Editorial

What does the future hold for immunotherapy in cancer?

Jarushka Naidoo[1], Bob T. Li[2], Katja Schindler[3], David B. Page[4]

[1]Upper Aerodigestive Division, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD 21224, USA; [2]Thoracic Oncology Service, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA; [3]Department of Dermatology, Division of General Dermatology and Dermatooncology, Medical University of Vienna, Vienna, Austria; [4]Providence Portland Medical Center, Earl A. Chiles Research Institute, Portland, OR 97213, USA

Correspondence to: Jarushka Naidoo, MB, BCH. Johns Hopkins Bayview, 301 Building Suite 4500, 4940 Eastern Avenue, Baltimore, MD 21224, USA. Email: jnaidoo1@jhmi.edu.

Submitted Mar 16, 2016. Accepted for publication Mar 22, 2016.
doi: 10.21037/atm.2016.04.05
View this article at: http://dx.doi.org/10.21037/atm.2016.04.05

Introduction

Cancer Immunotherapy—a therapeutic strategy of harnessing the immune system to recognize and clear cancer—was named by *Science* journal as 'Breakthrough of the Year' in 2013 (1). This was largely attributable to clinical successes in metastatic melanoma and lung cancer, whereby a class of immunotherapy called immune checkpoint antibodies demonstrated an improvement in survival outcomes compared to standard therapy. Since then, three immune checkpoint antibodies have been approved by the Food and Drug Association (FDA) and other regulatory agencies for the treatment of both metastatic melanoma and non-small cell lung cancer (NSCLC). These include ipilimumab—an antibody against cytotoxic T-lymphocyte antigen 4 (CTLA-4), as well as nivolumab and pembrolizumab—antibodies against programmed-death 1 (PD-1). There are now a number of other antibodies in development against both PD-1 and it's ligand, PD-L1, including pidilizumab, durvalumab, avelumab and atezolizumab, as well as antibodies against other immune checkpoint targets such as lymphocyte activation gene-3 (LAG-3), T-cell immunoglobulin mucin protein-3 (TIM-3), glucocorticoid-induced TNFR family related gene (GITR) and CD-137 (2,3). In addition to being clinically effective in melanoma and NSCSC, anti-PD-1/PD-L1 antibodies have demonstrated preliminary efficacy across a number of solid tumors, including: renal cell carcinoma (RCC) (4,5), urothelial carcinoma (6), hepatocellular carcinoma (HCC) (7), head and neck carcinoma (8), and mismatch-repair deficient colorectal cancer (CRC) (9); as well as hematologic malignancies including chronic lymphocytic leukemia with Richter's transformation (10) and Hodgkins lymphoma (11). Because of these successes, immune checkpoint antibodies are becoming incorporated into the standard treatment paradigm for a variety of cancers, alongside conventional therapies such as radiation, surgery and chemotherapy.

Immune checkpoint therapy is fundamentally distinct from traditional anti-cancer therapies and so-called "targeted" therapies, in that these agents modulate the host immune response, rather than directly targeting the aberrant or mutated features of tumor cells. With this in mind, Sharma and colleagues (12) published a position paper on the future of immune checkpoint therapy. Herein, we highlight the main elements of this article, including a summary of the current knowledge of how the immune system interacts with tumor cells in the tumor microenvironment (TME), the clinical development of immune checkpoint antibodies to date, and future directions with regard to the next wave of clinical studies and advances in both tissue-based and circulating biomarkers.

Activation of T-cells and the tumor microenvironment (TME)

Sharma and colleagues assert that two factors are central to the successful achievement of an immunologic anti-tumor response: activation of T-cells, and functional anti-tumor activity of T-cells in the TME. T-cells are the workhorses of the adaptive immune system, and have three unique capabilities that make them promising anti-cancer

agents. First, T-cells can be specific to an individual's tumor, in that they identify tumor-associated proteins called 'antigens' via cell-surface interactions of the T-cell receptor with major histocompatibility complex (MHC) molecules. Second, T-cells are capable of mediating long-lasting immune responses via a process called "immunologic memory": after an initial T-cell response is generated, the adaptive immune system produces long-lasting memory T-cell populations that circulate in the blood, and are capable of mounting efficient and sustained anti-tumor immune responses when re-exposed to the same antigen in the future. Because of this, anti-tumor immunity can be life-long, which is consistent with observations that clinical responses to immune checkpoint therapy can be durable. Finally, T-cell responses can evolve and improve over time, with new responses being mounted by T-cells in the face of intra-tumor heterogeneity or tumor clonal evolution. This adaptability of the immune response is mediated largely by the inherent vastness of antigen diversity and subsequent T-cell responses, as well as a process called "epitope spreading", whereby an initial immune response against a tumor-associated antigen may ultimately spread to epitopes distinct from the original or dominant epitope, leading to further immune responses against other tumor-associated antigens originating from the same tumor (13).

