Immunobiology and immunotherapy in genitourinary malignancies

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Abstract: Immunotherapy has traditionally been a critical component of the cancer treatment armamentarium in genitourinary (GU) cancers. It has an established role in the management of carefully selected patients with metastatic renal cell carcinoma (RCC) [e.g., high dose interleukin-2 (IL-2)] and non-muscle invasive bladder cancer (NMIBC) [e.g., intravesical Bacillus Calmette-Guérin (BCG)]. In 2010, the sipuleucel-T vaccine was approved by the FDA for the management of metastatic castration-resistant prostate cancer (mCRPC), based on a phase III trial showing overall survival (OS) benefit compared to placebo. The immune checkpoint inhibitor nivolumab (anti-PD-1) recently received FDA approval for the management of patients with advanced RCC patients previously treated with anti-angiogenic therapy, based on OS benefit compared to everolimus. Recently, large clinical trials demonstrated meaningful clinical benefit, including durable responses, as well as a good tolerability/safety profile with the use of immune checkpoint inhibitors in advanced RCC and chemotherapy-resistant advanced urothelial carcinoma (UC), while FDA just approved atezolizumab for platinum-treated advanced UC. Numerous interesting trials in different cancers are ongoing. Several combinations of immune checkpoint blockade with chemotherapeutics, vaccines, targeted tyrosine kinase inhibitors & monoclonal antibodies, epigenetic modifiers, anti-angiogenic agents, tumor microenvironment & myeloid cell targeting therapies, metabolic modification strategies, radiation, and others, are being tested in clinical trials. Comprehensive understanding of the factors underlying antitumor immune responses in physiologically relevant animal models and humans will refine further the clinical benefit of immunotherapy. Discovery and validation of appropriate molecular biomarkers via coordinated translational research efforts, rational clinical trial designs with suitable endpoints and well-defined eligibility criteria, prospective registries/databases, careful evaluation of cost-effectiveness and safety/tolerability, adequate funding and open continuous discussions among all stakeholders will support the revolutionary nature of immunotherapy in GU cancers.

Keywords: Bladder cancer (BC); immunotherapy; prostate cancer; renal cancer

Introduction

Immunotherapy has traditionally been a critical component of the cancer treatment armamentarium in genitourinary (GU) cancers. It has had an established role in the management of carefully selected patients with metastatic renal cell carcinoma (RCC) [e.g., high dose interleukin-2 (IL-2)] and non-muscle invasive bladder cancer (NMIBC) [e.g., intravesical Bacillus Calmette-Guérin (BCG)]. In 2010, the sipuleucel-T vaccine was approved by the FDA for the management of metastatic castration-resistant prostate cancer (mCRPC), based on a phase III trial showing overall survival (OS) benefit compared to placebo. Recently, large phase I and II clinical trials demonstrated a meaningful benefit with the use of immune checkpoint inhibitors in advanced RCC and chemotherapy-resistant

advanced urothelial carcinoma (UC), supporting the very recent FDA approval of atezolizumab in advanced UC. In the era of molecular medicine, deeper understanding in basic and translational immunology, as well as the discovery and validation of predictive biomarkers may further refine the clinical utility of immunotherapy in GU cancers. Here, we provide examples of clinical results with immunotherapeutic agents. Considering the plethora of data and space limitations, this review could not include all available data and is not exhaustive; however, it aims to provide an overview of the current landscape of clinical research in this exciting field.

Immunotherapy in prostate cancer

Prostate cancer is curable when it is diagnosed and treated early as localized disease. When it becomes metastatic, the cornerstone of treatment is androgen deprivation therapy (ADT), with or without chemotherapy. This approach offers durable disease control but eventually disease will progress and become castration resistant (1). Over the last decade, several therapeutic options were granted approval for mCRPC, each one providing a moderate OS benefit (2-8). Consequently, there is an unmet medical need for treatment strategies, which can transform mCRPC from a lethal to a chronic disease. Among those strategies, which are being tested today in clinical trials, are several immunotherapeutic approaches including cancer vaccines and immune checkpoint inhibition (9,10).

Cancer vaccines

The rationale behind vaccines in cancer is to mount a strong and effective immune response against tumor-related antigens, which can lead to the eradication of tumors. There several approaches to vaccine-based immunotherapy, which mainly include autologous or heterologous cell or peptide vaccines, viral- and DNA-based vaccines (11).

Sipuleucel-T (Provenge™) is a cell-based vaccine manufactured from the patient's own peripheral blood mononuclear cells, which are obtained by leukapheresis. These cells, which are enriched for antigen-presenting cells (APCs), are subsequently incubated with a recombinant fusion protein consisting of prostatic acid phosphatase (PAP) and granulocyte macrophage colony-stimulating factor (GM-CSF) (12,13). This process results in the activation of APCs and the final product is administered back to the patient by intravenous infusion. In early phase

I/II trials, Sipuleucel-T was demonstrated to be safe and well tolerated, as well as capable of producing significant antigen-specific responses (14,15). Sipuleucel-T was approved by the FDA in 2010, as the first therapeutic vaccine for cancer, after the completion of three phase III clinical trials, which showed a significant OS benefit for patients with mCRPC (16,17).

In the first two trials (D9901 and D9902A), a total of 225 patients with asymptomatic metastatic "hormone-refractory" (which was the term at that time) prostate cancer were randomized to receive, in a 2:1 ratio, three Sipuleucel-T infusions or placebo every 2 weeks. In the integrated analysis of the two studies, while the primary endpoint (median time to progression, TTP) was not met (11.1 vs. 9.7 weeks, P=0.11), a statistically significant OS benefit of 4.3 months was observed (23.2 vs. 18.9 months, P=0.011), suggesting that Sipuleucel-T may provide a survival advantage to these patients.

In the third, similar in design, phase III trial, the Immunotherapy for Prostate Adenocarcinoma Treatment (IMPACT) trial, a total of 512 patients were randomized to receive Sipuleucel-T or placebo. This trial showed a 22% relative reduction in the risk of death [hazard ratio (HR) 0.78], which was translated to a 4.1-month improvement in OS (25.8 and 21.7 months, in the Sipuleucel-T and placebo groups, respectively). Adverse events with Sipuleucel-T were mild and manageable with the most common being injection-site reactions, chills, fever and headache (2).

