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Genitourinary Cancers (S Gupta, Section Editor)

Future Strategies Involving Immune Checkpoint Inhibitors in Advanced Urothelial Carcinoma

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Opinion statement

Immune checkpoint inhibitors have importantly improved the outcome of patients with urothelial carcinoma. Different immune checkpoint inhibitors are currently approved and used in first- and second-line setting. The multiple agents currently approved in these setting make the choice sometimes difficult for clinicians. Furthermore, only a minority of patients present drastic response and long-term benefit with current immunotherapy. In this review, we describe the current use of immunotherapy in urothelial carcinoma but we also highlight the new strategies of treatment involving immune checkpoint inhibitors; we describe the place of immunotherapy with chemotherapy, targeted agents, and anti-angiogenic agents, incorporating the recent results presented at ASCO 2020. This review explores also the different action mechanisms of immune checkpoint inhibitors and the molecular rational to evaluate these agents in other strategies, such as maintenance and salvage strategies. The new advances in biomarker development are also presented.

Introduction

Urinary tract cancer is the ninth most common malignancy in the world, with an annual incidence of almost 275,000 new cases worldwide and an estimated 165,000 deaths each year [1–4]. Urothelial carcinoma (UC), also known as transitional cell carcinoma, accounts for 90% of all histologies; less frequent types are represented by squamous cell carcinoma, adenocarcinoma, and small cell carcinoma [5]. UC develops mainly in bladder but can also arise from other sites in the urinary tract such as the kidney, ureter, and urethra. Seventy percent of patients with bladder cancer present "superficial" or non-muscle invasive bladder cancer (NMIBC) and are treated with a curative intent. Conversely, muscle-invasive UC (MIUC) is an aggressive disease that requires multimodality therapy including cystectomy and chemotherapy. Despite aggressive management, around 50% of patients with MIUC will develop metastases with an estimated median survival of 14 months [6]. In metastatic setting, standard treatment in fit and cisplatin-eligible patients consists in platinum-based therapies, such as gemcitabine and cisplatin (GC) or methotrexate, vinblastine, adriamycin, cisplatin (MVAC), and results in an overall response rates (ORR) ranging from 40 to 70%, a median progression-free survival (PFS) of approximately 8 months and a 5-year overall survival (OS) of 15% [7–10]. Cisplatin-ineligible patients, elderly, and frail patients harbor a poorer prognosis due to unfeasibility to administer cisplatin. The better understanding of UC pathogenesis has led to development of multiple new strategies including immune checkpoint inhibitors (ICIs). Even if ICIs have importantly improved the outcome of patients, ORR is low and only a minority of patients presents a long-term benefit. In order to improve the efficacy of ICIs, new strategies are currently evaluated including the combinations with chemotherapy or targeted agents as well as developing biomarkers to predict response/resistance to ICIs. In this article, we will review the current use of ICI in advanced UC and the rational to combine ICIs with other anticancer agents.

Current use of immune checkpoint inhibitors

ICIs play a key role in the management of metastatic UC (mUC) patients. Immune system is able to detect and eliminate cancer cells, as they exhibit differences in antigenicity from healthy cells. Tumor cells release tumor-associated antigens, named neoantigens, that are captured by antigen-presenting cells (APC) through the major histocompatibility complex class I (MHC-I). APC migrate to lymphoid organs, where they activate effector T cells, which in turn infiltrate tumors and kill cancer cells. However, malignant cells have developed different mechanisms to evade immune recognition; one such strategy involves the expression of cell-surface molecules, named immune checkpoints, on tumor and tumor-specific lymphocytes that are able to inhibit activated T cells. The most commonly investigated immune checkpoints are cytotoxic T lymphocyte–associated protein 4 (CTLA-4)/B7 and programmed cell death 1 (PD-1)/programmed death-ligand 1 (PD-L1). Activation of T cells requires interaction between CD28 on T cell and B7 on APC. CTLA-4 expressed on T cell exerts its inhibitory effect by competing with CD28 and by binding to B7, resulting in T cell inactivation in lymphoid tissues. In the same way, PD-1 is an inhibitory receptor expressed on T cells. When binding to PD-1, PD-L1 expressed on tumor cells (TC) transmits an inhibitory signal into T cells [11–13]. UC pathogenesis and prognosis appears to be closely related to immune regulation. First, increased tumor-infiltrating CD8+ lymphocytes have been associated with better outcome in patients with MIUC. In addition, UC is associated with production of high amount of neoantigens, which is required

for antigenicity and effective immune response. Moreover, high levels of infiltrating immune cells (IC) exhibiting increased PD-L1/PD-1 expression in NMIBC have been correlated with higher stage, higher frequency of postoperative recurrence, and poorer survival [14–16]. As UC is one of the cancers with highest rate of somatic mutations and PD-L1 expression, there is a rational to evaluate IC in this cancer [17]. ICIs are monoclonal antibodies (mAbs) that target immune checkpoints such as PD-1 (nivolumab and pembrolizumab), PD-L1 (atezolizumab, durvalumab, and avelumab), and CTLA-4 (ipilimumab and tremelimumab), and thereby disrupt the inhibitory signals and reactivate immune system (Fig. 1).

ICIs after failure of platinum-based therapy

Five ICIs have showed efficacy in mUC after failure of platinum-based therapy. Key trials are summarized in Table 1.

