

At the Bedside: CTLA-4- and PD-1-blocking antibodies in cancer immunotherapy

Margaret K. Callahan^{*,†} and Jedd D. Wolchok^{*,†,‡,1}

^{*}Melanoma and Sarcoma Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York, USA; [†]Weill Medical College of Cornell University, New York, New York, USA; and [‡]Ludwig Institute for Cancer Research, New York, New York, USA

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► SEE CORRESPONDING ARTICLE ON PAGE 25

ABSTRACT

It is increasingly appreciated that cancers are recognized by the immune system, and under some circumstances, the immune system may control or even eliminate tumors. The modulation of signaling via coinhibitory or costimulatory receptors expressed on T cells has proven to be a potent way to amplify antitumor immune responses. This approach has been exploited successfully for the generation of a new class of anticancer therapies, “checkpoint-blocking” antibodies, exemplified by the recently FDA-approved agent, ipilimumab, an antibody that blocks the coinhibitory receptor CTLA-4. Capitalizing on the success of ipilimumab, agents that target a second coinhibitory receptor, PD-1, or its ligand, PD-L1, are in clinical development. Lessons learned from treating patients with CTLA-4 and PD-1 pathway-blocking antibodies will be reviewed, with a focus on concepts likely to inform the clinical development and application of agents in earlier stages of development. See related review *At the bench: Preclinical rationale for CTLA-4 and PD-1 blockade as cancer immunotherapy. J. Leukoc. Biol.* 94: 41–53; 2013.

Introduction

The development of an antigen-specific T cell response is a complex, highly regulated process. Early studies investigating T cell activation led to the “two-signal” model, wherein activation requires antigen-specific stimulation via the TCR (signal 1) and a costimulatory signal (signal 2) [1–4]. Subsequent studies have largely validated the two-signal model and added layers of complexity to this framework. It is now clear that a variety of immunomodulatory signals, both costimulatory and coinhibitory, are needed to orchestrate an antigen-specific immune response.

It is increasingly appreciated that cancers are recognized by the immune system, and under certain circumstances, the immune system may control or even eliminate tumors [5]. Studies in mouse models of transplantable tumors have demonstrated that manipulation of costimulatory or coinhibitory signals can amplify T cell responses against tumors [6]. This may be accomplished by blockade of coinhibitory molecules, such as CTLA-4, PD-1, and LAG-3, or by enhanced signaling of costimulatory molecules, such as GITR, OX40, and 4-1BB [7–19]. Based on robust preclinical activity in mouse models, antibodies targeting a variety of coinhibitory and costimulatory molecules are in clinical development as anticancer agents.

Building on the basic science and preclinical studies reviewed in the companion article by Intlekofer and Thompson [20], the present review focuses on the clinical development of antibodies that block signaling via the inhibitory molecules CTLA-4 or PD-1, including agents that are already approved by the FDA (anti-CTLA-4) or in the earlier stages of clinical development (anti-PD-1, anti-PD-L1). These checkpoint-blocking antibodies have demonstrated clinical activity in a variety of tumor types, including melanoma, RCC, and NSCLC. As novel anticancer agents, they have a distinct profile of antitumor activity and toxicity, underscoring their unique mechanism of activity. Whereas CTLA-4 and PD-1 function as negative regulators, each plays a nonredundant role in modulating immune responses. CTLA-4, through engagement with its ligands CD80 and CD86, plays a pivotal role in attenuating the early activation of naïve and memory T cells. In contrast, PD-1 is primarily involved in modulating T cell activity in peripheral tissues via its interaction with PD-L1 and PD-L2 [21]. Differences in the biological role of each molecule are likely to explain unique, clinical features of agents that target each pathway. The understanding of tumor-specific and patient-specific characteristics that shape tumor-immune interaction may allow selection of checkpoint-blocking strategies tailored to individual patients. Moreover, as CTLA-4 and PD-1 regulate immune re-

Abbreviations: ALC=absolute lymphocyte count, CR=complete response, CRC=colorectal cancer, FDA=U.S. Food and Drug Administration, GITR=glucocorticoid-induced TNFR family-related gene, irAE=immune-related adverse event(s), irRC=immune-related response criteria, LAG-3=lymphocyte-activation gene-3, MTD=maximum tolerated dose, NSCLC=non-small cell lung cancer, OS=overall survival, PD-1=programmed death-1, PD-L1=programmed death-1 ligand, PR=partial response(s), RCC=renal cell cancer, RECIST=Response Evaluation Criteria in Solid Tumors, SD=stable disease, WHO=World Health Organization

1. Correspondence: Dept. of Medicine, Melanoma and Sarcoma Service, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10065, USA. E-mail: wolchokj@mskcc.org.

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TABLE 1. Checkpoint-Blocking Antibodies in Clinical Development

Antibody	Target	Isotype	Clinical development
Ipilimumab (Bristol-Myers Squibb)	CTLA-4	IgG1k	FDA-approved
Tremelimumab (Pfizer, now Medimmune)	CTLA-4	IgG2	Completed Phase III
Nivolumab/BMS-936558/MDX1106 (Bristol-Myers Squibb)	PD-1	IgG4	Phase I interim results, Phase III open
CT-011 (CureTech, Yavne, Israel)	PD-1	IgG1	Completed Phase I
MK-3475 (Merck, Whitehouse Station, NJ, USA)	PD-1	IgG4	Phase I ongoing, interim results
AMP-224 ^a (Amplimmune, Gaithersburg, MD, USA)	PD-1	NA	Phase I ongoing
BMS-936559/MDX-1105 (Bristol-Myers Squibb)	PD-L1	IgG4	Completed Phase I
MEDI4736 (MedImmune/AstraZeneca, London, UK)	PD-L1	IgG1k	Phase I ongoing
MPDL3280A/RG7446 (Genentech, South San Francisco, CA, USA/Roche, Basel, Switzerland)	PD-L1		Phase I ongoing

^aFusion protein.

sponses in a nonredundant fashion, combined blockade of both pathways may achieve superior antitumor activity. Lessons learned from treating patients with CTLA-4 and PD-1 pathway-blocking antibodies are likely to inform the clinical development of the next generation of antibodies targeting T cells via a diversity of coinhibitory and costimulatory molecules.

