PERSPECTIVE

For reprint orders, please contact: reprints@futuremedicine.com

Systemic therapy for bladder cancer finally comes into a new age

Matthew Zibelman¹ & Elizabeth R Plimack^{*,1}

Systemic therapy for bladder cancer, both localized muscle-invasive disease and metastatic disease, has seen minimal progress over the past two decades. Current approaches rely upon cytotoxic chemotherapy combinations aimed at increasing cure rates or achieving palliation and disease control, but these regimens are fraught with short- and long-term toxicities and outcomes remain suboptimal. The emergence of systemic immunotherapies that can provide durable remissions in subsets of patients with other malignancies has the potential to transform the field, and early phase trials have begun to demonstrate activity in some patients with metastatic bladder cancer. In this article, we review the current state of systemic therapy for bladder cancer and discuss the current literature and ongoing trials utilizing various immunotherapies.

First draft submitted: 21 March 2016; Accepted for publication: 2 June 2016; Published online: 12 July 2016

Cancers of the urinary bladder are the sixth most commonly diagnosed malignancy in the USA and the most prevalent malignancy of the GI tract, with 74,000 new cases estimated from 2015 [1]. The predominant histologic subtype is urothelial carcinoma (UC), which accounts for more than 90% of diagnoses in the western world. For patients with muscle-invasive bladder cancer (MIBC) confined to the bladder and deemed surgical candidates, radical cystectomy (RC) with pelvic lymphadenectomy remains the gold standard, with bladder preservation techniques using combinations of radiation therapy (RT) and systemic therapy receiving increased attention [2–5]. Local therapy with surgery or radiation alone is suboptimal, with median overall survival (OS) ranging from 1.8 to 3.8 years and 5-year survival of only 43% after RC and less than 40% with RT, with the majority of deaths related to distant disease [6,7]. The addition of neoadjuvant chemotherapy (NAC) has significantly improved outcomes. For patients who present with metastatic disease, systemic chemotherapy with cisplatin-based regimens, long recognized as an active drug in this disease, is accepted as the standard of care. Thus, systemic therapy is an integral component of treatment for patients with UC, yet despite tremendous advances across a multitude of malignant conditions, no new therapies have entered the armamentarium in over decade. Fortunately, recent and ongoing research in the burgeoning field of cancer immunotherapy (IT) offers hope that new agents will finally demonstrate survival benefits in clinical trials. In this article, we review the definitive studies that have established the current standards of care for systemic treatment in UC, then focus on new and future trials aiming to bring the systemic treatment of UC into the IT age.

KEYWORDS

- bladder cancer
- checkpoint blockade
- CTLA-4 • immunotherapy
- neoadjuvant chemotherapy • PD-1
- urothelial carcinoma

¹Fox Chase Cancer Center, Temple Health, 333 Cottman Avenue, Philadelphia, PA 19111, USA

*Author for correspondence: Tel.: +1 215 728 3889; Fax: +1 215 728 3639; elizabeth.plimack@fccc.edu

Neoadjuvant/adjuvant therapy**• Standard cytotoxic chemotherapy in the neoadjuvant setting**

The National Comprehensive Cancer Network (NCCN) and the European Association of Urology (EAU) guidelines recommend the use of neoadjuvant, cisplatin-based chemotherapy for patients with organ-confined MIBC prior to RC for cisplatin-eligible patients [8,9]. This recommendation is based on high-level evidence from randomized controlled trials and uniform consensus among the Bladder Cancer Panel regarding the efficacy of this approach. Despite this recommendation, retrospective studies in the USA and Europe have reported that the majority of patients do not ultimately receive this potentially life-preserving therapy [10–12]. Reasons cited for this have varied, but include concerns about the toxicities of chemotherapy leading to worsening performance status and treatment delays, ultimately contributing to worse perioperative outcomes, though many of these concerns have proved to be unfounded [12,13]. Fortunately, there is some evidence that practice patterns may be changing, increasing the proportion of MIBC patients offered NAC [11,14]. Improvements over time in drug administration and supportive medications have improved patient tolerability to NAC, and more oncologists from all backgrounds have increasingly accepted NAC as standard practice. Furthermore, excitement about new agents to be combined with, or used instead of, standard chemotherapy offers promise for continued acceptance.

The first large, randomized study to demonstrate a benefit for NAC in patients with MIBC came from the BA06 30894 trial investigating three cycles of cisplatin, methotrexate and vinblastine (CMV) prior to definitive RC or RT versus definitive local therapy alone. While the initial results presented in 1999 revealed a non-statistically significant improvement in survival in the NAC cohort, the long-term follow-up results demonstrated a statistically significant 16% relative reduction in the risk of death and a 6% absolute benefit in OS at 10 years [15,16]. Given the results of the original study, this trial was not enough initially to tip the balance in favor of NAC. That changed in 2003 after publication of the Southwest Oncology Group (SWOG) 8710/Intergroup (INT)-0080 trial. This study used a similar regimen comprising methotrexate, vinblastine, doxorubicin, and

cisplatin (MVAC), and randomized patients to three cycles of MVAC followed by RC versus RC alone [6]. Final analysis revealed a clinically significant improvement in OS (77 vs 46 months; $p = 0.06$), and patients treated with NAC achieved a 38% complete response (no evidence of residual bladder disease, pT0) rate, leading to an OS of 85% at 5 years for those found to be pT0 at RC. This study established NAC as a viable, efficacious strategy for patients with nonmetastatic MIBC.

While the SWOG/INT study validated that NAC could improve outcomes, the ‘standard’ MVAC given in this study was administered over 28 days for three cycles, and this regimen was relatively toxic (33% grade 4 granulocytopenia), with a mean time from randomization to cystectomy in the NAC group of 115 days [6]. Consequently, strategies to improve tolerability and shorten the interval from needle to knife were needed to increase broad clinician acceptance and utilization. Borrowing from other malignancies and the metastatic UC setting, an accelerated regimen of MVAC (AMVAC) with granulocyte colony-stimulating factor (G-CSF) support given to allow hematologic recovery and facilitate dosing every 2 weeks was established [17–19]. For metastatic UC, AMVAC as compared with standard MVAC led to a statistically significant improvement in the complete response (CR: 21 vs 9%, $p = 0.009$) rate and median progression-free survival (PFS: 9.1 vs 8.2 months; $p = 0.037$) and a strong trend toward improved overall response rate (ORR: 62 vs 50%, $p = 0.06$) [18]. Importantly, it also resulted in less hematologic toxicity and shorter time to deliver the same dose concentrations. While AMVAC has not been studied in a randomized fashion in the neoadjuvant setting, two single-arm Phase II trials have demonstrated that NAC with AMVAC is safe, feasible and favorably comparable to historic trials [20,21].

Another treatment option for NAC is the combination of gemcitabine and cisplatin (GC). In the metastatic setting, GC has demonstrated similar efficacy with less toxicity compared with standard MVAC in a randomized trial, and these data have been extrapolated to support the use of GC in the neoadjuvant space [22]. However, to date NAC with GC has not been prospectively evaluated. Two contemporary, large, retrospective studies have evaluated the role of NAC in real-world settings to try to compare GC with MVAC [23,24]. Galsky *et al.* used an electronic

data-capture platform to evaluate usage and outcomes with NAC from 212 patients at 28 international centers [23]. GC was used most commonly (146 vs 66 patients who received MVAC), and 77% of the MVAC patients received in the accelerated fashion. Overall, the pT0 rates in the two arms were similar for MVAC and GC and hazard ratios for survival adjusted by the propensity scores yielded no statistically significant difference in survival. The largest retrospective study of NAC for UC compiled data from 935 patients across 19 institutions internationally that had received at least three cycles of preoperative chemotherapy [24]. In this study, NAC regimens included GC (64%), MVAC (20%, not specified if accelerated), and other (15%). The pT0 rate did not differ significantly between the MVAC and GC groups (24.5 and 23.9%, respectively). At this point, while little prospective data exists to support the use of GC as the NAC regimen of choice for patients with MIBC, it appears to be the most common regimen utilized based on retrospective data, extrapolation from the metastatic setting, and clinician perceptions of tolerability [23–25].

