

Review

Current Strategies and Novel Therapeutic Approaches for Metastatic Urothelial Carcinoma

Veronica Mollica ^{1,2,†}, Alessandro Rizzo ^{1,2,†}, Rodolfo Montironi ³, Liang Cheng ⁴,
Francesca Giunchi ⁵, Riccardo Schiavina ⁶, Matteo Santoni ⁷, Michelangelo Fiorentino ⁸,
Antonio Lopez-Beltran ⁹, Eugenio Brunocilla ⁶, Giovanni Brandi ^{1,2} and Francesco Massari ^{1,*}

¹ Division of Oncology, S.Orsola-Malpighi Hospital, 40138 Bologna, Italy; veronica.mollica7@gmail.com (V.M.); rizzo.alessandro179@gmail.com (A.R.); giovanni.brandi@unibo.it (G.B.)

² Department of Experimental, Diagnostic and Specialty Medicine, S.Orsola-Malpighi Hospital, 40138 Bologna, Italy

³ Section of Pathological Anatomy, Polytechnic University of the Marche Region, School of Medicine, United Hospitals, 60121 Ancona, Italy; r.montironi@staff.univpm.it

⁴ Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, USA; liang_cheng@yahoo.com

⁵ Pathology Service, Addarii Institute of Oncology, S-Orsola-Malpighi Hospital, 40138 Bologna, Italy; frachikka@virgilio.it

⁶ Department of Urology, University of Bologna, S-Orsola-Malpighi Hospital, 40138 Bologna, Italy; riccardo.schiavina3@unibo.it (R.S.); eugenio.brunocilla@unibo.it (E.B.)

⁷ Oncology Unit, Macerata Hospital, 62100 Macerata, Italy; mattymo@alice.it

⁸ Department of Pathology, University of Bologna, School of Medicine, 40126 Bologna, Italy; michelangelo.fiorentino@unibo.it

⁹ Unit of Anatomical Pathology, Faculty of Medicine, Cordoba University, 14071 Cordoba, Spain; eugenio.brunocilla@unibo.it

* Correspondence: fmassari79@gmail.com; Tel.: +39-051-214-2575

† These authors contribution is equally to this work.

Received: 21 May 2020; Accepted: 1 June 2020; Published: 2 June 2020

Abstract: Urothelial carcinoma (UC) is a frequent cause of cancer-related deaths worldwide. Metastatic UC has been historically associated with poor prognosis, with a median overall survival of approximately 15 months and a 5-year survival rate of 18%. Although platinum-based chemotherapy remains the mainstay of medical treatment for patients with metastatic UC, chemotherapy clinical trials produced modest benefit with short-lived, disappointing responses. In recent years, the better understanding of the role of immune system in cancer control has led to the development and approval of several immunotherapeutic approaches in UC therapy, where immune checkpoint inhibitors have been revolutionizing the treatment of metastatic UC. Because of a better tumor molecular profiling, FGFR inhibitors, PARP inhibitors, anti-HER2 agents, and antibody drug conjugates targeting Nectin-4 are also emerging as new therapeutic options. Moreover, a wide number of trials is ongoing with the aim to evaluate several other alterations and pathways as new potential targets in metastatic UC. In this review, we will discuss the recent advances and highlight future directions of the medical treatment of UC, with a particular focus on recently published data and ongoing active and recruiting trials.

Keywords: urothelial carcinoma; immunotherapy; immune checkpoint inhibitors; FGFR; antibody drug conjugates; clinical trials; PD-1; PD-L1

1. Introduction

Urothelial carcinoma (UC) is a common cancer worldwide, with nearly half a million of new diagnoses annually [1]. Although UC includes a group of tumors of the bladder, renal pelvis, ureter and urethra, more than 90% of UCs occurs in the lower urinary tract, therefore involving urinary bladder and, less often, urethra [2]. About 70–75% of patients at diagnosis are affected by non-muscle-invasive bladder cancer (NMIBC) while more than 25% of cases are already muscle-invasive bladder cancer (MIBC) or metastatic forms [3]. Despite recent improvements in the field of medical oncology, the prognosis of patients with advanced or metastatic UC remains dismal, with a median overall survival (OS) of approximately 15 months from diagnosis [4]. In the last twenty years, front-line cisplatin-based chemotherapy represented the mainstay of palliative treatment for UC, with combinations such as gemcitabine plus cisplatin (GC) and methotrexate, vinblastine, doxorubicin, and cisplatin (M-VAC) as the cornerstones of standard treatment in advanced or metastatic UC [5–7]. Although GC and M-VAC regimens showed similar outcomes in terms of OS and time to treatment failure (TTF), GC is commonly preferred over M-VAC on the basis of lower mucosal and hematological toxicity [5–7]. Nevertheless, most patients are cisplatin-ineligible because of inadequate renal function, poor Eastern Cooperative Oncology Group (ECOG) performance status, peripheral neuropathy, old age and/or other underlying comorbidities, and thus, cisplatin is usually replaced by carboplatin in unfit patients, as we shall see later [8]. Unfortunately, after the failure of first-line treatments, further therapies have yielded poor response rates and the overall results obtained with conventional cytotoxic agents (as monotherapy or in combination) have been far from being satisfactory since UC patients historically carried a median OS of approximately 12–17 months [9]. Thus, there is an urgent need for novel, more effective treatment options in advanced or metastatic UC.

Recent phase I to III studies with drugs targeting immune checkpoints and different molecular pathways of UC are ongoing and some were published in the last three years [10]. These novel agents primarily include immune checkpoint inhibitors (ICIs), tyrosine kinase inhibitors (TKIs) targeting fibroblast growth factor receptor (FGFR), and antibody-drug conjugates (ADCs) directed against Nectin-4 [11,12]; however, many other alterations and pathways are also emerging as new potential targets [13]. In this paper, we provide a comprehensive review of recent trials and the current state of ongoing active and recruiting Phase I, II, and III trials according to clinicaltrials.gov, looking into the future of the rapidly evolving landscape of medical treatment for advanced or metastatic UC.

We performed a research on Pubmed/Medline, Cochrane library and Scopus using the keyword “urothelial carcinoma” OR “bladder carcinoma” OR “bladder urothelial carcinoma” OR “bladder cancer” OR “bladder neoplasm.” We selected pivotal registration studies. We also selected the most relevant and pertinent studies considering quality of the studies in terms of their applicability, how they were conducted, statistical analysis, number of patients enrolled, outcomes. For ongoing clinical trials, we searched in the clinicaltrials.gov database for recruiting and active, not recruiting trials, using the following keywords: “urothelial carcinoma” OR “bladder carcinoma” OR “bladder urothelial carcinoma” OR “bladder cancer” OR “bladder neoplasm.” We restricted our research to phase 1, 2, or 3 trials focused on the metastatic/advanced setting.

2. Treatment Strategies: State-of-the-Art

2.1. Immune Checkpoint Inhibitors

The advent of ICIs blocking the interaction of Programmed Death 1 (PD-1) and Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) with their specific ligands has recently revolutionized the treatment of several hematological and solid malignancies (Figure 1) [14–16]. Outstandingly, ICIs have challenged previous treatment paradigms of most solid tumors, including the therapeutic decision-making approach to advanced or metastatic UC, in the first-line setting for cisplatin-ineligible patients as well as in the post-platinum setting [17,18]. Given the well-known activity of topical instillation of *Bacillus of Calmette-Guérin* (BCG) in high-risk, non-muscle invasive

disease, UC immediately appeared as a suitable candidate for modern immunotherapy [19]; moreover, UC is known to be a highly antigenic malignancy, given the high rates of DNA alterations and mutations leading to the formation of neoantigens, an element which further supports the application of ICIs in advanced or metastatic UC [20–22]. In light of data provided by a variety of recent trials, the therapeutic scenario of UC is rapidly changing but, unfortunately, several unmet clinical needs still persist [22,23].

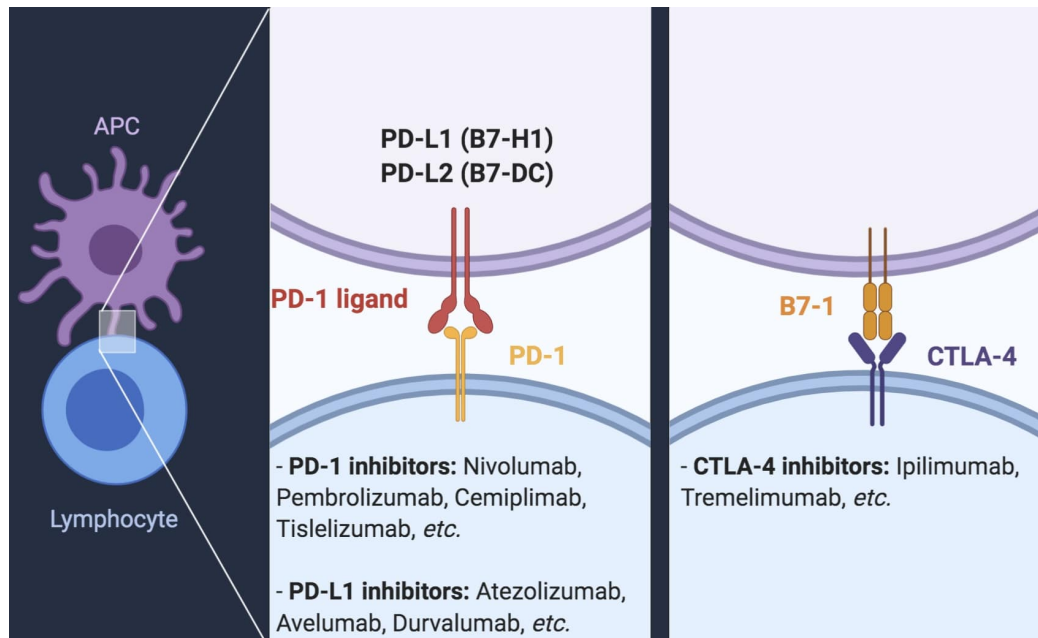


Figure 1. The interaction between PD-1/PD-L1 and CTLA-4/B7-1, a key mechanism exploited by immune checkpoint inhibitors. PD-1 inhibitors include nivolumab, pembrolizumab, cemiplimab, tislelizumab, and other agents currently in development; conversely, PD-L1 inhibitors encompass agents such as atezolizumab, avelumab, durvalumab, while CTLA-4 inhibitors encompass ipilimumab and tremelimumab.

Although cisplatin-based regimens are considered the standard first-line treatment in advanced or metastatic UC, more than 50% of patients are ineligible for cisplatin in clinical practice [24]. For this non-negligible group of patients, carboplatin plus gemcitabine has been considered the standard treatment based on the results of the EORTC 30,986 trial, with several other combinations and agents showing less favorable safety profiles and inferior outcomes compared to cisplatin-based first-line therapy [25–27]. Thus, the modest survival benefits observed with available treatment options highlighted the need for new effective strategies [28] and for this purpose, following small phase I trials, the role of ICIs as front-line treatment in cisplatin-ineligible patients was investigated in KEYNOTE-052 and IMvigor210 trials [29,30].

The KEYNOTE-052 [29] was a phase II trial aimed to evaluate the safety and efficacy of pembrolizumab monotherapy (200 mg flat dose every three weeks) in 370 chemo-naive, cisplatin-ineligible patients. In this setting pembrolizumab, a highly selective humanized monoclonal IgG4 isotype antibody against PD-1 protein, produced an overall response rate (ORR) of 24% with 5% of complete response (CR). Interestingly, the magnitude of ORR and survival benefit was related to programmed death ligand-1 (PD-L1) expression: in fact, in patients with PD-L1 expression combined positive score (CPS) $\geq 10\%$, pembrolizumab resulted in improved survival, with a median OS of 18.5 months versus 11.5 months in overall cohort. Finally, the CPS $\geq 10\%$ population reported higher ORR (37%) compared to the CPS $< 10\%$ subgroup of patients (ORR = 18%).

The IMvigor210 trial [30] was a 2-cohort Phase 2 study; while cohort 2 assessed atezolizumab in a post-platinum setting, in cohort 1 the anti-PD-L1 agent was tested as first-line treatment in

cisplatin-ineligible subjects. Total of 119 untreated patients were included in cohort 1 and received atezolizumab, 1200 mg flat dose every three weeks, achieving an ORR of 23% with CR and partial response (PR) of 9% and 12% respectively, regardless of PD-L1 expression. Clinical activity of atezolizumab was higher than those observed with systemic chemotherapies traditionally used in this setting, in respect of whom the anti-PD-L1 agent showed also a more manageable safety profile.

Based on the aforementioned studies, pembrolizumab and atezolizumab were approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) for front-line use in cisplatin unfit patients affected by advanced or metastatic UC. However, the use of pembrolizumab and atezolizumab has been subsequently restricted, following early data from KEYNOTE-361 and IMvigor130 phase III trials which, as we shall explain later, are currently investigating combination chemo-immunotherapy in advanced or metastatic UC. In these two trials, patients with low expression of PD-L1 receiving single-agent ICI experienced worse survival compared to patients receiving standard chemotherapy [31,32].

Following platinum-based chemotherapy, large proportions of patients are either non-responders or relapsed, and therefore proceed for second-line treatment [33]. Until some years ago, taxanes or vinflunine were considered standard second-line treatments, despite disappointing ORRs and an overall modest clinical benefit [34,35]. In this scenario, recent results of Phase I to III studies with agents targeting PD-1 and PD-L1 have led to fast approval of ICIs as second-line treatments. In particular, five ICIs (two anti-PD-1 agents—pembrolizumab and nivolumab—and three anti-PD-L1 agents—atezolizumab, durvalumab, and avelumab) have been granted approval by FDA for patients with advanced or metastatic UC whose disease progressed during or following platinum-based chemotherapy [36]. Conversely, despite FDA has granted approval for the aforementioned agents, pembrolizumab is the only ICI that showed a survival benefit in a phase III randomized clinical trial and whose activity is supported by higher levels of evidence [37].

The approval of pembrolizumab in post-platinum setting was granted based on the results of the KEYNOTE-045 trial [38]. This phase III, open-label, randomized trial compared pembrolizumab (flat dose of 200 mg every three weeks) with chemotherapy by investigators' choice, including vinflunine and taxanes, in patients who recurred or progressed after a platinum-based regimen. A higher ORR was observed in patients treated with pembrolizumab (21.1% vs. 11.4% of the chemotherapy arm); moreover, an OS benefit was observed, regardless of PD-L1 expression (in the overall population 10.3 and 7.4 months, in the immunotherapy and chemotherapy arm, respectively, hazard ratio (HR) 0.73; 95% confidence interval (CI) 0.59 to 0.91; $p = 0.002$). Finally, pembrolizumab was associated with fewer grade 3–4 adverse events compared to vinflunine, paclitaxel, and docetaxel.

Instead, the activity of atezolizumab was tested in the phase II IMvigor210 and the phase III IMvigor211 trials [39,40]. As stated above, the IMvigor210 trial was a 2-cohort phase II study aimed to evaluate the efficacy and safety of atezolizumab (1200 mg flat dose every three weeks) in untreated, cisplatin-ineligible patients (cohort 1) as well as in patients whose disease was refractory to platinum-based chemotherapy (cohort 2) [39]. In the cohort 2, including 315 eligible subjects, an ORR of 15% was observed, with a sustained response duration and an acceptable safety profile; moreover, in patients presenting PD-L1 expression $\geq 5\%$ the ORR was higher (27%) and the survival benefit longer compared to the PD-L1 ≥ 1 and $< 5\%$ cohort and the PD-L1 $< 1\%$ group. On the basis of these promising findings, the role of atezolizumab was further assessed in the confirmatory phase III, open-label, randomized IMvigor211 trial [40], which compared atezolizumab to chemotherapy by investigators' choice, including vinflunine and taxanes, in patients who recurred or progressed after a platinum-based regimen. The primary endpoint, OS in patients with PD-L1 expression $\geq 5\%$, did not significantly differ between the two arms, with a median OS of 11.1 and 10.6 months in atezolizumab and chemotherapy arms, respectively (HR 0.87; 95% CI 0.63–1.21; $p = 0.41$). Despite the negative primary endpoint, IMvigor211 provided useful data in terms of median duration of response, which was significantly higher in the ICI arm (15.9 vs. 8.3 months; HR 0.57; 95% CI 0.26–1.26) and in terms of toxicity, with the PD-L1 inhibitor confirming a manageable safety profile.

Finally, the exploratory analysis of the intention to treat population showed a survival benefit for atezolizumab (HR 0.85; 95% CI 0.73–0.99).

Nivolumab is a human monoclonal IgG4 antibody that blocks the human PD-1 receptor, whose efficacy in the post-platinum setting was explored in the CheckMate 275 trial [41]; in this phase II trial, nivolumab (240 mg flat dose every two weeks) showed an ORR of 20% with 2% CR in 270 patients affected by advanced or metastatic UC. With regard to PD-L1 expression, ORR was significantly higher in the subgroup of patients with PD-L1 expression $\geq 5\%$ (28.4%) compared to the PD-L1 $\geq 1\%$ (23.8%) and the PD-L1 negative (16.1%) cohorts.

A similar level of activity was observed with post-platinum avelumab and durvalumab in the multicohort phase Ib JAVELIN trial [42] and the single-arm, phase I/II Study 1108 [43], respectively. Avelumab (10 mg/kg every two weeks), an anti-PD-L1 antibody that blocks the binding of PD-L1 to PD-1, reported an ORR of 17% with 6% CR in platinum-refractory or cisplatin unfit patients; interestingly, in PD-L1 negative subgroup ORR fell to 9% while reached the 40% in PD-L1 $\geq 5\%$ patients.

Similarly, considering the cutoff of 25% of PD-L1 expression (assessed with immunohistochemistry (IHC) on tumor tissue via Ventana SP263 assay) in Study 1108, the subgroup of patients with PD-L1^{high} achieved higher response rates and survival benefit compared to PD-L1^{low} cohort (20 vs. 8 months) with the anti-PD-L1 human IgG1 durvalumab [43]. In Study 1108, patients were administered durvalumab intravenous infusion, 10 mg/kg every 2 weeks.

