CANCER IMMUNOLOGY AND IMMUNOTHERAPY – UNDERSTANDING AND ADAPTATION THE CURRENT EVIDENCE TO OPTIMIZE PATIENT THERAPY OUTCOMES

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ABSTRACT:
The aim of this publication includes the try to act as intermediary to the readers, which should be able to understand:
· The description of the cancer immunotherapy mechanisms in the context of current therapy decisions for the treatment of cancer
· The including criteria for those patients with cancer who could be appropriate candidates for immunotherapy
· And to optimize patient outcomes by using best practices to manage the adverse events associated with immunotherapy treatment
More than 15 promising immunotherapy approaches being tested in clinical trials with appropriate patients and colleagues for enrollment and peer-to-peer education purposes, respectively.

Keywords: cancer immunotherapy mechanisms

Schedule
· Current Immunotherapy in Melanoma, Lung, and Kidney Cancers
· Use of Ipilimumab in Metastatic Castration-Resistant Prostate Cancer That Progressed After Docetaxel
· Pembrolizumab Safe and Effective in Patients With Advanced Melanoma in a Phase I
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In this book chapter some important recent journal articles regarding advances in immunotherapy for cancer will be discussed. Topics include the role of the immunotherapy in melanoma, lung, and especially in kidney cancers, including discussion of the mechanism of action and immune-related adverse events associated with checkpoint blockade, CTLA-4 (cytotoxic T-lymphocyte-associated Protein 4) blockade in metastatic castration-resistant prostate cancer, but also the use of PD-L1 (programmed death-ligand 1) expression as a predictive biomarker in multiple tumor types.

An Overview of the actual Immunotherapy in Melanoma, Lung, and Kidney Cancers

Immunotherapy has become an expanding focus of cancer therapy and research in recent years. Since 2013 many article restart with a reviewing of the destinations of the activity of cancer vaccines, which act by exposing dendritic cells to an antigen, or set of cancer antigens, along with an adjuvant, activating CD4+ T cells and, subsequently, CD8+ T c cells. [1, 2] Unfortunately, cancer vaccines have, to date, been somewhat disappointing in their efficacy with very few objective responses. [3] The only approved vaccine for cancer is sipuleucel-T, at this time. This vaccine is approved for asymptomatic or minimally symptomatic castration-resistant prostate cancer based on demonstrating a significant improvement in overall survival in this setting but not response or progression-free survival compared with placebo. [4] The MAGRIT trial of the MAGE-A3, another cancer vaccine and its results in the adjuvant setting for lung cancer from another phase III trial were reported. MAGE-A3 is a cancer-testis antigen expressed in approximately one third of lung cancers. This largest phase III trial of a vaccine in non-small-cell lung cancer (NSCLC), failed to demonstrate a significant difference in the primary endpoint of disease-free survival. [5] The advent of immune checkpoint blockade, which overcomes immune tolerance by enhancing the immune response in the tumor microenvironment, has reinvigorated the field of cancer immunotherapies, despite the negative data seen from most of the cancer vaccine studies. Two pathways have been effectively targeted:

1. The cytotoxic T-lymphocyte antigen 4 (CTLA-4) and

2. The programmed cell death 1 receptor (PD-1) and one of its ligands on opposing cells, PD-L1.

Several antibodies targeting these immune checkpoints are in various phases of clinical development (Table 1).

Table 1. Checkpoint Inhibitors

Agent	Antibody type	Target point	Development / Phase
BMS 936559	Fully human, IG 4	PD-L1	Multiple tumors - phase I
Ipilimumab	Fully human, IgG1k	CTLA-4	Melanoma – approved for unresectable or metastatic disease NSCLC – phase III
MEDI-4736	Human engineered, IgG1k	PD-L1	NSCLC – phase III Colorectal – phase II HNSCC – phase II Multiple tumors – phase I/II
MPDL3082A	Human engineered, IgG1	PD-L1	NSCLC – phase III RCC – phase II Bladder urothel cancer – phase II Multiple tumors – phase I/II
MSB0010718C	Fully human, IgG1	PD-L1	Multiple tumors – phase I/II
Nivolumab	Fully human, IgG4	PD-1	Gliobastomamultiforme – phase III HNSCC – phase III NSCLC – phase III Metastatic RCC – phase III Melanoma – phase III completed
Pembrolizumab	Humanized, IgG4	PD-1	Melanoma – for ipilimumab-naïve patients phase III For progression after ipilumumab and BRAF-positive inhibitor aproved
Pidilizumab	Humanized, IgG1	PD-1	Multiple tumors – phase I/II

Legend:
HNSCC - Head and neck squamous cell carcinoma; **NSCLC** - Non small cell lung cancer; **RCC** – Renal cell carcinoma

Anti–CTLA-4 Therapy

Ipilimumab is a monoclonal antibody against CTLA-4. It has been approved for the treatment of metastatic melanoma based on 2 registration trials. The first trial included randomization of 502 treatment-naive patients to ipilimumab plus dacarbazine (DTIC) vs DTIC plus placebo. The reported median OS was 11.2 months with ipilimumab plus DTIC vs 9.1 months with DTIC alone ($P < .001$). [6] The other trial included 676 HLA-A*0201 - positive patients who had progressed on or during previous therapy who were randomized in a 3:1:1 ratio to either ipilimumab plus glycoprotein 100 peptide vaccine (gp100; n = 403) vs ipilimumab alone (n = 137) vs gp100 alone (n = 136). [7] The median OS in each arm was 10.0 months, 10.1 months, and 6.4 months, respectively, which yielded a significant difference between ipilimumab and gp100 ($P < .001$) and no significant difference between ipilimumab and ipilimumab plus gp100 ($P = .76$). There were registered immune-related toxicities, such as colitis and dermatitis, which were frequent in these studies and sometimes required management with corticosteroid or anti-tumor necrosis factor therapy. Autoimmune inflammation of multiple tissues in the body has been demonstrated, including hypophysitis, which can require lifelong pituitary hormone replacement. [6-8] Single agent ipilimumab was also evaluated in a phase II trial of 61 patients with metastatic renal cell carcinoma (RCC) at 2 different dose levels - 1 mg/kg or 3 mg/kg every 3 weeks, reporting an overall response rate (ORR) of 12.5% using a more intense, continuous dose than scheduled and used in melanoma. [9] However, this more intensive dosing resulted in one third of the patients developing grade 3/4 immune-related adverse events. It should be noted that ipilimumab is not currently under investigation in RCC, due to the competitive development of multiple targeted agents. A randomized, double-blind phase II trial of chemotherapy plus ipilimumab given either concurrently or in a phased manner vs standard chemotherapy in 204 patients with stage IIIB or IV NSCLC met its primary endpoint of improved progression-free survival (PFS) by immune-response criteria ($P = .05$) for the phased administration. The subset analyses revealed an OS benefit in patients with squamous histology. [10] Ipilimumab is currently being evaluated in late-phase trials in lung [11, 12] and prostate cancers. [13]

Anti–PD-1 Therapy

Antibodies that bind PD-1 and block the interaction between PD-1 on T cells and PD-L1 on tumor cell surfaces have demonstrated remarkable activity in several tumor types including melanoma, RCC, lung cancer and colorectal cancer (CRC), the last two are generally not considered to be immunogenic. [14] In a heavily pretreated population with NSCLC, a response rate of 17% was noted in the early-phase trials of the anti-PD-1 monoclonal antibody nivolumab. Its response durations lasting up to several years and a median OS of 9.6 months. [14, 15] Grade 3 or higher toxicities were relatively minimal in comparison with those observed with ipilimumab, although there were 2 deaths due to pneumonitis. Toxicities did not appear to be cumulative over time. Preliminary phase I data of nivolumab combined with platinum-based chemotherapy reported response rates of 33% to 50%. [16] Large phase III trials are currently ongoing, comparing nivolumab with Docetaxel in the second-line setting and beyond. [17, 18] Rapid and durable responses to PD-1 inhibition have also been observed in melanoma with a 31% ORR to nivolumab in phase I trials. 45% of those achieving response within 8 weeks of therapy. [19] Median duration of response was 2 years. Some responses persisting after discontinuation. Pembrolizumab, another anti-PD-1 antibody, has shown objective response rates of 38% by Response Evaluation Criteria in Solid Tumors (RECIST) in a phase I trial among patients with melanoma. [20] At the highest dose level (10 mg/kg every 2 weeks), a 52% ORR was observed and grade 3/4 toxicities were reported in 12.6% of patients. Previous treatment with ipilimumab did not influence response rates or toxicities. Pembrolizumab was approved in September 2014 by the US Food and Drug Administration (FDA) for patients with unresectable or metastatic melanoma who experienced disease progression following treatment with ipilimumab and, in those patients with BRAFV600 mutations, following a BRAF inhibitor. BRAF is a human gene that makes a protein called B-Raf. The B-Raf protein is involved in sending signals inside cells, which are involved in directing cell growth. In 2002, it was shown to be mutated in some human cancers. Early-phase studies of anti–PD-1 therapy have also demonstrated ORR of 30% to 35% in RCC, again with some responses appearing to be quite durable. [8, 21] Phase III trials are currently ongoing [22-24] including a potentially paradigm-changing, randomized phase III trial comparing nivolumab vs everolimus in the second-line setting with the primary endpoint of overall survival. [25]

Anti–PD-L1 Therapy

Additional monoclonal antibodies against PD-L1 are in the early stages of clinical investigation. BMS-936559 is a fully human monoclonal antibody that was well tolerated in a phase I study with activity observed in NSCLC and melanoma. [26] The most frequently observed treatment related adverse events were fatigue, infusion reaction, and diarrhea. MPDL3280A, an engineered PD-L1 antibody, has also demonstrated antitumor activity in NSCLC (ORR: 22%), melanoma (ORR: 29%), and RCC (ORR: 15%) across several dose levels. [27] Moreover, there was a 36% response rate observed among tumors positive for PD-L1 vs 13% in PD-L1-negative tumors. The antibody appeared to be well tolerated with 13% of patients experiencing grade 3/4 treatment-related adverse events. Another fully human antibody against PD-L1, MEDI-4736, has recently entered clinical testing and demonstrated antitumor activity in NSCLC, as well as head and neck, pancreatic, and gastro esophageal cancers in a phase I trial. It was well tolerated with 6% of patients experiencing grade 3/4 treatment-related adverse events. [28]

Combination Therapy with Immune Checkpoint Inhibitors

Attempts to combine immunotherapies with tyrosine kinase inhibitors are ongoing, although they appear hampered by high rates of hepatitis. [29] Wolchok and colleagues [30] evaluated the combination of nivolumab and ipilimumab in metastatic melanoma. He demonstrated more rapid responses with the dual immune checkpoint blockade than with either agent used as monotherapy, with approximately three quarters of responses occurring before 12 weeks.

