T Cell Co-regulatory Signals and Their Role in Cancer Therapy

Eitan R. Friedman, Lynn Feun, Pasquale Benedetto, and Joseph D. Rosenblatt

Abstract T cell activation is initiated by signaling through the TCR after binding to MHC-presented antigen. Both positive and negative co-regulatory signaling can modify this original activating signal. T cell co-regulation is provided by receptors on the T cell surface membrane. Inhibitory signals are provided by CTLA4 or PD-1, while co-stimulation is provided by CD28, 4-1BB, OX40, or GITR. These signals are being studied in the laboratory and at the clinical level in order to therapeutically modulate T cell responses to tumor cells. T cells can recognize tumor antigens in the same way that these immune cells recognize bacteria, viral antigens, and other foreign peptides. If appropriately activated by the tumor antigen, the immune system can mediate an antitumor gene response. Unfortunately, immune cells with antitumor specificity are not present in abundance and are often inhibited by tumor expression of CTLA4 or PD-1 ligands. Thus manipulation of co-regulatory signals can be used as a strategy by which to strengthen the immune response, via augmentation of T cell co-stimulation and/or blockade of inhibitory signals, in order to effectively treat cancer. In this chapter we review the basic principles and science as well as the ongoing clinical efforts in this area that have had recent success and offer additional promise.

1 Normal Biology of T Cell Activation and Checkpoint Signaling

T cells have long been a focus of translational oncology research, for in addition to their ability to dispose of foreign viruses, bacteria, and infected tissues, they may possess the ability to recognize cancer cells. Cell-mediated immunity may be

E.R. Friedman (✉) • L. Feun • P. Benedetto • J.D. Rosenblatt (✉)
School of Medicine, University of Miami, Miami, FL, USA
e-mail: EFriedman@med.miami.edu; JRosenblatt@med.miami.edu

J.D. Rosenblatt et al. (eds.), *Advances in Tumor Immunology and Immunotherapy*, Current Cancer Research, DOI 10.1007/978-1-4614-8809-5_14,

mediated by cytotoxic, CD8+ effector T lymphocytes, which recognize and kill cells expressing targeted antigens, and by CD4+ helper T cells that can facilitate CD8 T cell activation and induce B cells to mature into antibody-producing plasma cells [1].

The mounting of an immune response by the adaptive immune system involves coordination between several different cell types. This process begins in a lymph node where an antigen-presenting cell (APC), having phagocytized foreign antigen and processed it into smaller peptides intracellularly, presents it on its surface via the major histocompatibility complex (MHC) to a T cell for antigen sampling. CD4 T cells include TH1 cells which amongst their activities secrete cytokines such as interferon γ that activate macrophages and TH2 cells which generally secrete cytokines such as IL-4 to activate the B cells to produce antigen-specific antibodies. Alternatively, a virally infected somatic cell may directly present peptide antigen through MHC-I present on the cell surface. The T cell receptor (TCR), together with CD4 or CD8 co-receptors, recognizes cognate antigen presented on the MHC by the APC. A cognate antigen is an antigen recognized by both the T cell via the TCR complex and the APC or the somatic cell via the MHC complex. This provides the first step towards T cell activation, initiating a signaling cascade within the T cell known as signal 1, causing a naïve T cell, which has never been exposed to antigen, to become primed. However, to become an armed effector T cell and to allow its subsequent expansion, the T cell also requires a second signal. The requisite co-regulatory ligands are provided as surface molecules by the same APC presenting the MHC-bound antigen to the TCR. When cognate receptors are bound by these co-stimulatory ligands, positive signals are imparted to the activated T cell as signal 2.

In addition to these activating, co-stimulatory signals, other co-regulatory signals may dampen the T cell response [1]. Such inhibitory signals provided to the T cell are known as immune checkpoints, as they limit the extent to which an immune response is strengthened and prevent hyperactivity and autoimmunity. Examples of co-inhibitory receptors present on T lymphocytes include CTLA4 and PD-1, and examples of co-stimulatory receptors include CD28, 4-1BB, OX40, and GITR (Fig. 1).

Several of these immune checkpoints are being studied in the laboratory and in the clinical setting as potential targets of immunotherapy, with promising results. Cancer cells express tumor-specific antigens due to mutations that occur in their genome and due to epigenetic changes that alter normal expression of genes, which can theoretically be recognized and targeted by the immune system. Recent studies have shown that targeting T cell co-regulatory signals can slow, halt, or even reverse cancer growth. The hypothesis underlying these efforts is that blocking immune checkpoint inhibitory signals or strengthening T cell co-stimulatory signals with biologic therapeutics will strengthen the immune system's response against tumor antigens. In point of fact, the cancer cells themselves often express T cell inhibitory ligands on their surface that weaken the immune response [2]. Hence, efforts have been directed at blocking inhibitory signals in order to strengthen effector responses.