CTLA-4-BLOCKING ANTIBODIES

Based on the promising preclinical data generated in mouse models, two antibodies that block CTLA-4 in humans have been developed: ipilimumab (previously MDX-010; Medarex, Princeton, NY, USA, and Bristol-Myers Squibb, Princeton, NJ, USA) and tremelimumab (previously CP-675,206 or ticilimumab; Pfizer, New York, NY, USA, and now Medimmune, Gaithersburg, MD, USA; **Table 1**). Ipilimumab is a fully human IgG1 κ mAb, generated using mice transgenic for the human Ig heavy and light chains. Ipilimumab has high affinity for human and cynomolgus monkey CTLA-4 and not rodent CTLA-4 in vitro. Preclinical safety testing, performed in the cynomolgus monkey, showed a favorable profile. Ipilimumab has a half-life of 12–14 days. Tremelimumab, a fully human mAb belonging to the IgG2 antibody class, has a longer half-life of 22 days. It also has high affinity for human and cynomolgus monkey CTLA-4 in vitro.

CTLA-4-blocking antibodies in the clinic

Ipilimumab

—Fully human IgG1 κ mAb

—Half-life 12–14 days

Tremelimumab

—Fully human IgG2 mAb

—Half-life ~22 days

Research Question: How does the isotype impact antitumor activity of checkpoint-blocking antibodies?

IPILIMUMAB: PATHWAY TO FDA APPROVAL

Ipilimumab (commercial name Yervoy) was approved on March 25, 2011, by the FDA for the treatment of unresectable

or metastatic melanoma. The clinical testing of ipilimumab in patients began one decade earlier. Pilot studies of ipilimumab were first reported in 2002, with a Phase I study showing two PR in a cohort of 17 patients with unresectable melanoma treated with a single dose of ipilimumab, dosed at 3 mg/kg [22]. Treatment was well-tolerated, with only a mild rash noted. Subsequent studies focused on establishing appropriate dosing and schedule of the drug. A schedule of dosing every 3 weeks was adopted in several early studies, and the first evidence of a unique toxicity profile emerged from these trials. Collectively, these toxicities have been described as irAE, with the most common events including dermatitis, colitis, and hepatitis. These toxicities appeared to reflect a pattern of tissue-specific inflammation. A dose-response relationship was clearly defined in a double-blind Phase II study comparing ipilimumab at doses of 0.3, 3, and 10 mg/kg every 3 weeks, followed by maintenance doses administered every 12 weeks [23]. The highest dose cohort, 10 mg/kg, had the greatest response rate (11%), followed by 3 mg/kg (4.2%) and 0.3 mg/kg (0%). The rate of irAE was also higher with increased ipilimumab dose.

Ultimately, FDA approval was based on a benefit in OS seen in a randomized Phase III trial for patients with previously treated, unresectable Stage III or Stage IV melanoma [24]. This study randomized patients in a 3:1:1 ratio to receive ipilimumab at a dose of 3 mg/kg with a peptide vaccine (two HLA-A*0201-restricted peptides derived from the melanosomal antigen gp100 emulsified in Montanide), ipilimumab alone, or the peptide vaccine alone as the control arm. Median OS in the combination ipilimumab and peptide vaccine arm (10.0 months) was similar to the ipilimumab alone arm (10.1 months) but significantly higher than the peptide vaccine alone arm (6.4 months). Moreover, survival at 1 and 2 years was clearly superior in the ipilimumab-treated group when compared with peptide vaccine alone (45.6% vs. 25.3% at 1 year; 23.8% vs. 16.3% at 2 years), delivering on the promise of immunotherapy to establish durable disease control in a subset of patients. A second randomized, placebo-controlled Phase III trial comparing ipilimumab at a dose of 10 mg/kg plus dacarbazine chemotherapy versus dacarbazine alone confirmed the survival advantage of ipilimumab treatment in patients with treatment of naïve, unresectable Stage III or Stage IV mela-

noma [25]. A significant survival advantage for ipilimumab-treated patients was reported at 1 year (47.3% vs. 36.3%), 2 years (28.5% vs. 17.9%), and 3 years (20.8% vs. 12.2%), highlighting the long-term survival benefit to receiving ipilimumab.

Clinical development of tremelimumab suggests that this antibody may have similar activity to ipilimumab, although the two drugs have not been compared directly. In Phase I and II studies of tremelimumab, durable responses were seen in a significant minority of patients, along with a similar profile of irAE. The dosing and schedule chosen for tremelimumab, 15 mg/kg every 3 months, in part, reflect the difference in the half-life of tremelimumab versus ipilimumab. In one Phase II trial that enrolled 251 patients with metastatic melanoma, tremelimumab was associated with a durable overall response rate of 6.6%, lasting from 8.9 months to 29.8 months [26]. A number of additional patients had SD, and a total of 21% of patients achieved an objective response or prolonged SD. A randomized, open-label Phase III trial for patients with advanced melanoma, comparing tremelimumab with chemotherapy (dacarbazine or temozolomide) was conducted. After an interim analysis failed to demonstrate a benefit (OS 10.7 months vs. 11.7 months), the study was halted [27]. These results may have been complicated, however, by an unintentional crossover in the control arm that may have had access to ipilimumab. Additionally, the optimal dosing and schedule for tremelimumab have yet to be defined. Recently updated interim results demonstrate a nonsignificant trend, favoring an OS benefit for tremelimumab ($P=0.14$) in patients treated at locations where ipilimumab was not available [28].

