

Masterful Antibodies: Checkpoint Blockade

Nils Lonberg and Alan J. Korman



Abstract

Cancer therapeutics that target the immune system rather than the cancer cell itself are becoming standard of care in a growing number of different malignancies. Although cancer immunotherapy is not a new concept, the potential importance of this class of drugs was probably not fully appreciated as recently as a decade ago when much of the focus of cancer drug discovery was on cancer cell-targeted medicines. The authors

were lucky enough to be able to witness and participate in the discovery and development of ipilimumab and nivolumab, two relatively early examples of immune system-targeted drugs. The challenges associated with discovering and developing these molecules may be of historical interest and instructive for moving cancer immunotherapy forward for greater numbers of patients. *Cancer Immunol Res*; 5(4); 275–81. ©2017 AACR.

Historical Roots of Checkpoint Blockade

The possibility that our own immune systems might be harnessed to treat cancer has attracted many immunologists over many years (1). In 1957, Burnet (2, 3) outlined the logical framework for cancer immunotherapy as follows: (i) somatic mutation is the fundamental basis for cancer; (ii) somatic mutation can lead to antigenic differences that can be recognized by the host immune system; and (iii) the immune system, however, can acquire tolerance, allowing cancer cells to escape detection. Burnet proposed that "[a]ny therapeutic approach [to cancer] must be indirect and based on some exploitation of a physiological difference between the cancer cell and normal body cells (2)." He then pointed the way toward research that might lead to successful cancer therapy:

"What is to be sought is some means whereby the protective mechanism of the body has its reactivity against minor deviations from self-patterns made more sensitive—the converse of the effect of cortisone in damping down immunological reactivity. . . [and] research along these lines might be particularly valuable for its practical potentialities (2)."

Because of gaps that remained for decades in our fundamental understanding of the immune system, Burnet's suggestion did not exactly provide a roadmap. In particular, nondeletional mechanisms for acquired tolerance mediated by immune cell anergy, rather than selection of less immunogenic cancer cells or clonal deletion of immune cells, were not discovered for another 23 years (4). The first breakthroughs came from the identification of molecular components of the signaling pathways that control immune homeostasis. Krummel and Allison, Tivol and colleagues, and Walunas and colleagues identified CTLA-4 as a signaling molecule mediating attenuation of T-cell activity (5–7). Leach and

colleagues then exploited this finding to enhance antitumor immunity in a mouse model using a blocking hamster antibody to mouse CTLA-4 (8). Nishimura and colleagues identified PD-1 as a distinct signaling molecule mediating immune attenuation (9) and showed that, as with CTLA-4, blocking the PD-1 pathway could enhance antitumor immunity in a mouse model (10). Such negative signaling pathways were labeled "immunological checkpoints" by Pardoll in 1999 (11), and antagonistic targeting for cancer therapy is now commonly referred to as checkpoint blockade. Almost 60 years after Burnet, it is now clear that research along these lines was indeed valuable and that his hypothetical "converse cortisone" (cortisone blocks T-cell function) could be used to manipulate the immune system's protective mechanisms against cancers comprising somatic mutations (12, 13).

Drug Discovery

We entered this field not thinking of ourselves as cancer biologists or tumor immunologists, but rather as drug discoverers perched on the shoulders of the aforementioned. One of us (A.J. Korman, then at NeXstar Pharmaceuticals, a small biotech company) began collaborating with Jim Allison in 1996 with the goal of turning Allison's discovery into a practical therapy for treating cancer patients. The other one of us (N. Lonberg, then at Medarex, a small biotech company) joined the collaboration in 1998, bringing to the table a transgenic mouse platform for discovering fully human mAbs (14, 15). Since 1998, we have expanded our cancer drug discovery efforts (consolidated within Medarex in 2000) to include multiple targets associated with the immune system/tumor interface. Our goal was to discover novel molecules that could be practically manufactured, formulated, and stored, de-risk them as drugs in preclinical models and assays, and then get them tested in human patients as efficiently as possible, without trying the patience of investors.

One of the practical lessons we have learned over the past 20 years of drug discovery is the importance of noniterative processes that allow for rapid decision making. The human-immunoglobulin transgenic mouse platform directly provides drug lead candidates that frequently require little or no further optimization. Hybridoma isolation of mAbs allows for rapid production of small batches for use in *in vitro* assays and *in vivo* models, which in

Bristol-Myers Squibb, Biologics Discovery California, Redwood City, California.

Corresponding Author: Nils Lonberg, Bristol-Myers Squibb, 700 Bay Road, Redwood City, CA 94063. E-mail: nils.lonberg@bms.com

doi: 10.1158/2326-6066.CIR-17-0057

©2017 American Association for Cancer Research.

turn allows for rapid selection of a lead candidate and rapid entry into clinical testing. As an example, our first immune system, targeted cancer drug, ipilimumab, represented a significant toxicity risk going into the clinic. As CTLA-4 knockout mice develop a massive and fatal lymphoproliferative disorder (6), we were motivated to address this toxicity concern as rapidly as possible. Because ipilimumab was originally isolated from a transgenic mouse hybridoma and required no further optimization, we were able to generate phase I clinical material directly from cultured hybridoma calls. We were thus able to dose the first patient a mere 466 days after the spleen fusion that led to the isolation of the lead antibody. The results of the phase I trial allayed our toxicity concerns, and we then generated a recombinant CHO cell line for more efficient manufacture of material for further clinical studies and for eventual product launch.

