6 Current Landscape of Immunotherapy in Genitourinary Malignancies

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Abstract

The past decade has witnessed a revolution of immune checkpoint inhibitors in the treatment of multiple tumor types, including genitourinary cancers. Immune checkpoint inhibitors improved the treatment outcomes of patients with metastatic renal cell carcinoma and metastatic urothelial carcinoma. In prostate cancer, the role of immunotherapy with checkpoint inhibitors is not yet established, but clinical trials investigating their use are ongoing. Other immunotherapeutic approaches that have been explored in these malignancies include cytokines, vaccines, and cellular therapy. Ongoing studies are exploring the use of immunotherapy combinations as well as combination with chemotherapy and targeted therapy in these types of tumors. The use of immunotherapy beyond the metastatic setting is an active area of research. Moreover, there is a great interest in biomarker development to predict response to immunotherapy and risk of toxicity. This chapter is a comprehensive review of the immunotherapeutic approaches, both approved and investigational, for the treatment of renal cell carcinoma, urothelial carcinoma, and prostate cancer.

Keywords

Immunotherapy · Checkpoint inhibitors · Cellular therapy · Cytokines · Vaccines · Renal cell carcinoma · Urothelial carcinoma · Prostate cancer

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Immunotherapy for Renal Cell Carcinoma

Renal cell carcinoma (RCC) represents around 90% of all cancers of the kidney, with clear-cell renal cell carcinoma (ccRCC) being the most common subtype (accounting for approximately 85% of all RCC) [1]. Nearly one third of patients newly diagnosed with RCC have metastatic or

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advanced disease [2, 3]. Risk stratification of patients with newly diagnosed metastatic RCC is essential both to determine prognosis and to plan treatment as a key part of clinical decision-making. One tool for risk assessment for metastatic RCC was established by the International Metastatic Renal Cell Carcinoma Database (IMDC), which integrates six clinical factors that were shown to have an independent prognostic values in a multicenter study of 645 patients [4]. Those criteria include (1) anemia, (2) neutrophilia, (3) thrombocytosis, (4) hypercalcemia, (5) Karnofsky performance status <80, and [6] <1 year from diagnosis to first-line systemic therapy. Patients with none of these factors have favorable disease, while patients with 1–2 factors have an intermediate-risk disease, and patients with more than three factors have poor-risk disease. Another risk assessment tool is the Memorial Sloan Kettering Cancer Center (MSKCC) model in advanced RCC that similarly stratifies patients into favorable, intermediate, or poor risk [5]. Both clinical and laboratory data are included in this model: low Karnofsky performance status, high lactate dehydrogenase, low serum albumin, high corrected serum calcium, and time from diagnosis to systemic treatment [5]. Recently, the model was updated to incorporate genomic data, where the mutation status of *BAP1, PBRM1*, and *TP53* has been shown to have an independent prognostic value in patients with advanced or metastatic RCC treated with first-line tyrosine kinase inhibitors (TKIs) (Table 6.1).

The treatment of ccRCC has witnessed tremendous evolution over the past decade both with the introduction of targeted therapies and with the advent of immunotherapy. Multitargeted TKIs, which inhibit vascular endothelial growth factor receptors (VEGFR) and mammalian target of rapamycin (mTOR), have been standard therapies for the treatment of metastatic RCC (mRCC) [6, 7]. Within the past 3 years, immune checkpoint inhibitors (CPIs) have significantly changed the natural history of metastatic RCC. The combination of ipilimumab with nivolumab has shown significant efficacy in this setting and has been approved for first-line treatment of intermediate- to poor-risk patients with metastatic RCC (further detailed below) [8]. A more intricate understanding of the immune system and its interaction with the tumor microenvironment as well as the different pathways involved in tumorigenesis led to the investigation of new immunotherapeutic modalities in mRCC. Data from clinical trials exploring the combination of immune CPIs with TKIs also show promise for the expansion of available therapeutic options. However, it is important to be mindful of the potential for increased toxicity and cost with these combinations. Other exciting forms of immunotherapies are being investigated, including vaccines, adoptive cell therapy, and newer immunotherapy combinations. These combined efforts will likely continue to transform the field and offer novel options for patients with RCC. Strategies to extrapolate the success of immunotherapy from the metastatic setting to the adjuvant setting are underway. Herein, we present an overview of the various immunotherapies approved and being investigated in the treatment of ccRCC (Fig. 6.1).

Table 6.1 Memorial Sloan Kettering Cancer Center (MSKCC) and International Metastatic Renal Cell Carcinoma Database (IMDC) prognostic tools

Variable	MSKCC	IMDC
Karnofsky performance status	0–1	0–1
Time from diagnosis to systemic treatment <1 year	0–1	0–1
Anemia	0–1	0–1
Neutrophilia		0–1
Thrombocytosis		0–1
LDH > 1.5 × ULN	0–1	
Calcium >10 mg/dL	0–1	0–1

LDH lactate dehydrogenase, *ULN* upper limit of normal

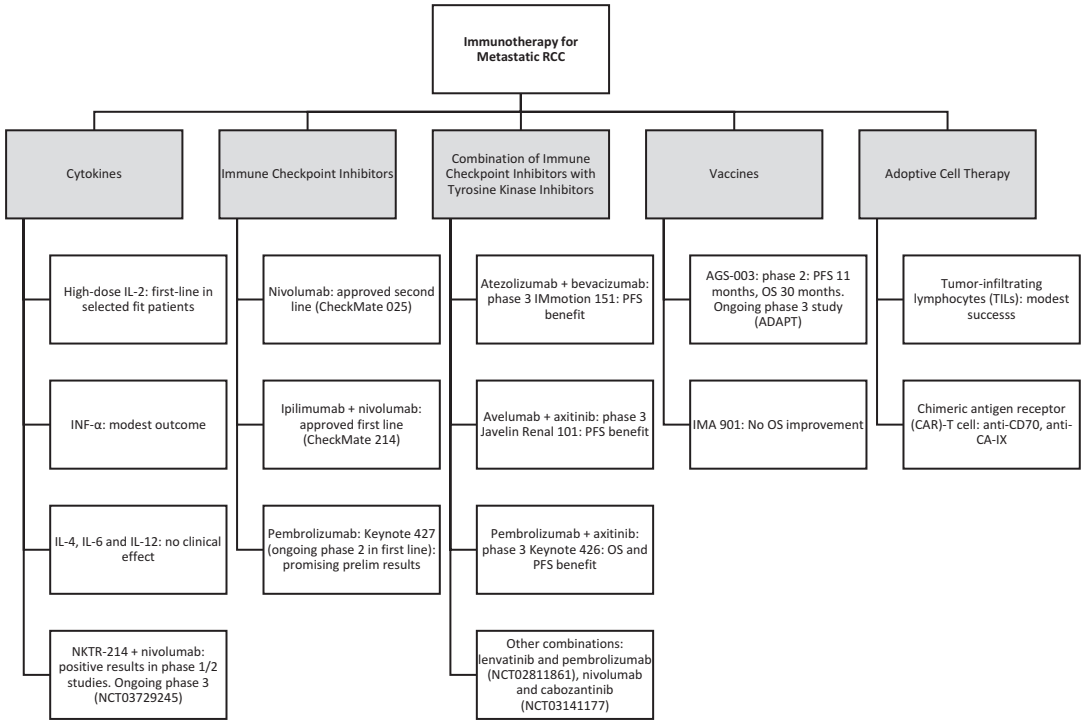

Fig. 6.1 Immunotherapy for the treatment of metastatic renal cell carcinoma. RCC renal cell carcinoma, IL interleukin, INF interferon, Prelim preliminary, PFS progression-free survival, OS overall survival

Rationale for Immunotherapy in RCC

RCC is known to be particularly resistant to chemotherapy, and this could be attributed to many features of this disease. First, RCC is derived from proximal tubules expressing high levels of multidrug-resistant (MDR) P-glycoprotein [9]. Moreover, a number of studies have identified cancer stem cells as a tumor subpopulation that has a self-renewal ability and confers resistance to chemotherapy [10]. However, RCC is exquisitely sensitive to immunotherapy relative to other tumor types. Early observations that removal of the primary tumor can trigger immune responses that could lead to spontaneous regression of metastatic RCC, particularly in the lung, were strong indicators that RCC could be amenable to immunotherapy [11]. Moreover, profuse tumor infiltration with T cells, natural killer (NK) cells, macrophages, and dendritic cells (DC) has been demonstrated in a number of studies, suggesting an inherent role of antitumor immunity [12, 13].

These observations were reinforced by the demonstrated clinical activity of the very first forms of immunotherapies for RCC with interleukin 2 (IL-2) and interferon-alpha (INF-α), although major clinical benefit was seen in only a minority of patients. In 1992, the US Food and Drug Administration (FDA)-approved high-dose intravenous IL-2 for the treatment of RCC [14–16]. This was based on preliminary data showing an overall response rate (ORR) of 15% as well as a 5% complete response (CR) [15]. In a follow-up study, CR was 7% and median duration of response was at least 80 months [17]. Its use, however, was limited by the significant side effect profile as well as the inability to predict response. In an attempt to decrease toxicity, low-dose IL-2 was also investigated and compared to high-dose IL-2, but ORR was much lower with low dose (21% with high dose vs 13% with low dose, $P = 0.048$) [18]. A recent prospective study of 352 patients [19] and another retrospective study of 391 patients [20] suggested an extended clinical

benefit of high-dose IL-2. Stable disease (SD) as a measure of best response was present in 39% and 32% of these cohorts, respectively, and was associated with survival benefit [19, 20]. INF-α, despite being better tolerated and having a broader applicability, had more modest outcomes (overall survival (OS) of 2.5 months greater than placebo) without the durable responses demonstrated with high-dose IL-2 [21].

Until 2005, IL-2 and INF-α were the only two approved therapies for RCC and the median survival was approximately 1 year [22]. Since then, a number of new therapies have been approved that led to a paradigm shift in the treatment of RCC including mTOR inhibitors (everolimus, temsirolimus), VEGF inhibitors (sunitinib, sorafenib, axitinib, pazopanib, cabozantinib, bevacizumab, lenvatinib), and more recently the revolutionary immunotherapies with immune CPIs [23, 24]. The use of high-dose IL-2 as first-line therapy is restricted to well-selected younger patients with a good performance status and without comorbidities.

While harnessing the immune system has long been on interest in the treatment of mRCC, the addition of CPIs to the therapeutic armamentarium was a breakthrough due to the unique immune-editing features they provide, which serve to alter the balance between tumor and immune system [25]. The immune-editing mechanism comprises three phases: elimination, equilibrium, and escape [26]. The elimination phase comprises killing of malignant cells through CD8+ T cells and NK cells. There are some cancer cells that elude the initial host defense mechanisms and survive in a constraint environment in the presence of immune cells in the equilibrium phase. Finally, evasion of the immune surveillance by cancer cells comprises the escape phase [26–28]. Under constant pressure from the immune system, tumor cells thrive through mechanisms that allow them to resist immune cells [29] such as downregulation of antigens, loss of major histocompatibility complex class I (MHC-I) to interfere with antigen presentation, or upregulation of inhibitory pathways and checkpoints such as programmed death-ligand 1 (PD-L1)/programmed death-1 (PD-1) [30–34]. Ongoing efforts to counteract these immune escape mechanisms are driving the scientific research and clinical trials in the exploration of the best treatment modalities for RCC.

Immune Checkpoint Blockade in Locally Advanced or Metastatic RCC (Fig. 6.2)

Nivolumab

Nivolumab is a fully humanized IgG4 anti-PD-1 antibody that blocks the interaction of PD-1 with its ligands PD-L1 and PD-L2 and thus interfering

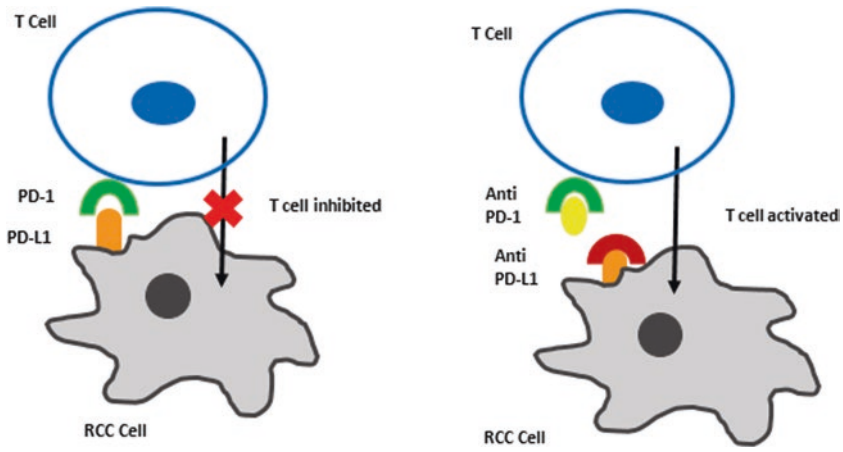

Fig. 6.2 Principle of immune checkpoint inhibition. RCC renal cell carcinoma, PD-1 programmed death 1, PD-L1 programmed death-ligand 1

with the immune response inhibitory pathways [35]. The first sign of efficacy of nivolumab in RCC was demonstrated in two phase 1 trials [36, 37]. A total of 296 patients with various metastatic solid tumors including 34 patients with heavily pretreated metastatic RCC received various doses of nivolumab [37]. At a minimum follow-up of 50.5 months, ORR was 29% and one patient had a CR in the 10 mg/kg cohort. For all doses, the ORR was 29.4%. Among the responders, 30% achieved objective response by 8 weeks (first assessment) and 70% achieved response by 16 weeks (second assessment). Median duration of response was 12.9 months (8.4–29.1). At the time of analysis, 40% of responses were ongoing [36]. These early data were very encouraging for the clinical benefit of immune checkpoint blockade in the treatment of RCC.