Additional data from a number of ongoing prospective clinical trials will help to confirm the activity of ICIs in previously treated and untreated patients [44]; in the era of precision, tailor-made oncology, several questions are still unanswered, including the identification of predictive biomarkers, sequential treatment strategies, and proper selection of patients in advanced or metastatic UC. A non-negligible unanswered question is how to assess PD-L1 expression. For example, in KEYNOTE-052 and IMvigor210 PD-L1 cutoff was different and it was assessed differently; in KEYNOTE-052 PD-L1 positive tumors were those presenting a CPS $\geq 10\%$ and PD-L1 expression in formalin-fixed, paraffine-embedded tissue was determined using the PD-L1 clinical trial assay (PD-L1 IHC 22C3 pharmDx assay; Agilent Technologies, Carpinteria, CA, USA). Differently, in the IMvigor210 trial the VENTANA SP142 immunohistochemistry assay (Ventana Medical Systems, Inc.; Tucson, AZ, USA) was used to evaluate PD-L1 expression on tumor-infiltrating immune cells (IC) and a scoring criteria designated tumors as IC0, IC1, or IC2/3 (PD-L1 expression on $<1\%$, $\geq 1\%$ and $<5\%$, or $\geq 5\%$ of IC, respectively).

2.2. Target Therapies

In the recent years, genomic characterization of advanced-stage UC has given an insight on which are molecular drivers at the basis of the oncogenesis and progression of UC and that could be potentially targetable (Figure 2) [45]. The Cancer Genome Atlas (TCGA) project for bladder cancer had the purpose to provide a comprehensive landscape of molecular alterations [46]. The first integrated analysis on 131 UC demonstrated statistically significant recurrent mutations in 32 genes. Furthermore, this analysis showed that 69% of the tumors presented potential therapeutic targets, of which 42% regarded the phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway and 44% in the receptor tyrosine kinase/MAPK pathway, and identified an in-frame activating *FGFR3-TACC3* fusion in three tumors [46]. Alterations in the PI3K/AKT/mTOR pathway consisted in point mutations in *PIK3CA* (17%), mutation or deletion of *TSC1* or *TSC2* (9%), and overexpression of *AKT3* (10%). Alterations in the receptor tyrosine kinase/RAS pathway included activation of *FGFR3* (17%), amplification of *EGFR* (9%), mutations of *ERBB3* (6%), and mutation or amplification of *ERBB2* (9%).

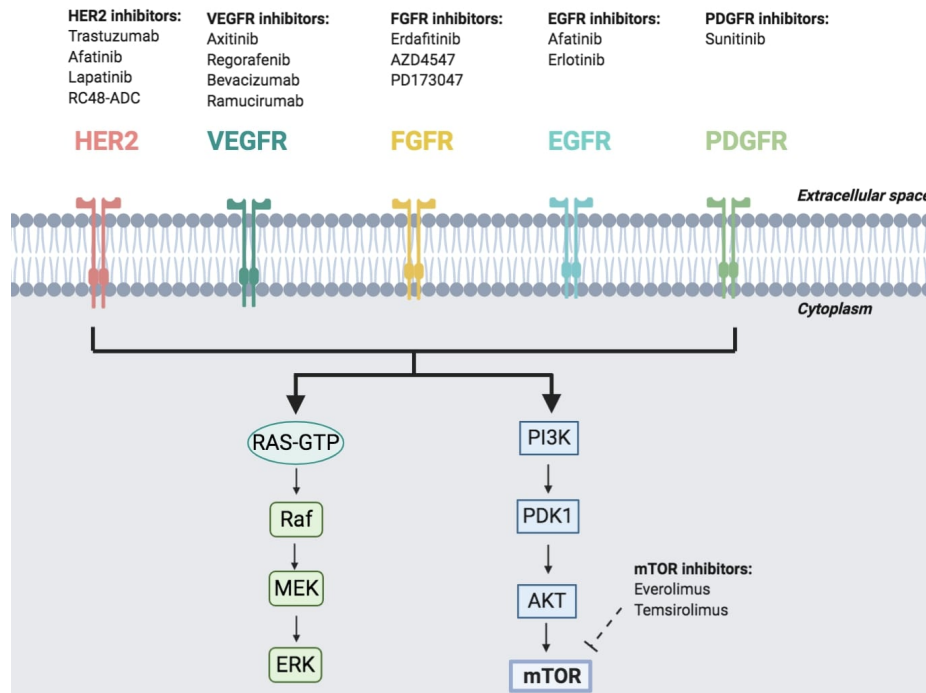


Figure 2. Frequent potentially actionable mutations and pathways involved in UC. EGFR: epidermal growth factor receptor; ERK: mitogen-activated protein kinase; FGFR: fibroblast growth factor receptor; HER2: receptor tyrosine-protein kinase ERBB2; MAPK: mitogen-activated protein kinase; MEK: dual-specificity mitogen-activated protein kinase; mTOR: mammalian target of rapamycin; PDGFR: platelet-derived growth factor receptor; PI3K: phosphoinositide 3-kinase; PTEN: phosphatase and tensin homologue; VEGFR: vascular endothelial growth factor receptor.

The TCGA expanded cohort analysis on 412 MIBC that identified 58 significantly mutated genes and confirmed the high mutation rate of MIBC [47]. Moreover, RNA expression analysis identified five expression subtypes that may stratify response to different treatments: luminal-papillary (35%), luminal (6%), basal-squamous (35%), luminal-infiltrated (19%), and neuronal (5%) [47]. Recently, a consensus molecular classification of MIBC has been proposed on the basis of 1750 MIBC transcriptomic profiles from 18 datasets comparing six molecular classification schemes. Six molecular classes were identified: luminal papillary, luminal nonspecified, luminal unstable, stroma-rich, basal/squamous, and neuroendocrine-like [48]. This consensus classification has possible therapeutic implications. In fact, the different consensus classes are associated with different stromal components and genetic alteration that could possibly identify a subset of patients more likely to respond to immunotherapy or to target therapy. The identification of molecular alterations is of great importance since many target therapies are being studied for the management of advanced UT [49].

FGFR1, FGFR2, FGFR3, FGFR4 are tyrosine kinases receptor that have been found altered in UC [50]. Activating FGFR3 mutations are most common in NMIBC, being identified in approximately two-third of these early stage tumors, while their frequency in MIBC is lower (less than 25%), including amplifications, mutations, and fusions in FGFR gene [51–54]. The activating FGFR3 mutation leads to ligand-independent receptor dimerization and constitutive downstream signal transduction [54]. The presence of activating point mutations in FGFR3 in early stage tumors is associated with favorable outcome [55]. Approximately 7% of UC present an amplification of FGFR1 [56]. FGFR1 has two splicing variants, FGFR1 α and FGFR1 β , that are equally expressed in normal urothelium, but the FGFR1 β variant is predominant in UC and its expression correlates with tumor grade and stage [57]. The luminal-papillary subtype of the consensus classification is characterized by a high rate of FGFR3 mutations and translocations, suggesting that these tumors may respond to FGFR inhibitors [48]. Moreover, FGFR3 pathway was found to be activated in non-T-cell-inflamed

tumors that are likely to present intrinsic resistance to ICIs [58]. Furthermore, immunotherapy seems to be less effective on TCGA luminal I subtype also based on an exploratory analysis of a phase 2 trial: luminal I cluster presented lower expression levels of CD8+ genes, lower PD-L1 immune cell or tumor cell expression, and lower responses to the anti-PD-L1 atezolizumab [39].

With these premises, multi-tyrosine kinase inhibitors targeting FGFR alterations have been studied in patients with metastatic UC [59]. The results of a phase 2 trial (BLC2001) testing the tyrosine kinase inhibitor of FGFR1–4 erdafitinib have been recently published [60]. In this trial, 99 patients with locally advanced or metastatic UC with FGFR3 mutation or FGFR2/3 fusion and progressed to at least one previous chemotherapy or treatment naïve if cisplatin ineligible were assigned to receive erdafitinib, 8 mg per day in a continuous regimen. The primary endpoint of the study was ORR. The treatment was found to be active with an ORR of 40% (3% with a complete response and 37% with a partial response). The median duration of progression-free survival (PFS) was 5.5 months and the median duration of OS was 13.8 months. Interestingly, the 22 patients previously treated with ICIs presented a response rate of 59%. Grade 3 or higher treatment-related adverse events were reported in nearly half the patients and the most common of any grade were hyperphosphatemia, stomatitis, diarrhea. FDA granted accelerated approval to erdafitinib for patients with FGFR3 or FGFR2 genetic alterations progressed during or following platinum-containing chemotherapy, including within 12 months of neoadjuvant or adjuvant platinum-containing chemotherapy.

Another pathway implicated in UC pathogenesis and progression is vascular endothelial growth factor receptors (VEGFR) 1 and 2 and their ligands (vascular endothelial growth factor, VEGF-A, -B, -C, and -D) [61,62]. Angiogenesis by microvessel quantification resulted to be an independent predictor of survival in patients with invasive bladder cancer and serum levels of VEGF have been correlated with tumor stage and grade, vascular invasion and presence of metastases [63–65].

VEGF/VEGFR inhibitors as single agents or in combination with chemotherapy have been investigated for the treatment of advanced UC. Single agent treatment with sorafenib, pazopanib, cabozantinib and sunitinib resulted to have limited activity and limited effect on clinical outcomes [66–69]. Similarly, combination therapies failed to be shown to be more active than chemotherapy alone: vandetanib combined with docetaxel or sunitinib associated with gemcitabine and cisplatin did not improve clinical activity and were more toxic [70,71].

The monoclonal antibody against VEGF Bevacizumab was evaluated in a phase II trial in association with gemcitabine and cisplatin in first line of therapy for metastatic UC: the combination treatment showed an ORR of 72% and an OS of 19.1 months [72]. Unfortunately, the subsequent phase III trial (CALGB-90601 Alliance) failed to show an advantage in OS, the primary endpoint of the study, for the combination regimen [73].

A phase III randomized trial investigated the combination of ramucirumab plus docetaxel versus placebo plus docetaxel in 530 patients with advanced or metastatic UC progressed during or after platinum-based chemotherapy [74]. The experimental arm was associated with a significantly longer PFS (4.07 months versus 2.76 months in the docetaxel-alone arm) with no OS benefit. Additional follow-up confirmed the advantage in PFS (4.1 months versus 2.8 months in the experimental arm versus the control arm, respectively; HR 0.696; $p = 0.0002$) and the lack of statistically significant advantage in OS for the combination treatment (9.4 months in the experimental arm versus 7.9 months in the placebo group; stratified HR 0.887; $p = 0.25$) [75].

2.3. Antibody-Drug Conjugates

Another interesting emerging class for the treatment for metastatic UC is antibody-drug conjugate (ADC), that consists in monoclonal antibody against a target expressed on cancer cell bounded to a cytotoxic agent with a protease-cleavable or non-cleavable linker [76]. When the monoclonal antibody binds to a tumor antigen, the drug is internalized and the active chemotherapeutic agent is released into the selected cells, leading to cell death. This mechanism of cell-killing is supposed to limit exposure and toxicity of cytotoxic agents. One of the most promising

antibody-drug conjugate currently under investigation in metastatic UC is enfortumab vedotin (ASG-22ME). This ADC is composed of an anti nectin-4 (a cell adhesion molecule highly expressed in UC) monoclonal antibody linked to a micro-tubule-disrupting agent (monomethyl auristatin E). The phase 1 EV-101 trial evaluated enfortumab vedotin in patients with Nectin-4-expressing solid tumors, including 155 heavily pretreated patients with metastatic UC [77]. Single-agent enfortumab vedotin resulted to be well tolerated and showed clinically meaningful and durable responses with an ORR of 43%, a duration of response of 7.4 months, a median OS of 12.3 months, and OS rate at 1 year of 51.8%.

The phase II EV-201 single-arm study investigated enfortumab vedotin in locally advanced or metastatic UC patients previously treated with ICI and platinum-containing chemotherapy (Cohort 1) or an ICI and no prior chemotherapy (Cohort 2) [78]. The preliminary data of cohort 1 enrolling 128 patients have been presented and showed an ORR of 42% with 9% complete responses. The safety profile was manageable with fatigue (50%), alopecia (48%), and decreased appetite (41%) as most common treatment-related adverse events. Of note, one death was reported as treatment related by the investigator (interstitial lung disease). Based on these results, the FDA granted accelerated approval to enfortumab vedotin for patients with locally advanced or metastatic urothelial cancer who have previously received a PD-1/PD-L1 inhibitor and a platinum-containing chemotherapy in the neoadjuvant/adjuvant, locally advanced or metastatic setting. A phase III trial evaluating enfortumab vedotin in patients progressed to previous ICI and platinum containing chemotherapy is ongoing (NCT03474107, EV-301).

Preliminary data for the combination of enfortumab vedotin with pembrolizumab for first line treatment of cisplatinum ineligible patients with metastatic UC are encouraging. The phase Ib study EV-103 (NCT03288545) demonstrated the efficacy of this combination approach in this subset of patients with a tolerable and manageable safety profile [79,80]. At the recent 2020 American Society of Clinical Oncology (ASCO) Genitourinary Cancer Symposium, the updated results were presented by Rosenberg: at a median follow-up of 11.5 months, investigator-assessed objective response rate was confirmed to be 73.3%, with 15.6% complete responses [81]. The most common adverse events were fatigue (58%), alopecia (53%), and neuropathy (53%). A phase III trial with this combination therapy is ongoing (NCT04223856, EV-302).

Another ADC that has been evaluated in metastatic UC is Sacituzumab govitecan, a humanized anti-Trop-2 (an epithelial cell surface antigen overexpressed in UC) monoclonal antibody linked with SN-38 (the active metabolite of irinotecan). Sacituzumab govitecan has been investigated in a phase I/II basket study in 45 patients progressed after at least one prior systemic therapy [82]. The ORR was 31%, including 2 CR and 12 PR. In patients with visceral involvement the ORR was 27% and in patients previously treated with ICIs it was 23%. Median PFS and OS were 7.3 months and 18.9 months, respectively. Among grade ≥ 3 adverse events there were neutropenia/neutrophil count decreased (38%), anemia (11%), hypophosphatemia (11%), diarrhea (9%), fatigue (9%), and febrile neutropenia (7%). A global, single-arm, phase II trial which is ongoing (TROPHY-U-01, NCT03547973) is evaluating the antitumor activity of Sacituzumab govitecan (10 mg/kg, days 1 and 8 of 21-day cycles) in patients with advanced UC. Cohort 1 [83] assessed the activity in 35 patients progressed to platinum-based regimens and ICIs while cohort 2 [84] enrolled 18 platinum-ineligible patients who progressed after first-line ICI. The interim results of cohort 1 demonstrated an ORR of 29% with 2 confirmed CR, 5 confirmed PR, and 3 unconfirmed PR. The preliminary results of cohort 2 showed an ORR of 28% with 4 confirmed PRs, and 1 PR pending confirmation. The safety profile was consistent with prior reports in both cohorts no treatment-related deaths were reported.

The evolution of practice changing treatments, including promising therapies approved by FDA, for metastatic UC is depicted in Figure 3. Current treatment scenario in metastatic UC is reported in Figure 4.

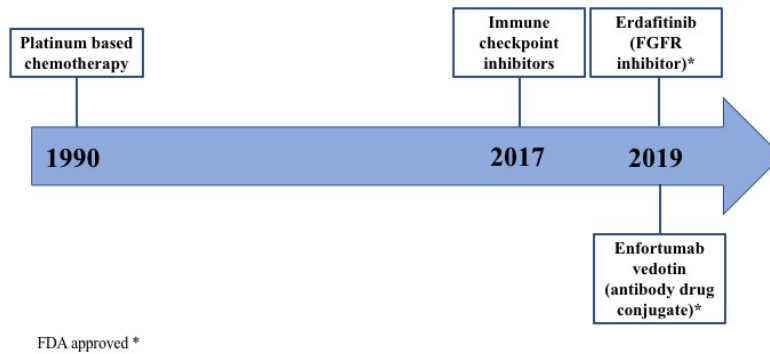


Figure 3. Evolution of practice changing treatments for metastatic urothelial carcinoma. For many years, platinum-based chemotherapy has been the gold standard treatment for patients with metastatic urothelial carcinoma. Since 2017, immune checkpoint inhibitors entered in the treatment scenario. In 2019, two new treatment strategies showed promising results and have been granted accelerated approval by the US Food and Drug Administration: erdafitinib and enfortumab vedotin.

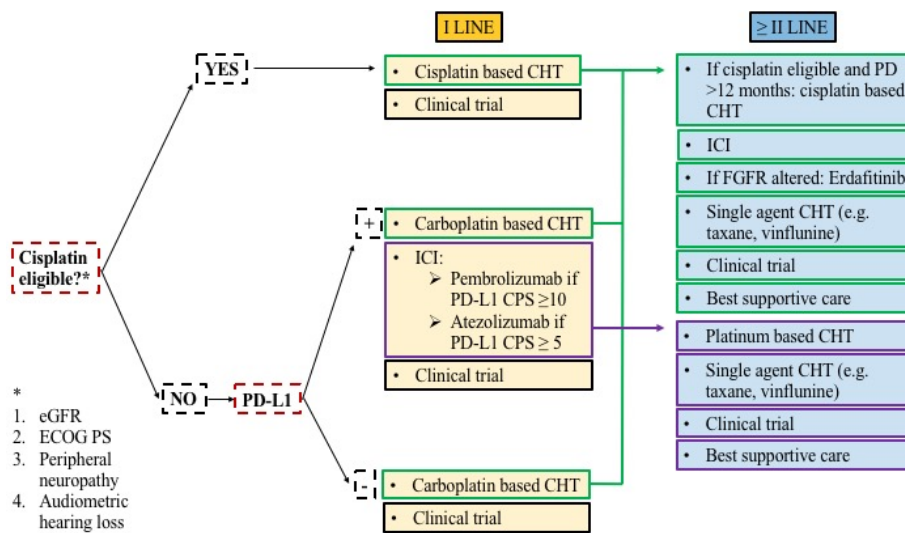


Figure 4. Current treatment algorithm for metastatic urothelial carcinoma. Based on cisplatin eligibility and PD-L1 positivity, patients are currently being treated as indicated in the figure. If cisplatin eligible (depending on eGFR, ECOG PS, peripheral neuropathy, audiometric hearing loss) patients should be treated with cisplatin-based chemotherapy. In cisplatin ineligible patients, the treatment changes according to PD-L1 positivity. If PD-L1 negative, patients should be treated with carboplatin-based chemotherapy. If PD-L1 positive, carboplatin-based chemotherapy or immunotherapy are the available options. Second and later lines of treatment depend on previous exposure to chemotherapy or immunotherapy. Enrollment in clinical trials should always be considered as a treatment option. CHT: chemotherapy. ICI: immune checkpoint inhibitor. PD: progressive disease. FGFR: fibroblast growth factor receptor. CPS: combined positive score. PD-L1: programmed death ligand-1. eGFR: estimated glomerular filtration rate. ECOG PS: eastern cooperative oncology group performance status.