A variety of biologic hallmarks of cancer may ultimately lead to the generation of antigens that are capable of facilitating anti-tumor immune responses. For example, some cancers are mediated by viral infection (for example, HPV-associated malignancies), and may produce virally-associated proteins that serve as tumor antigens. Other cancers are associated with tumor-specific differentiation antigens (such as proteins involved in melatonin production), fetal proteins (such as CEA in colon cancer) or cancer-tests (CT) antigens, which are expressed due to epigenetic dysregulation (such as NY-ESO-1). Importantly, cancers also generate tumor-specific peptides through somatic mutations that result in the production of mutation-associated-neoantigens, which can bind to MHC molecules and therefore be recognized by an individual's immune system (14,15). Studies assessing the epitope landscape of breast and colon carcinoma have demonstrated that neoantigens produced as a result of the activity of a selected number of mutant genes, have binding potential to HLA-A*0201. Since an individual may have up to 6 HLA class I genes, this equates to between 42 and 60 neoantigens that may be presented to T-cells.

Development of Immunotherapy: from vaccines to immune checkpoint antibodies

For many years, cancer immunotherapy research was focused on developing an anti-tumor vaccine against shared tumor antigens, such as gp100 for melanoma. These early trials had limited success in the clinic, in part attributable to a lack of understanding of the complexity of T-cell activation, inappropriate antigen selection, and a lack of appropriate co-stimulation (16). In the last 20 years, however, it has become known that successful T-cell activation, in addition to requiring antigen in the context of MHC, also requires a 'second signal' of activation. This second signal is generated by the interaction between B7 molecules (B7-1 and B7-2) on a tumor cell with CD-28 on a T-cell (17). It was later shown that, even if tumors lacked B7 molecules, they could still be targeted for immune destruction by an alternative T-cell activation pathway, in a process called cross-priming. During cross-priming, professional antigen-presenting cells (APCs) engulf tumor or tumor particles by phagocytosis, and then subsequently present tumor antigens to T-cells via MHC, in conjunction with the B7 co-stimulatory molecules, ultimately leading to T-cell activation. Once T-cells are activated through cross-priming, this generates a cascade of effects resulting in T-cell proliferation and functional differentiation.

In response to these discoveries, pioneers of anti-cancer vaccines began to incorporate antigen sources together with APCs and/or dendritic cells (DCs), in an effort to enhance co-stimulation via the cross-priming mechanism. From here, it also became clear that, converse to the B7/CD28 co-stimulatory pathway, a co-inhibitory signal is also generated which is capable of abrogating T-cell activation. This co-inhibitory signal is mediated by a related molecule called CTLA-4 (18). These findings were confirmed in studies of CTLA-4 knockout mice, where a rapid and lethal lymphadenopathy was demonstrated, thus indicating the ability of 'unleashed' T-cells to destroy normal tissue (19,20). Based on these findings, cancer immunologists began exploring the possibility that CTLA-4 and other checkpoint molecules could be targeted therapeutically.

Preclinical development of immune checkpoint antibodies

Murine models provided proof-of-concept that inhibiting CTLA-4 with an antibody could lead to tumor regression and long-standing anti-tumor immunity. In these models, anti-CTLA-4 therapy was associated with an increase in the

ratio of effector T-cells to regulatory T-cells (21). Since this therapy was effective pre-clinically across multiple tumor types and modified a subject's immune cells rather than tumor cells, it was postulated that the clinical benefits of anti-CTLA-4 antibodies may not be specific to a cancer's histologic subtype or genetic abnormality. Moreover, it was demonstrated that CTLA-4 blockade could be potentially synergistic with the effects of tumor vaccines, cryoablation (22), oncolytic viruses (23), and other agents aimed at enhancing other methods of T-cell activation in mouse models (24,25). These preclinical studies supported the clinical development of anti-CTLA-4 therapy, and spurred the development of a class of agents whose mode of action has been collectively termed, 'immune checkpoint blockade', with CTLA-4 classified as the first co-inhibitory immune checkpoint antibody.

A number of additional immune checkpoint molecules have been described (26), including another co-inhibitory T-cell checkpoint molecule called PD-1 (27). Similar to CTLA-4, PD-1 signaling may inhibit T-cell activation upon binding of PD-1 to either of two ligands, PD-L1 and PD-L2. PD-1 is expressed on T-cells that have been chronically exposed to antigen, whereas CTLA-4 is expressed on T-cells that have been acutely exposed to antigen. Furthermore, the PD-L1/2 ligands are expressed in a variety of cell types within the TME, including tumor cells that have been exposed to interferon-gamma, immune cells and epithelial cells (3). Similar to CTLA-4, PD-1 blockade with a therapeutic antibody has been effective in a variety of pre-clinical models. Furthermore, because of the unique roles of these two molecules, combination therapy with anti-CTLA-4 plus anti-PD-1 has been demonstrated to be synergistic in some models (28-30).