The authors reported a 40% confirmed objective response rate but did note that 53% of patients developed grade 3/4 treatment-related toxicities. Responses correlated with PD-L1 expression but not as strongly as seen in anti–PD-1 monotherapy studies. Patients were randomized to concurrent treatment with the 2 antibodies or 1 of 4 sequential cohorts of varying dose levels. Sznol and colleagues [31] presented updated survival data for the study at the 2014 meeting of the American Society of Clinical Oncology (ASCO). He reported unprecedented 2-year OS rates of 88% in the cohort of patients receiving nivolumab 1 mg/kg and ipilimumab 3 mg/kg sequentially (n = 17) and 79% among patients receiving concurrent dual-antibody therapy (n = 53). Other combinations of checkpoint blockade agents are being investigated, with preclinical work indicating potential synergies of PD-1 blockade combined with other immune checkpoint inhibiting antibodies targeting LAG-3 and TIM-3. [32, 33]

Discussion

The growing role of the checkpoint inhibitors in the practice of oncology is obvious. Many clinicians are aware that previous immunotherapy approaches to cancer therapy, such as vaccines, interleukin (IL) -2, and interferon were met with limited success. The mechanism of action of checkpoint blockade set them apart from the earlier therapies targeting the immune system. The difference is presented in the chronologic mechanism and differing act places. A vaccine must rely on the patient's endogenous dendritic cells and prime them to start an immune response with a target antigen. Cytokines such as IL-2 and interferon-á, which have demonstrated dramatic efficacy in a small percentage of patients with melanoma and RCC, induce antitumor activity via T-cell activation. [34] Checkpoint blockade works on the premise of an ongoing

antitumor immune response that is impeded by various molecular pathways known as immune checkpoints. Previous approaches to immunotherapy in cancer were formulated on mounting an immune response whereas immune checkpoint blockade act to facilitate a preexisting immune response by eliminating inhibition. Immunologically, the 2 approaches are actually quite different. Stimulating an antitumor immune response with a vaccine causes immune cells, particularly T cells, to target and migrate to the tumor and, ideally, produce yet another cytokine, interferon-γ. If interferon- γ is produced, the tumor cells upregulate compensatory mechanisms, particularly expression of immune checkpoint ligands on tumor cells and associated stromal cells in the tumor microenvironment. Therefore, even if the vaccine works optimally, the immune checkpoints will likely interfere with the intended antitumor response. Hence, thecheckpoint blockade has yielded superior objective responses in some cancers but not all cancers.

Activity of Immune Checkpoint Inhibitors across Tumor Types

*S*ome tumors, such as melanoma and RCC, seem to be immunogenic in that they respond better than other tumors to checkpoint inhibition and other immune-targeted therapies such as high-dose IL-2. Based on data presented at the 2014 ASCO annual meeting, many tumors that were not considered immune responsive, such as pancreatic, esophagogastric, head and neck, bladder, and lung cancers, have shown responses to immune checkpoint blockade (i.e. PD-1 and PD-L1 inhibition) in early-phase trials [28, 35-43]. It is very interesting speculation on what may make one cancer more immune responsive than another and why we are now seeing evidence that cancers previously unresponsive to other forms of immunotherapies are responding to these newer investigational immune checkpoint inhibitors. Some patients are clearly responsive and some are not. The difference in response either between tumor types or among patients within a specific tumor type is not clearly understood. There is one global explanation that has been discussed, and that is the notion that tumors with a higher mutational rate, such as melanoma, are more responsive because there is a greater number of potential neoantigens for the immune system to attack.

There are some data-some were presented at ASCO-suggesting that patients with a higher mutation rate may be more likely to respond. [44] At Johns Hopkins, Le and colleagues found that, in patients with micro satellite instable CRC, somatic mutations do seem to be responsive to PD-1, whereas CRC in general does not respond well to PD-1 blockade. So mutation rate might be an explanation for that phenomenon. However, this would not explain why kidney cancer, which has a much lower mutation rate than melanoma, responds quite favorably to checkpoint inhibition. That question currently remains unanswered. There are other cancers, such as small-cell lung cancer, that have very high mutational rates. But there are very few data on immunotherapy in small-cell lung cancer. Reck and colleagues [45] evaluated ipilimumab plus carboplatin/paclitaxel as first-line therapy in extensive-disease small cell lung cancer in a randomized phase II study. They reported a significant improvement in immune-related PFS with a phased combination regimen compared with control but no improvement in either PFS or OS. However, there is an ongoing trial in the United Kingdom of ipilimumab as maintenance after initial chemotherapy in small-cell lung cancer. There are multiple groups trying to start trials of PD-1 and PD-L1-related agents in small cell lung cancer as well. It certainly would seem to be a logical target.

Concerning other tumor types immune checkpoint inhibitors have demonstrated antitumor activity. For instance, it is expected that ovarian cancer would respond well to these agents due to preclinical experience in animal models. [46] However, in the multicenter phase I trial of anti–PD-L1 antibody BMS-936559, only 1 of 17 patients (6%) with ovarian cancer had a partial response and 3 (18%) had stable disease lasting 24 weeks or longer. Thus, there did not appear to be a robust response in that tumor.[26] Another example is prostate cancer, which has generally been non responsive to checkpoint inhibition. In the phase IB trial of the anti-PD-1 antibody nivolumab, 17 patients with metastatic castration-resistant prostate cancer were treated with no objective responses observed. [47] The response rate to ipilimumab is a prostate specific antigen response in the range of 10% to 20% at best.[48] Thus, despite the presence of infiltrating lymphocytes associated with prostate cancer, it has demonstrated a disappointing lack of response to immunotherapy with checkpoint blockade. On the other hand, Muro and colleagues [41] presented data from a phase Ib trial of 39 patients with PD-L1-positive carcinoma of the stomach or gastro esophageal junction treated with Pembrolizumab 10 mg/kg every 2 weeks for up to 24 months. They reported an ORR of 30% with the median response duration not yet reached. Another way for the cancer immunotherapies is to use them as initial therapy for many patients with various cancers. Much of the data that have been published or presented in tumors have been in the second line and beyond, for example, the anti-PD-1 (nivolumab) and anti-PD-L1 phase I data where all patients had at least 1 line of treatment, typically 3 or 4. [26, 47] There are some data that address that in the trial of nivolumab in kidney cancer, which was the dose finding trial presented by Motzer and colleagues [49] at ASCO 2014. There was a small cohort of treatment-naive patients. And in that small cohort of patients, the response rates appeared similar to what was observed for treatment-experienced patients. So, this may indicate that we will see responses in the first line.

Immune Checkpoint Inhibitor Response Characteristics

It appears that several different tumor types may be amenable to therapy with checkpoint inhibitors. Antitumor responses with immunotherapy can be different than those that we expect from chemotherapy and targeted agents, and as a result, some trials have assessed immune-related response criteria rather than RECIST. Do the immune-re-

lated response criteria differ from standard RECIST, and what advantages could we see for their use in immunotherapy trials? The first question with ipilimumab is the phenomenon that when patients are treated with immunotherapy, the tumor may enlarge before it shrinks. This is likely due to lymphocytic infiltration but is not yet fully understood. The other event that may occur after initiation of immunotherapy is the development of a new lesion. With standard RECIST, the presence of a new lesion indicates progression. In immune related response criteria, emergence of a new lesion on imaging would not indicate progression per se, unless there is a growth in the overall tumor burden. This is due to the possibility of a subclinical lesion that is not visible on initial imaging that becomes visible following immunotherapy due to increased T-cell migration and infiltration.[50] With anti-CTLA-4 therapy, this scenario of progression prior to response is fairly common, occurring 5% to 10% of the time. [51, 52] The same phenomenon is observed with anti-PD-1 antibodies although current data suggest that this occurs at a lower rate than with anti–CTLA-4. [26, 53] So whether the immune related response criteria will be as relevant in trials evaluating PD-1 blocking agents is yet to be determined. How durable are the responses to the immune therapy could be found in the FDA approval of both an anti-PD-1 (Pembrolizumab) and an anti-CTLA-4 antibody (ipilimumab) both in metastatic melanoma long-term data can be accumulated on the stability of these responses. It appears that an atypical response is often associated with a long-term response in the ipilimumab data. And this has not been addressed formally for PD-1-blockade agents. However, an atypical response is potentially a prognostic marker of durable response and a long-term outcome consistent with stable disease over time.