The ability to predict which patients would be most likely to benefit from NAC, would improve the therapeutic index of this treatment. Plimack *et al.* have reported their results of tissue sequencing specimens gleaned prospectively from MIBC patients who were treated on two separate trials with cisplatin-based NAC [26]. They determined that alterations in one or more of three DNA repair genes (*ATM*, *RBI*, *FANCC*) correlated with pT0 rate and OS, suggesting sensitivity to cisplatin-based chemotherapy. *ERCC2* has also emerged as a promising biomarker of cisplatin response [27]. Further studies of this genetic signature, as well as other predictive biomarkers, will help clinicians optimize patient selection and further improve outcomes.

• Adjuvant chemotherapy

Despite the clear evidence of feasibility and a survival benefit with NAC, the role of adjuvant therapy for MIBC remains controversial. For patients with $\geq T3$, node-negative disease, RC alone provides 10-year recurrence-free survival in only about 45–61%, so it is tempting to assume adjuvant chemotherapy will provide some benefit [28]. Yet, about 30% of patients suffer complications with RC that prevent subsequent receipt of chemotherapy, so feasibility for both clinical trial accruals and real-world

application is hampered [29]. Several large randomized trials have attempted to clarify the role of chemotherapy administered after RC, but most have been marked by poor accrual and flawed design.

The Spanish Oncology Genitourinary Group performed a Phase III trial of post-RC patients with pT3–4 and/or node-positive disease and randomized them to observation or chemotherapy with GC plus paclitaxel [30]. The study was closed after 7 years with less than half of planned accrual (142 of planned 340), thus was severely underpowered and has only been presented in abstract form at the 2010 American Society of Clinical Oncology (ASCO) Annual Meeting. Despite this, the 5-year OS significantly favored the chemotherapy arm (60 vs 31%; $p < 0.0009$). Although this study was positive, the accrual shortfall and choice of regimen begged for confirmation from more robust randomized trials. Accordingly, several large trials were planned to compare immediate adjuvant chemotherapy versus the same treatment administered at recurrence, or comparing adjuvant therapy versus sequential therapy. Like the preceding trials, all of these closed prematurely due to poor accrual, and thus they were unable to demonstrate a clear benefit of adjuvant chemotherapy [31,32].

With prospective randomized data lacking, meta-analytic methods have been utilized in an attempt to consolidate the information obtained from patients across the various adjuvant trials. The largest meta-analysis performed to date summarized trial-level data from 945 patients across nine studies [33]. The results found an improvement in OS with the use of adjuvant chemotherapy compared with RC alone (HR: 0.77; 95% CI: 0.59–0.99; $p = 0.049$), as well as an improvement in disease-free survival (DFS; HR: 0.66; 95% CI: 0.45–0.91) that was weighted toward the patients with node-positive disease ($p = 0.010$). More recently, Galsky and colleagues published their findings of a meticulous observational study of ‘real-world’ patients receiving adjuvant chemotherapy for high-risk MIBC, defined as pT3–4 and/or pathologically node positive [34]. Data from the National Cancer Data Base (NCDB) were used to compare OS for 5653 patients receiving adjuvant chemotherapy (23% of total) versus observation after curative-intent surgery. Their statistical analysis estimated propensity scores for all measured variables in an attempt to minimize confounding data across observed variables. Their stratified

results adjusted for propensity score revealed an OS benefit in the group receiving chemotherapy (HR: 0.70; 95% CI: 0.64–0.76) and appeared to persist across subset analyses. Although this trial is limited by the lack of patient specificity and selection bias inherent to observational studies, the size and statistical rigor offer further indirect support for the consideration of adjuvant chemotherapy. Across all of these attempts at clarifying a clouded issue, the guideline consensus is that NAC is standard, with adjuvant chemotherapy a reasonable consideration for patients with T3, T4 or node-positive disease who did not receive NAC prior.

• Immunotherapy

While NAC has a clear but controversial role in the treatment of MIBC, the emergence of novel treatment strategies across the landscape of oncology has instigated interest in potential alternatives. Specifically, IT with checkpoint blockade is being evaluated with and without chemotherapy for patients with MIBC in the preoperative setting. The primary pharmacotherapeutic targets currently include the PD-1 pathway and CTLA-4. PD-1 pathway blockade includes drugs targeting both the PD-1 receptor and its primary ligand (found on some tumor and immune cells) PD-L1. Immune checkpoint blockade in the metastatic setting for patients with advanced UC will be discussed later, but the neoadjuvant space is ripe for trials investigating these agents. Immune checkpoints are part of the innate immune system and refer to immune cell receptors that control downregulation of a targeted immune response against a foreign stimulus in order to protect against overactivation. Cancers are included as one of these stimuli and can hijack this system to evade immune surveillance. The mechanism of checkpoint blockade against solid tumors requires the presence of tumor antigens so that immune cells subsequently unleashed after inhibition of these key checkpoints have a recognizable target to attack. This may result in both direct cell-mediated antitumor toxicity by disinhibited immune effector cells, as well as instigating generation of immunologic memory that can lead to durable antitumor immunity [35]. Thus, the use of immunotherapeutic agents prior to definitive, curative-intent therapy for early-stage disease (i.e., when tumor is still present in the bladder) is appealing. Whether trials should be designed to continue checkpoint inhibition after

local treatment, and for how long, is a question of considerable interest.

Ipilimumab was the first checkpoint inhibitor studied in UC, and this initial study also marked the first time a checkpoint inhibitor was investigated in the preoperative setting [36]. In this neoadjuvant ‘window of opportunity’ study, patients with high-grade T1/T2 UC were treated with two doses of ipilimumab before proceeding to RC. This study was primarily exploratory and the most significant finding was a notable dose-dependent increase in CD4-positive inducible costimulator (ICOS^{hi}) cells in both the periphery and tumor microenvironment, which is being further explored as a potential biomarker. No correlation to outcomes was reported, but the study did establish the safety and feasibility of preoperative checkpoint blockade. With the emergence of drugs targeting the PD-1 pathway showing improved outcomes in a variety of malignancies, including in early studies of patients with advanced UC, trials including these agents was a logical next step.

Biologically, the optimal strategy to study checkpoint blockade in the neoadjuvant setting of UC is unknown and poses difficult design considerations. Given the proven benefits of NAC in chemotherapy eligible early-stage UC patients, there is a considerable lack of equipoise in treating ‘fit’ patients, whether in a randomized or single-agent setting, without NAC. Thus, selection of appropriate patient populations and the use of combination regimens are imperative. In a planned, straightforward, proof-of-concept neoadjuvant study, the PD-L1 inhibitor atezolizumab (MPDL3280A) will be administered as a single agent for two doses prior to RC, with relevant correlative studies embedded in the design (ABACUS) [37]. Eligible patients must be deemed clinically operable, but unable or unwilling to receive NAC. A separate, single-arm, Phase II study of atezolizumab is planned for patients with MIBC who refuse or are ineligible for NAC, and there is also an arm for patients with NMIBC that is refractory to standard intravesical bacillus Calmette–Guérin [38]. The study will accrue several cohorts of eligible patients at increasing doses and durations (one to three doses preoperatively) of atezolizumab administration prior to RC, with patients given an option to receive adjuvant atezolizumab. Alternatively, there is the option of adding IT to standard NAC. Two trials are currently exploring this approach combining the PD-1 inhibitor pembrolizumab

with GC prior to planned RC. Given that cytotoxic therapies can cause direct tumor cell death, potentially resulting in release of tumor antigens that become available for immune recognition, these combinations warrant clinical study. A planned Phase Ib/II study intends to establish a recommended Phase II dose (RP2D) combination of pembrolizumab with GC, followed by a Phase II expansion at the RP2D, as well as an arm for cisplatin-ineligible patients who will receive pembrolizumab with gemcitabine alone [39]. The second Phase II trial provides GC/pembrolizumab in a cisplatin-eligible, surgically operable population [40].

