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RESEARCH ARTICLE

Molecularly Targeted Therapeutics in Urothelial Cancer: Current Standards and Future Strategies

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ABSTRACT:
Urothelial carcinoma (UC) therapy is a rapidly evolving and expanding field. Traditional cytotoxic chemotherapy regimens have not produced optimal long-term outcomes, and many urothelial cancer patients have comorbidities that disqualify them as chemotherapy candidates. In recent years, a plethora of novel therapeutic agents that target diverse molecular pathways has emerged as alternative treatment modalities for not only metastatic urothelial carcinoma, but also for muscle-invasive bladder cancer and non-muscle invasive bladder cancer in adjuvant and definitive settings. This review paper aims to discuss the various categories of therapeutic agents for these different types of urothelial cancer. A particular focus was given to recent studies leading to new approvals and indications for checkpoint inhibitors (CPI), CPI combinations, antibody-drug conjugates (ADC), and inhibitors of fibroblast growth factor receptor (FGFR). Additionally, this article reviews novel therapeutics that will help shape the future of UC management. These new therapeutic options include immunotherapy, ADCs, kinase inhibitors, CAR-T cell therapy, peptide vaccination, and other drugs targeting pathways such as angiogenesis, DNA synthesis, mTOR/PI3K/AKT, and EGFR/HER-2.

Introduction

Bladder cancer or urothelial carcinoma (UC) of the bladder is a common and deadly malignancy worldwide, and chemotherapy has produced limited improvement in outcomes. There are 550,000 new cases of UC globally each year, and UC accounts for about 2.1% of all deaths due to cancer. Women have a 0.27% lifetime risk of acquiring UC, whereas this risk is 1.1% in men[1]. Management of UC depends on whether the malignancy is invasive or non-invasive. Low to intermediate risk non-muscle invasive bladder cancer (NMIBC) is treated with transurethral resection of bladder tumor (TURBT) followed by surveillance. For high risk NMIBC, cystectomy or intravesical induction therapy with chemotherapy followed by Bacille Calmette-Guerin (BCG) maintenance therapy or BCG therapy with maintenance therapy may also be considered. For NMIBC patients, refractory to BCG therapy, cystectomy is first-line management. Localized muscle-invasive bladder cancer (MIBC) is treated with neoadjuvant chemotherapy (NAC) followed by radical cystectomy. In patients, ineligible for chemotherapy, treatment is upfront cystectomy or bladder preservation strategies with concurrent chemoradiation. Standard chemotherapy for metastatic UC is platinum-based, and includes three different drug regimens: methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC), dose dense MVAC, and lastly gemcitabine with cisplatin (GC)[2]. Gemcitabine and Carboplatin is a regimen that has shown efficacy in patients who are Cisplatin ineligible[3].

However, metastatic UC still carries a particularly poor prognosis. After treatment with these chemotherapy regimens, the approximate median survival is only thirteen to fifteen months[4,5]. Considering this suboptimal response to chemotherapy, a growing body of research is exploring targeted molecular therapy as an alternative to traditional chemotherapy. Additionally, promising biomarkers such as tumor mutational burden and driver mutations continue to be investigated and can provide prognostic information to guide developing bladder cancer treatments such as immunotherapy[6].

This paper discusses multiple categories of novel therapy that each have diverse molecular targets, which can affect host immune response as well as tumor activity. There is a real unmet need for development of therapeutic strategies for BCG refractory NMIBC setting, perioperative setting in MIBC, and platinum unfit population. Literature for these various UC therapies will be reviewed in the context of localized and metastatic MIBC then NIMBC.

I. Therapeutic Agents for Locally Advanced or Metastatic Urothelial Carcinoma

Immunotherapy

A. Checkpoint Inhibitor Monotherapy

Checkpoint inhibitors (CPI) comprise one of the most promising fields for metastatic or advanced UC therapy. There are two main categories of checkpoint inhibitor therapy: agents targeting programmed cell death protein 1 (PD-1) or programmed cell death-ligand 1 (PD-L1) and agents targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). Although these agents are not first-line therapy for UC, multiple phase II and III trials have demonstrated CPI's significant roles in advanced/metastatic UC refractory to standard platinum-containing chemotherapy. Since 2016, the checkpoint inhibitors avelumab, atezolizumab, durvalumab, nivolumab, and pembrolizumab have received FDA (Food and Drug Administration) approval as treatment agents for bladder cancer[7]. However, as of 2021, two of these agents, atezolizumab and durvalumab, have since been withdrawn [8,9]. PD-L1 expression is a potential predictive biomarker for PD-1/PD-L1 immunotherapy efficacy, but this connection is not yet clearly established. For example, some malignancies demonstrate higher responses to immunotherapy with greater PD-L1 expression, whereas other tumors' responses to treatment have an inverse relationship with their PD-L1 expression. Additionally, the use of immunohistochemistry to quantify PD-L1 expression is limited by variation in tissue preparation and processing steps. There are also challenges in differentiating stained tumor cells from immune cells, primary tumor biopsies versus metastatic ones, and oncogenic PD-L1 expression versus induced expression[10].

Nivolumab targets the PD-1 receptor and was approved by the FDA in February 2017[11]. In the CheckMate 032 trial, a 2016 phase I/II study, nivolumab monotherapy in recurrent, metastatic UC produced an ORR of 24.4%[12]. The next year, Sharma et al. published the CheckMate 275 trial, a phase II study involving nivolumab monotherapy in patients with metastatic or surgically unresectable UC that was refractory to platinum-based therapy. 265 patients were treated with nivolumab, and 52 patients (19.6%) achieved objective response. Although there was a higher ORR in the patient groups with a higher percentage of PD-L1 expression, significant clinical improvement was observed in all groups regardless of PD-L1

expression. 48(18%) experienced grade 3-4 adverse events (AE), the most common being grade 3 fatigue and diarrhea[13]. A 2019 global analysis of the CheckMate 275 trial reached the same conclusions as those found in the original trial publication[14].

In the adjuvant setting, the CheckMate274 phase 3, randomized, double-blind, multicenter trial compared 353 patients in nivolumab arm and 356 patients in the placebo arm among patients with high-risk muscle-invasive urothelial carcinoma (with primary tumor sites including bladder, ureter, or renal pelvis) after radical surgery. Patients were allowed but not required to have received neoadjuvant cisplatin. At 20 months median follow up, median disease-free survival was significantly longer for patients receiving nivolumab at 21 months compared to placebo at 11 months (HR: 0.70, 95% C.I. 0.54-0.89). A similar effect was observed in the PD-L1 ≥ 1% population where percentage of patients was 74.5% and 55.7%, respectively (HR 0.53, 95% C.I. 0.34-0.84). Grade 3 or 4 treatment-related adverse events occurred in 17.9% and 7.2% of patients in the nivolumab and placebo arms, respectively[15]. On 8/19/2021, the FDA approved adjuvant nivolumab for patients with urothelial cancer who are at high risk of recurrence after radical resection.

Avelumab is a monoclonal IgG antibody that targets PD-L1. It received accelerated approval by the FDA in May 2017 for treatment of locally advanced/metastatic UC that progressed during or after platinum-containing chemotherapy or within 1 year of receiving neoadjuvant or adjuvant platinum-containing chemotherapy[16]. Avelumab then received approval in June 2020 for maintenance therapy in patients with locally advanced or metastatic UC that did not progress after first line platinum-containing chemotherapy[16]. The JAVELIN Bladder 100 phase III trial compared the outcomes of avelumab with best supportive care (BSC) versus BSC alone for maintenance treatment of advanced/metastatic UC that did not worsen from first-line chemotherapy. The median OS in all patients, regardless of PD-L1 status in the tumors, was 21.4 months in the arm receiving avelumab, compared to an OS of only 14.3 months in the BSC arm (HR 0.69, CI 0.40 to 0.79, P < 0.001). In the overall population, the median PFS in the avelumab group was 3.7 months, compared to only 2.0 months in the control (HR 0.62, CI 0.52 to 0.75). In the PD-1 positive population, the median PFS was 5.7 months in the avelumab group and 2.1 months in the control (HR 0.56, CI 0.43 to 0.73). 11.9% of patients in the avelumab arm had treatment discontinued due to AEs, and the most common AEs grade 3 or greater in the avelumab group were UTI and anemia. These results demonstrated avelumab's role as first-line maintenance therapy for advanced UC, thus establishing a new standard of care for this type of UC[17]. The benefit remains, regardless of whether patients got Carboplatin or Cisplatin.