Ipilimumab, the first checkpoint-blocking antibody approved by the FDA

—OS benefit demonstrated in two Phase III studies of patients with advanced melanoma

—Approved by the FDA in March 2011

—Approved dose of 3 mg/kg administered every 3 weeks for four doses

TOXICITIES ASSOCIATED WITH CTLA-4-BLOCKING ANTIBODIES

The clinical testing of the human CTLA-4-blocking antibodies ipilimumab and tremelimumab uncovered novel toxicities consisting of tissue-specific inflammation seen in a unique distribution of sites. These toxicities have been characterized as irAE based on their presumed link to the activation of the immune system by CTLA-4 blockade. Tissues most commonly affected include the skin (rash, pruritus, vitiligo), bowel (diarrhea, colitis), liver (hepatitis, elevated liver enzymes), the pituitary, and other endocrine glands (hypophysitis, hypothyroidism, thyroiditis, adrenal insufficiency; **Table 2**) [24]. Much more rarely reported irAE include uveitis, conjunctivitis, neuropathy, myopathy, pancreatitis, cytopenias, and nephritis [32–36]. irAE are typically responsive to interruption or discontinuation of CTLA-4 blockade in combination with immunosuppressive drugs, such as corticosteroids or occasionally TNF-blocking antibodies (for colitis) or mycophenolate mofetil (for hepatitis) [37, 38]. Irreversible damage to endocrine organs

during this period of inflammation may necessitate hormone supplementation [39–41].

Research Questions: What are the patient-related risk factors for the development of an irAE? What antibody-related factors determine the type(s) of irAEs that develop?

Curiously, this spectrum of irAE was not generally observed in the preclinical testing of ipilimumab or tremelimumab in animal models [42]. In some specific preclinical models, evidence that CTLA-4 blockade might lead to immune-related damage of non-tumor tissue was reported. For example, successful treatment of B16 tumor-bearing mice with a combination of CTLA-4 blockade plus the GVAX melanoma vaccine could lead to depigmentation, presumably through the targeting of normal melanocytic antigens [43]. However, more generalized evidence of tissue inflammation, such as colitis or dermatitis, was not seen in mice treated with CTLA-4 blockade as a monotherapy, perhaps reflecting differences in the half-life of the murine antibody, lack of genetic diversity, or the pathogen-free housing conditions of the mice. In a variety of mouse models of autoimmunity, CTLA-4 blockade can exacerbate the underlying disease [44]. Accordingly, patients with autoimmune disease have been excluded from clinical trials of ipilimumab or tremelimumab.

KINETICS OF TUMOR RESPONSE AND RE-EVALUATION OF TRADITIONAL RADIOGRAPHIC RESPONSE ENDPOINTS

Standard criteria for evaluating responses to chemotherapy have been developed to promote objectivity and to facilitate comparisons across trials. One commonly used response criteria is the RECIST, which is based on patterns of responses to chemotherapeutic agents that correlate with clinical outcomes. According to RECIST and the closely related, modified WHO criteria, an increase in tumor size and/or development of new lesions are defined as progressive disease, typically implicating that a change in therapy is warranted (**Table 3**). Observations in Phase I and Phase II clinical trials with CTLA-4 blockade suggested that patterns of response to immunotherapy may differ significantly from standard responses to chemotherapy. Assessing efficacy of immunotherapy by RECIST or WHO criteria, therefore, may not be appropriate.

To address this question, the radiographic responses of 487 patients treated on three multicenter Phase II clinical trials of ipilimumab were analyzed, and four patterns were identified: decreased baseline lesions without new lesions; durable SD; initial increase in total tumor burden but eventual response; and response of initial lesions with development of new lesions [29]. Interestingly, each of these patterns was associated with favorable survival. Based on these observations, irRC were proposed to evaluate the benefits of immunotherapy, previously, potentially misrepresented by traditional RECIST criteria. The main difference of irRC compared with traditional WHO or RECIST criteria is that irRC requires new lesions to be considered as part of the “total tumor burden” and not considered immediately as progressive disease. A detailed comparison of irRC and WHO criteria is presented in Table 3. The irRC is

TABLE 2. Selected Clinical Trials of Checkpoint-Blocking Antibodies in Patients with Advanced Melanoma

Agent tested	Patients	Treatment arms	Response rates	Median OS
CTLA-4 blockade				
Ipilimumab [24]	Six-hundred seventy-six patients with previously treated, unresectable Stage III or IV melanoma	Ipilimumab versus gp100 peptide vaccine versus combination Ipilimumab 3 mg/kg q3 weeks × 4 doses	Ipilimumab alone	
			BORR 10.9%	10.1 months
			DCR 28.5%	45.6% at 1 year
			two CR, 13 PR, 24 SD	23.5% at 2 years
Ipilimumab [29]	Two-hundred seventeen patients with previously treated metastatic melanoma	Ipilimumab 0.3 mg/kg versus 3 mg/kg versus 10 mg/kg q3 weeks × 4 doses, then q 3 months	Ipilimumab + gp100 peptide vaccine	
			BORR 5.7%	10.0 months
			DCR 20.1%	43.6% at 1 year
			one CR, 22 PR, 58 SD	21.6% at 2 years
			gp100 Peptide vaccine alone	
			BORR 1.5%	6.4 months
			DCR 11.0%	25.3% at 1 year
			zero CR, two PR, 13 SD	13.7% at 2 years
			10 mg/kg	
			BORR 11.1%	11.4 months
DCR 29.2%		48.6% at 1 year		
two CR, six PR, 13 SD				
3 mg/kg				
BORR 4.2%		8.7 months		
DCR 26.4%		39.3% at 1 year		
zero CR, three PR, 16 SD				
0.3 mg/kg				
BORR 9%		8.6 months		
DCR 13.7%		39.6% at 1 year		
zero CR, zero PR, 10 SD		39.6% at 1 year		
zero CR, zero PR, 10 SD				
PD-1 or PD-L1 blockade				
Nivolumab [30]	One-hundred four patients with advanced melanoma (94 evaluable for response)	Nivolumab (BMS-936558) 0.1, 0.3, 1, 3, or 10 mg/kg q 2 weeks for up to 96 weeks	ORR 28%	NR
BMS-936559 [31]	Fifty-five patients with advanced melanoma (52 evaluable for response)	BMS-936559 (MDX-1105) 0.3, 1, 3, or 10 mg/kg q 2 weeks for up to 96 weeks	ORR 17%	NR
			All dose levels three CR, six PR, 14 SD	

BORR, Best overall response rate; DCR, disease control rate; ORR, overall response rate; NR, not reported.

already being used in parallel with traditional criteria in current clinical protocols, and prospective validation of its correlation with additional clinical endpoints is ongoing.