A retrospective analysis of the data from the IMPACT trial showed that patients with the lowest tumor burden were likely to obtain greater benefit from Sipuleucel-T. Indeed, patients with lower PSA levels at baseline demonstrated a benefit of 13 months compared to placebo, whereas patients with higher PSA levels showed only a 2.8-month improvement with Sipuleucel-T (18). These results mirror the degree of immune suppression caused by the tumor (greater in higher tumor burden), as well as the fact that vaccine immunotherapy "may need" time to act and produce a sustained responses. Sipuleucel-T is currently under investigation in several trials, in combination with other approved drugs for mCRPC (abiraterone acetate, enzalutamide, or radium-223) or with other forms of immunotherapy (ipilimumab), (NCT01487863, NCT01981122, NCT02463799, NCT01832870 and NCT01804465).

GVAX is a whole cancer cell-based vaccine composed of whole tumor cells, derived from LNCaP and PC3 allogeneic prostate cancer cell lines. Cells are genetically modified to secrete the immune stimulatory cytokine GM-

CSF and are also irradiated for safety. In phase I/II trials, chemotherapy-naïve patients with "hormone-refractory" prostate cancer, received GVAX as a priming intradermal injection followed by boost injections every 2 weeks (five different dose levels). The results of these trials showed that GVAX was well tolerated with a safe toxicity profile, as well as effectiveness, demonstrating a median OS of 35 months in the high-dose group of patients (19). Following these results, two phase III trials were designed to evaluate GVAX in mCRPC patients. VITAL-1 enrolled asymptomatic patients to receive GVAX or docetaxel and VITAL-2 symptomatic patients to receive GVAX plus docetaxel or docetaxel alone. Unfortunately, a safety review of VITAL-2 showed an increase of deaths in the GVAX and docetaxel combination arm, compared with the docetaxel arm. This finding led to the termination of the VITAL-2 trial. A few months after the closure of VITAL-2, a futility analysis of VITAL-1 showed that there was a <30% chance of meeting its OS endpoint, so this trial was also terminated (11,20). GVAX is currently under investigation in combination with several agents in other cancers, such as pancreatic and colorectal (NCT02004262 and NCT01952730).

PROSTVAC is a recombinant viral vaccine, which consists of two poxviruses (vaccinia as priming and fowlpox as boosting agents). The two viruses are genetically engineered to express whole PSA as antigen, as well as three co-stimulatory molecules (B7.1, ICAM-1 and LFA-3; TRICOM) in order to enhance the PSA-targeted response (21). This form of immunotherapy utilizes the ability of viruses to infect cells and promote an immune response towards the cancer antigen they encode, PSA in the case of PROSTVAC. PROSTVAC was tested in a phase II trial where 125 mCRPC patients were randomized 2:1 to receive the vaccine or placebo. The study, although it did not meet its primary endpoint of progression-free survival (PFS), it showed a median OS benefit for PROSTVAC of 8.5 months (25.1 vs. 16.6 months, P=0.006) (22). This study prompted the design of a large phase III trial, which has completed enrollment of almost 1,300 asymptomatic or minimally symptomatic mCRPC patients, with OS being the primary endpoint (NCT01322490).

Immune checkpoint inhibition

Immune checkpoint inhibition has recently changed clinical practice in tumors, such as melanoma, lung cancer and Hodgkin lymphoma, and is now under investigation in the vast majority of solid and hematologic malignancies.

Antibodies against CTLA-4, PD-1, and its ligand PD-L1, are enhancing T cell activity by "releasing the brakes" of the T cell-mediated antitumor response (23).

Ipilimumab is a fully human IgG4 monoclonal antibody against CTLA-4. CTLA-4 has been shown to be up regulated upon T cell activation, in order to diminish this response. In the mCRPC setting, ipilimumab was tested in a phase I/II trial, where patients received ipilimumab in several dosing schedules plus radiation to a single bone metastasis (24). Results of this trial showed that ipilimumab has antitumor activity with tumor control and manageable toxicities. Following these results, two phase III trials were initiated, in mCRPC patients, using the dose of 10 mg/kg every 3 weeks for up to 4 doses plus bone-directed radiotherapy, after docetaxel failure or prior to docetaxel, respectively. The study protocol permitted the administration of ipilimumab as maintenance treatment every 12 weeks after completion of the first 4 doses. Results of the post-docetaxel trial showed no statistical difference in OS between ipilimumab and placebo (median OS 11.2 vs. 10.0 months, P=0.053) (25). Nevertheless, a subgroup analysis demonstrated that ipilimumab offers a survival advantage to patients with favorable baseline characteristics, such as alkaline phosphatase <1.5 times the upper limit of normal, hemoglobin >11.0 g/dL and no visceral metastases. These patients had a median OS of 22.7 months with ipilimumab vs. 15.8 months with placebo (P=0.004). At ESMO/ECCO 2015, the updated OS analysis, with an additional year of follow-up, was presented and was consistent with the primary analysis, with the same difference in OS between ipilimumab and placebo (11.2 vs. 10.0 months, P=0.030). Also consistent with previous reports, pre-specified subgroup analyses suggest greater activity in patients with lower disease burden. Another, similar in design trial, evaluated ipilimumab in chemotherapy-naïve mCRPC patients but results have not yet been reported (NCT02279862).

Several studies have shown that T cells, which infiltrate prostate tumors, express PD-1 in high levels. Nevertheless, in a pilot study of nivolumab (PD-1 antibody) there were no objective responses among 17 mCRPC patients and all those cases were negative for tumor PD-L1 expression (26,27). Nonetheless, these agents are currently under investigation in prostate cancer patients through combinatorial treatment strategies (NCT02601014 and NCT02499835).

Conclusions

In the future, the growing knowledge regarding prostate

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cancer biology and its interactions with the immune system may lead to more effective immunotherapeutic approaches. Moreover, the use of future novel validated biomarkers will aid in selecting those prostate cancer patients, which would benefit the most from immunotherapy.