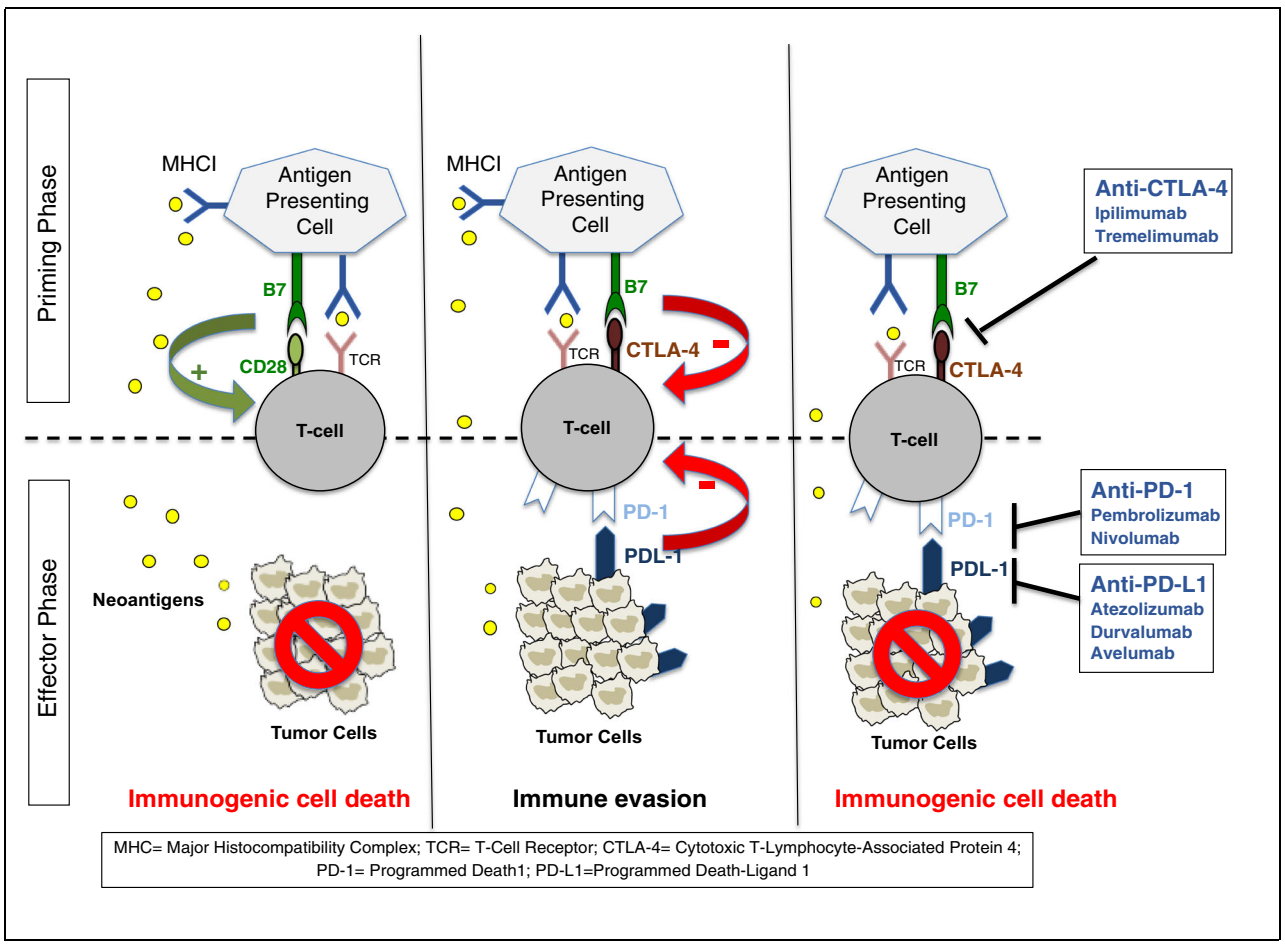

Fig. 1. Activation CTLA-4/B7 and PD-1/PD-L1 immune checkpoints lead to immune evasion of tumor cells. They could be inhibited by some specific monoclonal antibodies recently developed.

Table 1. Summary of key studies for immune checkpoint inhibitors in metastatic urothelial carcinoma after failure of platinum-based therapy. ***CPS***, **combined positive score;** ***CR***, **complete response;** ***DOR***, **duration of response;** ***n***, **number of patients;** ***NA***, **not assessed;** ***NR***, **not reached;** ***ORR***, **overall response rate;** ***OS***, **overall survival**

Study	Arm	Biomarker	n	ORR (%)		OS (months)	
				All patients	Positive biomarker	All patients	Positive biomarker
KEYNOTE-045, phase III (>2 years update) [18••]	Pembrolizumab	CPS≥10%	270	21.1 (CR: 9.3%)	20.3 (CR: NA)	10.1	NA
	Vinflunine or paclitaxel or docetaxel		272	11. (CR: 2.9%)	6.7 (CR: NA)	7.3	NA
IMvigor210 cohort 2, phase II [19]	Atezolizumab	PD-L1 in immune infiltrating cells ≥5%	310	15 (CR: 5%)	26 (CR: 11%)	11.4	7.9
IMvigor211, phase III [20•]	Atezolizumab	PD-L1 in immune infiltrating cells ≥5%	467	13.4 (CR: 3%)	23.0 (CR: 7%)	8.6	11.1
	Vinflunine or paclitaxel or docetaxel		464	13.4 (CR: 3%)	21.6 (CR: 7%)	8.0	10.6
CheckMate 275, phase II (update) [21]	Nivolumab	PD-L1 in tumor cells ≥1%	265	19.6 (CR: 2%)	23.8 (CR: 4%)	8.7	11.3
NCT01693562, phase I/II (update) [22]	Durvalumab	PD-L1 in tumor or immune infiltrating cells ≥25%	191.0	17.8 (CR: 3.4)	27.6 (CR: 4.1%)	18.2	20.0
Javelin, phase Ib [23]	Avelumab	PD-L1 in tumor cells ≥5%	44.0	17 (CR: 6)	24 (CR: 10)	6.5	8.2

Study	PFS (months)		DOR (months)		Treatment-related adverse events (%)	
	All patients	Positive biomarker	All patients	Positive biomarker	Any grade	Grade 3–4
KEYNOTE-045, phase III (>2 years update) [18••]	2.1	2.1	NR	NR	62.0	16.5
	3.3	3.1	4.3	4.4	90.6	50.2
IMvigor210 cohort 2, phase II [19]	2.7	4.0	NR	NR	69.0	16.0
IMvigor211, phase III [20•]	2.1	2.4	21.7	15.9	69	20
	4.0	4.2	7.4	8.3	89	43
CheckMate 275, phase II (update) [21]	2.0	NA	NR	NR	64	18.0
NCT01693562, phase I/II (update) [22]	1.5	2.1	NR	NR	60.7	6.8
Javelin, phase Ib [23]	6.3 weeks	11.9 weeks	NR	NR	58	8

Pembrolizumab received FDA approval in May 2017 with a level 1 of evidence in this setting based on the randomized phase III KEYNOTE-045 trial that compared efficacy of pembrolizumab (200 mg every 3 weeks) with chemotherapy (docetaxel, paclitaxel, or vinflunine) in 542 mUC patients who progressed during or after a platinum-based chemotherapy. Eighty percent of patients presented lower urinary tract UC, while 20% presented upper tract UC (UTUC). After a median follow-up of 14.1 months, OS was significantly improved in patients treated with pembrolizumab compared to patients treated with chemotherapy (10.3 vs 7.4 months, respectively; p=0.002). PFS was not significantly different between pembrolizumab and chemotherapy arms (2.1 vs 3.3 months, respectively; p=0.42). The ORR was significantly increased with pembrolizumab compared to chemotherapy (21.1% vs 11.4%, respectively; p=0.001); the complete response (CR) rate reached 7.0% in pembrolizumab arm and 3.3% in chemotherapy arm. Responses were durable with, at the time of data analysis, 18.4% of patients still receiving pembrolizumab compared with only 1.2% receiving chemotherapy. In this trial, PD-L1 status was measured based on a "Combined Positive Score" (CPS), which was measured as the percentage of PD-L1-positive IC and tumor cells (TC) compared with the total number of viable tumor cells. The CPS was not associated with a better OS, PFS, or ORR in pembrolizumab arm [24]. The 2-year follow-up confirmed the safety and the superiority of pembrolizumab compared to chemotherapy with a longer median duration of response (DOR) (not reached (NR) vs 4.4 months, respectively), and a greater proportion of responses lasting ≥12 months (68 vs 35%, respectively) [18••].

Atezolizumab was the first ICI to demonstrate clinical efficacy in mUC and received FDA approval in May 2016 as second-line treatment for mUC progressing during or after platinum-based chemotherapy, based on the results of the non-randomized phase II IMvigor210 cohort 2 [19]. This cohort enrolled 119 patients with inoperable locally advanced or mUC whose disease had progressed following platinum-based chemotherapy. Seventy-one percent had a bladder or urethra tumor and 29% presented UTUC. All these patients were stratified according to tumor-infiltrating IC PD-L1 expression in three subgroups: IC0 (<1%), IC1 (≥1% but <5%), and IC2/3 (≥5%). Importantly, 20% of patients were heavily pretreated with ≥3 previous chemotherapy regimens. Atezolizumab (1200 mg every 3 weeks) resulted in a 16% ORR, including 7% CR, in all treated patients; interestingly, ORR increased with PD-L1 expression, reaching 28%, including 15% CR in IC2/3 patients; however, up to 10% of responses were also seen in IC0 patients, suggesting that absence of PD-L1 expression does not exclude benefit from ICIs. Most responses were rapid, with a median time to response of 2.1 months (range 1.8–10.5). The responses tended to be durable with a median DOR that was not reached after a median follow-up of 17.5 months and 84% of ongoing responses. The median OS was 7.9 months for all patients and reached 11.4 months in IC2/3 patients, and the 1-year OS rate was 37% and 50%, respectively. Based on these promising results, the phase III IMvigor211 trial randomized 931 mUC patients between atezolizumab or chemotherapy (vinflunine, paclitaxel, docetaxel) after failure of platinum-based chemotherapy [20•]. The primary efficacy endpoint OS was to be tested in a hierarchical statistical model based on IC PD-L1 expression. Statistical significance needed to be first achieved in the IC2/3 population in order to evaluate the other subgroups of patients with any level of PD-L1