Pembrolizumab blocks PD-1, and in 2017 received accelerated FDA approval for patients with advanced/metastatic UC who are ineligible for cisplatin-containing chemotherapy. In 2018, the FDA modified this indication so that pembrolizumab could be used only in cisplatin-ineligible patients and high PDL1 expression with CPS (Combined Positive Score) >10, or in patients who are unfit for any platinum therapy, regardless of their PD-L1 expression as the KEYNOTE-361 trial showed decreased survival in patients with PD-L1-low status in the pembrolizumab monotherapy arm[18,19]. It also received regular FDA approval for advanced/metastatic UC progression during/after first-line platinum chemotherapy or within 12 months of receiving adjuvant/neoadjuvant platinum chemotherapy[20]. Pembrolizumab's accelerated approval was based upon the phase II KEYNOTE-052 trial's data, which treated 370 advanced/metastatic UC patients who were not eligible for cisplatin-based chemotherapy; median follow-up was 5 months, and the ORR was 28.6%[20,21]. Vuky et al.'s 2020 paper examined the long-term outcomes of the KEYNOTE-052 patients, with a minimum follow-up time of 2 years since the last patient was enrolled. The ORR was 28.6%, and the median response duration was 30.1 months. 33 (8.9%) of patients had complete response, and 73 (19.7%) achieved partial response[22]. Regular approval was given based upon the results of the phase III KEYNOTE-045 trial, where 542 patients with advanced UC that progressed or recurred following platinum-based chemotherapy were assigned to receive either pembrolizumab or the investigator's choice of paclitaxel, docetaxel, or vinflunine. The median OS was 10.3 months in the group receiving pembrolizumab, compared to 7.4 months in the chemotherapy group (HR 0.73, CI 0.59 to 0.91, P = 0.002)[23].

Atezolizumab targets PD-L1 and was initially FDA-approved in May 2016 for locally advanced/metastatic UC that worsened during or after platinum-based chemotherapy, or within 1 year of platinum-based chemotherapy, before or after surgical intervention[24]. In 2018, the FDA modified this indication to be used either for patients ineligible for cisplatin with PDL-1 stained tumor infiltrating cells >5%, or for patients ineligible for any platinum-based therapy,

regardless of PD-L1 expression[18]. However, in March 2021, Roche voluntarily withdrew the FDA indication for atezolizumab for advanced/ metastatic UC previously treated with platinum-based therapy[8]. This was based on the phase III IMvigor 211 trial, which involved patients with metastatic UC that progressed after platinum-based therapy. Patients were randomly assigned to receive either atezolizumab or the investigator's choice of chemotherapy (vinflunine, paclitaxel, or docetaxel). The study's primary endpoint of improved OS in patients with PD-L1 positive tumors was not achieved [25].

Durvalumab is another human monoclonal antibody that binds to PD-L1, initially approved by the FDA for advanced UC in February 2017[26]. In a 2017 phase I/II open-label study, Powles et al. assessed the safety profile and efficacy of durvalumab in patients with either locally advanced or metastatic UC[27]. Among 191 patients, the ORR was 17.8%, 3.7% of patients achieved CR, overall survival was 18.2 months, and the median PFS was 1.5 months. However, like atezolizumab, durvalumab was withdrawn by its developer AstraZeneca as therapy for locally advanced or metastatic UC refractory to chemotherapy in February 2021[9].

Ipilimumab is a CTLA-4 inhibitor that has not yet been FDA-approved for UC therapy; however, it is a promising therapy when combined with another checkpoint inhibitor such as nivolumab. Sharma et al.'s 2019 multicohort study CheckMate 032 suggested that combination treatment with ipilimumab and nivolumab is a safe regimen that produces greater antitumor outcomes than if either of these therapies were given alone [28]. Unfortunately, the phase III Checkmate 901 study did not show any significant improvement in OS for nivolumab and ipilimumab versus standard of care chemotherapy (NCT03036098) [29].

B. Checkpoint Inhibitor Combinations (CPI)

CPIs are being studied in combination with either chemotherapy or radiation for the treatment of patients in the perioperative and metastatic settings. These combinations aim to improve the efficacy and safety of treatment by moving CPIs into earlier lines of treatment. CPIs are also being studied in combination with antibody-drug conjugates (ADC), which is discussed in a separate section.

In an ongoing phase II trial, nivolumab is being studied as preoperative therapy concomitantly with radiation, in patients with locally advanced bladder cancer who then undergo radical cystectomy with lymphadenectomy (NCT03529890). Nivolumab 240 mg will be administered for a total of 4 cycles, every 2 weeks, with concomitant radiation therapy. The primary endpoint will be the rate of patients who completed the regimen (radiation with nivolumab, followed by radical cystectomy) after week 15 [30].

The SAKK 06/17 trial is a phase II trial looking at the addition of Durvalumab to neoadjuvant chemotherapy (NAC) in patients who are Cisplatin eligible. Patients were treated with 4 cycles of cisplatin/gemcitabine with durvalumab prior to surgery and continued durvalumab for 10 cycles thereafter[31]. 61 patients were included in the study and 91% of those patients underwent surgical resection. There were no new safety signals noted in this study. Of those patients, 98% achieved an R0 resection and 34% achieved a pathologic complete response (pCR). Similarly, the BLASST-1 trial investigated the addition of pembrolizumab to neoadjuvant chemotherapy in patients with MIBC getting cystectomy. Patients were treated with 4 cycles of gem/cis with pembrolizumab prior to radical cystectomy. 41 patients were included in the trial and 95% of them underwent radical cystectomy. Again, therapy was well tolerated, and no new safety signals were noted. Of those patients getting radical cystectomy, 65.8% had a pathologic response [32].

Several trials are studying the addition of immunotherapy to NAC in patients with MIBC who are, cisplatin ineligible. For example, The SWOG GAP TRIAL is a phase II study comparing neoadjuvant gemcitabine, carboplatin, and avelumab followed by surgery versus upfront surgery in patients with MIBC who are, cisplatin-ineligible (NCT04871529). This study is actively recruiting.

Pembrolizumab has recently been studied in combination with chemotherapy in the first-line metastatic setting. KEYNOTE-361 was a phase III randomized RCT comparing pembrolizumab + platinum containing chemotherapy versus pembrolizumab alone versus chemotherapy alone in patients with unresectable or metastatic MIBC[19]. In this study, 1010 patients were enrolled and were randomized to the above-mentioned groups in a 1:1:1 ratio. Patients getting chemotherapy could get gemcitabine with either cisplatin or carboplatin for a maximum of 6 cycles. Those in the immunotherapy group could get up to a maximum of 35 cycles of pembrolizumab. The study had dual primary end points of PFS and OS. There was no significant difference in PFS between the chemoimmunotherapy and immunotherapy alone groups, 8.3 months, and 7.1 months respectively (HR 0.86). Similarly, there was no significant difference in OS between the 2 groups, 15.6 months for the

chemoimmunotherapy group versus 14.3 months for the immunotherapy group (HR 0.92). There were no changes in findings when comparing the total population versus those with CPS of at least 10. The findings of this study do not support the addition of pembrolizumab to platinum-based chemotherapy in patients with mUC.

C. Chimeric Antigen Receptor (CAR) Therapy and Autologous Cytokine Induced Natural Killer T Cells

Chimeric Antigen Receptor (CAR) therapy is a promising therapy for the treatment of advanced or metastatic solid organ malignancies. However, CAR T-cell therapies are approved for acute lymphoblastic leukemia, B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, and multiple myeloma. The process for making CAR therapy is complex, but it relies on removing, modifying, and re-introducing modified immune cells that express receptors capable of identifying antigens expressed on tumors. When these modified immune cells recognize the tumor expressed antigen, an immune response is initiated that causes the immune system to fight the tumor[33,34]. A recent review highlighted active clinical trials involving CAR therapy and other cellular therapies in urogenital malignancies [35].