Immunotherapy in RCC

The prognosis for patients with metastatic RCC remains poor despite recent improvements in outcome with tyrosine kinase inhibitors, which mainly target the vascular endothelial growth factor (VEGF) pathway (28-34). Historically, immunotherapy with high-dose IL-2 has been an option for selected fit patients, with a minority of them achieving long-lasting remissions, including complete responses (35). Therefore, novel treatment approaches, including immunotherapeutic strategies, are warranted to further improve survival in RCC patients. There are currently several immunotherapeutic treatments being tested in clinical trials, alone or in combination, and these mainly include cancer vaccines and immune checkpoint inhibitors (36,37).

Cancer vaccines

AGS-003 is a dendritic cell (DC) immunotherapeutic vaccine constructed by autologous blood DCs and RNA from the tumor. DCs are co-electroporated with tumor RNA plus synthetic CD40L RNA and are administered via intradermal injections to patients after debulking nephrectomy (37). In a phase II trial, patients after nephrectomy were treated with sunitinib plus AGS-003 until disease progression. Median PFS was 11.2 months (95% CI, 6.0–19.4) and median OS from registration was 30.2 months (95% CI 9.4–57.1) for all patients (38). Based on these results, a phase III trial (ADAPT) is ongoing where AGS-003 plus sunitinib are tested against sunitinib monotherapy. The primary endpoint is OS and patients in the experimental arm are receiving eight injections of AGS-003 every 6 weeks followed by boost injections every 3 months. The accrual has been completed and results are pending (NCT01582672).

Another vaccine, IMA009, is a synthetic off-the-shelf vaccine consisting of 10 different tumor-associated peptides (39). Phase II results with the vaccine alone plus or minus cyclophosphamide co-administered to patients with GM-CSF as first line treatment showed an association between the magnitude of T cell responses and survival.

Patients given cyclophosphamide had a better immune response (40). Based on these results, a phase III trial was designed and enrolled 339 metastatic RCC patients (HLA-A*02-positive) who had favorable or intermediate risk status. Following one cycle of sunitinib, patients were randomized 3:2 to up to 10 intradermal IMA901 injections plus GM-CSF plus sunitinib vs. sunitinib alone. Patients in the vaccine arm were given a single infusion of cyclophosphamide three days before the first vaccination in order to achieve a reduced number of regulatory T cells. At ESMO/ECCO 2015, disappointing results of this study were presented with median OS, the primary endpoint, being 33.1 months in the vaccine arm versus not reached in the control arm that did not meet statistical significance (HR 1.34, P=0.080) (41).

Immune checkpoint inhibition

Nivolumab in phase I and II studies in RCC patients, who had progressed on or after conventional treatment with VEGF TKIs, demonstrated significant clinical activity, with response rates of almost 30% (26,27,42). This led to the design of a phase III trial (CheckMate 025) where 821 patients with advanced clear-cell RCC, for which they had received previous treatment with one or two regimens of antiangiogenic therapy, were randomly assigned (in a 1:1 ratio) to receive nivolumab or everolimus (43). The primary end point was OS and secondary end points included objective response rate and safety. Median OS was 25.0 months with nivolumab and 19.6 months with everolimus (HR 0.73, P=0.002). The objective response rate was 25% with nivolumab vs. 5% with everolimus; median PFS was 4.6 months with nivolumab and 4.4 months with everolimus. Treatment with nivolumab was better tolerated than with everolimus, with fewer patients suffering from grade 3 or 4 adverse events (19% vs. 37%). Analysis of tumor PD-L1 expression showed that it was not associated with greater responses to nivolumab and patients with ≥1% expression demonstrated a similar benefit to that of patients with <1%. These data resulted in FDA approval of nivolumab for RCC patients who have received first-line treatment with VEGF TKIs.

Pembrolizumab, another PD-1 antibody already approved in advanced melanoma and NSCLC, is currently being tested in two randomized phase II trials enrolling patients with advanced RCC. In the first trial, it is being tested as monotherapy or in combination with pegylated interferon-alfa (IFN-α) and in the other as monotherapy or

in combination with pazopanib. Results of these studies are pending (NCT02089685 and NCT02014636).

Atezolizumab, a PD-L1 antibody, has demonstrated encouraging results in a phase I monotherapy study in metastatic RCC patients (44). The objective response rate was 15% with the median duration of response being 17 months. The respective 1- and 2-year survival rates were 81% and 58%. An ongoing phase II study has recently completed enrollment of patients, which were randomized to receive atezolizumab plus bevacizumab vs. atezolizumab alone vs. sunitinib. Crossover was permitted from the monotherapy arms to atezolizumab plus bevacizumab arm at the time of progression. Results of this trial are awaited (NCT01984242). A larger phase III trial testing atezolizumab plus bevacizumab vs. sunitinib is currently completing accrual and results of this study will determine the efficacy of the combination in the first-line treatment of metastatic RCC (NCT02420821).

Immunotherapeutic combinations

The combined blockade of PD-1/PD-L1 along with CTLA-4 has been shown to further augment responses in patients with advanced melanoma compared to blocking either checkpoint alone. The combination of nivolumab plus ipilimumab has been tested in a phase I trial (CheckMate 016) where patients received the two agents for four doses in two dosing cohorts, nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (N3/I1) arm and nivolumab 1 mg/kg plus ipilimumab 3 mg/kg (N1/I3) arm. Subsequently, patients were administered nivolumab every 14 days as maintenance treatment. The results of this study were presented at the 2014 Annual ASCO Meeting; the objective response rate was 43% in the N3/I1 arm and 48% in the N1/I3 arm with a median duration of response of 31 weeks and not reached, respectively. Grade 3 or 4 adverse events occurred in 29% of the patients in the N3/I1 arm and in 61% of the patients in the N1/I3 arm, with the most common being diarrhea and elevated lipase, amylase and ALT. Following these results, a phase III study was initiated and recently completed accrual, where patients with previously untreated advanced or metastatic RCC were randomized to nivolumab plus ipilimumab vs. sunitinib (CheckMate 214; NCT02231749). Results of this study are forthcoming.