Antibodies that Block CTLA-4

Human antibodies to CTLA-4 were screened for their ability to inhibit the interaction between CTLA-4 and B7 ligands while showing no reactivity with CD28 and little to no cross-reactivity with normal tissues. This resulted in the selection of clone 10D1, now known as ipilimumab (16). *In vitro* activity assays for CTLA-4 blockade were limited. Assays using transfected cells and modified constituent molecules were used to show blockade in a cellular assay. Notwithstanding the phenotype of CTLA-4 knockout animals, antibodies to CTLA-4 were safe in mice (8) and were similarly safe in cynomolgus macaques (16). Ipilimumab potentiated a vaccine response in cynomolgus macaques, and this provided some functional evidence that CTLA-4 blockade could be achieved in a nonhuman primate. Antitumor activity of ipilimumab in a human CTLA-4 transgenic mouse was also confirmatory, but this result was obtained only after clinical trials had begun (unpublished data).

Rapid production of ipilimumab for clinical trials from the 10D1 hybridoma by perfusion did pose an issue. Ipilimumab is a human IgG1 that can participate in antibody-dependent cellular cytotoxicity (ADCC). This may have been considered a poor choice for an antagonistic antibody; however, we showed that ADCC of activated T cells was limited. Contemporaneous with the development of ipilimumab, the expression of constitutive CTLA-4 on the surface of T regulatory cells (Treg) and the role of CTLA-4 in Treg function began to be appreciated. The initial clinical trials of ipilimumab were in prostate cancer and melanoma (17). Early signs of clinical activity were hopeful (2/14 prostate cancer patients showed >50% reduction of PSA), and objective responses were observed in metastatic melanoma.

How to dose anti-CTLA-4 was not evident. The first trials of ipilimumab did not include standard dose escalation trials; these trials were performed later in the development of the antibody (18). The initial dosing regimen of 3 mg/kg given 4 times at 3-week intervals was based on extrapolations from mouse and monkey data. The development of "autoimmune-like" adverse events was observed early in the human trials and, although somewhat expected, was a continuous source of surprise and scientific speculation. These included rash, colitis, and endocrine abnormalities. Some of these adverse events had the potential to progress to fatalities, particularly if not managed aggressively (19). The potential for severe toxicities contributed to the natural skepticism surrounding a novel mechanism for tumor therapy. That skepticism placed considerable pressure on the internal and external support for the program. For example, in a presentation at

American Society of Clinical Oncology (ASCO) in 2005 (Abstract 2501), Jim Yang from the NCI (Rockville, MD) reported the results in renal cell cancer (RCC) patients that included 3 deaths, curtailing enthusiasm for continued development in that indication despite evidence of patient benefit. However, it was quickly discovered that cessation of ipilimumab therapy together with aggressive use of topical and intravenous corticosteroids, and in some cases TNF α -blocking antibodies, were largely effective and compatible with continuing clinical responses. As the variety and frequency of these inflammatory toxicities became better understood, specific management algorithms were developed (20) that are now routinely applied to most immunotherapy trials involving checkpoint blockade and other immune activators.

The pivotal trial of ipilimumab included three arms in HLA-A2–positive patients with stage III/IV unresectable metastatic melanoma. The trial was based on earlier clinical testing from Steve Rosenberg at the NCI (21). They included (i) a control arm containing two heteroclitic peptides from gp100 that bind to HLA-A2; (ii) ipilimumab alone; and (iii) ipilimumab together with gp100 peptides (1:1:3 ratio; ref. 22). This now iconic trial (Fig. 1) showed an improvement in median overall survival as well as durable survival ("the tail on the curve") for the ipilimumab-containing groups and led to the approval of ipilimumab in 2011. Long-term survival from a pooled analysis of more than 4,800 patients from multiple trials revealed the long-term durability of many of these responses (23). If the endpoint of the phase III trial had been based on objective response rate, the trial would have been considered a failure.

A phase III trial in metastatic melanoma of a competing antibody to CTLA-4, tremelimumab, an IgG2 isotype that binds poorly to Fc γ RIII, was also in progress during the ipilimumab trials (24). IgG2 antibodies generally have a low affinity for Fc γ RIII; however, in individuals carrying an R131H variant of Fc γ RIIa, that receptor has a significant affinity for IgG2 antibodies such as tremelimumab (25). The failure of this trial and low objective response rate of ipilimumab-treated patients created much uncertainty as to the viability of checkpoint blockade. Although the precise cause of the failure of this trial is unknown, the dosing schedule could have been a contributing factor. Tremelimumab was dosed at 15 mg/kg every 3 months, and the median duration on drug for the tremelimumab arm of the trial was only 3 months. Therefore, approximately half the patients only received a single dose of the drug. Other reasons for the lack of activity could be attributed to patient selection or biased crossover to additional lines of therapy (16% of patients in the control arm reported using ipilimumab subsequent to chemotherapy). However, a subsequent meta-analysis of patients from several trials showed evidence of long-term survival similar to that observed with ipilimumab (26).

