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Blocking Immune Checkpoints in Prostate, Kidney and Urothelial cancer: An Overview

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Abstract

Despite a long history of immunotherapeutic approaches to treatment, most genitourinary malignancies are not cured by existing immunotherapy regimens. More recently, cell-surface molecules known as immune checkpoints have become the focus of efforts to develop more effective immunotherapies. Interactions between these molecules and their ligands inhibit the proliferation and function of tumor-specific lymphocytes. A monoclonal antibody blocking one of these checkpoints was approved for the treatment of metastatic melanoma and is now being tested in other malignancies. The objective responses seen in these early trials of checkpoint blockade are driving renewed enthusiasm for cancer immunotherapy. There are several ongoing and planned trials in genitourinary malignancies of single-agent inhibitors, as well as combinations targeting multiple checkpoints or adding other types of therapies to checkpoint blockade.

Keywords

CTLA-4; PD-1; Prostate Cancer; Kidney Cancer; Bladder Cancer; PD-L1; LAG-3

I: Introduction

Genitourinary malignancies have a long history of immunotherapeutic approaches to treatment including high-dose interleukin-2 and interferon alpha for renal cell carcinoma[1], bacillus Calmette-Guerin (BCG) for bladder cancer[2]and, most recently, Sipuleucel-T for prostate cancer[3].While effective in many patients, these therapies are, in general, not curative. The development of more effective cancer immunotherapy has long been hampered

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by the multiple strategies that tumors use to evade destruction by the host immune system[4]. One such strategy involves the expression of cell-surface molecules, known as **immune checkpoints**, on tumor-specific lymphocytes[5-7]. The interactions between these molecules and their ligands inhibit the proliferation and function of cells with potentially important anti-tumor effect. The FDA approval of a monoclonal antibody that blocks the immune checkpoint CTLA-4 (Ipilimumab (BMS, Princeton, NJ)) for metastatic melanoma in 2011 marked a turning point for immunotherapy – especially for immune checkpoint blockade[8]. Here we review the various checkpoint inhibitors that are in the clinic and their particular importance in genitourinary (GU) malignancies.

II: CTLA-4 - A Prototypical Immune Checkpoint

The FDA approval in 2011 of Ipilimumab (Yervoy) for advanced melanoma opened a new chapter in the almost 20 year long story of CTLA-4 (Cytotoxic T Lymphocyte Antigen-4). Structurally homologous to the co-stimulatory molecule CD28, CTLA-4exerts its inhibitory role by binding to the same ligands (B7.1 and B7.2) CD28 does[9], though with a markedly higher affinity and avidity. This results in an effective “hijacking” of signal 2.While CD28 is found on the surface of naïve and activated T cells, CTLA-4 is only detectable after activation[10]. The therapeutic effects of CTLA-4 blockade seem to be due primarily to enhancing the effector function of T cells[11], though more recent data suggest that anti-CTLA-4 antibodies may function by depleting regulatory T cell (Treg) as well[12, 13]. The first reports of CTLA-4 blockade enhancing anti-tumor immunity in mice appeared in 1996 when the Allison group demonstrated enhanced rejection of established tumors after anti-CTLA-4 antibody treatment[14]. The broad and vital role of CTLA-4 in modulating the activation of T cells is underscored by the autoimmunity and early death seen in CTLA-4-deficient mice[15]and the significant rate of immune-related adverse events (IRAEs) seen in patients treated with CTLA-4 blockade[16].

IIA. Preclinical Studies of CTLA-4 Blockade in Prostate Cancer

The first studies showing that blockade of CTLA-4 could enhance anti-tumor immunity in a murine prostate cancer model used the TRAMP-C1 implantable tumor line. Growth of established sub-cutaneous tumors was significantly delayed, with some tumors regressing entirely[17]. Using an implanted model involving TRAMP-C2 cells, Kwon et al further demonstrated that administration of CTLA-4 blockade immediately following tumor resection (i.e. in the adjuvant setting) reduced metastatic spread to nearby lymph nodes from 97.4% to 44%[18]. Animal studies were extended to include combination treatment regimens – experiments in the TRAMP model of primary, autochronous prostate cancer tested the combination of CTLA-4 blockade along with an irradiated GM-CSF expressing whole tumor cell vaccine (similar to GVAX prostate)[19]. Assessment at 2 months post-treatment revealed a significant reduction in tumor incidence, lower tumor grade and increased accumulation of inflammatory cells when compared to the control monotherapy groups[20]. Our lab employed the antigen-bearing ProHA × TRAMP mouse to interrogate the optimal relative timing of CTLA-4 blockade and GM-CSF-secreting cell-based vaccine (GVAX) using antigen-specific adoptively transferred CD8 T cells to measure anti-tumor response. In these studies, maximum benefit was seen with anti-CTLA-4 antibody

