotherapy should not be overlooked in this subgroup, including in the advanced disease setting.

At the molecular level, global mRNA expression analysis in 43 patients with MPUC revealed that MPUC tumours were almost exclusively (42 of 43 patients) of luminal subtype, split equally between TCGA clusters I (urothelial-like) and II (p53-like,

infiltrated)[99]. Given these transcriptomic profiles, targeted therapies and immunotherapy should be tested in prospective molecularly driven studies.

[H2] Plasmacytoid

Plasmacytoid urothelial carcinoma (PUC) is a rare histopathological variant of urothelial carcinoma, comprising 1–3% of urothelial tumours. PUC was first described in 1991 and its histological appearance resembles plasma cells with a dyscohesive pattern, abundant eosinophilic cytoplasm, and eccentric nuclei[100] (**FIG. 3H**). The cells are highly proliferative, with the majority expressing the proliferation marker protein Ki-67 (REF.[101]). PUC tends to extensively involve the bladder wall and frequently extends into adjacent organs and the perivesical soft tissues[102]. PUC also seems to have a predilection for peritoneal surfaces and has a characteristic pattern of local spread along pelvic fascial planes, and often results in surgical upstaging and positive margins at the time of cystectomy owing to involvement of perirectal and periureteric tissues[102].

The rarity of PUC has led to a paucity of published data regarding treatment and outcomes of patients with this pathological variant, much of which is based on individual case reports or single institution experience. The reported outcomes in the literature suggest an aggressive clinical course with the potential for rapid systemic spread of disease[26]. However, it is clear that PUC can be a chemosensitive variant, but the duration of response can be short. In a 2017 case series of patients treated at Memorial Sloan Kettering Cancer Center in the US, 98 patients with PUC treated over a 19-year period were retrospectively identified[103]. Compared with patients with pure conventional urothelial carcinoma, patients with PUC were more likely to have higher stage disease, positive lymph nodes, and positive soft tissue surgical margins. In another US case series from MD Anderson Cancer Center published in 2013, 31 patients were identified with the PUC variant, a proportion of whom received platinum-based chemotherapy in the neoadjuvant setting[104]. Pathological downstaging was seen in 80% of patients who received neoadjuvant chemotherapy, but relapses were common and no difference in survival was seen between patients treated with neoadjuvant chemotherapy compared with initial surgery. The authors concluded that the survival outcomes of these patients was poor despite chemotherapy, and that the optimal chemotherapy regimen for this subtype remains unknown.

[H2] Sarcomatoid urothelial cancer

Sarcomatoid urothelial carcinoma (SUC; also known as carcinosarcoma) is a rare (0.1–0.3% of all bladder tumours) and aggressive variant of urothelial carcinoma that is

characterized by high-grade pathology with sarcomatous components (**FIG. 3J**). SUC has been associated with a history of cyclophosphamide therapy or radiation, presents as metastatic disease in ~50% of cases[105], and has a poor prognosis[89]. Evidence is limited, but mutational data are in favour a common clonal origin with divergent differentiation[106]. Treatment strategies in SUC are undefined and have been based on small case series. Froehner et al.[107] reported a single case study of a patient with metastatic SUC who had a complete response to GC, the same regimen commonly used to treat metastatic urothelial carcinoma. Extrapolating data from a phase II study of 55 patients with metastatic endometrial carcinosarcoma who were treated with carboplatin plus paclitaxel, which demonstrated an ORR of 54%, a median OS of 15 months, and reasonably good tolerability[108], we consider this regimen for patients with SUC.

[H1] Management of nonurothelial cancer

In addition to rare variant urothelial cancers, another rare subset of tumours with high morbidity compared with urothelial carcinomas are the nonurothelial subgroup of bladder cancer, including SCC of the bladder, neuroendocrine variants, adenocarcinoma and urachal carcinoma, mesenchymal tumours, and tumours of mullerian type.

[H2] Squamous cell carcinoma of the bladder

Pure SCC of the bladder is comprised of cohesive tumour cells with intervening intracellular bridges and central keratinization, and typically arises on a background of keratinizing squamous metaplasia **[G]** (**FIG. 3N**). The incidence of SCC of the bladder in Western bladder cancer cohorts is estimated to be ~2–3%[109], and SCC of the bladder was seen in 2.4% of patients in a large US cohort of >160,000 bladder cancers[110]. SCC of the bladder, although rare in Western countries, is more common in certain parts of Africa owing to its association with untreated *Schistosoma haematobium* infection and the resultant chronic fibrotic inflammation in the bladder wall[111]. This association with chronic infection or inflammation might explain the increased incidence of SCC of the bladder among patients with long-term indwelling catheters or spinal cord injury[112].