In the context of the CheckMate 016 trial, presented at the 2014 Annual ASCO Meeting, nivolumab was tested in dose-escalation cohorts of 2 or 5 mg/kg every 3 weeks, in combination with standard dosing schedules of sunitinib or pazopanib. Although in the nivolumab/sunitinib arm there was no dose-limiting toxicity (DLT), which led to the expansion in order to include more patients, in the nivolumab/pazopanib arm there were four DLTs (AST/ALT elevation in three patients and fatigue in one patient), which led to the discontinuation of this arm. The objective response rate in the nivolumab/sunitinib arm was 52%, which is higher than the response rate of either agent given as monotherapy in previous studies in RCC. Nevertheless, grade 3 or 4 adverse events occurred in 71% and 85% of the patients in the 2 and 5 mg/kg doses of nivolumab and these included fatigue, diarrhea and ALT/AST elevation. These high rates of grade 3/4 adverse events in the nivolumab/pazopanib and nivolumab/sunitinib arms precluded further development of this combinatorial strategy.

Conclusions

RCC is an immunogenic tumor and, in the past, immunotherapy with high-dose IL-2 was the only treatment option for a minority of patients, which could exhibit durable remissions. Results from recent clinical trials with immune checkpoint inhibitors suggest that immunotherapy with these agents, as monotherapy or in combination with other agents, is capable of producing durable responses and significant OS improvement. Thus, in the future, immunotherapy alone or together with other treatments, will likely cause a paradigm shift in the clinical management of RCC patients.

Immunotherapy in bladder cancer (BC)

BC is a very common malignancy, with significant morbidity and mortality and enormous financial burden for the healthcare systems worldwide. UC is the most common histological type of BC, accounting for >90% of the cases. It is more common in the elderly, with a median age at diagnosis of 73 years (45). Risk factors for the development of BC include tobacco smoking and exposure to various chemicals, e.g., aromatic amines, arsenic, rubber, leather, textiles, dyes (e.g., hair dyes), paint/printing products, aluminum, plastic, carpet, metal, cyclophosphamide, ifosfamide and radiation; machinists, firefighters and truck drivers may be at higher risk (46). Smoking is the most important environmental risk factor and may be associated with the relatively high mutation load noted in UC (47,48). Cancers with high mutational load may contain higher number of "neo-antigens" that can be targeted by the

immune system. The genomic instability of many BC cases may be the underlying driver of the high mutational load and thus merits further evaluation as potential indicator of response to immunotherapy.

BCG

BCG is an attenuated form of the bovine tuberculosis bacterium, mycobacterium bovis. The first reported clinical trial of BCG in BC, more than 35 years ago, showed a 20% reduction in recurrence rate (49). The mechanism of action of BCG is not fully understood but has recently been reviewed (50,51). BCG has been shown to reduce recurrence and progression rates in NMIBC. Studies comparing monthly, quarterly, and biannual BCG maintenance showed no evidence of greater efficacy compared to induction BCG alone (52-54); however, the 3-week maintenance schedule (SWOG) revealed significant benefit, with a recurrence-free survival (RFS) of 77 months with maintenance compared to 36 months with induction alone (55). The 5-year survival rate was 78% with induction alone compared to 83% with maintenance. Thus, only the 3-week BCG maintenance was shown to lower progression and improve overall and disease-specific mortality in randomized controlled studies (55,56). BCG remains a standard treatment after transurethral bladder tumor resection (TURBT) in patients with NMIBC (based on certain indications) and provides the backbone for immunotherapeutic combinatorial or sequential treatment strategies in this setting. Recently, the International Bladder Cancer Group has developed formal recommendations regarding definitions, endpoints and clinical trial designs, providing the necessary framework for uniform evaluation among trials in NMIBC (57). For example, the efficacy of BCG in BCG-naïve patients sets a high standard for novel comparators. Moreover, the type of failure (e.g., BCG unresponsive, refractory, relapsing, or intolerant) should be clearly defined in trial designs in order to allow comparisons across clinical trials.

Immune checkpoint inhibition

CTLA-4 blockade was evaluated in 12 patients with localized UC that received neoadjuvant ipilimumab either 3 or 10 mg/kg prior to cystectomy; ipilimumab was safe and led to an increase in CD4 and CD8 T cells in both the tumor and blood (58). A single arm phase II trial recently completed accrual of 36 patients treated with standard first-line chemotherapy [gemcitabine/cisplatin (GC)] plus ipilimumab. Galsky *et al.* presented data from this trial at the 2016 ASCO GU Symposium and noted that the 1-year OS rate was 59%, with median OS of 14.6 months (95% CI 10.5–18.6 months), which appears comparable to results from trials with GC alone (59). The trial did not reach the primary endpoint, since the lower bound of the 90% CI for OS (0.41) did not surpass the pre-specified criterion for further evaluation (0.60). The best objective response rate was 64%, according to RECIST 1.1 criteria; six patients (17%) demonstrated an improved response after the addition of ipilimumab to GC. GC alone did not deplete circulating regulatory T cells or myeloid-derived suppressor cells. There was an on-treatment increase in CD4 and CD8 T cells with an augmented inflammatory cytokine signature, including IL-2, IL-12 and GM-CSF with ipilimumab despite administration of concurrent cytotoxic chemotherapy. This justifies upcoming trials combining cytotoxic chemotherapy with PD-1 and PD-L1 blockade (60). Despite that CTLA-4 is a relevant checkpoint testing, anti-CTLA-4 into earlier stage UC or with combination regimens may be limited by its toxicity profile (61).

A phase I expansion trial (using an adaptive design that allowed for biomarker-positive enriched cohorts) with the anti-PD-L1 agent atezolizumab in 68 patients with chemotherapy-resistant advanced UC showed impressive results with high overall response rates (62,63). Overall, 55% of patients showed a reduction in tumor burden by RECIST criteria. Response was correlated with PD-L1 expression on infiltrating immune cells (IC), while rapid and durable responses were noted in a study population that included patients with poor prognostic features. Atezolizumab demonstrated good tolerability and a favorable safety profile compared to historical chemotherapy and received breakthrough designation status by the FDA in 2014. A multicenter, single-arm phase II trial evaluated atezolizumab (1,200 mg every three weeks) in 310 patients with locally advanced and metastatic UC that had progressed after platinum-based chemotherapy (64). PD-L1 expression on IC was evaluated; the co-primary endpoints were objective response rate by RECIST v1.1 and modified RECIST. Exploratory analyses included evaluation of potential associations between The Cancer Genome Atlas (TCGA) molecular subtypes, CD8+ T cell infiltration, mutation load, treatment response and outcomes. Based on independent evaluation, the objective response rates were 26% (95% CI, 18–36%) in the IC2/3 group, 18% (95% CI, 13–24%) in the IC1/2/3 group and