Clinical development of immune checkpoint antibodies

Currently, two anti-CTLA-4 therapeutic antibodies have been clinically developed: ipilimumab (Bristol Myers-Squibb LLC, NJ) and tremelimumab (MedImmune LLC, Gaithersburg, MD). Ipilimumab (3 mg/kg) was initially studied in a phase I trial in metastatic melanoma, with 2 of 17 patients enrolled in the study demonstrating durable partial responses (31). Ipilimumab was subsequently evaluated in a double-blind phase II trial at three dose levels of 0.3, 3, or 10 mg/kg for four doses administered every 21 days, followed by maintenance dosing every 3 months. The objective response rate (ORR) was 11%, with a median overall survival (OS) of 14 months in the 10 mg/kg cohort (32). Ipilimumab was then investigated in a randomized phase III trial (3 mg/kg) in metastatic melanoma, comparing gp100 peptide vaccine alone, versus ipilimumab alone, versus the combination (33). The ipilimumab and ipilimumab/gp100 groups improved survival compared with the gp100 vaccine alone (10.0 *vs.* 10.1 *vs.* 6.4 months, P<0.001). In a second phase III study, the combination of ipilimumab (10 mg/kg) and dacarbazine with maintenance 3-monthly ipilimumab was compared to dacarbazine plus placebo followed by maintenance placebo every 12 weeks, and demonstrated a 2-month improvement in median OS in favor of the ipilimumab combination (34). In addition, this study demonstrated a prolonged duration of response at 19.3 months with the ipilimumab combination. These clinical findings further substantiated the preclinical hypothesis that long-lived immunity may translate into a prolonged anti-tumor effect in selected patients with the use of immune checkpoint blockade. Ipilimumab was subsequently investigated in other solid tumors such as prostate cancer, RCC and NSCLC (35-37).

Tremelimumab (15 mg/kg every 3 months) was the second human immunoglobulin G2 (IgG2) monoclonal antibody directed against CTLA-4 to be clinically investigated (38). This agent was studied in a phase III trial in metastatic melanoma and compared to investigator's choice chemotherapy. In this trial, a prolonged median duration of response of 36 months was seen with tremelimumab compared with combination chemotherapy at 14 months (P=0.0011) (39). The lack of OS benefit demonstrated in this study may be attributable to subsequent ipilimumab treatment in the control arm, or either inadequate dose or schedule of therapy.

The first anti-PD-1 antibodies to be clinically investigated were nivolumab (BMS-936558, Bristol Myers-Squibb) and pembrolizumab (MK-3475, Merck, Sharpe and Dohme, Whitehouse Station, NJ), both human monoclonal IgG4 anti-PD-1 antibodies. Nivolumab was the first to be studied in a phase Ib study in multiple solid tumors (40). In this study, responses were reported in metastatic melanoma (28%), NSCLC (24%) and RCC (18%), with a median duration of response of 74 weeks in all tumor types, and an impressive median OS of 9.6 months in heavily-pretreated NSCLC patients. This study provided a basis for subsequent late phase clinical trials in all three solid tumor types, whereby nivolumab improved OS compared to standard-of-care (5,41-43). These findings resulted in FDA-approval in the first-line

setting for metastatic melanoma, and in the second-line setting for NSCLC and RCC. Similarly, pembrolizumab demonstrated adequate safety and tolerability at 1, 3, and 10 mg/kg dose levels in a phase I study (44). In melanoma, objective responses were 37-38%. In a subsequent phase II study of pembrolizumab for melanoma, ORR was 26% (45) leading to FDA-approval of pembrolizumab in ipilimumab-refractory metastatic melanoma. In a phase I pembrolizumab study of NSCLC patients with PD-L1-positive tumors as measured by immunohistochemistry (IHC) (at least 50% of tumor cells), ORR was 45.2%, with a median progression-free survival (PFS) of 6.2 months (46), leading to the FDA-approval of this agent for PD-L1 positive NSCLC.

In addition to anti-PD-1 antibodies, a number of agents against the PD-1 ligand, PD-L1, are in clinical development, for example, durvalumab (previously MEDI4736, MedImmune, Gaithersburg, MD) and atezolizumab (previously MPDL3280A, Genentech/Roche, San Francisco, CA). Atezolizumab is an anti-PD-L1 antibody that contains an engineered Fc portion that targets PD-L1, and was studied in a phase I trial in patients with metastatic melanoma, RCC and NSCLC, where ORR in these tumor types were 29%, 22% and 15% respectively (47). In addition, this agent demonstrated an ORR of up to 13% of patients with PD-L1 positive metastatic bladder carcinomas in a phase I study (6) and in PD-L1 positive NSCLCs (46), where PD-L1 positivity was measured on both tumor cells and immune cells (48).

The future of clinical trials with immune checkpoint antibodies

Combination studies of immune checkpoint antibodies, mainly anti-PD-1/PD-L1 antibodies combined with other immunotherapeutic agents as well as standard anti-cancer therapies, are currently underway in multiple tumor types. The combination of ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1) was first studied in a phase I trial in malignant melanoma, and demonstrated a 40% ORR, with 30% of patients (n=16) demonstrating shrinkage of more than 80% of their tumor (28). Subsequent phase II (29) and III studies (30) in advanced melanoma were undertaken, and the combination is now FDA-approved for the treatment of this disease. This combination is also under investigation in patients with advanced NSCLC in the first-line setting, as well as other combinations such as pembrolizumab plus ipilimumab (49), and durvalumab plus tremelimumab (50).