Localized SCC of the bladder is predominantly treated with surgery, and recurrence is predominantly locoregional (>80%)[113]. Given its rarity, the evidence for improved outcomes with current approaches is largely based on retrospective case series reviews, and historical data have been conflicting as to whether platinum-based chemotherapy is effective, both in the perioperative and metastatic setting. One small study reported poor outcomes following neoadjuvant chemotherapy, with 5 of 8

patients progressing following chemotherapy and becoming inoperable[114], which permeated a belief within the oncological community that SCC of the bladder does not respond to chemotherapy. However, this observation might simply be a reflection of the aggressive underlying biology of this variant rather than a measure of chemosensitivity per se, and perhaps responses are better than previously thought. In urothelial carcinoma, the Lund SCC-like subgroup (TCGA group III and Basal group) was the most likely tumour type to benefit from chemotherapy[42] but extrapolation is needed to apply this data to advanced nonurothelial carcinoma. However, a number of studies support the activity of platinum-based chemotherapy in advanced SCC of the bladder. A large study of >400 patients with advanced bladder cancer treated with platinum-based chemotherapy demonstrated no difference in response rate between conventional urothelial carcinoma (*n*=389), pure SCC of the bladder (*n*=15), and the SCC variant of urothelial carcinoma (*n*=41) (RR 44% versus 27% versus 34%; *P*=0.21)[115]. A phase II study of patients with advanced bilharzial-associated bladder cancer **[G]** , including 22 patients with urothelial carcinoma and 14 patients with SCC, demonstrated similarly high response rates to GC (RR 60% versus 50%; *P*=0.5)[116]. Some clinicians would advocate the use of taxanes in this population given its widespread use in SCC of the lung or head and neck cancer[117,118]. Additionally, in a prospective study of paclitaxel, ifosfamide and cisplatin (TIP) combination chemotherapy in 20 patients with nonurothelial carcinoma histology, including 8 patients with SCC of the bladder, clinical activity was seen with a median survival of 8.9 months and 2 complete responses for the whole cohort[119]. However, substantial toxicity (45% grade 3–4 myelosuppression) occurred, which has limited the use of the TIP regimen in the metastatic setting.

Dedicated molecular characterization of pure SCC of the bladder is limited. Studies comparing urothelial carcinoma with squamous cell cancers using fluorescence in situ hybridization (FISH) or comparative genomic hybridization (CGH) techniques, have reported differences, including a predilection for loss of chromosome 3p[120]. A preliminary dataset (in abstract form) comparing matched squamous and urothelial carcinoma areas in urothelial carcinoma with squamous cell differentiation concluded that, although the somatic mutation burden does not diverge, the gene expression signature for these two components of the same tumour can be extremely divergent[121]. This finding suggests that gene regulation mechanisms, such as epigenetics and noncoding RNAs, have a key role in the differentiation and phenotype of bladder cancer. Extrapolating data from SCCs located at a variety of other anatomical sites suggests an "essential commonality" in the genetic determinants of squamous differentiation — specifically, the *NOTCH, TP63*, and *SOX2* genes[122] Consideration of these shared

determinants learned from other tumour sites might enable progress in targeted and combinatorial therapeutics in rare entities such as SCC of the bladder.

[H2] Neuroendocrine variants

The bladder is the most common site for genitourinary extrapulmonary neuroendocrine (small cell) carcinoma. Accounting for <1% of primary bladder tumours, neuroendocrine carcinoma is a rare but aggressive histological subtype that is characterized by advanced stage at diagnosis and rapidly progressive disease[26]. Again, owing to its rarity, treatment is based on evidence obtained from case reports and retrospective analyses. Histologically, neuroendocrine tumours consist of sheets of small, round, blue (on H&E stain) hyperchromatic cells with a small cytoplasm, and stain positive for neuroendocrine markers (for example, chromogranin, synaptophysin,CD56 and neuron-specific enolase) (**FIG. 3Q**). Of clinical significance, 38–70% of cases also exhibit a co-existing non–small-cell carcinoma component (urothelial carcinoma, adenocarcinoma, or SCC) [123]. This observation supports the hypothesis that these neuroendocrine tumours arise from a single cancer stem cell that then differentiates into diverse cell types.

Opinion remains divided as to whether neuroendocrine tumours should be managed similarly to urothelial bladder tumours or other small cell tumours such as small-cell lung cancer (SCLC). Even with disease localized to the bladder, the median OS for neuroendocrine carcinoma is poor (<20 months), suggesting that integration of systemic therapy is crucial to optimize outcomes[124]. In addition, the potential quality-of-life benefit of a bladder-sparing approach should certainly be considered in this setting, as many patients will have distant recurrence and, therefore, might not necessarily benefit from an aggressive surgical approach.

Most chemotherapy approaches for neuroendocrine carcinoma of the bladder favour platinum and etoposide, which has been extrapolated from the standard management of SCLC. No prospective studies exist on the use of these treatments in neuroendocrine bladder cancer. Based on the limited retrospective experience in the literature, both neoadjuvant chemotherapy followed by radical cystectomy and neoadjuvant and/or concurrent chemotherapy with radiation therapy are considered reasonable treatment options for localized disease[125-127]. Optimization of systemic therapy is essential owing to the aggressive metastatic potential of this disease. Given that ICIs are now showing activity in SCLC[128], immunotherapy might have a future role in management of this aggressive variant.