Clinical and Research Questions: *What factor(s) determine the kinetics of an antitumor response in patients treated with checkpoint-blocking antibodies?
What features of the tumor or host immune system explain the cases of durable responses?
In cases of tumor relapse, what features of the tumor or host immune system allow the tumor to escape?*

Although radiographic response rate, whether by irRC or traditional criteria, is important, OS is perhaps the most important and least subjective study endpoint in therapeutic oncology trials. Results involving other forms of tumor immunotherapy, such as sipuleucel-T, an autologous cellular vaccine product, have demonstrated that immunotherapeutic strategies can confer a survival benefit in the absence of meaningful traditional disease re-

sponses [45–47]. Although many questions remain, the presence of a survival benefit without a convincing tumor response suggests that traditional methods of measuring disease response may need to be revised when considering immunotherapeutic approaches. Lastly, another unique aspect of the responses to CTLA-4 blockade has been the very slow kinetics of responses and the remarkable durability of responses. Prieto et al. [48] reported on the long-term follow-up for 177 patients treated in some of the earliest clinical trials of ipilimumab [49]. For the 15 patients who achieved a CR, a median of 30 months was required to achieve this endpoint. Encouragingly, all of these responses, except for one, are ongoing, with the longest lasting 99+ months.

DOSING AND SCHEDULE

The FDA-approved schedule for ipilimumab treatment is one dose every 3 weeks for a total of four doses. Some clinical tri-

TABLE 3. Comparison between irRC and WHO Criteria

Parameter-evaluated	irRC	WHO
CR	Disappearance of all lesions in two consecutive observations not less than 4 weeks apart	Disappearance of all lesions in two consecutive observations not less than 4 weeks apart
PR	≥50% Decrease in tumor burden compared with baseline in two observations at least 4 weeks apart	≥50% Decrease in SPD of all index lesions compared with baseline in two observations at least 4 weeks apart, in the absence of new lesions or unequivocal progression of nonindex lesions
SD	50% Decrease in tumor burden compared with baseline cannot be established, nor 25% increase compared with nadir	50% Decrease in SPD compared with baseline cannot be established, nor 25% increase compared with nadir, in the absence of new lesions or unequivocal progression of nonindex lesions
Progressive disease	At least 25% increase in tumor burden compared with nadir (at any single time-point) in two consecutive observations at least 4 weeks apart	At least 25% increase in SPD compared with nadir and/or unequivocal progression of nonindex lesions and/or appearance of new lesions (at any single time-point)

SPD, Sum of the products of the two largest perpendicular diameters. Tumor burden = SPD_{index lesions} + SPD_{new, measurable lesions}. Adapted from ref. [29].

als have permitted additional, so-called “maintenance” doses of ipilimumab, administered every 3 months after initial induction therapy. Alternatively, some trials have permitted repeat dosing or “reinduction” therapy using the original four-dose induction schedule. The Hodi study [24] provides some limited evidence that reinduction with ipilimumab may help patients who have benefitted previously but then ultimately developed disease progression. In this study, 31 patients with progressive disease after a confirmed PR, CR, or at least 3 months of SD were offered reinduction with subsequent evaluation showing one CR, five PRs, and 15 with SD. The use of the maintenance-dosing schedule has not been tested in a randomized fashion.

Clinical Questions: What is the optimal dose of ipilimumab?
Is there a role for maintenance dosing of ipilimumab?
Is there a role for reinduction dosing of ipilimumab?

The FDA has approved ipilimumab at a dose of 3 mg/kg. However, it is not clear that this reflects the best clinical activity for ipilimumab, and several important questions related to dosing and schedule have not been answered. What is the most effective dose of ipilimumab? Phase I studies did not identify a MTD. Furthermore, the randomized, double-blinded Phase II study, comparing ipilimumab at three dose levels—0.3, 3, and 10 mg/kg—demonstrated dose-dependent antitumor activity. The improved response rate (0% vs. 4.2% vs. 11.1%) must be balanced against an increased rate of Grade 3/4 irAE (0% vs. 7% vs. 25%). A double-blind Phase III study comparing the activity of ipilimumab at the 10 mg/kg versus the 3 mg/kg dose is ongoing (NCT01515189).

PD-1-BLOCKING ANTIBODIES

PD-1 is a second coinhibitory receptor expressed on T cells and a promising therapeutic target. Although CTLA-4 and PD-1 function as negative regulators, they play unique roles in the regulatory pathway of activated T cells. PD-1 appears to