Alternatively, the use of checkpoint blockade in the purely adjuvant space runs somewhat counterintuitive to the proposed mechanism of action, as post-surgery there would theoretically be a minimum amount of available tumor antigens from which to generate an enduring immune response. In RCC, surgical resection of the primary tumor resulted in a rapid and significant decline in expression of PD-1 receptors on immune cells, potentially eliminating the primary target of PD-1 inhibitors [41]. Yet in melanoma, the CTLA-4 inhibitor ipilimumab has received US FDA approval for the adjuvant treatment of high-risk, stage III patients after complete resection [42]. This study compared the use of ipilimumab to placebo, not the prior standard of care IFN- α , but offers hope that IT in this setting is viable. For patients with UC, where adjuvant therapy has not demonstrated an OS benefit in a properly powered randomized trial, this strategy is worthy of study. Currently, there are two purely adjuvant studies recruiting MIBC patients to receive checkpoint blockade after RC. IMvigor 010 is a randomized, Phase III trial of atezolizumab versus observation for patients who did not receive NAC and are unwilling or unable to receive adjuvant chemotherapy [43]. This study is open internationally with plans to enroll 440 patients. There is also a similarly planned randomized, double-blind, placebo-controlled Phase III trial investigating the PD-1 inhibitor nivolumab in the adjuvant setting [44].

Importantly, the toxicity profile with checkpoint blockade clearly differs from cytotoxic chemotherapy, and is arguably improved, but these agents are not without risk. The release of immune checkpoint control can lead to autoimmunity of almost any organ system as part of the innate mechanism protecting against recognition of self is inhibited. This can result in the

development of immune-related adverse events (irAEs), essentially the precipitation of the host immune system attacking itself. Common manifestations of this can include colitis, dermatitis, pneumonitis and various endocrinopathies. In the available data for patients with UC, albeit in the metastatic setting, the rate of grade 3–4 adverse events (AEs) has ranged from 8 to 16% [45–47]. Whether this event rate will persist in the early disease setting remains to be seen. Management of irAEs depends on prompt recognition of symptoms heralding an immune event, followed by aggressive suppressive therapy with corticosteroids along with supportive measures [48]. Once toxicity is stabilized, patients should be tapered from corticosteroids over at least 1 month and further therapy may need to be discontinued. For those patients that do not respond to corticosteroids within 2–3 days, administration of immunosuppressive agents such as infliximab or mycophenolate (in patients with hepatotoxicity) can be beneficial.

Checkpoint blockade is not the only investigational IT being evaluated in early-stage UC. DN24-02 is an autologous, HER2 targeted cellular IT modeled after sipuleucel-T (an FDA-approved treatment for metastatic prostate cancer) that combines antigen presenting cells (APCs) with a recombinant HER2 derived antigen known as BA7072 and linked to granulocyte macrophage colony-stimulating factor. The goal of therapy is to stimulate an enduring immune response against residual malignant, HER2-positive (considered $\geq 1+$ by immunohistochemistry) UC after RC, with patients receiving three infusions after leukapheresis and individualized drug preparation. As of the last update at the 2014 ASCO Annual Meeting, 38 patients had been treated and there was strong evidence of APC activation and a prime cytokine response. An AE occurred in approximately 15% of patients, with fatigue most common, and three patients experienced an AE $\geq$ grade 3. Accrual has since completed and results are expected.

Systemic therapy for metastatic disease

• Chemotherapy

Platinum-based regimens remain the backbone of chemotherapeutic treatment for patients with UC, with cisplatin first recognized as having activity in advanced UC in 1976 [49]. For patients who are eligible to receive cisplatin based on consensus criteria of performance status and relevant comorbidities, regimen selection depends on

varying side effect profiles as well as patient and physician preference [50,51]. Strategies to improve on standard MVAC have diverged in two major ways. With the emergence of growth factor support, it became feasible to give the MVAC agents in an accelerated, bi-weekly fashion to alleviate some of the toxicity while maintaining or even improving efficacy. AMVAC (also sometimes referred to as dose dense MVAC) was compared with standard MVAC in the European Organization for Research and Treatment of Cancer (EORTC) 30924 trial [18,19]. In long-term follow-up, AMVAC was associated with a strong trend toward improved ORR (64 vs 50%; $p = 0.06$) and an improvement in OS (15.1 vs 14.9 months; $p = 0.042$; HR: 0.76; 95% CI: 0.58–0.99) with survival at a mean follow-up of 7.3 years favoring the AMVAC arm (24.6 vs 13.2%). Grade ≥ 3 toxicity, driven mainly by the decrease in neutropenia with the addition of GM-CSF, favored AMVAC.

Standard MVAC has also been directly compared with the alternative regimen of GC in a randomized Phase III trial and there was no significant difference in OS (GC 14.0 months vs MVAC 15.2 months; $p = 0.66$) [22]. The toxicity profiles differed, with patients on the GC arm experiencing greater thrombocytopenia and anemia, while patients on the MVAC arm suffered from more neutropenia and mucositis [52]. Some have contended these differences favor GC as this regimen offers similar efficacy with less toxicity, which is desirable in the palliative setting. However, much of the toxicity of standard MVAC is mitigated with the addition of GM-CSF to AMVAC, and GC has never been compared head to head with AMVAC in a randomized setting. One trial performed in Greece randomized patients with treatment naïve advanced UC to AMVAC or GC given in a biweekly, dose-dense fashion with GM-CSF support (ddGC) [53]. Efficacy including median OS and PFS (mOS, mPFS) were similar between the two groups (AMVAC vs ddGC; mOS: 19 vs 18 months; $p = 0.98$, mPFS: 8.5 vs 7.8 months; $p = 0.36$). Toxicity marginally favored ddGC (grade ≥ 3 50% AMVAC vs 44% ddGC) and significantly more patients received at least six cycles of ddGC compared with AMVAC (85 vs 63%; $p = 0.011$). Regardless, both regimens remain viable options for cisplatin-eligible patients with metastatic UC.

Unfortunately, the epidemiology of patients who develop UC dictates that a substantial

proportion of patients are ineligible to receive cisplatin-based regimens due to performance status, renal dysfunction, cardiac dysfunction or hearing impairment [50]. A creatinine clearance ≥ 60 ml/min is generally used to identify patients safe to receive cisplatin in regards to renal function. For patients with borderline creatinine clearance (50–60 ml/min), alternative options that have been proposed include splitting the cisplatin dose over the first two days of each cycle (35 mg/m² on days 1–2 instead of 70 mg/m² on day 1) or changing to carboplatin. In the perioperative setting, carboplatin is deemed inferior to cisplatin and replacement is strongly discouraged, but in the palliative setting this is a reasonable alternative. For patients truly unfit for cisplatin, gemcitabine and carboplatin (GCa) has been extensively studied. The Hellenic Co-operative Oncology Group conducted a single-arm Phase II trial of GCa in previously untreated patients with ECOG PS of 3, age >75 years or GFR >50 ml/min [54]. The ORR was 36%, with a median overall survival (mOS) of 7.2 months, both reasonable for this population. A Phase II/III EORTC 30986 trial randomized cisplatin-ineligible patients to two different carboplatin-based regimens: GCa and methotrexate/carboplatin/vinblastine (MCAVI) [55]. The ORR showed a nonsignificant trend to improvement in the GCa group (41.2 vs 30.3%; $p = 0.08$), but there was no difference in mOS (9.3 vs 8.1 months; $p = 0.64$). Severe AEs were higher with MCAVI (21.2 vs 9.3%), thus the study favored GCa. GCa is a reasonable option for cisplatin-ineligible patients, though it is worth to mention that this regimen has never been compared in a randomized fashion to gemcitabine alone, which has demonstrated an ORR of 28% and mOS of 54 weeks in a similar population [56].

There is currently no standard chemotherapy regimen for the treatment of patients beyond first line in metastatic UC. Vinflunine is the only drug to have demonstrated a survival benefit in a Phase III trial; however, this was compared with best supportive care and was not a statistically significant improvement [57]. While this drug is used in some sites in Europe, it is not FDA approved. Generally, consensus guidelines support sequential single-agent therapies for treatment of patients in second line and beyond based on data from single arm, Phase II studies. Multi-agent regimens generally add toxicity without clinically meaningful

improvements in outcomes. If the chosen first-line regimen did not include gemcitabine, this is a viable second-line option as a single agent. In this setting, it has achieved an ORR of 22.5% and an mOS of 5 months [58]. Taxanes, including paclitaxel, nab-paclitaxel and docetaxel, have established activity for these patients, with ORRs ranging from 10 to 28% and including several durable responses [59–62]. The nontaxane microtubule modulator eribulin has more recently been evaluated in a single-arm, Phase II study that included treatment-naïve patients as well, and showed an ORR of 32% with an mOS of 9.6 months (for second line or beyond and taxane-naïve ORR was 28%, mOS 9.6 months) [63]. Further studies are planned or underway. Pemetrexed as a single agent has also demonstrated varying activity with ORRs ranging from 8 to 28% [64,65]. Currently, the NCCN guidelines list taxanes, (paclitaxel or docetaxel), gemcitabine and pemetrexed as standard options for second-line chemotherapy, with referral to clinical trials for novel agents as the preferred recommendation when applicable. Vinflunine is an additional option in Europe, but not available in the USA [8].