[H2] Adenocarcinoma and urachal carcinoma

Adenocarcinoma of the bladder can have a papillary, nodular, flat or ulcerated architecture and most commonly affects the trigone and posterior bladder wall[129]. Microscopically, these tumours show well-to-moderately differentiated colonic-type glandular morphology, with or without abundant mucin (**FIG. 3O**). In a Surveillance Epidemiology and End Results (SEER) database study of 306 patients with bladder adenocarcinoma, no statistically significant decrease in outcomes was reported in this population compared with a urothelial carcinoma cohort matched for age and stage[130]. Urachal carcinoma (**FIG. 3P**) comprises ~10% of bladder adenocarcinomas and affects the dome and anterior aspect of the bladder wall[129] In another SEER outcome study, patients with urachal carcinoma tended to be younger and had better survival outcomes than patients with non-urachal adenocarcinoma[131].

Nonurachal adenocarcinoma and urachal carcinoma are treated via surgical resection with wide excision margins and, in the case of urachal carcinoma, improved outcomes are associated with en bloc resection of the urachus and umbilicus[131]. Compared with nonurachal adenocarcinoma, patients with urachal carcinoma tend to present later as a result of the anatomical location of the site of the tumour, and have a higher risk of metastatic disease, particularly within the peritoneum, which is again thought to be related to site of origin. Although few data exist to guide optimal therapy in these patients, historical retrospective datasets suggest that patients with involved nodes, or positive margins, have a high risk of recurrence[132].

Studies have confirmed the similarities between colorectal adenocarcinoma and both bladder adenocarcinoma[133] and urachal carcinoma[134]. Cytoplasmic expression of β-catenin is seen histologically[135] and amplification of *EGFR* has been reported at the genomic level alongside mutations in MAPK pathway genes and *APC*[133,134]. These findings suggest that clinicians should perhaps treat advanced adenocarcinoma of the bladder akin to a colorectal cancer. Indeed, response rates for platinum–5-fluorouracil (5-FU)-based chemotherapy in metastatic urachal carcinoma or bladder adenocarcinoma, which form the backbone of systemic therapy in colorectal adenocarcinoma, have been reported to be as high as 48%[136]. Based on this finding, many practitioners would recommend treatment such as folinic acid, infusional 5-FU, and oxaliplatin (FOLFOX) or capecitabine–oxaliplatin (CAPOX). Given the rarity of nonurachal adenocarcinoma and urachal carcinoma, few clinical trials in bladder cancer are open to patients with this unusual subtype. However, a clinical trial of infusional 5-FU, gemcitabine and cisplatin in metastatic urachal carcinoma has recently completed accrual at the MD Anderson Cancer Center and the results are eagerly awaited[137]. The

molecular aberrations that characterize adenocarcinoma of the bladder also have implications for targeted approaches, and responses have been reported to appropriately selected molecular therapeutics such as cetuximab[134] and sunitinib[138].

[H2] Mesenchymal tumours

Pure sarcomas of the bladder are rare, comprising ~0.7% of high-grade urothelial cancers, and the most common subtypes are leiomyosarcoma **[G]** followed by rhabdomyosarcoma **[G]** and angiosarcoma **[G]** [139]. Leiomyosarcomas are characterized histologically by interwoven fascicles of malignant spindle cells[140] (**FIG. 3R**). The prevelance of leiomyosarcoma is thought to be increased in patients who have received pelvic radiotherapy or chemotherapy, who are treated according to sarcoma guidelines, independent of the anatomical location[140,141] . For patients with leiomyosarcoma, single-agent doxorubicin remains the standard of care following the observation of no improvement in OS in randomized controlled trials with either the addition of ifosfamide to doxorubcin[142] or the alternative regimen of gemcitabine and docetaxel[143].

[H2] Tumours of mullerian type

Tumours of Mullerian type, such as primary clear-cell carcinoma or endometrial cell carcinoma of the urinary bladder, are extremely rare, with true incidence figures unknown. Indeed, a study has highlighted that a total of only 47 patients with siuch tumours have been reported in the literature to date[144]. The gross histopathological features exactly replicate those of such tumours arising in the female genital tract, such as nuclear enlargement and hyperchromasia, brisk mitotic activity, and basophilic or eosinophilic secretions. Such tumours have been postulated to arise from mullerian origin tissue **[G]** in the bladder and, in keeping with this hypothesis, positivity of the ovarian carcinoma antigen CA125 (also known as mucin-16) has been reported[144]. The differential diagnosis includes nephrogenic metaplasia **[G]** and, importantly, local extension or metastasis from a pelvic gynaecological malignancy should be excluded[145]. Given the rarity of tumours of Mullerian type, little is known about optimal cytotoxic treatment strategies, but reported case series suggest that the clinical course is aggressive[145].

[H1] Future therapies

The next steps for drug development in urothelial cancer remain uncertain. The initial work with ICIs has not been universally successful in platinum-refractory disease[58], and recent restrictions by the FDA and European Medicines Agency (EMA) of ICI

monotherapy to PD-L1-high subgroups — following interim analyses of first-line atezolizumab and pembrolizumab studies in mUC — questions their use as single-agents in previously untreated patients with metastatic disease[146,147]. Thus, other avenues are required to take this group of drugs forward — three initial programmes are evaluating novel approaches.