Adverse Events Associated With Immune Checkpoint Inhibitors

The 4 major adverse events for ipilimumab are related to inflammation. Dermatitis is fairly common but is not typically dose limiting, and it can be managed with either topical antihistamines or simply moisturizing agents. Sometimes it does require some topical steroids. It is rare to see grade 3/4 dermatitis, but it can occur. The more common and more severe adverse event with ipilimumab is colitis and requires aggressive management. It has led to mortalities in some of the early ipilimumab trials. Grade 3/4 colitis is not particularly uncommon, and it is typically treated with doses of intravenous steroids, which depend on the severity of the colitis. The other adverse events that are worrisome with anti-CTLA-4 are hepatitis and hypophysitis. Hepatitis is usually asymptomatic and typically manifests itself as a lab abnormality. Hypophysitis, inflammation of the pituitary gland, can present in a number of ways. The more common presentation is a steady onset of unexpected fatigue. This can be confounded by fatigue from the cancer so it can be difficult to differentiate and attribute it to toxicity from the checkpoint inhibitor. Hypophysitis can also present other ways, for example, severe headache with visual changes, due to

the pituitary gland's proximity to the optic chiasm. Finally, men can present with new onset of impotence; new complaints of sexual dysfunction in a patient being treated with ipilimumab and doing well otherwise may be an early sign of hypophysitis. It is also important to treat hypophysitis aggressively because if it progresses, the patient can suffer permanent deficits in the associated hormones and require lifelong replacement with adrenocorticotropic hormones, thyroid hormones, and both glucocorticoids and mineralocorticoids. These 4 major toxicities can also occur, to some extent, with anti-PD-1 and anti–PD-L1 antibodies. In general, the colitis is far less common, but hypophysitis, hepatitis, and occasionally dermatitis have been observed. In general, the magnitude of the adverse events with anti-PD-1 blockade, both nivolumab and Pembrolizumab-and even from the anti-PD-L1 agents, MPDL3280A and MEDI3476-have generally appeared to be less severe. The phase I data have been presented for both Pembrolizumab [54] and nivolumab. [47] Both of these have a grade 3/4 adverse event rate that appears to be statistically significantly lower than that of ipilimumab (10% to 15% [54, 55] vs 20% to 30%, [7] respectively). The data that are emerging from ASCO seem to indicate that the anti-PD-1 agents as well as MPDL3280A [27] and MEDI4736 [28] appear to carry a fairly low rate of grade 3/4 adverse events. So one could conclude with some degree of confidence that the overall rate of these grade 3/4 toxicities will be significantly less with the anti-PD-1 and anti-PD-L1 agents than with the anti-CTLA-4 agents. However, we still await the release and publication of larger phase III data sets to reach a definitive conclusion about the adverse event rates in a broader patient population. Although, anecdotally, it does appear that the anti-PD-1 agents are slightly better tolerated and easier to administer. The manufacturers of these agents have generated algorithms that are used on the trials and that will be available if and when these agents are approved to manage these associated toxicities. These algorithms are a very important and useful tool when administering these agents and managing their respective toxicities.

Potential Combination Strategies to Enhance Immune Checkpoint Inhibitor Activity

Although monotherapy with checkpoint inhibitors is showing activity in many different types of tumors, there appear to be other tumor types that are not responding. There may be potential strategies to stimulate the response to checkpoint inhibitors in tumors that have been either modestly or relatively not immunogenic. A potential solution therefore might be to combine checkpoint inhibition with other therapy modalities. The example of prostate cancer that we mentioned earlier, combining checkpoint inhibitors with hormonal therapy, androgen ablation, which decreases the tumor volume, may lead to a pro immunogenic release of antigens.

Another option would be to combine checkpoint blockade with a vaccine and there are some trials ongoing using this approach. [57, 58] A third approach is the combination of radiotherapy with immunotherapy. Radia-

tion therapy also leads to pro immunogenic antigen release in addition to reducing tumor burden which mitigates tolerance. There have been preclinical data to support this approach in animal models but it has not yet reached the clinical trial phase of investigation.[59] What about the trials that are planned or ongoing that are combining cancer vaccines with checkpoint inhibition. Weber and colleagues [60] presented updated data from a phase I trial combining a peptide vaccine with PD-1 blockade in melanoma. The study enrolled 90 patients with unresectable stage III or IV melanoma, all had failed at least 1 previous therapy, and they were either ipilimumab naive or ipilimumab experienced. The response rate for both ipilimumab-refractory and ipilimumab-naive patients by RECIST was 25% with a median duration of response of 14.3 months. Unfortunately, the results did not yield the hoped-for synergistic boost in response above the 20% to 30% typically seen for PD-1 blockade in melanoma. Of note, however, is that peptide vaccines are generally fairly weak compared with some of the more modern vaccine technologies. In particular, Overwijk and colleagues [61] of the University of Texas M. D. Anderson Cancer Center published data suggesting that when a standard adjuvant is used with a peptide vaccine, the protein and the antigen are concentrated in a small focal area. T cells migrate to that small focus where they may be sequestered and undergo cell death rather than trafficking through the body and attacking tumor cells. Thus peptide vaccines with a standard depot oil emulsion plus adjuvant approach may not be an effective way to test whether vaccines and checkpoint blockade combine well. However, with the recent FDA approval of Pembrolizumab and initial report of phase III data for nivolumab, [62] I think there will be many more opportunities to try more combination trials with vaccines. Earlier results of studies combining 2 checkpoint inhibitors as in the dose-escalation phase I trial by Wolchok and colleagues, [30] combining nivolumab plus ipilimumab, appear to be very effective in patients with advanced melanoma. A total of 53 patients received concurrent therapy with the 2 antibodies, and 33 received sequential therapy. Overall response rates for patients receiving ipilimumab alone were 7% and 28% for nivolumab alone vs 53% in concurrent combination group. There are trials that are further exploring this approach in advanced melanoma and other tumor types. Hammers and colleagues [63] presented very similar data in patients with metastatic RCC. The response rate to ipilimumab plus nivolumab in kidney cancer appeared to be quite a bit higher than either nivolumab or ipilimumab monotherapy. [9, 47, 64] In the melanoma trial, [30] there were a fair number of patients who responded quite rapidly, before the first assessment, which was performed at 8 weeks. In the RCC trial, [62] however, this was not the case, although it was a small cohort from a phase II trial. In addition, the toxicity in patients with RCC was at least on the same order of magnitude and frequency as that reported in the melanoma trials with grade 3/4 adverse event rates of 53% in the melanoma trial and 29% to 61%, depending on the dosing schema, in the RCC trial. This raises the question

of whether these sorts of regimens will be broadly applicable in a community setting. Of note, in the presentation by Hammers and colleagues, [63] it appears that the dose of the anti-CTLA-4 is driving the toxicity. So with the dose of anti–PD-1 nivolumab at 3 mg/kg and then the anti-CTLA-4 ipilimumab at 1 mg/kg, there is significantly less toxicity (29.0% grade 3/4) vs the opposite dosing scheme of 1 mg/kg of nivolumab plus 3 mg/kg of ipilimumab (60.9%). One way to mitigate some of the toxicity of that combination in subsequent trials might be to use the lower dose of ipilimumab. Also, the response rate was similar between the 2 dosing schedules. What Motzer and colleagues [49] published about anti-PD-1 therapy is that globally dose does not significantly affect response.

But when anti-PD-1 and anti-CTLA-4 antibodies are combined, the relative ratio of the 2 drugs can make a substantial difference in terms of toxicity. So, this is something to be explored going forward.

Use of Ipilimumab in Metastatic Castration-Resistant Prostate Cancer That Progressed After Docetaxel
Study Design and study results

The article by Kwon and colleagues [48] describes the randomized, double-blind phase III CA184-043 trial that was designed to evaluate the use of ipilimumab following radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed following chemotherapy with Docetaxel. The rationale for this study was that a ≥50% decline in prostate-specific antigen had been observed in approximately 15% of patients in earlier-phase trials using ipilimumab, which targets CTLA-4 and thereby inhibits tumor immune tolerance. [65] It had also been noted that radiation therapy may synergize with immune checkpoint inhibition via CTLA-4 and may lead to increased tumor lymphocyte infiltration and the abscopal effect, which is the systemic response to immune therapy after local tumor radiation thought to be potentially due to tumor antigen release. [59, 66-68] Approximately 800 men with castration-resistant prostate cancer who had previously progressed on Docetaxel were randomized in a 1-to-1 fashion between placebo and ipilimumab 10 mg/kg intravenously every 3 weeks for up to 4 doses, with a single 8-Gy dose of radiation therapy to at least 1 bone lesion. Patients who had at least stable disease were allowed to continue placebo or ipilimumab maintenance at 10 mg/kg every 3 months until confirmed disease progression, intolerance, or clinical decline. Approximately 28% of these men had visceral metastases, and this was evenly distributed between the 2 groups. This ended up being a negative trial by the primary endpoint of OS. The median OS was 11.2 months for ipilimumab vs 10.0 months for placebo, which closely approached statistical significance with a P value of .053. The PFS was significantly improved with the addition of ipilimumab, with an HR of 0.70 ($P < .0001$). Notably, the proportional HRs were violated and the investigators performed a post hoc analysis that demonstrated improved HRs in favor of ipilimumab when the time from initiation of therapy was

> 5 months. This post hoc analysis also demonstrated that patients with less extensive disease-that is, those without visceral metastases, those with low alkaline phosphatase (< 1.5 x ULN), and those with hemoglobin concentration ≥ 110 g/L-benefited from ipilimumab, whereas those with visceral metastases and those with more advanced disease, overall, did not. The toxicities were approximately comparable with earlier trials of ipilimumab in prostate cancer and other tumor types. [65, 69-71] In this trial, diarrhea was particularly prevalent with 16% of patients experiencing grade 3 adverse events, and 4 patients died secondary to ipilimumab-related toxicities. Although this was a negative trial, the findings of improved outcomes in a less extensive disease setting suggest that there may be a role for ipilimumab in prostate cancer, and the upcoming results of the ongoing randomized, double-blind phase III CA184-095 trial investigating ipilimumab vs placebo in men with metastatic, chemotherapy naive, castration-resistant prostate cancer are anticipated. [13, 72] The treatment arms were well balanced and the trial was representative of a common patient population with few exclusion criteria. Important to note is that the suggestion of efficacy in smaller volume disease is supported by findings in metastatic melanoma. [73] In terms of weaknesses, not all the subgroup analyses were pre specified, and thus the results that suggest improved efficacy of ipilimumab in less extensive disease are hypothesis-generating only. The rationale for the chosen dose of 10 mg/kg was not specified in this paper. Notably, the dose used in melanoma is lower, at 3 mg/kg. And, lastly, this trial did not address the role of radiation in this population in combination with ipilimumab, and potential synergies with ipilimumab were not specifically addressed.