How Anti-CTLA-4 Antibody Works... Maybe?

While the studies of anti-CTLA-4 and the development of anti-PD-1 and PD-L1 antibodies (see below) were underway, we had an opportunity to revisit the mechanism of action of anti-CTLA-4 in the mouse. The isotype of the CTLA-4 antibody routinely used for murine tumor studies was murine IgG2b, which binds to activating Fc γ receptors (Fc γ R). Although the antibody is functional *in vivo*, we considered that it was possible that its activity could be limited by Fc γ R binding, that is, inadvertent delivery of an agonist signal

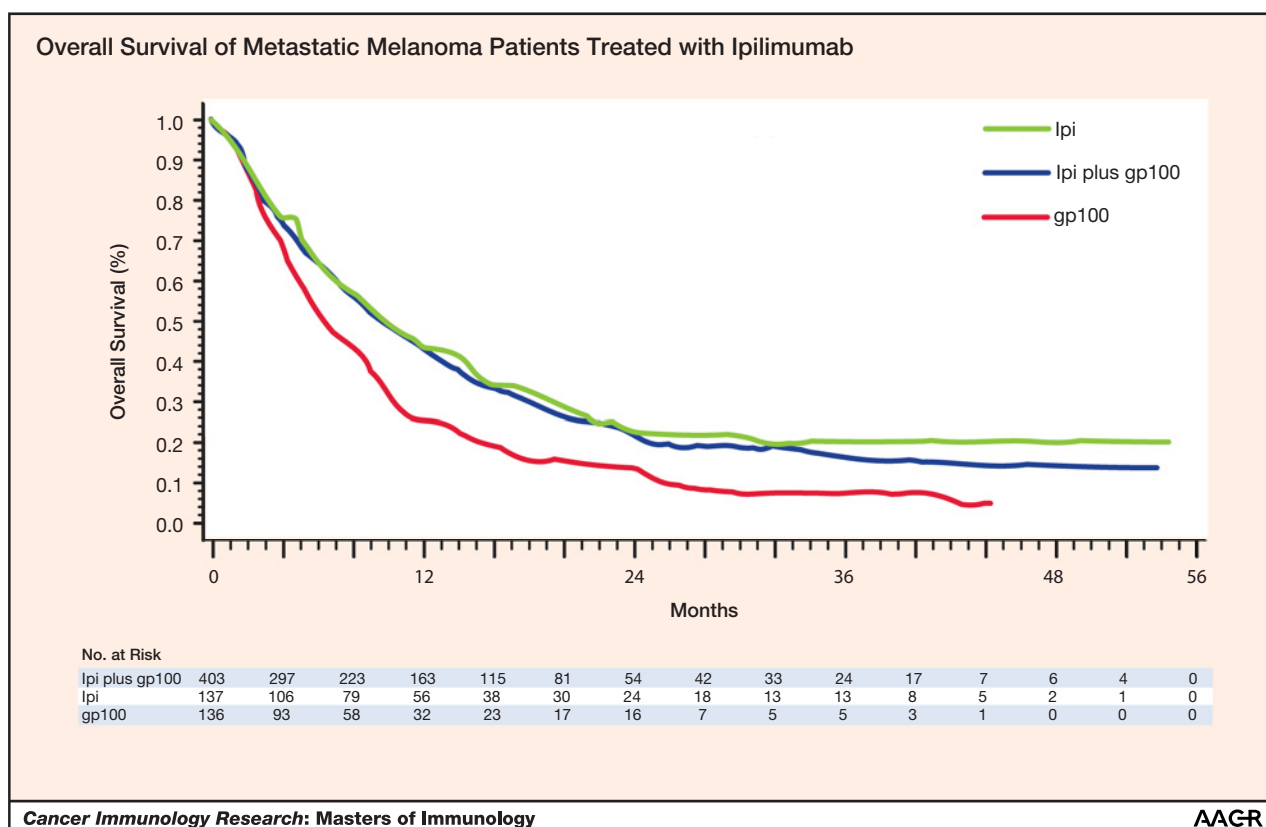


Figure 1.