The first approach is to explore combinations of PD1 or PDL1 inhibitors with other immune agents as the therapeutic backbone. Initial data with immune combinations of PD1 or PDL1 inhibitors and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) inhibition have not been ground-breaking[148]. DANUBE, a randomised phase III trial evaluating the combination of durvalumab and CTLA-4 antibody tremilimumab versus chemotherapy in patients with unresectable stage IV urothelial bladder cancer will be the first pivotal front-line trial to be reported[149]. Other randomized immune combination studies such as indoleamine 2,3-dioxygenase (IDO) inhibitor epicadostat plus pembrolizumab have been aborted following underwhelming results of the trials evaluating this combination in melanoma and pancreatic cancer[150]. Other combinations, including chemotherapy with PD1 or PDL1 inhibition seem promising, particularly in light of the positive results of this combination in metastatic non-small-cell lung cancer (NSCLC)[151], but this strategy remains experimental in urothelial carcinoma and is being investigated in ongoing trials[152,153]. BISCAY is a study exploring targeted therapy such as poly(ADP-ribose) polymerase (PARP) or FGFR3 inhibition with durvalumab in molecularly selected tumours with relevant mutations[154]. This trial design, whereby all patients are able to receive a novel therapy or novel combination therapy tailored to individualised molecular profiles, is attractive, but the strategy remains unproven.

The second approach is to test ICIs earlier in the disease course, such as in MIBC (T2–4aN0M0). Early data from two separate phase II studies suggest that 2–3 cycles of either atezolizumab or pembrolizumab prior to cystectomy is associated with a high pathological complete response (pCR) rates, comparable to the 25–41% historical pCR rates seen with neoadjuvant platinum-based chemotherapy[38,155]. The overall pCR rate in 88 patients treated with atezolizumab was 31%, which this was increased to 37% in patients who were PD-L1-positive[156]. Similarly, in the 50 patients treated with neoadjuvant pembrolizumab, the pCR rate was 41%, which was increased to 54% in the 35 patients who were PD-L1-positive (combined positive score (CPS) ≥10%)[157]. Perioperative trials will probably change the treatment paradigms if these data are reproduced in randomized trials

The third approach is selecting patients for therapy on the basis of composite biomarkers such as TMB and TGFβ gene signature expression, particularly as early data suggest that tumours with such aberrations can be targeted successfully[66,158].

Beyond immune checkpoint blockade, two current targets stand out — FGFR3, as its encoding gene (*FGFR3*) is commonly mutated, amplified, or translocated in urothelial carcinoma, and nectin-4, which is abundantly expressed in 60% of urothelial carcinomas[87,159]. Early studies in selected patients with metastatic or unresectable urothelial carcinoma and *FGFR* alterations treated with the pan-FGFR TKI erdafitinib have shown response rates of up to 35%[91]. Phase II data on efortumab vedotin (EV), an antibody–drug conjugate composed of an nectin-4 mAb attached to a cytotoxic agent, has shown promising response rates in patients with metastatic urothelial carcinoma who were previously treated with ICIs[160]. These promising data have led to FDA breakthrough status for EV and accelerated approval for erdafitinib[161], and randomised phase III trials are underway[162,163].

[H1] Conclusions

Chemotherapy remains the standard of care for advanced urothelial carcinoma, but the histological subtype and the presence of nonurothelial bladder cancer can influence the choice of chemotherapy. Conventional pathology augmented by genotyping and transcriptional profiling will increasingly direct choice of systemic therapy, particularly between chemotherapy, immunotherapy, or novel targeted agents. Technological and bioinformatic advances have identified molecular signatures and signalling pathways or complexes in urothelial cancer that could be exploited using targeted therapy and immune strategies. Future clinical trial design must reflect this molecular knowledge with the use of carefully selected biomarkers to help identify appropriate pharmacological inhibition of targets as well as putative predictive and prognostic factors. We would also argue for an effort to direct resources at some of the aforementioned rare bladder cancer tumours, which remain relatively poorly understood. Further mechanistic understanding of the molecular pathology of bladder cancer and how it changes through treatment will be required to enable the effective combination of chemotherapy, immunotherapy, and other targeted agents, and will be essential to improve future care for patients with bladder cancer.

Acknowledgements

M.L. and A.F. are supported by the National Institute for Health Research and the University College London Hospitals Biomedical Research Centre (no grant numbers apply). M.L. receives funding from Cancer Research UK, the Rosetrees trust, and a BMS II-ON grant (no grant numbers apply).

Author contributions

All authors researched data for the article, made substantial contributions to discussion of the article contents, wrote the manuscript, and reviewed and/or edited the manuscript before submission.

Competing interests

M.L. has received research funding from Bristol-Myers-Squibb, AstraZeneca/MedImmune and Astellas, and has received consulting fees from Janssen Pharmaceuticals. T.P. receives research funding from AstraZeneca/MedImmune, Genentech/Roche, and Novartis, and consulting fees from Genentech/Roche, Pfizer, GlaxoSmithKline and Merck.