expression (IC0, IC1, IC2/3) as well as the overall population (intention-to-treat [ITT] population). Surprisingly, this study failed to demonstrated superiority of atezolizumab over chemotherapy, with a median OS of atezolizumab that was not significantly superior to chemotherapy in IC2/3 patients (11.1 vs 10.6 months, respectively; p=0.41) and with a 1-year OS rate of 46% with atezolizumab compared to 41% with chemotherapy. A moderate but significant increase in median OS was observed in the ITT population with atezolizumab compared to chemotherapy (8.6 vs 8.0 months, respectively; p=0.038). These disappointing results for atezolizumab could be explained by the fact that the OS in the chemotherapy arm, and particularly in the vinflunine arm, appeared better than study design assumptions. Another explanation is that the selection of patients with high PD-L1 expression could had selected patients with better prognosis, explaining such impressive survival both in atezolizumab and in chemotherapy arm. However, the longer median DOR with atezolizumab compared to chemotherapy (21.7 vs 7.4 months, respectively, in the ITT population) and the OS observed with atezolizumab, which was indirectly similar to that observed with pembrolizumab, confirmed robust antitumoral efficacy of atezolizumab. Atezolizumab appears thus as an alternative to chemotherapy in second-line setting in mUC, although absence of level 1.

Nivolumab is also FDA-approved as second-line therapy for mUC patients. This approval was based on the non-randomized phase II CheckMate 275 study that evaluated the activity and efficacy of nivolumab (3 mg/kg every 2 weeks) in 265 previously treated patients with mUC [25]. The primary endpoint was ORR confirmed by blinded independent review committee in all treated patients and across the TC PD-L1 expression levels (≥5%, ≥1%, and<1%). The ORR reached 19.6% in all patients, 28.4% in PD-L1≥5%, 23.8% in PD-L1≥1%, and 16.1% in PD-L1<1% patients. The median time to response was 2.0 months (range 1.6–13.8). The median OS was 8.6 months in all patients and increased to 11.9 months in PD-L1≥1% patients. After 2 years of follow-up, the ORR was 20.7%, including 7% CR in all treated patients, 25.8% in PD-L1≥1% patients, and 16.4% in PD-L1<1% patients. The median DOR was 20.3 months and 59% of patients who initially responded had a DOR ≥12 months. The median OS reached 8.6 months in all treated patients, 11.9 months in PD-L1≥1% patients, and 6.0 months in PD-L1<1% patients. The 12-, 24-, and 36-month OS reached 40%, 30%, and 22%, respectively [21].

Two other PD-L1 inhibitors agents showed great efficacy in inoperable or mUC patients progressing on platinum-based treatment. A phase I/II study evaluated *durvalumab* monotherapy (10 mg/kg every 2 weeks) in cohort of 191 patients with advanced UC [22, 26]. Based on PD-L1 expression on TCs or ICs, patients were stratified as PD-L1 high (≥25%) or PD-L1 low (<25%) expression. The ORR was 17.8% including 3.4% CR in overall population. The ORR reached 27.6% in high PD-L1 and 5.1% in low/negative PD-L1 expression patients. The median time to response was 1.41 month (range 1.2–7.2) and the median DOR in the all population had not been reached at data cut-off (range 0.9 to 19.9 months). The median OS reached 18.2 months (95% confident interval (CI), 8.1 not estimable) in all treated patients, which appears very promising in such pretreated patients; it reached 20.0 months (95% CI, 11.6 not estimable) in high PD-L1 expression and only 8.1 months (95% CI, 3.1 not estimable) in low/negative PD-L1 expression patients. The 6-, 9-, and 12-month OS rates were 64%, 57%, and 55%, respectively, in all treated patients. Based on

these first promising results, FDA granted accelerated approval to durvalumab in May 2017 for patients with advanced UC who have disease progression during or following platinum-containing chemotherapy or who have disease progression within the 12 months after neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.

Avelumab can, in addition to PD-L1 inhibition, induce an antibody-dependent cell-mediated cytotoxicity (ADCC), which results in a direct lysis of tumor cells. The JAVELIN trial examined the role of avelumab in mUC and led to its FDA approval in 2017 [23]. Two hundred forty-nine patients with advanced or mUC progressing after platinum-based therapy were enrolled in two sequential cohorts, an initial cohort and an expansion cohort, and received avelumab (10mg/kg every 2 weeks); 77% had lower tract UC and 23% had UTUC. Of the 206 patients evaluable for PD-L1 expression level, 33% had PD-L1 positive tumors (defined as TC PD-L1≥5%) and 50% had PD-L1 low/negative tumors (defined as TC PD-L1<5%). The median duration of treatment was 12.0weeks and median follow-up was 9.9months (4.3–12.1). The ORR was 17%, including 6% CR and 11% PR, and stable disease (SD) rate was 23% resulting in a disease control rate (defined as CR+PR+SD) of 40%. The median PFS was 6.3weeks (95% CI 6.0–10.1), the median OS was 6.5months (95% CI 4.8–9.5), and the 6-month OS rate was 53%.

All these five ICIs are thus approved in advanced UC after failure of platinum-based therapy, resulting in improved ORR and OS compared to chemotherapy. Due to their better tolerance, these agents also improved the quality of life of such heavily pretreated patients.

ICIs in frontline setting

Among these five ICIs, both atezolizumab and pembrolizumab were FDA-approved for cisplatin-ineligible patients in first-line for mUC due to favorable outcomes (Table 2).

In April 2017, atezolizumab was approved as first-line agent in cisplatin-ineligible patients, based on the results of the IMvigor210. The cohort 1 of this trial study enrolled 119 patients who were ineligible for cisplatin-based chemotherapy and who had received no prior chemotherapy in metastatic setting. These patients had an Eastern Cooperative Oncology Group (ECOG) performance status (PS)≤2, and were ineligible for cisplatin by at least one of the following criteria: glomerular fraction rate (GFR) >30 and <60 mL/min, ≥ grade 2 hearing loss or peripheral neuropathy or ECOG performance status 2; 70% were cisplatin-ineligible due to renal impairment; and 20% had an ECOG PS 2. After 17.2 months of median follow-up, the ORR was 23%, including 9% CR in all treated patients. Patients were stratified based on IC PD-L1 expression as follows: IC0 (<1%), IC1 (≥1% but <5%), and IC2/3 (≥5%). Conversely to the cohort 2 (second-line setting after failure of platinum-based therapy), there was no association between high PD-L1 expression and ORR, i.e., 28% ORR in IC2/3, 24% ORR in IC1/2/3, 21% ORR in IC1, and 21% ORR in IC0 PD-L1 patients. The median DOR had not been reached in all treated patients or in the different PD-L1 subgroups (95% CI 3.7–21.0 months) and 70% of responses were still ongoing. The median time to onset of first response was 2.1 months. The median OS was 16.3 months (95% CI 10.4–24.5) in all treated patients, with a 12- and 24-month OS rate of 58% and 41%, respectively. The median OS