Certain cytotoxic chemotherapeutic agents are also thought to have immunogenic properties, such as 5-flurouracil, which may decrease myeloid derived suppressor cells (MDSC) (51) and increase effector T-cells at the TME (52), and oxaliplatin, which induces immunogenic cell death (53). These effects formed the basis for a phase I study of the combination of FOLFOX chemotherapy, bevacizumab and atezolizumab in metastatic CRC (54). A number of multi-arm phase I studies are currently underway in NSCLC and other solid tumors, aimed at investigating the safety and tolerability of combining anti-PD-1/PD-L1 antibodies with standard chemotherapeutic agents (NCT01454102, NCT02039674, NCT01633970).

Immune checkpoint antibodies have also been combined with 'targeted therapies' such as tyrosine kinase inhibitors for mutated oncogenes (55-57), as it is postulated that the PD-1/PD-L1 pathway may be an immune escape mechanism that mediates acquired resistance in these patients (58). Thus, patients with epidermal growth factor receptor (*EGFR*)-mutant NSCLC with the T790M resistance mutation are currently being studied in a phase I trial of the combination of a mutant-specific TKI to T790M, osimertinib (previously AZD9291), together with durvalumab (59). In both *BRAF*-mutant and wild-type metastatic melanoma, durvalumab has been combined with the *BRAF*-inhibitor dabrafenib and *MEK*-inhibitor trametinib either concomitantly or sequentially with trametinib alone (60).

Immune checkpoint antibodies have also been combined with anti-angiogenic agents such as vascular endothelial growth factor (VEGF) inhibitors (bevacizumab) and multi-targeted tyrosine kinase inhibitors (sunitinib, pazopanib). Blockade of VEGF may also lead to immunologic changes in the TME including, for example increasing effector T-cell populations (61,62) or reducing suppressive immune populations (such as regulatory T-cells and MDSCs) or immunosuppressive cytokines (63). In metastatic CRC and clear cell RCC, bevacizumab plus atezolizumab has been shown to be safe, and is being evaluated for efficacy in a phase II study (54,64).

Immune checkpoint antibodies have also been combined with ionizing radiotherapy, with the goal to enhance local control or to induce antigen release, cross-priming of T-cells against tumor-associated antigens, and systemic disease control, termed the "abscopal effect" (65,66). Radiation may induce immunogenic cell death (67), which is associated with an increase in effector T-cells at the TME, increased antigen presentation, and chemokine release. Conversely,

radiation may also lead to less effective DC function (68,69) and immunosuppression mediated by suppressive immune cell populations (70,71). A study of external-beam RT to between 1–3 osseous metastases combined with ipilimumab was found to be safe in a study in castration-resistant prostate cancer metastatic to bone (35). Studies exploring the combination of anti-PD-1/PD-L1 antibodies with definitive and palliative RT approaches are currently underway.

Next steps in biomarker discovery

Only a subset of patients treated with immune checkpoint antibodies experience durable disease control, highlighting the importance of identifying a predictive biomarker by which these patients may be selected. Biomarker studies can be sub-classified into either tissue-based or circulating biomarker studies. In the context of anti-CTLA-4 therapy, the absolute lymphocyte count (ALC) was identified as a potential pharmacokinetic circulating biomarker for ipilimumab, as absolute ALC level at 7 weeks (≥1,000 cells/μL) and magnitude of ALC increase was found to correlate with improved clinical outcomes in melanoma patients (72,73). In another study, the baseline absolute eosinophil and relative eosinophil counts were associated with improved survival in ipilimumab-treated patients (74,75). Sharma and colleagues postulate that pre-surgical clinical trial designs are an ideal platform with which to identify effective tissue or blood-based biomarkers. A pre-surgical trial creates an opportunity to examine a large amount of tumor tissue at the time of surgery, as well blood obtained at pre-specified time-points, both of which can be interrogated using multiple approaches. Thus, this group of investigators studied tumor tissue and serial blood samples from 12 patients with localized bladder carcinoma who received ipilimumab prior to cystectomy (76). Importantly, this study demonstrated that it was safe and tolerable to deliver immune checkpoint blockade with ipilimumab prior to surgery, and tissue from both pre and post treatment samples as well as stage-matched controls were interrogated using DNA, RNA and immune-based analyses. This study demonstrated that anti-CTLA-4 therapy was associated with an increase in a $CD4^+$ T-cell population that was positive for a cell-surface marker called inducible costimulator (ICOS) in the post-treatment cystectomy patients, a similar molecule to CD28/CTLA-4. In addition, the presence of this infiltrate was also accompanied by a demonstrable increase in ICOS+ T-cells in the circulation, detected by flow cytometry. These findings established ICOS as a pharmacodynamic biomarker for anti-CTLA-4 therapy, and was subsequently confirmed in mouse models as a co-stimulatory pathway integral to the achievement of therapeutic efficacy of CTLA-4 blockade (77). These findings also suggested that the combination of CTLA-4 blockade and increased ICOS activity could potentially improve upon the efficacy of CTLA-4 blockade alone (78).