• Immunotherapy

As discussed above, IT with checkpoint blockade is a promising research avenue in UC, and it offers the potential for durable remissions in a subset of metastatic UC (mUC) patients for which there are no standard options. Atezolizumab, a PD-L1 inhibitor, was US FDA approved for the treatment of patients with platinum refractory advanced UC in May 2016 based on results of the IMVigor 201 trial. IMVigor 210 trial is a two-arm parallel Phase II trial of atezolizumab including treatment-naïve cisplatin-ineligible patients with mUC (cohort 1) and patients with platinum-refractory mUC (cohort 2) [47]. Analysis of cohort 2 showed a modest ORR at 15%, though notably ORR was 26% in patients with high expression of PD-L1 in immune cells (IC) defined as 2+ or 3+ by IHC. The mOS was 7.9 months in the cohort overall, but notably 11.4 months in those with IC 2+ or 3+. Results of a large, multi-arm Phase I study of pembrolizumab (KEYNOTE-012) included a UC cohort and updated results were presented at the 2015 ASCO Annual Meeting [46]. Out of the 33 patients treated, the ORR was 28% with an mOS of 12.7 months. Toxicities with both pembrolizumab and atezolizumab were consistent

with what has been published for other tumor types, with grade 3–4 AEs at about 15% in both trials. Durvalumab is another PD-L1 inhibitor that has been tested in platinum-resistant mUC and results from its Phase I trial (Study 1108) have led to the drug being granted the Breakthrough Therapy designation by the FDA. Updated data from this trial were reported at the 2016 ASCO Annual Meeting [66]. A total of 42 patients were evaluable for response, with an ORR of 38.1%. Patients with $\geq 25\%$ PD-L1 expression on immune or tumor cells has a robust risk ratio (RR) of 53.6%, versus just 7.1% in the group low PD-L1 expression group. The fully human IgG1 mAb avelumab also reported its first results in mUC at the 2016 ASCO Meeting [67]. The ORR in the 44 patients reported on this trial was 18.2%, and was also higher in the group with higher PD-L1 expression (50.0 vs 4.3%), although using a cut-off of $\geq 5\%$ on tumor cells only. Finally, nivolumab reported its first data from previously treated mUC patients who participated in the Phase I/II CheckMate-032 study [68]. the results were generally comparable to other agents, with an ORR of 24.4%. Toxicities on all of these newly reported studies compared favorably to each other and to prior studies.

Ongoing studies will define the role of checkpoint blockade for mUC and intrigue remains. It is becoming clear in other malignancies that while a subset of patients will derive enduring benefit from single-agent therapy, the next steps involve discovery of biomarkers to prospectively identify patients likely to respond, as well as combinations that will increase ORRs. **Table 1** lists a selection of trials currently ongoing or not yet reported that are exploring checkpoint blockade as a single agent or in combination for patients with mUC.

Combination trials involve several different strategies. A few trials are combining checkpoint inhibitors with chemotherapy, as discussed previously. This provides a standard chemotherapeutic option along with IT with the hope that the release of tumor antigens induced by tumor cell lysis will enhance immune recognition after PD-1 blockade. The success of checkpoint blockade depends on the presence of cytotoxic T cells in the tumor microenvironment and tumor antigens that they can recognize. In the laboratory setting, various chemotherapeutic combinations have demonstrated the ability to generate a more immunogenic milieu that can

Table 1. Ongoing clinical trials with immune checkpoint blockade in metastatic urothelial carcinoma.

Trial number	Investigational drug(s)	Setting	Trial design	Phase	Expected completion	Notes
NCT02387996	Nivolumab	Second line and beyond	Single arm	II	April 2016	
NCT02553642	Nivolumab ± ipilimumab	Second line and beyond	Single-arm UC cohort (also melanoma cohort). Patients receive nivolumab up to second progression, then may add ipilimumab	II	September 2017	Also a melanoma cohort Correlatives for mutation burden and neoantigens
NCT01928394	Nivolumab ± ipilimumab	Any line metastatic	Randomized to nivolumab alone or various dose combinations	I/II	August 2017	Multiple tumor cohorts
NCT02496208	Nivolumab + cabozantinib ± ipilimumab	Second line and beyond	Part 1 will receive nivolumab + cabozantinib, while part 2 will receive all three drugs	I	December 2017	Expansion cohorts for each combinations at established doses
NCT02614456	Nivolumab + IFN- γ	Second line and beyond	Single arm for multiple tumors types, with expansion in UC and RCC	I	December 2017	Multiple biopsies embedded for correlative analyses
NCT02335424	Pembrolizumab	First-line, platinum-ineligible	Single arm	II	February 2017	KEYNOTE-052
NCT02500121	Pembrolizumab	First-line maintenance after chemotherapy	Randomized, double-blind, placebo controlled	II	November 2018	Hoosier Cancer Research Group Study Chemotherapy chosen by clinician
NCT02256436	Pembrolizumab	Second- or third-line metastatic	Randomized to pembrolizumab or chemotherapy with either paclitaxel, docetaxel or vinflunine	III	January 2017	KEYNOTE-045 Physician's choice chemotherapy
NCT02560636	Pembrolizumab + radiation therapy	Any line locally advanced or metastatic	Single-arm trial to receive pembrolizumab with 6 weeks of radiation to the bladder	I	February 2019	Not a candidate for definitive local therapy
NCT02437370	Pembrolizumab + docetaxel OR gemcitabine	Second- or third-line metastatic	Two parallel arms combining escalating doses of pembrolizumab with either docetaxel or gemcitabine	I	May 2017	Choice of chemotherapy depends on prior therapy received
NCT02619253	Pembrolizumab + vorinostat	Platinum-resistant metastatic	Dose escalation of vorinostat with fixed dose of pembrolizumab, followed by expansion cohorts	I/II	May 2018	RCC patients also included
NCT02636036	Pembrolizumab + enadenotucirev	Second- to fourth-line metastatic	Single-arm dose escalation followed by dose expansion	I	February 2017	SPICE study Enadenotucirev in an oncolytic adenovirus
NCT02351739	Pembrolizumab + acalabrutinib (ACP-196)	Second line and beyond	Randomized to pembrolizumab alone or the combination	II	May 2017	KEYNOTE 143 (RAPID CHECK) Acalabrutinib is a BTK inhibitor
NCT02443324	Pembrolizumab + ramucirumab	Second- to fourth-line metastatic	Single arm study with parallel arms for other tumor types	I	February 2017	Also being evaluated in gastric/GE junction cancer and NSCLC
NCT02581982	Pembrolizumab + paclitaxel	Second-line, platinum-refractory	Single arm	II	November 2018	

BTK: Bruton's tyrosine kinase; GC: Gemcitabine + cisplatin; GE: Gastroesophageal; mAb: Monoclonal antibody; NSCLC: Non-small-cell lung cancer; RCC: Renal cell carcinoma; TLR: Toll-like receptor; UC: Urothelial carcinoma.

Table 1. Ongoing clinical trials with immune checkpoint blockade in metastatic urothelial carcinoma (cont.).