Table 2. Summary of key studies for immune checkpoint inhibitors in metastatic urothelial carcinoma in platinum-ineligible patient. ***CPS***, **combined positive score;** ***CR***, **complete response;** ***DOR***, **duration of response;** ***n***, **number of patients;** ***NA***, **not assessed;** ***NR***, **not reached;** ***ORR***, **overall response rate;** ***OS***, **overall survival**

Study	Arm	Biomarker	n	ORR (%)		OS (months)	
				All patients	Positive biomarker	All patients	Positive biomarker
IMvigor210 Cohort 1, phase II [27]	Atezolizumab	PD-L1≥5%	119	23% (CR: 9.2%)	28% (CR: 12.5%)	15.9	12.3
KEYNOTE-052, phase II [28]	Pembrolizumab	CPS ≥10%	370	28.9% (CR: 8.1%)	47.3% (CR: NA)	11.5	18.5

Study	PFS (months)		DOR (months)		Treatment-related adverse events (%)	
	All patients	Positive biomarker	All patients	Positive biomarker	Any grade	Grade 3–4
IMvigor210 Cohort 1, phase II [27]	2.7	4.1	NR	NR	66.0	16.0
KEYNOTE-052, phase II [28]	NA	NA	NR	NA	67.6	20.3

reached 12.3 months in IC2/3 patients and 19.1 months in IC0/1PD-L1 patients. In exploratory subgroup analyses, responses were observed in patients with non-bladder UC, visceral metastases, and prior Bacillus Calmette-Guerin (BCG) treatment. Patients with high score Bajorin risk (as defined as a Karnofsky PS <80% and visceral metastases) had a lower response rate than those with low scores (11.1% vs 25.7%, respectively) [27, 29, 30].

In June 2017, pembrolizumab received accelerated approval for the treatment of cisplatin-ineligible mUC patients in first-line setting, based on the phase II trial KEYNOTE-052 that evaluated pembrolizumab as first-line agent in 370 cisplatin-ineligible patients [28, 31••]. The median age was 74 years; 10.8% of patients were ≥85 years old, 42.2% had ECOG PS 2, 49% had renal dysfunction, and 10% had both ECOG PS 2 and renal dysfunction. Furthermore, 85.1% had visceral disease, reflecting a frail and poor prognosis population. In the total population, ORR reached 28.9%, including 8.1% CR and 20.8% PR. The median DOR was 30.1 months (95% CI, 18.1 NR), and 67% and 52% of patients had DOR ≥12 and≥24 months, respectively. The median OS was 11.3 months and the 12- and 24-month OS rates were 47% and 31%, respectively. While the ORR was only 20% in CPS <10, it increased to 47.3% in patients with CPS ≥10. The median DOR for CPS <10 and CPS ≥10 patients was 18.2 months (95% CI, 9.7 NR) and NR (95% CI, 18.1 NR), respectively. Median OS for CPS <10 and CPS ≥10 patients was 9.7 months (95% CI, 7.6–11.5) and 18.5 months (95% CI, 12.2–28.5), respectively, and the 24-month OS rates were 24% and 47%, respectively. Pembrolizumab in the first-line setting is currently evaluated in the phase III KEYNOTE-361 trial (NCT02853305). Despite efficacy of these two ICIs in first-line setting in cisplatin-ineligible patients, the preliminary analysis by the Independent Data Monitoring Committee of the phase III IMvigor130 trial that randomized patients between atezolizumab alone and combination of chemotherapy plus atezolizumab/placebo showed that chemotherapy was more benefit in term of OS for patients with low/negative IC PD-L1 expression; this observation led to approval of ICIs in first-line mUC setting only in patients with positive PD-L1 expression [32•].

Future strategies implicating ICIs

Combination ICIs + chemotherapy

Some evidences defend the immunogenic potential of chemotherapy in synergizing efficacy of ICIs. Cytotoxic agents directly cause cancer cell death through multiple mechanisms, including lysis of tumor cells, DNA damage, inhibition of DNA replication, prevention of mitosis, and by this way, they induce a release of tumor neoantigens and enhance the immunogenic cell death. Chemotherapy may also induce direct immunoregulatory properties by promoting dendritic cell (DC) maturation and by enhancing the T cell activation by DCs. The chemotherapy-induced depletion of lymphocytes can also potentiate antigen-specific T cell responses, by eliminating immunosuppressive cells such as regulatory T cells (Treg), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages. Based on these observations, there is thus a rational to hypothesize that the addition of chemotherapy to ICI may further enhance the activities of cytotoxic T cells with improved clinical outcomes (Fig. 2) [33–43].

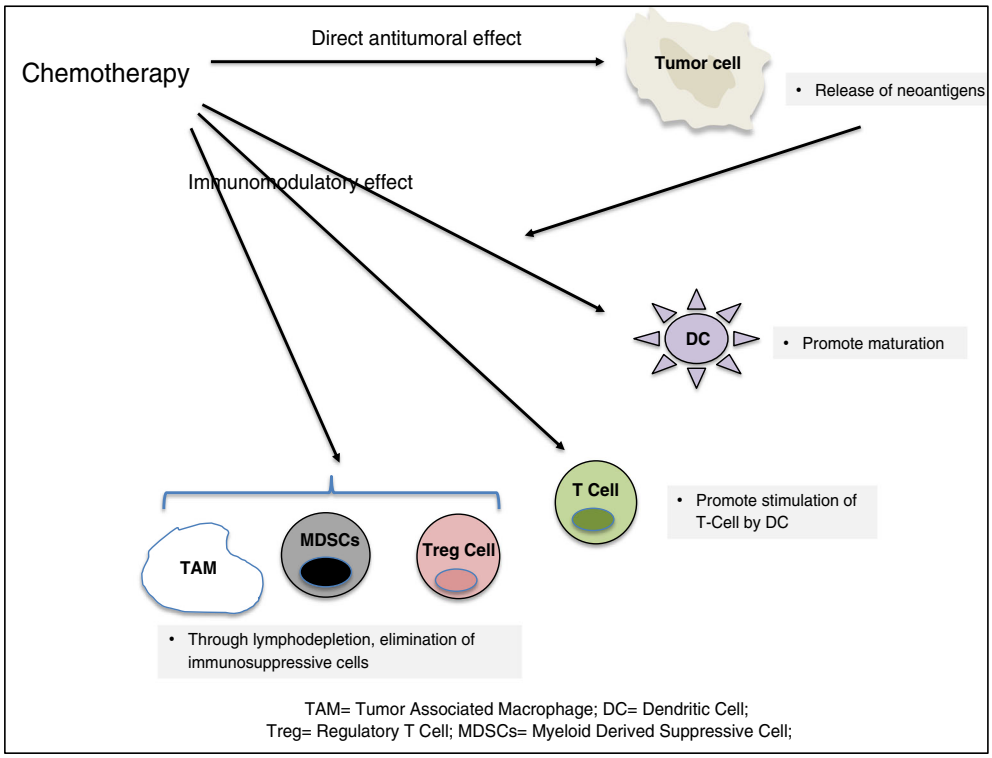

Fig. 2. Effect of cytotoxic agents on immune cells, suggesting a rational to evaluate chemotherapy in combination with immune checkpoint inhibitors.