administered one day post-vaccination[21]. The addition of low-dose cyclophosphamide to the combination regimen further enhanced the anti-tumor response by abrogating immune tolerance, augmenting prostatic CD8+ T-cell infiltration and mediating depletion of regulatory T cells (Tregs)[22]. Waitz et al observed an additive effect when combining anti-CTLA-4 antibody treatment with cryoablation of primary implanted TRAMP-C2 tumors to prevent the growth of distantly-implanted secondary TRAMP-C2 tumors. Taken together, these preclinical studies provided reasonable justification for clinical trials of CTLA-4 blockade in men with prostate cancer.

Additional translational data supporting that notion came from a small microarray study showing that CTLA-4 was up-regulated in CD4+ Tregs sorted from patient prostate infiltrating lymphocytes (PIL) vs. naïve CD4 T cells from peripheral blood[23]. Perhaps more intriguing was a recent report suggesting a role for CTLA-4+ cells on patient CD8+ T cells with regulatory function[24]. In that study, patients with biochemically recurrent prostate cancer (BCR) were immunized with a PAP DNA/GM-CSF vaccine. Analysis of PBMCs from the vaccinated patients revealed a population of antigen-specific CD8+CTLA-4+ Tregs that suppressed specific T cell responses via an IL-35 dependent mechanism; i.e.anti-CTLA-4 antibody added to *in vitro* PAP stimulation unmasked PAP-specific effector responses that had been inhibited by this CD8 Treg population, and *in vitro* CTLA-4 blockade also allowed the identification of pre-existing PAP-specific CD8+ Tregs in a portion of the patients. Taken together these two studies suggest a role for CTLA-4 in inhibiting prostate cancer specific immune responses in patients.

IIB. CTLA-4 Blockade in Prostate Cancer - Monotherapy

The first reported pilot trial of anti-CTLA-4 antibody in prostate cancer patients tested a single 3 mg/kg dose in fourteen patients with advanced mCRPC. While treatment at this dose was well-tolerated, only two patients demonstrated PSA declines of ≤50% before eventually progressing [25]. In a larger study,Slovin et al tested Ipilimumab alone or in combination with radiotherapy in 71 patients with mCRPC[26]. Of the fifty patients who received the highest dose of Ipilimumab (10 mg/kg) alone or in concert with radiotherapy, eight experienced PSA reduction of ≤50%, six had stable disease and one patient had an ongoing complete response. Across all groups, 80% of patients experienced IRAEs with grade 3/4 IRAEs reported in 32%. Fourteen patients (28%) in the 10 mg/kg cohorts discontinued treatment due to AEs. Until recently, this was the largest experience of Ipilimumab monotherapy in prostate cancer, and set the stage for two randomized Phase III trials, launched in 2009-2010. The first of these, CA184-043 (NCT00861614) randomized approximately 800 men with mCPRC who had progressed on chemotherapy to either placebo or to Ipilimumab at a dose of 10 mg/kg q 3 weeks × 4 doses, followed by q 3 month maintenance for non-progressing patients[27]. Based on preclinical data showing that treating animals with implanted tumors with radiation therapy plus anti-CTLA-4 was more effective than either treatment alone[28], this trial also included a low dose (8 Gy) of radiation therapy to at least one lesion in both groups. It should be noted that these men, in general, had multiple sites of disease, so this radiation treatment would not be expected to significantly reduce a tolerogenic tumor burden. Instead, the notion here was that antigen “liberation” might serve to prime an immune response which would then be boosted by anti-