Trial number	Investigational drug(s)	Setting	Trial design	Phase	Expected completion	Notes
NCT02661100	Pembrolizumab + CDX-1401 + poly-ICLC	Second line and beyond	Single arm	I/II	July 2018	Several disease-specific cohorts CDX-1401 is mAb vaccine specific for DEC-205 and fused to NY-ESO-1 tumor antigen POLY-ICLC is a TLR agonist
NCT02302807	Atezolizumab	Second-line, platinum-refractory	Randomized to atezolizumab or physician's choice chemotherapy	III	January 2017	IMvigor 211 Chemotherapy: docetaxel, paclitaxel or vinflunine
NCT02478099	Atezolizumab	Second-line, platinum-refractory	Single arm with embedded imaging	II	August 2017	Includes the use of investigational PET imaging as tool predictive of response
NCT02516241	Durvalumab + tremelimumab	First-line metastatic	Three-arm randomization to durvalumab alone, durvalumab + tremelimumab or chemotherapy with GC	III	November 2017	Carboplatin allowed in place of cisplatin if deemed ineligible
NCT02546661	Durvalumab + AZD4547	Second- or third-line metastatic	Randomized to durvalumab alone or the combination	I	June 2018	BISCAV study AZD4547 is an FGFR inhibitor Must have an FGFR mutation
NCT02318277	Durvalumab + epacadostat (INCB024360)	Second line and beyond	Single arm	I/II	March 2017	Multiple tumor types Epacadostat is an oral IDO inhibitor
NCT02527434	Durvalumab ± tremelimumab	First-line metastatic	Single arm with all patients starting on tremelimumab, then at progression patients can opt to either switch to or add durvalumab	II	May 2018	Multiple tumor types

BTK: Bruton's tyrosine kinase; GC: Gemcitabine + cisplatin; GE: Gastroesophageal; mAb: Monoclonal antibody; NSCLC: Non-small-cell lung cancer; RCC: Renal cell carcinoma; TLR: Toll-like receptor; UC: Urothelial carcinoma.

sensitize tumors to checkpoint inhibitors [69,70]. Whether these combinations will provide true synergy in the clinic and lead to more durable responses than would otherwise be predicted with chemotherapy alone remains to be seen. The CTLA-4 checkpoint inhibitor ipilimumab has been studied in mUC in combination with GC for first-line therapy for patients with mUC. The most recent update was presented at the 2016 Genitourinary Cancers Symposium [71]. The ORR in this combination with chemotherapy was 64%, with five CRs; however, the study did not meet its primary endpoint for proportion of patients alive at 1 year. PD-1 inhibitors are also being combined with chemotherapeutic agents for mUC. Examples include second line and beyond trials analyzing the combinations of pembrolizumab with gemcitabine or docetaxel and pembrolizumab plus paclitaxel [72,73].

A second strategy commonly employed involves adding a targeted therapy (TT) to IT. While this holds promise particularly in tumor types that have established sensitivity to a given TT, its utility in mUC is purely investigational. Currently, no TTs are approved for use in patients with mUC, although several drugs and targets are being investigated in clinical trials [13,74]. The advantage of this strategy in tumors that are identified to have certain driver mutations for which a specific drug is available is compelling, as it theoretically allows for target-directed tumor cell killing with release of neoantigens followed by the capacity to generate a durable antitumor response. Some examples of these combinations include the PD-L1 inhibitor durvalumab (MEDI4736) with the FGFR inhibitor AZD4547 and pembrolizumab added to the anti-VEGFR2 monoclonal antibody ramucirumab [75,76].

Combinations of two or more IT agents are another promising tactic attempting to harness the immune system on multiple levels. This can include dual checkpoint blockade, which involves inhibiting two known immune checkpoints with drugs simultaneously, such as anti-PD-1 and anti-CTLA-4. The combination of nivolumab and ipilimumab has garnered FDA approval for the treatment of metastatic melanoma based on impressive results in previously untreated patients compared with either drug alone (ORR: 53%), but at the cost of significantly increased toxicity [77]. Early results of this combination in RCC have also been presented, with ORR around 40% [78]. How this combination will fare in a pretreated mUC population that often have other medical co-morbidities is a cause for caution, but the potential is undeniable. Combinations being tested in mUC include nivolumab plus ipilimumab, and durvalumab with or without tremelimumab [79–81]. The PD-1 and CTLA-4 pathways are not the only immune checkpoints being targeted with potential anticancer agents [82]. Other T-cell targets such as LAG3, TIM3 as well as immunomodulatory enzymes from other immune cells such as IDO, are also being targeted with pharmacotherapeutic agents in early-stage clinical trials. These agents hold promise both alone and in conjunction with PD-1 blockade. In lieu of the known toxicity of dual checkpoint blockade, other IT combinations are being tested that hope to provide enhanced ORR compared with single-agent therapy, but mitigate the toxicity. One example of this is the combination of nivolumab with the cytokine IFN- γ which is being studied in a Phase I trial with an expansion cohort in mUC [83].

Future perspective

After decades of little to no progress in the management of patients with UC, hope abounds for new agents in both early-stage and advanced disease. IT offers the potential for durable remissions in a subset of patients with mUC, while time and trial outcomes will tell if these agents can increase the cure rates in patients undergoing definitive therapy for bladder-confined disease. Toxicity is generally manageable and compares favorably to chemotherapy, therefore full integration into clinical practice will depend on how broadly responses can be expected and predicted. It is still too early to predict which agents will win out and find a niche in the clinic, but in

Figure 1 we attempt forecasting one possibility of how the future systemic treatment landscape could change with the advent of immunotherapy. Looking ahead there are some questions that clearly warrant answers to optimize patient care and change practice.

The discovery of predictive biomarkers to determine the most effective therapeutic strategy for a given patient is critical. This holds true for cytotoxic chemotherapy as well as for IT. Chemotherapy is likely to maintain a central role in the treatment of patients with UC at all stages, so continued research to define chemosensitive populations by genetic signatures needs to be evaluated in large prospective studies. In many of the ongoing IT trials, tissue and blood correlatives are embedded to facilitate biomarker discovery, and the results may prove equally as practice-changing as the patient outcomes. PD-L1 expression on UC cells has been correlated with a worse prognosis, and given that this expression is measurable, early studies have stratified by this marker hoping to establish its role as a predictive biomarker [84]. Unfortunately, PD-L1 expression on tumor and immune cells has proven to be unreliable as the sole determinants of response to PD-1 blockade, and given our evolving understanding of the complexity of the immune milieu, this should not be surprising. Variability and lack of standardization of PD-L1 assays, tumor heterogeneity, and the dynamic nature of the immune system complicate precision determination of PD-L1 status with immunohistochemistry and its relevance to clinical decision-making. Additionally, a lack of consensus on the optimal cut-offs for PD-L1 expression as a biomarker exists. At a presentation at the 2015 Annual Meeting of the Society for Immunotherapy of Cancer, Powles *et al.* presented biomarker data derived from the first Phase Ia trial of atezolizumab in mUC [85]. They demonstrated using the NanoString platform that a baseline T-cell and natural killer cell genetic signature in the tumor was associated with clinical benefit, while the presence of a particular stromal signature correlated with disease progression. Prioritizing the inclusion of similar exploratory analyses in future and ongoing studies may help prospectively identify the patients most likely to benefit. Additionally, as combination therapies establish relevance, such biomarker signatures may help stratify patients that can receive single-agent therapy as opposed to a particular combination.

If and when randomized controlled trials prove superior efficacy for IT agents at the various stages

Figure 1. Proposed schema comparing present treatment algorithms with possible algorithms with possible algorithms in the future with immunotherapy for: (A) non-metastatic muscle-invasive bladder cancer and (B) metastatic urothelial carcinoma.

AMVAC: Accelerated methotrexate + vinblastine + adriamycin + cisplatin; GC: Gemcitabine + cisplatin; GCa: Gemcitabine + carboplatin; IT: Immunotherapy; NAC: Neoadjuvant chemotherapy; RC: Radical cystectomy; RT: Radiation therapy; SOC: Standard of care.

of UC, the question of optimal sequencing will become relevant. For patients with mUC, will IT regimens be best utilized in the treatment naïve setting when patients may have lower tumor burdens and have more time to generate an immune response? For patients who present with symptomatic disease or larger tumor burdens, would initial chemotherapy be preferred to attempt cytoreduction prior to initiating IT? It is as yet unknown whether patients are more or less likely to respond to IT before or after receiving chemotherapy and whether the timing has any bearing at all on response. Ideally, we will have data from randomized trials to help answer these questions, including studies that assess drug sequencing. Then consider the issue of systemic therapy choice upon disease recurrence if IT drugs enter

the clinic in the early disease setting. If a patient receives a checkpoint inhibitor in the adjuvant setting, but then has a recurrence, will further checkpoint inhibition yield benefit? And what if they have a serious AE in the adjuvant setting and later recur? Are they then denied the opportunity to get these agents in the metastatic setting, despite what may prove to be an even more efficacious time to use these agents? These are just a few of the many provocative questions that will continue to arise as more data regarding IT options emerge.