3. Therapeutic Approaches UNDER Evaluation

3.1. Ongoing Trials Evaluating ICIs

3.1.1. Combination of ICIs with Cytotoxic Chemotherapy

Several trials exploring the role of ICIs plus chemotherapy in different settings and combinations are currently ongoing. As regards the first-line setting, the randomized phase III KEYNOTE-361 trial (NCT02853305) is investigating the safety and efficacy of front-line pembrolizumab with or without chemotherapy (GC in eligible patients or gemcitabine–carboplatin combination in cisplatin unfit subjects) [85]. OS and PFS are the primary endpoints of this study, with ORR and safety assessed as secondary endpoints. Similarly, the phase III IMvigor130 trial (NCT02807636) has enrolled previously untreated patients affected by advanced or metastatic UC in a 1:1:1 ratio to either atezolizumab plus platinum-gemcitabine, atezolizumab monotherapy, or platinum-gemcitabine plus placebo [86]. The primary endpoints are OS and PFS; secondary endpoints are safety, ORR and DCR. As previously stated, preliminary findings of these two trials showing a close relation between PD-L1 expression, type of treatment and clinical outcomes have had a relevant impact on current indications of pembrolizumab and atezolizumab in UC. More specifically, FDA revised previous indications for the two ICIs, which are now limited for (1) first-line treatment in cisplatin-ineligible patients whose tumors express PD-L1 (CPS \geq 10% for pembrolizumab and PD-L1 stained tumor-infiltrating immune cells covering \geq 5% of the tumor area in the case of atezolizumab), (2) subjects which have disease progression during or following platinum-containing chemotherapy, or (3) patients unfit for any platinum-based chemotherapy, regardless of PD-L1 expression.

The anti-PD-L1 agent atezolizumab is being also investigated in a phase II trial (NCT03093922) aimed to evaluate the safety and efficacy of two different dosing schedules of atezolizumab in combination with GC as front-line treatment for advanced or metastatic UC. Regarding less commonly used ICIs, a randomized, placebo-controlled, phase III trial (NCT03967977) has been initiated to investigate the safety and efficacy of front-line tislelizumab plus standard chemotherapy (gemcitabine plus either cisplatin or carboplatin) versus placebo plus standard chemotherapy (gemcitabine plus either cisplatin or carboplatin). Tislelizumab (BGB-A317) is a humanized monoclonal PD-1 antibody which is being evaluating in several solid tumors [87].

With regard to second-line setting, atezolizumab is currently under evaluation in cisplatin-ineligible patients in an ongoing phase II trial (NCT03737123). In this study, subjects who previously received sequential or concurrent ICI and carboplatin-based chemotherapy will be treated with atezolizumab plus docetaxel combination; conversely, patients who have already received an ICI without prior platinum-based chemotherapy will be treated with atezolizumab plus carboplatin-gemcitabine.

Another anti-PD-L1 agent, avelumab, is being investigated in phase II trial on previously untreated, cisplatin-ineligible patients (NCT03390595). In this study, patients are randomized in a 1:1 ratio to receive avelumab in combination with carboplatin-gemcitabine chemotherapy versus carboplatin-gemcitabine alone. Avelumab is also under investigation in a phase II trial comparing avelumab plus GC versus GC in cisplatin fit, treatment naïve patients (NCT03324282). PT-112, a platinum-based agent belonging to the phosphaplatin family, is under evaluation in combination with avelumab in the ongoing phase I/II PAVE-1 trial (NCT03409458).

The combination of the anti-PD-1 antibody pembrolizumab with paclitaxel is currently under investigation in a phase II trial (NCT02581982) on platinum-refractory patients. Lastly, several other combinations of anti-PD-1/PD-L1 agents with cytotoxic agents such as pemetrexed, platinum, and etoposide are being evaluated in a series of ongoing trials (NCT03744793; NCT03582475).

Ongoing phase I/I/II trials, either recruiting or active not recruiting, of ICIs in combinations with cytotoxic chemotherapy in advanced or metastatic UC are summarized in Table 1.

Table 1. Ongoing phase I/II/III trials of immune checkpoint inhibitors in combination with cytotoxic chemotherapy. ORR: overall response rate. PFS: progression-free survival. OS: overall survival.

NCT (Clinicaltrials.gov)	Phase	Setting	Cisplatin Fit/Unfit	Arm A	Arm B	Arm C	Compounds Description	Number of Patients	Primary Outcome	Status	Estimated Study Completion Date
NCT03409458 (PAVE-1)	Ib/IIa	First- or later-line	All	Avelumab + PT-112			PT-112: platinum- based agent belonging to the phosphaplat in family	52	Recommen- ded dose of PT-112 to be used with avelumab	Recrui- ting	May 2020
NCT02437370	I	Second- or third-line	All	Pembrolizum ab + docetaxel	Pembrolizumab + gemcitabine		Pembrolizu- mab: anti-PD-1	38	Safety	Recrui- ting	December 2020
NCT03582475	I	First- or later-line	All	Pembrolizum ab + etoposide + cisplatin (or carboplatin)			Pembrolizu- mab: anti-PD-1	30	Durable response rate ORR Duration of response PFS OS Safety	Recrui- ting	September 2021
NCT02853305 (KEYNOTE-361)	III	First-line	All	Pembrolizum ab	Pembrolizumab + gemcitabine + cisplatin (or carboplatin)	Placebo + gemcitabine + cisplatin (or carboplatin)	Pembrolizu- mab: anti-PD-1	990	PFS OS	Active , not recruit- ing	May 2020
NCT02807636 (IMvigor130 trial)	III	First-line	All	Atezolizumab	Atezolizumab + gemcitabine +	Placebo + gemcitabine +	Atezolizum ab:	1200	PFS OS	Active , not	November 2020

					cisplatin (or carboplatin)	cisplatin (or carboplatin)	anti-PD-L1		Safety	recruiting	
NCT03093922	II	First-line	All	Atezolizumab + gemcitabine + cisplatin	Atezolizumab + gemcitabine + cisplatin (modified schedule)	Atezolizumab + gemcitabine + cisplatin (modified schedule)	Atezolizumab: anti-PD-L1	74	ORR	Recruiting	March 2021
NCT03967977	III	First-line	All	Tislelizumab + gemcitabine + cisplatin (or carboplatin)	Placebo + gemcitabine + cisplatin (or carboplatin)		Tislelizumab: humanized monoclonal PD-1 antibody	420	OS	Recruiting	July 2022
NCT03737123	II	Second-line (no prior platinum chemotherapy)	Cisplatin ineligible	Atezolizumab + chemotherapy (docetaxel or gemcitabine + carboplatin)			Atezolizumab: anti-PD-L1	33	PFS	Recruiting	January 2022
NCT03390595	II	First-line	Cisplatin ineligible	Avelumab + gemcitabine + carboplatin	Gemcitabine + carboplatin		Avelumab: anti-PD-L1	85	ORR	Active, not recruiting	August 2020
NCT03324282	II	First-line	All	Avelumab + gemcitabine + cisplatin	Gemcitabine + cisplatin		Avelumab: anti-PD-L1	90	ORR Safety	Recruiting	December 2022
NCT02581982	II	Second- or later-line	All	Pembrolizumab + paclitaxel			Pembrolizumab: anti-PD-1	27	ORR	Recruiting	April 2020
NCT03744793	II	Second-line or third-line	All	Avelumab + pemetrexed			Avelumab: anti-PD-L1	25	ORR	Recruiting	January 2021
NCT03575013	I	Second- or later-line	All	Avelumab + paclitaxel			Avelumab: anti-PD-L1	21	Safety	Recruiting	May 2020

3.1.2. Combination of ICIs with Other ICIs

In recent years, checkpoint-inhibition combination therapies have provided outstanding efficacy gains in several malignancies including melanoma, lung cancer and renal cell carcinoma [88,89]. The underlying rationale for these combinations lies in the synergistic effect provided by the inhibition of CTLA-4 and PD-1/PD-L1, resulting in an enhance of T-cell function through distinct pathways [90]. The results obtained in a number of cancer types have led to the recent attempt to translate these experiences in advanced or metastatic UC.

The phase 1/2 CheckMate-032 trial investigated ipilimumab plus nivolumab versus nivolumab alone in several malignancies, including platinum-refractory patients affected by advanced or metastatic UC [91,92]. In this cohort of subjects, the combination of the two immunotherapies yielded a promising response rate of 38%; moreover, subjects treated with the combination showed a median OS of 15.3 months versus 9.9 months in the nivolumab arm. The combination of an anti-PD-1 and a CTLA-4 antibody is being investigated also in the CheckMate-901 trial (NCT03036098) [93], aimed to evaluate the efficacy of nivolumab ± ipilimumab versus GC or carboplatin-gemcitabine chemotherapy. The same combination with modified schedules and additional nivolumab/ipilimumab “boost” cycles is under evaluation also in a Phase II trial (NCT03219775, TITAN-TCC) on treatment naïve and platinum-refractory patients with advanced or metastatic UC.

The anti-PD-L1 agent durvalumab, registered by FDA as monotherapy in previously treated patients affected by advanced or metastatic UC, is currently under investigation in combination with the CTLA-4 IgG2-kappa monoclonal antibody tremelimumab in the DANUBE (NCT02516241) and the NILE (NCT03682068) trials [94,95]. The DANUBE is an ongoing randomized, open-label, phase III trial aimed at ascertaining the value of front-line durvalumab ± tremelimumab versus platinum-gemcitabine chemotherapy in advanced or metastatic UC [90]. In the same setting of previously untreated patients, the NILE trial randomized subjects to three different cohorts: durvalumab plus tremelimumab plus platinum-gemcitabine; durvalumab plus platinum-gemcitabine; platinum-gemcitabine [95].

Ongoing phase II/III trials, either recruiting or active not recruiting, of ICIs in combinations with other ICIs in advanced or metastatic UC are summarized in Table 2.

Table 2. Ongoing phase II/III trials of immune checkpoint inhibitor combined with other immune checkpoint inhibitors. ORR: overall response rate. PFS: progression-free survival. OS: overall survival.

NCT (Clinicaltrials.gov)	Phase	Setting	Cisplatin Fit/Unfit	Arm A	Arm B	Arm C	Compounds Description	Number of Patients	Primary Outcome	Status	Estimated Study Completion Date
NCT03682068 (NILE)	III	First-line	All	Durvalumab + gemcitabine + cisplatin (or carboplatin)	Durvalumab + tremelimumab + gemcitabine + cisplatin (or carboplatin)	Gemcitabine + cisplatin (or carboplatin)	Durvalumab: anti-PD-L1. Tremelimumab: anti-CTLA-4.	885	PFS OS	Recruiting	April 2022
NCT03036098 (CheckMate-901)	III	First-line	All	Nivolumab + ipilimumab	Gemcitabine + cisplatin (or carboplatin)	Nivolumab + gemcitabine + cisplatin (or carboplatin)	Nivolumab: anti-PD-1. Ipilimumab: anti-CTLA-4	990	OS in cisplatin ineligible OS in PD-L1 \geq 1%	Recruiting	December 2022
NCT03219775, (TITAN-TCC)	II	First- or later-line	All	Nivolumab + ipilimumab			Nivolumab: anti-PD-1. Ipilimumab: anti-CTLA-4.	80	ORR	Recruiting	December 2020
NCT02516241 (DANUBE)	III	First-line	All	Durvalumab + tremelimumab	Durvalumab	Gemcitabine + cisplatin (or carboplatin)	Durvalumab: anti-PD-L1. Tremelimumab: anti-CTLA-4.	1126	OS	Active, not recruiting	May 2020
NCT03430895	II	First- or later-line	All	Durvalumab + tremelimumab			Durvalumab: anti-PD-L1. Tremelimumab: anti-CTLA-4.	15	ORR	Active, not recruiting	January 2021

3.1.3. Combination of ICIs with Antiangiogenic Agents

Given the importance of angiogenesis as a crucial process in the carcinogenesis and progression of UC, ICIs are under evaluation also in combination with VEGFR antibodies and TKIs, including bevacizumab, ramucirumab, lenvatinib, and several others [96,97].

As regards front-line treatment, the VEGF-A monoclonal antibody bevacizumab is being investigated in a phase II trial assessing bevacizumab plus atezolizumab in treatment-naïve, cisplatin-ineligible patients (NCT03272217).

Axitinib, a highly selective VEGFR-1, -2, and -3 inhibitor, is currently under investigation as front-line treatment in combination with avelumab in the ongoing phase II trial JAVELIN Medley VEGF (NCT03472560). Enrolled subjects are deemed ineligible for receiving cisplatin-containing first-line chemotherapy and the primary endpoint is ORR, defined as a confirmed CR or PR.

Ramucirumab is an IgG1 monoclonal antibody that binds VEGFR-2 preventing ligand binding and receptor-mediated pathway activation in endothelial cells [98]. An ongoing, phase I trial is assessing the safety of ramucirumab in combination with pembrolizumab in previously treated patients affected by a number of solid cancers, including UC (NCT02443324).

Lenvatinib is a small TKI able to inhibit VEGFR-1, FGFR1–4, stem cell factor receptor (KIT), platelet-derived growth factor receptor α (PDGFR α), and rearranged during transfection (RET) [99]. The combination of lenvatinib and pembrolizumab is being investigated as front-line treatment in the phase III LEAP-011 trial (NCT03898180) which is evaluating the combination in cisplatin-unfit subjects with PD-L1 CPS ≥ 10 or in patients deemed ineligible for any platinum-based regimen, regardless of PD-L1 expression.

Cabozantinib is another small TKI inhibiting a plethora of targets which play an important role in tumor growth, angiogenesis, and survival, such as VEGFR-2, MET, RET, KIT, AXL, and FLT3 [100,101]. Following the findings of a recent phase I trial where cabozantinib plus nivolumab plus ipilimumab yielded an ORR of 36% across all genitourinary cancers [102], this molecule is being evaluated in combination with pembrolizumab (NCT03534804), durvalumab (NCT03824691), atezolizumab (NCT03170960), and nivolumab plus ipilimumab (NCT03866382) in treatment-naïve and previously treated patients. Moreover, cabozantinib is also under investigation in a phase II trial (NCT04066595) which is enrolling previously treated subjects with platinum-based chemotherapy (cohort 1) and platinum-based chemotherapy plus ICIs (cohort 2).

The anti-VEGF recombinant EphB4-HSA fusion protein is currently under evaluation in combination with pembrolizumab in an ongoing phase II trial (NCT02717156). The study is enrolling treatment naïve patients affected by locally advanced or metastatic UC.

Apatinib, a small-molecule TKI which selectively inhibits VEGFR-2 resulting in a decrease in endothelial proliferation, migration, and tumor microvascular density, is under evaluation in combination with pembrolizumab in a phase I/IIa trial (NCT03407976; APPEASE). In this study, eligible subjects must have progressed during or following platinum-based chemotherapy.

Lastly, sitravatinib, a small TKI able to inhibit VEGFR, PDGFR, KIT, RET, and MET [103], is currently under investigation in combination with nivolumab in a non-randomized, Phase II trial (NCT03606174). Although all patients are planned to receive the same treatment (nivolumab 240 mg every 2 weeks or 480 mg every 4 weeks plus sitravatinib 120 mg orally once per day continuously in 28-day cycles), eligible subjects are assigned to eight different cohorts, based upon previous therapies for UC.

Ongoing phase I/II/III trials, either recruiting or active not recruiting, of ICIs in combinations with antiangiogenic agents in advanced or metastatic UC are summarized in Table 3.

Table 3. Ongoing phase I/I/II trials of combinations between immune checkpoint inhibitors with antiangiogenic agents. ORR: overall response rate. PFS: progression-free survival. OS: overall survival.

NCT (Clinicaltrials.gov)	Phase	Setting	Cisplatin Fit/Unfit	Arm A	Arm B	Arm C	Compounds Description	Number of Patients	Primary Outcome	Status	Estimated Study Completion Date
NCT02496208	I	Second- or later-line	All	Nivolumab + cabozantinib	Nivolumab + ipilimumab + cabozantinib		Nivolumab: anti-PD-1. Ipilimumab: anti-CTLA-4. Cabozantinib: tyrosine kinase inhibitor	152	Safety	Recruiting	September 2020
NCT02443324	I	First- or later-line	All	Pembrolizumab + ramucirumab			Ramucirumab: anti-VEGF. Pembrolizumab: anti-PD-1.	155	Safety	Active, not recruiting	November 2020
NCT03170960	I/II	First- or later-line	All	Atezolizumab + cabozantinib			Atezolizumab: anti-PD-L1 Cabozantinib: tyrosine kinase inhibitor	1723	Safety ORR	Recruiting	December 2021
NCT01552434	I	Second- or later-line	All	Temsirolimus + bevacizumab + cetuximab	Temsirolimus + bevacizumab + valproic acid	Temsirolimus + bevacizumab	Temsirolimus: mTOR inhibitor. Bevacizumab: anti-VEGF. Cetuximab: anti-EGFR.	216	Safety	Recruiting	March 2021
NCT03407976 (APPEASE)	I/IIa	Second- or later-line	All	Pembrolizumab + apatinib			Apatinib: tyrosine kinase inhibitor. Pembrolizumab: anti-PD-1.	119	Safety ORR	Active, not recruiting	June 2023
NCT03170960	I/II	Second- or later-line (with prior ICI)	All	Expansion Cohort 2, 3, 4, 5: atezolizumab plus cabozantinib	Expansion cohort 19: cabozantinib		Atezolizumab: anti-PD-L1. Cabozantinib: tyrosine kinase inhibitor.	1732	Safety ORR	Recruiting	December 2021
NCT03272217	II	First-line	Cisplatin ineligible	Atezolizumab + bevacizumab			Bevacizumab: anti-VEGF	70	OS	Recruiting	June 2021
NCT03472560	II	Second- or	Cisplatin	Avelumab +			Avelumab:	61	OR	Active,	September

(JAVELIN Medley VEGF)		later-line	ineligible	axitinib		anti-PD-L1. Axitinib: tyrosine kinase inhibitor.			not recruiting	2020
NCT03898180 (LEAP-011 trial)	III	First-line, PD-L1 \geq 10%	Cisplatin ineligible	Pembrolizumab + lenvatinib	Pembrolizumab + placebo	Lenvatinib: tyrosine kinase inhibitor	694	PFS OS	Recruiting	December 2022
NCT03534804 (PemCab)	II	First-line	Cisplatin ineligible	Pembrolizumab + cabozantinib		Cabozantinib: tyrosine kinase inhibitor	39	ORR	Recruiting	September 2023
NCT03824691 (ARCADIA)	II	Second-line or third-line	All	Durvalumab + cabozantinib		Durvalumab: anti-PD-L1. Cabozantinib: tyrosine kinase inhibitor.	122	OS	Recruiting	February 2023
NCT03866382	II	First- or later-line	All	Nivolumab + ipilimumab + cabozantinib		Nivolumab: anti-PD-1. Ipilimumab: anti-CTLA-4. Cabozantinib: tyrosine kinase inhibitor.	186	ORR	Recruiting	February 2023
NCT04066595 (CabUC)	II	Second-line or third-line	All	Cabozantinib		Cabozantinib: tyrosine kinase inhibitor	88	ORR 6-month	Recruiting	September 2024
NCT02717156	II	Second- or later-line	All	Pembrolizumab + EphB4-HSA		EphB4-HSA: A recombinant fusion protein composed of the full-length extracellular domain (soluble) of human receptor tyrosine kinase ephrin type-B receptor 4 and fused to full-length human serum albumin, with potential anti-angiogenic and	60	Safety	Recruiting	November 2021

						antineoplastic activities.				
						Pembrolizumab: anti-PD-1.				
NCT03606174	II	First- or later-line	All	Nivolumab + sitravatinib	Sitravatinib: tyrosine kinase inhibitor able to inhibit VEGFR, PDGFR, KIT, RET and MET. Nivolumab: anti-PD-1.	330	ORR	Recruiting	September 2021	

3.1.4. ICI Monotherapy

Although combination therapies are displaying the ability to broaden the anticancer activity of ICIs and the majority of ongoing trials are testing ICIs in combination with other anticancer agents, some trials are evaluating the role of monotherapy in different settings.