CTLA-4 treatment. As reported, the trial missed its primary endpoint of overall survival (O.S.) with treatment arm showing median OS of 11.2 months vs. 10 months in control arm (hazard ratio [HR] = 0.85, 95% confidence interval [CI] = 0.72–1.00, $P = .0530$). The secondary endpoint of progression free survival (PFS) was met, with a PFS of 4.0 months in the Ipilimumab arm as compared to 3.1 months in the placebo group. Pre-planned and exploratory subgroup analyses showed that patients with an alkaline phosphatase of < 1.5 times the upper limit of normal and a hemoglobin > 11 mg/dL might derive benefit. Perhaps most interestingly, analyses for interaction showed that the presence of visceral metastases strongly interacted with a treatment effect in that Ipilimumab appeared to have no effect on O.S. in patients with visceral metastases[29]. This surprising finding suggests that visceral metastases in prostate cancer might be immunologically different than bone lesions, and has profound implications for future immunotherapy trials in prostate cancer. It is worth noting that the pivotal trial of the prostate cancer vaccine Sipuleucel-T excluded patients with visceral metastases[30]; in retrospect this was likely a wise decision. Recently updated O.S. data of this trial was found to be consistent with the initial analysis, demonstrating larger benefits in patients with lower disease burden and especially when patients did not have visceral metastasis. Median OS was reported to be 11.2 months (9.6–12.6) in the ipilimumab arm vs. 10.0 months (8.4–11.2) in control arm (HR 0.84, p=0.03) [31]. A second large randomized Phase III trial of Ipilimumab in prostate cancer has completed accrual. This trial (CA184-095, NCT01057810) randomized 600 men who had not yet received chemotherapy to either Ipilimumab or placebo. This trial did not include “priming” radiotherapy, but did exclude men with visceral disease. Disappointingly, initial reports suggest that this trial is negative; results are expected to be released in 2016.

IIC. CTLA-4 Blockade in Prostate Cancer – Combination Regimens

Several combination trials involving anti-CTLA-4 have been completed thus far (Table 1). In one of these, Fong et al [32] treated 24 mCRPC patients with increasing doses of Ipilimumab plus a fixed dose of GM-CSF. Immunologically, the role of GM-CSF in this strategy is likely to be the activation and potentially the expansion of antigen presenting cells (APC) although an opposing inhibitory role cannot be excluded. Of the six patients receiving the highest Ipilimumab dose (3 mg/kg), 3 had confirmed PSA declines of >50%. One of these three responding patients also exhibited a partial response in visceral metastases. Correlative studies showed that patients treated with the two highest doses of Ipilimumab had increased levels of activated CD8+ T cells - above those previously seen with GM-CSF alone. These data are interesting; patients in previous trials who received CTLA-4 blockade alone demonstrated no reported increases in activated CD8 T cells. Additional combination trials have paired prostate cancer vaccines with Ipilimumab. In the first of these, patients were treated with an allogeneic GM-CSF secreting cell based prostate cancer vaccine (GVAX Prostate), along with escalating doses of Ipilimumab[33]. Of the seven patients (25%) who demonstrated PSA declines of ≤50%, all had received the two highest doses of Ipilimumab (3 or 5 mg/kg). Overall the combination was well tolerated, and patients appeared to have a longer than predicted overall survival (O.S.), although the trial was not powered to draw firm conclusions. In a second relevant trial, Ipilimumab was co-administered with the pox-virus based anti-PSA vaccine known as ProstVac VF[34]. In this Phase I dose escalation trial, 30 patients were treated with a fixed dose of a pox-viral PSA

vaccine (PSA-Tricom) and one of four different Ipilimumab doses[35]. Among the 24 chemotherapy naïve subjects, 14 had PSA declines from baseline but only 6 were >50%. Of the nine HLA-A2 patients, six demonstrated antigen-specific T cell responses via ELISpot. Taken together these trials show that combinations of Ipilimumab and other immunologically active agents can be well-tolerated, and suggest the potential for possible additive efficacy. Based on data from several groups showing that androgen-ablation increases T cell infiltration into the prostate gland[36, 37], and may mitigate tolerance[38], there are at least four ongoing trials combining Ipilimumab with androgen ablation (Table 1). In one of these trials (NCT01194271), the M.D. Anderson group is treating men with high-risk disease for 4 months with the combination of hormonal therapy and Ipilimumab, followed by radical prostatectomy. Tissue gathered at surgery will be critical in determining the tissue-level effects of combined treatment in the clinical setting.

IID. Blocking CTLA-4 in Kidney Cancer

A 2007 study found an association between CTLA-4 polymorphisms and the risk of developing RCC, as well as an association between a particular SNP and tumor grade in RCC patients[39]. Unfortunately, this finding did not translate to broad success in treating RCC with anti-CTLA-4[40]. A phase II trial conducted primarily at the National Cancer Institute (NCI) treated 61 patients with 3 mg/kg doses of Ipilimumab every 3 weeks, or with a single 3 mg/kg “loading dose” followed by 1 mg/kg doses every 3 weeks. In this trial, sequential cohorts were assessed [41]. Partial responses were observed in 5/40 (13%) patients receiving the higher dose. Grade 3 or 4 IRAEs were observed in 33% of patients, potentially a higher rate than that observed in melanoma patients, and likely reflecting the q 3 week dosing regimen used in this trial. At the current time, single-agent CTLA-4 blockade is not in clinical trials in RCC, most likely due to competition from the relative plethora of targeted agents, and other immunotherapy agents such as anti-PD-1. A phase I trial investigating CTLA-4 blockade in combination with anti-PD1 is ongoing and preliminary results are discussed below (section IIIB)