Publisher's note

-ONLINE ONLY-

Subject categories

Health sciences / Urology / Urogenital diseases / Urological cancer / Bladder cancer [URI /692/4025/2768/589/1336]

Health sciences / Oncology / Cancer / Cancer therapy / Cancer immunotherapy [URI /692/4028/67/1059/2325]

Health sciences / Oncology / Cancer / Cancer therapy / Chemotherapy [URI /692/4028/67/1059/99]

Health sciences / Oncology / Cancer / Cancer therapy / Targeted therapies [URI /692/4028/67/1059/602]

Health sciences / Health care / Diagnosis / Pathology [URI /692/700/139/422]

Health sciences / Oncology / Cancer / Cancer genomics [URI /692/4028/67/69]

Table of contents blurb

In this Review, the authors discuss the molecular and histopathological evidence supporting pathology-driven therapy for advanced bladder cancer, including rare histological subtypes of urothelial carcinoma and nonurothelial carcinomas, and highlight novel molecular taxonomies, relevant biomarkers, and promising future therapies.

Glossary

Angiosarcoma: A malignant tumour arising from blood vessels.

Basal: In gene expression studies, basal describes a group of bladder cancers lacking epithelial markers but expressing markers of mesenchymal or sarcomatoid differentiation.

Conventional urothelial carcinoma: The most common type of bladder carcinoma, arising from uroethelial cells lining the bladder and urinary tract, also known as the transitional epitheilium.

Keratinizing squamous metaplasia: A precancerous condition of squamous cells.

Blharzial-associated bladder cancer: Bladder cancer arising following chronic infection with schitosomiasis (infection also known as snail fever and bilharzia).

Leiomyosarcoma: A malignant tumour arising from smooth muscle.

Liquid biopsies: The use of circulating (dynamic) tumour-derived nucleic acids to inform tumour-specific somatic mutations.

Luminal: In gene expression studies, luminal describes a group of bladder cancers expressing epithelial markers.

Lynch syndrome: Also known as hereditary nonpolyposis colorectal cancer (HNPCC). An inherited autosomal dominant condition that increases the risk of certain solid tumours such as colorectal and endometrial cancer; caused by mutations in DNA mismatch repair genes, such as MLH1 and MSH2.

Nonurothelial carcinoma: The minority of urothelial carcinoma that arises from cells other than urothelial cells, most commonly pure squamous cell carcinoma or pure adenocarcinoma.

T-cell exhaustion: A state of T cell dysfunction that exists in many chronic diseases and cancer due to prolonged antigen stimulation; defined by expression of inhibitory receptors and loss of effector function in T cells.

Urothelial carcinoma with divergent differentiation: Otherwise known as variant urothelial carcinoma. A urothelial carcinoma with varying amounts of differentiation to other histological entities, of which 13 are currently recognised, including sarcomatoid and squamous differentiation.

Figure 1 | **Timeline of discoveries and therapeutics relevant to advanced bladder cancer.**

BCG, Bacillus Calmette–Guérin; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; MAGE-1, melanoma associated antigen 1; MVAC, mitomycin-C, vincristine, adriamycin and cisplatin chemotherapy; PD-1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; TCGA, The Cancer Genome Altas; UC, urothelial carcinoma; WHO, world health organisation.

Figure 2 | **The major classifications of urothelial carcinoma.**

Intersecting themes and commonalities of the major grouping classifications of urothelial carcinoma are shown. Studies have highlighted a number of phenotypically distinct groups within the broad basal and luminal classifications, which are indicated with the study title and the number of patients analyzed in each study[6-9]. The 2016 consensus study aimed to align some of these classifications, the result of which being an agreement on the existence of a basal-squamous-like classification[12]. UNCC, University of North Carolina at Charlotte; TCGA, The Cancer Genome Atlas.

Figure 3 | **Immunohistochemistry of bladder cancer.**

Representative haemotoxylin and eosin (H&E)-stained images (×200 magnification) of conventional urothelial carcinoma (UC), UC with divergent differentiation, UC variants, and nonurothelial carcinoma variants. **a |** Conventional UC; characterized by exophytic papillary structures covered by atypical urothelial cells. **b | UC with** squamous differentiation; predominantly features of UC but in areas the cells show evidence of intercellular bridges and keratinisation consistent with squamous differentiation. **c |** UC with glandular differentiation; predominantly features of UC but the tumour cells form gland-like structures consistent with glandular differentiation. **d |** Nested variant. The tumour cells are arranged as well-circumscribed nests of mildly pleomorphic cells with a rounded margin and may mimic von Brunn's nest. **e |** Micropapillary; small, cohesive clusters of tumour cells with a micropapillary appearance that are typically associated with lymphovascular invasion and show an infiltrative growth pattern. **f |** Microcystic, cords of tumour cells forming a network of small intervening cystic spaces. **g |** Lymphoepithelioma-like, small groups of tumour cells with florid chronic inflammatory cell infiltrate in the intervening stroma **h |** Plasmacytoid; single or small groups of discohesive tumour cells with abundant eosinophilic cytoplasm and an eccentrically placed nucleus. **i |** Signet ring**.** tumour cells are filled with abundant intracytoplasmic mucin that displaces the nucleus to one edge **j |** Sarcomatoid, tumour cells show marked pleomorphism with presence of elongated (spindle cell) nucle**i k |** Giant cell, tumour shows scattered epithelial tumour 'giant' cells with markedly enlarged, hyperchromatic nuclei that may be multinucleated. **l |** Lipid-rich, tumour cells contain abundant intracytoplasmic lipid imparting a 'foamy' appearance to the cytoplasm. **m |** Clear-cell, tumour cells contain intracytoplasmic glycogen that is dissolved out of the tissue during processing and results in the cells appearing to have 'vacuolated' or 'clear' cytoplasm. **n |**