play a more prominent role in modulating T cell activity in peripheral tissues via interaction with PD-L1 and PD-L2. The expression pattern of PD-L1, expressed broadly on hematopoietic and nonhematopoietic tissues, and PD-L2, expressed on DCs, macrophages, mast cells, and B cells, supports this notion. Furthermore, PD-L1 and PD-L2 are up-regulated under inflammatory conditions, mediated, in part, by type I and type II IFNs [50–54]. PD-L1 and to a lesser extent, PD-L2 are expressed on many human tumors, including urothelial, ovarian, breast, cervical, colon, pancreatic, gastric cancers, as well as melanoma glioblastoma and NSCLC [55–66]. In addition, these molecules have also been detected on hematologic malignancies, including Hodgkin lymphoma, primary mediastinal B cell lymphoma, angioimmunoblastic T cell lymphoma, multiple myeloma, acute myeloid leukemia, chronic lymphocytic leukemia, and adult T cell leukemia/lymphoma [54, 67–70]. Expression of PD-L1 has been correlated with prognosis in many of these malignancies, fueling the hypothesis that PD-L1 expression is a mechanism for tumor immune evasion [17, 58, 62, 71]. However, these findings must be interpreted with caution, given the technical difficulties with detecting PD-L1 in formalin-fixed tissues and the lack of a uniform assay. Additionally, PD-1 is highly expressed on lymphocytes infiltrating human tumors and circulating tumor-specific T cells, a phenotype that may be correlated with impaired T cell function in some settings [72–75]. Together, these findings suggest that interrupting the PD-1:PD-L1/PD-L2 interaction could be an effective anticancer therapy by blunting inhibition of immune responses in the tumor microenvironment [76, 77].

Research Question: How do the mechanisms of activity for CTLA-4 and PD-1 or PD-L1 blockade translate into clinical activity for each agent? What features are tumor-type-specific or specific to the individual tumor or host immune system?

Several antibodies that inhibit the PD-1 pathway, by blockade of the PD-1 molecule or PD-L1, have been developed, and their characteristics are described in Table 1 [78]. This area is

evolving rapidly; this review will focus on agents for which clinical data have been published. Nivolumab (BMS-936558) is a fully human IgG4 antibody. The largest experience with nivolumab reported to date comes from a Phase Ib dose-escalation study, including 269 patients with advanced solid tumors, including melanoma, NSCLC, prostate cancer, RCC, and CRC [79]. Patients received nivolumab at doses ranging from 0.1 to 10 mg/kg on an every-other-week schedule. In the initial reports, notable response rates were observed in patients with NSCLC (18%), melanoma (28%), and RCC (27%). These data have since been updated and presented recently at the European Society for Medical Oncology, now including a total of 304 patients. Updated results support the original findings; for example, in 106 evaluable patients with advanced melanoma, the response rate was 31% [80]. Responses in NSCLC were especially remarkable, as this cancer has not been described previously as amenable to immunologic approaches to disease control. In comparison with CTLA-4 blockade, rates of previously defined irAE (diarrhea, rash) appeared to be less frequent. However, a new and potentially serious irAE—pneumonitis—was observed in 3% of patients (Table 2).

MK-3475 (Merck) is a humanized IgG4 antibody directed against PD-1. A Phase I open-label, dose-escalation study tested doses of 1, 3, or 10 mg/kg in 27 patients with advanced solid tumors [81]. No dose-limiting toxicity was identified within this range, and clinical activity was noted. Notably, approximately one-half of the patients treated with MK-3475 in this study had been treated previously with ipilimumab. Thus, these data suggest that the PD-1-blocking antibodies may have activity in patients with advanced melanoma who are refractory to treatment with ipilimumab. More recently, the interim results of a Phase Ib study testing doses of 3 or 10 mg/kg were reported for 85 patients with advanced melanoma [31]. An objective response rate of 51% (43/85) and a CR rate of 9% (8/85) were reported. Adverse events appear consistent with irAE noted previously for nivolumab and included rash, diarrhea, itching, and arthralgias. CT-011 (CureTech) is a humanized IgG1 antibody that was tested initially in a Phase I open-label, dose-escalation, single-dose (0.2–6 mg/kg) study of 17 patients with hematological malignancies [30]. The MTD was not achieved, and the doses tested were well-tolerated. The most frequent adverse event was diarrhea (11.8%). Patients who achieved a response or sustained SD totaled 33% of the study population, and one patient with follicular lymphoma showed no evidence of malignancy following therapy. Based on these promising results, several Phase II protocols of CT-011 are being conducted.

Clinical Question: *How does the response (or lack of response) to one checkpoint-blocking antibody predict response (or lack of response) to another checkpoint-blocking antibody?*

Blockade of PD-L1 represents an alternative approach to disrupting PD-1 signaling, and three antibodies have been developed for clinical use (Table 1). BMS-936559 (previously MDX-1105; Bristol-Myers Squibb) is a fully human, PD-L1-spe-

cific IgG4 mAb. Results of a Phase I study evaluating the clinical activity of BMS-936559 in 207 patients with advanced solid tumors, including NSCLC, melanoma, CRC, RCC, ovarian cancer, pancreatic cancer, gastric cancer, and breast cancer, were reported recently [82]. As with PD-1 blockade, objective responses were seen in patients with melanoma (17%), RCC (12%), and NSCLC (10%). Additionally, one patient with ovarian cancer had an objective response. As with nivolumab, irAE appeared less common with PD-L1 blockade than with CTLA-4 blockade. In contrast to nivolumab, no cases of pneumonitis were reported with BMS-936559. The preservation of regulatory signaling via PD-L2 may be one explanation for the relative infrequency of irAE for PD-L1 blockade [83].

CANDIDATE BIOMARKERS OF RESPONSE

Monitoring of immunological parameters has been an integral component of completed and ongoing clinical trials of CTLA-4 and PD-1 blockade. Recent studies investigating the tumor microenvironment highlight the important differences in immune activity in the tumor compared with peripheral blood. The majority of putative biomarkers has been identified in small, retrospective analyses, and prospective validation is an area of active research.

Early studies of ipilimumab-treated patients identified the ALC as a marker of interest. In a retrospective review of 379 patients treated with ipilimumab, followed by prospective validation involving 64 additional patients, the rate of rise in ALC correlated with patients meeting a CR, PR, or SD, lasting >24 weeks [84]. A second, independent study of 51 patients showed that patients with ALC, >1000 after the second dose of ipilimumab, had a higher rate of CR, PR, or SD at Week 24 of therapy [85]. In this study, the high ALC group had higher OS at 6 and 12 months. Furthermore, it appears that CD8⁺ T cells may be the pertinent lymphocyte subset responsible for the beneficial response to ipilimumab, as suggested by analysis of lymphocyte subgroups [86].