One open-label, single group phase I study is exploring the combination of systemic CAR cytotoxic T cells targeting HER-2 and CAdVEC, an oncolytic adenovirus that is injected directly into the tumor. The oncolytic adenovirus expresses a PD-L1 blocking antibody which enhances the cytotoxic T-cell response to the tumor[36]. The study is actively recruiting individuals with HER2 positive solid tumors, including bladder cancer, that has progressed after standard first line therapy. The study will establish the dose limiting toxicity within 6 weeks after initiating CART therapy and CAdVEC injection. Secondary outcomes include 13-week ORR and, 15-year PFS and overall survival, and 30-day grade 3 or greater severity of treatment related adverse events (NCT03740256).

D. Bispecific Monoclonal Antibodies

XmAb22841 (Bavunalimab) is a bispecific monoclonal antibody which inhibits CTLA-4 and LAG-3 (CD223). LAG-3 is a surface molecule expressed on T-cells and Natural Killer cells and is important for regulating T-cell function[37]. Inhibition of LAG-3 is expected to activate T-cells and allow them to fight tumor cells. NCT03849469 is an open-label non-randomized study exploring the optimal dose and rates of immune-related or non-immune related treatment-related adverse events of XmAb22841 alone or in combination with pembrolizumab in individuals with advanced or metastatic solid organ malignancies, including urothelial carcinoma.

XmAb23104 is a bispecific monoclonal antibody that inhibits PD1 and Inducible Co-Stimulator (ICOS). One open-label, non-randomized actively recruiting phase 1 dose escalation and expansion study. The purpose of the study is to assess the dose and treatment-related adverse events of XmAb23104 alone or in combination with ipilimumab (NCT03752398). Individuals with urothelial carcinoma are only included in the dose escalation cohort and are not present in the dose expansion cohorts.

Targeting FGFR

One class of medications that has the potential to improve survival in metastatic urothelial carcinoma are Fibroblast Growth Factor Receptor (FGFR) inhibitors. Alterations in FGF/FGFR signaling pathway are common in urothelial carcinoma. For example, approximately 20% of individuals with advanced bladder cancer have over-activation of FGFR3[38]. FGFR is a member of the receptor tyrosine kinase family which are important for controlling basic cellular activities, for example, cell survival, proliferation, and differentiation[39,40]. Mutations or amplifications of the genes controlling the FGFR receptor could lead to overactivation of multiple signaling pathways resulting in tumor growth and proliferation.

Erdafitinib gained FDA approval as therapy for locally advanced or metastatic UC with an FGFR2 or FGFR3 mutation in April 2019[41]. Erdafitinib is a FGFR1-4 inhibitor that has a manageable safety profile in patients with advanced tumors such as UC[42]. A phase II trial published in 2019 found that erdafitinib produced a 40% ORR in patients with metastatic UC who progressed after least one course of chemotherapy or 12 months of adjuvant or neoadjuvant chemotherapy and possibly immunotherapy, with a median PFS of 5.5 months and median OS of 13.8 months. Out of the 99 patients, the most common grade $\geq$3 AEs included hyponatremia (11%), stomatitis (10%), asthenia (7%), nail dystrophy (6%), and urinary tract infection (5%)[43].

Early phase I study results suggested the activity of rogaratinib was low (ORR: ~15%) in individuals with advanced solid organ malignancies with FGFR mutations. However, rogaratinib had the highest activity in individuals with urothelial carcinoma (n=12/52, 24%)[44.47] The FORT-1 study was a randomized, open-label, multicenter phase II trial which compared the efficacy of rogaratinib versus

chemotherapy only (docetaxel, paclitaxel, or vinflunine) in individuals with advanced or metastatic urothelial carcinoma with FGFR1 or 3 mRNA overexpression[45]. The trial was stopped at the end of phase II study because the investigators determined the ORR (20.7% vs 19.3%), and overall survival (8.3 vs 9.8 months) was similar between rogaratinib and chemotherapy only treatment groups. A post-hoc analysis has suggested a potential benefit of rogaratinib in individuals with FGFR3 DNA mutations as the response was higher in individuals who received rogaratinib as compared to chemotherapy only (ORR: 52.4% vs 26.7%).

The FORT-2 study is an open-label phase Ib/II study that is designed to assess the safety and activity of combinatorial therapy of rogaratinib and atezolizumab in individuals with advanced or metastatic urothelial carcinoma with FGFR1/3 mRNA expression who are ineligible for platinum-based chemotherapy (NCT03473756). The primary outcome of the phase Ib study is to determine the dose-limiting toxicities and treatment-related adverse events, and the secondary outcome is to determine the ORR. The phase II study will begin if the phase Ib study supports continuation. The phase II study will be a randomized, placebo-controlled trial which will compare the PFS between the combinatorial therapy of rogaratinib and atezolizumab versus atezolizumab monotherapy.

Gunagratinib is a pan-FGFR irreversible inhibitor. An open-label, single group phase II study is recruiting individuals with locally advanced or metastatic urothelial carcinoma of the bladder with FGFR mutations/aberrations (NCT04492293). Individuals will receive gunagratinib monotherapy and will be followed for 3 years. The primary outcome of the study is the 3-year ORR and secondary outcomes include duration of response, disease control rate, and 3-year PFS and OS.

Futibatinib (TAS-120) is an irreversible pan-FGFR inhibitor. One open-label, non-randomized phase I/II study is exploring the safety and activity of futibatinib monotherapy in individuals with solid organ malignancies with FGF/FGFR aberrations (NCT02052778). The phase I trial is a dose-escalation and expansion study which includes individuals with urothelial carcinoma and aims to identify the optimal phase II dose and the potential ORR of Futibatinib. The phase II trial however will be limited to individuals with intrahepatic cholangiocarcinoma with FGFR2 gene rearrangements. One actively recruiting open-label, single-arm phase II study is investigating the 12-month ORR of futibatinib in combination with pembrolizumab in individuals with urothelial carcinoma with and without FGFR mutations (NCT04601857). Secondary outcomes include disease control rate, duration of response, PFS, OS, and incidence of treatment-emergent adverse events.

Antibody-Drug Conjugates

A. Enfortumab Vedotin

Enfortumab vedotin (EV) gained FDA approval in December 2019 for the treatment of locally advanced or metastatic UC in patients who had already received a PD-1 or PD-L1 inhibitor and adjuvant or neoadjuvant platinum-containing chemotherapy[46]. It is an antibody drug conjugate (ADC) that targets the adhesion molecule Nectin-4, which is expressed on many UC cells. After binding to Nectin-4, the ADC is internalized by the tumor cell and induces cytotoxic effects via disruption of microtubule function, which is accomplished by monomethyl auristatin E, the drug that is conjugated to the antibody[47]. The phase I trial EV-101 administered single-agent EV to patients with solid malignancies expressing Nectin-4, including metastatic UC. The therapy was well tolerated, the ORR was 43% with a median OS of 12.3 months, and OS was 51.8% at 1 year[48]. In a phase II single-arm EV-201 trial involving patients with advanced/metastatic UC previously treated with platinum chemotherapy and PD-1 or PD-L1 inhibitors, EV therapy produced an ORR of 44% with 12% having CR, median response duration of 7.6 months, and minimal adverse treatment related events[49]. The phase III EV-301 trial compared the outcomes of patients treated with EV versus chemotherapy (investigator's choice of docetaxel, paclitaxel, or vinflunine) following platinum and checkpoint inhibitor therapy. The OS in the EV group was 12.88 months, compared to only 8.97 months in the chemotherapy group (HR 0.7, CI 0.56 to 0.89, $P = 0.001$). The EV group also had a greater PFS than the chemotherapy group (5.55 months versus 3.71 months, HR 0.62, CI 0.51 to 0.75, $P < 0.001$). Among 296 patients in the EV arm, the most common grade $\geq$3 AEs were maculopapular rash (22[7.4%]), fatigue (19[6.4%]), and reduced neutrophil count (18[6.1%])[50].