The promising activity of the phase 1 trial led to the launching of a phase 2 study of nivolumab in metastatic ccRCC, which consisted of a randomized blinded multicenter clinical trial [38]. Three arms were included in the study with 1:1:1 randomization to three different doses of nivolumab: 0.3, 2, and 10 mg/kg. The randomization was stratified based on the number of prior therapies (1 vs >1 (70%)) and MSKCC risk group (favorable/intermediate vs poor (25%)). The primary endpoint was evaluation of the dose–response relationship as measured by progression-free survival (PFS); secondary end points included ORR, OS, and safety. One hundred sixty-eight patients were enrolled: 60 received nivolumab 0.3 mg/kg, 54 received nivolumab 2 mg/kg, and 54 received nivolumab 10 mg/kg. Median PFS was 2.7 months (80% CI: 1.9–3.0 months), 4.0 months (80% CI: 2.8–4.2 months), and 4.2 months (80% CI: 2.8–5.5 months) for the 0.3, 2, and 10 mg/kg groups, respectively. ORR was 20%, 22%, and 20% in the 0.3, 2, and 10 mg/kg arms, respectively. Continued response beyond 24 months was noted in 14 of the 35 (40%) responders. With a follow-up of at least 24 months, median OS was 18.2 months (80% CI: 16.2–24.0 months) in 0.3 mg/kg arm, 25.5 months (80% CI: 19.8–28.8 months) in the 2 mg/kg arm, and 24.7 months (80% CI: 15.3–26.0 months) in the 10 mg/kg arm. Adverse events (AE) were observed at similar rates between the three arms. The most common treatment-related AE was fatigue (24%, 22%, and 35%, respectively). Nineteen patients (11%) experienced grades 3–4 treatment-related AEs (nausea, arthralgia, and elevation of alanine and arginine transaminases), of which four of these patients were in the 0.3-mg/kg group, 14 patients were in the 1-mg/kg group, and 1 patient was in the 10-mg/kg group [38].

The successful phase 2 again led to the investigation of nivolumab in metastatic ccRCC in a phase 3, multicenter, international, open-label randomized study – CheckMate 025 trial [39]. This study compared the efficacy of nivolumab with everolimus, which is an approved second-line agent for the management of metastatic RCC after progression on an anti-VEGF agent [40]. The primary endpoint was OS rather than PFS, which had been the case in several prior phase 3 trials of new agents in metastatic RCC [41, 42]. This was based on the mechanism of action of nivolumab which enhances inflammation around the tumor causing a radiographic appearance of progression in the absence of true clinical progression, a phenomenon called "pseudoprogression." ORR was higher in the nivolumab group compared to everolimus (25% vs 5%, odds ratio, 5.98 [95% CI: 3.68–9.72]; $P < 0.001$). The median OS was significantly better in the nivolumab group at 25.0 months (95% CI: 21.8 to not estimable [NE]) compared to 19.6 months (95% CI: 17.6–23.1) in the everolimus group. However, the median PFS was not statistically significantly different between the nivolumab arm and the everolimus arm, 4.6 months (95% CI: 3.7–5.4) versus 4.4 months (95% CI: 3.7–5.5), respectively. The clinical benefit of nivolumab encompassed all the MSKCC risk groups. The AEs were similar to those seen in earlier trials.

A separate study investigated the health-related quality of life (HRQoL) in the different treatment groups of CheckMate 025 [43]. HRQoL measures analysis was performed using Functional Assessment of Cancer Therapy-Kidney Symptom Index–Disease-Related

Symptoms (FKSI-DRS) and European Quality of Life (EuroQol)-5 Dimensions (EQ-5D) questionnaires. More patients had a clinically meaningful (i.e., an increase of at least 2 points from baseline) HRQoL improvement with nivolumab (200 [55%] of 361 patients) versus everolimus (126 [37%] of 343 patients; $p < 0.0001$). Median time to HRQoL improvement was shorter in patients given nivolumab (4.7 months, 95% CI 3.7–7.5) than in patients given everolimus (median not reached, NE-NE) [43]. Based on the positive results of the CheckMate 025 study, the FDA approved nivolumab for the management of advanced metastatic RCC after progression on first-line therapy, on November 23, 2015. Limited data exist on the role of nivolumab monotherapy in the frontline treatment of advanced RCC.

Nivolumab Plus Ipilimumab

The increased effectiveness seen in advanced melanoma with the combination of nivolumab and ipilimumab, a cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) CPI, led to the investigation of this combination in RCC as well. The phase 3 CheckMate 214 trial established the efficacy and safety of ipilimumab and nivolumab combination in metastatic clear-cell RCC [8]. Previously untreated patients with advanced or metastatic clear-cell RCC were randomized to either sunitinib (50 mg per day for 4 weeks out of every 6-week cycle) or the combination of ipilimumab (1 mg/kg) and nivolumab (3 mg/kg) given every 3 weeks for four doses and were then followed by nivolumab (3 mg/kg). At a median follow-up of 25 months, OS was significantly higher in the combination group as opposed to the sunitinib group in the intention-to-treat population (median not reached with the combination vs 32.9 months in the sunitinib group, HR 0.68, 99.8% CI 0.49–0.95). The ORR was also significantly higher with ipilimumab and nivolumab (39% vs 32%), but there was no difference in PFS (median 12.4 vs 12.3 months, HR 0.98).

In the subgroup of 847 patients with intermediate- or poor-risk disease, the OS was significantly higher with the combination of ipilimumab and nivolumab compared to sunitinib (median not reached vs 26 months, HR 0.63, 95% CI 0.44–0.82). The ORR was also significantly higher in the combination group as opposed to sunitinib (42% vs 27%). The disease control rate (DCR) was 72%. While the median PFS was increased with the immunotherapy combination, statistical significance was not attained (11.6 vs 8.4 months, HR 0.82, 95% CI 0.64–1.05). However, PFS and response benefit appeared to be increased in patients with PD-L1 expression ≥1% (214 patients). More pronounced benefit was seen in patients with intermediate- or poor-risk disease as well as PD-L1 expression ≥1% (ORR 58% vs 25%, median PFS 22.8 vs 5.9 months, HR 0.48, 95% CI 0.28–0.82). The CR rate in this group was 16%. On the other hand, in the group of patients with intermediate- or poor-risk disease and PD-L1 expression <1% (562 patients), only OS was significantly increased (median not reached for either group, HR 0.73, 95% CI 0.56–0.96), while there was no significant difference between the combination and sunitinib in either the ORR (37% for the combination vs 28% for sunitinib) or median PFS (11 months for the combination vs 10.4 months for sunitinib, HR 1.0, 95% CI 0.74–1.36). While the study was underpowered to draw significant conclusions from the favorable-risk disease group, exploratory analyses showed that the response rate was lower with the ipilimumab-plus-nivolumab combination compared with sunitinib (29% vs 52%), and PFS was shorter (median 15.3 vs 25.1 months, HR 2.17, 95% CI 1.46–3.22). Survival data are not yet available for the favorable-risk group; however, the maturing data suggest that the nivolumab–ipilimumab combination has better outcomes in the favorable-risk group than initially presented [44].

The toxicity profile of the combination of nivolumab and ipilimumab was consistent with that observed with the use of the combination for other indications and favored the combination group over sunitinib. Grade 3 or 4 AEs occurred in 46% of patients in the immunotherapy combination group versus 63% in the sunitinib group. The most common grade 3 or 4 AEs on the immunotherapy combination group were increased lipase

(10%), diarrhea (4%), and fatigue (4%). The most common AEs in the sunitinib group were hypertension (16%), palmar-plantar erythrodysesthesia (9%), and increased lipase (7%). Immune-related AEs of any grade occurred in 80% of patients who received ipilimumab with nivolumab, among those 35% received high-dose corticosteroids. It is important to note, however, that the treatment was discontinued due to treatment-related AEs in 22% of the patients who received the immunotherapy combination and in 12% of patients who received sunitinib. Moreover, death due to treatment-related AEs occurred in eight patients in the ipilimumab and nivolumab group (causes of death in each patient were pneumonitis, bronchitis, pneumonia and aplastic anemia, lower gastrointestinal hemorrhage, hemophagocytic syndrome, sudden death, lung infection, and liver toxicity) and in four patients in the sunitinib group (two due to cardiac arrest, one due to heart failure, and one due to multiorgan failure).

A separate study reported on patient-reported outcomes (PROs) from the CheckMate 214 study [45]. PROs were assessed according to three measurement tools: the Functional Assessment of Cancer Therapy–Kidney Symptom Index-19 (FKSI-19), which is validated for kidney cancer; Functional Assessment of Cancer Therapy-General (FACT-G), which is validated for cancer in general; and EuroQol Five-Dimensional, Three-Level (EQ-5D-3L), which is validated for general health status. Patients in the immunotherapy combination arm reported better PROs than those who received sunitinib for the two of the three assessment tools, from the start of treatment through about 2 years. The average change in the overall FKSI-19 score between baseline and 103 weeks was 4.00 (95% CI 1.91–6.09) for the combination arm compared with −3.14 (95% CI –6.03 to −0.25) for the sunitinib arm ($P < 0.0001$) and the average change in overall FACT-G score was 4.77 (95% CI 1.73–7.82) for the combination arm versus −4.32 (95% CI −8.54 to −0.11) for the sunitinib arm ($P = 0.0005$). EQ-5D-3L scores, however, were not significantly different between treatment groups.

Based on the results from the CheckMate 214 clinical trial, the combination of ipilimumab and nivolumab was approved by US FDA for the treatment of previously untreated patients with intermediate- to poor-risk advanced or metastatic RCC, on April 16, 2018.

Pembrolizumab

Pembrolizumab, a humanized anti-PD1 IgG4 antibody, is being investigated as single-agent CPI for advanced or metastatic RCC in the Keynote 427 phase 2 trial [46]. Preliminary results from cohort A of this trial were presented at the 2018 American Society of Clinical Oncology (ASCO) annual meeting. One hundred ten patients with previously untreated advanced or metastatic clear-cell RCC were enrolled and received pembrolizumab 200 mg every 3 weeks for 2 years or until confirmed progressive disease, unacceptable toxicity, or patient's decision to withdraw. At a median follow-up of 12.1 months (range 2.5–16.8), pembrolizumab demonstrated an ORR of 38.2% (95% CI 29.1–47.9), with a CR rate of 2.7% and a partial response (PR) rate of 35.5%. The DCR was 59%. The median time to response was 2.8 months, and 74.8% of patients had responses lasting for 6 months or more. Median PFS was 8.7 months (95% CI 6.7–12.2), and the 6-month PFS rate was 60.2%. OS was not reached, and the 6-month OS rate was 92.7%. In the subgroup of 69 patients with intermediate- and poor-risk disease, ORR was 42% (95% CI 30.2–54.5) compared to 31.7% (95% CI 18.1–48.1) in the subgroup of 41 patients with favorable-risk disease. In an analysis based on PD-L1 expression, ORR was 50% (95% CI 34.9–65.1), the CR rate was 6.5%, and the PR rate was 43.5% in the subgroup of 46 patients with tumors overexpressing PD-L1 (combined positive score (CPS) ≥1; tumor and immune cell PD-L1 expression) compared to an ORR of 26.4% (95% CI 15.3–40.3) and all responses being partial in the 53 patients who had low tumor expression of PD-L1 (CPS < 1).

The safety profile of pembrolizumab was consistent that seen in pembrolizumab used for other indications. Treatment-related grade 3–5 AEs

occurred in 22.7% of patients. The most common treatment-related AEs were pruritus (27.3%), fatigue (24.5%), diarrhea (19.1%), rash (15.5%), arthralgia (12.7%), and hypothyroidism (10%). The most common immune-mediated AEs of any grade were hypothyroidism (10.9%), pneumonitis (4.5%), hyperthyroidism (4.5%), colitis (2.7%), hepatitis (1.8%), severe skin reaction (1.8%), and myositis (1.8%). Treatment-related AEs led to the discontinuation of treatment in 12 patients, and treatment-related death due to pneumonitis occurred in 1 patient.

Combined Antiangiogenic Plus CPI Immunotherapy in Locally Advanced or Metastatic RCC

Pembrolizumab with Axitinib

The combination of immune checkpoint blockade with pembrolizumab and VEGF receptor tyrosine kinase inhibition with axitinib has shown antitumor activity in patients with previously untreated advanced RCC [46, 47]. This was confirmed in a phase 1b trial of the combination in the front-line setting of metastatic RCC with ORR of 73% (95% CT 59–84) [48].

The phase 3 Keynote-426 trial demonstrated an OS and PFS benefit of the combination of pembrolizumab and axitinib in the front-line treatment of advanced or metastatic RCC [49]. This study included 861 patients who were randomly assigned to oral sunitinib once daily or to combination therapy. Pembrolizumab was given every 3 weeks along with oral axitinib twice daily. At a median follow-up of 12.8 months, the median OS was not reached in either arm, and the 12-month survival rates were 90% in the combination arm versus 78% in the sunitinib arm (HR for death 0.53, 95% CI 0.38–0.74). Median PFS was 15.1 months in the pembrolizumab plus axitinib arm versus 11.1 months in the sunitinib arm (HR for progression or death 0.69, 95% CI 0.57–0.84), and ORR was 59% versus 36%, respectively. The DCR with the immunotherapy combination was 83.8%. The benefit of the combination of pembrolizumab with axitinib was observed irrespective of the PD-L1 expression or the disease risk category. Grade 3 or higher AEs of any cause occurred in 75.8% of patients in the pembrolizumab–axitinib group and in 70.6% in the sunitinib group. Based on the results of this trial, the combination of pembrolizumab with axitinib was recently FDA approved as a first-line treatment in advanced RCC on April 19, 2019, regardless of IMDC risk score or PD-L1 status. This recent approval poses interesting considerations in the frontline treatment of mccRCC. As compared to historic data in mccRCC, the data from CheckMate-214 and Keynote-426 suggest that OS is the new benchmark for approval of frontline therapies. Furthermore, endpoints such as CR rate, DCR, and treatment-free survival (TFS) may nuance the choice of which therapy to choose in case-specific circumstances. The role of PD-L1 status yet remains indeterminate in therapy selection in mccRCC.