The IMvigor130 trial was the first phase III trial to evaluate ICI and chemotherapy in mUC; this trial evaluated the association atezolizumab plus chemotherapy in 1200 cisplatin-eligible mUC patients in frontline setting, randomizing patients in a 1:1:1 fashion to atezolizumab plus platinum/gemcitabine (arm A) vs atezolizumab monotherapy (arm B) vs placebo plus platinum/gemcitabine (arm C). The two co-primary endpoints were PFS (arm A vs arm C) and OS (arm B vs arm C). The stratification factors were IC PD-L1 expression (IC0 vs IC1 vs IC2/3), Bajorin risk factors score, and investigator's choice of platin (cisplatin vs carboplatin). The PFS endpoint was met with a statistical difference between arm A and C. After a median follow-up of 11.8 months, atezolizumab plus chemotherapy combination improved significantly the PFS compared to placebo plus chemotherapy (8.2 vs 6.3 months, respectively; p=0.007). Exploratory subgroup analysis suggested that the benefit of atezolizumab plus chemotherapy combination was more important in patients with ECOG PS 0 (HR 0.69; 0.54–0.89), with IC2/3 PD-L1 expression (HR 0.68; 0.49–0.95) and in patients who received cisplatin (HR 0.73; 0.55–0.97). The interim analysis showed a trend for a better median OS in atezolizumab plus chemotherapy compared to placebo plus chemotherapy (16.0 vs 13.4 months, respectively) but it did not reach statistical significance. Combination therapy (atezolizumab or placebo combination) increased the ORR, including the CR rate, compared to atezolizumab alone; the ORR was 47% (CR 13%), 23% (CR 6%), and 44% (CR 7%) for arms A, B and C, respectively. There was, however, no difference in the ORR between atezolizumab plus chemotherapy and placebo plus chemotherapy. The OS co-primary endpoint was not met; when

comparing OS in arm B and C, atezolizumab monotherapy did not improve significantly OS compared to chemotherapy (15.7 vs 13.1 months, respectively; HR 1.07 (95% CI 0.86–1.33)). Although no formal statistical testing has been performed given the hierarchical design, the analysis of subgroups showed that IC2/3 PD-L1 patients presented a median OS improvement with atezolizumab monotherapy compared to placebo plus chemotherapy (NR vs 17.8 months, respectively). In IC0/1 PD-L1 patients, the median OS was similar between atezolizumab monotherapy and placebo plus chemotherapy combination (13.5 vs 12.9 months, respectively), but atezolizumab monotherapy was felt to be inferior to chemotherapy with a higher proportion of patients dying more rapidly when receiving atezolizumab compared to placebo plus chemotherapy. Based on this result, the trial was amended by the Independent Data Monitoring Committee to exclude enrollment of PD-L1 low patients to single agent atezolizumab but the majority of the patients had already been accrued. The combination atezolizumab plus chemotherapy was thus not considered as superior to chemotherapy alone; beyond the hierarchical statistical method to reach significance in the different subgroups, another explanation is that a large number of patients received carboplatin although they were cisplatin-eligible (25% in arm A and 31% in arm C), while it was demonstrated that in both combination arms (atezolizumab and placebo combinations), a non-significant survival benefit was observed when using cisplatin compared to carboplatin (HR 0.83, 95% CI 0.69–1.00, one-sided p=0.027). This suggests that cisplatin has to be used instead of carboplatin when proposing combination with ICI. In regards of these results and the absence of improvement in OS, there is no matter to date to consider atezolizumab plus chemotherapy combination as frontline standard treatment in mUC [32•, 44]. Another interesting point is that atezolizumab monotherapy was better tolerated than chemotherapy with a discontinuation rate of 34% in the chemotherapy arm vs 6% in the atezolizumab arm. Therefore, this confirms, in the same way than the IMvigor210 cohort 1 trial that patients with IC2/3 PD-L1 cisplatin-ineligible patients, atezolizumab monotherapy could be the best choice for these patients instead of carboplatin-based chemotherapy regimens.

Pembrolizumab is currently evaluated in combination with cisplatin/carboplatin plus gemcitabine in mUC patients in the phase III KEYNOTE-361 trial (NCT02853305) and results are expected soon.

The PEANUT trial evaluated the association of pembrolizumab and nanoparticle albumin-bound paclitaxel (Nab-paclitaxel) as salvage therapy for 65 mUC. Patients progressing after 1–2 platinum-based regimen for mUC were included and received pembrolizumab on day 1 and nab-paclitaxel on day 1 and 8 every 3 weeks. These patients were heavily pretreated as 25% of patients had ≥2 prior lines of systemic therapy and 33% of patients had liver metastases. The ORR in this cohort was 39%, including 14.3% CR. The median DOR has not been reached and 7% of patients had a DOR ≥12 months. Importantly, 72.5% of patients presented a shrinkage in target lesions. This treatment was feasible and well tolerated, with 30% of patients presenting grade 3 treatment-related adverse events (TRAEs) that were most commonly alopecia, neutropenia, and fatigue [45]. These results are promising as the ORR commonly observed in second-line ICIs range from 20 to 25%, as previously seen in CheckMate 275 or KEYNOTE-045 [18••, 21].

Other strategies combining ICIs and other cytotoxic agents are under investigation in different trials (Table 3).

Table 3. Ongoing phase II/III trials for combination of immune checkpoint inhibitors and chemotherapy in advanced/metastatic urothelial carcinoma. ***ICI*****, immune checkpoint inhibitor;** ***UC*****, urothelial carcinoma;** ***MTAP*****, methylthioadenosine phosphorylase**

Protocol	Phase	Anti-PD-1/PD-L1	Combined agent	Setting	Primary outcomes
NCT03737123	II	Atezolizumab	Carboplatin + Gemcitabine Docetaxel	Advanced or metastatic UC in ICI refractory patients	Progression-free survival
NCT03179943	II	Atezolizumab	Guadecitabine	Advanced or metastatic UC in ICI refractory or resistant patients	Response rate
NCT03390595	II	Avelumab	Carboplatin + gemcitabine	Advanced or metastatic UC in first-line platinum-ineligible patients	Response rate
NCT03744793	II	Avelumab	Permetrexed	MTAP-deficient metastatic urothelial cancer in platinum or anti-PD-1 /PD-L1 refractory patients	Response rate
NCT03324282	II	Avelumab	Carboplatin + gemcitabine	First-line for locally or metastatic bladder carcinoma	Response rate and safety
NCT02581982	II	Pembrolizumab	Paclitaxel	Metastatic UC in platinum refractory patients	Response rate
NCT03464734	II	Pembrolizumab	Nab-paclitaxel	Metastatic UC in platinum refractory patients	Progression-free survival
NCT02853305 (KEYNOTE-367)	III	Pembrolizumab	Cisplatin-gemcitabine Carboplatin-gemcitabine	First-line for locally or metastatic bladder carcinoma	Progression-free survival and overall survival

Combining ICI and ICI

CheckMate 032 is an open-label, multicohort study that evaluated the association of nivolumab and ipilimumab through different regimens in mUC patients: nivolumab 3 mg/kg every 2 weeks (NIVO3), nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every 3 weeks for 4 doses followed by nivolumab monotherapy 3 mg/kg every 2 weeks (NIVO3+IPI1), or nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 weeks for 4 doses followed by nivolumab monotherapy 3 mg/kg every 2 weeks (NIVO1+IPI3). Seventy-eight patients were treated with NIVO3 (minimum follow-up of 37.7 months), 104 with NIVO3+IPI1 (minimum follow-up of 38.8 months), and 92 with NIVO1+IPI3 (minimum follow-up of 7.9 months). The primary endpoint, ORR, was 25.6%, 26.9%, and 38.0% in the NIVO3, NIVO3+IPI1, and NIVO1+IPI3 arms, respectively. The median DOR was superior to 22 months in all arms. Grade 3–4 TRAEs occurred in 26.9%, 30.8%, and 39.1% of patients treated with NIVO3, NIVO3+IPI1, and NIVO1+IPI3, respectively [46]. These results confirmed the robust effect of ICI combinations in mUC but need further confirmation. The ongoing CheckMate 901 study (NCT03036098) is comparing the combination ipilimumab plus nivolumab to platinum-based chemotherapy in first-line treatment of mUC patients [47].