Another approach used to examine potential predictive biomarkers to CTLA-4 blockade has focused on assessing the characteristics of the tumor cells in patients who have demonstrated long-term responses to this therapy, compared to those patients who did not sustain a durable response. In a study of melanoma patients receiving ipilimumab, whole-exome sequencing was conducted to evaluate the prognostic utility of measuring the number of non-synonymous somatic mutations. It was speculated that these non-synonymous mutations might be translated into non-self-proteins capable of stimulating anti-tumor immune responses. It was found that patients with a "high mutational load" in their tumors exhibited improved survival compared to patients with a low mutational load. From here, the investigators conducted patient-specific HLA typing and used prediction algorithms to identify which mutations were capable of being presented by MHC. A neoantigen landscape was identified in patients who demonstrated a deep and prolonged response to CTLA-4 blockade, and was validated in a second set of 39 melanoma patients treated with anti-CTLA-4 antibodies (79). In a similar assessment of NSCLC patients receiving pembrolizumab, patients with a higher mutational load had improved ORR and PFS (80). Other predictors of favorable outcome included an increased number of predicted mutation-associated neoantigens, as well as mutations in DNA repair pathways. Such observations contributed to the theory that tumors with deficiencies in DNA mismatch-repair might respond to immunotherapy. In patients with both CRCs and non-CRCs with mismatch-repair deficiency, pembrolizumab demonstrated improved immune-related ORR and PFS (CRC: n=4/10, 40%; non-CRC: n=7/8, 88%) compared to colorectal tumors with intact mismatch-repair enzymes (n=0/18, 0%; and n=2/18, 11%) (81). In this study, mismatch repair-deficient tumors exhibited a mean of 1,782 somatic mutations per tumor, versus 73 mutations seen in mismatch repair-proficient tumors (P=0.007).

Based upon the assumption that anti-PD-1 therapy functions principally by blocking interactions of PD-1 with PD-L1 on tumors, the first proposed biomarker for

PD-1/PD-L1 therapy was tumor PD-L1 expression as measured by IHC. In the phase I nivolumab trial, patients with tumors expressing PD-L1, using a cutoff of 5% membranous staining of tumor cells, demonstrated an ORR of 36% *vs.* 0% for PD-L1-negative patients (40). However, in the phase I ipilimumab plus nivolumab combination study, membranous PD-L1 expression was not predictive of response, with similar ORRs regardless of PD-L1 expression (8/17 responses in PD-L1 negative tumors *vs.* 4/10 responses in PD-L1 positive tumors) (82). Subsequent late phase clinical trials have revealed that PD-L1 expression—using a variety of cut-off values including 1% and 5% of tumor membranous staining—was neither predictive of response nor OS benefit in the second-line setting in both NSCLC (42) or pre-treated RCC (4). In a phase III trial of nivolumab in metastatic melanoma, PD-L1 positivity (cutoff: 5%) enriched for response, however patients with PD-L1 negative tumors still demonstrated a 33% ORR to nivolumab (41).

PD-L1 may also be expressed on immune cells present in the TME. In a trial of atezolizumab in metastatic bladder carcinoma, PD-L1-positivity was defined as greater than 5% positivity of intra-tumoral immune cells. With this definition, PD-L1-positive tumors exhibited a higher response (43.3%, n=13/30) compared to PD-L1 negative tumors (11.7%, n=4/13). While provocative, the PD-L1 biomarker may be less clinically useful in this setting, as ORRs even for PD-L1 negative tumors are appealing compared to standard-of-care second-line chemotherapy.

There are inherent challenges in developing and standardizing the PD-L1 biomarker for clinical use. To date, there is no consensus regarding the optimal PD-L1 antibody stain, threshold for positivity, standard for cell scoring, or biopsy methodology. Furthermore, PD-L1 expression may be dynamic and heterogeneous across space and time, changing as a result of tumor plasticity (for example, loss of the PTEN tumor suppressor gene), TME factors, or treatment effects (for example, chemotherapy-induced interferon gamma production) (83). To overcome these challenges, a PD-L1 working group has been developed, as well as dedicated studies comparing different PD-L1 antibodies and biomarker evaluation techniques. Proposed future IHC biomarker research directions include assessment of other immune cell populations, such as $CD8^+$ T-cell infiltration at the invasive margin by conventional IHC, or novel methods such as multiplex quantitative immunofluorescence (84,85).

Conclusions

Immune checkpoint antibodies are an exciting class of agents that are being incorporated into the treatment paradigm for several cancers. These antibodies harness anti-tumor immunity, which can be specific to one's tumor, yet adaptive and long-lasting, thus generating durable clinical benefit across a variety of malignancies. However, because of the inherent complexity of the immune response, patient selection and biomarker discovery and development has been a challenge.