The anti-PD-1 agent pembrolizumab is being evaluated in a randomized, double-blinded phase II trial (NCT02500121) assessing the role of maintenance pembrolizumab (200 mg flat dose every three weeks, for up to 24 months) versus placebo after front-line chemotherapy in patients affected by metastatic UC. Eligible subjects must have achieved CR, PR or stable disease (SD) after 4 to 6 cycles of first-line platinum-based chemotherapy; six-month PFS assessment, regardless of PD-L1 expression, is the primary outcome. Maintenance treatment with ICIs is also under investigation in an ongoing phase III trial (NCT02603432) comparing avelumab maintenance plus best supportive care versus best supportive care alone in patients whose disease did not progress after first-line platinum-based chemotherapy.

Atezolizumab treatment is being tested in the real-world phase III SAUL trial (NCT02928406) and preliminary results of this study assessing the role of atezolizumab in a pretreated population of 1004 UCs have been recently published [104]. Median OS and PFS were 8.7 and 2.2 months respectively, with an ORR of 13%. The trial enrolled patients who experienced progression during or after one to three prior therapies, of which 10% had ECOG-PS 2 and 98% were platinum pretreated.

Toripalimab (JS001), a recombinant, humanized PD-1 monoclonal antibody capable of preventing the binding of PD-1 with PD-L1 and PD-L2, is being evaluated as monotherapy in pretreated advanced or metastatic UC in an ongoing phase II trial (NCT03113266). The primary outcome is ORR, with duration of response, PFS, OS, and safety as secondary outcomes.

The anti-CTLA-4 agent tremelimumab is currently being evaluated as monotherapy in a phase II trial (NCT03557918) assessing ORR in patients with metastatic UC which previously received PD-1/PD-L1 blockade.

The novel anti-PD-L1 CK-301 (Cosibelimab) is being tested in a phase I trial (NCT03212404) on a number of advanced malignancies, including UC. Lastly, a phase I trial (NCT03053466) is studying the role of the anti-PD-1 agent APL-501 in patients affected by advanced solid tumors presenting at least 1% of PD-L1 expression by IHC.

Ongoing phase I/II/III trials, either recruiting or active not recruiting, of ICI monotherapy in advanced or metastatic UC are summarized in Table 4.

Table 4. Ongoing phase I/II/III trials of monotherapy immune checkpoint inhibitors. ORR: overall response rate. PFS: progression-free survival. OS: overall survival.

NCT (Clinicaltrials.gov)	Phase	Setting	Cisplatin Fit/Unfit	Arm A	Arm B	Arm C	Compounds Description	Number of Patients	Primary Outcome	Status	Estimated Study Completion Date
NCT02500121	II	Maintenance after SD, RP or RC to first-line chemotherapy	All	Pembrolizumab	Placebo		Pembrolizumab: anti-PD-1	108	6-month PFS	Active, not recruiting	January 2020
NCT02603432 (JAVELIN Bladder 100)	III	Maintenance after SD, RP or RC to first-line chemotherapy	All	Avelumab	Best supportiv e care		Avelumab: anti-PD-L1	700	OS	Active, not recruiting	June 2021
NCT02928406 (SAUL)	III	Second-, third or fourth- line	All	Atezolizumab			Atezolizumab: anti-PD-L1	1004	Safety	Active, not recruiting	March 2022
NCT03113266	II	Second- or later-line	All	Toripalimab (JS001)			Toripalimab: anti-PD-1	370	ORR	Recruiting	February 2022
NCT03557918	II	Second- or later-line	All	Tremelimumab			Tremelimumab: anti-CTLA-4	28	ORR	Recruiting	July 2021
NCT03212404	I	Second- or later-line (no prior ICI)	All	Cosibelimab (CK-301)			Cosibelimab: anti-PD-L1	500	Safety ORR	Recruiting	December 2021
NCT03053466	I	First- or later-line, PD-L1 \geq 1%	All	APL-501			APL-501: anti-PD-1	114	Safety	Active, not recruiting	December 2021

3.1.5. Novel Immunotherapy Approaches

With the aim to enhance the response to ICIs and other anticancer agents, a number of novel immunomodulatory molecules and brand-new combinations are being evaluated in UC [105,106].

A recently emerging immunotherapeutic target is represented by the indoleamine 2,3-dioxygenase-1 (IDO1), an enzyme playing a crucial role in immunosuppression, angiogenesis, and metastasis [107]; in fact, IDO1 is an immune regulatory enzyme which promotes tryptophan depletion, a mechanism necessary for T-cell survival [108]. More specifically, IDO1 enhances the activity of CD4⁺ T regulatory cells and myeloid-derived suppressor cells and, conversely, is able to suppress CD8⁺ T effector and natural killer (NK) cells [109]. Despite early promising results, the combination of pembrolizumab plus the IDO-1 inhibitor epacadostat came up short against its primary endpoints of OS and PFS; thus, the two trials assessing the role of the anti-IDO-1 ± pembrolizumab in treatment-naïve, cisplatin ineligible subjects (NCT03361865) and in platinum-refractory patients (NCT03374488) arrested recruitment. Currently, the safety of the combination of pembrolizumab plus KHK2455, a long-active selective IDO-1 inhibitor, is being evaluated in an ongoing Phase I study on platinum-refractory patients affected by metastatic UC (NCT03915405).

Another attracting target is represented by the tumor necrosis factor receptor OX40 (CD134) [110,111]; when activated by its ligand OX40L, OX40 is involved in T-cell signaling activation, promoting T-cell survival and enhancing the expression of several molecules such as Bcl-2 anti-apoptotic molecules, cytokines, cyclin A, and cytokine receptor [112]. Therefore, as OX40 may promote proliferation and survival of CD4⁺ and CD8⁺ T cells, immunostimulatory agonistic agents are currently under investigation in several solid malignancies [113]. The OX40 agonist PF-04518600 is being evaluated as monotherapy or in combination with the cytokine modulator utomilumab (PF-05082566)—a monoclonal antibody with agonist activity toward 4-1BB (CD137), a receptor expressed on NK, CD8⁺, and CD4⁺ T cells [114]. Preliminary results of this trial, which includes also a cohort of patients affected by UC, have shown an ORR of 5.4% across all cancer types; nevertheless, the promising 50% of ORR reported in the UC subgroup has led to the NCT03217747 and the Javelin Medley (NCT02554812) ongoing phase I/II trials which are evaluating the OX40 agonist PF-04518600 in combination with ICIs, radiation therapy, utomilumab, and cytotoxic chemotherapy. Finally, the hexavalent OX40 agonist INBRX-106 is currently under investigation as monotherapy or in combination with pembrolizumab for previously treated patients in a phase I trial (NCT04198766).

Other immunotherapeutic strategies currently under investigation include cytokine agonists such as NKTR-214 (bempegaldesleukin)—an IL-2 pathway agonist which targets CD122, a protein expressed in NK and CD8 T cells—ALT-803 and YT107 [115,116]. Following promising early results from a Phase I trial across several solid tumors (NCT02983045, PIVOT-02), NKTR-214 is currently being evaluated in combination with nivolumab in treatment-naïve, cisplatin ineligible patients affected by locally advanced or metastatic UC (NCT03785925, PIVOT-10). Conversely, NKTR-214 is now being investigated in the phase I PROPEL trial (NCT03138889) assessing the combination of atezolizumab plus NKTR-214 in platinum-refractory UC. Similarly, the recombinant human interleukin-7 CYT-107 is under evaluation in combination with atezolizumab versus atezolizumab alone in platinum-refractory UCs (NCT03513952); the IL-15 superagonist ALT-803 is being investigated as combination therapy with pembrolizumab, nivolumab, atezolizumab, or avelumab in previously treated patients (NCT03228667). Finally, an ongoing open-label, non-randomized phase I study (NCT03809624) is testing the role of INBRX-105 in advanced solid tumors. INBRX-105, a next generation bispecific antibody targeting PD-L1 and 4-1BB, blocks inhibitory PD-1/PD-L1 axis and simultaneously activates essential co-stimulatory activity via 4-1BB. Other bispecific antibodies such as GEN1046, XmAb20717, XmAb22841, and XmAb23104 are currently under investigation in ongoing phase I (NCT03752398, NCT03849469, NCT03517488) and phase I/II (NCT03917381) trials.

Another potential target is lymphocyte activation gene-3 (LAG-3, CD223), a co-inhibitory receptor able to suppress T-cell activation and cytokines secretion [117]; more specifically, LAG-3 overexpression in tumor cells is involved in the phenomenon of immune exhaustion, with

suppression of T-cell function [118,119]. Thus, LAG-3 inhibitors as monotherapy or in combination with anti-PD-1 agents are currently being explored in several phase I and II trials in advanced malignancies, including pretreated UC (NCT03538028, NCT03250832).

T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) is another co-inhibitory receptor expressed on regulatory T cells, effective T cells, tumor cells, and innate immune cells (macrophage and dendritic cells) [120]. TIM-3 expression has been recently associated with poor prognosis in a number of cancer types, including UC [121–123]; because of the implication of TIM-3 overexpression in T-cell dysfunction and exhaustion, several TIM-3 inhibitors are currently being studied in advanced cancer. Among them, INCAGN02390 is under evaluation in a phase I trial (NCT03652077) assessing its role as monotherapy in previously treated metastatic malignancies including UC.

Glucocorticoid-induced TNF receptor family related receptor (GITR) represents a co-stimulatory receptor that binds the GITR ligand (GITRL) [124]; the activation of GITR can result in signals influencing the activity of CD4+, CD8+ and regulatory T cells, playing an important role in autoimmune and inflammatory diseases as well as in anticancer immune response [125,126]. Thus, GITR seems to be a promising target for novel immunotherapy agents. A phase I/II trial analyzing the combination of nivolumab, ipilimumab, and the GITR agonist INCAGN01876 (NCT03126110) in patients with metastatic malignancies including UC is recruiting at present.

Chimeric antigen receptor (CAR)-T immunotherapy has shown impressive responses in a number of B cell malignancies and is currently being tested in several solid tumors, including advanced or metastatic UC (NCT03185468) [127]. CAR-T action is based on engineered T cells expressing a CAR; current second-generation CAR are receptors composed of (1) an extracellular, epitope-specific binding domain, (2) a transmembrane domain, (3) and an intracellular domain of the T cell receptor; this last domain consists in its turn of costimulatory molecules such as CD28, 4-1BB, and the CD3 ζ chain, and is involved in a massive activation of T cells which is independent from T-cell receptor (TCR)—major histocompatibility complex (MHC) interactions [128,129].

Lastly, another promising immunotherapeutic strategy lies in tumor vaccines (TVs), which are currently under investigation in many solid tumors [130,131]. As regards UC, the majority of developing TVs concerns BCG-relapsing, non-muscle invasive disease, where neo-antigens are being studied in combination with immune-stimulating adjuvant agents, cytotoxic agents, and/or mTOR inhibitors (NCT01353222, NCT02015104, NCT01498172). Cancer vaccines are also under investigation in combination with ICIs, as in the case of the NCT03689192 and the NCT03639714 trial. In the NCT03639714 Phase 1/2 Study, two vaccines vectors (GRT-C901 and GRT-R902)—used as immune boosts—are being investigated in combination with nivolumab plus ipilimumab in patients affected by a number of solid cancers including previously treated, metastatic UC.

Ongoing phase I/II/III trials, either recruiting or active not recruiting, of novel immunotherapy approaches in advanced or metastatic UC are summarized in Table 5.

Table 5. Ongoing phase I/II/III trials of novel immunotherapy approaches. ORR: overall response rate. PFS: progression-free survival. OS: overall survival.

NCT (Clinicaltrials.gov)	Phase	Setting	Cisplatin Fit/Unfit	Arm A	Arm B	Arm C	Compounds Description	Number of Patients	Primary Outcome	Status	Estimated Study Completion Date
NCT03361865 (KEYNOTE-672/EC HO-307)	III	First-line	Cisplatin ineligible	Pembrolizumab + epacadostat	Pembrolizumab + placebo		Epacadostat: IDO1 inhibitor. Pembrolizumab: anti-PD-1.	93	ORR	Active, not recruiting	September 2020
NCT03374488	III	Second- or later-line	All	Pembrolizumab + epacadostat	Pembrolizumab + placebo		Epacadostat: IDO1 inhibitor. Pembrolizumab: anti-PD-1.	84	ORR	Active, not recruiting	August 2020
NCT02554812 (JAVELIN Medley)	II	Second- or third-line	All	6 cohorts, different combinations with avelumab, PF-04518600 and utomilumab (PF-05082566)			PF-04518600: OX40 agonist. Utomilumab: 4-1BB agonist. Avelumab: anti-PD-L1.	620	Safety OR	Recruiting	December 2022
NCT03785925 (PIVOT-10)	II	First-line	Cisplatin ineligible	Nivolumab + Bempegaldesleuki n (NKTR-214)			Bempegaldesleukin (NKTR-214): IL-2 pathway agonist designed to target CD122. Nivolumab: anti-PD-1.	205	ORR	Recruiting	March 2022
NCT03513952	II	Second- or later-line (prior platinum chemothera py)	All	Atezolizumab + CYT-107	Atezolizumab		CYT-107: glycosylated recombinant human interleukin-7. Atezolizumab: anti-PD-L1.	54	ORR	Recruiting	December 2020
NCT03915405	I	Second- or later-line (no prior ICI, prior platinum chemothera py)	All	Avelumab + KHK2455			KHK2455: IDO1 inhibitor. Avelumab: anti-PD-L1.	44	Safety	Recruiting	February 2022
NCT03217747	I/II	First- or later-line	All	6 cohorts, different combinations with utomilumab (PF-05082566), PF-04518600, and radiation therapy			PF-04518600: OX40 agonist Utomilumab: 4-1BB agonist	184	Safety CD8 immune markers	Recruiting	September 2023
NCT04198766	I	Second- or	All	Pembrolizumab ±			INBRX-106: OX40 agonist.	150	Safety	Recruiting	March 2023

		later-line		INBRX-106			Pembrolizumab: anti-PD-1.					
NCT02983045 (PIVOT-02)	I/II	First- or later-line	All	Nivolumab + Beppegaldesleukin (NKTR-214)	Nivolumab + ipilimumab + NKTR-214		NKTR-214: IL-2 pathway agonist designed to target CD122. Nivolumab: anti-PD-1. Ipilimumab: anti-CTLA-4.	780	ORR	Recruiting	December 2021	
NCT03138889 (PROPEL)	I/II	First- or later-line	All	Pembrolizumab + Beppegaldesleukin (NKTR-214)			Beppegaldesleukin (NKTR-214): an interleukin-2 pathway agonist that targets CD122. Pembrolizumab: anti-PD-1.	135	ORR	Recruiting	June 2023	
NCT03809624	I	Second- or later-line	All	INBRX-105			INBRX-105: next generation bispecific antibody targeting PD-L1 and 4-1BB, blocks inhibitory PD-1/PD-L1 axis and simultaneously activates essential co-stimulatory activity via 4-1BB	90	Safety	Recruiting	December 2021	
NCT03538028	I	Second- or later-line	All	INCAGN02385			INCAGN02385: LAG-3 inhibitor	40	Safety	Recruiting	September 2020	
NCT03652077	I	First- or later-line	All	INCAGN02390			INCAGN02390: TIM-3 inhibitor	41	Safety	Recruiting	January 2021	
NCT03126110	I/II	First- or later-line	All	Nivolumab + INCAGN01876	Ipilimumab + INCAGN01876	Nivolumab + ipilimumab + INCAGN01876	INCAGN01876: GITR agonist. Nivolumab: anti-PD-1. Ipilimumab: anti-CTLA-4.	285	ORR	Recruiting	October 2021	
NCT03185468	I/II	First- or later-line	All	CAR-T				20	OS Safety	Recruiting	December 2020	
NCT03639714	I/II	Second-line	All	Nivolumab + ipilimumab + GRT-C901 + GRT-R902			GRT-C901, GRT-R902: tumor vaccines. Nivolumab: anti-PD-1. Ipilimumab: anti-CTLA-4.	214	ORR	Recruiting	March 2023	
NCT03228667 (QUILT-3.055)	II	Second- or later-line	All	ALT-803 + ICI (nivolumab or pembrolizumab or avelumab or atezolizumab)			ALT-803: ALT-803: Superagonist Interleukin-15. Pembrolizumab: anti-PD-1. Nivolumab: anti-PD-1. Avelumab: anti-PD-L1. Atezolizumab: anti-PD-L1.	611	ORR	Recruiting	August 2020	
NCT03639714	I	Second- or later-line	All	ARG1-18, 19, 20			ARG1-18, 19, 20: Arginase-1 Peptide Vaccine	10	Safety	Recruiting	June 2021	
NCT03917381	I/II	Second- or later-line	All	GEN1046			GEN1046: bispecific	192	Safety	Recruiting	February	