The phase III DANUBE trial (NCT02516241) compared standard chemotherapy (cisplatin or carboplatin plus gemcitabine) to durvalumab monotherapy and the combination durvalumab plus tremelimumab in the first-line metastatic treatment of both cisplatin-eligible and ineligible UC patients. One thousand four patients were enrolled and results are pending but early results showed that this trial did not meet its co-primary endpoints of improving OS compared with chemotherapy for durvalumab monotherapy in PD-L1≥25% patients or for combination durvalumab and tremelimumab regardless of PD-L1 expression [48].

The randomized phase III NILE trial (NCT03682068) is investigating durvalumab ± tremelimumab with standard chemotherapy followed by durvalumab vs standard of care alone as first-line treatment for mUC patients. The primary endpoints are OS and PFS. Secondary endpoints are ORR, duration of response, and time to second progression.

Maintenance strategies

Due to their low toxicity profile, the long-term benefit of ICIs and the non-cross resistance mechanism with chemotherapy, maintenance immune checkpoint blockade could potentially confer benefit after response to chemotherapy in mUC patients. A phase II trial randomized 108 mUC patients achieving at least SD on first-line platinum-based chemotherapy in double-blind 1:1 to switch to maintenance pembrolizumab (200 mg every 3 weeks) vs placebo for up to 24 months. Patients with disease progression on placebo could cross over to pembrolizumab. After a median follow-up of 12.9 months, the median PFS was longer with pembrolizumab compared to placebo (5.4 vs 3.0 months, respectively; p=0.04). The ORR was 23% with pembrolizumab and 10% with placebo. Despite the cross-over possibility, the median OS tended to be longer with pembrolizumab maintenance compared to placebo (22 vs 18.7 months, respectively; p=0.74). Among the 52 patients initially randomly assigned to placebo who experienced disease progression, 27 crossed over to receive

pembrolizumab; the ORR reached 22% in these patients with pembrolizumab, the median PFS and OS from cross-over were 2.7 months and 15.8 months, respectively. There was no significant interaction between PD-L1 CPS ≥10 and treatment arm on PFS or OS (p=0.8 and 0.9, respectively) [49].

The JAVELIN Bladder 100 is a randomized phase III study for maintenance avelumab therapy in patients who experiment disease response or stabilization from first-line platinum-based chemotherapy for advanced UC. Patients had to have received at least 4 cycles of frontline chemotherapy and have had at minimum SD on therapy. They were then randomized to best supportive care (BSC, n=350) or avelumab maintenance therapy (n=350). The primary endpoint of this study was OS in the ITT population as well as in the PD-L1-positive population. Cross-over was not allowed. Patient baseline characteristics were well balanced between groups, though the PD-L1-positive group had higher rates of non-visceral metastases. After a 19.6-month median follow-up in the avelumab arm and 19.2-month median follow-up in the BSC arm, 24% of avelumab patients and 7% of BSC arm were still receiving therapy. Avelumab maintenance prolonged significantly median OS compared to BSC (21.4 vs 14.3 months, respectively; p<0.001). At 12 months, 71% of avelumab patients were alive compared to 58% of BSC patients. This OS benefit was also seen in PD-L1 patients with avelumab compared to BSC (NR vs 17.1 months, respectively; p<0.001). Independent radiology review confirmed a radiographic PFS advantage for the avelumab treatment arm compared to BSC in the overall population (3.7 vs 2.0 months, respectively; p<0.001) as well as in the PD-L-positive patients (5.7 vs 2.1 months, respectively; p<0.001) [50•]. Maintenance with ICIs appears thus as a promising strategy for improving outcome of mUC patients. However, this should be confirmed; in one hand, it induced longer PFS and OS in some patients, but in other hand, it could overtreat some patients who have naturally durable response after initial chemotherapy. It will be important to identify which patients are more likely to benefit from maintenance therapy.

Combining ICI with anti-angiogenic

In order to enhance the continuous growth of tumors, an adequate vascular framework has to be maintained for the nutrient and oxygen supply to tumor cells. This angiogenic switch, leading to an uncontrolled proliferation of endothelial cells (EC), results from an excessive production of different angiogenic factors, such as vascular endothelial factor (VEGF), platelet-derived growth factor, placental growth factor, angiogenin. VEGF-A is one of the major drivers of angiogenesis that binds to VEGF receptor (VEGFR) family. VEGFR-2 is a highly active receptor that mediates broad signaling cascades and regulates many EC functions including proliferation, migration, and differentiation. Several studies have suggested that VEGF plays an important role in the growth of UC cells, as excessive expression of VEGF was observed in UC cells and altered VEGF expression was associated with advanced pathological stage and lymph node metastasis; furthermore, inhibition of VEGF transcripts significantly reduced growth and invasion of these cells [51–54]. Anti-angiogenic agents such as sunitinib, sorafenib, pazopanib, bevacizumab, and aflibercept showed modest ORR, PFS, and OS benefit in monotherapy or in association with chemotherapy in mUC [55–61]. A new role could emerge for anti-angiogenic

agents in the era of ICIs, as it is now well demonstrated that angiogenic factors can play a direct and indirect role in immunosuppressive tumor microenvironment. First, VEGF may inhibit the maturation of DC and the differentiation of hematopoietic progenitor cells into CD8+ and CD4+ T cells [62]. Furthermore, VEGF-A may directly upregulate PD-L1 expression on CD8+ T cells, decreasing their cytotoxic function. VEGF-A can also increase the expression of Fas ligand, a member of the tumor necrosis factor on tumor EC, activating the cascade of multiple caspases that lead to apoptosis of cytotoxic T cells [63]. Excessive amounts of VEGF-A can also modulate the expression of adhesion molecules such as vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, creating a specific barrier that blocks ICs. In addition, sustained and uncontrolled expression of VEGF, by increasing vascularization and tumor growth, may lead to an activated cellular consumption of oxygen and a subsequent hypoxia, which is known to modulate negatively the immune recognition of tumor cells, by enhancing the recruitment of T regulatory cells into the tumor and by promoting the shift of pro-inflammatory M1 tumor–associated macrophages towards the M2-like phenotype which are anti-inflammatory (Fig. 3) [64, 65]. There is thus a rational to hypothesize that anti-angiogenic therapy could, by decreasing the VEGF levels, by normalizing blood vessels, and by reducing hypoxia, increase T cells infiltration within the tumor, decrease recruitment of immunosuppressive cells, and improve efficacy of ICIs. Different trials are evaluating the association of anti-angiogenic and ICIs.