Breakthrough clinical data from the pivotal phase III trial of ipilimumab, demonstrating long-term overall survival for stage III/IV metastatic melanoma patients treated with ipilimumab (ipi) alone or with a gp100 peptide vaccine, versus vaccine alone (adapted from The New England Journal of Medicine: F. Stephen Hodi, Steven J. O'Day, David F. McDermott, et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. Volume 363, Page 716. Copyright © 2010 Massachusetts Medical Society). Reprinted with permission from Massachusetts Medical Society.

via CTLA-4. When we tested Fc variants of the murine anti-CTLA-4, we made two surprising observations: CTLA-4 blockade alone, through the use of a non-Fc γ R-binding antibody, resulted in no antitumor activity; and improving the binding of antibody to Fc γ R receptors increased the activity of the antibody so that monotherapy activity with anti-CTLA-4-mIgG2a resulted in nearly complete antitumor activity in two sensitive tumor models (27). We and other laboratories reported (28, 29) that binding to Fc γ R resulted in specific depletion of CTLA-4-high Treg cells at the tumor site, without loss of these cells in the periphery. Treg cell depletion likely together with blockade of CTLA-4 on T effectors resulted in superior antitumor activity. This phenomenon also applied to targets of agonist antibodies whose receptors are expressed on Tregs as well as on T effector cells (e.g., GITR, OX40).

Does ipilimumab deplete Treg cells? Although ipilimumab has been through many clinical trials, whether Treg depletion occurs at the tumor site in man has not been rigorously determined. A study addressing this question suggests that ipilimumab can lyse CTLA-4⁺ Treg cells through nonclassical CD16⁺ monocytes (30). Despite the fact that tremelimumab and ipilimumab differ with respect to their Fc γ R binding, they appear to have only minor differences in activity and ability to induce immune-related adverse events (26). This suggests that in man, as opposed to mouse, CTLA-4 blockade alone may result in antitumor activity through T-effector cell

activation and perhaps a functional role for CTLA-4 blockade in Treg activity. Augmenting the ADCC activity of ipilimumab may provide for a novel therapeutic with enhanced activity if it is capable of differentially depleting Treg cells at the tumor.

Targeting the PD-1/PD-L Pathway

In 2002, we initiated a program focused on the PD-1 pathway. This led very quickly to the identification of nivolumab (31), an antibody that targets PD-1 and blocks PD-L1 and PD-L2 binding, and MDX-1105, an antibody that targets PD-L1 and blocks PD-1 and B7-1 binding. In contrast to CTLA-4 blockade, where *in vitro* functional assays using human T cells proved difficult to execute, lead selection for the PD-1 pathway programs was aided by a robust *in vitro* assay, the mixed lymphocyte reaction (MLR). PD-1 blockade in a human MLR assay resulted in the induction of IFN γ and other cytokines.

The decision to take nivolumab into clinical testing was not an easy one for a small biotech company like Medarex. We were expending considerable resources on the clinical testing of ipilimumab that, although the initial results were promising, was still considered a high-risk program. Nivolumab represented a doubling down on the mechanistic class of checkpoint inhibitors. The arguments that eventually prevailed were as follows: (i) we had

established a network of experienced and motivated clinical investigators through the ipilimumab program who were willing to test nivolumab; (ii) targeting PD-1 with nivolumab represented a potentially preferable and differentiated mechanism of action from ipilimumab; (iii) the observed expression of the PD-1 ligands in human tumor samples (10, 32) suggested that in some cases, patient antitumor immune responses might be preexisting and specifically impeded by PD-1 signaling; and finally (iv), simultaneous blockade of CTLA-4 and PD-1 in a mouse model provided synergistic antitumor activity (see below), suggesting that the combination of ipilimumab and nivolumab might also provide improved efficacy over monotherapy in human patients.

Nivolumab entered phase I clinical testing in 2006 and MDX-1105 in 2009 (33, 34). The initiation of human clinical trials for nivolumab was complicated by the disastrous phase I clinical trial of TGN1412, an agonist antibody (developed by TeGenero, a small biotech company) directed to the T-cell costimulatory molecule CD28 (35), that had taken place only a few months earlier and had resulted in multiple severe inflammatory reactions, including catastrophic systemic organ failure. Nivolumab was probably the first T-cell-activating molecule to come before the FDA for an IND decision after the TGN1412 trial, and a very cautious dose escalation was selected. Unlike the first-in-human

trial for ipilimumab, in which the drug was dosed at 3 mg/kg, the trial protocol for nivolumab involved a dose escalation with the initial patient cohort receiving 0.3 mg/kg and additional cohorts progressing to 1, 3, and 10 mg/kg.

The initial phase I clinical trial of nivolumab included 39 patients with a variety of different solid tumors. In this first-in-human experiment, we and our clinical collaborators at Johns Hopkins (Baltimore, MD) and other sites were encouraged by the relative safety (one serious adverse event) and preliminary evidence of activity, which included a durable complete response in a patient with microsatellite instability–high colorectal cancer and two partial responses in melanoma and renal cancer. In addition, one patient with non–small cell lung cancer (NSCLC) experienced significant tumor regression in a single lesion. We were particularly intrigued by this evidence of activity in lung cancer, for which good treatment options were limited. Data from our first multidosing trial confirmed the potential for the drug. At doses of 3 mg/kg and above given every 2 weeks, we saw objective response rates of 41%, 31%, and 22%, in patients with melanoma, RCC, and NSCLC, respectively (36). Also, the drug was relatively well tolerated. Grade 3 or 4 toxicities occurred in 14% of the 296 treated patients across all dosing cohorts, and 3 deaths were due to pneumonitis, a significant toxicity not observed at this frequency with ipilimumab. Mario Sznol's presentation of the preliminary