		later-line				antibody targeting PD-L1 and 4-1BB				2022
NCT03517488 (DUET-2)	I	Second- or later-line	All	XmAb20717		XmAb20717: A Fc-engineered bispecific antibody directed against the human negative immunoregulatory checkpoint receptors PD-1 and CTLA-4	154	Safety	Recruiting	March 2021
NCT04044859	I	Second- or later-line	All	Autologous genetically modified ADP-A2M4CD8 cells		Autologous genetically modified ADP-A2M4CD8 cells, directed to MAGE-A4, a member of the MAGE family expressed in a number of solid tumor types.	30	Safety	Recruiting	January 2021
NCT03894618	I	First-line (in platinum unfit) or second- or later-line	All	SL-279252		SL-279252: agonist redirected checkpoint fusion protein consisting of the extracellular domains of PD-1 and OX40L, linked by a central Fc domain (PD1-Fc-OX40L).	87	Safety	Recruiting	April 2022
NCT03849469 (DUET-4)	I	Second- or later-line	All	XmAb*22841	Pembrolizumab + XmAb*22841	XmAb*22841: a Fc-engineered bispecific antibody directed against CTLA-4 and LAG-3. Pembrolizumab: anti-PD-1	242	Safety	Recruiting	March 2027
NCT03752398 (DUET-3)	I	Second- or later-line	All	XmAb*23104		XmAb23104: bispecific monoclonal antibody directed against PD-1 and inducible T-cell co-stimulator CD278	144	Safety	Recruiting	March 2025
NCT03758781	I	First- or later-line	All	IRX-2 Regimen plus Nivolumab		IRX-2 Regimen: cyclophosphamide and subcutaneous IRX-2, a cell-free mixture comprising a variety of naturally derived cytokines obtained from normal, unrelated donor lymphocytes with potential immunostimulatory activity. The cytokines in IRX-2 include interleukin-1, -2, -6, -8, -10, -12, tumor necrosis factor alpha, interferon-gamma and	100	Safety	Recruiting	February 2022

						colony stimulating factors. Nivolumab: anti-PD-1.				
NCT03841110	I	First- or later-line	All	FT500	FT500 + nivolumab or pembrolizumab or atezolizumab	FT500: natural killer cell product that can bridge innate and adaptive immunity. Pembrolizumab: anti-PD-1. Nivolumab: anti-PD-1. Atezolizumab: anti-PD-L1.	76	Safety	Recruiting	June 2022
NCT03329950	I	Second- or later-line	All	CDX-1140	CDX-1140 + CDX-301	CDX-1140: anti-CD40, a key activator of immune response which is found on dendritic cells, macrophages and B cells and is also expressed on many cancer cells. CDX-301: recombinant human FMS-like tyrosine kinase-3 ligand that acts by uniquely binding FMS-like tyrosine kinase-3 (CD135). Pembrolizumab: anti-PD-1.	220	Safety	Recruiting	November 2021
NCT03674567	I/II	First-line (in platinum unfit) or second- or later-line	All	FLX475	FLX475 + pembrolizumab	FLX475: antagonist of C-C chemokine receptor type 4 with potential immunomodulatory and antineoplastic activities. FLX475 inhibits the binding of CCR4 to its signaling molecules, thereby blocking the recruitment of regulatory T cells to the tumor microenvironment. Pembrolizumab: anti-PD-1.	375	ORR	Recruiting	August 2021
NCT03970382	I	Second- or later-line	All	NeoTCR-P1	NeoTCR-P1 + nivolumab	NeoTCR-P1: A preparation of autologous CD4- and CD8-positive T lymphocytes that have been engineered with site-specific nucleases to suppress the expression of most endogenous forms of the T-cell receptor and promote expression of a single, native T-cell receptor targeting a neoepitope	148	Safety	Recruiting	December 2023

						presented on the surface of a patient's tumor cells, with potential immunostimulating and antineoplastic activities. Nivolumab: anti-PD-1.				
NCT03277352	I/II	Second- or later-line	All	INCAGN01876 + Pembrolizumab + Epacadostat		Epacadostat: IDO1-inhibitor. INCAGN01876: anti-human glucocorticoid-induced tumor necrosis factor receptor agonistic humanized monoclonal antibody, with potential immune checkpoint modulating activity. Pembrolizumab: anti-PD-1.	10	Phase 1: safety. Phase 2: ORR and complete response rate	Active, not recruiting	May 2020
NCT03250832 (CITRINO)	I	Second- or later-line	All	TSR-033	TSR-033 + an anti-PD-1 agent	TSR-033: anti-LAG-3 monoclonal antibody	200	Safety	Recruiting	May 2021
NCT03693612	I/II	Second- or later-line	All	GSK3359609 plus tremelimumab		GSK3359609: agonistic antibody for the inducible T-cell co-stimulator (ICOS; CD278), with potential immune checkpoint inhibitory and antineoplastic activities. Tremelimumab: anti-CTLA-4.	114	Safety	Recruiting	April 2023
NCT03739931	I	First line in cisplatin ineligible and PD-L1 negative patients; second-line after platinum-containing chemotherapy	All	mRNA-2752	mRNA-2752 plus durvalumab	mRNA-2752: lipid nanoparticle encapsulating mRNAs encoding human OX40L, IL-23, and IL-36γ. Durvalumab: anti-PD-L1.	126	Safety	Recruiting	July 2021

3.2. PARP Inhibitors

One of the new promising therapeutic approaches is the use of Poly(ADP-ribose) polymerase (PARP) inhibitors that target DNA repair gene mutations and have been proven active in other type of cancer like ovarian, breast, and prostate cancer [132,133].

Regarding UC, genomic alterations in DNA repair genes like *ATM*, *ERCC2*, *RAD51B* were found in 2–14% and in *BRCA 1/2*, *PALB2*, *FANCD2*, *ERCC2*, *ATM* in 3.7–12.3% of MIBC [47,134]. Moreover, patients with DNA damage response and repair (DDR) gene alterations treated with platinum based chemotherapy resulted to have better PFS and OS [135]. In fact, in multiple tumors the presence of DDR gene aberrations correlates with an enhanced sensibility to platinum compounds [136]. Based on these results, PARP inhibitors have been studied in UC as well [137–140]. At the recent ASCO Genitourinary Cancers Symposium 2020, the results of the study ATLAS (NCT03397394) were presented [136]. This phase II trial assessed the efficacy and safety of the PARP inhibitor rucaparib in 97 patients with locally advanced or metastatic UC with or without homologous recombination deficiency (HRD), progressed to one or two prior treatments. Total of 20.6% of patients were HRD-positive, 30.9% were HRD-negative, and 48.5% had unknown HRD status. Among patients with sequencing results (64 patients), deleterious alterations in *BRCA1*, *BRCA2*, *RAD51C*, *PALB2* were infrequent (9.4%). Common alterations were found in *TP53* (52.4%) and in *FGF/FGFR* pathway (77.6%). The results showed that there were no confirmed responses to rucaparib, 28.1% of patients achieved a stable disease as best response with no difference in efficacy between HRD-negative and HRD-positive patients. The trial was discontinued because protocol-defined continuance criteria were not met. Two phase II trials are investigating the PARP inhibitor olaparib in monotherapy in chemotherapy naïve cisplatin ineligible patients or progressed to first line treatment selected for DDR mutations (NCT03448718) and in patients with DNA-repair defects progressed to 1 or 2 prior treatment regimens (NCT03375307).

A phase II trial is currently investigating the PARP inhibitor niraparib as maintenance therapy until disease progression or unacceptable toxicity or death in patients unselected for DDR mutational status not progressing to first line platinum-based chemotherapy (NCT03945084).

Another strategy being tested is combination therapy of PARP inhibitors with ICIs or target therapies.

Indeed, the presence of alteration in DDR genes has been associated with higher mutational load and higher response to ICIs in patients with UC [141,142]. Based on these observations, several combinations of PARP inhibitors and PD-1/PD-L1 inhibitors are currently being tested: durvalumab plus olaparib (module B, NCT02546661, active not recruiting; NCT03459846, active not recruiting), rucaparib plus nivolumab (NCT03824704, active not recruiting), niraparib plus atezolizumab (NCT03869190, recruiting).

The phase I BISCAY (NCT02546661) trial is evaluating the combination of durvalumab with olaparib or a FGFR1-3 inhibitor (AZD4547) or a TORC 1 and 2 inhibitor (vistusertib) in platinum refractory, immuno-therapy naïve UC patients allocated depending on tumour DNA alterations determined by next generation sequencing. Total of 391 patients were screened and NGS analysis showed the following absolute frequency of biomarkers: *FGFR1–3* fusions or *FGFR3* activating mutations in 21% of cases (83 patients in the AZD4547 arm/391), HRR deleterious gene alterations (*ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCI*, *FANCL*, *PALB2*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD54L*) in 14% of cases (54 patients in the olaparib arm/391), *RICTOR* amplification and *TSC1/TSC2* loss or inactivating mutations in 15% cases (60 patients in the Vistusertib arm/391). The preliminary results available on 14 patients with homologous recombination repair genomic alterations treated with olaparib and durvalumab showed a high tumor mutation burden and a confirmed ORR of 35.7%, a 6-months PFS rate of 42%, 1-years OS rate of 54% [143].

A phase Ib-II trial (NCT03992131) is evaluating the combination between the PARP inhibitor rucaparib and lucitanib, a VEGFR1-2-3, FGFR1-2, and PDGFR α - β inhibitor, or sacituzumab govitecan.

Ongoing phase I/II/III trials, either recruiting or active not recruiting, of PARP inhibitors in advanced or metastatic UC are summarized in Table 6.

Table 6. Ongoing phase I/II/III trials of PARP inhibitors. ORR: overall response rate. PFS: progression-free survival. OS: overall survival.

NCT (Clinicaltrials.gov)	Phase	Setting	Cisplatin Fit/Unfit	Arm A	Arm B	Arm C	Compounds Description	Number of Patients	Primary Outcome	Status	Estimated Study Completion Date
NCT03682289	II	Third- or later-line	All	AZD6738	AZD6738 plus olaparib		AZD6738: an orally available morpholino-pyrimidine-based inhibitor of ataxia telangiectasia and rad3 related kinase. Olaparib: PARP inhibitor.	68	ORR	Recruiting	March 2023
NCT03945084	II	Maintenance after first-line treatment	All	Niraparib + best supportive care	Best supportive care		Niraparib: PARP inhibitor	77	PFS	Recruiting	June 2024
NCT03375307	II	Second- or third-line	All	Olaparib			Olaparib: PARP inhibitor	150	ORR	Recruiting	August 2023
NCT03448718	II	Second- or later-lines; cisplatin ineligible; chemotherapy naïve	All	Olaparib			Olaparib: PARP inhibitor	30	ORR	Recruiting	March 2023
NCT03459846 (BAYOU)	II	First-line	Cisplatin ineligible	Durvalumab + olaparib	Durvalumab + placebo		Durvalumab: anti-PD-L1. Olaparib: PARP inhibitor.	154	PFS	Active, not recruiting	September 2021
NCT03992131	I/II	Second- or later-line	All	Rucaparib and Lucitanib (phase Ib)	Rucaparib and Sacituzumab govitecan (phase Ib and II)		Sacituzumab govitecan: humanized anti-Trop-2 monoclonal antibody linked with SN-38, the active metabolite of irinotecan. Lucitanib: VEGFR 1, 2 and 3, FGFR 1 and 2, and PDGFR alpha and beta inhibitor. Rucaparib: PARP inhibitor.	329	Safety and tolerability, dose limiting toxicities (phase Ib), ORR (phase II)	Recruiting	March 2024
NCT02546661 (BISCAY)	I	Second- or third-line	All	8 cohorts: AZD4547; AZD4547 + durvalumab; durvalumab + olaparib;			AZD4547: FGFR-1, 2 and 3 inhibitor. AZD1775 (adavosertinib): inhibitor of the tyrosine kinase WEE1.	156	Safety	Active, not recruiting	March 2020

durvalumab + AZD1775;	Durvalumab: anti-PD-L1.
durvalumab; durvalumab + vistusertib;	Vistusertib: mTOR inhibitor.
durvalumab + AZD9150;	AZD9150 (danvatirsen): an antisense oligonucleotide targeting signal transducer and activator of transcription 3 (STAT3).
durvalumab + Selumetinib	Selumetinib: MEK or MAPK/ERK kinase 1 and 2 inhibitor.

3.3. Target Therapy

As already discussed, the FGFR inhibitor erdafitinib is a promising treatment strategy in patients with FGF/FGFR alterations. In these subgroup of patients, other therapies directed at inhibiting FGFR are currently being tested: PRN1371, a FGFR 1-4 inhibitor, in a phase I trial in previously treated patients (NCT02608125); Pemigatinib, a FGFR1-3 inhibitor, in phase II trial in patients progressed to at least one prior treatment (NCT02872714, FIGHT-201); Rogaratinib (BAY1163877), a FGFR 1-4 inhibitor, in a phase II/III trial in patients progressed to at least one platinum-containing regimen (NCT03410693).

Moreover, FGFR inhibitors are being evaluated in combinations with PD-1/PD-L1 inhibitors. A study by Sweis et al. showed that FGFR3 pathways were activated in non-T-cell-inflamed UC, characterized by an absence of intratumoral T cells, thus identifying a potential targetable pathway that could help to overcome tumor-intrinsic immunotherapy resistance [58]. The updated results of the interim analysis of the phase II study FIERCE-22 (NCT03123055) evaluating the combination of the FGFR3 inhibitor vofatamab (a human IgG1 monoclonal antibody directed against FGFR3) in combination with pembrolizumab in 28 patients (20 wild-type) progressed following platinum-based chemotherapy have been presented at European Society for Medical Oncology (ESMO) Congress 2019: the combination therapy resulted to be well tolerated with encouraging ORR (29.6%) and a median PFS is 4.7 months [144].

Combination of FGFR inhibitors and ICIs currently under investigation in ongoing clinical trials are: Rogaratinib ± atezolizumab in first-line treatment of cisplatin ineligible patients with FGFR 1-3 alterations (NCT03473756, FORT-2); pemigatinib ± pembrolizumab versus standard of care (chemotherapy or pembrolizumab) in first-line treatment cisplatin-ineligible patients with FGFR3 mutation or rearrangement (NCT04003610, FIGHT-205); derazantinib (a FGFR 1-3 inhibitor) ± atezolizumab in cisplatin ineligible patients with FGFR alteration in first-line or progressed to prior FGFR inhibitor treatment (NCT04045613); erdafitinib plus cetrelimab (an IgG4 monoclonal antibody directed against PD-1) in pretreated (phase Ib) or previously untreated cisplatin-ineligible patients (phase II) (NCT03473743).

Other target therapy treatments under evaluation are PI3K/mTOR inhibitors since this pathway resulted to be frequently altered in UC, as already discussed [46]. mTOR inhibitors and PI3K inhibitors are currently being tested alone (everolimus: NCT00805129, sapanisertib: NCT03047213, Buparlisib: NCT01551030), and in combination with anti-PD-1/PD-L1 (nivolumab plus nab-rapamycin: NCT03190174, durvalumab plus vistusertib: NCT02546661, module E) or chemotherapy (paclitaxel plus TAK-228: NCT03745911).

Another interesting pathway being investigated is targeting human epidermal growth factor receptor 2 (HER2, ERBB2) considering that mutation or amplification of *ERBB2* gene has been identified in 9% of MIBC [46]. Trastuzumab deruxtecan is an ADC composed of trastuzumab, a monoclonal antibody targeting HER2 conjugated to deruxtecan, a derivative of the camptothecin analog exatecan, a DNA topoisomerase 1 inhibitor. This compound is being tested in combination with nivolumab in a phase I/II trial in patients with HER2 expression of IHC 1+, 2+ or 3+, progressed to prior platinum-based therapy (NCT03523572). RC48-ADC, an anti-HER2 monoclonal antibody, is under evaluation in two phase II trial in previously treated patients, one in HER2 negative (IHC 0 or 1+, NCT04073602) and one in HER2 overexpressed tumors (IHC 2+ or 3+, NCT03809013). PRS-343, a bivalent, bispecific fusion protein composed of an anti-HER2 monoclonal antibody linked to a CD137-targeting anticalin, is being investigated in HER2 positive solid tumor malignancy, including UC, for which standard therapies are not available.

Ongoing phase I/II/III trials, either recruiting or active not recruiting, of target therapies in advanced or metastatic UC are summarized in Table 7, while miscellanea therapies are reported in Table 8.

Table 7. Ongoing phase I/II/III trials of target therapies. ORR: overall response rate. PFS: progression-free survival. OS: overall survival.