Discussion: Disease Characteristics and Outcomes

In this trial, patients with less extensive disease appeared to benefit whereas those with more advanced disease did not. All of these patients had metastatic castration-resistant disease that had progressed on chemotherapy. They all had a good performance status-Eastern Cooperative Oncology Group 0 or 1.

Thus, they were all at a fairly similar stage in the timeline of their disease. It is to emphasize that the differences in the characteristics that came out in the subgroup analysis do not indicate earlier vs more advanced stage disease, but rather the aggressiveness of the tumors. For instance, lower hemoglobin and visceral metastases are characteristic of a more aggressive biology, perhaps even a different phenotype. My overall hypothesis is that in the setting of prostate cancer with visceral involvement, there is a systemic release of anti-inflammatory or immunosuppressive cytokines, such that the patient's microenvironment or systemic milieu is rendered anti-inflammatory or less amenable to an immune response. Specific cytokines that may be involved in this process include TGF-β, IL-8, IL-6, and tumor necrosis factor-α. My colleagues and I are in the process of analyzing the cytokines that are available from the serum samples from this trial

in order to determine if patients who had a high baseline level of these immunosuppressive cytokines were ones who failed to benefit. An important feature of this trial was that it allowed enrollment of men with visceral metastases, whereas all the other immunotherapy trials for castration-resistant prostate cancer-the trial of sipuleucel-T, [4] the ongoing trial of PROSTVAC [74] and even the ongoing CA184-095 trial of chemotherapy-naïve treatment with ipilimumab [13, 72] discussed above-did not enroll men with visceral metastases. Based on the post hoc analysis results, there are other biomarkers and patient selection factors that would be incorporated to better identify which groups of men with castration-resistant prostate cancer may benefit from ipilimumab. Excluding visceral metastases would almost certainly have led to a positive trial. But at the time the trial was designed, neither abiraterone nor enzalutamide were treatment options, hence the placebo as a comparator. Treatment options were very limited for men when the disease progressed following Docetaxel chemotherapy. One felts at the time that it was important to include men with visceral metastases, who had not been permitted to enroll on other trials in the post chemotherapy setting. However, if I had to go back, I would absolutely have excluded men with visceral metastases. The updated OS from this trial were recently presented at the 2014 European Society of Medical Oncology (ESMO) annual meeting and indicated a 3-year survival rate of 12% among ipilimumab-treated patients vs 6% among the placebo group in the intent-to-treat population with a median OS of 11.2 months and 10.0 months, respectively (HR: 0.84; 95% CI: 0.72-0.98). [75] The data that speak to that with higher patient numbers were presented by Schadendorf and colleagues [76] at the 2013 European Cancer Conference and involve a pooled analysis from 2985 patients with melanoma treated with ipilimumab in 12 retrospective and prospective phase II and III trials, observing the long-term survival. Results showed a flattening of the curve and 22% and 17% 3- and 7-year OS rates, respectively, which was quite compelling. The follow-up survival data in prostate cancer presented at ESMO demonstrate 2- and 3-year OS rates in prostate cancer of 25% and 12%, respectively. [75] These data corroborated those shown with melanoma.

Patient Selection and Immune Checkpoint Inhibitors

In terms of patient selection and tumor types beyond prostate cancer, there are clinical characteristics or biomarkers that would help us identify patients who may be more likely to benefit from CTLA-4 inhibition. It is likely that the same characteristics that predict response to anti-PD-1 and anti-PD-L1 will also help predict response to anti-CTLA-4. Tumors that are infiltrated with CD8+ cells which are dividing and producing effectors cytokines are primed to respond to checkpoint blockade. So, how can be this measured. Production of interferon-ã from the infiltrating CD8+ cells leads to up regulation of PD-L1 on the tumor cells and in the microenvironment. Hence, PD-L1 will likely be a reasonable marker for re-

sponse to CTLA-4, as well. The challenge is obtaining a biopsy that can be adequately analyzed by immunohistochemistry for these types of markers. This will need to be resolved to effectively apply these markers in clinical practice. Data have also been shown with other checkpoint inhibitors, such as nivolumab, Pembrolizumab, MEDI4736, and MPDL3280A demonstrating the predictive ability of PD-L1 expression. [27, 28, 47, 77-79] This lends further support to the idea that tumor inflammation and infiltration with CD8+ T cells holds across different checkpoint inhibitors. There are some immunochemistry and other biomarkers for anti-PD-1/anti-PD-L1 therapy that appear promising.

There are multiple competing assays available with varying levels of sensitivity and specificity due to different staining protocols and different antibodies. A biomarker that could be widely used in trials would require a more standardized assay, either developed globally to be used across multiple trials, or developed by one company to be used as a companion diagnostic. However, it is important to note that the response rate in PD-L1-negative patients is never zero. In all of the trials that have evaluated this, the response rate in PD-L1-negative tumors is in the range of approximately 10% to 15% for checkpoint inhibitor monotherapy. Going forward, having a standardized test is the only way it can be used as a predictive biomarker. And even then some of the PD-L1-negative tumors will respond to checkpoint inhibition, so how to manage this in the clinical setting is yet to be determined. The next questions are how we can better identify patients who may have an unfavorable risk-to-benefit ratio to checkpoint inhibitors, aside from a history of autoimmune disease, and as a follow-up, are there autoimmune diseases in which you would still feel comfortable administering checkpoint inhibitors. Some of the trials that have been running for a long time broadly excluded previous autoimmune disease in general. However, particularly with the PD-1 and PD-L1 agents, these checkpoint inhibitors appear to be less toxic and some autoimmune disease can be permitted. For instance, many patients have had some history of some type of psoriasis in their lifetime. And as long as this is not a severe steroid-requiring psoriasis, they will likely be able to be treated successfully. And one needs to consider the degree to which arthritis is inflammatory vs osteoarthritis. If a patient has been receiving immunomodulatory agents for arthritis, then they definitely need to be excluded. So the entry criteria for PD-1 and PD-L1 inhibitors are likely to expand going forward. And autoimmune disease may be less of a clear exclusion criterion in clinical practice.

Future Directions with Immunotherapy in Prostate Cancer

The phase III CA184-095 trial of ipilimumab in patients with chemotherapy-naive, metastatic, castration-resistant prostate cancer is currently ongoing and excludes patients with visceral metastases. [13] The initial results of this very interesting trial will be presented sometime in 2015. If the difference between success and failure in the

CA184-043 trial was the patients with the visceral metastases, then one can anticipate that the CA184-095 trial will, in fact, be positive. However, there is no way to know with absolute certainty. For instance, one drug that was felt to be very promising for prostate cancer by many investigators, cabozantinib (XL184), recently failed in a phase III trial that enrolled men with castration-resistant prostate cancer who progressed after Docetaxel as well as abiraterone and/or enzalutamide; these results have not been presented yet. So, although the inclusion of patients with visceral metastases is a good explanation for the lack of success of ipilimumab in the post chemotherapy setting, we will not know for sure until the results of the CA184-095 trial are reported. Another factor to consider with the CA184-095 trial is that it was started before the approvals of abiraterone and enzalutamide. So there is a possibility that post hoc or post treatment usage of abiraterone and enzalutamide in this setting could complicate the results of this trial. Thus prostate cancer, perhaps unlike some of the other cancers, absolutely requires a combination approach. However, despite that, there have been some case reports of prostate cancer that have had impressive responses to immunotherapy. Graff and colleagues [80] reported a complete response to sipuleucel-T in a patient treated with enzalutamide. The same group also reported a patient treated with ipilimumab who had a complete response. [81] Thus, in prostate cancer, there are a select few individuals who are likely to respond to monotherapy, but in general, the combination approach will be optimal in this tumor. Also, the radiation therapy may still be a viable approach. Moreover, the dose and schedule of radiation that is optimal to prime an immune response has yet to be elucidated. The single 8-Gy dose is one palliative regimen that is used somewhat commonly. However, there are other palliative regimens such as 6 Gy x 5, 9.5 Gy x 3. [82] Some institutions will use a single stereotactic dose of 20 Gy for select patients with oligometastatic disease. [83] So what is the exact dose of radiation that would combine optimally with blockade of either PD-1 or CTLA-4? This has been an open clinical question, and further investigation is warranted to advance the treatment of metastatic prostate cancer.