15% (95% CI, 11–19%) in all patients (*Table 1*). With a median follow-up of approximately 12 months, ongoing responses were observed in 84% of responding patients. The median duration of response was not reached (range, 2.0*–13.7* months, *censored). The median OS was 11.4 months (95% CI, 9.0–not estimable) in the IC2/3 group, 8.8 months (95% CI, 7.1–10.6) in the IC1/2/3, and 7.9 months (95% CI, 6.6–9.3) in all patients. The 12-month landmark OS rate was 48% in the IC2/3 (95% CI, 38–58%) group, 39% in the IC1/2/3 (95% CI, 32–46%) group and 36% (95% CI, 30–41%) in the intent to treat population. Grade 3–4 treatment-related adverse events were noted in 16% and grade 3–4 immune-mediated adverse events in 5% of treated patients. PD-L1 expression on IC, TCGA molecular subtypes, and mutation load were independently associated with response to atezolizumab. In particular, response to atezolizumab occurred in all TCGA subtypes but was significantly higher in the luminal cluster II subtype than in other subtypes (objective response rate of 34%, P=0.002). The mutational load was estimated in 150 patients by examining a representative panel of 315 cancer-related genes. The median mutation load was significantly increased in responders (12.4/Mb) compared to non-responders (6.4/Mb) (P<0.001). The relationship between mutational load and response was unrelated to TCGA subtype (P=0.22). Data from patients who were treatment-naïve for advanced UC but cisplatin-ineligible and were treated with atezolizumab in the same trial (cohort 1) are expected at the 2016 Annual ASCO Meeting. These results led to FDA approval of atezolizumab on May 18, 2016. Atezolizumab is currently being tested in trials, mostly as single agent, in several settings, including BCG-unresponsive NMIBC (S1605), neoadjuvant, adjuvant and advanced disease settings (including two randomized large phase III registration trials: NCT02450331 and NCT02302807).

The anti-PD-1 agent pembrolizumab also showed impressive anti-tumor efficacy and a good safety profile in a phase I trial of 33 patients with pretreated advanced UC (65). The ORR of 28% was similar to that for PD-L1 inhibition, with 64% of patients having reduction in tumor lesions. Median PFS and OS were 2 and 12.7 months, respectively, while 1-year landmark OS was 53%. PD-L1 expression correlated with response; patients with negative PD-L1 measured in both the tumor and infiltrating cells did not respond. A phase III study comparing pembrolizumab to either paclitaxel or docetaxel or vinflunine in patients with pretreated advanced UC recently completed accrual

(NCT02256436). Moreover, pembrolizumab is being evaluated in several UC clinical trials, either as single agent or combined with other therapies. For example, pembrolizumab is tested alone or combined with ACP-196 (Btk inhibitor) in a randomized phase II trial (KEYNOTE 143; NCT02351739) in platinum-resistant patients with advanced UC; as single agent in patients without prior systemic chemotherapy for advanced disease who cannot tolerate cisplatin (KEYNOTE 052; NCT02335424); as single agent in patients with BCG-unresponsive NMIBC (NCT02625961); and also in other trials in the neoadjuvant (NCT02365766), adjuvant (planned US intergroup trial), and advanced disease settings.

The anti-PD-L1 agent avelumab was evaluated in a phase Ib trial of 44 patients with advanced UC who either progressed after platinum chemotherapy or were cisplatin-ineligible (given at 10 mg/kg every 2 weeks) (66). The overall response rate was 16% by RECIST criteria with one complete response; disease-control rate was 59%. The median duration of response was not reached and six of the seven responses were ongoing at the time of data analysis. The proportion of patients alive and progression-free at 12 weeks was 47%. Overall, 18% had tumor shrinkage of ≥30%, including patients with visceral metastases. Anti-tumor activity was associated with PD-L1 expression. The objective response rate was 40% in PD-L1-positive patients (≥5% cut-off) compared to 9% in PD-L1-negative patients. The median PFS was not reached for the PD-L1-positive patients and was 12 weeks for the PD-L1-negative patients; PFS at 12 weeks was 70% *vs*. 46%, respectively. Avelumab is currently being compared to best supportive care, as switch maintenance strategy in patients who received 4–6 cycles of standard first-line gemcitabine plus either cisplatin or carboplatin for advanced UC (NCT02603432).

There are at least two other immune checkpoint inhibitors, nivolumab (anti-PD-1) and durvalumab (anti-PD-L1) under clinical testing either alone or combined with other therapies in UC trials, e.g., NCT01928394 and NCT02516241, confirming the very high interest in the extensive investigation of this treatment approach in UC.

Other immunotherapeutic strategies

There is a plethora of immunotherapeutic strategies, e.g., combinations, sequences, switch maintenance, as well as numerous agents that are being tested alone, together with immune checkpoint inhibitors, or other (immune and/or non-immune) therapies. A comprehensive review,

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Table 1 Selected immunotherapeutic clinical trials in genitourinary cancers