NCT (Clinicaltrials.gov)	Phase	Setting	Cisplatin Fit/Unfit	Arm A	Arm B	Arm C	Compounds Description	Number of Patients	Primary Outcome	Status	Estimated Study Completion Date
NCT03980041 (MARIO-275)	II	First- or later-line	All	Nivolumab + IPI-549	Nivolumab + placebo		IPI-549: PI3K inhibitor. Nivolumab: anti-PD-1.	160	ORR	Recruiting	November 2022
NCT03745911	II	Second- or later-line	All	Paclitaxel + TAK-228			TAK-228: PI3K/AKT/mTOR inhibitor	52	ORR	Recruiting	November 2020
NCT00805129	II	Second-, third or fourth- line	All	Everolimus			Everolimus: mTOR inhibitor	46	PFS Safety	Active, not recruiting	December 2020
NCT02567409	II	First-line or second-line (only with prior ICI)	All	Gemcitabine + cisplatin + berzosertib (M6620)	Gemcitabine + cisplatin		Berzosertib (M6620): ATR kinase inhibitor	90	PFS	Active, not recruiting	August 2020
NCT03047213	II	Second- or later-line	All	Sapanisertib			Sapanisertib: mTORC1 and mTORC2 inhibitor	209	ORR	Recruiting	June 2020
NCT02535650	II	Second- or later-line	All	Tipifarnib			Tipifarnib: farnesyltransferase inhibitor	18	6-month PFS	Recruiting	March 2020
NCT01551030	II	Second- or later-line	All	Buparlisib			Buparlisib: PI3K inhibitor	35	PFS	Active, not recruiting	March 2020
NCT04073602	II	Second- or later-line; HER-2 negative	All	RC48-ADC			RC48-ADC: anti-HER2 monoclonal antibody	20	ORR	Recruiting	October 2020
NCT03809013	II	Second- or later-line; HER2 overexpressed	All	RC48-ADC			RC48-ADC: anti-HER2 monoclonal antibody	60	ORR	Recruiting	December 2021
NCT02795156	II	Second-line	All	Afatinib or Regorafenib or			Afatinib, Regorafenib, Cabozantinib:	160	ORR	Recruiting	December 2020

				Cabozantinib (based on specific genomic alterations on next-generation sequencing)			tyrosine kinase inhibitor				
NCT02872714 (FIGHT-201)	II	Second- or later-line	All	Pemigatinib			Pemigatinib: FGFR inhibitor	240	ORR	Recruiting	August 2020
NCT03410693 (FORT-1)	II/III	Second- or later-line	All	Rogaratinib (BAY1163877)	Chemotherapy		Rogaratinib: FGFR inhibitor	171	OS	Active, not recruiting	November, 2020
NCT04003610 (FIGHT-205)	II	First-line	Cisplatin ineligible	Pemigatinib + Pembrolizumab	Pemigatinib	Chemotherapy or pembrolizumab	Pemigatinib: FGFR inhibitor. Pembrolizumab: anti-PD-1.	372	PFS	Recruiting	June, 2024
NCT03190174	I/II	Second- or later-line	All	Nivolumab + ABI-009 (Nab-rapamycin)			ABI-009 (Nab-rapamycin): mTOR inhibitor. Nivolumab: anti-PD-1.	40	Safety	Recruiting	April 2021
NCT03523572	I/II	Second- or later-line	All	Trastuzumab Deruxtecan (DS-8201a) + Nivolumab			Trastuzumab Deruxtecan (DS-8201a): antibody drug conjugated composed of trastuzumab, a monoclonal antibody targeting HER2 conjugated to deruxtecan, a derivative of the camptothecin analog exatecan, a DNA topoisomerase 1 inhibitor. Nivolumab: anti-PD-1.	99	Safety, ORR	Recruiting	September 2020
NCT03330561	I	Third- or later-line	All	PRS-343			PRS-343: anti-HER2 monoclonal antibody linked to a	78	Safety	Recruiting	September 2020

						CD137-targeting anticalin				
NCT02546661 (BISCAY)	I	Second- or third-line	All	8 cohorts: AZD4547; AZD4547 + durvalumab; durvalumab + olaparib; durvalumab + AZD1775; durvalumab; durvalumab + vistusertib;		AZD4547: FGFR-1, 2 and 3 inhibitor.	156	Safety	Active, not recruiting	March 2020
				durvalumab + AZD9150; durvalumab + Selumetinib		AZD1775 (adavosertinib): inhibitor of the tyrosine kinase WEE1. Durvalumab: anti-PD-L1. Vistusertib: mTOR inhibitor. AZD9150 (danvatirsen): an antisense oligonucleotide targeting signal transducer and activator of transcription 3 (STAT3). Selumetinib: MEK or MAPK/ERK kinase 1 and 2 inhibitor.				
NCT02608125	I	Third- or later-line	All	PRN1371		PRN1371: FGFR inhibitor	50	Safety	Recruiting	February 2021
NCT03473756 (FORT-2)	I/II	First-line	Cisplatin ineligible	Rogaratinib + Atezolizumab	Placebo + Atezolizumab	Rogaratinib: FGFR inhibitor	210	Safety, PFS	Recruiting	September, 2024
NCT04045613	I/II	First-line; later-lines after prior FGFR inhibitor	Cisplatin ineligible	Derazantinib	Derazantinib + Atezolizumab	Derazantinib: FGFR inhibitor. Atezolizumab: anti-PD-L1.	303	ORR; recommended phase 2 dose	Recruiting	May, 2022
NCT03473743	I/II	Phase 1b: second- or	Phase 2: cisplatin	Erdafitinib + Cetrelimab		Erdafitinib: FGFR inhibitor.	150	Safety, ORR	Recruiting	September, 2021

later-line.
Phase 2:
first-line

ineligible

Cetrelimab:
anti-PD-1.

Table 8. Ongoing phase I/II/III trials of miscellanea therapies. ORR: overall response rate. PFS: progression-free survival. OS: overall survival.

NCT (Clinicaltrials.gov)	Phase	Setting	Cisplatin Fit/Unfit	Arm A	Arm B	Arm C	Compounds Description	Number of Patients	Primary Outcome	Status	Estimated Study Completion Date
NCT03854474	I/II	Second- or later-line	All	Pembrolizumab + tazemetostat			Tazemetostat: Enhancer of zeste homolog 2 (EZH2) methyltransferase inhibitor. EZH2 is a histone-lysine N-methyltransferase enzyme participating in histone methylation and, ultimately, transcriptional repression. Pembrolizumab: anti-PD-1.	30	ORR	Recruiting	June 2020
NCT04200963	I	Second- or later-line	All	KYN-175			Aryl Hydrocarbon Receptor antagonist	53	Safety	Recruiting	September 2022
NCT04007744	I	First-line (in platinum unfit) or second- or later-line	All	Pembrolizumab + sonidegib			Sonidegib: Hedgehog signaling pathway inhibitor. Pembrolizumab: anti-PD-1.	45	Safety	Recruiting	June 2021
NCT03829436	I	First- or later-line	All	TPST-1120; TPST-1120 + nivolumab; TPST-1120 + docetaxel; TPST-1120 + cetuximab			TPST-1120: selective antagonist of peroxisome proliferator activated receptor alpha. Nivolumab: anti-PD-1.	338	Safety	Recruiting	June 2024

										Cetuximab: anti-EGFR.					
NCT02420847	I/II	Second- or later-line	All	Ixazomib + Gemcitabine + Doxorubicin			Ixazomib: second generation proteasome inhibitor with potential antineoplastic activity		50	Safety	Active, not recruiting	July 2022			
NCT00365157	I/II	Second- and third-line	All	Eribulin			Eribulin: microtubule-targeting agent		132	Maximum tolerated dose and recommended phase II dose, ORR	Active, not recruiting	December 2020			
NCT03179943	II	First-line (in platinum unfit) or second- or later-line (with prior platinum-based chemotherapy or ICI)	All	Atezolizumab + guadecitabine			Guadecitabine: DNA methyltransferase (DNMT) inhibitor. Atezolizumab: anti-PD-L1.		53	Safety ORR	Recruiting	July 2022			
NCT03547973	II	First-line (in platinum unfit) or second- or later-line (with prior platinum-based chemotherapy or ICI)	All	Sacituzumab govitecan (IMMU-132)			Sacituzumab govitecan (IMMU-132): Anti-Trop-2/SN-38 Antibody-Drug Conjugate		201	ORR	Recruiting	September 2021			
NCT04223856	III	First-line	Cisplatin or carboplatin eligible	Enfortumab vedotin + pembrolizumab		Enfortumab vedotin + pembrolizumab + cisplatin or carboplatin		Gemcitabine + cisplatin or carboplatin		Enfortumab vedotin: anti nectin- 4 monoclonal antibody linked to a micro-tubule-disrupting agent (monomethyl auristatin E). Pembrolizumab: anti-PD-1.		1095	PFS, OS	Recruiting	November 2023

NCT03474107										
(EV-301)	III	Third- or later-line	All	Enfortumab vedotin	Chemotherapy (docetaxel, vinflunine, paclitaxel)	Enfortumab vedotin: anti nectin- 4 monoclonal antibody linked to a micro-tubule-disrupting agent (monomethyl auristatin E)	608	OS	Active, not recruiting	September 2021

4. Conclusions

In the recent years, the treatment scenario of metastatic UC has been enriched with several new therapeutic options. Immunotherapy is a very promising approach for this disease, but a high percentage of patients are still resistant to this type of treatment. In the future years, the results of the ongoing trials investigating ICIs in combination with target therapy or chemotherapy will assess if resistance to ICIs alone can be overcome. Promising treatment approaches are FGFR inhibitors and enfortumab vedotin. These two treatment strategies already showed good results in monotherapy and combination therapies with ICIs being tested. Other compounds, such as PARP inhibitors, mTOR inhibitors, anti-VEGF, tyrosine kinase inhibitors, HER2 targeting therapies, either alone or in several types of combinations, are being investigated in clinical trials.

The therapeutic approach to UC, which for many years has been dominated by platinum containing chemotherapy based on clinical and laboratory variable defining cisplatin eligibility, is now shifting toward a more personalized approach, based on the presence of molecular alteration (e.g., FGFR alterations) or PD-L1 expression.

Funding: This research received no financial support.

Conflicts of Interest: The authors declare no competing interests.

References

1. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2019. *CA Cancer J. Clin.* **2019**, *69*, 7–34.
2. Smith, A.B.; Deal A.M.; Woods, M.E.; Wallen E.M.; Pruthi R.S.; Chen R.C.; Milowsky, M.I.; Nielsen M.E. Muscle-invasive bladder cancer: evaluating treatment and survival in the National Cancer Data Base. *BJU Int.* **2014**, *114*, 719–726.
3. Antoni, S.; Ferlay, J.; Soerjomataram, I.; Znaor, A.; Jemal, A.; Bray, F. Bladder cancer incidence and mortality: a global overview and recent trends. *Eur. Urol.* **2017**, *71*, 96–108.
4. Burger, M.; Catto, J.W.; Dalbagni, G.; Grossman, H.B.; Herr, H.; Karakiewicz, P.; Kassouf, W.; Kiemeny, L.A.; La Vecchia, C.; Shariat, S.; et al. Epidemiology and risk factors of urothelial bladder cancer. *Eur. Urol.* **2013**, *63*, 234–241.
5. Necchi, A.; Pond, G.R.; Raggi, D.; Giannatempo, P.; Vogelzang, N.J.; Grivas, P.; Galsky M.D.; Bellmunt, J.; Sonpavde, G. Efficacy and safety of gemcitabine plus either taxane or carboplatin in the first- line setting of metastatic urothelial carcinoma: a systematic review and meta- analysis. *Clin. Genitourin. Cancer.* **2016**, *15*, 331–340.
6. Sternberg, C.N.; de Mulder, P.; Schornagel, J.H.; Theodore, C.; Fossa, S.D.; van Oosterom, A.T.; Witjes, J.A.; Spina, M; van Groeningen C.J.; Duclos, B.; et al. Seven years 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.* **2006**, *42*, 50–54.
7. Galsky, M.D.; Chen G.J.; Oh W.K.; Bellmunt, J.; Roth, B.J.; Petrioli, R.; Dogliotti, L.; Dreicer, R.; Sonpavde, G. Comparative effectiveness of cisplatin-based and carboplatin-based chemotherapy for treatment of advanced urothelial carcinoma. *Ann. Oncol* **2012**, *23*, 406–410.
8. Galsky, M.D.; Hahn N.M.; Rosenberg, J.; Sonpavde, G.; Hutson, T.; Oh, W.K.; Dreicer, R.; Vogelzang, N.; Sternberg, C.N.; Bajorin, D.F.; et al. Treatment of patients with metastatic urothelial cancer “unfit” for Cisplatin-based chemotherapy. *J. Clin. Oncol.* **2011**, *29*, 2432–2438.
9. Raggi, D.; Miceli, R.; Sonpavde, G.; Giannatempo, P.; Mariani, L.; Galsky, M.D.; Bellmunt, J.; Necchi, A. Second-line single-agent versus doublet chemotherapy as salvage therapy for metastatic urothelial cancer: a systematic review and meta-analysis. *Ann. Oncol.* **2016**, *27*, 49–61.
10. Lattanzi, M.; Balar, A.V. Current status and future direction of immunotherapy in urothelial carcinoma. *Curr. Oncol. Rep.* **2019**, *21*, 24.
11. Godwin, J.L.; Hoffman-Censits, J.; Plimack, E. Recent developments in the treatment of advanced bladder cancer. *Urol. Oncol.* **2018**, *36*, 109–114.
12. Rouanne, M.; Roumiguié, M.; Houédé, N.; Masson-Lecomte, A.; Colin, P.; Pignot, G.; Larré, S.; Xylinas, E.; Roupret, M.; Neuzillet, Y. Development of immunotherapy in bladder cancer: present and future on targeting PD(L)1 and CTLA-4 pathways. *World J. Urol.* **2018**, *36*, 1727–1740.

13. Hanna, K.S. Updates and novel treatments in urothelial carcinoma. *J. Oncol. Pharm. Pract.* **2019**, *25*, 648–656.
14. Parry, R.V.; Chemnitz, J.M.; Frauwirth, K.A.; Lanfranco, A.R.; Braunstein, I.; Kobayashi, S.V.; Linsley, P.S.; Thompson, C.B.; Riley, J.L. CTLA- 4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Mol. Cell. Biol.* **2005**, *25*, 9543–9553 (2005).
15. Motzer, R.J.; Escudier, B.; McDermott, D.F.; George, S.; Hammers, H.J.; Srinivas, S.; Tykodi, S.S.; Sosman, J.A.; Procopio, G.; Castellano, D.; et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. *N. Engl. J. Med.* **2015**, *373*, 1803e13.
16. Massari, F.; Di Nunno, V.; Mollica, V.; Montironi, R.; Cheng, L.; Cimadamore, A.; Blanca, A.; Lopez-Beltran, A. Immunotherapy in renal cell carcinoma from poverty to the spoiled of choice. *Immunotherapy*. **2019**, *11*, 1507–1521.
17. Powles, T.; Smith, K.; Stenzl, A.; Bedke, J. Immune checkpoint inhibition in metastatic urothelial cancer. *Eur. Urol.* **2017**, *72*, 477–481.
18. Massari, F. & Di Nunno, V. Atezolizumab for platinum-treated metastatic urothelial carcinoma. *The Lancet*. **2017**, *S0140- 6736*, 33298–33301.
19. Smith, A.B.; Deal, A.M.; Woods, M.E.; Wallen, E.M.; Pruthi, R.S.; Chen, R.C.; Milowsky, M.I.; Nielsen, M.E. Muscle-invasive bladder cancer: evaluating treatment and survival in the National Cancer Data Base. *BJU Int.* **2014**, *114*, 719–726.
20. Ciccicarese, C.; Iacovelli, R.; Bria, E.; Mosillo, C.; Bimbatti, D.; Fantinel, E.; Bisogno, I.; Brunelli, M.; Tortora, G. Second-line therapy for metastatic urothelial carcinoma: defining the best treatment option among immunotherapy, chemotherapy, and antiangiogenic targeted therapies. A systematic review and meta-analysis. *Semin. Oncol.* **2019**, *46*, 65–72.
21. Rijnders, M.; de Wit, R.; Boormans, J.L.; Lolkema, M.P.J.; van der Veldt, A.A.M. Systematic review of immune checkpoint inhibition in urological cancers. *Eur. Urol.* **2017**, *72*, 411–423.
22. Di Nunno, V.; De Luca, E.; Buttigliero, C.; Tucci, M.; Vignani, F.; Gatto, L.; Zichi, C.; Ardizzoni, A.; Di Maio, M.; Massari, F. Immune-checkpoint inhibitors in previously treated patients with advanced or metastatic urothelial carcinoma: a systematic review and meta-analysis. *Crit. Rev. Oncol. Hematol.* **2018**, *129*, 124–132.
23. Galsky, M.D.; Hahn, N.M.; Rosenberg, J.; Sonpavde, G.; Hutson, T.; Oh, W.K.; Dreicer, R.; Vogelzang, N.; Sternberg, C.; Bajorin, D.F.; et al. A consensus definition of patients with metastatic urothelial carcinoma who are unfit for cisplatin-based chemotherapy. *Lancet Oncol.* **2011**, *12*, 211–214.
24. Sonpavde, G.; Galsky, M.D.; Latini, D.; Chen, G.J. Cisplatin-ineligible and chemotherapy- ineligible patients should be the focus of new drug development in patients with advanced bladder cancer. *Clin. Genitourin. Cancer.* **2014**, *12*, 71–73.
25. De Santis, M.; Bellmunt, J.; Mead, G.; Kerst, J.M.; Leahy, M.; Maroto, P.; Gil, T.; Marreaud, S.; Daugaard, G.; Skoneczna, I.; 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.* **2012**, *30*, 191–199.
26. von der Maase, H.; Sengelov, L.; Roberts, J.T.; Ricci, S.; Dogliotti, L.; Oliver, T.; Moore, M.J.; Zimmermann, A.; Arning, M. 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.* **2005**, *23*, 4602–4608.
27. Sternberg, C.N.; de Mulder, P.H.; Schornagel, J.H.; Théodore, C.; Fossa, S.D.; van Oosterom, A.T.; Witjes, F.; Spina, M.; van Groeningen, C.J.; de Balincourt, C.; 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.* **2001**, *19*, 2638–2646.
28. Massari, F.; Di Nunno, V.; Cubelli, M.; Santoni, M.; Fiorentino, M.; Montironi, R.; Cheng, L.; Lopez-Beltran, A.; Battelli, N.; Ardizzoni, A. Immune checkpoint inhibitors for metastatic bladder cancer. *Cancer Treat. Rev.* **2018**, *64*, 11–20.
29. Balar, A.V.; Castellano, D.; O'Donnell, P.H.; Grivas, P.; Vuky, J.; Powles, T.; Plimack, E.R.; Hahn, N.M.; de Wit, R.; Pang, L.; et al. First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. *Lancet Oncol.* **2017**, *18*, 1483–1492.