The EV-103/KEYNOTE-869 trial (NCT03288545) was a phase Ib/II multicenter study that assessed the safety and tolerability of first-line EV + pembrolizumab in patients with advanced urothelial cancer who are cisplatin ineligible. This was a multi-cohort study with three groups; dose escalation cohort, Cohort A, and Cohort K. The dose escalation cohort and cohort A were treated with EV + pembrolizumab, while Cohort K was randomized to either the combination or EV alone. The primary

endpoint was safety and secondary endpoints included duration of response (DOR), confirmed ORR, PFS, and OS. In 121 evaluable patients, confirmed ORR was 68% (95% CI: 59,76) with 12% of patients achieving a CR. The median DOR for the dose escalation group and Cohort A was 22 months. The median DOR for Cohort K was not reached. The combination was well tolerated, and no new safety signals were identified. In April 2023, based on these results, the FDA granted accelerated approval for the combination of EV + pembrolizumab for patients with locally advanced or metastatic US who are ineligible for cisplatin-containing chemotherapy-Citation. The ongoing phase III EV-302 trial will compare the outcomes of previously untreated UC patients who are given either EV with pembrolizumab or gemcitabine with platinum-based chemotherapy (NCT04223856).

Enfortumab Vedotin is being studied in combination with immunotherapy in the neoadjuvant setting for cisplatin-ineligible patients. The VOLGA trial is an actively enrolling phase III trial looking at EV + durvalumab + tremelimumab versus EV + durvalumab alone in this population. The goal is to enroll 830 patients and primary outcomes will be pCR rate and event free survival (NCT04960709)[51].

B. Sacituzumab Govitecan

Sacituzumab govitecan (SG) is an antibody-drug conjugate containing SN-38, a metabolite in irinotecan which inhibits the activity of topoisomerase (Figure 1). In a phase I trial, 6 patients with metastatic UC refractory to platinum therapy were treated with IMMU-132. This agent was well-tolerated overall, and three patients had significant response, with a PFS 6.7-8.2 months, and OS 7.5-11.4 months [52]. Phase II of this trial was completed in April 2021 (NCT01631552). Additionally, the phase II TROPHY trial administered IMMU-132 to locally advanced or metastatic UC who previously received platinum-based chemotherapy and either a PD-1 or PD-L1 inhibitor. The ORR was 27.7%, and median DOR of 7.2 months (NCT03547973). In April 2021,SG received accelerated FDA approval for treatment of advanced UC[53].

Sacituzumab govitecan is currently being studied in the phase III TROPiCS-04 trial (NCT04527991). This trial is recruiting patients with advanced or mUC who progressed on platinum-based and CPI therapies [54]. Patients will be randomized to either GC or physicians' choice of single agent chemotherapy (paclitaxel, docetaxel, or vinflunine). This trial is powered for OS as the primary endpoint. SG is also being studied in the neoadjuvant setting for patients who are platinum-ineligible or refuse platinum-based chemotherapy. The phase II SURE trial compares neoadjuvant SG and SG plus pembrolizumab prior to radical cystectomy.[55]

C. Her2 Directed Antibody Drug Conjugate

Antibodies linked to drug conjugates have been developed to target HER-2 expressing urothelial carcinomas. The advantage of antibody drug conjugates like Enfortumab Vedotin, Sacituzumab govitecan, and monoclonal antibodies against HER-2 is the precise delivery of anti-tumor therapies to tumors and limiting off-target adverse effects. It is estimated that 9-12% of individuals with bladder carcinomas overexpress HER-2 [56,57]. Therefore, the addition of HER-2 antibody drug conjugates may provide substantial benefit to some individuals.

Trastuzumab deruxtecan is one antibody drug candidate being studied in the setting of metastatic urothelial carcinoma. Deruxtecan, a derivative of exatecan, inhibits topoisomerase I, resulting in DNA damage and cell death. One phase I trial is exploring the activity of trastuzumab deruxtecan with nivolumab in a dose-escalation and expansion trial (NCT03523572). The study is recruiting individuals with unresectable or metastatic treatment refractory breast or urothelial carcinoma. The primary outcome is the dose-limiting toxicity in the first part of the study. The second part of the study will explore the activity of trastuzumab deruxtecan by assessing the ORR. In a primary analysis of 34 patients who received trastuzumab deruxtecan and nivolumab, the activity in 30 individuals was 36.7% (13.3% were complete responses) and grade 3 or greater treatment emergent adverse events occurred in 73.5% of patients, requiring discontinuation in 32.4% of patients. Common side effects related to treatment were nausea, fatigue, and vomiting[58]. The DESTINY-PanTumor02 is an open-label, single-arm Phase II study exploring the activity of Trastuzumab Deruxtecan monotherapy in 7 cohorts of individuals with locally advanced or metastatic HER-2 expressing cancers, including one cohort of bladder cancer (NCT04482309). The primary outcome is the 12-month ORR, and the secondary outcomes include the 18-month duration of response, disease control rate, and PFS, 30-month OS, and adverse events experienced over 24 months.

Disitamab vedotin is a HER-2 targeting monoclonal antibody covalently linked with monomethyl auristatin E (MMAE). MMAE's mechanism of action is to inhibit the polymerization of tubulin which causes microtubules to unwind. In September 2020, the FDA designated disitamab vedotin as a

breakthrough therapy for individuals with HER-2 positive locally advanced or metastatic urothelial carcinoma who previously were treated with first-line platinum-based therapy. Published results of a phase II study have shown the ORR of disitamab vedotin was 51.2% in individuals with locally advanced or metastatic urothelial carcinoma [59].The study identified 58% of individuals who experienced grade 3 treatment related adverse events, in which hypoesthesia and neutropenia were the two most common adverse events. Disitamab vedotin alone or in combination with pembrolizumab is being trialed in an actively recruiting open-label, multi-center, multi-cohort phase II study consisting of individuals with locally advanced or metastatic urothelial cancer, including bladder as primary site (NCT04879329). The primary outcome is the confirmed ORR, and the secondary outcomes include the DOR, PFS, DCR, 3-year OS, adverse events, and pharmacokinetic parameters.

A166 is a HER2 antibody drug conjugate consisting of trastuzumab covalently linked with duostatin-5. The mechanism of action of duostatin-5 is to stop mitosis and tumor proliferation through inhibition of the polymerization of tubulin. A166 monotherapy is being studied in an open-label phase I trial to identify the maximum tolerated dose in individuals with relapsed or treatment-refractory HER-2 expressing solid organ malignancies, including bladder cancer (NCT03602079).

Miscellaneous

A. Tyrosine Kinase inhibitors

Cabozantinib inhibits a diverse set of tyrosine kinase receptors including VEGFr, KIT, TRKB, FLT-3, AXL, RET, MET, and TIE-2. The ARCADIA trial is an open-label, nonrandomized phase II trial which assessed the safety and efficacy of cabozantinib with durvalumab in individuals with advanced urothelial carcinoma who progressed after platinum-based chemotherapy and had not previously received immune checkpoint inhibitor therapy. In a published planned interim and safety analysis after recruiting 16 individuals (out of a planned 122) the study reported an ORR of 37.5% (n = 6). Grade 1 or 2 treatment related adverse effects were common (n = 14) with fatigue (43.8%), diarrhea (31.3%), and dysphonia (31.3%) being the most common[60]. The study is currently recruiting and is estimating to complete the trial in February 2023 (NCT03824691).

Sitravatinib has inhibitory activity against a spectrum of receptor tyrosine kinase, including MET, Axl, MERTK, VEGFR family, PEDGFR family, KIT, FLT3, Trk, RET, DDR2, and Eph. A recently published phase I trial studying the safety and activity of sitravatinib in individuals with a variety of advanced solid organ malignancies (bladder cancer, n=4) found the response was 11.8%[61]. Most common experienced adverse events were diarrhea, fatigue, hypertension, and nausea. The activity of sitravatinib is being assessed in a nonrandomized, open-label phase II (NCT03606174) consisting of 9 cohorts of individuals with urothelial carcinoma, including bladder as the primary site. Sitravatinib + nivolumab will be administered in 8 cohorts of individuals with various checkpoint inhibitor treatment history and eligibility of platinum-based chemotherapy. The ninth cohort is a dose-escalation and expansion study of Sitravatinib, pembrolizumab, and EV. The primary outcome is 36-month ORR and secondary outcomes include adverse events, duration of response, clinical benefit rate, 36-month PFS, and 36-month OS.