Ipilimumab, nivolumab and pembrolizumab are FDA-approved as single agents for the treatment of metastatic melanoma as well as the combination of ipilimumab and nivolumab, and nivolumab and pembrolizumab are also approved for NSCLC. Most recently, nivolumab was approved for pre-treated metastatic RCC. Furthermore, atezolizumab has been granted breakthrough designation for the second-line treatment of bladder carcinoma. These advancements are examples of how years of immunotherapy research has come to fruition in the clinic.

The next challenge is to determine how best to optimize response by rationally combining immune checkpoint antibodies with other immunotherapies or standard anti-cancer agents that may augment the immune response. These approaches are also being evaluated as methods of overcoming drug resistance to immune checkpoint monotherapy. The mechanisms of primary and acquired resistance to immune checkpoint blockade are under active investigation.

Biomarker discovery in this field has focused thus far on both tumor-based and circulating biomarkers. ICOS+ $CD\text{-}4^+$ T-cells were identified as a pharmacodynamic biomarker to CTLA-4 blockade in a pre-surgical study of ipilimumab in bladder carcinoma. A pre-surgical study of PD-1 blockade in non-small lung cancer is currently underway, led by Johns Hopkins investigators, and may glean similar insights in this population. PD-L1 expression using IHC is currently being employed as a biomarker for PD-1 blockade, and has demonstrated the ability to enrich for response, in some but not all clinical contexts. Other predictive biomarkers such as tumor mutational burden and multiplex methods are under active study.

Acknowledgements

Funding: BT Li has received research funding support from the NIH/NCI Cancer Center Support Grant P30

CA008748.

Footnote

Provenance: This is a Guest Editorial commissioned by Section Editor Xiaozheng Kang, MD (Department of Thoracic Surgery, Beijing Cancer Hospital, Peking University, Beijing, China).
Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Couzin-Frankel J. Breakthrough of the year 2013. Cancer immunotherapy. Science 2013;342:1432-3.
2. Page DB, Postow MA, Callahan MK, et al. Immune modulation in cancer with antibodies. Annu Rev Med 2014;65:185-202.
3. Naidoo J, Page DB, Wolchok JD. Immune checkpoint blockade. Hematol Oncol Clin North Am 2014;28:585-600.
4. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med 2015;373:1803-13.
5. Motzer RJ, Rini BI, McDermott DF, et al. Nivolumab for metastatic renal cell carcinoma: results of a randomized phase II trial. J Clin Oncol 2015;33:1430-7.
6. Powles T, Eder JP, Fine GD, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature 2014;515:558-62.
7. El-Khoueiry AB, Melero I, Crocenzi TS, et al. Phase I/II safety and antitumor activity of nivolumab in patients with advanced hepatocellular carcinoma (HCC): CA209-040. J Clin Oncol 2015;33:abstr LBA101.
8. Segal NH, Ou SI, Balmanoukian AS, et al. Safety and efficacy of MEDI4736, an anti-PD-L1 antibody, in patients from a squamous cell carcinoma of the head and neck (SCCHN) expansion cohort. J Clin Oncol 2015;33:abstr 3011.
9. Pruett JR Jr, Kandala S, Hoertel S, et al. Accurate age classification of 6 and 12 month-old infants based on resting-state functional connectivity magnetic resonance imaging data. Dev Cogn Neurosci 2015;12:123-33.
10. Ding W, Dong H, Call TG, et al. PD-1 Blockade with pembrolizumab (MK-3475) in relapsed/refractory CLL including richter transformation: an early efficacy report from a phase 2 trial (MC1485). Orlando: 57th Annual Meeting & Exposition, 2015:AB834.
11. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med 2015;372:311-9.
12. Sharma P, Allison JP. The future of immune checkpoint therapy. Science 2015;348:56-61.
13. Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. Nature 1988;334:395-402.
14. Sjöblom T, Jones S, Wood LD, et al. The consensus coding sequences of human breast and colorectal cancers. Science 2006;314:268-74.
15. Segal NH, Parsons DW, Peggs KS, et al. Epitope landscape in breast and colorectal cancer. Cancer Res 2008;68:889-92.
16. Melero I, Gaudernack G, Gerritsen W, et al. Therapeutic vaccines for cancer: an overview of clinical trials. Nat Rev Clin Oncol 2014;11:509-24.
17. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. Annu Rev Immunol 2005;23:515-48.
18. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med 1995;182:459-65.
19. Waterhouse P, Penninger JM, Timms E, et al. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. Science 1995;270:985-8.
20. Tivol EA, Borriello F, Schweitzer AN, et al. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity 1995;3:541-7.
21. Quezada SA, Peggs KS, Curran MA, et al. CTLA4 blockade and GM-CSF combination immunotherapy alters the intratumor balance of effector and regulatory T cells. J Clin Invest 2006;116:1935-45.
22. Waitz R, Fassò M, Allison JP. CTLA-4 blockade synergizes with cryoablation to mediate tumor rejection. Oncoimmunology 2012;1:544-6.
23. Zamarin D, Holmgaard RB, Subudhi SK, et al. Localized oncolytic virotherapy overcomes systemic tumor resistance to immune checkpoint blockade immunotherapy. Sci Transl Med 2014;6:226ra32.
24. Hurwitz AA, Yu TF, Leach DR, et al. CTLA-4 blockade synergizes with tumor-derived granulocyte-macrophage colony-stimulating factor for treatment of an experimental mammary carcinoma. Proc Natl Acad Sci U S A 1998;95:10067-71.
25. van Elsas A, Hurwitz AA, Allison JP. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune

depigmentation. J Exp Med 1999;190:355-66.
26. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252-64.
27. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 2000;192:1027-34.
28. Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013;369:122-33.
29. Kiess AP, Wolchok JD, Barker CA, et al. Stereotactic radiosurgery for melanoma brain metastases in patients receiving ipilimumab: safety profile and efficacy of combined treatment. Int J Radiat Oncol Biol Phys 2015;92:368-75.
30. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med 2015;373:23-34.
31. Tchekmedyian S, Glaspy J, Korman A, et al. MDX-010 (human anti-CTLA4): a phase I trial in malignant melanoma. Proc Am Soc Clin Oncol 2002;21:223(Abstract 56).
32. Wolchok JD, Neyns B, Linette G, et al. Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. Lancet Oncol 2010;11:155-64.
33. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711-23.
34. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011;364:2517-26.
35. Slovin SF, Higano CS, Hamid O, et al. Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. Ann Oncol 2013;24:1813-21.
36. Yang JC, Hughes M, Kammula U, et al. Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. J Immunother 2007;30:825-30.
37. Lynch TJ, Bondarenko I, Luft A, 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;30:2046-54.
38. Ribas A, Hanson DC, Noe DA, et al. Tremelimumab (CP-675,206), a cytotoxic T lymphocyte associated antigen 4 blocking monoclonal antibody in clinical development for patients with cancer. Oncologist 2007;12:873-83.
39. Ribas A, Kefford R, Marshall MA, et al. Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. J Clin Oncol 2013;31:616-22.
40. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366:2443-54.
41. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 2015;372:320-30.
42. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med 2015;373:123-35.
43. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 2015;373:1627-39.
44. Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 2013;369:134-44.
45. Robert C, Ribas A, Wolchok JD, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet 2014;384:1109-17.
46. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 2015;372:2018-28.
47. Hamid OS, Lawrence DP. Clinical activity, safety, and biomarkers with MPDL32801A, an engineered anti-PDL1 antibody in patients with locally advanced or metastatic malignant melanoma. J Clin Oncol 2012;31:abstr 9010.
48. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 2014;515:563-7.
49. Patnaik A, Socinski MA, Gubens MA, et al. Phase 1 study of pembrolizumab (pembro; MK-3475) plus ipilimumab (IPI) as second-line therapy for advanced non-small cell lung cancer (NSCLC): KEYNOTE-021 cohort D. J Clin Oncol 2015;33:abstr 8011.
50. Antonia SJ, Goldberg SB, Balmanoukian AS, et al. Phase Ib study of MEDI4736, a programmed cell death ligand-1 (PD-L1) antibody, in combination with tremelimumab, a cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) antibody, in patients (pts) with advanced NSCLC. J Clin Oncol 2015;33:abstr 3014.
51. Vincent J, Mignot G, Chalmin F, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived

suppressor cells resulting in enhanced T cell-dependent antitumor immunity. Cancer Res 2010;70:3052-61.
52. Michaud M, Martins I, Sukkurwala AQ, et al. Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. Science 2011;334:1573-7.
53. Tesniere A, Schlemmer F, Boige V, et al. Immunogenic death of colon cancer cells treated with oxaliplatin. Oncogene 2010;29:482-91.
54. Bendell JC, Powderly JD, Lieu CH, et al. Safety and efficacy of MPDL3280A (anti-PDL1) in combination with bevacizumab (bev) and/or FOLFOX in patients (pts) with metastatic colorectal cancer (mCRC). J Clin Oncol 2015;33:abstr 704.
55. Sierra JR, Cepero V, Giordano S. Molecular mechanisms of acquired resistance to tyrosine kinase targeted therapy. Mol Cancer 2010;9:75.
56. Jancarikova D, Pesek M, Benesova L, et al. Acquired resistance of pulmonary adenocarcinoma to initially successful targeted therapy due to EGFR mutation T790M. Anticancer Res 2007;27:1879-82.
57. Jackman D, Pao W, Riely GJ, et al. Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. J Clin Oncol 2010;28:357-60.
58. Garrido G, Rabasa A, Garrido C, et al. Preclinical modeling of EGFR-specific antibody resistance: oncogenic and immune-associated escape mechanisms. Oncogene 2014;33:3129-39.
59. Oxnard GR, Ramalingam SS, Ahn M, et al. Preliminary results of TATTON, a multi-arm phase Ib trial of AZD9291 combined with MEDI4736, AZD6094 or selumetinib in EGFR-mutant lung cancer. J Clin Oncol 2015;33:abstr 2509.
60. Ribas A, Butler M, Lutzky J, et al. Phase I study combining anti-PD-L1 (MEDI4736) with BRAF (dabrafenib) and/or MEK (trametinib) inhibitors in advanced melanoma. J Clin Oncol 2015;33:abstr 3003.
61. Manning EA, Ullman JG, Leatherman JM, et al. A vascular endothelial growth factor receptor-2 inhibitor enhances antitumor immunity through an immune-based mechanism. Clin Cancer Res 2007;13:3951-9.
62. Shrimali RK, Yu Z, Theoret MR, et al. Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. Cancer Res 2010;70:6171-80.
63. Roland CL, Lynn KD, Toombs JE, et al. Cytokine levels correlate with immune cell infiltration after anti-VEGF therapy in preclinical mouse models of breast cancer. PLoS One 2009;4:e7669.
64. Sznol M, McDermott MF, Jones SF, et al. Phase Ib evaluation of MPDL3280A (anti-PDL1) in combination with bevacizumab (bev) in patients (pts) with metastatic renal cell carcinoma (mRCC). J Clin Oncol 2015;33:abstr 410.
65. Mole RH. Whole body irradiation; radiobiology or medicine? Br J Radiol 1953;26:234-41.
66. Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med 2012;366:925-31.
67. Vatner RE, Cooper BT, Vanpouille-Box C, et al. Combinations of immunotherapy and radiation in cancer therapy. Front Oncol 2014;4:325.
68. Merrick A, Errington F, Milward K, et al. Immunosuppressive effects of radiation on human dendritic cells: reduced IL-12 production on activation and impairment of naive T-cell priming. Br J Cancer 2005;92:1450-8.
69. Liao YP, Wang CC, Butterfield LH, et al. Ionizing radiation affects human MART-1 melanoma antigen processing and presentation by dendritic cells. J Immunol 2004;173:2462-9.
70. Ahn GO, Tseng D, Liao CH, et al. Inhibition of Mac-1 (CD11b/CD18) enhances tumor response to radiation by reducing myeloid cell recruitment. Proc Natl Acad Sci U S A 2010;107:8363-8.
71. Lugade AA, Moran JP, Gerber SA, et al. Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor. J Immunol 2005;174:7516-23.
72. Ku GY, Yuan J, Page DB, et al. Single-institution experience with ipilimumab in advanced melanoma patients in the compassionate use setting: lymphocyte count after 2 doses correlates with survival. Cancer 2010;116:1767-75.
73. Postow MA, Yuan J, Panageas K, et al. Evaluation of the absolute lymphocyte count as a biomarker for melanoma patients treated with the commercially available dose of ipilimumab (3mg/kg). J Clin Oncol 2012;30:abstr 8575.
74. Delyon J, Mateus C, Lefeuvre D, et al. Experience in daily practice with ipilimumab for the treatment of patients with metastatic melanoma: an early increase in lymphocyte and eosinophil counts is associated with improved survival. Ann Oncol 2013;24:1697-703.
75. Schindler K, Harmankaya K, Postow MA, et al. Pretreatment levels of absolute and relative eosinophil count to improve overall survival (OS) in patients with metastatic melanoma under treatment with ipilimumab, an

anti CTLA-4 antibody. J Clin Oncol 2013;31:abstr 9024.
76. Carthon BC, Wolchok JD, Yuan J, et al. Preoperative CTLA-4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial. Clin Cancer Res 2010;16:2861-71.
77. Fu T, He Q, Sharma P. The ICOS/ICOSL pathway is required for optimal antitumor responses mediated by anti-CTLA-4 therapy. Cancer Res 2011;71:5445-54.
78. Fan X, Quezada SA, Sepulveda MA, et al. Engagement of the ICOS pathway markedly enhances efficacy of CTLA-4 blockade in cancer immunotherapy. J Exp Med 2014;211:715-25.
79. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 2014;371:2189-99.
80. Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015;348:124-8.
81. Le DT, Uram JN, Wang H, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med 2015;372:2509-20.
82. Grosso J, Horak CE, Inzunza D, et al. Association of tumor PD-L1 expression and immune biomarkers with clinical activity in patients (pts) with advanced solid tumors treated with nivolumab (anti-PD-1; BMS-936558; ONO-4538). J Clin Oncol 2013;31:abstr 3016.
83. Callahan MK, Curran MA. Peripheral and tumor immune correlates in patients with advanced melanoma treated with combination nivolumab (anti-PD-1, BMS-936558, ONO-4358) and ipilimumab. The Annual Meeting of the American Society of Clinical Oncology, Chicago, USA. J Clin Oncol 2013;31:abstr 3003.
84. Schalper KA, Brown J, Carvajal-Hausdorf D, et al. Objective measurement and clinical significance of TILs in non-small cell lung cancer. J Natl Cancer Inst 2015;107.
85. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014;515:568-71.

Cite this article as: Naidoo J, Li BT, Schindler K, Page DB. What does the future hold for immunotherapy in cancer? Ann Transl Med 2016;4(9):177. doi: 10.21037/atm.2016.04.05