Pure SCC; tumour is entirely composed of cohesive nests of cells with presence of intercellular bridges and keratinisation. **o |** Adenocarcinoma; tumour shows prominent gland formation and may show mucin production similar to that seen in adenocarcinoma from other sites (particularly GI tract). **p |** Urachal carcinoma; tumour arising from embryological urachal remnants of the bladder, predominantly at the dome, and usually showing features of a mucin-producing adenocarcinoma**. q |** Small cell; solid sheets of tumour cells with hyperchromatic nuclei and scant cytoplasm resulting in a 'small, round blue' cell appearance microscopically, positive for neuroendocrine markers and usually requiring systemic therapy **r |** Mesenchymal differentiation; tumour composed of interlacing fascicles of malignant spindle cells with immunocytochemical or molecular evidence of sarcomatous transformation..

Figure 4 | **Signalling pathways for targeted therapies in urothelial carcinoma.**
Receptor tyrosine kinases (RTKs), such as epidermal growth factor receptor (EGFR), HER2, and fibroblast growth factor receptor 3 (FGFR3), are often activated in UC through mutations, amplification or, for FGFR3, translocations[74].. Small molecule inhibition of the intracellular domain of FGFR (Erdafitinib, Dovitinib and BGJ398) or EGFR/HER2 (Laptinib) leads to decreased bladder cancer cell proliferation via the RAS/MEK/ERK pathway and to reduced protein synthesis and growth via the PI3K/AKT/MTOR pathway. Vascular epithelial growth factor (VEGF), which is found at high levels in bladder cancer, binds to VEGFR2 on endothelial cells. Treatment with the human monoclonal antibody to VEGFR2, Ramucirumab, has been shown to reduce bladder tumour angiogenesis. Normal signalling functions of VEGFR2 upon exposure to VEGF in endothelial cells, which likely account for the anti-angiogenic function of Ramucirumab, include the reduced endothelial cell permeability via SRC dependent activation of vascular endothelial (VE) cadherin and nitric oxide (NO). Furthermore, VEGFR2 becomes internalised into early endosome antigen 1 (EEA1)-positive early endosomes and subsequent PLCγ and PKC dependent RAF/MEK/ERK signalling resulting in proliferation[164]. PLCγ, Protein Lipase C γ; DAG, diacylglycerol; Ca^{2+}, calcium; cPKC, conventional protein kinase C; eNOS, endothelial nitric oxide synthatase; IP_3, inositol 1,4,5-triphosphate; endothelial cells, including the conversion of endothelial nitric oxide synthatase (eNOS)-dependent production of nitric oxide (NO). This, combined with VEGFR2–SRC-dependent activation of vascular endothelial (VE) cadherin, leads to increased endothelial permeability; GRB2, Growth factor receptor-

bound protein 2; SOS, son of sevenless (SOS); $PIP_{2,}$ phosphatidylinositol 4,5-biphosphate, $PIP_{3,}$ phosphatidylinositol-3,4,5-triphosphate; PDK1, 3-phosphoinositide-dependent protein kinase 1; MTORC, mTOR complex; AKT, RAC-alpha serine/threonine-protein kinase.

Table 1 | **WHO classification of invasive tumours of the urothelial tract.**

Histological classification			**ICD code**
Histological group	**Main histology**	**Submorphology**	
Urothelial (transitional cell) tumours	Conventional UC	NOS	8120/3
	UC with divergent differentiation	With squamous cell differentiation	[a]
		With glandular differentiation	[a]
		With trophoblastic differentiation	[a]
		Other	[a]
	Nested	-	[a]
	Microcystic	-	[a]
	Micropapillary	-	8131/3
	Lymphoepithelioma-like	-	8082/3
	Plasmacytoid/signet ring/diffuse	-	[a]
	Sarcomatoid	-	8122/3
	Giant cell	-	8031/3
	Poorly differentiated	-	8020/3
	Lipid-rich	-	[a]
	Clear-cell	-	[a]
Squamous cell neoplasms	Pure squamous cell carcinoma	-	8070/3
	Verrucous carcinoma	-	8051/3
Glandular neoplasms	Adenocarcinoma	NOS	8140/3
		Enteric	8144/3
		Mucinous	8480/3
		Mixed	8140/3
Urachal carcinoma	-	-	8010/3
Tumours of Mullerian type	Clear cell carcinoma	-	8310/3
	Endometrioid carcinoma	-	8380/3
Mesenchymal tumours	Rhabdomyosarcoma	-	8900/3
	Leiomyosarcoma	-	8890/3
	Angiosarcoma	-	9120/3
	Perivascular epitheloid cell tumour	-	8714/3
	Solitary fibrous tumour	-	8815/1
	Granular cell tumour	-	9580/0
Urothelial tract haematopoietic and lymphoid tumours	-	-	[a]
Miscellaneous tumours	Carcinoma of Skene, Cowper, and Littre glands	-	8140/3
	Metastatic tumours and tumours from other organs	-	[a]
	Epithelial tumours of the upper urinary tract	-	[a]
	Tumours arising in a bladder diverticulum	-	8144/3
	Urothelial tumours of	-	[a]

	the urethra		

UC, urothelial carcinoma; NOS, not otherwise specified; [a]Currenty no recognised ICD-10 code. Adapted from REF.[3].