IIE. Blocking CTLA-4 in Bladder Cancer

To date, the only reported trial of Ipilimumab in bladder cancer is a relatively small pre-operative trial with the primary endpoints of safety/tolerability and immune monitoring[42]. In this study, 12 patients received 2 doses of Ipilimumab (either 10 mg/kg or 3 mg/kg) at 7 weeks and 4 before radical cystectomy. While the majority of IRAE's were Grade 1/2 and did not delay surgery, in the higher dose cohort, one patient did not receive the second Ipilimumab dose due to Grade 3 diarrhea and 2 patients had their surgery delayed due to IRAEs. This small trial led to the somewhat unexpected finding of a significantly higher number of $ICOS^{high}$ CD4 T-cells, both circulating and in the tumor tissue, and an increased ratio of ICOS+ to FoxP3+ CD4 T-cells in treated patients. ICOS (Inducible COStimulator) is related to CD28 and CTLA-4 and plays a role in immune cell responses and proliferation. These $ICOS^{high}$ CD4 T-cells produced IFN-γ upon stimulation, suggesting that they have the potential to be involved in an anti-tumor immune response. This important clinical observation has also led to interesting preclinical studies suggesting the importance of ICOS as a checkpoint, as well as a potential biomarker for the efficacy of anti-CTLA-4. In further trials, an ongoing multi-center Phase II trial(NCT01524991) is currently recruiting patients

with advanced/metastatic urothelial carcinoma for treatment with a regimen combining Gemcitabine/Cisplatin with Ipilimumab (10 mg/kg every 3 weeks). The primary endpoint is one-year overall survival with progression-free survival, overall response rate and safety/AEs as secondary outcome measures. Taken together these data suggest that bladder cancer is an intriguing target for immune checkpoint blockade. Additional trials blocking PD-L1 and PD-1 in bladder cancer are ongoing as well(Table I).

III: The Immune Checkpoint PD-1 (Programmed Death–1)

In contrast to the early, widespread and lethal autoimmunity seen in mice lacking CTLA-4, PD-1 knockout mice exhibit late-onset, milder, strain-specific autoimmunity that is generally limited in scope[43, 44]. Together with its expression on the “exhausted” CD8 cells seen in chronic infections[45], these data speak to a role for PD-1 in modulating T cell responses to prevent autoimmunity and restrain inflammatory responses in the face of persistent antigen. First described by the Honjo group in 1992[46], PD-1 can be found on T cells, B cells, natural killer T cells, dendritic cells and activated monocytes[6], and is well-described on tumor-infiltrating lymphocytes in numerous human cancers[47-51]. T cell expression of PD-1 occurs upon activation via the TCR[52]. Given the role of PD-1 in limiting inflammatory responses, it is no surprise that its ligands, PD-L1/B7-H1 and PD-L2/B7-DC, are up-regulated on multiple cell types by pro-inflammatory cytokines[5]. PD-L1 is widely expressed in numerous human carcinomas, including lung, ovary, colon, melanoma, kidney and bladder and has been shown to correlate with progression and poor prognosis for some malignancies[53, 54]. The IFNγ-induced expression of PD-L1 on tumors is a mechanism of “adaptive resistance” in response to the immune infiltrate, as opposed to an oncogene-driven, constitutive means of escape. In melanoma, immunohistochemical(IHC) examination of 150 benign and cancerous lesions revealed a highly significant association of B7-H1 expression with inflammatory infiltrates ($P < 0.0001$)[55], suggesting that here the expression of PD-L1 is primarily adaptive. In terms of genitourinary cancers, expression of PD-L1 is common in kidney cancer and bladder cancer, but exceedingly rare in prostate cancer. In terms of translation, multiple preclinical studies using murine tumor models demonstrated success with antibody-mediated blockade of the PD-1/PD-L1 interaction[56, 57]. In 2010 the results of the first pilot study of anti-PD-1[58]were reported, followed by Phase Ib multi-dose results for PD-1[59]and anti-PD-L1 in 2012[60].In comparison with anti-CTLA-4, grade 3 or 4 IRAEs were less frequent, and with the exception of a small number of serious pneumonitis cases, proved manageable. In general, 15-30% of patients with kidney cancer, melanoma and lung cancer showed objective responses to these agents, providing important clinical proof of concept for monotherapy.