Pembrolizumab Safe and Effective in Patients with Advanced Melanoma in a Phase I Dose-Escalation Study
Study Design and results

Hamid and colleagues [20] evaluated the safety and efficacy of Pembrolizumab in a phase I dose escalation trial. In total, 135 patients with metastatic or locally advanced melanoma who had progressed following previous treatment with ipilimumab or up to 2 previous systemic regimens were administered Pembrolizumab 10 mg/kg either every 2 weeks or every 3 weeks or, for advanced melanoma, 2 mg/kg every 3 weeks. Treatment continued until disease progression, unacceptable toxicity, or withdrawn consent. Those who experienced disease progression at the first evaluation were allowed to continue treatment until confirmatory imaging was performed at least 1

month later. The rationale behind this trial was that no standard of care treatment option was available for patients with advanced melanoma who had progressed on ipilimumab, and BRAF/MEK inhibitors, if BRAF mutated. Pembrolizumab, formerly known as lambrolizumab or MK-3475, is a monoclonal IgG4ê antibody against PD-1. There was previously a dose escalation study of Pembrolizumab in multiple solid tumors to 10 mg/kg every 2 weeks that was reported separately, [84] followed by the expansion cohort of patients with advanced melanoma reported here as an open-label, multicenter phase I trial.[20] Pembrolizumab, in previous reports from the phase I trial, was well tolerated with relatively infrequent autoimmune toxicity and had impressive and durable activity in a heavily pretreated cohort of patients with metastatic melanoma. The IgG4 immunoglobulin subtype does not activate complement or bind Fc receptors, thereby avoiding cytotoxic effects on T cells. In this study, there was a 37% ORR by immune-related response criteria and 38% confirmed response rate by RECIST across all tested doses.

There was no evidence of cross-resistance between ipilimumab and Pembrolizumab as the response rates were similar in the ipilimumab-naive and ipilimumab-experienced cohorts. Responses were seen as late as 36 weeks after initiation of therapy. Drug-related adverse events of any grade occurred in 79% of patients and grade ¾ events occurred in 13%. Toxicity included rare pneumonitis (all < grade 3), rash (2% grade 3/4), diarrhea (1% grade 3/4), fatigue (1% grade 3/4), and autoimmune hepatitis. In a follow-up paper, Robert and colleagues [6] described an expansion cohort of patients with ipilimumab-refractory melanoma. In this open-label phase I trial, 173 patients with advanced melanoma who experienced disease progression after at least 2 doses of ipilimumab were randomized in a 1-to-1 ratio to receive treatment with Pembrolizumab at either 2 mg/kg every 3 weeks (n = 89) or 10 mg/kg every 3 weeks (n = 84) until disease progression, unacceptable toxicity, or withdrawal of consent. The primary endpoint was ORR assessed with RECIST by independent central review. Investigators noted an ORR of 26% at each dose level compared with 6% response rates from previous treatments of ipilimumab in the same patients. Reductions in tumor burden were seen in approximately 70% of patients at both doses. Treatment with Pembrolizumab was well tolerated, and toxicity profiles were similar between the groups. The median PFS was 22 weeks for the 2-mg/kg cohort in this study and 14 weeks for the 10-mg/kg cohort by independent central review, and were 31 weeks and 35 weeks, respectively, as assessed by the investigators using immune-related criteria. Overall survival at 1 year was 58% for the 2-mg/kg cohort and 63% for the 10-mg/kg cohort, respectively. These data from the ipilimumab-refractory expansion cohort led to FDA approval of Pembrolizumab for patients with unresectable or metastatic melanoma and disease progression following ipilimumab, and a BRAF inhibitor if BRAFV600-mutation positive. The major strength of this trial was that it was a rational expansion of the population into melanoma and then ipilimumab-refractory

melanoma. However, there was no discussion of toxicity in those who had previous ipilimumab-related toxicity, and there was no discussion of predictive biomarkers, although these will presumably be reported separately.

Discussion: Checkpoint Inhibitors and Cross-Resistance

An important finding from these studies was the activity of Pembrolizumab in patients who were previously treated with ipilimumab. There is a proven lack of cross-resistance between ipilimumab and Pembrolizumab therapy that was seen in these studies [20, 54]

This is a fascinating observation and it speaks to the notion that the negative signals mediated by PD-1/PD-L1 and CTLA-4 are actually biochemically and molecularly different. In a retrospective microarray analysis presented by Weber and colleagues, [85] purified peripheral blood CD4+ and CD8+ T cells were isolated from patients with melanoma who were treated with ipilimumab both pre-treatment and post treatment. When patients were treated with ipilimumab, the majority of the gene expression changes appeared to be in the CD4+ T-cell compartment-the helper T cells-whereas biopsies on patients treated with anti-PD-1 reveal predominately a CD8+ infiltrate. One explanation is that the biochemical pathways mediated by CTLA-4 and PD-1/PD-L1 are biochemically and molecularly distinct. The other more broad-level approach is perhaps CTLA-4 preferentially augments CD4+ T cells and PD-1 acts predominately on CD8+ T cells, which may also explain the lack of cross-resistance observed in this study. [20]

Activity in Brain Metastases

Patients who had a history of treated brain metastases were allowed to enroll on this study. The activity of immunotherapy in sanctuary sites such as the brain has not been examined comprehensively in any trial. However, there is a combination trial of ipilimumab and nivolumab in patients with recurrent glioblastoma. [86] So, it is currently an open question; however, anecdotally, more than one response has been observed in patients with brain lesions, and the traditional idea of the brain as an immunologically privileged sanctuary site is languishing. Jackson and colleagues [87] published a review about immunotherapy for glioblastoma multiforme that discusses this concept, citing reports of a limited number of native antigen-presenting cells in the central nervous system and the lack of a lymphatic system, as well as an inability of antibodies and lymphocytes to cross the blood–brain barrier. However, when there is ongoing inflammation, as in the case of a tumor, it appears that the blood-brain barrier is readily crossed by activated lymphocytes. [88, 89] Lipson and colleagues [90] also published 3 case reports, one of whom was treated initially with anti-PD-1 antibody for RCC and later developed a brain lesion which, upon surgical resection, contained no tumor cells, only fibrovascular tissue with inflammation, CD8+ macrophages, and T cells. This was deemed a resolving lesion of undetermined origin. Hence, it appears that brain lesions

Differences between PD-1 and PD-L1 Agents

Concerning the differences between therapies that target PD-1 and PD-L1 there are several different viewpoints about it. However, from a purist, evidence-based point of view, it is unfair to say there is any difference between these 2 types of agents because they have not been investigated in a head-to-head comparison trial. So unless a cross-comparison trial is performed that is adequately powered to see a difference, one should refrain from concluding that there is a difference. Alternatively, if we were to compare the phase I data from nivolumab with objective response rates ranging from 18% to 28% vs the phase I data from the anti–PD-L1 agent BMS-936559 with objective response rates ranging from 6% to 17%, it would appear that the anti-PD-1 agent nivolumab was slightly more active-albeit these are phase I data. [26, 47] However, we know that the anti-PD-L1 agents have activity. MPDL3280A has clear activity in melanoma, bladder cancer, lung cancer, and RCC. [27, 39] There are also data showing activity of MEDI-4736 in lung, colorectal, and head and neck cancers. [28] Blocking PD-1 in turn blocks the interaction of PD-1 with its 2 ligands, PD-L1 and PD-L2. So if PD-L2 is important, then blocking PD-1 is likely more beneficial. Alternatively, there is some suggestion that PD-1 interacts with B7-1, which is another co stimulatory molecule on T cells. In this case, blocking PD-L1 may be preferential so as to avoid collateral interference with other PD-1-mediated signals. One could derive reasonable immunologic rationales for blocking either pathway.

Cross-comparisons based on the current evidence in humans are unfair due to the disparate nature of the patients, the different design of phase I trials, and differences in the individual antibodies in terms of their affinity, their half-life, and the epitope they bind. Perhaps the safest thing to say right now is both PD-1 and PD-L1 blockade have activity in different cancer types and we await the phase III data.

Pembrolizumab in Practice

Pembrolizumab has been approved by the FDA as a single agent and is well tolerated. It has a high response rate so it will likely be used immediately in the on-label setting, that is, in late-stage melanoma, which has progressed following treatment with ipilimumab and, if BRAFV600 mutation positive, a BRAF inhibitor. However, owing to the wealth of data that have been published on PD-1 blockade, in multiple tumor types and in a variety of settings, there is a real concern, and among physicians a real enthusiasm, to use Pembrolizumab off label. It is yet to be seen how this enthusiasm will affect clinical practice over the next several months in terms of reimbursement policy. We know from data that we presented at ASCO [15, 35, 63] and the published data [47] that anti-PD-1 therapy has clear activity in RCC.

The toxicity associated with the anti-PD-1 drugs and the use of steroids and other immunosuppressive drugs decrease the response rate or worsen the outcome in patients who develop toxicity. There is an algorithm for each potential toxicity (eg, hepatitis, dermatitis, hypophysitis, colitis) which indicates the range of steroids that is appropriate for these patients. That is one clear management concept that should really be promulgated going forward. The big question is, if you have a patient who is having a good antitumor response to anti-PD-1 or anti-PD-L1, or even with anti-CTLA-4, who then develops a toxicity that requires treatment with a reasonably high dose of steroids, what happens to that autoimmune response? Sometimes, or perhaps, even most of the time, the antitumor immune response continues and the antitumor response is now so robust that it continues through the steroid course. One thing that needs to be done is a quantitative analysis to determine how often an antitumor response is turned off as a result of high-dose steroid treatment for autoimmune-related events. Those data will be critical for clinical practice because it can drive the decision about which scenarios clinicians can feel comfortable about administering high-dose steroids without interfering with the antitumor immune response. For instance, if the likelihood of turning off the antitumor response is exceedingly low, then when an autoimmune event, such as pneumonitis, is suspected, we can feel confident about treating it aggressively with steroids. Alternatively, if the data indicate that approximately 50% of the time, administration of high-dose steroids will turn off an antitumor immune response, then we may be more judicious in the approach. The general impression is that many of the antitumor responses proceed and that the optimal approach is to be cautious and administer steroids to try to mitigate an immune-related adverse event. However, this is not supported by hard data and it will be important to be mindful of these data as they emerge.