Avelumab with Axitinib

Another combination of antiangiogenesis inhibition with immunotherapy composed of avelumab and axitinib showed promising results in phase 3 study. The Javelin Renal 101 phase 3 trial involved 886 treatment-naive patients with advanced clear-cell RCC, and the patients were randomly assigned to the combination of avelumab and axitinib versus sunitinib [50]. In the group of patients with PD-L1-positive tumors (560 patients), the median PFS was 13.8 months with avelumab with axitinib compared to 7.2 months with sunitinib (HR for progression or death 0.61; 95% CI 0.47–0.79; $P < 0.001$), and ORR was 55.2% compared to 25.5%, respectively. In the overall population, the DCR with the avelumab and axitinib arm was 81%. The median PFS was higher in the combination arm at 13.8 months compared to 8.4 months (HR 0.69; 95% CI, 0.56–0.84; $P < 0.001$). At a median follow-up for OS of 11.6 months and 10.7 months in the two groups, 37 patients and 44 patients had died,

respectively; the role of the regimen in the treatment landscape of mccRCC will become clearer as OS data mature. AEs during treatment occurred in 99.5% of patients in the avelumab and axitinib group and in 99.3% of patients in the sunitinib group. Grade 3 or higher AEs were similar between the two groups, occurring in 71.2% and 71.5% of patients, respectively.

Atezolizumab with Bevacizumab

Positive results of the phase 2 trial of bevacizumab and atezolizumab [51] led to a phase 3 trial of this combination in 915 untreated patients with metastatic RCC (IMmotion151). Patients were randomized to either receive atezolizumab with bevacizumab or sunitinib [52]. Median PFS was longer in the combination arm as opposed to the sunitinib arm (11.2 vs 8.4 months, HR 0.83, 95% CI 0.70–0.97), ORR was 37% and 33%, and CR rates were 5% and 2%, respectively. In the PD-L1-positive population, median PFS was longer with atezolizumab with bevacizumab than with sunitinib (11.2 vs 7.7 months, HR 0.74, 95% CI 0.67–0.96). ORR was 43% (9% CRs) compared with 35% (4% CRs) in the combination and the sunitinib groups, respectively. OS data are immature to analyze in both the overall intention-to-treat and the PD-L1-positive populations.

Other Combinations

Other phase 3 trials are currently ongoing that investigate different combinations including a trial comparing three arms: the combination of lenvatinib and pembrolizumab versus the combination of lenvatinib with everolimus versus sunitinib (NCT02811861). Another phase 3 trial is comparing the combination of nivolumab and cabozantinib with sunitinib (NCT03141177). Other combination studies of sunitinib in combination with nivolumab and pazopanib in combination with either nivolumab or pembrolizumab were stopped early because of increased toxicity with synergistic fatigue and liver toxicity [53, 54]. Table 6.2 summarizes phase 3 combination trials.

Other Immunotherapy Approaches in Locally Advanced or Metastatic RCC

Vaccines

The use of vaccines to enhance the immune recognition of tumor has been investigated in RCC. AGS-003 is an autologous immunotherapy prepared from fully matured and optimized monocyte-derived DCs, which are co-electroporated with amplified tumor RNA from nephrectomy specimens plus synthetic CD40L RNA. AGS-003 was evaluated in combination with sunitinib in an open-label phase 2 study of 21 patients with intermediate and poor risk, treatment-naive metastatic RCC [55]. The median PFS was 11 months (95% CI 6.0–19.4), and the median OS was 30 months (95% CI 9.4–57.1). These results lead to the currently ongoing phase 3 ADAPT study (NCT01582672) where patients with metastatic RCC undergoing debulking nephrectomy are randomly assigned to either sunitinib with AGS-003 or sunitinib alone. AGS-003 was given as eight intradermal injections in the first year followed by boosters every 3 months.

Another cancer vaccine IMA901 that is based on tumor-associated peptides was administered in the front-line setting to patients with metastatic RCC who were positive for HLA-A∗02 antigen and have positive results in a phase 2 study [56]. A phase 3 study, IMPRINT, investigated its addition to sunitinib [57]. Three hundred thirty-nine patients were randomly assigned to sunitinib or sunitinib plus IMA901. The vaccine was given as an intradermal injection in conjunction with 75 μg of granulocyte macrophage colony-stimulating factor (GM-CSF) for up to 10 doses. There was no improvement in median OS, the primary endpoint of the study, with the addition of the vaccine (33.2 months vs not reached, HR 1.34, 95% CI 0.96–1.86, $P = 0.08$).

Other Cytokines

Multiple interleukins have been studied for the use in RCC, including IL-4 [58], IL-6 [59], and IL-12 [60, 61], but their antitumor activities were modest or toxicities of some were concerning.

Table 6.2 Phase 3 trials of the combination of immune checkpoint inhibitors with tyrosine kinase inhibitors in metastatic renal cell carcinoma

Trial name/ clinical trial number	Treatment arm	Control arm	Primary end point	Treatment arm vs control arm					Grades 3–4 adverse events
				PFS (months)	OS (months)	CR	ORR	DCR	
Clear/ NCT02811861	Lenvatinib/ pembrolizumab vs everolimus/ lenvatinib	Sunitinib	PFS	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
IMmotion 151/ NCT02420821	Bevacizumab/ atezolizumab	Sunitinib	PFS in PD-L1 positive; OS in all patients	11.2 vs 8.4 PD-L1 positive: 11.2 vs 7.7	Immature to analyze	5% vs 2% PD-L1 positive: 9% vs 4%	37% vs 33% PD-L1 positive: 43% vs 35%	75% vs 72% PD-L1 positive: 75% vs 69%	Grades 3–4: LFT abnormalities (3%) in treatment arm vs LFT abnormalities (4%) in control arm Notably 16% of patients on treatment arm received systemic steroids
Javelin Renal 101/ NCT02684006	Axitinib/avelumab	Sunitinib	PFS	13.8 vs 8.4 PD-L1 positive: 13.8 vs 7.2	11.6 vs 10.7	3.4% vs 1.8% PD-L1 positive: 4.4% vs 2.1%	51.4% vs 25.7% PD-L1 positive: 55.2% vs 25.5%	81% vs 71.2% PD-L1 positive: 81.8% vs 68.6%	irAEs: 38.2% in treatment arm Grades 3–4: 4% in treatment arm (HTN, HFS, diarrhea, increase ALT) vs 7% in control arm (fatigue, thrombocytopenia, anemia)
Keynote 426/ NCT02853331	Axitinib/ pembrolizumab	Sunitinib	PFS and OS	15.1 vs 11.1 (no difference in PD-L1 expression or risk category)	Not reached in either arm	5.8% vs 1.9%	59.3% vs 35.7%	83.8% vs 75.1%	Grades 3–4: diarrhea (9%), HTN (22%), HFS (5%), increased ALT (13%), increased AST (7%) in treatment arm vs diarrhea (5%), HTN (19%), fatigue (7%) in control arm
CheckMate 9ER/ NCT03141177	Cabozantinib/ nivolumab	Sunitinib	PFS	Ongoing	Ongoing	Ongoing		Ongoing	Ongoing

vs versus, *PFS* progression-free survival, *OS* overall survival, *CR* complete response, *ORR* objective response rate, *DCR* disease control rate, *LFT* liver function tests, *irAE* immune-related adverse events, *HTN* hypertension, *HFS* hand–foot syndrome, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

The combination of IL-2 and IL-12 was shown to be efficacious in preclinical studies, but this was not reproduced in human clinical trials [62].

A novel prodrug of pegylated IL-2, NKTR-214, has gained recent interest due to promising results. NKTR-214 preferentially binds to CD122 on the surface of immune cells and stimulates their proliferation. In both preclinical and clinical studies, NKTR-214 was shown to result in the expansion of these cells and mobilization into the tumor microenvironment [63]. The PIVOT phase 1/2 study is currently evaluating the combination of nivolumab with NKTR-214 in advanced solid malignancies. The preliminary results were presented at the ASCO 2018 annual meeting [64] and reported safety, efficacy, and biomarker data for patients enrolled in the phase 1 dose-escalation stage of the study and for the first patients consecutively enrolled in select dose expansion cohorts in phase 2. In metastatic treatment-naive RCC, prespecified efficacy criteria were met for ORR in stage 1 with 7/11 (64%) patients achieving a PR. Median time on study for 26 patients in stage 2 was 5.6 months. ORR was 46%. ORR in 17 patients with PD-L1-negative tumors was 53% and in 7 patients with PD-L1-positive tumors was 29%. One of two patients (50%) with unknown PD-L1 baseline status experienced a PR. The most common treatment-related AEs in the overall population including 283 patients with various solid malignancies were flu-like symptoms (58.7%), rash (44.5%), fatigue (42.0%), and pruritus (31.4%). Grade 3 or higher AEs occurred in 14.1% of patients, and treatment was discontinued in 2.1% of patients due to treatment-related AEs. Treatment-related immune-mediated AEs occurred in 3.5% of patients. One nivolumab-related grade 5 pneumonitis was reported.

The positive results of the phase 1/2 study led to phase 3 studies including a clinical trial comparing the combination of NKTR-214 with nivolumab to oncologist choice of either sunitinib or cabozantinib for the front-line treatment of metastatic RCC (NCT03729245). Work is also being done to evaluate the role of triplet therapy with nivolumab, ipilimumab, and NKTR-214 in mRCC (NCT02983045).

Adoptive Cell Therapy

The generation and adoptive transfer of tumor-infiltrating lymphocytes (TILs) has demonstrated durable complete responses in metastatic melanoma [65], but the success rates of this strategy are much lower in other cancers [66]. A number of studies have shown that the tumor microenvironment in RCC harbors tumor-reactive T cells [66, 67], but the magnitude and quality of responses generated by these cells and compared to other tumor types remain to be determined. Only modest success was elucidated with TIL therapy in RCC in previous clinical trials [68]. It is important to note, however, that these early trials did not use current advanced methods of TIL harvest and expansion and preoperative chemotherapy regimens, opening the horizon to revisit TIL therapy in RCC. This is especially true with the tremendous success achieved with immunotherapy in RCC, proving that immunologic control of this disease is feasible.

The use of chimeric antigen receptor (CAR)-T cells was also investigated in preclinical and clinical studies. CAR-T cells are generally T cells isolated from the patient and engineered to target TAAs [69]. Second- and third-generation CARs are engineered to express a co-stimulatory molecule, such as CD28, 4-1BB, CD27, ICOS, or OX40, to increase the antitumor effect, proliferation, and survival of CAR-T cells [70]. The greatest challenge in solid tumors is the identification of antigen targets. Many TAAs are also expressed at low level on healthy tissue so that an immune response could have serious toxicities. Carboxy-anhydrase-IX (CA-IX) expression in metastatic RCC was exploited for CAR-T cell therapy [71]. CA-IX is a metalloprotease that is considered a tumor-associated antigen (TAA) in RCC. However, it is also expressed on several normal tissues, such as the epithelium of the gastric mucosa, small intestine, duodenum, and biliary tree [72, 73]. Preclinical studies of first generation of CA-IX-directed T cells in RCC showed a robust cytokine production and cytotoxic activity was demonstrated [74]. Lamers et al. treated three patients with CA-IX-positive

metastatic RCC with first-generation anti-CA-IX CAR-T cells along with IL-2 administration but no prior lymphodepletion [75]. Two of these patients developed grades 2–4 liver toxicity, and liver biopsies showed T-cell infiltration around bile ducts causing cholangitis. CA-IX was overexpressed on the biliary ductal epithelium. Antibodies against the murine-derived scFv were detected in all three patients. In a subsequent study, the investigators preadministered unmodified antibody from which scFv was derived to saturate the liver before CAR-T cell administration and abrogate liver toxicity [71]. With this approach, no hepatotoxicity was observed in all four patients who received antibody pretreatment. No human anti-mouse antibodies against the cellular product were detected in patients who received the pretreatment, suggesting that the inflammation caused by the cholangitis possibly contributed to the generation of human anti-mouse antibodies. Unfortunately, no meaningful clinical responses were seen despite CAR-T cell persistence for 3–5 weeks.

Other antigens are being investigated for the exploitation of corresponding CAR-T cells including CD70 that is significantly overexpressed in RCC. Preclinical evaluation of CD70-targeting CD27-containing CAR in CD70-expressing tumors including RCC supported its safety and efficacy [76]. A clinical trial of anti-CD70 CAR in CD70-expressing solid tumors including RCC is currently recruiting (NCT02830724).

Multiple mechanisms are involved in T-cell suppression and are mediated via myeloid-derived suppressor cells (MDSCs) [56, 77], through arginase-mediated downregulation of the T-cell receptor ζ chain [78] as well as circulatory regulatory T cells (Tregs) [79, 80]. Sunitinib is a multikinase inhibitor for the treatment of metastatic RCC, and it has been shown to decrease MDSCs [81], enhance type-I INF responses, and decrease Treg function [82]. It would be intriguing to investigate the role of VEGFR-TKI in preconditioning and maintenance after CAR-T cell therapy in RCC [83].