IIIA. Blocking PD-1 in Prostate Cancer

Despite evidence from at least two groups showing that the CD8 T cells that infiltrate prostate tumors express PD-1[47, 61], no objective responses to single-agent PD-1 blockade were reported in 17 patients with prostate cancer treated on the Phase Ib multi-dose trial of anti-PD-1[59]. The precise mechanisms underlying this lack of response are not immediately obvious, but may involve the phenotype of prostate infiltrating lymphocytes, which are generally refractory to stimulation [62]. Another possible explanation involves the

expression of PD-L1, which is generally associated with an increased response to anti-PD-1 monotherapy[63], and which is generally absent in prostate cancer. Indeed, the relative paucity of PD-L1 expression in human prostate tumors is somewhat puzzling, given pre-clinical data suggesting that loss of PTEN appears to be associated with PD-L1 up-regulation in both prostate cancer[64] and glioblastoma[65], and the notion that between 10 and 70% of prostate tumors lose PTEN[66]. Regardless of the precise mechanism, clinical data thus far suggest that PD-1 blockade is not as likely to be as effective as monotherapy for prostate cancer as it is for kidney cancer, lung cancer or melanoma.

IIIB. Blocking PD-1 in Kidney Cancer

Given the presence of PD-1+ mononuclear cells and PD-L1+ tumor cells in RCC patients, it was not especially surprising that phase I dose-escalation trials of anti-PD-1 monotherapy yielded objective responses in RCC[58, 67]. Perhaps more impressive, however, is the case of an advanced RCC patient who demonstrated a stable partial response that, over time, evolved into a documented complete response. In 2013, Lipson et al reported that the patient has remained off-treatment for over 5 years[68]. Longer-term follow-up data from a phase Ib study of nivolumab showed that the objective response rate to anti-PD-1 monotherapy in RCC patients was in the 30-35% range with prolonged stable disease in another 10% of patients [69]. Similar long term follow-up study of MPDL3280A in RCC patients showed durable median response of 54 weeks (2.7+ to 68.1+ weeks) and positive association between PD-L1 intensity and response to MPDL3280A [70]. Based on these long term efficacy activities and safe tolerability profile, further studies of anti PD-1 agents especially Nivolumab in RCC were undertaken and have been successfully completed (Table 1).

Phase II study of nivolumab in previously treated RCC patients involved three randomized cohorts treated every three weeks at doses of 0.3, 2 or 10 mg/kg respectively. Median PFS was reported as 2.7, 4.0, and 4.2 months respectively in three cohorts (P = 0.9) with median OS of 18.2 months (80% CI, 16.2 to 24.0 months), 25.5 months (80% CI, 19.8 to 28.8 months), and 24.7 months (80% CI, 15.3 to 26.0 months) across 3 cohorts. 19/168 (11%) of patients experienced grade3/4 study drug related toxicities [71]. In a Phase I “biomarker” trial of nivolumab, immunomodulatory activity of this drug was assessed using pre- and post-treatment biopsies as well as peripheral blood samples. Study included 3 cohorts that received nivolumab every three weeks at doses of 0.3, 2 or 10 mg/kg respectively. An additional cohort in this study enrolled treatment-naïve RCC patients. PD-L1+ patients demonstrated better ORR of 22% (4/18) as compared to 8% (3/38) seen in PD-L1– patients. During the course of treatment from baseline to cycle 2 day 8, T cell infiltrates increased by a median of 70% (CD3+; range 53– 220%) and 88% (CD8+; 61–257%). Such transition in biomarkers along the study drug treatment course proved immunomodulatory effects of nivolumab [72].

Nivolumab and MPDL3280A have also been investigated in combination with FDA approved drugs. A Phase I dose escalation study(NCT01472081) of Nivolumab (anti-PD-1 agent) in combination with tyrosine kinase inhibitors sunitinib or pazopanib in mRCC patients has been reported. No dose limiting toxicity was observed in sunitinib arm at starting dose of nivolumab (2 mg/kg IV Q3W) leading to expansion of higher dose arm

(5mg/kg IV Q3W). But DLT's were observed in pazopinib arm at starting dose of nivolumab leading to closure of the arm. Overall both combinations demonstrated safe toxicity profiles along with anti-tumor activity [73]. Another phase I study of nivolumab in combination with ipilimumab in mRCC patients has demonstrated acceptable safety with ongoing anti-tumor responses. 16% patients in the study experienced dose limiting AEs that most commonly included increased lipase or ALT. Objective response rate (ORR) was reported in 29% pts. (nivolumab 3 mg/kg + ipilimumab 1 mg/kg cohort) and 39% patients (nivolumab 1 mg/kg + ipilimumab 3 mg/kg cohort) [74]. MPDL3280Ahas also been investigated along with bevacizumab in mRCC patients with good tolerability of the treatment combination [75].

