Immunomodulation: checkpoint blockade etc.

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The immune microenvironment is considered a major obstacle to generating an effective antitumor immune response. Checkpoint inhibitors manipulate the co-stimulatory response between antigen-presenting cells and immune cells—or between the tumor and immune cells—to elicit an antitumor immune response that would have otherwise been suppressed. Checkpoint inhibitors have shown great promise in the clinics, and some inhibitors such as anti-CTLA-4 antibodies and anti-PD-1 antibodies have gained FDA approval for certain tumors. Here we will discuss the current state of checkpoint inhibitors, biomarker strategies, and management of associated toxicities in glioblastoma.

Keywords: biomarker, checkpoint blockade, clinical trial, glioblastoma, immunotherapy.

On June 1, 2015, the lead story on CNN.com proclaimed “New cancer treatment hailed...” in response to results simultaneously presented at the American Society of Clinical Oncology (ASCO) Annual Meeting and published in the New England Journal of Medicine demonstrating that combining nivolumab (an inhibitor of programmed cell death protein 1 [PD-1]) and ipilimumab (an inhibitor of cytotoxic T-lymphocyte-associated protein 4 [CTLA-4]) improved progression-free survival (PFS) in patients with stage 3 and 4 melanoma to 11.5 months as compared with 2.9 months in patients treated with ipilimumab alone and 6.9 months in patients treated with nivolumab as monotherapy.1 In the same issue of the New England Journal of Medicine, researchers showed improvement in overall survival (OS) for squamous cell lung cancer patients when treated with nivolumab versus docetaxel in a randomized clinical study.2 These phase 3 clinical studies and the accompanying press are the most recent entries documenting the notable advancement of immune checkpoint inhibition as cancer immunotherapy over the past 5 years, beginning with the demonstration in 2010 that CTLA-4 blockade with ipilimumab yielded OS of 10 months in patients with advanced melanoma, which was a significant improvement when compared with patients in the control arms.3 On the basis of this study, ipilimumab gained FDA approval for patients with advanced unresectable melanoma in August 2011, and the clinical development of immune checkpoint inhibitors as cancer therapeutics has continued to accelerate.

“Immune checkpoint inhibition” refers to the manipulation of interactions that most often occur at the immune synapses where antigen-presenting cells bind to and influence the behavior of T-lymphocytes. The generation of a clonal and proliferative antigen-specific immune response requires interaction of antigen-bearing major histocompatibility complex (MHC) molecules with the T cell receptor (TCR). It is well established that MHC-TCR ligation alone is insufficient for establishing protective immunity, which requires simultaneous co-stimulation by CD80 and CD86 (also known as B7-1 and B7-2) on the antigen-presenting side with CD28 expressed on the T cell surface. T-lymphocyte activation ensues, and in temporally delayed fashion, there is upregulation of checkpoint molecules on the T cell side of the immune synapse, the binding of which serves to either enhance T cell proliferation and activation (eg, 4-1BB or OX40) or inhibit the activated clonal response (Fig. 1). The latter, which includes CTLA-4 and PD-1, serves as negative immune regulators, which are part of normal immune homeostasis. For instance, genetic diseases characterized by CTLA-4 deficiency are associated with autoimmunity and lymphoproliferation—hence, the concept that functional ligation of CTLA-4 by CD80 and CD86 “applies the brakes” to the normal immune response (a process that may be required to balance immune surveillance of the environment but may be disadvantageous when taking on cancer). CTLA-4 expression is induced by the initial activation of effector T-lymphocytes, particularly CD4+ cells, and is constitutively expressed on immunosuppressive regulatory T-lymphocytes (Tregs). CTLA-4 binds CD80 and CD86 on antigen-presenting cells with roughly 10 times greater affinity than does CD284,5 and thereby outcompetes it at the immune synapse. The mechanisms by which CTLA-4 upregulation...
inhibit antigen-specific responses are not fully understood but likely involve ligand competition, both extracellularly and intercellularly, by effector and regulatory T lymphocytes. Suppression of T cell responses through CTLA-4 is most pertinent at sites wherein antigen-presenting cells stimulate cellular responses, such as in lymph nodes.

PD-1 was identified as a negative regulator of lymphocyte activation in 2000 by Gordon Freeman et al. Like CTLA-4, PD-1 is expressed by activated CD4+ and CD8+ T-lymphocytes after MHC-TCR engagement and CD80/86-CD28 co-stimulation. PD-1 is bound by PD-Ligand 1 (PD-L1) or PD-Ligand 2 (PD-L2), which are expressed on a variety of cell types including cancer. PD-L1/L2–PD-1 interactions predominate in the periphery (eg, in the tumor microenvironment after T cell activation has occurred in lymph nodes). The complementary, but distinct, actions between CTLA-4 and PD-1 set up potential synergies for combination treatment, as seen in the recent nivolumab/ipilimumab study for patients with melanoma.