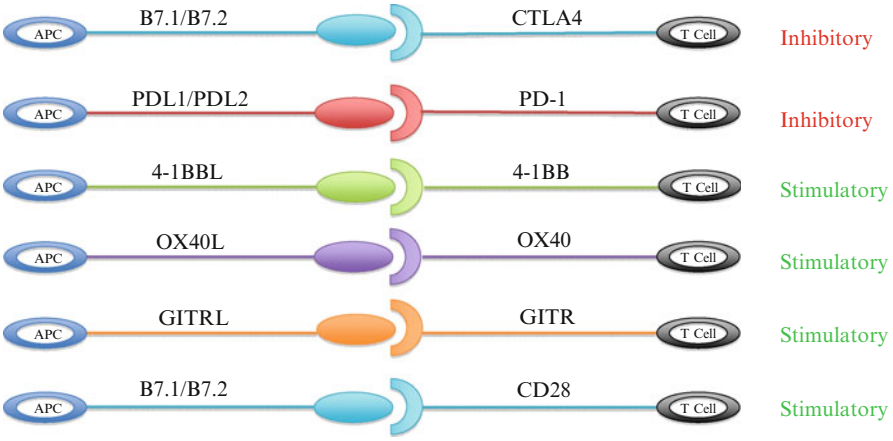

Fig. 1 APC and T cell with their ligands and bound co-regulatory receptors. The ligands on the APC bind to the T cell co-regulatory receptors and can alter the signaling of these receptors. CTLA4 and PD-1 are inhibitory receptors, and positive signals are sent from 4-1BB, OX40, GITR, and CD28

2 CTLA4 and CD28

CTLA4 is one of the better studied and well-defined inhibitory immune checkpoint receptors. It is a member of the immunoglobulin superfamily, and its gene is located on chromosome 2q33. CTLA4 is expressed on the surface membrane of activated T cells and counters the stimulatory effect of the CD28 receptor, which is another member of the immunoglobulin superfamily and is present on naïve CD4 and most naïve CD8 T cells [3]. The CD28 gene is also found on chromosome 2q33, and its protein on the T cell surface membrane binds to its ligands B7.1 (CD80) or B7.2 (CD86) expressed on APCs. The resulting co-stimulatory signals induce T cells to proliferate and differentiate into effector and memory T cells. However, once activated the T cells up-regulate the expression of CTLA4, providing negative feedback to dampen the T cell activation signals [1]. CTLA4 is an alternate receptor for B7, and its amino acid sequence is very similar to that of CD28. In fact, B7 binds CTLA4 with an affinity 20 times stronger than CD28. Once bound, CTLA4 signal transduction activates inhibitory phosphatases, including SHP2 and PP2A, which counter the stimulatory kinase signaling of the B7:CD28 interaction. CTLA4 signaling has an inhibitory effect on CD8 effectors, preventing their cytotoxic effects, and on CD4 helper T cells, preventing these cells from activating B cells. In contrast, CTLA4 activation has been found to have a stimulatory effect on T regulatory cells (Tregs) where it is expressed constitutively, as its signaling causes increased immunosuppressive Treg activity. The mechanism of Treg stimulation by CTLA4 signaling is not known. The normal role of CTLA4 is to keep the immune system from becoming over-activated by preventing the uncontrolled activation of naïve T cells, and CTLA4 knockout mice develop fatal T cell hyperactivity and autoimmunity [2].

CTLA4 has been well studied in the laboratory in preclinical studies. Partial blockade of the CTLA4 receptor with antibodies in mice has demonstrated significant antitumor activity. Researchers predicted that antibody blockade of the CTLA4 receptor would cause increased immune activity and autoimmunity [2]. However, although CTLA4 knockout is lethal in mice, anti-CTLA4 antibodies are better tolerated as they only partially inhibit CTLA4 activity and can be employed following the development of a normal T cell repertoire. Notably, such partial blockade is sufficient for an inhibitory effect on tumor growth [4]. Studies have been done showing no effect on melanoma tumors in mice treated with CTLA4 antibody alone; however, when treated with antibody in addition to a GM-CSF-expressing tumor vaccine, inhibition was observed in 80 % of mice. GM-CSF vaccines consist of cancer cells that secrete GM-CSF, which causes migration and accumulation of APCs at the injection site, allowing increased antigen presentation and thus increased activation of the T cell immune response [5]. In addition, there was rejection of the melanoma following rechallenge, suggesting the establishment of immunological memory.