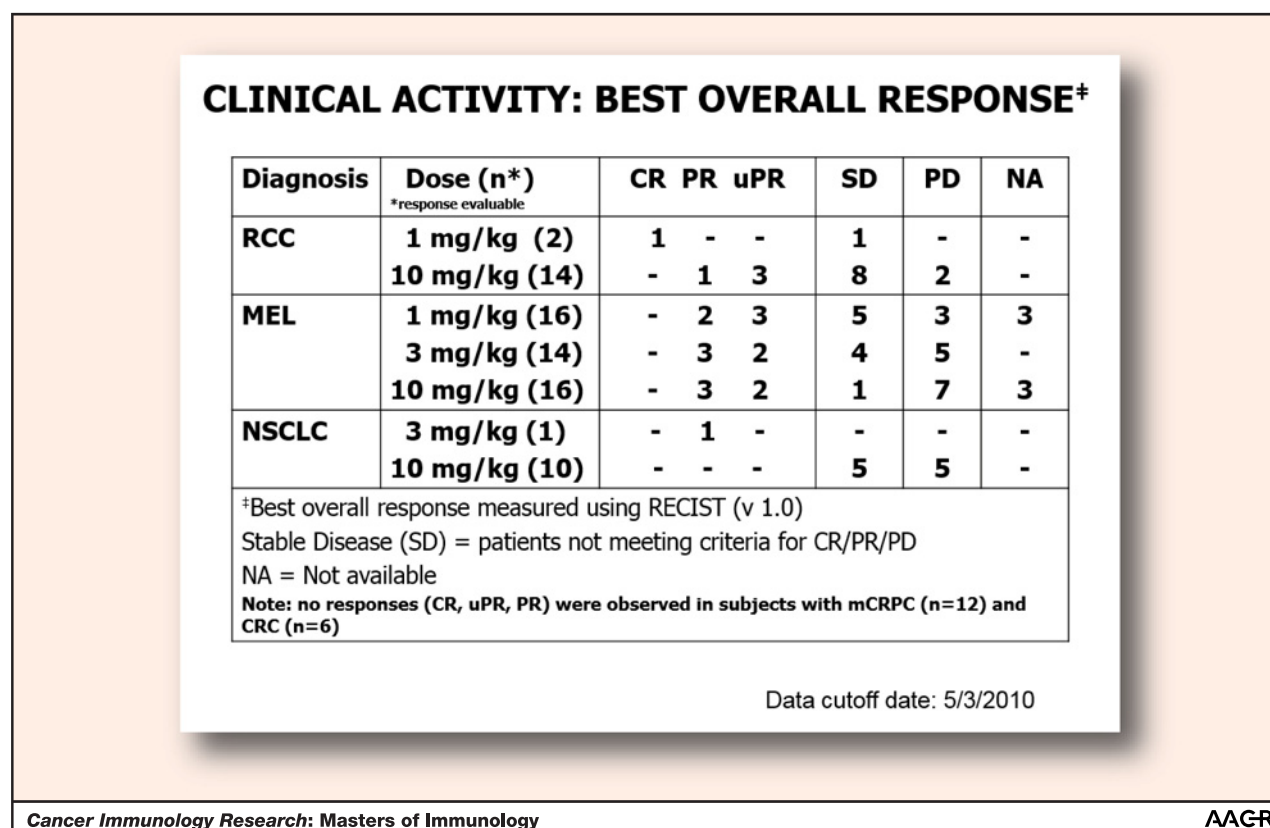


Figure 2.

First public presentation of the broad response of multiple tumor types to nivolumab in a multidose escalation trial as presented by M. Sznol at ASCO 2010 [J Clin Oncol 2010; 28:15s, (suppl; abstr 2506)]. RECIST, response evaluation criteria in solid tumors; MEL, melanoma; mCRPC, metastatic castration-resistant prostate cancer; CRC, colorectal carcinoma; CR, complete response; PR, partial response; uPR, unconfirmed partial response; PD, progressive disease; SD, stable disease; NA, not available.

Table 1. Explaining differential responses to immunotherapy across patient populations