30. Balar, A.V.; Galsky, M.D.; Rosenberg, J.E.; Powles, T.; Petrylak, D.P.; Bellmunt, J.; Loriot, Y.; Necchi, A.; Hoffman-Censits, J.; Perez-Gracia, J.L.; et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. *Lancet*. **2017**, *7*, 67–76.
31. Nadal, R.; Bellmunt, J. Management of metastatic bladder cancer. *Cancer Treat. Rev.* **2019**, *76*, 10–21.
32. Zarrabi, K.; Paroya, A.; Wu, S. Emerging therapeutic agents for genitourinary cancers. *J. Hematol. Oncol.* **2019**, *12*, 89.
33. Lorusso, V.; Pollera, C.F.; Antimi, M.; Luporini, G.; Gridelli, C.; Frassinetti, G.L.; Oliva, C.; Pacini, M.; De Lena, M. A phase II study of gemcitabine in patients with transitional cell carcinoma of the urinary tract previously treated with platinum. Italian Co-operative Group on Bladder Cancer. *Eur. J. Cancer.* **1998**, *34*, 1208–1212.
34. Bellmunt, J.; Théodore, C.; Demkov, T.; Komyakov, B.; Sengelov, L.; Daugaard, G.; Caty, A.; Carles, J.; Jagiello-Gruszfeld, A.; Karyakin, O.P.; 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.* **2009**, *27*, 4454–4461.
35. Vaughn, D.J.; Broome, C.M.; Hussain, M.; Gutheil, J.C.; Markowitz, A. Phase II trial of weekly paclitaxel in patients with previously treated advanced urothelial cancer. *J. Clin. Oncol.* **2002**, *20*, 937–940.
36. Al Harthy, M.; Redman, J.; Madan, R.A. Novel immunotherapy combinations for genitourinary cancers. *Expert Opin. Biol. Ther.* **2020**, *27*, 1–10.
37. Pierantoni, F.; Maruzzo, M.; Gardi, M.; Bezzon, E.; Gardiman, M.P.; Porreca, A.; Basso, U.; Zagonel, V. Immunotherapy and urothelial carcinoma: An overview and future perspectives. *Crit Rev Oncol Hematol.* **2019**, *143*, 46–55.
38. Bellmunt, J.; de Wit, R.; Vaughn, D.J.; Fradet, Y.; Lee, J.L.; Fong, L.; Vogelzang, N.J.; Climent, M.A.; Petrylak, D.P.; Choueiri, T.K.; et al. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. *N. Engl. J. Med.* **2017**, *16*, 1015–1026.
39. Rosenberg, J.E.; Hoffman-Censits, J.; Powles, T.; van der Heijden, M.S.; Balar, A.V.; Necchi, A.; Dawson, N.; O'Donnell, P.H.; Balmanoukian, A.; Loriot, Y.; 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*. **2016**, *7*, 1909–1920.
40. Powles, T.; Duran, I.; van der Heijden, M.S.; Loriot, Y.; Vogelzang, N.J.; De Giorgi, U.; Oudard, S.; Retz, M.M.; Castellano, D.; Bamias, A.; 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. *Lancet*. **2018**, *391*, 748–757.
41. Sharma, P.; Callahan, M.K.; Bono, P.; Kim, J.; Spiliopoulou, P.; Calvo, E.; Pillai, R.N.; Ott, P.A.; De Braud, F.; Morse, M.; et al. Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): A multicentre, single-arm, phase 2 trial. *Lancet Oncol.* **2017**, *18*, 312–322.
42. Patel, M.R.; Ellerton, J.; Infante, J.R.; Agawal, M.; Gordon, M.; Aljumaily, R.; Britten, C.D.; Dirix, L.; Lee, K.W.; Taylor, M.; et al. Avelumab in metastatic urothelial carcinoma after platinum failure (JAVELIN Solid Tumor): Pooled results from two expansion cohorts of an open-label, phase 1 trial. *Lancet Oncol.* **2018**, *19*, 51–64.
43. Powles, T.; O'Donnell, P.H.; Massard, C.; Arkenau, H.T.; Friedlander, T.W.; Hoimes, C.J.; Lee, J.L.; Ong, M.; Sridhar, S.S.; Vogelzang, N.J.; et al. Efficacy and safety of durvalumab in locally advanced or metastatic urothelial carcinoma: Updated results from a phase 1/2 open-label study. *JAMA Oncol.* **2017**, *14*, 3.
44. Crist, M.; Iyer, G.; Hsu, M.; Huang, W.C.; Balar, A.V. Pembrolizumab in the treatment of locally advanced or metastatic urothelial carcinoma: clinical trial evidence and experience. *Ther. Adv Urol.* **2019**, *11*, 1756287219839285.
45. Messina, C.; Buzzatti, G.; Dellepiane, C.; Cavo, A.; Tolomeo, F.; Cattrini, C.; Boccardo, F. Genitourinary tumours in the targeted therapies era: new advances in clinical practice and future perspectives. *Anticancer Drugs.* **2016**, *27*, 917.
46. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature.* **2014**, *507*, 315–322.

47. Robertson, A.G.; Kim, J.; Al-Ahmadie, H.; Bellmunt, J.; Guo, G.; Cherniack, A.D.; Hinoue, T.; Laird, P.W.; Hoadley, K.A.; Akbani, R.; et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. *Cell*. **2017**, *171*, 540–56.e25.
48. Kamoun, A.; de Reyniès, A.; Allory, Y.; Sjobahl, G.; Robertson, A.G.; Seiler, R.; Hoadley, K.A.; Groeneveld, C.S.; Al-Ahmadie, H.; Choi, W.; et al. A Consensus Molecular Classification of Muscle-invasive Bladder Cancer. *Eur Urol*. **2020**, *77*, 420–433.
49. Alifrangis, C.; McGovern, U.; Freeman, A.; Powles, T.; Linch, M. Molecular and histopathology directed therapy for advanced bladder cancer. *Nat Rev Urol*. **2019**, *16*, 465–483.
50. Knowles, M.A.; Hurst, C.D. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. *Nat. Rev. Cancer*. **2015**, *15*, 25–41 (2015).
51. Billerey, C.; Chopin, D.; Aubriot-Lorton, M.H.; Ricol, D.; Gil Diez de Medina, S.; Van Rhijn, B.; Bralet, M.P.; Lefrere-Belda, M.A.; Lahaye, J.B.; Abbou, C.C.; et al. Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors. *Am J Pathol*. **2001**, *158*, 1955–1959.
52. van Rhijn, B.W.; Lurkin, I.; Radvanyi, F.; Kirkels, W.J.; van der Kwast, T.H.; Zwarthoff, E.C. The fibroblast growth factor receptor 3 (FGFR3) mutation is a strong indicator of superficial bladder cancer with low recurrence rate. *Cancer Res*. **2001**, *61*, 1265–1268.
53. Tomlinson, D.C.; Baxter, E.W.; Loadman, P.M.; Hull, M.A.; Knowles, M.A. FGFR1-induced epithelial to mesenchymal transition through MAPK/PLCgamma/COX-2-mediated mechanisms. *PLoS One*. **2012**, *7*, e38972.
54. Cheng, T.; Roth, B.; Choi, W.; Black, P.C.; Dinney, C.D.; McConkey, D.J. Fibroblast growth factor receptors-1 and -3 play distinct roles in the regulation of bladder cancer growth and metastasis: implications for therapeutic targeting. *PLoS One*. **2013**, *8*, e57284.
55. Cappellen, D.; De Oliveira, C.; Ricol, D.; de Medina, S.; Bourdin, J.; Sastre-Garau, X.; Chopin, D.; Thiery, J.P.; Radvanyi, F. Frequent activating mutations of *FGFR3* in human bladder and cervix carcinomas. *Nature Genet*. **1999**, *23*, 18–20.
56. Helsten, T.; Elkin, S.; Arthur, E.; Tomson, B.N.; Carter, J.; Kuzrock, R. The FGFR landscape in cancer: analysis of 4,853 tumors by next-generation sequencing. *Clin. Cancer Res*. **2016**, *22*, 259–267.
57. Tomlinson, D.C.; Knowles, M.A. Altered splicing of *FGFR1* is associated with high tumor grade and stage and leads to increased sensitivity to *FGF1* in bladder cancer. *Am J Pathol*. **2010**, *177*, 2379–2386.
58. Sweis, R.F.; Spranger, S.; Bao, R.; Paner, G.P.; Stadler, W.M.; Steinberg, G.; Gajewski, T.F. Molecular Drivers of the Non-T-cell-Inflamed Tumor Microenvironment in Urothelial Bladder Cancer. *Cancer Immunol Res*. **2016**, *4*, 563–568.
59. Casadei, C.; Dizman, N.; Schepisi, G.; Cursano, M.C.; Basso, U.; Santini, D.; Pal, S.K.; De Giorgi, U. Targeted therapies for advanced bladder cancer: new strategies with FGFR inhibitors. *Ther. Adv. Med. Oncol*. **2019**, *11*, 1758835919890285.
60. Llorca, Y.; Necchi, A.; Park, S.H.; Garcia-Donas, J.; Huddart, R.; Burgess, E.; Fleming, M.; Rezazadeh, A.; Mellado, B.; Varlamov, S.; et al. Erdafitinib in Locally Advanced or Metastatic Urothelial Carcinoma. *N Engl J Med*. **2019**, *381*, 338–348.
61. Mazzola, C.R.; Chin, J. Targeting the VEGF pathway in metastatic bladder cancer. *Expert Opin. Investig. Drugs*. **2015**, *24*, 913–927.
62. Aldebasi, Y.H.; Rahmani, A.H.; Khan, A.A.; Aly, S.M. The effect of vascular endothelial growth factor in the progression of bladder cancer and diabetic retinopathy. *Int. J. Clin. Exp. Med*. **2013**, *6*, 239–251.
63. Dickinson, A.J.; Fox, S.B.; Persad, R.A.; Hollyer, J.; Sibley, G.N.; Harris, A.L. Quantification of angiogenesis as an independent predictor of prognosis in invasive bladder carcinomas. *Br. J. Urol*. **1994**, *74*, 762–766.
64. Bochner, B.; Cote, R.J.; Weidner, N.; Groshen, S.; Chen, S.C.; Skinner, D.G.; Nichols, P.W. Angiogenesis in bladder cancer: relationship between microvessel density and tumour prognosis. *J. Natl. Cancer Inst*. **1995**, *87*, 1603–1612.
65. Bernardini, S.; Fauconnet, S.; Chabannes, E.; Henry, P.C.; Adessi, G.; Bittard, H. Serum levels of vascular endothelial growth factor as a prognostic factor in bladder cancer. *J. Urol*. **2001**, *166*, 1275–1279.
66. Dreicer, R.; Li, H.; Stein, M.; DiPaola, R.; Eleff, M.; Roth, B.J.; Wilding, G. Phase 2 trial of sorafenib in patients with advanced urothelial cancer: a trial of the Eastern Cooperative Oncology Group. *Cancer*. **2009**, *115*, 4090–4095.
67. Bellmunt, J.; Gonzalez-Larriba, J.L.; Prior, C.; Maroto, P.; Carles, J.; Castellano, D.; Mellado, B.; Gallardo, E.; Perez-Gracia, J.L.; Aguilar, G.; et al. Phase II study of sunitinib as first-line treatment of urothelial

- cancer patients ineligible to receive cisplatin-based chemotherapy: baseline interleukin-8 and tumor contrast enhancement as potential predictive factors of activity. *Ann. Oncol.* **2011**, *22*, 2646–2653.
68. Necchi, A.; Mariani, L.; Zaffaroni, N.; Schwartz, L.H.; Giannatempo, P.; Crippa, F.; Morosi, C.; Lanocita, R.; Sava, T.; Ortega, C.; et al. Pazopanib in advanced and platinum-resistant urothelial cancer: an open-label, single group, phase 2 trial. *Lancet. Oncol.* **2012**, *13*, 810–816.
 69. Apolo, A.B.; Parnes, H.L.; Francis, D.C.; Cordes, L.M.; Berninger, M.; Lamping, E.; Costello, R.; Trepel, J.B.; Merino, M.J.; Folio, L.; et al. A phase II ^[18F]study of cabozantinib in patients (pts) with relapsed or refractory metastatic urothelial carcinoma (mUC). *J Clin Oncol.* **2016**, *34*(15 suppl), abstr 4534.
 70. Choueiri, T.K.; Ross, R.W.; Jacobus, S.; Vaishampayan, U.; Yu, E.Y.; Quinn, D.I.; Hahn, N.M.; Hutson, T.E.; Sonpavde, G.; Morrissey, S.C.; et al. Double-blind, randomized trial of docetaxel plus vandetanib versus docetaxel plus placebo in platinum-pretreated metastatic urothelial cancer. *J. Clin. Oncol.* **2012**, *30*, 507–512.
 71. Galsky, M.D.; Hahn, N.M.; Powles, T.; Hellerstedt, B.A.; Lerner, S.P.; Gardner, T.A.; Yu, M.; O'Rourke, M.; Vogelzang, N.J.; Kocs, D.; et al. Gemcitabine, Cisplatin, and sunitinib for metastatic urothelial carcinoma and as preoperative therapy for muscle-invasive bladder cancer. *Clin. Genitourin. Cancer.* **2013**, *11*, 175–181.
 72. Hahn, N.M.; Stadler, W.M.; Zon, R.T.; Waterhouse, D.; Picus, J.; Nattam, S.; Johnson, C.S.; Perkins, S.M.; Waddell, M.J.; Sweeney, C.J.; Hoosier Oncology Group. Phase II trial of cisplatin, gemcitabine, and bevacizumab as first-line therapy for metastatic urothelial carcinoma: Hoosier Oncology Group GU 04-75. *J. Clin. Oncol.* **2011**, *29*, 1525–1530.
 73. Rosenberg, J.E.; Ballman, K.V.; Halabi, S.; Watt, C.; Hahn, O.M.; Steen, P.D.; Dreicer, R.; Flaig, T.W.; Stadler, W.M.; Sweeney, C.; et al. CALGB 90601 (Alliance): Randomized, double-blind, placebo-controlled phase III trial comparing gemcitabine and cisplatin with bevacizumab or placebo in patients with metastatic urothelial carcinoma. *J. Clin. Oncol.* **2019**, *37*, no. 15_suppl, 4503-4503.
 74. Petrylak, D.P.; de Wit, R.; Chi, K.N.; Drakaki, A.; Sternberg, C.N.; Nishiyama, H.; Castellano, D.; Hussain, S.; Flechon, A.; Bamias, A.; et al. Ramucirumab plus docetaxel versus placebo plus docetaxel in patients with locally advanced or metastatic urothelial carcinoma after platinum-based therapy (RANGE): a randomised, double-blind, phase 3 trial. *Lancet.* **2017**, *390*, 2266–2277.
 75. Petrylak, D.P.; de Wit, R.; Chi, K.N.; Drakaki, A.; Sternberg, C.N.; Nishiyama, H.; Castellano, D.; Hussain, S.; Flechon, A.; Bamias, A.; et al. Ramucirumab plus docetaxel versus placebo plus docetaxel in patients with locally advanced or metastatic urothelial carcinoma after platinum-based therapy (RANGE): overall survival and updated results of a randomised, double-blind, phase 3 trial. *Lancet Oncol.* **2020**, *21*, 105–120.
 76. Sarfaty, M.; Rosenberg, J.E. Antibody-Drug Conjugates in Urothelial Carcinomas. *Curr. Oncol. Rep.* **2020**, *22*, 13.
 77. Rosenberg, J.; Sridhar, S.S.; Zhang, J.; Smith, D.; Ruether, D.; Flaig, T.W.; Baranda, J.; Lang, J.; Plimack, E.R.; Sangha, R.; 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. *J. Clin. Oncol.* **2020**, *38*, 1041-1049.
 78. Petrylak, D.P.; Balar, A.V.; O'Donnell, P.H.; McGregor, B.A.; Heath, E.I.; Yu, E.Y.; Galsky, M.D.; Hahn, N.M.; Gartner, E.M.; Pinelli, J.; et al. EV-201: Results of enfortumab vedotin monotherapy for locally advanced or metastatic urothelial cancer previously treated with platinum and immune checkpoint inhibitors. *J. Clin. Oncol.* **2019**, *37*, no. 18_suppl, 4505-4505.
 79. Hoimes, C.J.; Rosenberg, J.E.; Srinivas, S.; Petrylak, D.P.; Milowsky, M.; Merchan, J.R.; Bilen, M.A.; Gupta, S.; Carret, A.S.; Yuan, N.; et al. EV-103: initial results of enfortumab vedotin plus pembrolizumab for locally advanced or metastatic urothelial carcinoma. *Ann. Oncol.* **2019**, *30*(suppl_5), v356-v402.
 80. Rosenberg, J.E.; O'Donnell, P.H.; Balar, A.V.; McGregor, B.A.; Heath, E.I.; Yu, E.Y.; Galsky M.D.; Hahn, N.M.; et al. Pivotal Trial of Enfortumab Vedotin in Urothelial Carcinoma After Platinum and Anti-Programmed Death 1/Programmed Death Ligand 1 Therapy. *J. Clin. Oncol.* **2019**, *37*, 2592-2600.
 81. Rosenberg, J.E.; Flaig, T.W.; Friedlander, T.W.; Milowsky, M.I.; Srinivas, S.; Petrylak, D.P.; Merchan, J.R.; Bilen, M.A.; Carret, A.S.; Yuan, N.; et al. Study EV-103: Preliminary durability results of enfortumab vedotin plus pembrolizumab for locally advanced or metastatic urothelial carcinoma. *J. Clin. Oncol.* **2020**, *38*, no. 6_suppl 441-441.
 82. Tagawa, S.T.; Morris Faltas, B.; Tat Lam, E.; Saylor, P.J.; Bardia, A.; Hajdenberg, J.; Morgans, A.K.; Lim, E.A.; Kalinsky, K.; Simpson, P.S.; et al. Sacituzumab govitecan (IMMU-132) in patients with previously treated metastatic urothelial cancer (mUC): Results from a phase I/II study. *J. Clin. Oncol.* **2019**, *37*, 2019 (suppl 7S; abstr 354).