Regimen	Phase	N	Disease setting	Control	PFS	OS	References
Sipuleucel-T	III	127	mCRPC (asymptomatic)	Placebo	11.7 *vs.* 10 weeks (P=0.052)	25.9 *vs.* 21.4 months (P=0.010)	(16,17)
Sipuleucel-T	III	98	mCRPC (asymptomatic)	Placebo	10.9 *vs.* 9.9 weeks (P=0.72)	19.0 *vs.* 15.7 months (P=0.33)	(16,17)
Sipuleucel-T	III	512	mCRPC (asymptomatic)	Placebo	14.6 *vs.* 14.4 weeks (P=0.63)	25.8 *vs.* 21.7 months (P=0.032)	(2)
GVAX	I/II	80	mCRPC (asymptomatic)	NR	NR	23.1 months (low-dose); 20.0 months (mid-dose); 35.0 months (high-dose)	(19)
GVAX	III	626	mCRPC (asymptomatic)	Docetaxel	NR	20.7 *vs.* 21.7 months (P=0.78)	(9,11,20)
GVAX plus docetaxel	III	408	mCRPC (symptomatic)	Docetaxel	NR	12.2 *vs.* 14.1 months (P=0.008)	(9,11,20)
PROSTVAC-VF	II	125	mCRPC (minimal symptomatic)	Placebo	3.8 *vs.* 3.7 months (P=0.60)	25.1 *vs.* 16.6 months (P=0.006)	(22)
PROSTVAC-VF plus GM-CSF	III	1,300	mCRPC (asymptomatic or minimal symptomatic)	Placebo/GM-CSF	Ongoing	Ongoing	NCT01322490
Ipilimumab ± RT	I/II	71	mCRPC	NR	NR	17.4 months	(24)
Ipilimumab ± RT (CA184-043)	III	799	mCRPC (post-docetaxel)	Placebo	4.0 *vs.* 3.1 months (P<0.001)	11.2 *vs.* 10.0 months (P=0.053)	(25)
Ipilimumab ± RT (CA184-043)	III	799	mCRPC (post-docetaxel)	Placebo	4.0 *vs.* 3.1 months (P<0.001)	11.2 *vs.* 10.0 months (P=0.053)	(25)
AGS-003	II	21	mRCC (intermediate- and poor-risk)	NR	11.2 months	30.2 months	(38)
IMA901 plus GM-CSF plus sunitinib	III	339	mRCC (favorable- and intermediate-risk)	Sunitinib	NR	33.1 months *vs.* NR (P=0.080)	(41)
Nivolumab	III	821	mRCC	Everolimus	4.6 *vs.* 4.4 months (P=0.11)	25.0 *vs.* 19.6 months (P=0.002)	(43)
Atezolizumab	I	70	mRCC	NR	5.6 months	28.9 months	(45)
Atezolizumab	II	310	mUC	NA	2.1 months	11.4 months (IC2/3); 8.8 months (IC1/2/3); 7.9 months (all comer); 12.7 months	(64)
Pembrolizumab	I	33	mUC	NA	2 months		(65)
Avelumab	I	44	mUC	NA	47.2% progression-free survival at 12 weeks		(66)

N, number; PFS, progression-free survival; OS, overall survival; mCRPC, metastatic castration-resistant prostate cancer; NR, not reported; mRCC, metastatic renal cell cancer; RT, radiotherapy; GM-CSF, granulocyte macrophage colony-stimulating factor; mUC, metastatic/advanced urothelial cancer; NA, not applicable; IC, immune cells.

underlying several immunotherapeutic agents, their mechanism of action and early clinical data, was recently published (67). One example is the IgG1 monoclonal antibody against B7-H3 (MGA271, MacroGenics Inc., USA) that is currently being tested in a phase I trial in patients with different advanced cancers including UC (NCT01391143). Another example is cancer vaccines, which attempt to initiate T cell responses against tumor antigens by inducing activated APC expressing tumor-associated or specific antigens (68). Activated APCs can drive the proliferation and function of specific T cells. The ability of immune checkpoint inhibitors to reverse T cell exhaustion has important implications in the testing of therapeutic cancer vaccines. Two cancer vaccines in clinical testing include vesigenurtacel-L (Heat Biologics Inc.), which is a cell-based vaccine being tested in a phase I/II trial (NCT02010203) and lapuleucel-T, which is a DC-based vaccine being tested in a phase II trial evaluating survival, safety and immune responses in the adjuvant setting in patients with high-risk HER2-positive UC (NCT01353222) (69-72). Another approach involves the use of "agonist antibodies" that activate IC via co-stimulatory molecules. Examples include anti-OX40 on T cells (MEDI6469, Medimmune), anti-4-1BB on T cells (urelumab, Bristol-Myers Squibb; PF05082566, Pfizer Inc.) and anti-CD40 on APCs (CP-870,893). Last but not least, targeting the tumor immune microenvironment is another rational treatment strategy. CSF1R is a cell surface receptor expressed predominantly on macrophages and monocytes and may promote the M2 immunosuppressive phenotype of macrophages (73,74). A specific small molecule inhibitor of CSF1R (PLX3397, Plexxikon) and two monoclonal antibodies have been developed (FPA008, Five Prime Therapeutics; emactuzumab, Hoffmann-La Roche). PLX3397 is being tested combined with pembrolizumab in a phase I/II trial (NCT02452424). Moreover, indoleamine 2,3-dioxygenase 1 (IDO1) is a cytosolic enzyme that mediates the rate-limiting step of tryptophan metabolism, thus suppressing T cells (75-77). The small molecule INCB024360 (Incyte Corp.) is a selective IDO1 inhibitor currently being tested with pembrolizumab in a phase I/II trial (NCT02178722), while GDC0919 (Genentech Inc.) is now in phase I trials, including one in which it is combined with atezolizumab (NCT02471846).

Conclusions

Immune checkpoint inhibition recently changed the

treatment paradigm in platinum-treated advanced UC, while PD-L1 expression appears to be a candidate predictive biomarker (not optimal). However, there is a plethora of anti-PD-L1 antibodies, various assays/methodologies and percent cut-off levels to define biomarker positivity, tested in different cancer types and sponsored by different companies. This creates difficulty in the standardization and uniform applicability of tested biomarkers, which need to demonstrate not only clinical validity but also clinical utility in order to be incorporated in clinical practice.

Future perspectives

Several immune checkpoint blockade agents, alone or in combination with chemotherapeutics, vaccines, targeted tyrosine kinase inhibitors and monoclonal antibodies, epigenetic modifiers, anti-angiogenic agents, tumor microenvironment and myeloid cell targeting therapies, metabolic modification strategies, radiation and others, are being tested in clinical trials. Comprehensive understanding of the factors underlying antitumor immune responses in relevant animal models and in the clinical setting will further refine the clinical benefit of immunotherapy in GU malignancies. Discovery and validation of appropriate molecular biomarkers via coordinated translational research efforts, rational clinical trials, prospective registries/databases, careful evaluation of cost-effectiveness and safety/tolerability, adequate funding and open continuous discussions among all stakeholders will support the revolutionary nature of immunotherapy in this disease.