Sources of variation	Examples	Practical impact
Somatic neoantigens	Tumor mutational load and mutations that affect antigen presentation (MHC and antigen processing)	<ul style="list-style-type: none"> • Patient selection • Vaccines (neoantigen or shared antigens for low mutational load tumors)
Somatic driver mutations	Driver mutations that correlate with tumor immune infiltration	<ul style="list-style-type: none"> • Patient selection • Targeted therapies • Drug combination selection • New drug target identification
Inflammatory status/ phenotype of tumor	<ul style="list-style-type: none"> • Checkpoint ligand expression on tumor or TIL (e.g., PD-L1, PD-L2) • Expression of checkpoint molecules on T effector cells in TILs vs. periphery (immunotherapy naïve and immunotherapy failures/nonresponders) • Differential expression of molecules in Treg cells in TILs vs. periphery • Innate immune cell infiltrate (e.g., NK, macrophage, dendritic cells) 	<ul style="list-style-type: none"> • Patient selection • Drug combination selection (e.g., PD-1 pathway blockade + anti-LAG-3, TIGIT, TIM-3, GITR, OX40, CD137, ICOS, CSF1R, KIR2DL) • New drug target identification
Patient immune status including the microbiome	Factors impacting immune response to cancer [age, history of infection, vaccination, and microbiome(s)]	<ul style="list-style-type: none"> • Patient selection • New drug target identification
Germline allelic variation	Polymorphisms in target and ligand(s), target pathway genes, FcγR	<ul style="list-style-type: none"> • Patient selection • Drug combination selection • New drug target identification

results from this trial at ASCO in 2010 (Abstract 2506; Fig. 2) was probably a catalyst for the race that ensued to develop PD-1 pathway blockers in multiple cancers. The enthusiastic embrace of the PD-1/PD-L1 pathway as a target for cancer therapy has now led to three distinct marketed drugs: two PD-1 antibodies [pembrolizumab (37) and nivolumab] and a PD-L1 antibody (atezolizumab; refs. 38 and 39). Several other antibodies that target this pathway have also entered clinical testing (40, 41). It is not yet clear from the available data whether there is a difference in safety or efficacy between targeting PD-1 or PD-L1. The impact of different Fc formats for these antibodies is also not yet clear; however, preclinical models suggest format could be important (42).

Since its discovery only 15 years ago, nivolumab has now been tested in successful monotherapy registrational trials in a variety of cancer types and therapeutic settings, including metastatic melanoma, RCC, squamous and nonsquamous NSCLC, Hodgkin lymphoma, and squamous cell carcinoma of the head and neck. This rapid expansion into multiple indications, to the benefit of thousands of patients, was made possible through the 2009 acquisition of Medarex by Bristol-Myers Squibb. The acquisition kept our drug discovery team intact and put the portfolio of immune system-targeted drugs into the hands of the experienced development team at Bristol-Myers Squibb.

Combination Immunotherapy

The use of combinations in immuno-oncology is predicated on the need to impact multiple pathways of immunosuppression. Ideally, the combination of antibodies targeting different cell types [activating cytolytic T or natural killer (NK) cells and inhibiting suppressive cell types, such as Treg cells or macrophage/myeloid cells] would promote enhanced activity. As many of the targets of immune checkpoint blockade or agonist antibodies to costimulatory receptors are present on the same cell as well as on multiple cell types, antibodies to these targets may combine in unexpected ways. Combination chemotherapy pro-

vides a historical example of addressing multiple targets of cancer cell growth as well as assessment of the limits of tolerability in treatment. Similarly, tolerability is also an issue in combinations with multiple immuno-oncology agents.

In 2005, we (Mark Selby and colleagues at Medarex) conducted a set of mouse tumor model experiments that demonstrated a significant enhancement of antitumor activity by combining PD-1 and CTLA-4 blockade (see US patent no. 8008449). Other laboratories also tested the combination of these antibodies in various tumor models (43, 44). Our analysis of this combination in mice showed that it improved tumor-infiltrating lymphocyte (TIL) function in a variety of ways, including Treg depletion and T-effector cell activation mediated by anti-CTLA-4 and activation of PD-1⁺ CD8⁺ TILs through PD-1 blockade (45). A role for tumor antigen-specific CTLA-4⁺PD-1⁺ T cells was also suggested in this work. Observations in man suggest that the activity of anti-PD-1 may depend on the frequency of CTLA-4⁺PD-1⁺ CD8⁺ T cells in melanoma TILs (46). A theoretical framework for the relevance of multiple negative regulators contributing to T-cell nonresponsiveness was developed in the field of chronic virus infection (47). Unlike anti-PD-1, anti-CTLA-4 was never shown to play a role in exhausted T-cell responses in lymphocytic choriomeningitis virus (LCMV; ref. 48). In contrast, the effectiveness of a combination of anti-PD-1 and anti-LAG-3 (a negative regulator with homology to CD4) was suggested by results in LCMV and borne out in tumor models (49, 50).

The toxicology studies for the ipilimumab/nivolumab combination in cynomolgus macaques revealed additional limited adverse events as compared with individual antibodies (44). Although these signals may have provided a cautionary note, we viewed these adverse events positively as indicative of the potency of this combination. The first patients treated with the combination of anti-CTLA-4 and anti-PD-1 in a dose-ranging study at MSKCC (New York, NY) and Yale (New Haven, CT; ref. 51) beginning in 2009 revealed that the combination could elicit dramatic responses in tumor reduction, albeit with an adverse event rate that was elevated over what had been observed with

ipilimumab or nivolumab monotherapy in that setting. This initial study has been extended in larger subsequent trials (52, 53), and the combination was approved in the United States for metastatic melanoma in 2015. The combination of ipilimumab and nivolumab is now undergoing testing in a variety of additional indications and employing different dose and schedule regimens to reduce the toxicities observed in these earlier trials.