Pneumonitis and therapy

Concerning the management of pneumonitis the evidence suggests that pneumonitis may be more common with PD-1- blocking agents than with anti-CTLA-4 and anti-PD-L1 agents. Pneumonitis has been observed both with nivolumab and Pembrolizumab. [15, 16, 47, 84] A great challenge with pneumonitis is recognizing it, particularly when treating patients with lung cancer who already have evident lesions in their lung. Moreover, on imaging after the initiation of treatment, an antitumor immune response against a previously occult lesion can confound interpretation. Scott Antonia, MD, PhD, from the Moffitt Cancer Center, and Scott N. Gettinger, MD, from Yale University, both thoracic oncologists, have advised that in the management of patients with lung cancer, it is important to be cognizant of each patient's clinical status. Their practice is to measure patients' baseline O2 saturations, at rest and on exertion. While on checkpoint blockade therapy, if the patient experiences a decrease in exertional O2 saturation from baseline, that would be functionally worri-

some, and they suggest that this indicates the need to intervene and treat an incidental radiologic lesion with corticosteroids.

Most clinicians are uncomfortable treating asymptomatic pneumonitis during checkpoint blockade therapy. When an incidental finding appears on imaging and a patient is feeling otherwise well, it can be quite challenging to determine the appropriate management strategy going forward. However, if a lesion appears on imaging and the patient is symptomatic (eg, worsening shortness of breath or cough), all clinicians are comfortable treating a patient with symptomatic pneumonitis with appropriate, algorithm-specified, doses of steroids. The other advice received from the thoracic oncologists is to be more aggressive about imaging.

For instance, if a patient has an incidental finding on imaging but is asymptomatic and appears to be doing well otherwise on an immune checkpoint blockade agent, it is prudent to be more proactive about re-imaging more frequently, rather than waiting for the next routinely scheduled imaging visit, and if progression is observed that may indicate a pneumonitis, consider intervening at that time. Undoubtedly, pneumonitis is one of the most worrisome of all the potential toxicities with anti-PD-1 agents and also one of the more difficult to both diagnose and to manage. However, the algorithms are helpful as well as paying more careful attention to the patient's clinical status.

Combining Checkpoint Inhibitors With Other Therapies

Moving forward to further improvement of the efficacy of anti-PD-1 therapy could affect autoimmune toxicity. It is going to be a challenge. It is known from a study that combined the BRAF inhibitor with anti-CTLA-4. [91] This trial was halted early due to autoimmune toxicity, mostly hepatitis. It is a speculation that when agents that have subclinical toxicity to any organ are administered, that organ upregulates PD-L1 to protect itself from an autoimmune response. As an example, pazopanib is a tyrosine kinase inhibitor used in RCC that, by itself, has a very low rate of hepatic function abnormalities. However, when combined with an anti-PD-1 agent, as was done in a phase II trial presented at ASCO 2014 that evaluated nivolumab in combination with sunitinib or pazopanib in patients with metastatic RCC, a fairly high incidence of liver toxicity is observed. [29] In fact, the arm that contained the nivolumab and pazopanib combination was closed prematurely due to the occurrence of 4 dose-limiting toxicities. Perhaps in a fair number of patients, pazopanib induces a low-level hepatotoxicity-a low level of cellular damage, which by itself is clinically inconsequential. The body protectively upregulates PD-L1 and, as a result, prevents inflammation. However, if PD-1 is blocked with nivolumab or another anti–PD-1 antibody, quite possibly an autoimmune or immune-related process proceeds culminating in the development of clinically relevant inflammation. Autoimmune nephritis and other inflammatory toxicities have been described as well, [26, 27, 47, 84] Due to the surprisingly benign toxicity profile of all the PD-1 and PD-L1 blocking agents, investigators will be eager to combine them with existing treatments. In reality, the toxicities of the combinations of checkpoint inhibitors and other agents may well prove to be more profound than you would predict from the checkpoint inhibitors alone.

REFERENCES:

1. Medzhitov R, Janeway C Jr. Innate immune recognition: mechanisms and pathways. *Immunol Rev.* 2000 Feb;173:89-97. [PubMed] [CrossRef]

2. Masopust D, Schenkel JM. The integration of T cell migration, differentiation and function. *Nat Rev Immunol.* 2013 May;13(5):309-320. [PubMed] [CrossRef]

3. Drake CG, Lipson EJ, Brahmer, JR. Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer. *Nat Rev Clin Oncol.* 2014 Jan;11(1):24-37. [PubMed] [CrossRef]

4. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med.* 2010 Jul;363(5):411-422. [PubMed] [CrossRef]

5. Vansteenkiste J, Cho B, Vanakesa T, De Pas T, Zielinski M, Kim MS, et al. MAGRIT, a double-blind, randomized, placebocontrolled phase III study to assess the efficacy of the recMAGE-A3 + AS15 cancer immunotherapeutic as adjuvant therapy in patients with resected MAGE-A3-positive non-small cell lung cancer (NSCLC). Program and abstracts from the 2014 ESMO. Abstract 1173O.

6. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med.* 2011 Jun;364(26):2517-2526. [PubMed] [CrossRef]

7. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med.* 2010 Aug; 363(8):711-723. [PubMed] [CrossRef]

8. Lipson EJ, Sharfman WH, Drake CG, Wollner I, Taube JM, Anders RA, et al. Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. *Clin Cancer Res.* 2013 Jan;19(2):462-468. [PubMed] [CrossRef]

9. Yang JC, Hughes M, Kammula U, Royal R, Sherry RM, Topalian SL, et al. Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. *J Immunother.* 2007 Nov-Dec;30(8):825-830. [PubMed] [CrossRef]

10. Lynch TJ, Bondarenko I, Luft A, Serwatowski P, Barlesi F, Chacko R, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter

phase II study. *J Clin Oncol.* 2012 Jun;30(17):2046-2054. [PubMed] [CrossRef]

11. ClinicalTrials.gov. Randomized, multicenter, double-blind, phase 3 trial comparing the efficacy of ipilimumab in addition to paclitaxel and carboplatin versus placebo in addition to paclitaxel and carboplatin in subjects with stage IV/recurrent non small cell lung cancer (NSCLC). Available at: http://clinicaltrials.gov/ct2/show/NCT01285609

12. ClinicalTrials.gov. Randomized, multicenter, double-blind, phase 3 trial comparing the efficacy of ipilimumab plus etoposide/platinum versus etoposide/platinum in subjects with newly diagnosed extensive-stage disease small cell lung cancer (ED-SCLC). Available at: http://clinicaltrials.gov/ct2/show/NCT01450761

13. ClinicalTrials.gov. Randomized, double-blind, phase 3 trial to compare the efficacy of ipilimumab vs placebo in asymptomatic or minimally symptomatic patients with metastatic chemotherapynaive castration resistant prostate cancer. Available at: http://clinicaltrials.gov/ct2/show/NCT01057810

14. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, 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 ClinOncol.* 2010 Jul;28(19):3167-3175. [PubMed] [CrossRef]

15. Topalian SL, Sznol M, Brahmer JR, McDermott DF, Smith DC, Gettinger SN, et al. Nivolumab (anti-PD-1; BMS-936558; ONO-4538) in patients with advanced solid tumors: survival and long-term safety in a phase I trial. Program and abstracts from the 2013 ASCO, Abstract 3002.

16. Rizvi NA, Antonia SJ, Chow LQ, Brahmer JR, Juergens RA, Borghaei H, et al. A phase I study of nivolumab (anti-PD-1; BMS-936558, ONO-4538) plus platinum-based doublet chemotherapy (PT-doublet) in chemotherapy-naive nonsmall cell lung cancer (NSCLC) patients (pts). Program and abstracts from the 2013 ASCO, Abstract 8072.

17. ClinicalTrials.gov. An open-label randomized phase III trial of BMS-936558 (nivolumab) versusdocetaxel in previously treated advanced or metastatic squamous cell non-small cell lung cancer(NSCLC). Available at: http://clinicaltrials.gov/ct2/show/NCT01642004

18. ClinicalTrials.gov. An open-label randomized phase III Trial of BMS-936558 (nivolumab) versusdocetaxel in previously treated metastatic non-squamous non-small cell lung cancer (NSCLC).Available at: http://clinicaltrials.gov/ct2/show/NCT01673867 .

19. Sznol M, Kluger HM, Hodi S, et al. Survival and long-term follow-up of safety and response inpatients (pts) with advanced melanoma (MEL) in a phase I trial of nivolumab (anti-PD-1; BMS-936558; ONO-4538). Program and abstracts from the 2013 ASCO, Abstract CRA9006

20. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) inmelanoma. *N Engl J Med.* 2013; 369:134-144. [CrossRef]

21. Drake CG, McDermott DF, Sznol M, et al. Survival, safety and response duration results ofnivolumab (anti-PD-1; BMS-936558; ONO-4538) in a phase I trial in patients with previouslytreated metastatic renal cell carcinoma (mRCC): long-term patient follow-up. Program andabstracts from the 2013 ASCO, Abstract 4514.