Finally, it is important to maintain a healthy sense of cautious optimism as we await results from randomized trials. The oncology history books are littered with promising but ultimately unsuccessful drugs that failed to demonstrate superiority when subjected to the

rigors of randomization, despite encouraging signals from single-arm trials. Case in point, single-arm studies of second-line chemotherapy agents, as reviewed above for mUC, generally have reported RRs in the 10–15% range, but some have demonstrated RRs as high as 28%. These results do not differ much from what has thus far been reported for atezolizumab in the IMvigor 210 study in a similar setting. Certainly the potential of durability remains

enticing, but we need to await longer follow-up and randomized trial results before assuming this or similar agents will ultimately change practice. In the meantime, chemotherapy agents still hold a central role in the management of patients at all stages of UC. The unprecedented pace of enrollment to mUC clinical trials will help answer these questions and hopefully provide new alternatives to current standard-of-care therapies for mUC.

EXECUTIVE SUMMARY

Standard neoadjuvant chemotherapy for muscle-invasive bladder cancer

- Randomized trials have demonstrated a survival benefit for patients with muscle-invasive bladder cancer who receive neoadjuvant chemotherapy, but routine use in clinical practice is still lacking.
- Accelerated methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) and gemcitabine/cisplatin are the consensus best options for neoadjuvant chemotherapy in cisplatin-eligible patients, although they have not been compared in a randomized clinical trial.

Adjuvant chemotherapy for bladder cancer

- Randomized prospective data supporting the use of standard adjuvant chemotherapy after radical cystectomy is lacking, owing primarily to premature closure due to poor accrual of several large randomized trials attempting to answer this question.
- Meta-analyses have suggested a benefit from adjuvant chemotherapy across several trials and analyzing ‘real-world’ data.

Immunotherapy for early-stage disease

- Immunotherapeutic strategies, including checkpoint blockade with agents targeting the CTLA-4 and PD-1 pathways, are being investigated in the neoadjuvant and adjuvant settings.
- Neoadjuvant immunotherapy offers the potential for a clinical response prior to definitive local therapy with the ability to maintain an immunogenic antitumor response.

Chemotherapy for metastatic disease

- The standard first-line treatment for patients with metastatic urothelial carcinoma remains cisplatin-based chemotherapy regimens for those eligible to receive cisplatin.
- No second-line regimen has demonstrated a survival benefit over another active agent in a randomized trial; however, several drugs are options in this setting and may be used as sequential single agent based on toxicity profiles.

Immunotherapy in the metastatic setting

- Phase I and II nonrandomized data demonstrate the potential of PD-1/PD-L1 blockade for patients with metastatic urothelial carcinoma, and atezolizumab is the first immunotherapeutic agent to achieve US FDA approval for this disease.
- Ongoing trials will attempt to establish that these novel agents can provide an overall survival benefit for patients in a variety of settings.
- Combinations of checkpoint inhibitors combined with other immunotherapies, chemotherapies and targeted therapies all are being explored to increase response rates with acceptable toxicity profiles.

Future perspective

- In order to optimize the use of immunotherapy, the discovery of predictive biomarkers will be essential.
- The precise role and benefit of immunotherapeutic agents for bladder cancer is still undefined, so patients should continue to be referred for clinical trials to inform future clinical paradigms.

Conclusion

Systemic treatment for bladder cancer has long been an integral component of the treatment of muscle-invasive, organ confined disease through advanced disease. Cytotoxic chemotherapy regimens have been the mainstay for decades, and combinations including aMVAC and GC have marginally improved outcomes for patients. Patients with MIBC should continue to be referred for NAC when feasible, and consideration of adjuvant therapy is warranted in patients with T3–4 or N1 disease if they have not received NAC. However, as much of oncology moves into a new era of IT, changes in the therapeutic dogma that has defined this disease for decades seem attainable. Immunotherapies, highlighted by drugs targeting immune checkpoint blockade, have shown promise in mUC and have entered clinical trials in the perioperative space. Single-agent therapies may provide durable disease control in a subset of patients, and combination therapies are being explored to extend response rates while mitigating toxicity. With the proliferation of clinical trials and

agents being tested, referring patients for trials is paramount, and incorporating trials with correlative assessments is essential to gain insight into possible predictive biomarkers. Undoubtedly, as trial results yield some answers, new questions will arise. Regardless, the potential availability of new drugs on the horizon for all stages of this disease provides hope that the systemic treatment for UC is finally coming of age.

Financial & competing interests disclosure

M Zibelman has received institutionally directed clinical trial support from Horizon Pharma. ER Plimack has served on advisory boards and as consultant for Genentech, Eli Lilly, Novartis, Pfizer and Bristol-Myers Squibb and has received institutionally directed clinical trial support from Bristol-Myers Squibb, AstraZeneca and Merck. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:

• of interest; •• of considerable interest

- 1 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J. Clin.* 65(1), 5–29 (2015).
- 2 Ploussard G, Daneshmand S, Efstathiou JA *et al.* Critical analysis of bladder sparing with trimodal therapy in muscle-invasive bladder cancer: a systematic review. *Eur. Urol.* 66(1), 120–137 (2014).
- 3 Stein JP, Lieskovsky G, Cote R *et al.* Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients. *J. Clin. Oncol.* 19(3), 666–675 (2001).
- 4 Gakis G, Efstathiou J, Lerner SP *et al.* ICUD-EAU International Consultation on Bladder Cancer 2012: radical cystectomy and bladder preservation for muscle-invasive urothelial carcinoma of the bladder. *Eur. Urol.* 63(1), 45–57 (2013).
- 5 Chen RC, Shipley WU, Efstathiou JA, Zietman AL. Trimodality bladder preservation therapy for muscle-invasive bladder cancer. *J. Natl Comp. Canc. Netw.* 11(8), 952–960 (2013).
- 6 Grossman HB, Natale RB, Tangen CM *et al.* Neoadjuvant chemotherapy plus cystectomy compared with cystectomy alone for locally advanced bladder cancer. *N. Engl. J. Med.* 349(9), 859–866 (2003).
- Landmark article establishing the role of neoadjuvant chemotherapy for patients with muscle-invasive bladder cancer.
- 7 Coppin C, Gospodarowicz MK, James K *et al.* Improved local control of invasive bladder cancer by concurrent cisplatin and preoperative or definitive radiation. The National Cancer Institute of Canada Clinical Trials Group. *J. Clin. Oncol.* 14(11), 2901–2907 (1996).
- 8 Clark PE, Spiess PJ, Agarwal N *et al.* NCCN Clinical Practice Guidelines in Oncology: Bladder Cancer. 1.2016 (2016). www.nccn.org
- 9 Witjes J, Comperat E, Cowan N *et al.* EAU guidelines on muscle-invasive and metastatic bladder cancer. European Association of Urology. (2015). <https://uroweb.org>
- 10 Burger M, Mulders P, Witjes W. Use of neoadjuvant chemotherapy for muscle-invasive bladder cancer is low among major European centres: results of a feasibility questionnaire. *Eur. Urol.* 61(5), 1070–1071 (2012).
- 11 Zaid HB, Patel SG, Stimson C *et al.* Trends in the utilization of neoadjuvant chemotherapy in muscle-invasive bladder cancer: results from the National Cancer Database. *Urology* 83(1), 75–80 (2014).
- 12 Cowan NG, Chen Y, Downs T *et al.* Neoadjuvant chemotherapy use in bladder cancer: a survey of current practice and opinions. *Adv. Urol.* 2014 (2014).
- 13 Knollman H, Godwin JL, Jain R, Wong YN, Plimack ER, Geynisman DM. Muscle-invasive urothelial bladder cancer: an update on systemic therapy. *Ther. Adv. Urol.* 7(6), 312–330 (2015).
- 14 Reardon ZD, Patel SG, Zaid HB *et al.* Trends in the use of perioperative chemotherapy for localized and locally advanced muscle-invasive bladder cancer: a sign of changing tides. *Eur. Urol.* 67(1), 165–170 (2015).
- 15 International Collaboration of Trialists; Medical Research Council Advanced Bladder Cancer Working Party (now the National Cancer Research Institute Bladder Cancer Clinical Studies Group); European Organisation for Research and Treatment of Cancer Genito-Urinary Tract Cancer Group; Australian Bladder Cancer Study Group; National Cancer Institute of Canada Clinical Trials Group; Finnbladder; Norwegian Bladder Cancer Study Group; Club Urologico Espanol de Tratamiento Oncologico Group; Griffiths G, Hall R, Sylvester R, Raghavan D, Parmar MK. 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(16), 2171–2177 (2011).
- 16 Neoadjuvant cisplatin, methotrexate, and vinblastine chemotherapy for muscle-invasive bladder cancer: a randomised controlled trial. International collaboration of trialists. *Lancet* 354(9178), 533–540 (1999).
- 17 Citron ML, Berry DA, Cirincione C *et al.* Randomized trial of dose-dense versus conventionally scheduled and sequential versus concurrent combination chemotherapy as postoperative adjuvant treatment of node-positive primary breast cancer: first report of Intergroup Trial C9741/Cancer and Leukemia Group B Trial 9741. *J. Clin. Oncol.* 21(8), 1431–1439 (2003).
- 18 Sternberg C, De Mulder P, Schornagel J *et al.* Randomized Phase III trial of high-dose-intensity methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) chemotherapy and recombinant human granulocyte colony-stimulating factor versus classic MVAC in advanced urothelial tract tumors: European Organization for Research and Treatment of Cancer Protocol No. 30924. *J. Clin. Oncol.* 19(10), 2638–2646 (2001).
- 19 Sternberg C, De Mulder P, Schornagel J *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(1), 50–54 (2006).
- **Final update establishing the benefits of accelerated MVAC versus conventional MVAC for patients with advanced urothelial carcinoma, which has significant implications for the neoadjuvant setting as well.**
- 20 Plimack ER, Hoffman-Censits JH, Viterbo R *et al.* Accelerated methotrexate, vinblastine, doxorubicin, and cisplatin is safe, effective, and efficient neoadjuvant treatment for muscle-invasive bladder cancer: results of a multicenter Phase II study with molecular correlates of response and toxicity. *J. Clin. Oncol.* 32(18), 1895–1901 (2014).
- 21 Choueiri TK, Jacobus S, Bellmunt J *et al.* Neoadjuvant dose-dense methotrexate, vinblastine, doxorubicin, and cisplatin with pegfilgrastim support in muscle-invasive urothelial cancer: pathologic, radiologic, and biomarker correlates. *J. Clin. Oncol.* 32(18), 1889–1894 (2014).
- 22 Von Der Maase H, Sengelov L, Roberts JT *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(21), 4602–4608 (2005).
- **Landmark trial that established the chemotherapeutic regimen of gemcitabine and cisplatin for metastatic urothelial carcinoma.**
- 23 Galsky MD, Pal SK, Chowdhury S *et al.* Comparative effectiveness of gemcitabine plus cisplatin versus methotrexate, vinblastine, doxorubicin, plus cisplatin as neoadjuvant therapy for muscle-invasive bladder cancer. *Cancer* 121(15), 2586–2593 (2015).
- 24 Zargar H, Espiritu PN, Fairey AS *et al.* Multicenter assessment of neoadjuvant chemotherapy for muscle-invasive bladder cancer. *Eur. Urol.* 67(2), 241–249 (2015).
- 25 Apolo AB, Kim JW, Bochner BH *et al.* Examining the management of muscle-invasive bladder cancer by medical oncologists in the United States. *Urol. Oncol.* 32(5), 637–644 (2014).
- 26 Plimack ER, Dunbrack RL, Brennan TA *et al.* Defects in DNA repair genes predict response to neoadjuvant cisplatin-based chemotherapy in muscle-invasive bladder cancer. *Eur. Urol.* 68(6), 959–967 (2015).
- 27 Van Allen EM, Mouw KW, Kim P *et al.* Somatic *ERCC2* mutations correlate with cisplatin sensitivity in muscle-invasive urothelial carcinoma. *Cancer Discov.* 4(10), 1140–1153 (2014).
- 28 Stein JP, Lieskovsky G, Cote R *et al.* Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients. *J. Clin. Oncol.* 19(3), 666–675 (2001).
- 29 Donat SM, Shabsigh A, Savage C *et al.* Potential impact of postoperative early complications on the timing of adjuvant chemotherapy in patients undergoing radical cystectomy: a high-volume tertiary cancer center experience. *Eur. Urol.* 55(1), 177–185 (2009).
- 30 Paz-Ares L, Solsona E, Esteban E *et al.* Randomized Phase III trial comparing adjuvant paclitaxel/gemcitabine/cisplatin (PGC) to observation in patients with resected invasive bladder cancer: results of the Spanish Oncology Genitourinary Group (SOGUG) 99/01 study. Presented at: *ASCO Annual Meeting Proceedings*. Chicago, IL, USA, 4–8 June 2010.
- 31 Sternberg CN, Skoneczna I, Kerst JM *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(1), 76–86 (2015).
- 32 Cognetti F, Ruggeri E, Felici A *et al.* Adjuvant chemotherapy with cisplatin and gemcitabine versus chemotherapy at relapse in patients with muscle-invasive bladder cancer submitted to radical cystectomy: an Italian, multicenter, randomized Phase III trial. *Ann. Oncol.* 23(3), 695–700 (2011).
- 33 Leow JJ, Martin-Doyle W, Rajagopal PS *et al.* Adjuvant chemotherapy for invasive bladder cancer: a 2013 updated systematic review and meta-analysis of randomized trials. *Eur. Urol.* 66(1), 42–54 (2014).
- 34 Galsky MD, Stensland KD, Moshier E *et al.* Effectiveness of adjuvant chemotherapy for locally advanced bladder cancer. *J. Clin. Oncol.* doi:10.1200/JCO.2015.64.1076 (2016) (Epub ahead of print).
- **Thorough real-world analysis of the utility and outcomes with adjuvant chemotherapy for resected bladder cancer, which may be the best evidence of a potential benefit of adjuvant chemotherapy in a space that has been unable to complete randomized trials.**
- 35 Guibert N, Delaunay M, Mazières J. Targeting the immune system to treat lung cancer: rationale and clinical experience. *Ther. Adv. Respir. Dis.* 9(3), 105–120 (2015).
- 36 Carthon BC, Wolchok JD, Yuan J *et al.* Preoperative CTLA-4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial. *Clin. Cancer Res.* 16(10), 2861–2871 (2010).
- **First trial of checkpoint inhibition in urothelial carcinoma and the first presurgical trial utilizing checkpoint blockade.**
- 37 Clinical Trials Database: NCT02662309. <https://clinicaltrials.gov>
- 38 Clinical Trials Database: NCT02451423. <https://clinicaltrials.gov>
- 39 Clinical Trials Database: NCT02365766. <https://clinicaltrials.gov>
- 40 Clinical Trials Database: NCT02690558. <https://clinicaltrials.gov>
- 41 Macfarlane AWT, Jillab M, Plimack ER *et al.* PD-1 expression on peripheral blood cells increases with stage in renal cell carcinoma patients and is rapidly reduced after surgical tumor resection. *Cancer Immunol. Res.* 2(4), 320–331 (2014).
- 42 Eggermont AMM, Chiarion-Sileni V, Grob J-J *et al.* Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): a randomised, double-blind, Phase 3 trial. *Lancet Oncol.* 16(5), 522–530 (2015).