The Limits of Immunotherapy?

The limits of currently available immunotherapies in certain tumor types are beginning to emerge. Defining the cellular, molecular, and mutational parameters that characterize responsive tumor types and individuals (e.g., tumor infiltrate and location, PD-L1 expression, and tumor mutational burden) is under intense study (Table 1). We are now confronted with the possibility that existing immunotherapies may not be effective in certain settings and that novel therapies are needed. As we have argued in combining anti-PD-1 and anti-CTLA-4, multiple mechanisms of nonresponsiveness may be alleviated by targeting multiple molecules on T cells and other immunosuppressive cell types and soluble mediators of suppression. A variety of combinations of checkpoint inhibitors together with agonist antibodies to costimulators on T cells, as well as additional antagonists of attenuating pathways are under investigation (54). In addition, antibodies targeting other cell types, such as myeloid cells, antigen-presenting cells, and NK cells, molecules

targeting other immunosuppressive pathways, and tumor-targeted agents including standard-of-care therapies such as radiation, chemotherapy, and targeted therapy, are also being explored together with checkpoint inhibitors.

The enormity of the potential combinations, and the proliferation of trials exploring this landscape, has precipitated a backlash by some commentators. However, there is persuasive preclinical rationale for many of these combinations, and rather than decry this exploration, we believe that we should promote them in a manner in which on-treatment biopsies allow us to understand the science and guide us to those combinations that offer the greatest promise of efficacy and tolerability. It is only through this clinical exploration that we can understand the limits of cancer immunotherapy. We remain optimistic that these limits are far beyond the borders defined by existing therapies.

Disclosure of Potential Conflicts of Interest

N. Lonberg has ownership interest in Bristol-Myers Squibb stock. A.J. Korman has ownership interest in Bristol-Myers Squibb stock.

Acknowledgments

N. Lonberg and A.J. Korman are indebted to their dedicated colleagues and collaborators who contributed to the discovery and development of nivolumab and ipilimumab over the past two decades, as well as to the many clinical investigators and the patients who volunteered for clinical trials to test these molecules and turn them into cancer therapies.