Based on promising phase II study results, phase III randomized trial comparing Nivolumab to the mTOR inhibitor everolimus (1:1 randomization) in RCC patients was undertaken. While overall survival was the study's primary endpoint, numerous secondary outcomes such as safety, progression-free survival, objective responses and disease-related symptom progression were also measured. A total of 821 RCC patients (clear cell histology) who had previously received one or two lines of anti-angiogenic regimens were accrued. After a minimum follow-up period of 14 months, median OS was reported at 25.0 months (95% confidence interval [CI], 21.8 to not estimable) in the nivolumab arm vs. 19.6 months (95% CI, 17.6 to 23.1) in the everolimus arm. The objective response rate was 25% with nivolumab and 5% with everolimus (odds ratio 5.98; 95% CI, 3.68 to 9.72; P<0.001). The median progression-free survival was found to be 4.6 months in the nivolumab group and 4.4 months in the everolimus group (hazard ratio, 0.88; 95% CI, 0.75 to 1.03; P=0.11) [76]. These results eventually led to FDA approval of nivolumab for advanced RCC patients.

IV: Other Checkpoint Molecules – LAG-3, TIM-3,B7-H3 and B7-H4/B7-Hx

IVA. Blocking LAG-3

LAG-3 is a cell surface molecule found on several different types of immune cells that is structurally quite similar to CD4[77]. Both molecules bind to Class II MHC but LAG-3's affinity for Class II is likely higher than that of CD4[78]. Though LAG-3 is up-regulated upon activation of either CD4 or CD8 T-cells[79], it serves as a negative regulator of homeostatic proliferation[80]and can be found on CD8+ TILs as well as CD4+ induced Treg[50, 51, 81]. Analysis of tumor-infiltrating lymphocytes (TILs) from both primary and metastatic RCC tumors showed significant expression of LAG-3 on CD8 T cells. Expression, particularly in concert with other checkpoint molecules, has been observed in melanoma as well[51]. An interesting synergy between PD-1 and LAG-3 blockade has been shown in murine tumor models[82]and ovarian cancer patients[50]. Both studies demonstrated that combined antibody blockade of PD-1 and LAG-3 was more effective than either alone and may be a promising future checkpoint blockade strategy for some malignancies[82, 73].One LAG-3 related molecule currently in clinical trials is a recombinant, soluble, dimeric LAG-3-Ig fusion protein known as IMP321 or ImmuFact (Immutep, Orsay, France) that is intended to condition dendritic cells without inducing inflammation. There have been several Phase I trials of IMP321 in various cancers, including a non-randomized, fixed dose-escalation Phase I study of 21 patients with advanced or metastatic RCC (NCT00351949)[84]. The only adverse events attributed to

IMP321 in this trial were grade 1 local reactions at the injection sites. Increases in activated CD8+ T cells were seen at the two highest dosages but there were no objective responses. Progression-free survival was significantly better in the higher doses vs. the lower doses with 7/8 (87.5%) high dose patients experiencing stable disease at 3 months vs. 3/11 (27%) in lower dose cohort (P=0.015). At this time, there is only one IMP321 study recruiting patients – a multi-peptide vaccine for melanoma with IMP321 as adjuvant. A LAG-3 blocking monoclonal antibody, BMS-986016, (BMS, Princeton NJ) has very recently entered a Phase I dose escalation trial, both as a monotherapy and in combination with PD-1 blockade (NCT01968109).