Clinical and Research Question: *What features of individual patients (i.e., their immune system) determine the likelihood of response to checkpoint blockade?*

Antigen-specific immune responses during CTLA-4 blockade have been evaluated for a number of cancer-related antigens, such as NY-ESO-1, MAGE, Melan-A, melanoma antigen recognized by T cell 1, gp-100, tyrosinase, prostate-specific antigen, prostate acid phosphatase, and prostate-specific membrane antigen. Immune responses to the cancer-testis antigen NY-ESO-1 have been characterized the most extensively to correlate with clinical activity following CTLA-4 inhibition. In one study of 15 patients with metastatic melanoma treated with ipilimumab, five of eight (62.5%) with tumor shrinkage or stabilization had developed NY-ESO-1 antibodies [87]. All five of these seropositive patients had clearly detectable CD4⁺ and CD8⁺ T cells against NY-ESO-1. In contrast, none of the seven

TABLE 4. Planned or Ongoing Studies of Combination Therapies with Checkpoint-Blocking Antibodies

Combinations	Tumor type(s)	NCT number
Combinations with targeted inhibitors		
Ipilimumab + vemurafenib	Melanoma	NCT01673854
		NCT01400451
Ipilimumab + dabrafenib ± trematenib	Melanoma	NCT01767454
Ipilimumab + BMS-908662	Melanoma	NCT01245556
Ipilimumab + imatinib	Ckit mutant tumors	NCT01738139
Ipilimumab + radiation	Melanoma	NCT01565837
		NCT01689974
		NCT01703507
Ipilimumab + androgen-deprivation therapy	Prostate cancer	NCT01377389
		NCT01688492
		NCT01498978
Ipilimumab + dasatinib	Gastrointestinal stromal tumor	NCT01643278
Combinations with immunotherapies		
Ipilimumab + nivolumab	Melanoma	NCT01783938
		NCT01024231
Ipilimumab + anti-OX40	Melanoma	NCT01689870
Ipilimumab + anti-KIR antibody	Advanced cancers	NCT01750580
Ipilimumab + rIL-21	Melanoma	NCT01489059
Ipilimumab + GM-CSF	Melanoma, prostate cancer	NCT01134614
		NCT01530984
		NCT00064129
		NCT01363206
Ipilimumab + adoptive cell transfer	Melanoma	NCT01701674
Ipilimumab after allogeneic stem cell transplant	Advanced cancers	NCT00060372
Ipilimumab + IFN- α -2b	Melanoma	NCT01496807
		NCT01708941
Ipilimumab + autologous DC vaccine	Melanoma	NCT01302496
Ipilimumab + IDO inhibitor	Melanoma	NCT01604889
Ipilimumab + intratumoral IL-2	Melanoma	NCT01672450
		NCT01480323
Ipilimumab + OncoVex	Melanoma	NCT01740297
Other combinations		
Ipilimumab + bevacizumab	Melanoma	NCT00790010
Ipilimumab + radiation	Melanoma and other tumor types	NCT01565837
		NCT01689974
		NCT01703507
		NCT01769222
		NCT01449279
		NCT01557114
		NCT01497808
Ipilimumab + lenolidamide	Advanced cancers	NCT01750983
Ipilimumab + isolated limb infusion	Melanoma	NCT01323517
Ipilimumab + doxycycline + temozolamide	Melanoma	NCT01590082
Ipilimumab + paclitaxel + carboplatin	NSCLC	NCT01165216
		NCT01285609
Ipilimumab + cryoablation	Breast cancer	NCT01502592
Ipilimumab + gemcitabine	Pancreatic cancer	NCT01473940
Ipilimumab + rituximab	Lymphoma	NCT01729806
Ipilimumab + carboplatin + etoposide	Small cell lung cancer	NCT01331525
Ipilimumab + radioembolization	Melanoma	NCT01730157
Nivolumab + combination chemotherapy	NSCLC	NCT01454102
Ipilimumab + gemcitabine + cisplatin	Urothelial carcinoma	NCT01524991
Ipilimumab + low-dose cyclophosphamide	Melanoma	NCT01740401
Ipilimumab + fomustine	Melanoma	NCT01654692

NCT, National Clinical Trial.

patients with progressive disease was seropositive for NY-ESO-1. There was, however, one patient with progressive disease who had a CD4⁺ T cell response. These findings imply that the development of polyfunctional NY-ESO-1-specific T cells, as a

surrogate of a more developed antitumor immune response or as direct mediators of antitumor immunity, may identify patients likely to benefit from ipilimumab. This connection was also supported by the findings from a larger, retrospective

study of 144 ipilimumab-treated patients with melanoma [88]. In this study, NY-ESO-1-seropositive patients were more likely to have a CR, PR, or prolonged SD after treatment with ipilimumab, with an even stronger positive correlation in patients that were seropositive and positive for NY-ESO-1 CD8⁺ T cells. This correlation between ipilimumab and NY-ESO-1 serology was not found, however, in another study [89].

Clinical and Research Question: *What features of individual tumors determine the likelihood of response to checkpoint blockade?*

ICOS is a costimulatory molecule thought to play an especially important role in T cell survival, proliferation, and generation of memory [90]. ICOS is up-regulated on activated T cells, and ICOS expression on T cells has been proposed as a potential biomarker for clinical activity in patients treated with checkpoint-blocking antibodies. The first studies correlating ICOS expression with clinical activity came from patients with bladder cancer, treated with neoadjuvant ipilimumab. In this setting, ICOS was identified as a biomarker up-regulated on tumor-infiltrating T cells [91]. Subsequent studies extended these findings to patients with prostate or breast cancer, treated with CTLA-4 blockade [92, 93]. Finally, in a retrospective analysis of melanoma patients treated with ipilimumab, increased frequency of CD4⁺ICOS^{high} T cells, sustained over a period of 12 weeks, correlated positively with increased OS [94].