83. Tagawa, S.T.; Balar, A.; Petrylak, D.P.; Grivas, P.; Agarwal, N.; Sternberg, C.N.; Hong, Q.; Gladden, A.; Kanwal, C.; Siemon-Hryczyk, P.; et al. Initial Results From Trophy-U-01: A Phase 2 Open-Label Study Of Sacituzumab Govitecan In Patients (Pts) With Metastatic Urothelial Cancer (Muc) After Failure Of Platinum-Based Regimens (Plt) Or Immunotherapy. *Ann. Oncol.* **2019**, *30* (suppl_5), v851-v934.
84. Petrylak, D.P.; Tagawa, S.T.; Jain, R.K.; Bupathi, M.; Balar, A.V.; Rezaadeh, A.; et al. Early results of TROPHY-U-01 Cohort 2: Sacituzumab govitecan (SG) in platinum-ineligible patients (pts) with metastatic urothelial cancer (mUC) who progressed after prior checkpoint inhibitor (CPI) therapy. *J. Clin. Oncol.* **2020**, *38* (suppl; abstr 5027).
85. Powles, T.; Gschwend, J.E.; Loriot, Y.; Bellmunt, J.; Geczi, L.; Vulsteke, C.; Abdelslam, M.; Gafanov, R.; Bae, W.K.; Revesz, J.; et al. Phase 3 KEYNOTE-361 trial: Pembrolizumab (pembro) with or without chemotherapy versus chemotherapy alone in advanced urothelial cancer. *J. Clin. Oncol.* **2017**, *35*, e15.
86. Galsky, M.D.; Grande, E.; Davis, I.D.; De Santis, M.; Arranz Arijia, J.A.; Kikuchi, E.; Mecke, A.; Thastrom, A.C.; Bamias, A. IMvigor130: A randomized, phase III study evaluating first-line (1L) atezolizumab (atezo) as monotherapy and in combination with platinum-based chemotherapy (chemo) in patients (pts) with locally advanced or metastatic urothelial carcinoma (mUC). *J. Clin. Oncol.* **2018**, *36*, e15.
87. Qin, S.; Finn, R.S.; Kudo, M.; Meyer, T.; Vogel, A.; Ducreux, M.; Macarulla, T.M.; Tomasello, G.; Boisserie, F.; Hou, J.; Li, X.; et al. RATIONALE 301 study: tislelizumab versus sorafenib as first-line treatment for unresectable hepatocellular carcinoma. *Future Oncol.* **2019**, *15*, 1811-1822.
88. Mollica, V.; Di Nunno, V.; Gatto, L.; Santoni, M.; Cimadamore, A.; Cheng, L.; Lopez-Beltran, A.; Montironi, R.; Pisconti, S.; Battelli, N.; et al. Novel Therapeutic Approaches and Targets Currently Under Evaluation for Renal Cell Carcinoma: Waiting for the Revolution. *Clin. Drug Investig.* **2019**, *39*, 503-519.
89. Hassel, J.C.; Heinzerling, L.; Aberle, J.; Bahr, O.; Eigentler, T.K.; Grimm, M.O.; Grunwald, V.; Leipe, J.; Reinmuth, N.; Tietze, J.K.; et al. Combined immune checkpoint blockade (anti-PD-1/anti-CTLA-4): Evaluation and management of adverse drug reactions. *Cancer Treat Rev.* **2017**, *57*, 36-49.
90. Larkin, J.; Chiarion-Sileni, V.; Gonzalez, R.; Grob, J.J.; Cowey, C.L.; Lao, C.D.; Schadendorf, D.; Dummer, R.; Smylie, M.; Rutkowski, P.; et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med.* **2015**, *373*, 23-34.
91. Sharma, P.; Siefker-Radtke, A.; De Braud, F.; Basso, U.; Calvo, E.; Bono, P.; Morse, M.A.; Ascierto, P.A.; Lopez-Martin, J.; Brossart, P.; 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. *J Clin. Oncol.* **2019**, *37*, 1608-1616.
92. Rosenberg, J.E.; Sharma, P.; De Braud, F.; Basso, U.; Calvo, E.; Bono, P.; Morse, M.A.; Ascierto, P.A.; Lopez-Martin, J.; Brossart, P.; et al. Nivolumab(N) alone or in combination with ipilimumab (I) in patients (pts) with platinum-pretreated metastatic urothelial carcinoma (mUC), including the nivolumab 1 mg/kg + ipilimumab 3 mg/kg expansion from CheckMate 032. *Ann. Oncol.* **2018**, *29*, e32.
93. Galsky, M.D.; Powles, T.; Li, S.; Hennicken, D.; Sonpavde, G. A phase 3, open-label, randomized study of nivolumab plus ipilimumab or standard of care (SoC) vs. SoC alone in patients (pts) with previously untreated unresectable or metastatic urothelial carcinoma (mUC.; CheckMate 901). *J. Clin. Oncol.* **2018**, *36*, TPS4588.
94. Powles, T.; Galsky, M.D.; Castellano, D.; Van Der Heijden, M.S.; Petrylak, D.P.; Armstrong, J.; Belli, R.; Ferro, S.; Ben., Y.; Bellmunt, J. A phase 3 study of first-line durvalumab (MEDI4736) ± tremelimumab versus standard of care (SoC) chemotherapy(CT) in patients (pts) with unresectable stage IV urothelial bladder cancer (UBC): DANUBE. *J. Clin. Oncol.* **2016**, *34*, TPS4574.
95. Galsky, M.D.; Necchi, A.; Sridhar, S.S.; Ogawa, O.; Angra, N.; Hois, S.; He, P.; Ghiorghiu, D.C.; Bellmunt, J. A phase III, randomized, open label, multicenter, global study of first-line (1L) durvalumab in combination with standard of care (SOC) chemotherapy and durvalumab in combination with tremelimumab and SOC chemotherapy versus SOC chemotherapy alone in patients with unresectable locally advanced or metastatic urothelial cancer (UC). *J. Clin. Oncol.* **2019**, *37*, e15.
96. Mazzola, C.R.; Chin, J. Targeting the VEGF pathway in metastatic bladder cancer. *Expert Opin. Investig. Drugs.* **2015**, *24*, 913-927.
97. Geldart, T.; Chester, J.; Casbard, A.; Crabb, S.; Elliott, T.; Protheroe, A.; Huddart, R.A.; Mead, G.; Barber, J.; Jones, R.J.; et al. SUCCINCT: an open-label, single-arm, non-randomised, phase 2 trial of gemcitabine and cisplatin chemotherapy in combination with sunitinib as first-line treatment for patients with advanced urothelial carcinoma. *Eur. Urol.* **2015**, *67*, 599-602.

98. Petrylak, D.P.; Arkenau, H.-T.; Perez-Gracia, J.L.; Krebs, M.; Santana- Davila, R.; Yang, J.; Rege, J.; Mi, G.; Ferry, D.; Herbst, R.S. A multicohort phase I study of ramucirumab (R) plus pembrolizumab (P): Interim safety and clinical activity in patients with urothelial carcinoma. *J. Clin. Oncol.* **2017**, *35*, e349.
99. Baxter, M.A.; Glen, H.; Evans, T.R. Lenvatinib and its use in the treatment of unresectable hepatocellular carcinoma. *Future Oncol.* **2018**, *14*, 2021-2029.
100. Abdelaziz, A.; Vaishampayan, U. Cabozantinib for the treatment of kidney cancer. *Expert Rev. Anticancer Ther.* **2017**, *17*, 577-584.
101. Di Nunno, V.; Massari, F.; Mollica, V.; Cimadamore, A.; Santoni, M.; Cheng, L.; Lopez-Beltran, A.; Scarpelli, M.; Montironi, R. Another one in the chamber: cabozantinib for patients with metastatic non clear cell renal cell carcinoma. *Ann. Transl. Med.* **2019**, *7*(Suppl 3), S137.
102. Apolo, A.B.; Mortazavi, A.; Stein, M.N.; Davarpanah, N.N.; Nadal, R.M.; Parnes, H.L.; Ning, Y.M.; Francis, D.C.; Cordes, L.M.; Berniger, M.A.; et al. A phase I study of cabozantinib plus nivolumab (CaboNivo) and cabonivo plus ipilimumab (CaboNivoIpi) in patients (pts) with refractory metastatic (m) urothelial carcinoma (UC) and other genitourinary (GU) tumors. *J. Clin. Oncol.* **2017**, *35*, e4562.
103. Dolan, M. Mastri, M.; Tracz, A.; Christensen, J.G.; Chatta, G.; Ebos, J.M.L. Enhanced efficacy of sitravatinib in metastatic models of antiangiogenic therapy resistance. *PLoS One.* **2019**, *14*, e0220101.
104. Sternberg, C.N.; Loriot, Y.; James, N.; Choy, E.; Castellano, D.; Lopez-Rios, F.; Banna, G.L.; De Giorgi, U.; Masini, C.; Bamias, A.; et al. Primary Results from SAUL, a Multinational Single-arm Safety Study of Atezolizumab Therapy for Locally Advanced or Metastatic Urothelial or Nonurothelial Carcinoma of the Urinary Tract. *Eur. Urol.* **2019**, *76*, 73-81.
105. Khalil, D.N.; Smith E.L.; Brentjens, R.J.; Wolchok, J.D. The future of cancer treatment: immunomodulation, CARs and combination immunotherapy. *Nat. Rev. Clin. Oncol.* **2016**, *13*, 273-290.
106. Zhai, L.; Ladomersky, E.; Lenzen, A.; Nguyen, B.; Patel, R.; Lauing K.L.; Wu, M.; Wainwright, D.A. IDO1 in cancer: a Gemini of immune checkpoints. *Cell. Mol. Immunol.* **2018**, *15*, 447-457.
107. Theate, I.; van Baren, N.; Pilotte, L.; Moulin, P.; Larrieu, P.; Renauld, J.C.; Hervé, C.; Gutierrez-Roelens, I.; Marbaix, E.; Sempoux, C.; et al. Extensive profiling of the expression of the indoleamine 2,3-dioxygenase 1 protein in normal and tumoral human tissues. *Cancer Immunol. Res.* **2015**, *3*, 161-172.
108. Meireson, A.; Chevolet, I.; Hulstaert, E.; Ferdinande, L.; Ost, P.; Geboes, K.; De Man, M.; Van de Putte, D.; Verset, L.; Kruse, V.; et al. Peritumoral endothelial indoleamine 2, 3-dioxygenase expression is an early independent marker of disease relapse in colorectal cancer and is influenced by DNA mismatch repair profile. *Oncotarget.* **2018**, *9*, 25216-25224.
109. Dill, E.A.; Dillon, P.M.; Bullock, T.N.; Mills, A.M. IDO expression in breast cancer: an assessment of 281 primary and metastatic cases with comparison to PD-L1. *Mod. Pathol.* **2018**, *31*, 1513-1522.
110. Wing, J.B.; Tay, C.; Sakaguchi, S. Control of Regulatory T Cells by Co-signal Molecules. *Adv. Exp. Med. Biol.* **2019**, *1189*, 179-210.
111. So, T.; Ishii, N. The TNF-TNFR Family of Co-signal Molecules. *Adv. Exp. Med. Biol.* **2019**, *1189*, 53-84.
112. Kashima, J.; Okuma, Y.; Hosomi, Y.; Hishima, T. High Serum OX40 and OX40 Ligand (OX40L) Levels Correlate with Reduced Survival in Patients with Advanced Lung Adenocarcinoma. *Oncology.* **2020**, *25*, 1-8.
113. Piconese, S.; Valzasina, B.; Colombo, M.P. OX40 triggering blocks suppression by regulatory T cells and facilitates tumor rejection. *J. Exp. Med.* **2008**, *205*, 825-839.
114. Redmond, W.L.; Linch, S.N.; Kasiewicz, M.J. Combined targeting of co-stimulatory (OX40) and co-inhibitory (CTLA-4) pathways elicits potent effector T cells capable of driving robust anti-tumor immunity. *Cancer Immunol. Res.* **2014**, *2*, 142-153.
115. Bentebibel, S.E.; Hurwitz, M.E.; Bematchez, C.; Haymaker, C.; Hudgens, C.W.; Kluger, H.M.; Tetzlaff, M.T.; Tagliaferri, M.A.; Zalevsky, J.; Hoch, U.; et al. A first-in-human study and biomarker analysis of NKTR-214, a novel IL-2-receptor beta/gamma (betagamma)-biased cytokine, in patients with advanced or metastatic solid tumors. *Cancer Discov.* **2019**, *9*, 711-721.
116. Sharma, M.; Khong, H.; Fa'ak, F.; Bentebible, S.E.; Janssen, L.M.E.; Chesson, B.C.; Creasy, C.A.; Forget, M.A.; Kahn, L.M.S; Pazdrak, B.; et al. Bempegaldesleukin selectively depletes intratumoral Tregs and potentiates T cell-mediated cancer therapy. *Nat. Commun.* **2020**, *11*, 661.
117. Long, L.; Zhang, X.; Chen, F.; Pan, Q.; Phiphatwatchara, P.; Zeng, Y.; Chen, H. The promising immune checkpoint LAG-3: from tumor microenvironment to cancer immunotherapy. *Genes Cancer.* **2018**, *9*, 176-189.

118. Lichtenegger, F.S.; Rothe, M.; Schnorfeil, F.M.; Deiser, K.; Krupka, C.; Ausberger, C.; Schalter, M.; Neitz, J.; Subklewe, M. Targeting LAG-3 and PD-1 to Enhance T Cell Activation by Antigen-Presenting Cells. *Front. Immunol.* **2018**, *9*, 385.
119. Fujio, K.; Yamamoto, K.; Okamura, T. Overview of LAG-3-Expressing, IL-10-Producing Regulatory T Cells. *Curr. Top. Microbiol. Immunol.* **2017**, *410*, 29–45 (2017).
120. Das, M.; Zhu, C.; Kuchroo, V.K. Tim-3 and its role in regulating antitumor immunity. *Immunol. Rev.* **2017**, *276*, 97–111.
121. Piao, Y.R.; Piao, L.Z.; Zhu, L.H.; Jin, Z.H.; Dong, X.Z. Prognostic value of T cell immunoglobulin mucin-3 in prostate cancer. *Asian Pac. J. Cancer. Prev.* **2013**, *14*, 3897–3901.
122. Zhou, E.; Huang, Q.; Wang, J.; Fang, C.; Yang, L.; Zhu, M.; Chen, J.; Chen, L.; Dong, M. Up-regulation of Tim-3 is associated with poor prognosis of patients with colon cancer. *Int. J. Clin. Exp. Pathol.* **2015**, *8*, 8018–8027.
123. Yang, M.; Yu, Q.; Liu, J.; Fu, W.; Cao, Y.; Yu, L.; Shao, S.; Wang, X.; Niu, H.; Wang, Y. T-cell immunoglobulin mucin-3 expression in bladder urothelial carcinoma: clinicopathologic correlations and association with survival. *J. Surg. Oncol.* **2015**, *112*, 430–435.
124. Knee, D.A.; Hewes, B.; Brogdon, J.L. Rationale for anti-GITR cancer immunotherapy. *Eur. J. Cancer.* **2016**, *67*, 1–10.
125. Zappasodi, R.; Sirard, C.; Li, Y.; Budhu, S.; Abu-Akeel, M.; Liu, C.; Yang, X.; Zhong, H.; Newman, W.; Qi, J.; et al. Rational design of anti-GITR-based combination immunotherapy. *Nat. Med.* **2019**, *25*, 759–766.
126. Riccardi, C.; Ronchetti, S.; Nocentini, G. Glucocorticoid-induced TNFR-related gene (GITR) as a therapeutic target for immunotherapy. *Expert Opin. Ther. Targets.* **2018**, *22*, 783–797.
127. Sermer, D.; Brentjens, R. CAR T-cell therapy: Full speed ahead. *Hematol. Oncol.* **2019**, *37 Suppl 1*, 95–100.
128. Wang, Z.; Wu, Z.; Liu, Y.; Han, W. New development in CAR-T cell therapy. *J. Hematol. Oncol.* **2017**, *10*, 53.
129. Brown, C.E.; Mackall, C.L. CAR T cell therapy: inroads to response and resistance. *Nat. Rev. Immunol.* **2019**, *19*, 73–74.
130. Aldous, A.R.; Dong, J.Z. Personalized neoantigen vaccines: A new approach to cancer immunotherapy. *Bioorg. Med. Chem.* **2018**, *26*, 2842–2849.
131. Criscitiello, C.; Viale, G.; & Curigliano, G. Peptide vaccines in early breast cancer. *Breast.* **2019**, *44*, 128–134.
132. Turner, N.; Tutt, A.; Ashworth, A. Hallmarks of ‘BRCAness’ in sporadic cancers. *Nat. Rev. Cancer.* **2004**, *4*, 814–819.
133. Mateo, J.; Lord, C.J.; Tutt, A.; Balmana, J.; Castroviejo-Bermejo, M.; Cruz, C.; Oaknin, A.; Kaye, S.B.; de Bono, J.S. A decade of clinical development of PARP inhibitors in perspective. *Ann. Oncol.* **2019**, *30*, 1437–1447.
134. Yap, K.L.; Kiyotani, K.; Tamura, K.; Antic, T.; Jang, M.; Montoya, M.; Campanile, A.; Yew, P.Y.; Ganshert, C.; Fujioka, T.; et al. Whole-exome sequencing of muscle-invasive bladder cancer identifies recurrent mutations of UNC5C and prognostic importance of DNA repair gene mutations on survival. *Clin. Cancer Res.* **2014**, *20*, 6605–6617.
135. Teo, M.Y.; Bambury, R.M.; Zabor, E.C.; Jordan, E.; Al-Ahmadie, H.; Boyd, M.E.; Bouvier, N.; Mullane, S.A.; Cha, E.K.; Roper, N.; et al. DNA damage response and repair gene alterations are associated with improved survival in patients with platinum-treated advanced urothelial carcinoma. *Clin. Cancer Res.* **2017**, *23*, 3610–3618.
136. Fong, P.C.; Yap, T.A.; Boss, D.S.; Carden, C.P.; Mergui-Roelvink, M.; Gourley, C.; De Greve, J.; Lubinski, J.; Shanley, S.; Messiou, C.; et al. Poly(ADP-ribose) polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. *J. Clin. Oncol.* **2010**, *28*, 2512–2519.
137. Rimar, K.J.; Tran, P.T.; Matulewicz, R.S.; Hussain, M.; Meeks, J.J. The emerging role of [5E]homologous recombination repair and PARP inhibitors in genitourinary malignancies. *Cancer.* **2017**, *123*, 1912–1924.
138. Criscuolo, D.; Morra, F.; Giannella, R.; Visconti, R.; Cerrato, A.; Celetti, A. New combinatorial strategies to improve the PARP inhibitors efficacy in the urothelial bladder Cancer treatment. *J. Exp. Clin. Cancer Res.* **2019**, *38*, 91.
139. Garje, R.; Vaddepally, R.K.; Zakharia, Y. PARP Inhibitors in Prostate and Urothelial Cancers. *Front Oncol.* **2020**, *10*, 114.
140. Grivas, P.; Loriot, Y.; Feyerabend, S.; Morales-Barrera, R.; Teo, M.Y.; Vogelzang, N.J.; Grande, E.; Zakharia, Y.; Adra, N.; Alva, A.S.; et al. Rucaparib for recurrent, locally advanced or metastatic urothelial

- carcinoma (mUC): results from ATLAS, a phase 2 open-label trial. *J. Clin. Oncol.* **2020**, *38*, no. 6_suppl, 440-440.
141. Galsky, M.D.; Uzilov, A.V.; McBride, R.B.; Wang, H.; Patel, V.G.; Sfakianos, J.; Wang, L.; Akers, N.; Iyer, G.; Solit, D.B.; et al. DNA damage response (DDR) gene mutations (mut), mut load, and sensitivity to chemotherapy plus immune checkpoint blockade in urothelial cancer (UC). *J. Clin. Oncol.* **2017**, *35* (6_suppl), 300.
 142. Teo, M.Y.; Seier, K.; Ostrovnaya, I.; Regazzi, A.M.; Kania, B.E.; Moran, M.M.; Cipolla, C.K.; Bluth, M.J.; Chaim, J.; Al-Ahmadie, H.; et al. Alterations in DNA Damage Response and Repair Genes as Potential Marker of Clinical Benefit From PD-1/PD-L1 Blockade in Advanced Urothelial Cancers. *J. Clin. Oncol.* **2018**, *36*, 1685-1694 (2018).
 143. Powles, T.; Balar, A.; Gravis, G.; Jones, R.; Ravaud, A.; Florence, J.; Grivas, P.; Petrylak, D.P.; Galsky, M.; Carles, J.; et al. An open-label, randomised, biomarker-directed, multi-arm study in metastatic urothelial cancer. Presented by Powles, T. at ESMO Congress 2019.
 144. Siefker-Radtke, A.O.; Lugowska, I.; Tupikowski, K.; Andric, Z.G.; Rezazadeh Kalebasty, A.; Curigliano, G.; Vaena, D.; Vogl, F.D; Currie, G.; Abella, S.; et al. Clinical activity of vofatamab (V), an FGFR3 selective antibody in combination with pembrolizumab (P) in metastatic urothelial carcinoma (mUC), updated interim analysis of FIERCE-22. *Ann. Oncol.* **2019**, *30* (suppl_5), v356-v402.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).