Eganelisib is an antagonist of the phosphatidylinositol-3-kinase (PI3K). The PI3K/AKT/mTOR pathway is important for tumor growth, proliferation, and angiogenesis[62]. Therefore, inhibition of the PI3K/AKT/mTOR signaling pathway could slow tumor growth and reduce proliferation and metastatic potential. One 2:1 randomized; triple-blinded, placebo-controlled phase II study is investigating the relative efficacy of eganelisib with nivolumab versus nivolumab alone in individuals with advanced urothelial carcinoma who progressed on platinum-based chemotherapy but are checkpoint inhibitor therapy naïve (NCT03980041). The primary outcome is 24-month ORR, and the secondary outcomes are the time to response, duration of response, and PFS.

B. Agents Altering Tumor Microenvironment

The tumor microenvironment is an area characterized by complex interactions between a tumor and the surrounding immune, stromal, and endothelial cells. This area serves to help provide the tumor with nutrients to sustain its growth and enhance proliferation and to suppress the immune system from mounting an attack against the tumor.

KHK2455 is an inhibitor of IDO, an enzyme with an immunosuppressive effect on T cells in the tumor microenvironment[63]. One dose escalation and dose-expansion multicenter open-label phase I study is assessing the treatment-related adverse events of KHK2455 in combination with avelumab in individuals with mUC who progressed after platinum-based therapy, those who are platinum ineligible, and those who progressed after treatment with CPI (NCT03915405).

NO synthase is an important enzyme for nitric oxide

production and is upregulated in the tumor microenvironment. Nitric oxide creates an immunosuppressive tumor microenvironment by preventing T-cell proliferation, inhibiting the activity of antigen presenting cells, and attracting regulatory T cells into the local environment[64]. NG-monomethyl-L-arginine (L-NMMA) is a nitric oxide synthase inhibitor. L-NMMA in combination with pembrolizumab is being trialed in a dose-escalation and expansion phase Ib study (NCT03236935). The study recruited 12 individuals with metastatic solid organ malignancies, including UC. The purpose of the study is to assess the safety and maximum tolerated dose of L-NMMA and pembrolizumab.

TTX-030 is a monoclonal antibody that targets and inhibits CD39, Ectonucleoside triphosphate diphosphohydrolase-1. CD39 is an important enzyme to produce adenosine via degradation of ATP. High concentrations of adenosine in the tumor microenvironment are associated with suppression of the immune system[65]. Inhibition of CD39, therefore, causes ATP to accumulate and promote an immune response against a tumor. One phase I study is assessing the safety and dosing of TTX-030 in individuals with a variety of metastatic solid tumors, including UC and lymphomas (NCT03884556). The study has three arms which will study the safety profiles of TTX-030 monotherapy, TTX-030 and pembrolizumab, and TTX-030, gemcitabine, and nab-paclitaxel. A second open-label phase I study is set to explore the safety of TTX-030 in combination with budigalimab or pembrolizumab in combination with various chemotherapy regimens (NCT04306900).

Arginase depletes the tumor environment of arginine, leading to suppressed function of the immune cells. Therefore, therapies which inhibit arginase provide an important mechanism for restoring the immune system's ability to fight a growing and proliferating tumor. The arginase inhibitor INCB001158 is currently being studied in a phase I/II trial for patients with advanced/metastatic malignancies, such as UC (NCT02903914). The primary outcome is the safety and tolerability of INCB001158 alone and in combination with pembrolizumab.

II. Novel Molecular Targets for Management for Non-Muscle Invasive Bladder Cancer

A. Checkpoint Inhibitors

In January 2020, pembrolizumab also gained FDA approval as treatment for NMIBC with carcinoma in situ that is unresponsive to Bacillus Calmette-Guerin (BCG)[66]. The phase II KEYNOTE-057 trial (NCT02625961) administered pembrolizumab monotherapy in high-risk NMIBC (T1, high grade Ta and / or carcinoma in situ [CIS] only) patients, involving one cohort of CIS patients with or without papillary tumors that failed BCG therapy. The published results of the cohort indicate that pembrolizumab has significant activity, with an appropriate safety profile. The CRR at 3 months was 38.8%. In 80.2% of the patients, CR was durable at 6 months, and grade 3 or 4 treatment related AEs only occurred in 12.6% of the cohort. The CRRs were 44.6%, 41.7%, and 28.0% for patients with CIS alone, CIS with T1 tumors, and CIS with high-grade Ta tumors, respectively. 75% of patients demonstrated complete response for $\geq$ 6 months, and 53% for $\geq$ 9 months. Out of 101 patients, the most common grade 3-4 treatment related AEs were arthralgia (2%) and hyponatremia (3%)[67]. The KEYNOTE-676 trial, which will study the efficacy of pembrolizumab with BCG treatment in high-risk NMIBC patients, is currently in the enrollment phase (NCT03711032)

B. Nadofaragene firadenovec

Nadofaragene firadenovec is a novel gene-therapy agent recently approved for management of BCG unresponsive NMIBC. It relies on a non-replicating adenovirus to deliver interferon-alfa 2b gene copy that is translated in the bladder urothelium. Interferon alfa 2b expression is thought to exert an antitumor effect via different mechanisms, including, promoting apoptosis and stimulating an immune response against tumor cells[68]. A phase II study involving patients with relapsed or BCG refractory NMIBC were given intravesical nadofaragene firadenovec. Out of 40 patients, 14 remained recurrence-free for 12 months after receiving treatment, and the therapy was well-tolerated. The most common treatment-related AEs were micturition urgency (16[40%]), dysuria (16[40%]), and fatigue (13[32.5%])[69]. There is an ongoing phase III study evaluating this agent in patients with high grade NMIBC refractory to intravesical BCG therapy, and CR rate will be assessed (NCT02773849). On December 16, 2022 the FDA approved nadofaragene firadenovec for the treatment of high-risk BCG-unresponsive non-muscle-invasive bladder cancer with carcinoma in situ with or without papillary tumors. In 98 individuals who had BCG-unresponsive carcinoma in situ with or without papillary tumors followed for a median duration of 9.7 months, 51% of individuals on nadofaragene firadenovec achieved a complete response with 46% of individuals who had a complete response for at least one year.

C. TAR-200

The TAR-200 is a novel intravesical drug delivery system which continuously delivers pre-loaded

gemcitabine through a silicone tube locally into the bladder[70]. The FDA granted Fast Track designation to the TAR-200 delivery system for individuals with organ-confined or locally advanced muscle-invasive bladder cancer who are ineligible for curative intent therapy. Recently published phase I data (NCT02722538) has demonstrated the safety, tolerability, and initial activity of neoadjuvant gemcitabine administered continuously through the TAR-200 system. They recruited 23 individuals with newly diagnosed MIBC who were ineligible for cisplatin-based chemotherapy regimens and assigned them to two treatment arms. Both arms received two cycles of TAR-200 prior to radical cystectomy. Common side effects experienced included frequent abnormal urination throughout the day (n=3) and urinary incontinence (n=2). Among arm 1 participants (n=10), 4 patients experienced pathologic downstaging. Among the 10 patients in arm 2, 6 had downstaging and 3 had CR. Further studies on TAR-200 are also ongoing. The SunRISe-2 trial is an actively recruiting phase III study (NCT04658862) of patients with MIBC who are ineligible for radical cystectomy. Individuals will be randomly assigned to receive either TAR-200 and Cetrelimab or Chemoradiation and will be followed for 8 years to assess the time to first bladder intact event-free survival event. The SunRISe-4 trial is an open-label, randomized trial investigating if TAR-200 in addition to cetrelimab improves the pathologic complete response rate in individuals with MIBC who are ineligible for platinum-based neoadjuvant chemotherapy (NCT04919512).

Conclusion

In conclusion, this article provides a substantive update to our previous publication on current and prospective treatment options for urothelial carcinoma. Ongoing trials continue to explore which combination of established agents have the potential to improve the health of individuals with urothelial carcinoma. Recent trials of FDA approved therapies have been launched further exploring efficacy in different lines of therapies or in combination with other agents. The innovation in identification of drug targets and development of novel therapies has driven an expansion in the number of clinical trials testing the efficacy of novel agents for urothelial carcinoma. Further long-term studies are needed assessing the improvement of survival, quality of life, and side effect profiles for these potential therapies. These agents, however, have the potential to transform management of urothelial carcinoma and provide options for individuals with metastatic disease who are ineligible for platinum-based therapies.