Translation to Oncology Clinic

Seminal preclinical work by Leach, et al in 1996 demonstrated that antibody blockade of CTLA-4 enhanced antitumor immunity in melanoma-bearing mice. In subsequent years as PD-1 was identified, preclinical studies confirmed the potential anticancer immunotherapeutic benefit of blocking either PD-1 or PD-L1. With the creation of a humanized anti-CTLA-4 monoclonal antibody by Medarex, Inc., cancer clinical studies began and led to the landmark 2010 phase 3 ipilimumab study by Hodi, et al. A full review of immune checkpoint clinical trials is beyond the scope of this manuscript, but a few consistent themes have emerged. CTLA-4, PD-1, and PD-L1 inhibition all seem to have activity against melanoma and other cancers, although not all patients respond. In fact, recent genomic analysis of colorectal cancer patients treated with PD-1 inhibition via pembrolizumab demonstrated that patients with mismatch repair-deficient tumors had significantly better clinical responses than patients with mismatch-repair proficient tumors, most likely because of much higher somatic mutation loads. Tumor expression of PD-L1 is associated with more frequent antitumor activity blocking the PD-1/PD-L1 pathway, but this is not requisite. For patients with melanoma, PD-1 inhibition with pembrolizumab is more effective than CTLA-4 blockade with ipilimumab as measured by 6-month PFS (46%–26%), 12-month survival (74%–58%), and response rate (33%–12%). In addition, consistent with previous studies, anti-PD-1 immunotherapy is milder and has less high-grade toxicity than CTLA-4 blockade. Finally, as mentioned at the outset of this article, combination therapy with nivolumab (anti-PD-1) and
iplimumab is highly efficacious against melanoma, albeit with high rates of toxicity (55%).

Immune Checkpoints and Glioma

With these clinical successes in melanoma, lung cancer, and other solid malignancies, immune checkpoint manipulation is now being examined in patients with glioblastoma. A number of preclinical studies provide specific rationale for using checkpoint inhibitors against glial tumors. Glioblastomas, particularly those with PTEN loss and resulting PI3kinase activation, are known to drive local immunoresistance, partially by expression of PD-L1. In fairly immunoresistant tumor models, investigators have demonstrated activity against intracranial gliomas via CTLA-4 blockade both alone and in combination with vaccination. Similarly, the combination of anti-PD-1 blockade and stereotactic radiation produces long-term survival in mice with intracranial gliomas.

As of this writing, the 3 clinical studies below are enrolling patients with glioblastoma.

NCT02017717

A Randomized phase 3 Open-Label Study of Nivolumab versus Bevacizumab and Multiple Phase I Safety Cohorts of Nivolumab or Nivolumab in Combination with Ipiilimumab Across Different Lines of Glioblastoma.

This large international multicenter and multipurpose clinical trial is sponsored by Bristol-Meyers Squibb and examines the efficacy and safety of its 2 significant immune checkpoint inhibitors in participants with recurrent glioblastoma.

The purpose of this study is to compare the efficacy and safety of nivolumab alone versus treatment with bevacizumab alone in participants with recurrent glioblastoma and to evaluate the safety of nivolumab alone versus the combination of nivolumab and ipilimumab. Participants are being enrolled into 2 main cohorts, the first of which is principally set up to measure safety and tolerability of the interventions, and the second of which is to understand OS in recurrent glioblastoma patients treated with nivolumab versus bevacizumab. Built in is a randomized phase 3 analysis of nivolumab versus bevacizumab in participants with recurrent glioblastoma. Also, building on the demonstrated efficacy of nivolumab in combination with ipilimumab in patients with advanced melanoma, the safety and tolerability of this checkpoint combination has been examined in recurrent glioblastoma participants at any point of recurrence that is not limited to first evidence of progression.

Preliminary results from cohort 1 have been presented at ASCO 2015. In participants with recurrent glioblastoma, nivolumab monotherapy at 3 mg/kg every 2 weeks was well tolerated without treatment-related grade 3–4 adverse events. When nivolumab 1 mg/kg was combined with ipilimumab 3 mg/kg at 3-week intervals, significant toxicity ensued, with 4 of 10 participants discontinuing treatment because of adverse events prior to completing 4 doses. One confirmed partial response was observed in the nivolumab arm as well as 2 cases of pseudoprogression.

The study has now moved forward to the Phase 3 portion examining nivolumab monotherapy (3 mg/kg) with bevacizumab in participants with recurrent glioblastoma. The estimated primary completion date is June 2017.