References

1. Srivastava PK. Neoepitopes of cancers: looking back, looking ahead. *Cancer Immunol Res* 2015;3:969–77.
2. Burnet M. Cancer: a biological approach. III. Viruses associated with neoplastic conditions. IV. Practical applications. *Br Med J* 1957;1:841–7.
3. Burnet M. Cancer: a biological approach. I. The processes of control. *Br Med J* 1957;1:779–86.
4. Nossal GJ, Pike BL. Clonal anergy: persistence in tolerant mice of antigen-binding B lymphocytes incapable of responding to antigen or mitogen. *Proc Natl Acad Sci U S A* 1980;77:1602–6.
5. 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.
6. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. 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.
7. Walunas TL, Lenschow DJ, Bakker CY, Linsley PS, Freeman GJ, Green JM, et al. CTLA-4 can function as a negative regulator of T cell activation. *Immunity* 1994;1:405–13.
8. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science* 1996;271:1734–6.
9. Nishimura H, Minato N, Nakano T, Honjo T. Immunological studies on PD-1 deficient mice: implication of PD-1 as a negative regulator for B cell responses. *Int Immunol* 1998;10:1563–72.
10. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc Natl Acad Sci U S A* 2002;99:12293–7.
11. Pardoll DM. Inducing autoimmune disease to treat cancer. *Proc Natl Acad Sci U S A* 1999;96:5340–2.
12. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med* 2014;371:2189–99.
13. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology: Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015;348:124–8.
14. Lonberg N. Fully human antibodies from transgenic mouse and phage display platforms. *Curr Opin Immunol* 2008;20:450–9.
15. Lonberg N, Taylor LD, Harding FA, Trounstein M, Higgins KM, Schramm SR, et al. Antigen-specific human antibodies from mice comprising four distinct genetic modifications. *Nature* 1994;368:856–9.
16. Keler T, Halk E, Vitale L, O'Neill T, Blanset D, Lee S, et al. Activity and safety of CTLA-4 blockade combined with vaccines in cynomolgus macaques. *J Immunol* 2003;171:6251–9.
17. Small EJ, Tchekmedyan NS, Rini BI, Fong L, Lowy I, Allison JP. A pilot trial of CTLA-4 blockade with human anti-CTLA-4 in patients with hormone-refractory prostate cancer. *Clin Cancer Res* 2007;13:1810–5.
18. Wolchok JD, Neyns B, Linette G, Negrier S, Lutzky J, Thomas L, 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.
19. Beck KE, Blansfield JA, Tran KQ, Feldman AL, Hughes MS, Royal RE, et al. Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. *J Clin Oncol* 2006;24:2283–9.
20. Naidoo J, Page DB, Li BT, Connell LC, Schindler K, Lacouture ME, et al. Toxicities of the anti-PD-1 and anti-PD-L1 immune checkpoint antibodies. *Ann Oncol* 2016;27:1362.
21. Phan GQ, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, et al. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc Natl Acad Sci U S A* 2003;100:8372–7.
22. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010;363:711–23.
23. Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, et al. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. *J Clin Oncol* 2015;33:1889–94.
24. Ribas A, Kefford R, Marshall MA, Punt CJ, Haanen JB, Marmol M, 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.
25. Schneider-Merck T, Lammerts van Bueren JJ, Berger S, Rossen K, van Berkel PH, Derer S, et al. Human IgG2 antibodies against epidermal growth factor receptor effectively trigger antibody-dependent cellular cytotoxicity but, in contrast to IgG1, only by cells of myeloid lineage. *J Immunol* 2010; 184:512–20.
 26. Eroglu Z, Kim DW, Wang X, Camacho LH, Chmielowski B, Seja E, et al. Long term survival with cytotoxic T lymphocyte-associated antigen 4 blockade using tremelimumab. *Eur J Cancer* 2015;51:2689–97.
 27. Selby MJ, Engelhardt JJ, Quigley M, Henning KA, Chen T, Srinivasan M, et al. Anti-CTLA-4 antibodies of IgG2a isotype enhance antitumor activity through reduction of intratumoral regulatory T cells. *Cancer Immunol Res* 2013;1:32–42.
 28. Simpson TR, Li F, Montalvo-Ortiz W, Sepulveda MA, Bergerhoff K, Arce F, et al. Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. *J Exp Med* 2013;210:1695–710.
 29. Bulliard Y, Jolicoeur R, Windman M, Rue SM, Ettenberg S, Knee DA, et al. Activating Fc gamma receptors contribute to the antitumor activities of immunoregulatory receptor-targeting antibodies. *J Exp Med* 2013;210: 1685–93.
 30. Romano E, Kusio-Kobialka M, Foukas PG, Baumgaertner P, Meyer C, Ballabeni P, et al. Ipilimumab-dependent cell-mediated cytotoxicity of regulatory T cells *ex vivo* by nonclassical monocytes in melanoma patients. *Proc Natl Acad Sci U S A* 2015;112:6140–5.
 31. Wang C, Thudium KB, Han M, Wang XT, Huang H, Feingersh D, et al. *In vitro* characterization of the anti-PD-1 antibody nivolumab, BMS-936558, and *in vivo* toxicology in non-human primates. *Cancer Immunol Res* 2014; 2:846–56.
 32. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* 2002;8:793–800.
 33. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol* 2010;28:3167–75.
 34. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* 2012;366:2455–65.
 35. Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* 2006;355:1018–28.
 36. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012;366:2443–54.
 37. Khoja L, Butler MO, Kang SP, Ebbinghaus S, Joshua AM. Pembrolizumab. *J Immunother Cancer* 2015;3:36.
 38. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet* 2016;387:1909–20.
 39. Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. *Lancet* 2017;389:67–76.
 40. Sunshine J, Taube JM. PD-1/PD-L1 inhibitors. *Curr Opin Pharmacol* 2015;23:32–8.
 41. Homet Moreno B, Ribas A. Anti-programmed cell death protein-1/ligand-1 therapy in different cancers. *Br J Cancer* 2015;112:1421–7.
 42. Dahan R, Segal E, Engelhardt J, Selby M, Korman AJ, Ravetch JV. Fc gammaRs modulate the anti-tumor activity of antibodies targeting the PD-1/PD-L1 axis. *Cancer Cell* 2015;28:285–95.
 43. Duraiswamy J, Freeman GJ, Coukos G. Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors–response. *Cancer Res* 2014;74:633–4.
 44. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. *Proc Natl Acad Sci U S A* 2010;107:4275–80.
 45. Selby MJ, Engelhardt JJ, Johnston RJ, Lu LS, Han M, Thudium K, et al. Preclinical development of ipilimumab and nivolumab combination immunotherapy: mouse tumor models, *in vitro* functional studies, and cynomolgus macaque toxicology. *PLoS One* 2016;11: e0161779.
 46. Daud AI, Loo K, Pauli ML, Sanchez-Rodriguez R, Sandoval PM, Taravati K, et al. Tumor immune profiling predicts response to anti-PD-1 therapy in human melanoma. *J Clin Invest* 2016;126:3447–52.
 47. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol* 2015;15:486–99.
 48. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 2006;439:682–7.
 49. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol* 2009;10:29–37.
 50. Woo SR, Turnis ME, Goldberg MV, Bankoti J, Selby M, Nirschl CJ, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res* 2012;72: 917–27.
 51. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med* 2013;369:122–33.
 52. Larkin J, Hodi FS, Wolchok JD. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med* 2015;373:1270–1.
 53. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N Engl J Med* 2015;372:2006–17.
 54. Melero I, Berman DM, Aznar MA, Korman AJ, Pérez Gracia JL, Haanen J, et al. Evolving synergistic combinations of targeted immunotherapies to combat cancer. *Nat Rev Cancer* 2015;15:457–72.