Name of Drug	Class	FDA Approval Date	Studies Supporting Approval
Atezolizumab	PD-L1 inhibitor	5/2016: advanced or metastatic UC after platinum. 6/2018 for cisplatin-ineligible advanced. Withdrawn 3/2021	[71], Necchi et al.[72], Galsky et al[73]. (2016-2020)
Durvalumab	PD-L1 inhibitor	2/2017 for advanced or metastatic UC after platinum), withdrawn 2/2021	Massard et al.[74], Powles et al[27].
Nivolumab	PD-1 inhibitor	2/2017 for advanced or metastatic UC after platinum	CheckMate trials[12,13,28]
Avelumab	PD-L1 inhibitor	5/2017: advanced UC that progressed after platinum 6/2020: for maintenance	Apolo et al.[75], Patel et al. (2017)[76], JAVELIN Bladder 100 (NCT02603432)
Pembrolizumab	PD-L1 inhibitor	5/2017: for advanced or metastatic UC after platinum, 6/2018: cisplatin-ineligible advanced or metastatic UC 1/2020 for high-risk BCG-unresponsive NMIBC), 8/2021 adjuvant	KEYNOTE trials (2017-21)[21,67,77-79]
Erdafitinib	FGFR inhibitor	4/2019: for advanced UC with FGFR 2 or 3 that after platinum	Bahleda et al.[42],Loriot et al.[43]
Enfortumab vedotin	ADC targeting Nectin-4	12/2019: advanced UC that progressed after two lines 4/2023: in combination with pembrolizumab as first-line in cisplatin-ineligible patients	Rosenburg et al.[48,49] (2019-20), EV-103/KEYNOTE-869 study (NCT03288545)

Name of Drug	Class	FDA Approval Date	Studies Supporting Approval
Sacituzumab govitecan	ADC targeting Trop-2	4/2021: for advanced or metastatic UC after platinum or PD-1/PD-L1	TROPHY trial [52](NCT03547973)
nadofaragene firadenovec-vncg	Novel adenovirus vector-based gene therapy	12/2022: for high-risk BCG-unresponsive NMIBC with CIS with or without papillary tumors	NCT02773849

References

1. Richters A, Aben KKH, Kiemeney LALM. The global burden of urinary bladder cancer: an update. *World J Urol*. 2020;38(8):1895-1904. doi:10.1007/s00345-019-02984-4
2. Lenis AT, Lec PM, Chamie K, Mshs M. Bladder Cancer. *JAMA*. 2020;324(19):1980. doi:10.1001/jama.2020.17598
3. 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. Clinical Trial, Phase II, Clinical Trial, Phase III, Multicenter Study, Randomized Controlled Trial. Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't. *J Clin Oncol*. Jan 10 2012;30(2):191-9. doi:10.1200/JCO.2011.37.3571
4. Von Der Maase H, Hansen SW, Roberts JT, 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. *Journal of Clinical Oncology*. 2000;18(17):3068-3077. doi:10.1200/jco.2000.18.17.3068
5. Sternberg CN, De Mulder P, Schornagel JH, 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. *European journal of cancer*. 2006;42(1):50-54. doi:10.1016/j.ejca.2005.08.032
6. Lv J, Zhu Y, Ji A, Zhang Q, Liao G. Mining TCGA database for tumor mutation burden and their clinical significance in bladder cancer. *Bioscience Reports*. 2020;40(4)doi:10.1042/bsr20194337
7. Wołącewicz M, Hrynkiewicz R, Grywalska E, et al. Immunotherapy in Bladder Cancer: Current Methods and Future Perspectives. *Cancers*. 2020;12(5):1181. doi:10.3390/cancers12051181
8. Roche. Roche provides update on Tecentriq US indication in prior-platinum treated metastatic bladder cancer. https://www.roche.com/media/releases/med-cor-2021-03-08.htm (accessed on 13 March 2021)
9. AstraZeneca. Voluntary withdrawal of Imfinzi indication in advanced bladder cancer in the US. Accessed May 3, 2021. https://www.astrazeneca.com/content/astraz/media-centre/press-releases/2021/voluntary-withdrawal-imfinzi-us-bladder-indication.html (accessed on 13 March 2021)
10. Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. *Molecular cancer therapeutics*. 2015;14(4):847-856. doi:10.1158/1535-7163.mct-14-0983
11. FDA. Nivolumab for treatment of urothelial carcinoma. Accessed May 3, 2021. https://www.fda.gov/drugs/resources-information-approved-drugs/nivolumab-treatment-urothelial-carcinoma
12. Sharma P, Callahan MK, Bono P, et al. Nivolumab monotherapy in recurrent metastatic urothelial carcinoma (CheckMate 032): a multicentre, open-label, two-stage, multi-arm, phase 1/2 trial. *The Lancet Oncology*. 2016;17(11):1590-1598. doi:10.1016/s1470-2045(16)30496-x
13. Sharma P, Retz M, Siefker-Radtke A, et al. Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): a multicentre, single-arm, phase 2 trial. *The Lancet Oncology*. 2017;18(3):312-322. doi:10.1016/s1470-2045(17)30065-7
14. Ohyama C, Kojima T, Kondo T, et al. Nivolumab in patients with unresectable locally advanced or metastatic urothelial carcinoma: CheckMate 275 2-year global and Japanese patient population analyses. *International Journal of Clinical Oncology*. 2019;24(9):1089-1098. doi:10.1007/s10147-019-01450-w
15. Bajorin DF, Witjes JA, Gschwend JE, et al. Adjuvant Nivolumab versus Placebo in Muscle-Invasive Urothelial Carcinoma. research-article.

https://doiorg/101056/NEJMoa2034442. 2021-06-02 2021;doi:NJ202106033842207

16. FDA. FDA approves avelumab for urothelial carcinoma maintenance treatment. Accessed May 3, 2021. https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-avelumab-urothelial-carcinoma-maintenance-treatment
17. Powles T, Park SH, Voog E, et al. Avelumab Maintenance Therapy for Advanced or Metastatic Urothelial Carcinoma. *New England Journal of Medicine*. 2020;383(13):1218-1230. doi:10.1056/nejmoa2002788
18. Suzman DL, Agrawal S, Ning YM, et al. FDA Approval Summary: Atezolizumab or Pembrolizumab for the Treatment of Patients with Advanced Urothelial Carcinoma Ineligible for Cisplatin-Containing Chemotherapy. *The Oncologist*. 2019;24(4):563-569. doi:10.1634/theoncologist.2018-0084
19. Powles T, Csoszi T, Ozguroglu M, et al. Pembrolizumab alone or combined with chemotherapy versus chemotherapy as first-line therapy for advanced urothelial carcinoma (KEYNOTE-361): a randomized, open-label, phase 3 trial. *The Lancet Oncology*. 2021;doi:10.1016/s1470-2045(21)00152-2
20. FDA. Pembrolizumab (Keytruda): advanced or metastatic urothelial carcinoma. Accessed May 3, 2021. https://www.fda.gov/drugs/resources-information-approved-drugs/pembrolizumab-keytruda-advanced-or-metastatic-urothelial-carcinoma
21. Balar AV, Castellano D, O'Donnell PH, et al. First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. *The Lancet Oncology*. 2017;18(11):1483-1492. doi:10.1016/s1470-2045(17)30616-2
22. Vuky J, Balar AV, Castellano D, et al. Long-term outcomes in KEYNOTE-052: Phase II study investigating first-line pembrolizumab in cisplatin-ineligible patients with locally advanced or metastatic urothelial cancer. *Journal of Clinical Oncology*. 2020;38(23):2658-2666.
23. Bellmunt J, De Wit R, Vaughn DJ, et al. Pembrolizumab as Second-Line Therapy for Advanced Urothelial Carcinoma. *New England Journal of Medicine*. 2017;376(11):1015-1026. doi:10.1056/nejmoa1613683
24. FDA. FDA approves new, targeted treatment for bladder cancer. Accessed May 3, 2021. https://www.fda.gov/news-events/press-announcements/fda-approves-new-targeted-treatment-bladder-cancer
25. Powles T, Durán I, Van Der Heijden MS, et al. Atezolizumab versus chemotherapy in patients with platinum-treated locally advanced or metastatic urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised controlled trial. *The Lancet*. 2018;391(10122):748-757. doi:10.1016/s0140-6736(17)33297-x
26. FDA. Durvalumab (Imfinzi). Accessed May 3, 2021. https://www.fda.gov/drugs/resources-information-approved-drugs/durvalumab-imfinzi
27. Powles T, O'Donnell PH, Massard C, et al. Efficacy and Safety of Durvalumab in Locally Advanced or Metastatic Urothelial Carcinoma. *JAMA Oncology*. 2017;3(9):e172411. doi:10.1001/jamaoncol.2017.2411
28. Sharma P, Siefker-Radtke A, De Braud F, et al. Nivolumab Alone and With Ipilimumab in Previously Treated Metastatic Urothelial Carcinoma: CheckMate 032 Nivolumab 1 mg/kg Plus Ipilimumab 3 mg/kg Expansion Cohort Results. *Journal of Clinical Oncology*. 2019;37(19):1608-1616. doi:10.1200/jco.19.00538
29. Squibb BM. Bristol Myers Squibb Provides Update on CheckMate -901 Trial Evaluating Opdivo (nivolumab) Plus Yervoy (ipilimumab) as First-Line Treatment for Patients with Unresectable or Metastatic Urothelial Carcinoma. News. 2022;
30. Schmid SC, Koll FJ, Rödel C, et al. Radiation therapy before radical cystectomy combined with immunotherapy in locally advanced bladder cancer – study protocol of a prospective, single arm, multicenter phase II trial (RACE IT). *BMC Cancer*. 2020;20(1)doi:10.1186/s12885-019-6503-6
31. Cathomas R, Rothschild S, Hayoz S, et al. Safety and efficacy of perioperative cisplatin/gemcitabine (cis/gem) and durvalumab (durva) for operable muscle-invasive urothelial carcinoma (MIUC): SAKK 06/17. *Journal of Clinical Oncology*. 2021;39(6_suppl):430-430. doi:10.1200/JCO.2021.39.6_suppl.430
32. Gupta S, Sonpavde G, Weight CJ, et al. Results from BLASST-1 (Bladder Cancer Signal Seeking Trial) of nivolumab, gemcitabine, and cisplatin in muscle invasive bladder cancer (MIBC) undergoing cystectomy. *Journal of Clinical Oncology*. 2020;38(6_suppl):439-439. doi:10.1200/JCO.2020.38.6_suppl.439
33. June CH, Sadelain M. Chimeric Antigen Receptor Therapy. *New England Journal of*