Table 2 | **Therapeutic algorithm for advanced bladder cancer.**

Pathology	First-line systemic therapy	Level of evidence	references
Algorithm for advanced disease according to pathologic review			
Urothelial carcinomas	• Platinum and gemcitabine • Anti-PDL1 or anti-PD1 antibody if patients are platinum-ineligible and express high levels of PD-L1 protein	1 a 2 b	27 31 56
Squamous cell carcinoma	• Platinum and gemcitabine • Carboplatin and paclitaxel	3b	115,116 117-119
Neuroendocrine	• Platinum and etoposide	4	125-127
Adenocarcinoma and urachal Carcinoma	• Oxaliplatin and 5-fluorouracil	4	136
Sarcomatoid urothelial cancer	• Platinum and gemcitabine • Carboplatin/paclitaxel	4	107 108
Sarcoma of the bladder	• Doxorubicin and Ifosfamide • Gemcitabine and Docetaxel	4	142 143
Horizon scanning: molecular pathology-driven therapy for UC			
TCGA cluster I	Novel targeted therapy	5	
TCGA cluster II	Immunotherapy	5	
TCGA cluster III	Platinum and gemcitabine (or chemoimmunotherapy)	5	
TCGA cluster IV	Novel targeted therapy	5	

The levels of evidence are based on the Oxford (UK) CEBM Levels of evidence[165]. 1a, systematic reviews of randomised controlled trials; 2b, individual cohort study or low quality RCT; 3b, individual case controlled studies; 4, case series; 5, expert opinion.

Table 3 | **PDL1 and PD1 checkpoint inhibitor trials in advanced urothelial cancer.**

Name	Phase	End points	Patient selection	Treatment Arms	Patients (*n*)	ORR (%)	RR by PDL1 expression (%)	Survival (months)	Grade 3–4 toxicity (%)	IHC mAb and comments	Refs
KEYNOTE-012	Ib	Safety, tolerability, ORR	aUC, post-PLT setting	Pembrolizumab	33 (27 evaluable)	26	PD-L1^{+}: 38	mPFS: 2 mOS: 13	15	DAKO 22C3 mAb	[64]
KEYNOTE-045	III	Co-primary: OS and PFS	aUC, second-line setting	Pembrolizumab or physicians choice of chemotherapy (vinflunine, paclitaxel, or docetaxel)	542	21.1 versus 11.4	CPS ≥10%: 21.6 versus 6.7	OS:10.3 versus 7.4 (HR 0.73; *P*=0.002) PFS: 2.1 versus 3.3 (HR 0.98, *P*=0.42) mOS for CPS ≥10%: : 8 versus 5.2 (HR 0.57; *P*=0.005)	15.0 versus 49.4	DAKO 22C3 mAb Benefit of pembrolizumab in all subgroups, including the PD-L1 <1% group and patients with liver metastasis	[51]
KEYNOTE-052	II	ORR	mBC, PLT-ineligible setting	Pembrolizumab	374 (370 treated)	24	CPS validation cohort (*n*=270) CPS ≥10%: 39 CPS 1–<10%: 20 CPS <1% : 11	mPFS: 2 (6 month OS: 67%)	15	DAKO 22C3 mAb PD-L1 centrally reviewed Durable response rate	[52]
CheckMate 032	I/II	ORR	aUC, post-PLT setting	Nivolumab	86 (78 treated)	24.4	≥1% on TCs: 24.0 <1% on TCs: 26.2	mPFS: 2.8 mOS: 9.7 mDR: 9.4	22	DAKO 28-8 mAb Unselected on PDL1	[53]
CheckMate 275	II	ORR	aUC, post-PLT setting	Nivolumab	270 (265 evaluable)	19.6	≥5% on TCs: 28.4 1–4% on TCs: 23.8 <1% on TCs: 16.1	mPFS: 2 mOS: 8.7	18	DAKO 28-8 mAb Unselected on PDL1 25-gene INFγ response signature	[54]
PCD4989g	I	Safety, tolerability, ORR	mUBC, any line; 72% ≥2 lines	Atezolizumab	68 (67 evaluable)	26.2	PDL1 IHC 0–1: 11 PDL1 IHC 2–3: 43	Not presented	4	Ventana SP142 Initially PDL1^{+} only and then expanded to all	