- 43 Clinical Trials Database: NCT02450331. <https://clinicaltrials.gov>
- 44 Clinical Trials Database: NCT02632409. <https://clinicaltrials.gov>
- 45 Petrylak DP, Powles T, Bellmunt J *et al.* A Phase Ia study of MPDL3280A (anti-PDL1): Updated response and survival data in urothelial bladder cancer (UBC). Presented at: *ASCO Annual Meeting Proceedings*. Chicago, IL, USA, 29 May–2 June 2015.
- 46 Plimack ER, Bellmunt J, Gupta S *et al.* Pembrolizumab (MK-3475) for advanced urothelial cancer: updated results and biomarker analysis from KEYNOTE-012. *J. Clin. Oncol. (Meeting Abstracts)* 33(15 Suppl.), 4502 (2015).
- 47 Rosenberg JE, Hoffman-Censits J, Powles T *et al.* Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, Phase 2 trial. *Lancet* 387(10031), 1909–1920 (2016).
- **First published Phase II data demonstrating the activity of checkpoint blockade in urothelial carcinoma.**
- 48 Weber JS, Kähler KC, Hauschild A. Management of immune-related adverse events and kinetics of response with ipilimumab. *J. Clin. Oncol.* 30(21), 2691–2697 (2012).
- 49 Yagoda A, Watson RC, Gonzalez-Vitale JC, Grabstald H, Whitmore W. Cis-dichlorodiammineplatinum (II) in advanced bladder cancer. *Cancer Treat. Rep.* 60(7), 917–923 (1976).
- 50 Galsky MD, Hahn NM, Rosenberg J *et al.* A consensus definition of patients with metastatic urothelial carcinoma who are unfit for cisplatin-based chemotherapy. *Lancet Oncol.* 12(3), 211–214 (2011).
- 51 Pal SK, Milowsky MI, Plimack ER. Optimizing systemic therapy for bladder cancer. *J. Natl Comp. Canc. Netw.* 11(7), 793–804 (2013).
- 52 Von Der Maase H, Hansen S, Roberts J *et al.* Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin, and cisplatin in advanced or metastatic bladder cancer: results of a large, randomized, multinational, multicenter, Phase III study. *J. Clin. Oncol.* 18(17), 3068–3077 (2000).
- 53 Bamias A, Dafni U, Karadimou A *et al.* Prospective, open-label, randomized, Phase III study of two dose-dense regimens MVAC versus gemcitabine/cisplatin in patients with inoperable, metastatic or relapsed urothelial cancer: a Hellenic Cooperative Oncology Group study (HE 16/03). *Ann. Oncol.* 24(4), 1011–1017 (2013).
- 54 Linardou H, Aravantinos G, Efstathiou E *et al.* Gemcitabine and carboplatin combination as first-line treatment in elderly patients and those unfit for cisplatin-based chemotherapy with advanced bladder carcinoma: Phase II study of the Hellenic Co-operative Oncology Group. *Urology* 64(3), 479–484 (2004).
- 55 De Santis M, Bellmunt J, Mead G *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.* JCO. 3571(2011) 2037 (2011).
- 56 Stadler WM, Kuzel T, Roth B, Raghavan D, Dorr FA. Phase II study of single-agent gemcitabine in previously untreated patients with metastatic urothelial cancer. *J. Clin. Oncol.* 15(11), 3394–3398 (1997).
- 57 Bellmunt J, Théodore C, Demkov T *et al.* Phase III trial of vinflunine plus best supportive care compared with best supportive care alone after a platinum-containing regimen in patients with advanced transitional cell carcinoma of the urothelial tract. *J. Clin. Oncol.* 27(27), 4454–4461 (2009).
- 58 Lorusso V, Pollera C, Antimi M *et al.* A Phase II study of gemcitabine in patients with transitional cell carcinoma of the urinary tract previously treated with platinum. *Eur. J. Cancer* 34(8), 1208–1212 (1998).
- 59 McCaffrey JA, Hilton S, Mazumdar M *et al.* Phase II trial of docetaxel in patients with advanced or metastatic transitional-cell carcinoma. *J. Clin. Oncol.* 15(5), 1853–1857 (1997).
- 60 Vaughn DJ, Broome CM, Hussain M, Gutheil JC, Markowitz AB. Phase II trial of weekly paclitaxel in patients with previously treated advanced urothelial cancer. *J. Clin. Oncol.* 20(4), 937–940 (2002).
- 61 Ko Y-J, Canil CM, Mukherjee SD *et al.* Nanoparticle albumin-bound paclitaxel for second-line treatment of metastatic urothelial carcinoma: a single group, multicentre, Phase 2 study. *Lancet Oncol.* 14(8), 769–776 (2013).
- 62 Lee J-L, Ahn J-H, Park SH *et al.* Phase II study of a cremophor-free, polymeric micelle formulation of paclitaxel for patients with advanced urothelial cancer previously treated with gemcitabine and platinum. *Invest. New Drugs* 30(5), 1984–1990 (2011).
- 63 Quinn DI, Ruel N, Twardowski P *et al.* Eribulin in advanced urothelial cancer (AUC patients (pts): a California Cancer Consortium trial – NCI/CTEP 7435. *J. Clin. Oncol. (Meeting Abstracts)* 33(15 Suppl.), 4504 (2015).
- 64 Galsky MD, Mironov S, Iasonos A, Scattergood J, Boyle MG, Bajorin DF. Phase II trial of pemetrexed as second-line therapy in patients with metastatic urothelial carcinoma. *Invest. New Drugs* 25(3), 265–270 (2007).
- 65 Sweeney CJ, Roth BJ, Kabbinnar FF *et al.* Phase II study of pemetrexed for second-line treatment of transitional cell cancer of the urothelium. *J. Clin. Oncol.* 24(21), 3451–3457 (2006).
- 66 Massard C, Gordon MS, Sharma S *et al.* Safety and efficacy of durvalumab (MDA436), a PD-L1 antibody, in urothelial bladder cancer, June 2016. . Presented at: *ASCO Annual Meeting*. Chicago, IL, USA, 3–7 June 2016.
- 67 Apolo AB, Infante JR, Hamid O *et al.* Avelumab (MSB0010718C; anti-PD-L1) in patients with metastatic urothelial carcinoma from the JAVELIN solid tumor Phase Ib trial: analysis of safety, clinical activity, and PD-L1 expression. *ASCO Annual Meeting*. Chicago, IL, USA, 4–7 June 2016.
- 68 Sharma P, Bono P, Kim JW *et al.* Efficacy and safety of nivolumab monotherapy in metastatic mUC: results from the Phase I/II CheckMate 032 study. *ASCO Annual Meeting*. Chicago, IL, USA, 4–7 June 2016.
- 69 Pfirschke C, Engblom C, Rickelt S *et al.* Immunogenic chemotherapy sensitizes tumors to checkpoint blockade therapy. *Immunity* 44(2), 343–354 (2016).
- 70 Zamarin D, Postow MA. Immune checkpoint modulation: rational design of combination strategies. *Pharmacol. Ther.* 150, 23–32 (2015).
- 71 Galsky MD. Phase II trial of gemcitabine + cisplatin + ipilimumab in patients with metastatic urothelial cancer. Presented at: *2016 Genitourinary Cancer Symposium*. San Francisco, CA, USA, 7–9 January 2016.
- 72 Clinical Trials Database: NCT02437370. <https://clinicaltrials.gov>
- 73 Clinical Trials Database: NCT02581982. <https://clinicaltrials.gov>
- 74 Abbosh PH, McConkey DJ, Plimack ER. Targeting signaling transduction pathways in bladder cancer. *Curr. Oncol. Rep.* 17(12), 1–10 (2015).
- 75 Clinical Trials Database: NCT02546661. <https://clinicaltrials.gov>
- 76 Clinical Trials Database: NCT02443324. <https://clinicaltrials.gov>

- 77 Larkin J, Chiarion-Sileni V, Gonzalez R *et al.* Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N. Engl. J. Med.* 373(1), 23–34 (2015).
- 78 Hammers HJ, Plimack ER, Infante JR *et al.* Expanded cohort results from CheckMate 016: a Phase I study of nivolumab in combination with ipilimumab in metastatic renal cell carcinoma (mRCC). Presented at: *ASCO Annual Meeting Proceedings*. Chicago, IL, USA, 2–5 June 2015.
- 79 Clinical Trials Database: NCT02553642. <https://clinicaltrials.gov>
- 80 Clinical Trials Update: NCT01928394. <https://clinicaltrials.gov>
- 81 Clinical Trials Database: NCT02516241. <https://clinicaltrials.gov>
- 82 Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* 12(4), 252–264 (2012).
- 83 Clinical Trials Database: NCT02614456. <https://clinicaltrials.gov>
- 84 Huang Y, Zhang S-D, Mccrudden C, Chan K-W, Lin Y, Kwok H-F. The prognostic significance of PD-L1 in bladder cancer. *Oncol. Rep.* 33(6), 3075–3084 (2015).
- 85 Powles T, Nickles D, Van Allen E *et al.* Immune biomarkers associated with clinical benefit from atezolizumab (MPDL3280a; anti-PD-L1) in advanced urothelial bladder cancer (UBC). *J. Immunother. Cancer* 3(Suppl. 2), P83 (2015).