Medicine. 2018;379(1):64-73. doi:10.1056/NEJMra1706169
34. Feins S, Kong W, Williams EF, Milone MC, Fraietta JA. An introduction to chimeric antigen receptor (CAR) T-cell immunotherapy for human cancer. *Am J Hematol*. 2019/05// 2019;94(S1):S3-S9. doi:10.1002/ajh.25418
35. Alkhouli MA, Bazargan S, Pilon-Thomas S, Poch M, Chahoud J. Current State of Cell Therapies for Genitourinary Malignancies. *Cancer J*. 2022 Jul-Aug 2022;28(4):294-300. doi:10.1097/ppo.0000000000000604
36. Tanoue K, Rosewell Shaw A, Watanabe N, et al. Armed Oncolytic Adenovirus-Expressing PD-L1 Mini-Body Enhances Antitumor Effects of Chimeric Antigen Receptor T Cells in Solid Tumors. *Cancer Res*. Apr 15 2017;77(8):2040-2051. doi:10.1158/0008-5472.Can-16-1577
37. Goldberg MV, Drake CG. LAG-3 in Cancer Immunotherapy. *Curr Top Microbiol Immunol*. 2011;344:269-78. doi:10.1007/82_2010_114
38. Xiao JF, Caliri AW, Duex JE, Theodorescu D. Targetable Pathways in Advanced Bladder Cancer: FGFR Signaling. *Cancers (Basel)*. Sep 29 2021;13(19)doi:10.3390/cancers13194891
39. Zhou Y, Wu C, Lu G, Hu Z, Chen Q, Du X. FGF/FGFR signaling pathway involved resistance in various cancer types. *J Cancer*. 2020;11(8):2000-2007. doi:10.7150/jca.40531
40. Chioni AM, Grose RP. Biological Significance and Targeting of the FGFR Axis in Cancer. *Cancers (Basel)*. Nov 13 2021;13(22)doi:10.3390/cancers13225681
41. FDA. FDA approves first targeted therapy for metastatic bladder cancer. Accessed May 3, 2021. https://www.fda.gov/news-events/press-announcements/fda-approves-first-targeted-therapy-metastatic-bladder-cancer
42. Bahleda R, Italiano A, Hierro C, et al. Multicenter Phase I Study of Erdafitinib (JNJ-42756493), Oral Pan-Fibroblast Growth Factor Receptor Inhibitor, in Patients with Advanced or Refractory Solid Tumors. *Clinical Cancer Research*. 2019;25(16):4888-4897. doi:10.1158/1078-0432.ccr-18-3334
43. Loriot Y, Necchi A, Park SH, et al. Erdafitinib in Locally Advanced or Metastatic Urothelial Carcinoma. *New England Journal of Medicine*. 2019;381(4):338-348. doi:10.1056/nejmoa1817323
44. Schuler M, Cho BC, Sayehli CM, et al. Rogaritinib in patients with advanced cancers selected by FGFR mRNA expression: a phase 1 dose-escalation and dose-expansion study. *Lancet Oncology*. 2019;20(10):1454-1466. doi:10.1016/S1470-2045(19)30412-7
45. Sternberg CN, Petrylak DP, Bellmunt J, et al. FORT-1: Phase II/III Study of Rogaratinib Versus Chemotherapy in Patients With Locally Advanced or Metastatic Urothelial Carcinoma Selected Based on FGFR1/3 mRNA Expression. *J Clin Oncol*. Jan 20 2023;41(3):629-639. doi:10.1200/jco.21.02303
46. FDA. FDA grants accelerated approval to enfortumab vedotin-ejfv for metastatic urothelial cancer. Accessed May 3, 2021. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-enfortumab-vedotin-ejfv-metastatic-urothelial-cancer
47. Heath EI, Rosenberg JE. The biology and rationale of targeting nectin-4 in urothelial carcinoma. *Nature reviews Urology*. Feb 2021;18(2):93-103. doi:10.1038/s41585-020-00394-5
48. Rosenberg J, Sridhar SS, Zhang J, et al. EV-101: A Phase I Study of Single-Agent Enfortumab Vedotin in Patients With Nectin-4–Positive Solid Tumors, Including Metastatic Urothelial Carcinoma. *Journal of Clinical Oncology*. 2020;38(10):1041-1049. doi:10.1200/jco.19.02044
49. Rosenberg JE, O'Donnell PH, Balar AV, et al. Pivotal Trial of Enfortumab Vedotin in Urothelial Carcinoma After Platinum and Anti-Programmed Death 1/Programmed Death Ligand 1 Therapy. *Journal of Clinical Oncology*. 2019;37(29):2592-2600. doi:10.1200/jco.19.01140
50. Powles T, Rosenberg JE, Sonpavde GP, et al. Enfortumab Vedotin in Previously Treated Advanced Urothelial Carcinoma. *New England Journal of Medicine*. 2021;384(12):1125-1135. doi:10.1056/nejmoa2035807
51. Powles T, Drakaki A, Teoh JY-C, et al. A phase 3, randomized, open-label, multicenter, global study of the efficacy and safety of durvalumab (D) + tremelimumab (T) + enfortumab vedotin (EV) or D + EV for neoadjuvant treatment in cisplatin-ineligible muscle-invasive bladder cancer (MIBC) (VOLGA). *Journal of Clinical Oncology*. 2022;40(6_suppl):TPS579-TPS579. doi:10.1200/JCO.2022.40.6_suppl.TPS579
52. Faltas B, Goldenberg DM, Ocean AJ, et al. Sacituzumab Govitecan, a Novel Antibody–Drug Conjugate, in Patients With Metastatic Platinum-Resistant Urothelial Carcinoma. *Clinical genitourinary cancer*. 2016;14(1):e75-e79. doi:10.1016/j.clgc.2015.10.002
53. FDA. FDA grants accelerated approval to sacituzumab govitecan for advanced urothelial

cancer. Accessed May 3, 2021. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-sacituzumab-govitecan-advanced-urothelial-cancer