PCD4989g	I	Safety, tolerability, ORR	mUBC, any line; 72% ≥2 lines	Atezolizumab	95	10.1	≥5% on ICs: 40 <5% on ICs: 11	mPFS: 2.7 mOS: 10.1 mDR: 22.1 mPFS for PD-L1 ≥5% on ICs: 5.5 mOS for PD-L1 ≥5% on ICs: 14.6	9	Ventana SP142 Similar OS in patients aged ≥65 years and <65 years	
IMvigor 210 (Cohort 1)	II	ORR	aUC, PLT-ineligible	Atezolizumab	123 (119 evaluable)	23	<1% on ICs: 21 1–<5% on ICs: 21 ≥5% on ICs: 28	mPFS: 2.7 mOS: 15.9	7	Ventana SP263 mAb High ORR in UTUC TMB predicts ORR	[75]
IMvigor 210 (Cohort 2)	II	ORR	aUC, post-PLT setting	Atezolizumab	315 (310 treated)	15	<1% on ICs: 8 1–<5% on ICs: 10 ≥5% on ICs: 26	mPFS: 2.1 mOS: 11.4 mDR: 13.7 (not reached)	16	Ventana SP263 mAb TCGA-T and TMB predictive of ORR	[56]
IMvigor 211	III	ORR in PDL1+ patients (≥5% PDL1 expression of immune cells)	aUC	Atezolizumab versus physicians choice of chemotherapy (vinflunine, paclitaxel, or docetaxel)	931	13 versus 13	≥5% on ICs: 23 versus 22	OS for PD-L1 ≥5% on ICs: 11.1 versus 10.6 (HR 0.87, *P*=0.41) OS for PD-L1 ITT population: 8.6 versus 8.0 (HR 0.85; NS)	20 versus 43	Ventana SP142 mAb PDL1 expression not predictive	[58]
Massard et al. 2016	I/II	Safety, ORR	mUBC, any line; 31.1% ≥3 lines	Durvalumab	61 (42 evaluable)	31	≥25% on TCs and ICs: 46.4 <25% on TCs and ICs: 0	Not reported	G3: 4.9	SP263 mAb Median FU 4.3 months	[59]
Powles et al. 2017	I/II	Safety, ORR	aUBC, any line; 95.3% post-PLT setting	Durvalumab	191	17.8	≥25% on TCs and ICs: 27.6 <25% on TCs and ICs: 5.1	mPFS: 1.5 mOS: 18.2 (Median FU only 4.3 months)	6.8	SP263 mAb High ORR in LN only disease	[60]
JAVELIN	I	Safety, tolerabi	mUC, post	Avelumab	44	18.2	≥5% on TCs: 50	mPFS: 2.9	6.8	Dako 73-10	[61]

		lity, ORR	PLT setting				<5% on TCs: 4.3	mOS: 13.7		mAb 5 CRs	
JAVELIN (updated)	I	Safety, tolerability, ORR	aUC, post-PLT setting or PLT-ineligible	Avelumab	249 (161 second-line)	16	≥5 on TCs %: 24 <5% on TCs: 13	mPFS: 1.6 mOS: 6.5	8	Dako 73-10 mAb Pooled analysis of 2 cohorts	

ORR, overall response rate; RR, response rate; mPFS, median progression free survival; mOS, median overall survival; mDR, median duration of response; CR, complete response; aUC, advanced (locally recurrent, locally advanced & metastatic) urothelial carcinoma (including renal pelvis, ureter & bladder); mUC, metastatic urothelial carcinoma; mUBC, metastatic urothelial cancer of bladder origin only; UTUC, upper tract urothelial carcinoma; PLT, platinum chemotherapy; IC, inflammatory cells; TCs, tumour cells; CPS, combined positive score of PD-L1 on immune and tumour cells; TCGA-T, Cancer genome atlas bladder subgroup taxonomy; TMB, tumour mutational burden; ITT, intention to treat; NS, not significant; NIVO, nivolumab; DURVA, durvalumab; AVELU, avelumab; mAb, monoclonal antibody for PD-L1; CT, chemotherapy; FU, follow up; LN, lymph node;

Table 4 | **Targeted therapy trials in advanced urothelial cancer**

Study	**Phase**	**Targeted agent**	**End points**	**Patient selection**	**Treatment Arms**	**Patients (*n*)**	**Response and survival**	**Refs**
Milowsky et al. 2013	II	mTOR inhibitor evorolimus	PFS	mUC, post-chemotherapy	Single arm: evorolimus	45	1 PR, 1 CR, 12 minor regressions Median PFS 2.6 months	82
Powles et al. 2017	III	HER family (EGFR/HER2) inhibitor lapatinib	OS, PFS	mUC, post-first-line chemotherapy, $EGFR^+$ or $HER2^+$	Lapatinib versus placebo	232	RR 14% versus 8%	84
Nogova et al. 2017	I	FGFR mutation inhibitor BGJ398	Determination of MTD and RP2D	Advanced solid tumours with *FGFR* alterations	Escalating doses of BGJ398	132	7 PR in patients with *FGFR1* amplified NSCLC and *FGFR3* mutant UC. ORR 38%	90
Loriot, Y. et al. 2018	II	FGRF2/3 inhibitor erdafitinib	RR	mUC with specific *FGFR2/3* mutations, chemorefractory	Continuous or intermittent dosing of erdafitinib	78	RR 35% in continuous arm and 24% in intermittent	91

RANGE; Petrylak et al. 2018	III	VEGF2 inhibitor ramucirumab	PFS	mUC, platinum-refractory	Docetaxel plus ramucirumab versus docetaxel plus placebo.	530	RR 24.5% versus 14%, PFS 4.07 months versus 2.76 months	[92]

PFS, progression free survival; mUC, metastatic urothelial carcinoma; RR, response rate; PR, partial response; RP2D, recommended phase 2 dose; CR, complete response; OS, overall survival; MTD, maximum tolerated dose

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