Acknowledgements

Footnote

References

1. Parker C, Gillessen S, Heidenreich A, et al. Cancer of the prostate: ESMO Clinical Practice Guidelines for diagnosis,

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treatment and follow-up. Ann Oncol 2015;26 Suppl 5:v69-77.

2. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010;363:411-22.

3. de Bono JS, Oudard S, Ozguroglu M, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. Lancet 2010;376:1147-54.

4. de Bono JS, Logothetis CJ, Molina A, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011;364:1995-2005.

5. Scher HI, Fizazi K, Saad F, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 2012;367:1187-97.

6. Ryan CJ, Smith MR, de Bono JS, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med 2013;368:138-48.

7. Beer TM, Armstrong AJ, Rathkopf DE, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med 2014;371:424-33.

8. Parker C, Nilsson S, Heinrich D, et al. Alpha emitter radium-223 and survival in metastatic prostate cancer. N Engl J Med 2013;369:213-23.

9. Noguchi M, Koga N, Moriya F, et al. Immunotherapy in prostate cancer: challenges and opportunities. Immunotherapy 2016;8:69-77.

10. Wei XX, Fong L, Small EJ. Prospects for the use of ipilimumab in treating advanced prostate cancer. Expert Opin Biol Ther 2016;16:421-32.

11. Gerritsen WR, Sharma P. Current and emerging treatment options for castration-resistant prostate cancer: a focus on immunotherapy. J Clin Immunol 2012;32:25-35.

12. Rini BI. Technology evaluation: APC-8015, Dendreon. Curr Opin Mol Ther 2002;4:76-9.

13. Patel PH, Kockler DR. Sipuleucel-T: a vaccine for metastatic, asymptomatic, androgen-independent prostate cancer. Ann Pharmacother 2008;42:91-8.

14. Small EJ, Fratesi P, Reese DM, et al. Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. J Clin Oncol 2000;18:3894-903.

15. Burch PA, Breen JK, Buckner JC, et al. Priming tissue-specific cellular immunity in a phase I trial of autologous dendritic cells for prostate cancer. Clin Cancer Res 2000;6:2175-82.

16. Small EJ, Schellhammer PF, Higano CS, et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic,

asymptomatic hormone refractory prostate cancer. J Clin Oncol 2006;24:3089-94.

17. Higano CS, Schellhammer PF, Small EJ, et al. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. Cancer 2009;115:3670-9.

18. Schellhammer PF, Chodak G, Whitmore JB, et al. Lower baseline prostate-specific antigen is associated with a greater overall survival benefit from sipuleucel-T in the Immunotherapy for Prostate Adenocarcinoma Treatment (IMPACT) trial. Urology 2013;81:1297-302.

19. Higano CS, Corman JM, Smith DC, et al. Phase 1/2 dose-escalation study of a GM-CSF-secreting, allogeneic, cellular immunotherapy for metastatic hormone-refractory prostate cancer. Cancer 2008;113:975-84.

20. Antonarakis ES, Drake CG. Current status of immunological therapies for prostate cancer. Curr Opin Urol 2010;20:241-6.

21. Madan RA, Arlen PM, Mohebtash M, et al. Prostvac-VF: a vector-based vaccine targeting PSA in prostate cancer. Expert Opin Investig Drugs 2009;18:1001-11.

22. Kantoff PW, Schuetz TJ, Blumenstein BA, et al. Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. J Clin Oncol 2010;28:1099-105.

23. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature 2011;480:480-9.

24. Slovin SF, Higano CS, Hamid O, et al. Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. Ann Oncol 2013;24:1813-21.

25. Kwon ED, Drake CG, Scher HI, et al. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. Lancet Oncol 2014;15:700-12.

26. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366:2443-54.

27. Brahmer JR, Drake CG, Wollner I, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol 2010;28:3167-75.

28. Yang JC, Haworth L, Sherry RM, et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med 2003;349:427-34.

29. Motzer RJ, Hutson TE, Tomczak P, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 2007;356:115-24.

30. Motzer RJ, Escudier B, Oudard S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 2008;372:449-56.

31. Escudier B, Eisen T, Stadler WM, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med 2007;356:125-34.

32. Hudes G, Carducci M, Tomczak P, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 2007;356:2271-81.

33. Motzer RJ, Hutson TE, Cella D, et al. Pazopanib versus sunitinib in metastatic renal-cell carcinoma. N Engl J Med 2013;369:722-31.

34. Rini BI, Escudier B, Tomczak P, et al. Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): a randomised phase 3 trial. Lancet 2011;378:1931-9.

35. Rosenberg SA, Yang JC, Topalian SL, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin 2. Jama 1994;271:907-13.

36. Weinstock M, McDermott D. Targeting PD-1/PD-L1 in the treatment of metastatic renal cell carcinoma. Ther Adv Urol 2015;7:365-77.

37. Thomas JS, Kabbinavar F. Metastatic clear cell renal cell carcinoma: A review of current therapies and novel immunotherapies. Crit Rev Oncol Hematol 2015;96:527-33.

38. Amin A, Dudek AZ, Logan TF, et al. Survival with AGS-003, an autologous dendritic cell-based immunotherapy, in combination with sunitinib in unfavorable risk patients with advanced renal cell carcinoma (RCC): Phase 2 study results. J Immunother Cancer 2015;3:14.

39. Rausch S, Kruck S, Stenzl A, et al. IMA901 for metastatic renal cell carcinoma in the context of new approaches to immunotherapy. Future Oncol 2014;10:937-48.

40. Walter S, Weinschenk T, Stenzl A, et al. Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. Nat Med 2012;18:1254-61.

41. Rini B, Stenzl A, Zdrojowy R, et al. An open-label, randomized, phase 3 study investigating IMA901 multipeptide cancer vaccine in combination with sunitinib vs. sunitinib alone as first-line therapy for metastatic RCC. Proceedings of the European Cancer Congress 2015; LBA17. Available online: www.esmo.org

42. Motzer RJ, Rini BI, McDermott DF, et al. Nivolumab for Metastatic Renal Cell Carcinoma: Results of a Randomized Phase II Trial. J Clin Oncol 2015;33:1430-7.

43. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. N Engl J Med 2015;373:1803-13.

44. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366:2455-65.