Adjuvant Immunotherapy

The success of immunotherapy in advanced and metastatic RCC led to its investigation as adjuvant therapy. Adjuvant IL-2 and INF-α in locally advanced, nonmetastatic RCC following nephrectomy were investigated in multiple clinical trials. A randomized phase 3 study compared INF-α to observation following nephrectomy for pT3–4 M0 and/or pathologically lymph node-positive disease and involved 283 patients [84]. At a median follow-up of 10.4 years, OS was 7.4 years in the INF arm compared to 5.2 years in the observation arm, but this difference was not statistically significant ($P = 0.09$). There was also no difference in recurrence-free survival (RFS) between the two arms (3 vs 2.2 years, $P = 0.33$). The treatment-related toxicity was prominent in this study with 12% of patients experiencing grade 4 AEs (most commonly neutropenia and myalgias). No treatment-related deaths occurred.

Another phase 3 trial was conducted by the Cytokine Working Group which randomized patients to either receive single administration of high-dose bolus IL-2 or observation following complete resection of pT3–T4 Nx or pTany N1–3, and/or M1 RCC [85]. The study was stopped after a per protocol interim analysis showed no improvement in disease-free survival (DFS), which was initially anticipated to be 30% improved in the IL-2 group, despite full accrual. Again, IL-2 toxicity was severe. Eighty-eight percent of patients experienced at least grade 3 or 4 AEs, most commonly hypotension (52% required vasopressor support).

Vaccines were also investigated as potential adjuvant immunotherapeutic agents. Reniale®, an autologous RCC tumor vaccine derived from a lysate of a patient's own renal tumor, has been investigated in the adjuvant setting. A phase 3 trial randomized 379 patients with suspected RCC undergoing nephrectomy to either receive the tumor vaccine or observation postoperatively if the disease was high risk (pT2-T3b, pN0–3) [86]. The vaccine was administered every 4 weeks for a total of six doses. There was a mod-

est 5-year PFS improvement in the vaccine arm (77.4% vs 67.8%, $P = 0.02$). The survival benefit was more pronounced in pT3 tumors. Despite the positivity of this phase 3 trial, concerns about its applicability arose as the pathologic staging was based on the 1993 UICC classification, the lack of blinding, the fact that patients in the control arm did not receive placebo injections, and the exclusion of a large number of patients (179 patients) after randomization due to non-RCC histology, loss to follow-up within 6 months, and other reasons.

Vitespen (HSPPC-96) is a vaccine derived from heat shock protein-peptide complex from autologous tumor [87]. Its use in the adjuvant setting was investigated in a multicenter phase 3 randomized trial of patients with cT1b-T4N0M0 or TanyN1-2M0 RCC and planned to undergo curative nephrectomy [88]. The vaccine was administered weekly for 4 weeks and then every 2 weeks as long as the Vitespen supply lasted or until disease progression. There was no statistically significant difference in RFS or OS between the experimental and control groups. Preplanned and post hoc subgroup analyses suggested that vitespen improves RFS in patients with lower stage (T1b-T2) high-grade tumors. Therapy was well tolerated and no grade 3 or 4 AEs occurred.

Immune checkpoint blockade is also being actively investigated in the adjuvant setting. The PROSPER trial (NCT03055013) is currently exploring nivolumab in both the neoadjuvant and adjuvant settings. Patients with cT2–T4 and/or cN+ disease are randomized to observation or to two courses of nivolumab prior to radical or partial nephrectomy, followed by 9 months of adjuvant nivolumab. This design took advantage of the robust antitumor immune responses elicited in the presence of the primary tumor and, hence, allows for nivolumab administered neoadjuvantly to amplify its efficacy in the adjuvant setting.

The IMmotion 010 (NCT03024996) phase 3 trial is evaluating the efficacy of atezolizumab in the adjuvant treatment of RCC. Patients with pT2 Fuhrman grade 4, pT3a Fuhrman grade 3 or 4, and pT3b-4, or any N+ disease were included. The study is limited to clear-cell or clear-cell component RCC and RCC with or without sarcomatoid dedifferentiation. Primary endpoint is DFS.

Additional clinical trials of other immune CPIs in the adjuvant setting are ongoing, including pembrolizumab (KEYNOTE-564, NCT03142334) and the combination of ipilimumab with nivolumab (CheckMate914, NCT03138512). To date, there are no data on the use of CPIs in the adjuvant setting in RCC.

Biomarkers for Response

The research of biomarkers to predict response to immunotherapy, in general, and in RCC, in particular, is critical but remains challenging. Different trials of immune CPIs in RCC used different assays for the assessment of tumor expression of PD-L1. The CheckMate 025 and 214 trials used Dako PD-L1 IHC 28-8 pharmDx test to assess for PD-L1 expression. While nivolumab efficacy was not affected by PD-L1 expression in CheckMate 025, patients with tumor expressing PD-L1 more than 1% showed a worse OS suggesting rather a prognostic more than a predictive role of PD-L1 [39]. On the other hand, CheckMate 214 showed that PFS benefit was more pronounced in patients expressing PD-L1 (more or equal to 1%) [8]. OS was maintained in all categories. Results from the two trials suggest that PD-L1 IHC expression is not a predictor of response in patients with metastatic RCC receiving immune CPIs. Not only did different trials use different tests for the detection of PD-L1 expression with varying results, but the inconsistencies seen in results across trials make PD-L1 a challenging marker to rely on in predicting response in RCC. Intratumoral heterogeneity of PD-L1 expression was demonstrated by a multisite tumor sampling strategy [89], which identified a greater number of positive cases than those detected by current sampling protocols as the same tumor exhibited multiple regions with positive and negative expressions.

Another biomarker used in other diseases to predict response to immunotherapy is tumor

mutational burden (TMB) and nonsynonymous expression where higher tumor expression of neoantigens was linked to a favorable response to immunotherapy [90, 91]. In RCC, immunotherapy was shown to be effective in higher risk categories where tumor mutational load is high, which warrants additional investigation of the role of TMB as a biomarker of response with immunotherapy [92]. In CheckMate-214, subgroup analysis showed significantly better results of the combination of ipilimumab with nivolumab in the intermediate- to poor-risk disease category, which could be partly related to higher TMB and abundance of neoantigens in these worse risk categories [8]. Contrary to these thoughts, however, TMB across different IMDC or MSKCC prognostic criteria was not shown to be different [92]. Moreover, TMB did not differ between clear-cell and sarcomatoid components of different tumor samples, suggesting that TMB is not associated with worst clinical features, although this hypothesis needs to be further investigated [93]. Another study carried out whole exome and transcriptome sequencing of nine patients with metastatic RCC receiving nivolumab [94]. They found out that RCC had relatively few nonsynonymous mutations and neoantigens. Interestingly, among the nivolumab-treated patients, neoantigen load was significantly higher in nonresponders compared to responders ($P = 0.048$), but nonsynonymous mutation load was not. An exceptional responder who experienced CR (PFS > 30 months) had outlying higher expression of selected immune-related genes compared to the eight other patient samples ($P < 0.05$ for PD-L1, PD-L2; $P < 0.01$ for CTLA4, PD-1, PRF1; $P < 0.001$ for GZMA, BTLA, CD8A) and was in the top 1–5% of expression of these genes among all The Cancer Genome Atlas (TCGA) data. While the sample size of this study is too small to draw a generalizable conclusion, this study could suggest that TMB role in predicting response to immunotherapy is RCC is different from that seen in other tumor types.

Other biomarkers are being actively investigated. An analysis of the phase 3 IMmotion151 trial identified gene signatures in RCC that correlate with improved PFS in patients treated with atezolizumab plus bevacizumab compared to sunitinib [95]. These findings were presented at the European Society for Medical Oncology (ESMO) 2018 Congress. In the study by Rini et al., a group of patients with a gene signature showing high expression of T-effector cells had improved PFS with the combination of atezolizumab and bevacizumab compared with sunitinib (12.45 vs 8.34 months). On the other hand, in patients with low expression of T-effector cell genes, a smaller increase in PFS was seen with the combination compared to sunitinib (9.72 vs 8.41 months). Moreover, they studied a signature of angiogenesis-associated genes and found that in the group of patients with low expression of these genes, median PFS was higher in patients treated with the combination of atezolizumab with bevacizumab as opposed to sunitinib (8.94 vs 5.95 months). The improvement in PFS in the group of patients with high expression of angiogenesis-associated genes was not as robust in patients treated with the combination compared to sunitinib, 12.45 versus 10.2, respectively. They also demonstrated that in the sunitinib-treated group of patients, sunitinib was associated with higher PFS in the high versus low expression of angiogenesis-related genes (10.12 vs 5.95 months, respectively).

Other markers are being explored including PD-L2 expression, the gastrointestinal microbiome composition, and others. This is an active area of research, and the future, perhaps, involves a combination of biomarkers used together to predict response.

Future Directions for Immunotherapy in RCC

Current immunotherapeutic indications in advanced RCC include nivolumab monotherapy after prior antiangiogenic use in metastatic RCC, the combination of nivolumab and ipilimumab in the frontline setting of intermediate- to poor-risk disease metastatic RCC, and the combination of pembrolizumab and axitinib in frontline mRCC. More recent trials of immunotherapy-based treatment approaches combining CPIs

with antiangiogenesis agents show promise and could be approved soon to add to the current immunotherapy landscape. Many other ongoing trials will help elucidate more therapeutic options. No data currently exist on the role of immunotherapy in the adjuvant setting after curative nephrectomy, but this is an area of current investigation. Other immunotherapeutic strategies in the management of RCC are being investigated, including vaccines, adoptive cell transfer, cytokines, etc.

The breakthrough of immunotherapy in RCC is promising, but it is essential to realize that maximal clinical benefit will be hard to achieve without continuous efforts to optimize immune-related toxicities that have been shown to hinder the widespread use and applicability of these treatments. A multidisciplinary approach with assistance from specialists such as pulmonologists, endocrinologists, cardiologists, gastroenterologists, and others is necessary. Moreover, evidence-based and algorithmic approaches in handling toxicity need to be standardized in the management of immune-related toxicities. More research is required in the field of stratifying and prioritizing patients who will draw maximum gain from the use of immunotherapies as well as those who are predisposed to higher toxicities. The discovery and development of newer ways to manipulate the immune system so to potentiate T-cell and immune cell responses in the presence of immune CPIs or other immunotherapies will lead to increase in the scope of benefit from these breakthrough treatments.

Immunotherapy for Urothelial Carcinoma

Bladder cancer is the sixth most common cancer with an estimate of 80,470 new cases to be diagnosed in the United States in 2019 and 17,760 deaths during the same year [96]. Urothelial carcinoma (UC) is the most common subtype in the United States and Europe [97, 98]. Bladder cancer is most frequently diagnosed among people aged 65–74 [99]; therefore, it is important to factor other medical comorbidities into treatment choices. Approximately, 75% of new cases are nonmuscle invasive and characterized by a tendency to recur [100, 101]. On the other hand, muscle-invasive disease (extension past the basement membrane) and metastatic UC represent the other 25% and have a significantly worse outcome [102]. Despite the effectiveness of platinum-based therapies, metastatic UC still has a modest median OS of around 15 months [100, 103]. Similarly, second-line chemotherapies provide a suboptimal OS [104, 105]. CPIs flipped the equation for both platinum-refractory and platinum-ineligible patients [106–113]. Actionable genetic alterations, which are found in >50% of high-grade UCs, are gaining interest as well especially fibroblast growth factor receptor (FGFR) alterations [114]. Additionally, several TAAs in UC are attractive targets for antibody drug conjugate (ADC) development, which are being studied alone and in combinations with CPIs [115, 116]. Here, we describe the FDA-approved immune-oncology (I-O) modalities and the prominent investigational strategies for early or advanced stage UC.

Rationale for Immunotherapy in UC

In 1976, immune modulation was found to be helpful in the management of nonmuscle-invasive bladder cancer (NMIBC) with the use of Bacillus Calmette–Guerin (BCG) [117]. Forty years later, genomic studies showed that bladder cancer ranks third after melanoma and non-small-cell lung cancer in terms of somatic mutation rate [118, 119]. This high mutational burden and genomic instability seem to determine sensitivity to immunotherapy [120, 121]. Genomic alterations are translated into foreign proteins that could be recognized by cytotoxic T cells and potentiate cancer cells response to CPI [122]. However, infiltrating CD4+ and CD8+ T cells expresses high levels of PD-1 in UC [123], rendering them ineffective at eradicating tumors. Furthermore, expression of PD-L1 on UC cells is associated with higher grade, stage, rate of postoperative recurrence, and risk of death after cystectomy [123–125]. These findings provide

the rationale for using anti-PD1 and anti-PD-L1 immunotherapies to treat patients with UC.

Immunotherapy for NMIBC

Following endoscopic removal of tumors, size, multifocality, grade, and other risk factors help determine the further steps of management of NIMBC. Risk of recurrence determines the type and duration of intravesicular therapy or even cystectomy if needed [126].

BCG Vaccine

The first trial to show the benefit of BCG in NMIBC was done by Lamm et al. in 1980 and showed reduction in tumor recurrence [127]. This was followed by the FDA approval for this indication in 1990 [128]. In terms of reducing recurrences, BCG post resection of high-grade NMIBC is superior to observation and superior to intravesicular chemotherapy [129–131]. Based on SWOG8507, BCG is commonly given as an induction phase (6 weekly instillations) followed by maintenance (BCG each week for 3 weeks given 3, 6, 12, 18, 24, 30, and 36 months) [132]. BCG failure can be classified into BCG refractory disease (persistence of high-grade tumors after induction and one maintenance course) and BCG-relapsing disease (reappearance of disease after a disease-free state). Understanding the mechanism of BCG immune response is essential to develop strategies for BCG refractory disease. BCG is thought to invade the urothelium inducing an innate immune response followed by a T helper 1-based adaptive immune response that prevents tumor recurrence. It is unclear if this immune response is tumor specific or BCG specific with a side effect of antitumor activity [128]. A combination of intravesicular pembrolizumab + intravesicular BCG is being investigated in BCG naive high-risk NIMBC and BCG-relapsing NIMBC (NCT02808143).