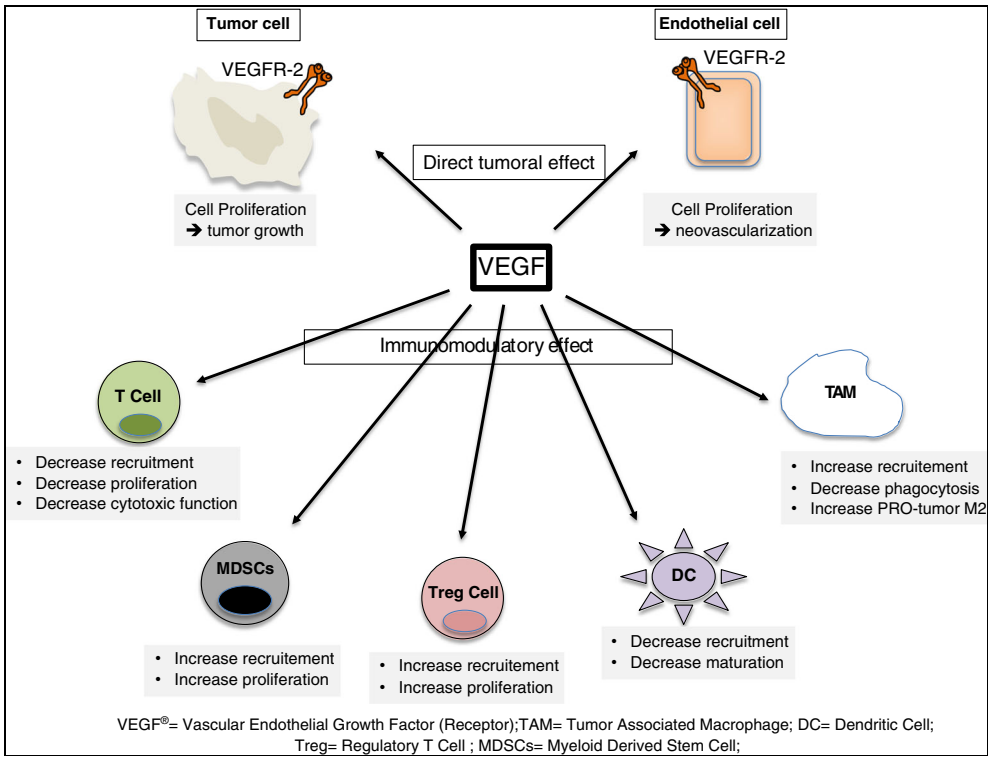

Fig. 3. In addition to the direct action on tumor cell proliferation and neovascularization, vascular endothelial growth factor can modulate some other important immunologic functions.

Cabozantinib is a potent inhibitor of c-MET and VEGFR-2 but it also inhibits other tyrosine kinase receptors such as RET, c-Kit, AXL, and FLT3, all being implicated in tumor pathogenesis as well as angiogenesis. In the same way than VEGF-A, c-MET and AXL can facilitate immune evasion of tumor cells by increasing PD-L1 expression on tumor cells [66] and by interacting and decreasing the expression of MHC class 1 receptors [67]. Cabozantinib has been shown to promote an immune-permissive tumor environment which may enhance response to ICI [68]. Cabozantinib is currently evaluated in association with atezolizumab in a phase Ib trial (COSMIC-021 trial) in cohorts of patients with genitourinary malignancies including UC. The study comprises a dose escalation and an expansion stage. The dose escalation stage (3+3 design) is completed and cabozantinib (40 mg daily) plus atezolizumab (1200 mg every 3 weeks) was the recommended dose for the expansion stage. In the expansion stage, 18 cohorts (each 30 patients) will be enrolled including one cohort with cisplatin-ineligible UC in first-line setting, one cohort with UC progressing after prior platinum-based therapy, one cohort with cisplatin-eligible UC in first-line setting, one cohort with UC progressing after ICI therapy. In addition, an exploratory cohort (30 patients) will evaluate single agent cabozantinib 60 mg daily in mUC patients progressing after ICI therapy.

The cohort 2 of the COSMIC-021 trial has been reported at American Society of Clinical Oncology (ASCO) 2020 meeting; 30 patients with advanced UC were enrolled with a median follow-up of 16.5 months. This was a high-risk and heavily pretreated population with 67% having a Bellmunt risk score of ≥2 and 47% having progressed on ≥2 systemic therapy. The ORR reached 27%, including 7% CR, and 55% of patients presented a reduction in tumor size. The median DOR has not been reached in one patient with an ongoing response at 15.6 months. Preliminary results suggest no association between PD-L1 expression and response rate. The median PFS was 5.4 months (range 0–17.3). Fifty-seven percent of patients presented grade 3/4 TRAEs, including 37% fatigue, 27% diarrhea, 23% decreased appetite, and 23% increased transaminases [69].

In a phase II trial, cabozantinib is evaluated with pembrolizumab in first-line for cisplatin-ineligible patients in the PemCab trial (NCT03534804) or with nivolumab ± ipilimumab in genitourinary malignancies including UC (NCT02496208). *Axitinib*, a selective inhibitor of VEGF receptors, is under evaluation for the combined treatment with avelumab in the JAVELIN Medley VEGF trial (NCT03472560), a phase II study involving cisplatin-ineligible patients with non-small cell lung carcinoma and mUC in the first-line setting. *Apatinib*, a molecule inhibitor of VEGFR-2, is being evaluated for clinical efficacy in combination with pembrolizumab for the treatment of patients with platinum refractory mUC in the APPEASE trial (NCT03407976). Lastly, *ramucirumab*, a monoclonal antibody that also targets VEGFR2, is currently being evaluated with pembrolizumab in different types of cancer, including in UC (NCT02443324) (Table 4).

Combining ICIs and targeted therapies

Nectin-4 is a transmembrane protein involved in cellular adhesion and is overexpressed in many solid tumors including UC. Enfortumab vedotin (EV) is a monoclonal antibody targeting Nectin-4 and is combined with a potent cytotoxic microtubule inhibitor, monomethyl auristatin E (MMAE); the release

Table 4. Ongoing trials for combination of immune checkpoint inhibitors and vascular endothelial growth factor in advanced/metastatic urothelial carcinoma. ***ICI,*** **immune checkpoint inhibitor;** ***NSCLC,*** **non-small cell lung carcinoma;** ***UC,*** **urothelial carcinoma**

Protocol	Phase	Anti-PD-1/PD-L1	Combined agent	Setting	Primary outcomes
NCT03472560 (Javelin Medley VEGF)	II	Avelumab	Axitinib	Advanced or metastatic NSCLC and UC in first-line platinum-ineligible patients	Objective response
NCT03170960 (COSMIC-021)	Ib	Atezolizumab	Cabozantinib	Advanced or metastatic UC in platinum refractory patients (+ expansion cohort: advanced or metastatic UC in ICI refractory patients)	Dose escalation and response rate
NCT02496208	I	Nivolumab Nivolumab + ipilimumab	Cabozantinib	Metastatic genitourinary tumors in second line	Dose and toxicity
NCT03534804 (PemCab)	II	Pembrolizumab	Cabozantinib	Metastatic UC in first-line platinum-ineligible patients	Response rate
NCT03407976 (APPEASE)	I/IIA	Pembrolizumab	Apatinib	Various advanced or metastatic solid tumors including UC platinum refractory patients	Dose and response rate
NCT02443324	I	Pembrolizumab	Ramucirumab	Various advanced or metastatic solid tumors including UC platinum refractory patients	Dose and toxicity

of MMAE disrupts the microtubule network within the cell, inducing cell cycle arrest and apoptotic cell death. In the end of 2019, FDA accelerated approval of EV in advanced UC, based on the results of the phase II EV-201 trial in which 125 previously treated mUC patients received EV. These patients were heavily pretreated; 46% received a prior PD-1 inhibitor, 42% received a prior PD-L1 inhibitor, and 13% received both; 66% received prior cisplatin-based regimens, 26% received prior carboplatin-based regimens, and 8% received both. EV achieved an ORR of 44%; the median PFS reached 5.8 months and the median OS was 11.7 months. The median DOR was 7.6 months. The ORR was 56% in patients who previously responded to anti-PD-1/PD-L1 and 41% in patients who not responded to PD-1/PD-L1 inhibition. Pembrolizumab was associated with EV in the phase 1b dose escalation and expansion study (NCT03288545) in mUC patients. This is a multicohort trial (A to G) evaluating EV in first- or second-line setting combination with pembrolizumab, chemotherapy, or chemotherapy plus pembrolizumab. The cohort A consists of first-line cisplatin-ineligible patients and enrolled 45 patients (91% with visceral metastases) who received EV (1.25 mg/kg days 1 and 8 of every 3-week cycle) plus pembrolizumab (200 mg every 3 weeks). The ORR reached 73.3% including 15.6% CR and 22% had SD, resulting in a disease control rate of 93.3%. Benefit was observed irrespective of PD-L1 expression as measured by the CPS, with an ORR of 78.6% in high PD-L1 and 63.2% in low PD-L1 expression. Responses were observed at a median of 2.0 months from the time of treatment initiation, and response duration ranged from 1.0 to 10.5 months. One patient in the study died due to multiple organ failure. Immune-mediated AEs of at least grade 3 were observed in 11% of patients [70]. This impressive rate of control disease rate, exceeding 90% in such heavily pretreated patients is particularly promising and needs, of course, further confirmation.