NCT02337491


This single center randomized phase 2 study opened at the Dana-Farber Cancer Institute in February 2015 and randomizes glioblastoma participants at first and second recurrence to Merck’s PD-1 inhibitor, pembrolizumab, or to pembrolizumab in combination with bevacizumab. The primary outcome measure is 6-month progression-free survival (PFS-6).

NCT02336166

Phase 2 Study of MED14736 in Patients with Glioblastoma.

This is an open-label, nonrandomized multicenter phase 2 study examining the activity of this PD-L1 inhibitor in 3 cohorts of participants with glioblastoma: (i) newly diagnosed patients (cohort A) in combination with the standard of care; (ii) as monotherapy for bevacizumab-naïve patients (cohort B) with recurrent glioblastoma; and (iii) in combination with bevacizumab in recurrent glioblastoma patients (cohort C) who have been bevacizumab refractory. Primary outcome measures differ slightly for each of the 3 cohorts. For newly diagnosed glioblastoma patients, the primary endpoint is the OS rate at 12 months. In cohort B, the primary endpoint is the PFS-6, and in cohort C, the primary endpoint is OS at 6 months.

Biomarkers

Certain subsets of cancer patients respond to checkpoint inhibitors, while others are at higher risks for toxic side effects. Identification of biomarkers that can predict these subsets is critical to maximizing the benefits of checkpoint inhibitors. An ideal biomarker would be highly sensitive and specific in predicting response, resistance, and safety. General strategies for identifying potential biomarkers include understanding the mechanism(s) of action and pharmacodynamics of the checkpoint inhibitors and measuring the markers of inflammation.

Current techniques to identify candidate biomarkers include quantifying expression of checkpoint molecules, evaluating immune cell activation status, and assessing genetic mutations. The most common approaches to measuring protein expression of checkpoint molecules are immunohistochemistry (IHC) or flow cytometry. IHC is appealing because the assay can be performed on paraffin-embedded tissues. Issues such as technique and antigen reactivity can affect the sensitivity and specificity of this assay. Flow cytometry, on the other hand, is better at quantifying the number of positive cells compared with IHC, but the technique requires fresh tissue. One of the most established biomarkers for checkpoint inhibitors, PD-L1 expression on the tumor, is more commonly measured by IHC. Assessment for PD-L1 expression through the use of IHC appears to be a promising predictive biomarker. As an example, the overall response rate (ORR) to anti-PD-1 therapy directly correlated to PD-L1 positivity in melanoma. Interestingly, PD-L1 expression is not predictive of ORR in patients who were treated with concurrent ipilimumab (anti-CTLA antibody).
and nivolumab (anti-PD-1 antibody). What is more remarkable is if participants were given sequential sequenced therapy, (ie, give nivolumab after ipilimumab), the ORR to anti-PD-1 therapy was again predicted by PD-L1 status. While this finding is important for patient care, it also raises the possibility that there are separate mechanisms of action with anti-PD-1 therapy when it is given alone versus given in combination. The correlation with anti-PD-L1 therapy and PD-L1 tumor expression appears to hold with other solid tumor types such as non-small cell lung cancer (NSCLC), renal, colon, colorectal, and prostate cancer.

Assessing the activation status of the immune system is another approach to measuring response to checkpoint molecules. Strategies can be divided into quantifying the immune cell populations or assessing the activation status of the immune cells. Grossman et al have suggested that lymphocyte counts alone are predictive of prognosis, with lower counts correlating with shorter survival in patients with GBM. Thus far, absolute lymphocyte counts have not been predictive of response rates in checkpoint trials. Immune cell activation status has also been proposed as a biomarker for checkpoint inhibitor activity. In general, activation status is quantified by measuring cytokine expression, proliferative capacity of immune cells, or activation markers of immune cells. As an example, assessment of CD8+ T cell activation status defined by eomesodermin (EOMES) positivity was predictive of relapse-free survival in patients treated with ipilimumab. Other markers of immune cell activation include expression of Helios and ICOS in Tregs, PD-L1, PD-L2, LAG-3, and TIM-3, and PD-1 expression in lymphocytes. Of note, the checkpoint molecules themselves can be considered biomarkers.

Another exciting biomarker strategy involves measuring DNA-based markers. Approaches have included quantification of DNA mutations shed by tumor in the plasma, while other groups have measured the number of mutations in the tumors themselves. As an example, Lipson et al assessed circulating tumor DNA in melanoma patients undergoing checkpoint blockade therapies as a marker for tumor burden. Patient plasma samples were assessed for hotspot mutations in BRAF, CKIT, NRAS, and TERT. They found that circulating tumor DNA levels correlated with tumor progression both radiographically and clinically. Another approach has been to quantify the mutation burden tumors. A recent study by Rizvi et al demonstrated that a higher nonsynonymous mutational burden of NSCLCs correlated with PFS in patients treated with anti-PD-1 therapy. TCR repertoires are also another area of intense interest. Recently, a group found that an increased number of TCR repertoires correlated with an increased immune response in patients who were treated with radiation, anti-CTLA-4 therapy, and anti-PD-L1.