BCG Refractory Population

Several years prior to anti-PD-1/PD-L1 clinical use in UC, Inman et al. reported that PD-L1 expression was abundant in the BCG-induced bladder granulomata in 11 of 12 patients failing BCG treatment. SWOG1605 (NCT02844816) is a phase 2 trial based on the reported efficacy of atezolizumab in metastatic UC and the known expression of PD-L1 expression in NMIBC after BCG therapy. This trial will evaluate the activity of atezolizumab in BCG-unresponsive high-risk NMIBC [133]. Two similar ongoing clinical trials with pembrolizumab + BCG (NCT02324582) and nivolumab + BCG (CheckMate 9UT; NCT03519256) in BCG-refractory patients are aiming to address this question as well.

Immunotherapy for Muscle-Invasive Bladder Cancer (MIBC)

In addition to the resection of MIBC, most patients require further treatment with cystectomy, partial cystectomy, neoadjuvant, adjuvant therapy, or a combination of these modalities [134, 135]. Neoadjuvant cisplatin-based chemotherapy prior to cystectomy for MIBC patients who are resectable provides 5% improved 5-year OS and 9% improved 5-year DFS [136]. Therefore, neoadjuvant chemotherapy followed by radical cystectomy is a category 1 recommendation for MIBC.

Neoadjuvant Immunotherapy in Cisplatin-Ineligible Patients

Patients with hearing loss, neuropathy, poor performance status, and cardiac or renal insufficiency are typically deemed cisplatin ineligible. It is estimated that 50% of patients are cisplatin ineligible [137, 138]. Neoadjuvant therapy with anti-CTLA-4 showed a measurable immunologic effects, consisting of an increased frequency of CD4 + $ICOS^{hi}$ T cells in tumor tissues and the systemic circulation [139]. PURE-01 (NCT02736266) is an open-label, single-arm, phase 2 study that assessed pembrolizumab in the neoadjuvant setting for MIBC for cisplatin-eligible patients. Fifty patients were enrolled, all underwent cystectomy and 42% had pathological complete response (PCR). A TMB of 15 mutations/Mb was significantly correlated with higher

likelihood of PCR [140]. Atezolizumab is being studied in a similar fashion (ABACUS; NCT02662309). Interim analysis showed that 39% of patients underwent downstaging. However, 10% did not undergo cystectomy [141]. An ongoing trial (NCT02812420) at M. D. Anderson Cancer Center, Houston, TX, is evaluating neoadjuvant durvalumab (anti-PD-L1) plus tremelimumab (anti-CTLA-4) in patients with MIBC who are ineligible for cisplatin-based neoadjuvant chemotherapy. Preliminary data show that of the six patients who underwent cystectomy, three had PCR [142]. DUTRENEO (NCT03472274) is comparing the durvalumab plus tremelimumab combination to cisplatin in the neoadjuvant setting for cisplatin-eligible patients. CPI plus cisplatin chemotherapy is also being investigated (NCT02690558).

Immunotherapy in Combination with Radiotherapy for Localized Bladder Cancer

Several trials are assessing combining radiotherapy with CPIs alone for cisplatin-ineligible MIBC (NCT02891161, NCT03419130) or radiotherapy with CPIs plus chemotherapy for MIBC cisplatin-eligible patients (NCT02662062, NCT03170125, NCT02621151). Of these studies, NCT02621151 gains particular interest as it is a pilot study for MIBC patients who either wish for bladder preservation or are ineligible for cystectomy. This trial is expected to take 2 years to accrue planned 30 patient enrollment [143].

Adjuvant Immunotherapy in High-Risk Patients

Following standard neoadjuvant therapy and cystectomy, in patients with pT3, pT4 disease, or positive nodes, there is an unclear role for additional adjuvant chemotherapy. CheckMate 274 (NCT02632409) is a randomized phase 3 trial comparing nivolumab as adjuvant treatment versus placebo in patients with high-risk invasive UC of the bladder, ureter, or renal pelvis post resection. The IMvigor010 (NCT02450331) and AMBASSADOR (NCT03244384) are similar randomized phase 3 adjuvant trials studying atezolizumab and pembrolizumab, respectively (Table 6.3). NIAGARA (NCT03732677) is a phase 3 study of neoadjuvant durvalumab + cisplatin-based chemotherapy followed by durvalumab adjuvant therapy.

Immunotherapy for Advanced Stage UC

To date, the US FDA has approved five CPI agents as a frontline or second-line treatment for patients with advanced bladder cancer who are either ineligible or progressed after cisplatin [106–113].

Platinum Ineligible

Pembrolizumab

KEYNOTE-052 is the phase 2 trial that studied pembrolizumab as first-line treatment for cisplatin-ineligible patients with metastatic UC [112]. Overall, ORR was 24% (CR 6%), but it was higher at 38% (CR 13.3%) in patients with ≥10% CPS. KEYNOTE-361 trial (NCT02853305) is the phase 3 study for frontline pembrolizumab in metastatic UC. Arms of treatment are pembrolizumab monotherapy, pembrolizumab plus cisplatin-based chemotherapy, or chemotherapy alone [144, 145]. Cisplatin was replaced by carboplatin in cisplatin-ineligible patients. Based on KEYNOTE-052 results, the US FDA approved the use of pembrolizumab for cisplatin-ineligible population in 2017. However, in June 2018, the FDA announced that treatment-naive patients with <10% CPS have lower OS with the use of pembrolizumab as monotherapy compared to carboplatin chemotherapy. Therefore, the FDA changed the prescribing label for pembrolizumab to include cisplatin-ineligible patients with CPS ≥ 10% by an FDA-approved test. If patients are cisplatin and carboplatin ineligible, then pembrolizumab is still indicated regardless of PD-L1 status (Fig. 6.3).

Atezolizumab

The phase 2 IMvigor210 trial included two cohorts (treatment-naive and previously treated patients). Cohort 1 studied atezolizumab in treatment-naive cisplatin-ineligible metastatic

Table 6.3 Ongoing phase 3 trials studying adjuvant checkpoint therapy for invasive UC

NCT identifier (trial)	Intervention	Phase	Population	Estimated sample	Results
NCT02632409 (CheckMate 274)	Nivolumab	3	Adjuvant therapy high-risk MIBC	640	NR
NCT02450331 (IMvigor010)	Atezolizumab	3	Adjuvant therapy high-risk MIBC	800	NR
NCT03244384 (AMBASSADOR)	Pembrolizumab	3	Adjuvant therapy high-risk MIBC and locally advanced UC	739	NR

MIBC muscle-invasive bladder cancer, *NR* not reported

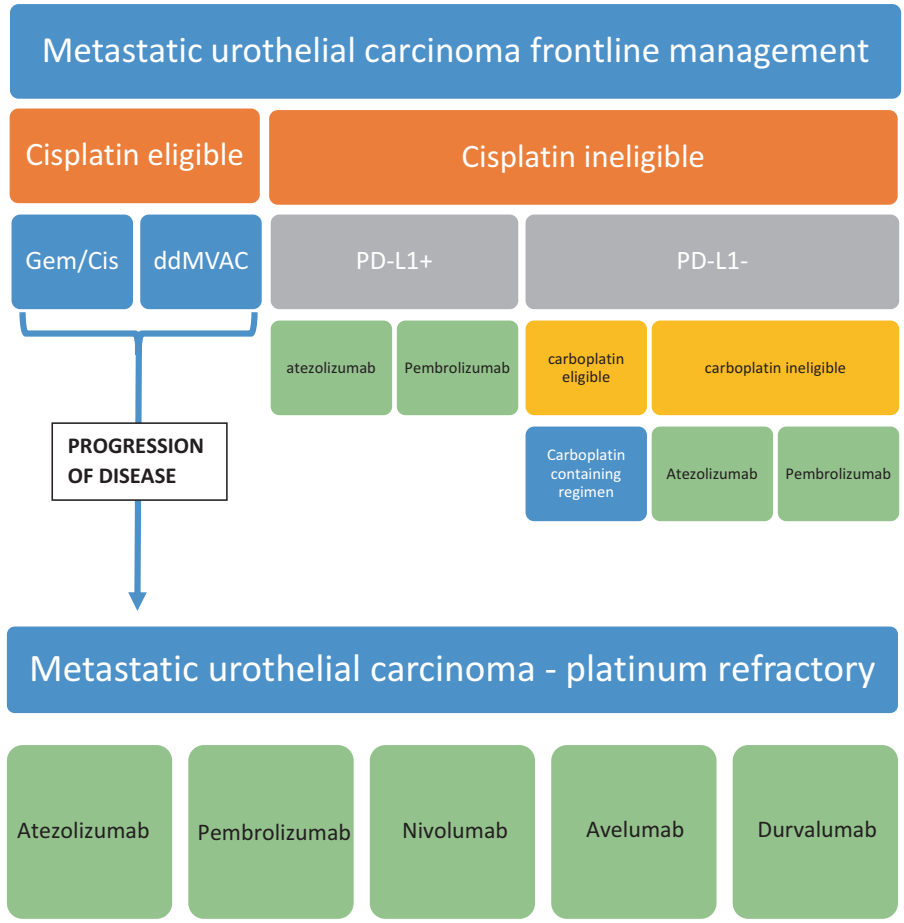

Fig. 6.3 Current treatment algorithm for metastatic urothelial carcinoma

UC patients [146]. This cohort had a different breakdown of patients deemed cisplatin ineligible: 70% had renal impairment; 20% had ECOG PS 2, and 14% had hearing loss. They were stratified based on PD-L1 expression on immune cells (IC) into IC0 (<1%), IC1 (≥1% but <5%), and IC2/3 (≥5%). ORR in unselected patients was 23%, and in contrast to prior results, ORR did not correlate with PD-L1 expression. Similar to pembrolizumab, the FDA approved atezolizumab in 2017 as first line for cisplatin-ineligible patients. IMvigor130 is an ongoing phase 3 trial randomizing treatment-naive patients to three arms: atezolizumab

plus platinum-based chemotherapy, atezolizumab alone, and chemotherapy alone [147]. Stratification is similar to the IMvigor210. Similar to pembrolizumab, in June 2018, the FDA announced that treatment-naive patients with IC0/1 PD-L1 status have lower OS with the use of atezolizumab compared to carboplatin chemotherapy. Therefore, the FDA changed the prescribing label for atezolizumab to include cisplatin-ineligible patients with IC2/3 by an FDA-approved test. If patients are cisplatin and carboplatin ineligible, then atezolizumab is still indicated regardless of PD-L1 status (Fig. 6.3).

Platinum Refractory

Five agents nivolumab, pembrolizumab, atezolizumab, durvalumab, and avelumab, with the first two being PD-1 antibodies and the last three being PD-L1 antibodies, demonstrated clinical activity following platinum in metastatic UC with ORRs ranging from 15% to 25% [106–111].

Pembrolizumab

Pembrolizumab for UC was first studied in the phase 1b KEYNOTE-12 trial [148], which required ≥1% PD-L1 expression. ORR was 26% in unselected patients with good tolerance, that is, only 15% with grade ≥3 AEs. The phase 3 KEYNOTE-45 compared pembrolizumab to second-line chemotherapy in platinum-refractory UC [113]. Control arm was investigator's choice of chemotherapy with paclitaxel, docetaxel, or vinflunine. Pembrolizumab had a survival advantage over chemotherapy (10.3 vs 7.4 months) and a better response rate (21% vs 11%). These results showed for the first time in 30 years an agent that improves survival in the second-line setting. The FDA approved pembrolizumab (May, 2017) for metastatic UC progressing during or following platinum-containing chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy. For pretreated UC, several trials are attempting combinations of pembrolizumab plus chemotherapy (NCT02437370).

Atezolizumab

Atezolizumab was the first FDA-approved CPI for locally advanced or metastatic UC patients who progressed on platinum therapy. In a phase 1 trial, which enrolled 68 patients with previously treated metastatic UC, atezolizumab had an ORR ranging from 11% to 43% [110]. The higher ORR was seen in tumors expressing high levels of PD-L1, defined as ≥5% in tumor cells or tumor-infiltrating immune cells. Cohort 2 (previously treated) from the abovementioned IMvigor210 had an ORR in all-comers of 15% versus historical control of ORR with second-line cytotoxic chemotherapy of 10%. However, ORR was 27% for IC2/3 and 18% for IC1/2/3 [108]. This provided the basis for the FDA to approve atezolizumab as second-line in May 2016. IMvigor211 was the phase 3 trial that randomized patients who progressed after platinum therapy to receive either atezolizumab or chemotherapy (physician's choice between taxanes or vinflunine). Similar to IMvigor210, PD-L1 on ICs was used to stratify patients. The primary endpoint of OS was tested in hierarchical fixed-sequence procedure: in the IC2/3 population, followed by IC1/2/3, followed by the intention-to-treat. Statistical significance was required at each step before formal testing of the subsequent population. The IC2/3 population failed to show improved survival; therefore, the other populations were not evaluated [141]. Nonetheless, atezolizumab is approved by the FDA for post platinum therapy of metastatic UC based on improvement of ORR in comparison to historic rates for second-line chemotherapy.