Recent studies have highlighted the important role that the tumor microenvironment plays in modulating antitumor immune responses and the diversity of mechanisms that may interfere with effective elimination of tumor cells [95]. In a prospective Phase II study of patients with melanoma treated with ipilimumab, several immune-related biomarkers were identified in pretreatment tumor biopsies that correlated with favorable clinical outcomes [96]. High baseline expression levels of forkhead box P3 and IDO in the tumor, as well as increased levels of tumor-infiltrating lymphocytes during treatment, were all associated with clinical activity. In a further analysis of this study using gene-expression profiling, pretreated expression of immune-related genes, especially IFN- γ -responsive genes, was correlated positively with clinical activity [97].

Consistent with this emphasis on the clinical relevance of the tumor microenvironment, Topalian et al. [30] have suggested that tumor expression of PD-L1 may correlate favorably with clinical responses to nivolumab. In the Phase I study of nivolumab, PD-L1 expression, detected by immunohistochemistry, was positive in tumor biopsy samples from 25 patients and negative in 17 cases. For patients with PD-L1-positive tumors, the objective response rate was 36% (nine of 25), whereas the response rate in the PD-L1-negative group was 0%. Larger, prospective studies will be necessary to explore this further. It is worthwhile to remark that PD-L1 expression in the tumor may be dynamic and heterogeneous, and this may pose a challenge in the use of PD-L1 as a biomarker. In a retrospective review of 150 melanoma tumor samples, tumor PD-L1 expression was heterogeneous and highly concordant in colocalizing with immune infiltrate and the presence of IFN- γ , supporting the concept that tumor PD-L1 expression is a dynamic, adaptive response to immune cells in the microenvironment [98].

AREAS OF PROMISE FOR CLINICAL DEVELOPMENT OF CHECKPOINT BLOCKADE

Activity for checkpoint blockade outside of melanoma

Checkpoint-blocking antibodies have been tested most extensively in patients with advanced melanoma, but evolving data support testing the clinical activity of these agents more widely. In mouse models of transplantable tumors, CTLA-4 blockade has demonstrated activity in diverse tumor types, including glioma, sarcoma, ovarian carcinoma, and bladder carcinoma (reviewed by Grosso and Jure-Kunkel [98]). In some tumor types, such as breast, prostate, and colorectal tumors, CTLA-4 blockade was not active in the monotherapy setting, but activity was seen in combination with other agents. Recent Phase I studies of the PD-1-blocking antibody (BMS-936558) and the PD-L1-blocking antibody (BMS-936559) have underscored the wider potential of checkpoint blockade. In a Phase I study of BMS-936558, clinical activity was seen in patients with NSCLC (18% objective response rate; 14/76 patients) and RCC (27% objective response rate; nine of 33 patients), in addition to patients with melanoma (28% objective response rate; 26/94 patients) [30]. No objective responses were seen in patients with prostate or CRC. In a Phase I study evaluating BMS-936559, evidence of clinical activity was seen in patients with NSCLC, RCC, and melanoma, and one patient with ovarian cancer also had a response [31]. No objective responses were seen in patients with pancreatic or CRC. Phase III studies of BMS-936558 in NSCLC, RCC, and melanoma are ongoing. Ipilimumab has also been evaluated in nonmelanoma tumors with modest evidence of clinical activity in some tumor types. Objective radiographic and biochemical responses have been reported in patients with prostate cancer [99–102]. At present, two Phase III trials of ipilimumab in prostate cancer are ongoing. A study

TABLE 5. irAE Associated with Checkpoint Blockade

Toxicity	Any grade	Grade 3/4
Ipilimumab ^a (data from Phase III study; ref. [23])		
Rash	18% (93/511)	1% (six/511)
Pruritus	19% (99/511)	<1% (one/511)
Diarrhea	30% (151/511)	4% (20/511)
Hepatitis	1% (five/511)	<1% (two/511)
Hypophysitis	<1% (four/511)	<1% (four/511)
Hypothyroidism	2% (eight/511)	<1% (one/511)
Pneumonitis	NR	NR
Nivolumab ^b (data from Phase I study; ref. [30])		
Rash	12% (31/259)	NR
Pruritus	10% (26/259)	<1% (one/259)
Diarrhea	12% (30/259)	1% (three/259)
Hepatitis (increased alanine aminotransferase)	4% (11/296)	1% (two/296)
Hypophysitis	<1%	<1%
Hypothyroidism	2% (six/259)	<1% (one/259)
Pneumonitis	3% (nine/259)	1% (three/259)

^aIpilimumab monotherapy or ipilimumab plus peptide vaccine at a dose of 3 mg/kg; patients with advanced melanoma. ^bNivolumab monotherapy at doses of 0.1–10 mg/kg; patients with advanced solid tumors.

TABLE 6. Summary and Future Questions for the Clinical Development of Checkpoint-Blocking Antibodies