54. Grivas P, Tagawa ST, Bellmunt J, et al. TROPiCS-04: Study of sacituzumab govitecan in metastatic or locally advanced unresectable urothelial cancer that has progressed after platinum and checkpoint inhibitor therapy. *Journal of Clinical Oncology*. 2021;39(6_suppl):TPS498-TPS498. doi:10.1200/JCO.2021.39.6_suppl.TPS498
55. Necchi A, Lo Vullo S, Mariani L, et al. An open-label, single-arm, phase 2 study of the Aurora kinase A inhibitor alisertib in patients with advanced urothelial cancer. *Investigational new drugs*. 2016;34(2):236-242. doi:10.1007/s10637-016-0328-9
56. Yan M, Schwaederle M, Arguello D, Millis SZ, Gatalica Z, Kurzrock R. HER2 expression status in diverse cancers: review of results from 37,992 patients. *Cancer Metastasis Rev*. Mar 2015;34(1):157-64. doi:10.1007/s10555-015-9552-6
57. Laé M, Couturier J, Oudard S, Radvanyi F, Beuzeboc P, Vieillefond A. Assessing HER2 gene amplification as a potential target for therapy in invasive urothelial bladder cancer with a standardized methodology: results in 1005 patients. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. Apr 2010;21(4):815-819. doi:10.1093/annonc/mdp488
58. Galsky MD, Conte GD, Foti S, et al. Primary analysis from DS8201-A-U105: A phase 1b, two-part, open-label study of trastuzumab deruxtecan (T-DXd) with nivolumab (nivo) in patients (pts) with HER2-expressing urothelial carcinoma (UC). *Journal of Clinical Oncology*. 2022;40(6_suppl):438-438. doi:10.1200/JCO.2022.40.6_suppl.438
59. Sheng X, Yan X, Wang L, et al. Open-label, Multicenter, Phase II Study of RC48-ADC, a HER2-Targeting Antibody–Drug Conjugate, in Patients with Locally Advanced or Metastatic Urothelial Carcinoma. *Clinical Cancer Research*. 2021;27(1):43-51. doi:10.1158/1078-0432.Ccr-20-2488
60. Marandino L, Raggi D, Calareso G, et al. Cabozantinib Plus Durvalumab in Patients With Advanced Urothelial Carcinoma After Platinum Chemotherapy: Safety and Preliminary Activity of the Open-Label, Single-Arm, Phase 2 ARCADIA Trial. *Clinical genitourinary cancer*. Oct 2021;19(5):457-465. doi:10.1016/j.clgc.2021.04.001
61. Bauer T, Cho BC, Heist R, et al. First-in-human phase 1/1b study to evaluate sitravatinib in patients with advanced solid tumors. *Investigational new drugs*. Oct 2022;40(5):990-1000. doi:10.1007/s10637-022-01274-y
62. Yang J, Nie J, Ma X, Wei Y, Peng Y, Wei X. Targeting PI3K in cancer: mechanisms and advances in clinical trials. *Molecular Cancer*. 2019/02/19 2019;18(1):26. doi:10.1186/s12943-019-0954-x
63. Munn DH, Mellor AL. IDO in the Tumor Microenvironment: Inflammation, Counter-Regulation, and Tolerance. *Trends Immunol*. Mar 2016;37(3):193-207. doi:10.1016/j.it.2016.01.002
64. Vedenko A, Panara K, Goldstein G, Ramasamy R, Arora H. Tumor Microenvironment and Nitric Oxide: Concepts and Mechanisms. *Adv Exp Med Biol*. 2020;1277:143-158. doi:10.1007/978-3-030-50224-9_10
65. Moesta AK, Li XY, Smyth MJ. Targeting CD39 in cancer. *Nat Rev Immunol*. Dec 2020;20(12):739-755. doi:10.1038/s41577-020-0376-4
66. FDA. FDA approves pembrolizumab for BCG-unresponsive, high-risk non-muscle invasive bladder cancer. Accessed May 3, 2021. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-pembrolizumab-bcg-unresponsive-high-risk-non-muscle-invasive-bladder-cancer
67. Balar AV, Kulkarni GS, Uchio EM, et al. Keynote 057: Phase II trial of Pembrolizumab (pembro) for patients (pts) with high-risk (HR) nonmuscle invasive bladder cancer (NMIBC) unresponsive to bacillus calmette-guérin (BCG). *Journal of Clinical Oncology*. 2019;37(7_suppl):350-350. doi:10.1200/JCO.2019.37.7_suppl.350
68. Asmana Ningrum R. Human interferon alpha-2b: a therapeutic protein for cancer treatment. *Scientifica (Cairo)*. 2014;2014:970315. doi:10.1155/2014/970315
69. Boorjian SA, Alemozaffar M, Konety BR, et al. Intravesical nadofaragene firadenovec gene therapy for BCG-unresponsive non-muscle-invasive bladder cancer: a single-arm, open-label, repeat-dose clinical trial. *The Lancet Oncology*. Jan 2021;22(1):107-117. doi:10.1016/s1470-2045(20)30540-4
70. Daneshmand S, Brummelhuis ISG, Pohar KS, et al. The safety, tolerability, and efficacy of a neoadjuvant gemcitabine intravesical drug delivery system (TAR-200) in muscle-invasive bladder cancer patients: a phase I trial. *Urologic oncology*. Jul 2022;40(7):344.e1-344.e9. doi:10.1016/j.urolonc.2022.02.009
71. 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*. May 7 2016;387(10031):1909-20. doi:10.1016/s0140-6736(16)00561-4

72. Necchi A, Joseph RW, Loriot Y, et al. Atezolizumab in platinum-treated locally advanced or metastatic urothelial carcinoma: post-progression outcomes from the phase II IMvigor210 study. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. Dec 1 2017;28(12):3044-3050. doi:10.1093/annonc/mdx518
73. Galsky MD, Arija JÁ A, Bamias A, et al. Atezolizumab with or without chemotherapy in metastatic urothelial cancer (IMvigor130): a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet*. May 2020;395(10236):1547-1557. doi:10.1016/s0140-6736(20)30230-0
74. Massard C, Gordon MS, Sharma S, et al. Safety and Efficacy of Durvalumab (MEDI4736), an Anti-Programmed Cell Death Ligand-1 Immune Checkpoint Inhibitor, in Patients With Advanced Urothelial Bladder Cancer. *J Clin Oncol*. Sep 10 2016;34(26):3119-25. doi:10.1200/jco.2016.67.9761
75. Apolo AB, Infante JR, Balmanoukian A, et al. Avelumab, an Anti-Programmed Death-Ligand 1 Antibody, In Patients With Refractory Metastatic Urothelial Carcinoma: Results From a Multicenter, Phase Ib Study. *J Clin Oncol*. Jul 1 2017;35(19):2117-2124. doi:10.1200/jco.2016.71.6795
76. Patel MR, Ellerton J, Infante JR, et al. Avelumab in metastatic urothelial carcinoma after platinum failure (JAVELIN Solid Tumor): pooled results from two expansion cohorts of an open-label, phase 1 trial. *The Lancet Oncology*. Jan 2018;19(1):51-64. doi:10.1016/s1470-2045(17)30900-2
77. Plimack ER, Bellmunt J, Gupta S, et al. Safety and activity of pembrolizumab in patients with locally advanced or metastatic urothelial cancer (KEYNOTE-012): a non-randomised, open-label, phase 1b study. *The Lancet Oncology*. Feb 2017;18(2):212-220. doi:10.1016/s1470-2045(17)30007-4
78. Vaughn DJ, Bellmunt J, Fradet Y, et al. Health-Related Quality-of-Life Analysis From KEYNOTE-045: A Phase III Study of Pembrolizumab Versus Chemotherapy for Previously Treated Advanced Urothelial Cancer. *J Clin Oncol*. Jun 1 2018;36(16):1579-1587. doi:10.1200/jco.2017.76.9562
79. Nishiyama H, Yamamoto Y, Sassa N, et al. Pembrolizumab versus chemotherapy in recurrent, advanced urothelial cancer in Japanese patients: a subgroup analysis of the phase 3 KEYNOTE-045 trial. *Int J Clin Oncol*. Jan 2020;25(1):165-174. doi:10.1007/s10147-019-01545-4