Nivolumab

Nivolumab was first studied in the CheckMate 032, which was a phase 1/2 single-arm trial. The trial showed an ORR of 24.4% in patients with locally advanced or metastatic UC who progressed after platinum-based therapy. PD-L1 high (≥1% on tumor cells) and PD-L1 low (<1% on tumor cells) had similar responses (24% vs 26%). However, PD-L1 high median OS was longer (16.2 months vs 9.9 months) [109]. CheckMate 275 was the phase 2 study to

verify these findings [149]. The primary endpoint was ORR in all treated patients and used slightly different stratification for tumor PD-L1 expression (≥5%, ≥1%, and <1%). ORR was 19% for unselected patients. However, when analyzed by tumor PD-L1 expression, ORR was 28.4% in PD-L1 of ≥5%, 23.8% in PD-L1 of ≥1%, and 16.1% in PD-L1 of <1%. Nivolumab was well tolerated with 18% of grade ≥3 AEs. The FDA approved nivolumab in 2017 for use in metastatic UC as second-line post cisplatin therapy.

Avelumab

Avelumab has the additional ability (beside checkpoint inhibition) to lyse PD-L1 expressing tumor cells by an antibody-dependent cell-mediated cytotoxicity [150]. In a phase 1b trial, avelumab showed an ORR of 18.2% in post platinum UC and tolerable profile with only 6.8% grade ≥3 AEs. In a pooled analysis post platinum cohort from the phase 1 dose-expansion JAVELIN Solid Tumor study, avelumab had an OR of 17%. Patients in the JAVELIN trial were not selected based on PD-L1 expression. Maintenance avelumab compared to supportive care in patients with metastatic UC that did not progress after 4–6 cycles of platinum-based chemotherapy is the focus of the JAVELIN Bladder 100 phase 3 trial (NCT02603432) [151]. GCISAVE (NCT03324282) is a phase 2 study that is studying the safety and efficacy of gemcitabine, cisplatin (GC) +/− avelumab in first-line treatment for locally advanced or metastatic UC patients.

Durvalumab

A phase 1 trial of durvalumab in platinum-resistant UC showed an ORR of 46.4% in the PD-L1-positive (defined as ≥25% of tumor cells or tumor-infiltrating immune cells) subgroup and 0% in the PD-L1-negative subgroup [152]. A phase 1/2 trial for metastatic UC patients followed and 95.3% of enrolled patients had failed platinum therapy [107]. ORR was 17.8% across all patients, 27.6% for PD-L1 high, and 5.1% for PD-L1 low. These results led the FDA to grant accelerated approval in 2017 to durvalumab in the second-line setting after failing cisplatin.

Predictive Biomarkers for Response and Resistance

As detailed above, only a minority of patients respond to CPIs. Therefore, several efforts are aimed at identifying biomarkers that predict response. As detailed previously, PD-L1 expression in UC is associated with higher grade of tumor [123], worse clinical outcomes, and less postoperative survival [124]. Intuitively, PD-L1 was predicted as a potential predictive biomarker for CPI therapy. In the IMvigor210 trial, higher PD-L1 expression was associated with an increased response [108]. In contrast, the CheckMate 275 showed nivolumab responses irrespective of tumor PD-L1 expression [149]. Using PD-L1 as a predictive marker faces several critiques. First, staining PD-L1 by immunohistochemistry assays is not yet reproducible. For example, the IMvigor210 used the Ventana SP142 assay to measure PD-L1 on tumor-infiltrating ICs, the durvalumab trial utilized the Ventana SP263 assay to measure PD-L1 on both tumor cells and ICs, and the CheckMate 275 used the Dako PD-L1 28-8 pharmDx kit to measure PD-L1 on tumor cells only [108, 149, 152]. Second, the cutoffs used to define low or high expression are not universal. Third, PD-L1 expression is dynamic, and a single biopsy is unlikely to provide a complete assessment of PD-L1 status for the entire duration of disease [153]. In the CheckMate 275, a 25-gene interferon-γ (IFN-γ) signature was associated with response PD-L1 expression [149]. Genomic defects in IFN-γ pathway genes are linked to anti-PD-1 and anti-CTLA-4 resistance [154–158]. An exploratory subgroup analysis of IMvigor210 Cohort II showed a significant increase in TMB in responding patients relative to nonresponding patients (12.4 mutation/megabase vs 6.4 mutation/megabase) [108]. Smoking status and TCGA subtype did not correlate with TMB. Unified depth of sequencing, comprehensive sequencing panels, and silencing of germline

variants are among the challenges to clinical use of TMB. Other possible biomarkers include the four mRNA subtype clusters I–IV (luminal I, luminal II, basal I, and basal II) elucidated by TCGA project [119]. Sampling the primary tumor, lymph nodes, or metastatic lesions for TCGA subtyping may lead to inappropriate tumor classification, and this limits its utility as a marker. TCGA subtype has not proven to be a strong predictive biomarker for immunotherapy at this time.

Future Directions and Ongoing Trials

Although CPI offers an effective alternative option in a disease that had very few treatment options, objective responses with CPI remain low and more than 75% of patients do not respond. Unfortunately, the majority of patients with UC do not have an elevated PD-L1 expression [159], and many patients in the front line are also cisplatin ineligible [137]. Thus, additional therapies are necessary, and research is ongoing to investigate combinations of CPIs along with other agents that target the immune microenvironment [144].

Combination of Anti-PD-L1 + Anti-CTLA4

DANUBE (NCT02516241) is an ongoing phase 3 trial of durvalumab as monotherapy or combined with tremelimumab versus standard-of-care (SOC) chemotherapy for patients with metastatic or unresectable UC. OS is the primary endpoint for this three-arm trial. CheckMate 901 (NCT03036098) is a similar phase 3 trial evaluating nivolumab + ipilimumab and nivolumab + SOC chemotherapy versus SOC chemotherapy in treatment-naive patients with metastatic UC [160].

Combination of CPI + Chemotherapy (Table 6.4)

It is unclear if CPI therapy will replace current chemotherapy or add a synergetic effect. Currently, IMvigor130 (NCT02807636), KEYNOTE-361 (NCT02853305), and CheckMate 901 (NCT03036098) are addressing whether combination of immunotherapy and chemotherapy will be more effective than immunotherapy alone [144, 145, 147, 160]. Interestingly, cohort 2 of the IMvigor210 study demonstrated high PD-L1 expression corresponded with higher ORR, while in cohort 1, there was no correlation between PD-L1 expression and ORR. The major difference between cohorts was the exposure of cohort 1 patients to chemotherapy prior to receiving atezolizumab [108]. This suggests that prior chemotherapy can modulate the immune microenvironment and expression of PD-L1. Indeed, a recent retrospective study demonstrated that PD-L1 tumor expression was significantly higher on postneoadjuvant chemotherapy specimens than in matched preneoadjuvant specimens, supporting this hypothesis [161].

Table 6.4 Ongoing phase 3 studies assessing frontline CPIs conbined with chemotherapy in patients with metastatic or unresectable UC

NCT identifier (trial)	Intervention	Comparator	Phase	Primary outcome	Results
NCT02516241 (DANUBE)	Durvalumab as monotherapy or combined with tremelimumab	Standard-of-care (SOC) chemotherapy	3	OS	NR
NCT02807636 (IMvigor130)	Atezolizumab plus platinum-based chemotherapy or atezolizumab alone	Platinum-based chemotherapy	3	PFS, OS, AEs	NR
NCT02853305 (KEYNOTE-361)	Pembrolizumab plus cisplatin-based chemotherapy or pembrolizumab alone	Platinum-based chemotherapy	3	PFS, OS	NR
NCT03036098 (CheckMate 901)	Nivolumab + ipilimumab or nivolumab + SOC chemotherapy	SOC chemotherapy	3	PFS, OS	NR

OS overall survival, *PFS* progression-free survival, *AEs* percentage of patients with adverse events, *NR* not reported

Other Combinations

Several trials are investigating immunotherapy with novel agents including other I-O drugs, ADCs, FGFR inhibitors, and others. Frontline combination trial (EV-103) of enfortumab vedotin (ADC against nectin-4) combined with pembrolizumab for cisplatin-ineligible patients with locally advanced or metastatic UC has been launched (NCT03288545). On April 12, 2019, the FDA granted erdafitinib approval for metastatic platinum-refractory UC with susceptible fibroblast growth factor receptor (FGFR) 2 or 3 genetic alterations. The promising results with FGFR-targeted therapies led to the investigation of using them in combination with immunotherapy. FORT-2 (NCT03473756) is a phase 1b/2 trial of the FGFR inhibitor rogaratinib plus atezolizumab in untreated FGFR-positive metastatic UC. FIERCE-22 (NCT03123055) is a phase 1/2 study for combination of FGFR3 inhibitor vofatamab plus pembrolizumab in platinum refractory UC. M7824 is a novel first-in-class bifunctional fusion protein consisting of the extracellular domain of the human transforming growth factor beta (TGFβ) receptor 2, which functions as a "trap" for all three TGFβ isoforms, covalently linked to the C terminus of the heavy chain of the anti-PD-L1 antibody derived from avelumab [162]. Preliminary data from a phase 1 dose-escalation study suggest that M7824 has clinical activity and manageable safety profile in patients with heavily pretreated advanced solid tumors [163]. This is being further explored in UC. NKTR-214, a CD122-preferential IL-2 pathway agonist, is being studied in combination with nivolumab in the phase 1/2 PIVOT-2 (NCT02983045) for cisplatin-ineligible patients. Siefker-Radtke et al. presented promising data during the GU malignancy symposium 2019 showing ORR of 48% in 27 evaluable patients [164].

Cellular Therapy

Cellular therapy for bladder cancer is still in its infancy. NCT02153905 was a phase 1 trial using autologous T-cell receptor immunotherapy targeting MAGE-A3 for patients with metastatic solid tumor who are HLA-A∗01 positive. However, trial was terminated early. NCT03389438 is a phase 1 study with autologous central memory T cells for metastatic bladder UC treated with first-line gemcitabine plus cisplatin. NCT02457650 is an ongoing phase 1 T-cell receptor-transduced T cells targeting NY-ESO-1 for treatment of patients with NY-ESO-1-expressing malignancies.

Future Directions in Immunotherapy for UC

Metastatic UC has a poor prognosis, and immunotherapy was a significant advancement that offered new treatment options to patients with metastatic UC. However, response rates from CPI monotherapy remain low, and it is important to understand mechanisms of resistance, identify biomarkers to choose potential responders, and develop more effective combination therapies. Immunotherapy, currently being investigated in the perioperative setting, offers the promise of improving outcomes by reducing the risk of recurrence.

Immunotherapy for Prostate Cancer

Prostate cancer (PC) is the most common cancer expected to be diagnosed in men in 2019 accounting for nearly one in five new diagnoses. In the United States, it is estimated that PC will still be the second leading cause of death from cancer in men in 2019 [96]. PC deaths have been increasing from an estimate of 26,739 in 2017 and 29,430 in 2018 to 31,620 in 2019 [165, 166]. Perhaps, this could be explained by the recommendations against screening and as a result an increased rate of distant metastases at diagnosis [167, 168]. Androgen deprivation therapy (ADT), commonly using medical castration, remains the current standard of care for initial treatment of patients with metastatic PC [169]. More recently, in February 2018, the FDA approved abiraterone

with prednisone to be added to ADT for newly diagnosed castration-sensitive PC (CSPC) [170, 171]. Additionally, chemotherapy (docetaxel) added to ADT (chemohormonal therapy) is also an option for metastatic CSPC based on the CHAARTED and STAMPEDE phase 3 trials [172, 173]. Despite the effectiveness of the previously mentioned therapies, eventually, all CSPC patients will progress to castrate-resistant PC (CRPC) [170–173]. Per the National Comprehensive Cancer Network (NCCN) guidelines, CRPC patients can be considered for microsatellite instability/mismatch repair (MSI/MMR) testing. Furthermore, they can be considered for mutational testing of homologous recombination genes in germline and tumor tissue [174]. This information is useful for counseling families at increased risk of malignancy, utilizing platinum early in the course of the disease, or guiding enrollment in targeted and immunotherapeutic clinical trials. Current approved therapies for metastatic CRPC include abiraterone, enzalutamide, radium-223, sipuleucel-T, and chemotherapy including docetaxel and cabazitaxel (Fig. 6.4) [175–182]. For men with metastatic CRPC, the median survival in recent phase 3 studies has ranged from 12.2 to 21.7 months [175–181]. The inevitable resistance to hormonal and chemotherapy indicates the need to develop novel therapeutic approaches [183] such as immunotherapies. Here, we discuss the basic immune biology of PC. We then highlight approved and investigational immunotherapy approaches that have advanced to later stage clinical trials.