IVB. TIM-3 as an Immune Checkpoint

TIM-3 (T-cell immunoglobulin mucin-3) was identified in 2002 by the Kuchroo lab as part of an effort to find a reliable marker for T_H1 CD4 T-cells[85]. Originally thought to be expressed only on differentiated T_H1 and T_c1 cells as a means of restraining T_H1 responses, later studies demonstrated expression on innate immune cells including dendritic cells[86]. Murine studies found that the majority of CD8 TILs expressing TIM-3 also expressed PD-1, and that these double-expressors were characterized by the most severe inhibition of effector cytokine secretion (IL-2, IFN-γ and TNF-α). Few studies have addressed the expression of TIM-3 on human TIL; in one relevant study it was shown that advanced melanoma patients exhibited co-expression of TIM-3 and PD-1 on tumor infiltrating, antigen-specific CD8 T cells[87]. Combined *in vitro* blockade with both TIM-3 and PD-1 was able to restore effector cytokine secretion. A recent study of benign and cancerous tissue from 137 treatment-naïve prostate cancer patients found weak TIM-3 expression in benign tissue compared to up-regulation in both PIN and invasive carcinomas[88]. Univariate analysis showed a significant association with TNM stage, nuclear grade and recurrence-free or progression-free survival. In RCC patients, percentages of TIM-3+ and TIM-3+PD-1+ CD8 T cells and CD4 T cells were significantly higher in tumor-infiltrate than in peripheral blood[89]. Despite ample preclinical interest, at this time, there are no ongoing clinical trials of TIM-3 blockade in cancer patients.

IVC. B7-H3

First described by the Chen lab in 2001[90], B7-H3 is a member of the B7 superfamily with inducible cell surface expression on T cells, monocytes and dendritic cells as well as low-level constitutive expression on many non-immune cells and tissues. It was originally characterized as a co-stimulatory molecule that, when combined with anti-CD3, could induce IFNγ production in T cells. Later work suggestedthat ligation of B7-H3 could mediate suppression of T_H1 responses[91]. Interestingly, B7-H3 may have a dual role in the immune response to cancer, in some conditions up-regulating a response and in others down-modulating immune responsiveness[92]. Multiple studies of prostate cancer patient samples showed strong immunohistochemical staining of B7-H3 on adenocarcinomas and high-grade PIN as well as some cell lines[93, 94]. Of 823 prostatectomy samples, 93% showed high expression which was correlated with metastases at time of surgery as well as a significantly higher risk of recurrence and death attributable to prostate cancer. More intense staining of B7-H3 in prostate cancer was also associated with greater risk of biochemical recurrence after salvage radiation therapy[95],staining of the proliferation marker Ki-67[96] and lower

numbers of intratumoralCD4 T cells, CD8 T cells and DCs[97]. Interestingly, B7-H3 expression was not affected by androgen deprivation before radical prostatectomy[98]. In 743 RCC patients, only 17% had tumoral B7-H3 expression while 95% were positive for expression in the tumor vasculature[99]. Both tumor expression and diffuse vascular expression were associated with greater risk of disease progression and death due to RCC. In UCC of the bladder, B7-H3 was found to be widely expressed (>70% of samples) across all tumor stages, though BCG recipients tended to have increased expression[100]. Clinically, Loo and colleagues developed an Fc-enhanced monoclonal antibody that targets B7-H3 expressing tumors via ADCC[101]. This antibody exhibited potent anti-tumor activity in both in vitro and xenograft studies with no adverse events seen in primate studies. At the current time, a B7-H3 targeted antibody (MGA271, MacroGenics, Frederick MD), is being clinically evaluated in a Phase I trial in patients with melanoma or prostate cancer (NCT01391143).

IVD. B7-H4/B7x/B7S1

B7-H4, also known as B7x/B7S1, is a member of the B7 super family first described in 2003 by three independent research groups and is considered to be an inhibitor of proliferation and cytokine production in CD4 and CD8 T-cells[102-105]. Primarily expressed on activated T cells, B cells, dendritic cells and monocytes, surface expression is low in most non-lymphoid tissues, though somewhat higher in prostate, testis and a small number of other sites. B7-H4 expression has been described in numerous malignancies including prostate and renal cancers. In both prostate cancer and RCC, more robust expression is associated with a higher risk of death, metastatic disease and recurrence[94, 106]. B7-H4 expression is not confined to the tumor cells themselves – in one study 211 of 259 RCC patient specimens (81.5%) were positive for tumor vasculature endothelium expression via IHC[105]. In preclinical studies, a recombinant human antibody delayed tumor growth in an ovarian cancer model involving humanized mice with established sub-cutaneous tumors[107].vAt this time, there are no ongoing clinical trials targeting B7-H4.