Improving the prediction of response to ICIs

Although ICIs have shown to improve outcome of mUC patients, a majority of patients responds poorly or moderately to these agents. Many efforts are done to identify biomarkers able to predict response or resistance to ICIs. PD-L1 is not an ideal biomarker and its role is controversial [16, 71, 72]. IMvigor210 was the first trial to show ICI efficacy in mUC; in the cohort 2, focusing on previously treated patient, high PD-L1 expression was associated with a high response rate [26]. However, in the cohort 1, focusing on previously untreated patients, there was no relation between PD-L1 expression and response. No relation was also seen in the CheckMate 275 trial that demonstrated efficacy of nivolumab in previously treated mUC patients [19]. These discrepancies between these trials could have different explanations: first, the PD-L1 staining and interpretation methods are not yet reproducible due to the large heterogeneity in PD-L1 assays across the trials: PD-L1 antibodies, expressing cells (IC or TC or both), are different as well as the cut-off for positivity (1% vs 5%). Furthermore, PD-L1 expression is heterogeneous in the tumor and dynamic during the cancer evolution, meaning that a single biopsy is unlikely to provide a complete assessment of status for the entire duration of disease.

The interferon-gamma (IFN-γ) signature appears also as a promising assay; defects in this pathway leads to acquired resistance to ICIs [73, 74]. In the CheckMate 275 trial, higher values of the 25-gene IFN-γ signature were

associated with a greater proportion of responders (CR or PR) to nivolumab compared to low value IFN-γ expression score (34% compared to 16%, respectively; p =0.0003) [25]. The level of MDSCs was also associated with OS; low level of baseline MDSCs were associated with longer OS compared to high baseline levels of MDSCs in all patients regardless the tumor PD-L1 expression (22.3 vs 3.7 months). Patients with both high tumor IFN-γ and low circulating MDSC levels experienced the longest OS with nivolumab compared to patients with both higher IFN-γ scores and high baseline MDSC levels (p<0.05). This requires, of course, confirmation in further clinical prospective trials. Transforming growth factor-ß (TGF-β) has been shown to be associated with poor clinical outcome in UC patients and could also be implicated in resistance to ICIs. Indeed, TGF-β is produced by cancer cells to prevent T cells from penetrating the tumor and in the IMvigor210 trial, high levels of TGF-ß were associated with low response to PD-L1 blockade by contributing to exclusion of T cells (p =0.00019) [27].

Tumor mutational burden (TMB) represents the total number of somatic coding mutations found in cancer cells, reflecting the deficiency in the mismatch repair mechanism and the neoantigen burden. In the IMvigor210 cohort 2, responding patients presented a significant level of TMB compared to non-responding patients (12.4 per megabase vs 6.4 per megabase, respectively; p<0.0001) [29]. This requires also confirmation in prospective trials.

Another approach to predict responses to ICIs could involve a model regrouping 3 factors including TMB and 2 poor prognosis factors, peripheral blood neutrophil/lymphocyte ratio and visceral metastasis. [75, 76]. Recent publication showed promising results for this predicting 3-factor model in 58 mUC patients treated with atezolizumab as the presence of all 3 factors was associated with PR or CR in 15/21 patients (71.4%), while no factor at all was associated with PR or CR in only 2/37 patients (5.4%) [77].

Based on the results of the IMvigor210 trial and translational analysis, correlative molecular analysis of pre-treatment samples from patients treated on the IMvigor130 trial were presented at ASCO meeting 2020 [78]. When stratifying by PD-L1 expression, IC2/3 PD-L1 patients appeared to have numerically longer survival when treated with atezolizumab either alone or in combination with chemotherapy compared to IC0/1 PD-L1 patients. Higher TMB (greater than 10 mutations/megabase) and the presence of the apolipoprotein B mRNA editing enzyme, catalytic polypeptide–like (APOBEC) mutational signature were also associated with numerically improved OS in patients treated with atezolizumab monotherapy or in combination with chemotherapy. Mutation in the APOBEC gene results some cluster mutations along genome, contributing to the overall mutation burden in the spectrum of human cancers, especially in bladder [79]. Conversely, expression of a TGF-β response signature in tumor fibroblasts conferred resistance and poor survival to patients treated with atezolizumab monotherapy.

Finally, the Tumor Cancer Genome Atlas (TCGA) project categorized UC into 4 microRNA subtype clusters I–IV (luminal I, luminal II, basal I, and basal II) [80, 81]. These cluster subtypes could be associated with different sensitivities to ICIs as luminal I have low T-effector gene expression ("immune desert" tumor), luminal II have high T-effector gene expression, and low stromal gene expression ("inflamed tumor") and basal are inflamed but immune suppressed (high T-effector and high stromal gene expression). In IMVigor 210 cohort 2,

TCGA classification divided patients into luminal or basal subtypes [29, 82]. Enrichment in IC PD-L1 expression was noted in the basal compared to luminal subtype (60% vs 23%, respectively), while TC PD-L1 expression was noted almost exclusively in basal subtypes compared to luminal subtype (39% vs 4%, respectively). Higher response rate to atezolizumab was seen in luminal cluster II subtype (34%) compared to luminal cluster I (10%), basal cluster I (16%), and basal cluster II (20%). A similar trend was noted in cohort 1 of IMvigor210 with the highest percentage of responses in the luminal cluster II group [29]. By contrast, the CheckMate 275 study showed a higher ORR with nivolumab in basal I subtype tumors compared to luminal subtype (30% vs 25%, respectively) [25].The reasons for these discrepancies is multifactorial and could be explained by the low number of patients, the retrospective analysis of these trials, the differences in the assays to classify the tumor subtypes, and the differences in the tumor biopsies (primary tumors vs metastasis). The TCGA classification has to be evaluated in large prospective trials with genomic-related stratification of patients.

Conclusion

Immune checkpoint inhibitors have importantly improved the outcome of advanced UC patients. However, only a minority present significant response rate and long-term benefits. Due to their better tolerability and their different mechanisms of anticancer action, ICIs could play a role in combination strategies, in association with chemotherapy, targeted agents, or anti-angiogenic molecules. However, it is important to better identify patients who are more likely to benefit from ICIs in clinical practice. We have to improve our clinical trials design, incorporating large biomarker-driven cohorts and exploring different strategies, such as combination and maintenance strategies.

Compliance with Ethical Standards

Conflict of Interest

Guillaume Grisay declares that he has no conflict of interest. Julien Pierrard declares that he has no conflict of interest. Caterina Confente declares that she has no conflict of interest. Emmanuel Seront declares that he has no conflict of interest.

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- •• Of major importance

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