Rationale for Immunotherapy in PC

Several reasons make immunotherapy an attractive option to target PC. In the 1990s, PC cells were reported to express specific TAAs such as the prostate-specific antigen (PSA), prostatic acid phosphatase (PAP), and prostate-specific membrane antigen (PSMA) [184–186]. These unique proteins to the prostate can serve as immunogenic antigens toward which the immune system can attack. The slow-growing nature of PC and its expression of TAAs allow the immune system time to mount a response [187, 188]. In fact, effector T cells responsive to PC TAAs have been identified in the peripheral blood of patients with PC especially those with CRPC [189, 190]. Preclinical data showed that antiprostate immune responses can exclusively target normal as well as cancerous prostate tissues without affecting other tissues that lack PC TAAs [191–193]. Additionally, histological evaluation of PC tissue

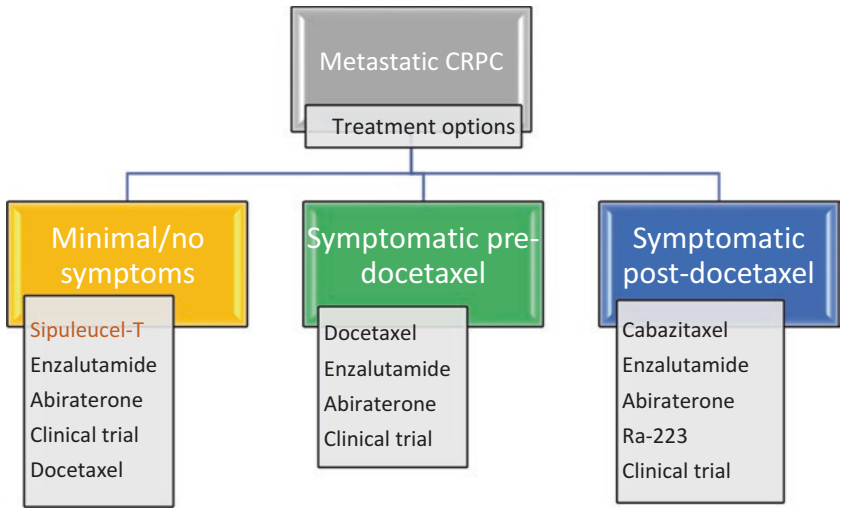

Fig. 6.4 Current treatment options for metastatic CRPC including the only approved immunotherapy sipuleucel-T

has identified infiltrating CD4+ and CD8+ lymphocytes (TILs) that are oligoclonally expanded, suggesting that their presence is due to specific antigenic stimulation [194]. Treatment with ADT modulates the immune microenvironment by inducing infiltration of CD8+ TILs as well as CD68+ macrophages into prostate tumors [195, 196]. CD68+ macrophages seem to be associated with increased risk of biochemical recurrence [196], indicating the complex nature of immune changes driven by ADT. Despite the clonal expansion of TILs, the high expression of PD-1 makes them likely incapable of mounting an effective immune response [194]. Coinhibition of TILs, generated mainly by the interaction between the B7 family and their receptor CD28 family, is another principal immune evasion pathway for PC [197]. Based on these findings, effective immunotherapy strategies against PC, especially CRPC, have focused on training the immune system against PC TAAs (via therapeutic vaccines) [198] and antagonizing immune checkpoints.

Vaccines

"Vaccines" is the broad term for mechanisms designed to stimulate the immune cells to ultimately target specific TAAs and destroy PC cells. Vaccines for PC can be divided into ex vivo processed (e.g., sipuleucel), vector-based (e.g., PROSTVAC), and whole tumor-cell vaccines (e.g., GVAX) [199]. Ex vivo processed vaccines are usually personalized (i.e., generated from the patient's own tumor-reactive immune cells), such as sipuleucel-T. Conversely, vector-based and whole tumor-cell vaccines are commonly generic (i.e., created or engineered to deliver selected TAAs known to be immunogenic) [200]. Several vaccines were developed to target PC, but they failed to show clinical efficacy [201]. We will be discussing agents that reached FDA approval or a late-stage clinical trial.

Sipuleucel-T

Sipuleucel-T is an example of personalized, cell-based, ex vivo processed DC vaccine against PC. Patient's peripheral blood mononuclear cells including antigen presenting cells (APCs) are activated ex vivo with recombinant fusion protein (PAP fused to GM-CSF) and reinfused into the patient (Fig. 6.5). D9901 was a placebo controlled phase 3 of 127 men with metastatic CRPC showed a survival advantage of 4.5 months but no significant delay in time to progression (TTP), which was the intended primary outcome [202, 203]. D9902A was an identical study that showed a trend toward increased survival with sipuleucel-T, although it was not statistically significant with no advantage in the primary outcome, TTP [202]. D9902B or the Immunotherapy for Prostate Adenocarcinoma Treatment (IMPACT)

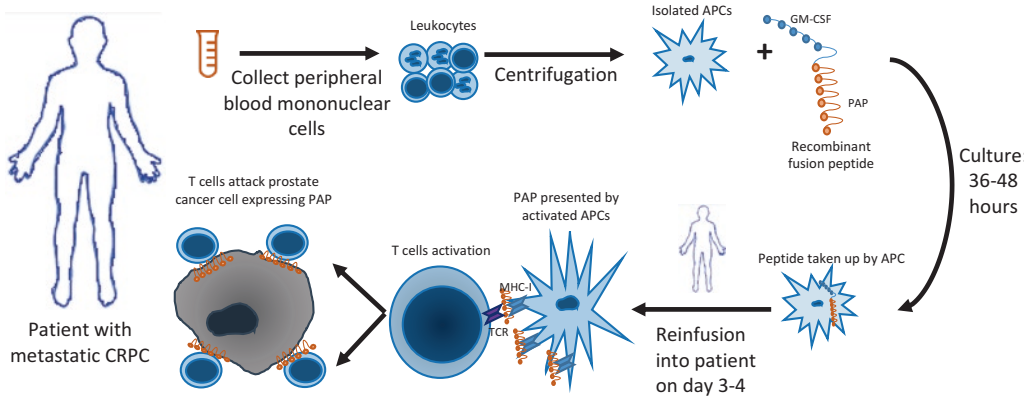

Fig. 6.5 The manufacturing process and proposed mechanism of action for sipuleucel-T

trial was a larger phase 3 that made OS its primary outcome. A total of 512 men with metastatic CRPC were randomized to either sipuleucel-T or placebo. There was a 4.1-month improvement in median survival (25.8 months in the sipuleucel-T group vs 21.7 months in the placebo group) but again no effect on TTP [179]. Based on these findings, sipuleucel-T was the first anticancer immunotherapy to be approved by the FDA. Despite sipuleucel-T approval, the IMPACT is critiqued as two thirds of the cells harvested were lost and not reinfused in the placebo arm. This large cell loss could provide an alternative explanation for the survival improvement [204]. However, these concerns were not credited during the FDA review due to the careful consideration given to the leukapheresis procedures in the placebo arm [205]. Sipuleucel-T is being studied in different combinations with other vaccines, antiandrogens, chemotherapy, cytokines, or CPIs. Examples of added agents include a DNA vaccine encoding PAP (NCT01706458) [206] after sipuleucel-T; however, PAP-specific T-cell responses, median TTP, and median OS were not statistically different from giving sipuleucel-T alone. STRIDE (NCT01981122) is a study that compared concurrent versus sequential enzalutamide with sipuleucel-T in metastatic CRPC, but it is not powered enough for difference in OS or PFS [207]. STAMP (NCT01487863) is a similar study to STRIDE using abiraterone instead of enzalutamide, and it is not powered to report differences in clinical outcomes as well [208]. Combinations of sipuleucel-T with chemotherapy were either terminated or withdrawn (NCT01420965, NCT02793765, and NCT02793219). On the other hand, NCT01804465 is a phase 2 study comparing immediate versus delayed addition of ipilimumab to sipuleucel-T and is still recruiting as of April 2019. Finally, it is worth mentioning that radiographic or PSA progression does not accurately reflect survival with sipuleucel-T, and finding an immune biomarker that can accurately reflect clinical benefit is urgently needed [209]. The absence of objective parameters to judge whether or not sipuleucel-T is benefitting the patients poses a major difficulty in determining when to consider sipuleucel-T ineffective and switch treatment.

GVAX

GVAX is an off-the-shelf allogeneic whole-cell vaccine that is made from irradiated PC lines and is genetically transduced to express GM-CSF. Two phase 1/2 studies established the safety of GVAX in CSPC and CRPC and suggested clinical response by reducing PSA [210, 211]. However, phase 2 and phase 3 trials are so far not promising. NCT00771017, a phase 2 combination with ADT trial for nonmetastatic biochemically relapsed PC was withdrawn. VITAL-1 (NCT00089856) was a phase 3 trial comparing GVAX to docetaxel in chemo-naive metastatic CRPC, but was terminated based on futility analysis showing <30% chance of meeting primary endpoint. VITAL-2 (NCT00133224) was another phase 3 trial with GVAX combined with docetaxel that was terminated due to an independent data monitoring committee recommendation reporting excess deaths in the experimental arm [201].

PROSTVAC

PROSTVAC is a recombinant vaccinia virus, modified to express PSA. It is safe and can induce stable PSA levels in half of treated patients, but it was not effective in inducing enough PSA-specific T-cell population [212, 213]. Therefore, PROSTAVAC-*VF* was developed as a prime/boost strategy using *v*accinia (primer) and *f*owlpox (booster) recombinant viral vectors. The vectors were engineered to express three co-stimulatory molecules (CD80, CD54, and CD58), hence, the name PROSTVAC-VF/TRICOM. Despite showing 8.5-month OS benefit, the phase 2 trial with this vaccine failed to show PFS benefit in metastatic CRPC which was its primary endpoint [214]. Consequently, the phase 3 trial, PROSPECT, was conducted to further investigate these findings but failed to show the benefit in OS. In fact, the trial was stopped early after meeting criteria for futility [215, 216]. Nonetheless, combination trials with

PROSTVAC-VF are underway. For example, the phase 2 trial NCT03315871 an anti-PD-L1 antibody (avelumab) with TGF beta-Trap molecule is added to PROSTVAC. Additionally, PROSTVAC is being studied in combination with other CPIs (NCT03532217, NCT02933255), enzalutamide (NCT01867333, NCT01875250), and chemotherapy (NCT02649855).

CPIs

CPIs have revolutionized the management of solid tumors in the past few years [217, 218]. Unfortunately, CPIs have not been as successful in PC perhaps due to its multifaceted and pleotropic immune tumor microenvironment [219]. Particularly, the sole use of CPIs has shown limited evidence of antitumor activity, likely due to the immunologically "cold" nature of the tumor and low PD-L1 expression on tumor cells. However, if existing PC treatments can trigger an adaptive immune response, attracting infiltrating immune cells and increasing tumor PD-L1 expression, there is a rationale for combinations improve outcomes [220] (Tables 6.5 and 6.6).

Anti-CTLA-4 for Metastatic PC

Ipilimumab blocks the T-cell-negative regulator CTLA-4 allowing CD28 and B7 interactions, which result in T-cell activation, proliferation, tumor infiltration, and ultimately, cancer cell death. In a phase 1/2 study (NCT00323882), escalating doses of ipilimumab (3–10 mg/kg) were used with and without radiation for metastatic CRPC. The 10 mg/kg with radiation cohort suggested activity and had similar rate of irAEs to the previously reported rates [221]. Therefore, 10 mg/kg was the dose chosen for phase 3 trials. NCT00861614 was a phase 3 trial in post docetaxel CRPC that involved bone-directed radiotherapy followed by randomization to either ipilimumab or placebo [222]. NCT01057810 was the second phase 3 trial that randomized patients with chemotherapy-naive metastatic CRPC without visceral metastases to ipilimumab alone versus placebo [223]. In both studies, ipilimumab did not improve OS, and when given alone, it increased PFS and had a higher PSA RR, suggesting antitumor activity in a patient subset. A small phase 2 trial using ipilimumab plus chemotherapy did not show any improvement in the activity of ipilimumab [224]. Another phase 2 trial evaluated ipilimumab combined with ADT early on for CSPC and established the safety of the combination [225]. Combination trials of ipilimumab with abiraterone (NCT01688492), ADT (NCT01194271, NCT01377389, NCT00170157), and sipuleucel-T (NCT01832870) are ongoing.

Anti-PD-1 in Metastatic PC

Pembrolizumab is another CPI that blocks the interaction of PD-1 and its ligand PD-L1, leading to T-cell activation and antitumor activity in PD-L1-positive mCRPC based on the phase 1b KEYNOTE-028 trial ($n = 23$) [226]. PD-L1 positivity was defined as expression in ≥1% of tumor or stromal cells. ORR was 17.4% with a median duration of response of 13.5 months. KEYNOTE-199 was a phase 2 that enrolled 258 patients with docetaxel-refractory mCRPC in cohorts 1 through 3 (C1–3). A total of 131 patients had measurable PD-L1+ disease (C1), 67 patients had measurable PD-L1- disease (C2), and 60 patients had nonmeasurable, bone-predominant disease (C3). Chemotherapy-naive subjects with mCRPC either having failed or showing signs of failure with enzalutamide in Cohorts 4 and 5 received pembrolizumab monotherapy in addition to their current regimen of enzalutamide. ORR ranged from 3% to 5%, and DCR lasting ≥6 months was 11%. ORR was not different between C1 and C2, indicating antitumor activity and disease control regardless of PD-L1 status. The RR was numerically higher in patients with somatic BRCA1/2 or ATM mutations (12%), supporting further investigation in patients with homologous recombination defects (HRD) [227]. A small phase 2 single-arm clinical trial demonstrated activity of pembrolizumab + enzalutamide in CRPC patients after progression with enzalutamide. Of the 10 patients enrolled,

Table 6.5 Later stage clinical trials for checkpoint inhibitors combined with oral therapies for metastatic CRPC (mCRPC)