While there is much excitement about biomarkers, this should be tempered by the knowledge that tumors are often heterogeneous, both at the population level and intrinsically. As a result, immune responses may be heterogeneous within a tumor type. In addition, we must also remember that the readouts of the biomarkers are often not binary but rather are based on thresholds that are constantly being redefined. Hence, it is not likely that we will find only one biomarker for each immunotherapy that will be adequately predictive to guide clinical practice. Rather, we will require multiple complementary biomarkers to identify patients who will respond.

Toxicity

While checkpoint blockade has shown great potential for tumor control, inhibiting mechanisms that normally function to protect healthy tissues from immune attack carry an inherent risk of significant toxicity. In theory, releasing the brakes on the immune system could make treated individuals more prone to autoimmune reactions. The immune-related toxicities from these drugs can cause significant morbidity and, in some cases, cause mortality if unrecognized. As such, the clinician should have a low threshold for working up symptoms. Oftentimes, the diagnosis of drug-related toxicities is made by exclusion of other causes such as tumor progression, infectious etiologies, and other drugs.

When working up suspected immune-related toxicities, it is important to remember that toxicities can affect different organs, are time dependent, and are drug specific. In assessing a potential adverse event in a patient being treated with checkpoint inhibitors, it is critical to recognize that autoimmune reactions can affect any organ. Common examples of immune-related toxicities are colitis, hypophysitis, thyroiditis, dermatitis, pancreatitis, pneumonitis, hepatitis, arthritis, nephritis, and neuritis. In making the diagnosis, timing often plays a key role in establishing a link to the various checkpoint inhibitors. As an example, colitis and diarrhea can start any time after 4–10 weeks from the first infusion. A rash, however, can start 3 weeks after infusion. Interestingly, hepatitis or hypophysitis typically starts later (about 7 weeks after infusion). Furthermore, it is important to note that once patients present with symptoms, these symptoms can crescendo quickly, so it is in the best interest of the patient to proceed rapidly with workup and intervention. In addition, the characteristics of the specific agents should be considered in the workup and treatment of toxicities. As an example, in comparing the colitis rates from the large trials using anti-CTLA-4 versus the trials using anti-PD-1 therapies, there is a higher rate of colitis in the anti-CTLA-4 trials. Finally, there appears to be a correlation of toxicities to the dose of the checkpoint molecules. As an example, higher doses of ipilimumab have resulted in higher rates of colitis in patients.

The treatment algorithm for patients with side effects is based on the Common Terminology Criteria for Adverse Events (CTCAE) grading system. General principles for effective management of toxicities are to recognize symptoms early by having a low threshold for working up symptoms, having regular monitoring intervals, using corticosteroids when necessary, and assembling a multidisciplinary team. In general, toxicities are assigned on a scale of 1 to 4. For example, the CTCAE grades the toxicity of colitis based on either number of diarrhea episodes per day or colitis symptoms. Patients with grade 1 toxicities usually continue the checkpoint inhibitor with symptom management, grade 2 toxicities are generally treated by delaying the checkpoint inhibitors and management of symptoms, and grade 3 or 4 toxicities are treated by discontinuing the checkpoint inhibitors and starting corticosteroids. In cases of severe side effects that are refractory to steroids, infliximab
has been used. Workup usually entails the aid of a specialist. In the example of colitis, a gastroenterologist is often involved, and he/she may need to perform endoscopy as part of the workup. As such, it is best to have a team of specialists who are readily available and familiar with the range of toxicities seen with checkpoint inhibitors. This approach ensures consistency in diagnosis and treatment of these toxicities for patients.

**Future Directions**

While studies of immune checkpoint molecules have yielded exciting results preclinically and in clinical trials with some agents already gaining FDA approval, the next advancements are likely to come with combination strategies. While dual PD-1 and CTLA-4 blockade studies have already demonstrated synergy, there are many exciting combination strategies yet to be tested. Furthermore, combination strategies using checkpoint molecules with other modalities such as radiation, chemotherapy, and other immunotherapy strategies have thus far been promising. One eminent challenge is integrating immune checkpoint therapy with current standard-of-care therapies such as steroids, chemotherapy, and radiation. This is one of many areas of active investigation that will define the role of immune checkpoint blockade in cancer treatment.

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**References**