22. ClinicalTrials.gov. A randomized, blinded, phase 2 dose-ranging study of BMS-936558 (MDX-1106) in subjects with progressive, advanced/metastatic clear-cell renal cell carcinoma who havereceived prior anti-angiogenic therapy. 2015; Available at: http://clinicaltrials.gov/ct2/show/NCT01354431

23. ClinicalTrials.gov. An exploratory study to investigate the immunomodulatory activity of variousdose levels of anti programmed-death-1 (PD-1) antibody (BMS-936558) in subjects withmetastatic clear cell renal cell carcinoma (RCC). Available at: http://clinicaltrials.gov/show/NCT01358721

24. Clinical Trials.gov. A phase 1 study of nivolumab (BMS-936558) plus sunitinib, pazopanib oripilimumab in subjects with metastatic renal cell carcinoma. 2014; Available at: http://clinicaltrials.gov/ct2/show/NCT01472081

25. ClinicalTrials.gov. A randomized, open-label, phase 3 study of nivolumab (BMS-936558) vseverolimus in subjects with advanced or metastatic clear-cell renal cell carcinoma who havereceived prior anti-angiogenic therapy. Available at:http://clinicaltrials.gov/ct2/show/NCT01668784.

26. Brahmer JR, Tykodi SS, Chow LQ, Hwu W-J, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patientswith advanced cancer. *N Engl J Med.* 2012; 366(26):2455-2465. [PubMed] [CrossRef]

27. Herbst RS, Gordon MS, Fine GD, et al. A study of MPDL3280A, an engineered PD-L1 antibody inpatients with locally advanced or metastatic tumors. Program and abstracts from the 2013 ASCO, Abstract 3000.

28. Segal NH, Antonia SJ, Brahmer JR, et al. Preliminary data from a multi-arm expansion study ofMEDI4736, an anti-PD-L1 antibody. Program and abstracts from the 2014 ASCO, Abstract 3002.

29. Amin A, Plimack ER, Infante JR, et al. Nivolumab (anti-PD-1; BMS-936558, ONO-4538) incombination with sunitinib or pazopanib in patients (pts) with metastatic renal cell carcinoma(mRCC). Program and abstracts from the 2014 ASCO. Abstract 5010.

30. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med.* 2013 Jul;369(2):122-133. [PubMed] [CrossRef]

31. Sznol M, Kluger HM, Callahan MK, et al. Survival, response duration, and activity by BRAFmutation (MT) status of nivolumab (NIVO, anti-PD-1, BMS-936558, ONO-4538) and ipilimumab(IPI) concurrent therapy in advanced melanoma (MEL). Program and abstracts from the 2014 ASCO, Abstract LBA9003.

32. Woo SR, Turnis ME, Goldberg MV, Bankoti J, Selby M, Nirschl CJ, et al. Immune inhibitory molecules LAG-3 and PD-1synergistically regulate T-cell function to promote tumoral im-

mune escape. *Cancer Res.* 2012 Feb 15;72(4):917-27. [PubMed] [CrossRef]

33. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. Targeting Tim-3 andPD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J Exp Med.* 2010 Sep 27;207(10):2187-2194. [PubMed] [CrossRef]

34. McDermott DF. Immunotherapy of metastatic renal cell carcinoma. Cancer. 2009 May 15;115(10 Suppl):2298-2305. [PubMed] [CrossRef]

35. Brahmer JR, Horn L, Gandhi L, et al. Nivolumab (anti-PD-1, BMS-936558, ONO-4538) in patients(pts) with advanced non-small-cell lung cancer (NSCLC): survival and clinical activity by subgroupanalysis. Program and abstracts from the 2014 ASCO, Abstract 8112.

36. Rizvi NA, Garon EB, Patnaik A, et al. Safety and clinical activity of MK-3475 as initial therapy inpatients with advanced non-small cell lung cancer (NSCLC). Program and abstracts from the 2014 ASCO, Abstract 8007.

37. Garon EB, Leighl NB, Rizvi NA, et al. Safety and clinical activity of MK-3475 in previously treatedpatients (pts) with non-small cell lung cancer (NSCLC). ASCO, Abstract 8020.

38. Soria JC, Cruz C, Bahleda R, et al. Clinical activity, safety and biomarkers of PD-L1 blockade innonsmall cell lung cancer (NSCLC). Program and abstracts from the 2013 European CancerCongress, Abstract 3408.

39. Powles T, Vogelzang NJ, Fine GD, et al. Inhibition of PD-L1 by MPDL3280A and clinical activityin pts with metastatic urothelial bladder cancer (UBC). 2014 ASCO, Abstract 5011.

40. Seiwert TY, Burtness B, Weiss J, et al. A phase Ib study of MK-3475 in patients with humanpapillomavirus (HPV)-associated and non-HPV–associated head and neck (H/N) cancer.Program and abstracts from the 2014 ASCO; Abstract 6011.

41. Muro K, Bang Y, Shankiaran V, et al. A phase 1b study of pembrolizumab (Pembro; MK-3475) inpatients (Pts) with advanced gastric cancer. Program and abstracts from the 2014 ESMO.Abstract LBA15.

42. Rizvi N, Chow LQ, Borghaei H, et al. Safety and response with nivolumab (anti-PD-1; BMS-936558, ONO-4538) plus erlotinib in patients (pts) with epidermal growth factor receptor mutant(EGFR MT) advanced NSCLC. Program and abstracts from the 2014 ESMO, Abstract 8022.

43. Gettinger SN, Shepherd FA, Antonia SJ, et al. First-line nivolumab (anti-PD-1; BMS-936558,ONO-4538) monotherapy in advanced NSCLC: safety, efficacy, and correlation of outcomes withPD-L1 status. Program and abstracts from the 2014 ASCO, Abstract 8024.

44. Charen, AS, Makarov V, Merghoub, et al. The neoantigen landscape underlying clinical responseto ipilimumab.ASCO, Abstract 3003.

45. Reck M, Bondarenko I, Luft, A, Serwatowski P, Barlesi F, Chacko R, et al. Ipilimumab in combination with paclitaxel and carboplatin asfirst-line therapy in extensive-disease-small-cell lung cancer: results from a randomized, doubleblind, multicenter phase 2 trial. *Ann Oncol.* 2013 Jan;24(1):75-83. [PubMed] [CrossRef]

46. Guo Z, Wang X, Cheng D, Xia Z, Luan M, Zhang S. PD-1 blockade and OX40 triggering synergistically protectsagainst tumor growth in a murine model of ovarian cancer. *PLoS One.* 2014 Feb;9(2):e89350. [PubMed] [CrossRef]

47. Topalian SL, Hodi FS, Brahmer JR. Safety, activity, and immune correlates of anti-PD-1 antibodyin cancer. *N Engl J Med.* 2012 Jun;366(26):2443-2454. [PubMed] [CrossRef]

48. Kwon ED, Drake CG, Scher HI, Fizazi K, Bossi A, van den Eertwegh AJ, et al. Ipilimumab versus placebo after radiotherapy in patientswith metastatic castration-resistant prostate cancer that had progressed after docetaxelchemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. *Lancet Oncol.* 2014 Jun;15(7):700-712. [PubMed] [CrossRef]

49. Motzer RJ, Rini BI, McDermott DF, et al. Nivolumab for metastatic renal cell carcinoma (mRCC): results of a randomized, dose-ranging phase II trial. Program and abstracts from the 2014 ASCO, Abstract 5009.

50. Ribas A, Chmielowski B, Glaspy JA. Do we need a different set of response assessment criteriafor tumor immunotherapy? *Clin Cancer Res.* 2009 Dec;15(23):7116-7118. [PubMed] [CrossRef]

51. Di Giacomo AM, Danielli R, Guidoboni M, Calabrò L, Carlucci D, Miracco C, et al. Therapeutic efficacy of ipilimumab, an anti-CTLA-4 monoclonal antibody, in patients with metastatic melanoma unresponsive to prior systemictreatments: clinical and immunological evidence from three patient cases. *Cancer Immunol Immunother.* 2009 Aug;58(8):1297-1306. [PubMed] [CrossRef]

52. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbé C, et al. Guidelines for the evaluation of immune therapy activity insolid tumors: immune-related response criteria. *Clin Cancer Res.* 2009 Dec;15(23):7412-7420. [PubMed] [CrossRef]

53. Harvey RD. Immunologic and clinical effects of targeting PD-1 in lung cancer. *Clin Pharmacol Ther.* 2014 Aug;96(2):214-223. [PubMed] [CrossRef]

54. Robert C, Ribas A, Wolchok JD, Hodi FS, Hamid O, Kefford R, et al. Anti-programmed-death-receptor-1 treatment withpembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparisoncohort of a phase 1 trial. *Lancet.* 2014 Sep;384(9948):1109-1117. [PubMed] [CrossRef]

55. Topalian SL, Sznol M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH, et al. Survival, durable remission, and long-term safety inpatients with advanced melanoma receiving nivolumab. *J ClinOncol.* 2014 Apr;32(10):1020-1030. [PubMed] [CrossRef]

56. Ipilimumab adverse reaction management guide. Available at: https://www.hcp.yervoy.com/pages/rems.aspx

57. ClinicalTrials.gov. Phase 1 study of sipuleucel-T and ipilimumab in combination for advancedprostate cancer. Available at: http://clinicaltrials.gov/ct2/show/NCT01832870

58. Clinical Trials. gov. A randomized phase 2 trial of immediate vs. delayed anti-CTLA4 blockadefollowing sipuleucel-T treatment for prostate cancer immunotherapy. Available at: http://clinicaltrials.gov/ct2/show/NCT01804465

59. Wada S, Harris TJ, Tryggestad E. Combined treatment effects of radia-

tion and immunotherapy:studies in an autochthonous prostate cancer model. *Int J Radiat Oncol Biol Phys.* 2013 Nov; 87(4):769-776. [PubMed] [CrossRef]

60. Weber, JS, Kudchadkar RR, Gibney GT, et al. Updated survival, toxicity, and biomarkers of nivolumab with/without peptide vaccine in patients naive to, or progressed on, ipilimumab (IPI). Program and abstracts from the 2014 ASCO. Abstract 3009.