45. National Cancer Institute. SEER Stat Fact Sheets: Bladder Cancer. Available online: http://seer.cancer.gov/statfacts/html/urinb.html

46. Ploeg M, Aben KK, Kiemeney LA. The present and future burden of urinary bladder cancer in the world. World J Urol 2009;27:289-93.

47. Pashos CL, Botteman MF, Laskin BL, et al. Bladder cancer: epidemiology, diagnosis, and management. Cancer Pract 2002;10:311-22.

48. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer associated genes. Nature 2013;499:214-8.

49. Lamm DL, Thor DE, Harris SC, et al. Bacillus Calmette-Guerin immunotherapy of superficial bladder cancer. J Urol 1980;124:38-40.

50. Redelman-Sidi G, Glickman MS, Bochner BH. The mechanism of action of BCG therapy for bladder cancer–a current perspective. Nat Rev Urol 2014;11:153-62.

51. Gandhi NM, Morales A, Lamm DL. Bacillus Calmette-Guerin immunotherapy for genitourinary cancer. BJU Int 2013;112:288-97.

52. Badalament RA, Herr HW, Wong GY, et al. A prospective randomized trial of maintenance versus non maintenance intravesical bacillus Calmette-Guerin therapy of superficial bladder cancer. J Clin Oncol 1987;5:441-9.

53. Hudson MA, Ratliff TL, Gillen DP, et al. Single course versus maintenance bacillus Calmette-Guerin therapy for superficial bladder tumors: a prospective, randomized trial. J Urol 1987;138:295-8.

54. Palou J, Laguna P, Mill an-Rodríguez F, et al. Control group and maintenance treatment with bacillus Calmette-Guerin for carcinoma in situ and/or high grade bladder tumors. J Urol 2001;165:1488-91.

55. Lamm DL, Blumenstein BA, Crissman JD, et al. Maintenance bacillus Calmette-Guerin immunotherapy

for recurrent TA, T1 and carcinoma in situ transitional cell carcinoma of the bladder: a randomized Southwest Oncology Group Study. J Urol 2000;163:1124-9.

56. Sylvester RJ, Brausi MA, Kirkels WJ, et al. Long-term efficacy results of EORTC genito-urinary group randomized phase 3 study 30911 comparing intravesical instillations of epirubicin, bacillus Calmette-Gu erin, and bacillus Calmette-Guerin plus isoniazid in patients with intermediate- and high-risk stage Ta T1 urothelial carcinoma of the bladder. Eur Urol 2010;57:766-73.

57. Kamat AM, Sylvester RJ, Böhle A, et al. Definitions, End Points, and Clinical Trial Designs for Non-Muscle-Invasive Bladder Cancer: Recommendations From the International Bladder Cancer Group. J Clin Oncol 2016;34:1935-44.

58. Carthon BC, Wolchok JD, Yuan J, et al. Preoperative CTLA-4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial. Clin Cancer Res 2010;16:2861-71.

59. Galsky MD, Hahn NM, Albany C, et al. Phase II trial of gemcitabine + cisplatin + ipilimumab in patients with metastatic urothelial cancer. J Clin Oncol 2016;34:abstr 357.

60. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252-64.

61. Weber JS, Kahler KC, Hauschild A. Management of immune-related adverse events and kinetics of response with ipilimumab. J Clin Oncol 2012;30:2691-7.

62. Powles T, Eder JP, Fine GD, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature 2014;515:558-62.

63. Petrylak DP, Powles T, Bellmunt J, et al. A phase Ia study of MPDL3280A (anti-PDL1): Updated response and survival data in urothelial bladder cancer (UBC). J Clin Oncol 2015;33:abstr 4501.

64. Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 2016;387:1909-20.

65. Plimack ER, Bellmunt J, Gupta S, et al. Pembrolizumab (MK-3475) for advanced urothelial cancer: updated results and biomarker analysis from KEYNOTE-012. J Clin Oncol 2015;33:abstr 4502.

66. Apolo AB, Infante JR, Hamid O, et al. Safety,

clinical activity, and PD-L1 expression of avelumab (MSB0010718C), an anti-PD-L1 antibody, in patients with metastatic urothelial carcinoma from the JAVELIN Solid Tumor phase Ib trial. J Clin Oncol 2016;34:abstr 367.

67. Ghasemzadeh A, Bivalacqua TJ, Hahn NM, et al. New Strategies in Bladder Cancer: A Second Coming for Immunotherapy. Clin Cancer Res 2016;22:793-801.

68. Drake CG, Lipson EJ, Brahmer JR. Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer. Nat Rev Clin Oncol 2014;11:24-37.

69. Vabulas RM, Braedel S, Hilf N, et al. The endoplasmic reticulum-resident heat shock protein Gp96 activates dendritic cells via the Toll-like receptor 2/4 pathway. J Biol Chem 2002;277:20847-53.

70. Raez L, Walker G, Baldie P, et al. CD8 T cell response in a phase I study of therapeutic vaccination of advanced NSCLC with allogeneic tumor cells secreting endoplasmic reticulum-chaperone gp96-Ig-peptide complexes. Adv Lung Cancer 2013;2:9-18.

71. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. Nat Rev Cancer 2012;12:265-77.

72. Peethambaram PP, Melisko ME, Rinn KJ, et al. A phase I trial of immunotherapy with lapuleucel-T (APC8024) in patients with refractory metastatic tumors that express HER-2/neu. Clin Cancer Res 2009;15:5937-44.

73. Stanley ER, Chitu V. CSF-1 receptor signaling in myeloid cells. Cold Spring Harb Perspect Biol 2014;6:1-21.

74. Lima L, Oliveira D, Tavares A, et al. The predominance ofM2-polarized macrophages in the stroma of low-hypoxic bladder tumors is associated with BCG immunotherapy failure. Urol Oncol 2014;32:449-57.

75. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nat Rev Immunol 2004;4:762-74.

76. Moretti S, Menicali E, Voce P, et al. Indoleamine 2,3-dioxygenase 1 (IDO1) is up-regulated in thyroid carcinoma and drives the development of an immunosuppressant tumor microenvironment. J Clin Endocrinol Metab 2014;99:E832-40.

77. Munn DH. Blocking IDO activity to enhance anti-tumor immunity. Front Biosci (Elite Ed) 2012;4:734-45.