NCT identifier (trial)	Phase	Outcome measures	Intervention	Population	Anticipated sample size	Preliminary results
NCT01688492	1/2	Primary: safety, PFS Secondary: PSA kinetics, bone scan changes	Ipilimumab + abiraterone + prednisone	Chemotherapy-naive mCRPC	57	NR
NCT02861573 (KEYNOTE-365)[a]	1b/2 umbrella trial	Primary: PSA RR, AEs, discontinuation rate Secondary: DCR, OS, DOR, ORR, rPFS (PCWG3 RECIST)	Cohort A: Pembrolizumab + olaparib	Post docetaxel mCRPC	70	Enrolled 41 patients. DCR ≥ 6 months: 29%. ORR: 7% (28 evaluable patients) [248]
			Cohort C: Pembrolizumab + enzalutamide	Post abiraterone acetate but prechemotherapy mCRPC	70	Enrolled 69 patients. DCR ≥ 6 months: 33%. ORR: 20% (25 evaluable patient) [235]
			Cohort D: Pembrolizumab + abiraterone + prednisone	Post enzalutamide acetate but prechemotherapy mCRPC	70	NR
NCT02787005 (KEYNOTE-199: cohorts 4 and 5)	2	Primary: ORR Secondary: DCR, PSA RR, AEs, discontinuation rate	Pembrolizumab + enzalutamide	Chemotherapy-naive mCRPC	370 (for all cohorts)	Results for single-agent pembrolizumab in postdocetaxel cohorts 1, 2, and 3 show ORR 3–5% [227]
NCT03338790 (CheckMate-9KD)[b]	2	Primary: ORR, PSA RR Secondary: rPFS, TTR, DOR, TTP-PSA, OS, AEs.	Arm A: Nivolumab + rucaparib	Post docetaxel mCRPC	330 (for all arms)	NR
			Arm C: Nivolumab + enzalutamide	Post abiraterone acetate but prechemotherapy mCRPC		NR
NCT03016312 (IMbassador 250)	3	Primary: OS Secondary: rPFS, PSA RR, TTP PSA, AEs, ORR	Atezolizumab + enzalutamide	Post abiraterone acetate and failure of taxane in mCRPC	730	NR
NCT03330405 (JAVELIN PARP MEDLEY)	2	Primary: toxicity, ORR Secondary: avelumab and talazoparib kinetics, TTR, DOR, PFS	Avelumab + talazoparib	mCRPC	242 (for all arms)	NR
NCT02484404	1/2	Primary: RP2D, ORR	Durvalumab with olaparib and/or cediranib	mCRPC	384 (for all arms)	17 patients enrolled; 47% had PSA responses >50% [240]

PFS progression-free survival, *PSA RR* prostatic-specific antigen response rate, *AE* adverse events, *ORR* objective response rate, *DCR* disease control rate, *OS* overall survival, *DOR* duration of response, *rPFS* radiographic progression-free survival, *PCWG3 RECIST* Prostate Cancer Working Group 3 modified RECIST 1.1, *NR* not reported, *TTP-PSA* time to prostate-specific antigen progression, *TTR* time to tumor response

[a]Cohort B of the KEYNOTE-365 is pembrolizumab + docetaxel + prednisone

[b]Arm B of the CheckMate-9KD is nivolumab in combination with docetaxel

Table 6.6 Selected combination trials of vaccines with checkpoint inhibitors for prostate cancer

NCT identifier (trial)	Phase	Status	Outcome measures	Intervention	Population	Anticipated sample size	Preliminary results
NCT02933255	1/2	Recruiting	Primary: safety Secondary: peripheral PSA-specific T cells, Treg prostate infiltration, PSA/PD-L1/MRI changes	PROSTVAC-VF + nivolumab	Chemotherapy-naive mCRPC	29	NR
NCT03315871	2	Recruiting	Primary: PSA RR Secondary: AEs, PSA slope over time	PROSTVAC-VF + avelumab linked with TGF beta-Trap molecule	Biochemically recurrent prostate cancer	34	NR
NCT03532217	1	Recruiting	Primary: safety, neoantigen-reactive T cells among other correlatives Secondary: PSA RR, OS, rPFS	PROSTVAC-VF + ipilimumab + nivolumab + neoantigen DNA vaccine	mCSPC	20	NR
NCT01832870	1	Completed	Primary: PAP/PA2024-specific immune response. Secondary: PSA, radiographic, clinical response, T-cell activity	Sipuleucel-T + ipilimumab	Chemotherapy-naive mCRPC	Actual enrollment of 9	Increased PAP/PA2024-specific immune response [249]
NCT01804465	2	Recruiting	Primary: PAP/PA2024-specific immune response. Secondary: PSA, radiographic, clinical response, T-cell activity	Sipuleucel-T + immediate or delayed ipilimumab	Chemotherapy-naive mCRPC	54	NR

mCRPC metastatic castrate-resistant prostate cancer, *mCSPC* metastatic castrate-sensitive prostate cancer, *PFS* progression-free survival, *PSA RR* prostatic-specific antigen response rate, *OS* overall survival, *DOR* duration of response, *rPFS* radiographic progression-free survival, *PCWG3 RECIST* Prostate Cancer Working Group 3 modified RECIST 1.1, *NR* not reported

three experienced a biochemical response and two a radiological response. Genetic analysis revealed markers of MSI in one patient [228]. MSI has been shown to be a predictive factor for response to pembrolizumab [229].

Pembrolizumab in High MSI

The prevalence of MMR deficiency in metastatic CRPC is estimated at 2–5% [230, 231]. In one series from MSKCC, 20 of 839 PC patients (2.4%) were found to have MSI-H/dMMR tumors, defined as an MSI sensor score of ≥3 and TMB of ≥10, confirmed by IHC and mutational signature analysis. Of 13 of 20 MSI-H patients who consented to germline analysis, 3 of 13 (23%) had a germline MMR gene mutation. In total, 10 patients with MSI-H tumors received a PD-1/PDL-1-targeting agent. Of 10 patients, five had radiographic PR or PSA decline of >60%, one had SD for 6 months, and four had no response or were inevaluable [232]. In fact, pembrolizumab is FDA approved for a variety of advanced solid tumors (including CRPC) that are MSI-H or dMMR, after progressing on a prior treatment, and no satisfactory alternative treatment options are available.

Combination of Anti-CTLA-4 Plus Anti-PD-1

At the 2019 Genitourinary Cancers Symposium, Sharma et al. presented a preplanned interim efficacy/safety analysis for nivolumab + ipilimumab in patients with mCRPC from the phase 2 CheckMate 650 [233]. Asymptomatic/minimally symptomatic patients with mCRPC were divided into pretaxane therapy (cohort 1) and after taxane (cohort 2). Treatment was nivolumab 1 mg/kg + ipilimumab 3 mg/kg Q3W for four doses, and then nivolumab 480 mg every 4 weeks. Coprimary endpoints were ORR and radiographic PFS per PC working group 2 [234]. Sixty-two patients were enrolled, and ORR was 26% and 10% in cohorts 1 and 2, respectively. Higher activity in the chemotherapy-naive cohort is consistent with data from other immunotherapy modalities such as sipuleucel-T. In both cohorts, ORR was higher in patients with PD-L1 ≥ 1%, DNA damage repair (DDR), HRD, or above-median TMB. Careful interpretation is recommended, given the small number of subgroups. Grade 3–4 TRAEs occurred in 39% and 51% of patients in cohorts 1 and 2, respectively.

CPIs Plus Enzalutamide

KEYNOTE-365 is a phase 1b/2 umbrella trial [235] that is based on the activity seen with pembrolizumab in KEYNOTE-199 and following reports of adding enzalutamide [227, 228]. This study is assessing different combinations of pembrolizumab, either with olaparib (poly ADP ribose polymerase [PARP] inhibitor) (cohort A), docetaxel (cohort B), enzalutamide (cohort C), or abiraterone (cohort D). Cohort C enrolled a total of 69 patients and had a DCR ≥ 6 months of 33%. ORR was 20% in 25 evaluable patient, that is, having measurable disease [235]. CheckMate 9KD (NCT03338790) is another phase 2 umbrella trial evaluating nivolumab in combination with rucaparib (PARP inhibitor), docetaxel, or enzalutamide [220]. IMbassador 250 (NCT03016312) is a phase 3 multicenter trial evaluating atezolizumab with enzalutamide versus enzalutamide alone for CRPC [236].

Other Ongoing Immunotherapeutic Trials in PC

CPIs Plus PARP Inhibitors

Data suggest that 25–30% of sporadic mCRPC patients have somatic or germline defects in DNA repair pathways, which may confer sensitivity to PARP inhibition (PARPi) [174]. Data from the above-mentioned CheckMate 650, KEYNOTE-199, and other reports suggest that there may be improved activity in CRPC with DDR mutations when treated with CPIs [227, 233, 237]. NCT02484404 is phase 1/2 trial based on the hypothesis that increased DNA damage by olaparib will complement antitumor activity of the anti-PD-L1 durvalumab, in part due to increased signaling through STING

(stimulator of interferon (INF) genes) pathway and enhanced IFN production [238]. Of 17 CRPC patients, eight (47%) had PSA responses >50%, and six of the eight responders had mutations in the DDR pathways [239, 240]. This was the first study to demonstrate activity for the PARPi+CPI combination in PC patients without having to have defects in DDR genes. While this study is limited by a small patient cohort, the 12-month PFS of 51.5% in a taxane-refractory population is promising. As mentioned above, the KEYNOTE-199 and CheckMate 9KD are aiming to answer this question.

PSMA Radioligand Therapy and Combinations with Immunotherapy

PSMA's expression is upregulated in dedifferentiated and CRPC making it an attractive target for therapy [241]. ^{177}Lu-PSMA-617 is composed of the therapeutic radionuclide Lutetium-177 attached to the high-affinity PSMA ligand called PSMA-617. ^{177}Lu-PSMA-617 has shown a promising activity in metastatic CRPC based on a meta-analysis that included 455 patients [242]. PSMA-lutetium Radionuclide Therapy and ImmuNotherapy in Prostate CancEr (PRINCE) is an Australian phase 1/2 trial (NCT03658447) that is assessing the safety and efficacy of pembrolizumab in conjunction with ^{177}Lu-PSMA-617. NCT03805594 is a similar study conducted in the United States.

Chemokine Receptor 2 (CXCR2) Antagonist in Combination with Enzalutamide

ACE (NCT03177187) is a phase 1/2 study studying AZD5069 (CXCR2 antagonist) + enzalutamide in metastatic CRPC to reverse enzalutamide resistance. CXCR2 antagonism is reported to stop recruitment of MDSCs to the premetastatic niche and, as a result, reduce the chance of developing cancer metastasis [243].

Cellular Therapy

In CRPC, two groups reported developing a CAR construct targeting PSMA [244, 245]. NCT01140373 is a phase 1 trial that started in 2010 using PSMA CAR T cell and has not reported results yet. A major concern is the immune suppressive microenvironment; therefore, TGFβ-insensitive PSMA-directed CAR-T cells were developed. This newer construct resulted in increased proliferation, enhanced cytokine secretion, resistance to exhaustion, and long-term in vivo persistence in a human PC mouse models [246]. NCT03089203 is a phase 1 clinical trial conducted at the University of Pennsylvania to assess the safety and preliminary efficacy of this lentivirally transduced PSMA-directed/TGFβ-insensitive CAR-T cells in men with metastatic CRPC [247].

Future Directions for Immunotherapy in PC

PC has evident potential to induce immune responses, and clinical data have proven the principle that immune modulation can prolong survival [179]. However, developing immunotherapies for PC has faced several challenges. Perhaps, immunotherapies may be most effective when used earlier in the course of disease or in a combinatorial fashion. Identifying the beneficial combinations of hormonal therapy, chemotherapy, CPIs, and vaccines is the current goal of several clinical trials (Fig. 6.6). Another important consideration for immunotherapy is identifying patients who are most likely to benefit from therapy. Most intriguing is the possibility of identifying patients with high-risk, localized PC with a preexisting antitumor immune response and treating them with immunotherapy in a neoadjuvant or adjuvant setting to maximize the benefit. There is currently substantial evidence that immunotherapy may be active and beneficial in PC, and continued evaluation of this treatment is surely warranted.

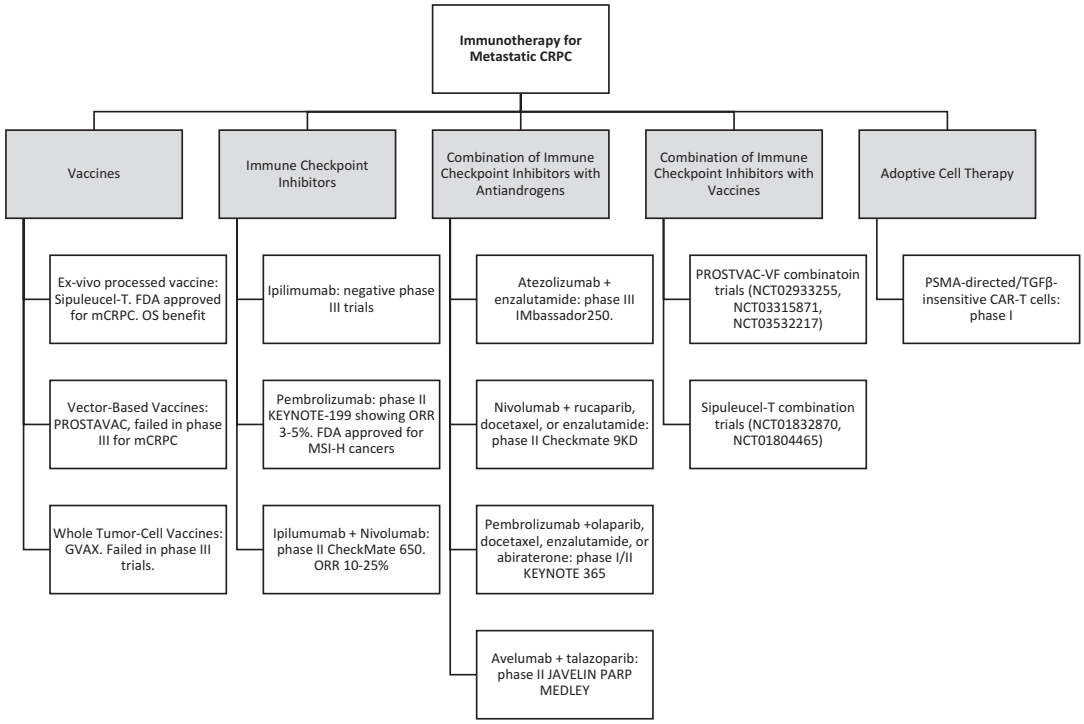

Fig. 6.6 Selected categories of current immunotherapy landscape for prostate cancer. Sipuleucel-T remains the only FDA-approved agent

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