61. Hailemichael Y, Dai Z, Jaffarzad N, Ye Y, Medina MA, Huang XF, et al. Persistent antigen at vaccination sites induces tumorspecificCD8+ T cell sequestration, dysfunction, and deletion. *Nat Med.* 2013 Apr;19(4):465-472. [PubMed] [CrossRef]

62. Weber J, Minor D, D'Angelo S, et al. A phase 3 randomized, open-label study of nivolumab (anti-PD-1; BMS-936558; ONO-4538) versus investigator's choice of chemotherapy in patients withadvanced melanoma after prior anti-CTLA-4 therapy. Program and abstracts from the 2014 ESMO. Abstract LBA3.

63. Hammers HJ, Plimack ER, Infante JR, et al. Phase I study of nivolumab in combination with ipilimumab in metastatic renal cell carcinoma (mRCC). Program and abstracts from the 2014ASCO. Abstract 4504.

64. Motzer RJ, Rini BI, McDermott DF, et al. Nivolumab for metastatic renal cell carcinoma (mRCC):results of a randomized, dose-ranging phase II trial. Program and abstracts from the ASCO. Abstract 5009.

65. Slovin S, Higano CS, Hamid O, Tejwani S, Harzstark A, Alumkal JJ, et al. Ipilimumab alone or in combination with radiotherapy inmetastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/IIstudy. *Ann Oncol.* 2013 Jul;24(7): 1813-1821. [PubMed] [CrossRef]

66. Harris TJ, Hipkiss EL, Borzillary S, Wada S, Grosso JF, Yen HR, et al. Radiotherapy augments the immune response toprostate cancer in a time-dependent manner. *Prostate.* 2008 Sep;68(12):1319-1329. [PubMed] [CrossRef]

67. Chakraborty M, Abrams SI, Camphausen K, Liu K, Scott T, Coleman CN, et al. Irradiation of tumor cells up-regulates Fas andenhances CTL lytic activity and CTL adoptive

immunotherapy. *J Immunol.* 2003 Jun;170(12):6338-6347. [PubMed]

68. Postow MA, Callahan MK, Barker CA, Yamada Y, Yuan J, Kitano S, et al. Immunologic correlates of the abscopal effect in apatient with melanoma. *N Engl J Med.* 2012 Mar; 366 (10):925-931. [PubMed] [CrossRef]

69. Weber JS, O'Day S, Urba W, Powderly J, Nichol G, Yellin M, et al. Phase I/II study of ipilimumab for patients with metastatic melanoma. *J Clin Oncol.* 2008 Dec;26(36):5950-5956. [PubMed] [CrossRef]

70. Lynch TJ, Bondarenko I, Luft A, Serwatowski P, Barlesi F, Chacko R, et al. Ipilimumab in combination with paclitaxel and carboplatinas first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized,double-blind, multicenter phase II study. *J Clin Oncol.* 2012 Jun;30:2046-2054.   [PubMed] [CrossRef]

71. Wolchok JD, Neyns B, Linette G, Negrier S, Lutzky J, Thomas L, et al. Ipilimumab monotherapy in patients with pretreatedadvanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. *Lancet Oncol.* 2010 Feb;11(2):155-164. [PubMed] [CrossRef]

72. Beer TM, Logothetis C, Sharma P, et al. CA184-095: a randomized, double-blind, phase III trial tocompare the efficacy of ipilimumab versus placebo in asymptomatic or minimally symptomaticpatients (pts) with metastatic chemotherapy-naive castration-resistant prostate cancer (CRPC). Program and abstracts from the 2012 ASCO. Abstract TPS4691.

73. Joseph RW, Elassaiss-Schapp J, Wolchok JD, et al. Baseline tumor size as an independentprognostic factor for overall survival in patients with metastatic melanoma treated with the anti-PD-1 monoclonal antibody MK-3475. Program and abstracts of the 2014 ASCO. Abstract 3015.

74. ClinicalTrials.gov. A randomized, double-blind, phase 3 efficacy trial of PROSTVAC-V/F +/- GMCSFin men with asymptomatic or minimally symptomatic metastatic castrate-resistant prostatecancer. Available at: http://clinicaltrials.gov/ct2/show/NCT01322490

75. Fizazi K, Drake C, Kwon E, et

al. Updated overall survival (OS) from the phase 3 trial, CA184-043: Ipilimumab (Ipi) vs placebo (Pbo) in patients with post-docetaxel metastatic castrationresistantprostate cancer (mCRPC). Program and abstracts from the 2014 ESMO. Abstract 763PD.

76. Schadendorf D, Hodi FS, Roberts C, et al. Pooled analysis of long-term survival data from phaseII and phase III trials of ipilimumab in metastatic or locally advanced, unresectable melanoma.Program and abstracts from the 2013 European Cancer Congress. Abstract LBA24

77. Grosso J, Horak CE, Inzunza D, et al. Association of tumor PD-L1 expression and immunebiomarkers with clinical activity in patients (pts) with advanced solid tumors treated with nivolumab (anti-PD-1; BMS-936558; ONO-4538). Program and abstracts from the 2013 ASCO. Abstract 3016.

78. Sznol M, Kluger HM, Callahan MK, et al. Survival, response duration, and activity by BRAF mutation (MT) status of nivolumab (NIVO, anti-PD-1, BMS-936558, ONO-4538) and ipilimumab (IPI) concurrent therapy in advanced melanoma (MEL). Program and abstracts from the 2014 ASCO. Abstract LBA9003.

79. Kefford R, Ribas A, Hamid O, et al. Clinical efficacy and correlation with tumor PD-L1 expressionin patients (pts) with melanoma (MEL) treated with the anti-PD-1 monoclonal antibody MK-3475.Program and abstracts from the 2014 ASCO. Abstract 3005.

80. Graff JN, Drake CG, Beer TM. Complete biochemical (prostate-specific antigen) response to sipuleucel-T with enzalutamide in castration-resistant prostate cancer: a case report with implications for future research. *Urology.* 2013 Feb;81(2):381-383. [PubMed] [CrossRef]

81. Graff JN, Puri S, Bifulco CB, Fox BA, Beer TM. Sustained complete response to CTLA-4blockade in a patient with metastatic, castration-resistant prostate cancer. *Cancer Immunol Res.* 2014 May;2(5):399-403. [PubMed] [CrossRef]

82. Hartsell WF, Scott CB, Bruner DW, Scarantino CW, Ivker RA, Roach M 3rd, et al. Randomized trial of short-versus long-courseradiotherapy for palliation of painful bone metastases. *J*

Natl Cancer Inst. 2005 Jun;97(11):798-804. [PubMed]

83. Ahmed KA, Barney BM, Davis BJ, Park SS, Kwon ED, Olivier KR. Stereotactic body radiationtherapy in the treatment of oligometastatic prostate cancer. *Front Oncol.* 2012 Jan; 2:215. [PubMed] [CrossRef]

84. Patnaik A, Kang SP, Tolcher AW, et al. Phase I study of MK-3475 (anti-PD-1 monoclonalantibody) in patients with advanced solid tumors. Program and abstracts from the 2012 ASCO. Abstract 2512.

85. Weber JS, Yu B, Hall D, et al. Pharmacodynamic and predictive markers of ipilimumab onmelanoma patients' T-cells. Program and abstracts from the 2011 Annual Meeting of theAmerican Society of Clinical Oncology. June 3-6, 2011; Chicago, Illinois.

Abstract 2503.

86. Clinical Trials.gov. A randomized phase 3 open label study of nivolumab versus bevacizumab anda safety study of nivolumab or nivolumab in combination with ipilimumab in adult subjects withrecurrent glioblastoma (GBM). Available at: http://www.clinicaltrials.gov/ct2/show/NCT02017717

87. Jackson C, Ruzevick J, Phallen J, Belcaid Z, Lim M. Challenges in immunotherapy presented bythe glioblastoma multiforme microenvironment. *Clin Dev Immunol.* 2011; 2011:732413. [PubMed] [CrossRef]

88. Pachter JS, De Vries HE, Fabry Z. The blood-brain barrier and its role in immune privilege in thecentral nervous system. *J Neuropath Exper Neurol.*

2003 Jun;62(6):593-604. [PubMed]

89. Fabry Z, Raine CS, Hart MN. Nervous tissue as an immune compartment: the dialect of theimmune response in the CNS. *Immunol Today.* 1994 May;15(5):218-224. [PubMed] [CrossRef]

90. Lipson EJ, Sharfman WH, Drake CG, Wollner I, Taube JM, Anders RA, et al. Durable cancer regression off-treatment and effectivereinduction therapy with an anti-PD-1 antibody. *Clin Cancer Res.* 2013 Jan;19(2):462-468. [PubMed] [CrossRef]

91. Ribas A, Hodi FS, Callahan M, Konto C, Wolchok J.. Hepatotoxicity with combination of vemurafenib andipilimumab. *N Engl J Med.* 2013 Apr;368(14):1365-1366. [PubMed] [CrossRef]

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