Lessons learned	Outstanding questions
CBA can generate potent antitumor immune responses with evidence of clinical activity demonstrated in patients with melanoma, RCC, and NSCLC and anecdotal reports of activity in other solid tumors.	What tumor types are most (or least) amenable to treatment with CBA and why?
Many patients do not have objective responses to CBA monotherapy based on the dosing and schedule of antibodies tested thus far.	What features of individual tumors determine the likelihood of response to checkpoint blockade?
	What features of individual patients (i.e., their immune system) determine the likelihood of response to checkpoint blockade?
	Can the type of CBA be customized based on tumor or patient characteristics to enhance the likelihood of response?
Compared with CTLA-4 blockade, early studies suggest that PD-1-blocking antibodies have a favorable response rate; data on long-term survival for larger numbers of patients treated with PD-1-blocking antibodies are awaited.	How do the mechanisms of activity for CTLA-4 and PD-1 or PD-L1 blockade translate into clinical activity for CBA? What features are tumor-type-specific or specific to the individual tumor or patient?
	Does response (or lack of response) to one CBA predict response (or lack of response) to another CBA?
CBA can generate immune activation leading to tissue-specific inflammation and consequent toxicities in a variety of organs.	What determines which patients develop toxicity and which type(s) of toxicity develop?
In early studies, PD-1- and PD-L1-blocking antibodies appear to have an acceptable toxicity profile.	How can toxicity be uncoupled from antitumor activity (some patients have a tumor response without toxicity and others, vice versa)?
CTLA-4- and PD-1-blocking antibodies generate overlapping and unique toxicities.	How do the mechanisms of activity for CTLA-4 blockade and PD-1 or PD-L1 blockade translate into the toxicity profile for each agent (what explains unique toxicities such as pneumonitis)?
Compared with traditional cytotoxic anticancer agents, CBA may have unique and delayed kinetics of response and remarkable durability of responses.	What factors determine the kinetics of an antitumor response in patients treated with CBA, and what explains the cases of delayed responses?
	In cases where durable responses are seen, what features of the tumor or the immune system determine durable disease control?
	In cases of tumor relapse, what features of the tumor or the immune system allow the tumor to escape?
Preclinical studies suggest that CBA may be combined successfully with a diversity of anticancer agents, generating superior antitumor activity.	Which combinations are feasible and safe in the clinic?
	Which combinations generate the most robust antitumor activity and in what setting?
	Can combinations of CBA be tailored to an individual's tumor and/or immune system to improve clinical activity?

CBA, Checkpoint-blocking antibodies.

combining chemotherapy with ipilimumab for NSCLC suggested a modest benefit [103]. A Phase II study in patients with metastatic RCC also demonstrated some clinical activity [104]. In a study of metastatic pancreatic cancer, no responses were seen in patients treated with ipilimumab [105]. Additional studies will be necessary to understand the diversity in responses to checkpoint blockade across the spectrum of tumor types and evaluate whether novel combinations of therapies may demonstrate activity in tumors where robust activity has not been seen thus far.

Clinical and Research Question: *What tumor types are most (or least) amenable to treatment with checkpoint-blocking antibodies and why?*

Novel combinations

As the clinical activity of checkpoint-blocking antibodies in the monotherapy setting is established, the next step will be to as-

sess the safety, feasibility, and activity of a variety of combinations (Table 4). CTLA-4-blocking antibodies and PD-1-blocking antibodies have demonstrated clinical antitumor activity against melanoma and other solid tumors. Preclinical studies suggest that CTLA-4 and PD-1- or PD-L1-blocking antibodies may be combined to achieve superior antitumor activity [106, 107]. The nonredundant functions of CTLA-4 and PD-1 support the concept that combined blockade could have favorable clinical activity. However, similarities in the toxicity profile for CTLA-4-blocking and PD-1/PD-L1-blocking agents (Table 5) highlight the importance of evaluating toxicity of combined blockade. A first clinical trial in humans, combining ipilimumab with nivolumab in a concomitant schedule, is presently ongoing (NCT01024231). Anecdotal experience with individuals who have responded to PD-1 blockade after failing to respond to ipilimumab, or vice-versa, supports the notion that these agents have distinct mechanisms of action.

However, additional studies will be needed to better delineate the optimal sequencing of these two agents.

Clinical Question: Which combinations are feasible and safe in the clinic?

CTLA-4 blockade and PD-1 blockade are also being tested in combination with traditional anticancer modalities. In the context of these combination studies, traditional therapies are being looked at through the lens of immunology. The potential for cytotoxic therapies, including chemotherapy, radiotherapy, and cryotherapy, to modulate immune activity is increasingly being appreciated, adding a layer of complexity to the design of combination regimens [108–110]. With the use of radiation therapy as an example, systemic antitumor effects from local radiation (described as “abscopal” effects) and are thought to be mediated via changes in the immune system [111]. Preclinical mouse model studies provide evidence that radiation-induced changes impact the antitumor immune response and that radiation may partner favorably with immunotherapies [112–114]. In case reports, patients treated with ipilimumab have been described to benefit from the abscopal effect of local radiation [115, 116]. These observations have provided the rationale for several clinical trials, now ongoing, combining radiation therapy with ipilimumab. Another area of active exploration is the combination of checkpoint blockade with targeted inhibitors, a topic of particular interest in melanoma, where the BRAF inhibitor vemurafenib and ipilimumab are the two therapies that have demonstrated a survival benefit.

Clinical Question: Can combinations of checkpoint-blocking antibodies be tailored to an individual's tumor and/or immune system to improve clinical activity?

SUMMARY AND FUTURE DIRECTIONS

The immune system has established an elaborate system of self-regulation to prevent against excessively damaging immune responses. Although essential for immunologic homeostasis, in the presence of active malignancy, inhibitory signaling may predominate and prevent effective antitumor immune responses. Research over the past two decades has highlighted the important components of several immune-regulatory circuits, and insight into these mechanisms has led to novel approaches to tumor immunotherapy. Antibodies that block inhibitory pathways, such as those directed against CTLA-4 and PD-1, have been developed for clinical use. These checkpoint-blocking antibodies are revitalizing interest in solid tumor immunotherapy and have resulted in promising clinical outcomes, exemplified by the recent FDA approval of ipilimumab. Lessons learned from studies of CTLA-4 and PD-1 blockade provide a foundation for the further development of checkpoint-blocking antibodies but highlight a number of outstanding questions that remain (Table 6). These studies have opened the door for clinical development of agents targeting a variety of additional coinhibitory and costimulatory molecules, including LAG-3, T cell Ig and mucin domain-3, GITR, OX-40, and 4-1BB, among others.

AUTHORSHIP

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