V: Conclusions and Future Directions

The objective responses seen with anti-PD-1 and anti-CTLA-4 antibodies are driving renewed enthusiasm for cancer immunotherapy. While single-agent CTLA-4 blockade shows efficacy in multiple tumor types, the high rates of serious IRAEs cannot be overlooked. The results of ongoing Phase III trials will clarify how Ipilimumab can best be used going forward, especially in prostate cancer where a randomized Phase III trial in the pre-chemotherapy space has accrued and is maturing. PD-1 blockade monotherapy, in contrast, results in durable responses in multiple tumor types. With a potentially lower incidence of serious IRAEs, Nivolimumab and other PD-1 / PD-L1 targeting agents like MK-3475 (Merck) and MPDL3280A (Roche / Genentech) have great potential, though simultaneous inhibition of more than one checkpoint may be likely to be more effective than targeting a single pathway[108]. Checkpoint blockade in combination with other therapies, such as vaccines, androgen ablation, targeted therapies and radiation, has proved effective in murine models. With several ongoing or planned trials to explore these approaches, the field is eager to see the same efficacy in patients.

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Table 1

Kidney cancer						
Agent	**Phase**	**Trial Status**	**Design/Description**	**Number Of Subjects**	**Results/Comments**	**Reference or NCCT Identifier**
Nivolumab Monotherapy	III	Completed	Randomized, open- label study of anti-PD-1 versus the mTOR inhibitor everolimus in patients with advanced / metastatic RCC. Patients have received prior anti-angiogenic therapy.	821	Primary endpoint = OS 25 Vs.19.6 months	Motzer RJ et al NEJM 2015
MPDL3280A (anti-PD-L1) +/- Avastinvs. Sunitinib	III	Ongoing	Randomized, open- label study comparing MPDL3280A + avastin to sunitinib alone in patients with untreated locally advanced or metastatic RCC	550	Primary endpoint = PFS, Secondary Endpoints = Overall RR/duration, OS, DOR, Adverse Event Incidence & pharmacokinetics	NCT02420821
Nivolumab + Ipilimumab	III	Ongoing	Randomized open label study of nivolumab + ipilimumab compared with sunitinib monotherapy in previously untreated advanced or mRCC	1070	Primary Outcome Measures: Progression-free survival (PFS), Overall survival (OS) Secondary Outcome Measures: Objective response rate (ORR) & Adverse Event Incidence	NCT02231749
Prostate cancer						
Agent	Phase	Trial Status/PC	Design/Description	Number of Subjects	Results/Comments	Reference or NCCT Identifier
Ipilimumab Monotherapy	III	Completed	Randomized, double blind trial of ipilimumabvs. placebo in pts with chemotherapy-naïve mCRPC	600	Primary endpoint- OS Secondary endpoints = PFS, pain progression, time to subsequent therapy and safety. Results- not yet reported	NCT01057810
Ipilimumab Following radiotherapy	III	Completed	Randomized, double blind trial of ipilimumabvs placebo following radiotherapy in patients previously treated with docetaxel	799	Primary endpoint = OS Secondary endpoints = PFS, pain response and safety OS- 11.2 vs 10 months (p=0.05)	Kwon ED et al Lancet Onc. 2014
Bladder cancer						
Agent	Phase	Trial Status	Design/Description	Number Of Subjects	Results/Comments	Reference or NCCT Identifier
Pembrolizumab (anti-PD-1) monotherapy	III	Ongoing	Randomized open label stud of Pembrolizumab (MK-3475) Versus Paclitaxel, Docetaxel or Vinflunine in Recurrent or Progressive Metastatic UC patients	470	Primary Outcome Measures: Overall survival (OS), Progression-free survival (PFS) per RECIST 1.1	NCT02256436
Atezolizumab (MPDL3280A)	III	Ongoing	Open-Label study in Patients With PD-L1-Selected, High-Risk Muscle Invasive UC patients after	440	Primary Outcome: Disease-Free Survival (DFS)	NCT02450331

Kidney cancer						
Agent	**Phase**	**Trial Status**	**Design/Description**	**Number Of Subjects**	**Results/Comments**	**Reference or NCCT Identifier**
			Cystectomy Randomized to Atezolizumab Vs. Observation as Adjuvant Therapy		Secondary Outcome: Overall Survival (OS), Disease-Specific Survival (DSS), Distant Metastasis-Free Survival (DMFS), Adverse Events (AEs), Percentage of Anti-Therapeutic Antibody (ATA) Response	
Atezolizumab (MPDL3280A)	III	Ongoing	Open label randomized study of Atezolizumab vs. chemotherapy in metastatic UC patients who have failed platinum based chemotherapy	767	Primary Outcome: Overall survival Secondary Outcome: Objective response rate (ORR), Progression-free survival (PFS), Duration of response (DOR), Incidence of adverse events (AEs), Incidence of anti-therapeutic antibodies to Atezolizumab	NCT02302807