B7 and CD28 have also been the focus of research efforts. T cells in the tumor microenvironment become anergic secondary to receiving signaling through only the TCR:MHC complex as the co-stimulatory ligands B7.1 and B7.2 are not present on the tumor cells and receipt of signal 1 in the absence of signal 2 induces T cell anergy. In preclinical experiments B7 was shown to be important when its exogenous expression in tumor cells induced a CD8 T cell response allowing for tumor rejection. These experiments were performed in several different tumor models, and rejection was seen primarily with relatively immunogenic tumors [6].

Anti-CD28 agonistic antibodies have been used to expand T cell populations. These antibodies allow for T cell proliferation, survival, and cytokine secretion. In preclinical experiments in which humanized anti-CD28 antibodies were injected into monkeys, peripheral T cells were activated, with secretion of low levels of proinflammatory cytokines. However, in a phase I clinical trial with a super-agonist humanized antibody TGN1412, CD28 resulted in severe cytokine storm which was life threatening for many patients [7]. Subsequent efforts have largely involved use of anti-CD28 antibodies for purposes of ex-vivo T cell expansion.

3 PD-1

PD-1 is another immune checkpoint inhibitory receptor on the T cell surface membrane. The PD-1 receptor is a 50–55 kDa transmembrane glycoprotein receptor of the immunoglobulin superfamily. The ligands for PD-1 are PDL1, also referred to as B7-H1 or CD274, and PDL2, also referred to as B7-DC or CD273. These ligands are up-regulated on APCs during the inflammatory response. PDL1 is up-regulated in response to interferon γ and is expressed on hematopoietic, endothelial, and epithelial cells. PDL2 is expressed on macrophages and dendritic cells in response to IL-4 and other cytokines. The PD-1 receptor is expressed on B cells, NK T cells,

and Tregs. PD-1 limits autoimmunity and the T cell response during inflammation and primarily inhibits activated effector T cells. PD-1 dampens the T cell response in the peripheral tissues during inflammation, and it also limits the immune response to prevent autoimmunity. Since its ligands are expressed on peripheral surrounding tissues, these areas are protected from inflammation spreading to areas outside the main focus of inflammation [8]. PD-1 also plays a role in induction of T cell anergy and tolerance [9]. Its expression is increased following T cell activation. Binding of PDL1 or PDL2 to PD-1 results in activation of the inhibitory phosphatase SHP2 and decreased TCR signaling. B7.1 (CD80) on the T cell has also been shown to bind PDL1, sending additional inhibitory signals into the T cell [2]. PD-1 knockout mice also develop significant autoimmunity suggesting that, as with CTLA4, PD-1 prevents the immune system from becoming overactive [8]. Other studies in mice infected with lymphocytic choriomeningitis virus have shown that CD8 T cells in a chronic infectious setting become "exhausted" and less active and that antibody blockade of PD-1 restores the CD8 T cell function and reverses such exhaustion [10]. PD-1 has also been shown to deplete memory B cells, as shown in experiments in which rhesus macaques infected with the SIV virus had rapid depletion of memory B cells that specifically expressed PD-1. The researchers then blocked PD-1 using antibodies in vitro, which prevented the depletion of the memory B cells. Inhibiting PD-1 using antibodies in macaques resulted in increased humoral immunity, presumed to be due to increased survival of memory B cells [11]. PDL1 has also been shown to promote Treg inhibitory functions, as PDL1-coated beads promote Treg proliferation in vitro and Tregs are significantly reduced in number in dual $PDL1^{-/-}/PDL2^{-/-}$ double-knockout mice [12].

Malignant tumors often contain tumor-infiltrating CD8 and Treg lymphocytes that express PD-1, and tumor cells at times express PDL1 or PDL2 leading to T cell anergy. In malignant cells, PDL1 expression increases when the PTEN tumor-suppressor gene is deactivated or when the cells are exposed to interferon γ. Tumor expression of inhibitory T cell checkpoint ligands is an example of the tumor co-opting natural immune functions to protect against immune attack and induce tolerance. Inhibiting PD-1 signaling therefore has the potential to increase immunity against cancer [2].

There have been several preclinical mouse studies showing that antibody blockade of the PD-1 receptor or its ligand inhibits tumor growth. Anti-PDL1 antibodies were administered to mice with myeloma and inhibited cancer cell growth transiently; however, in mice deficient for PD-1, tumor growth was inhibited completely without the addition of anti-PDL1 antibodies [13]. Mice lacking PD-1, PDL1, or PDL2 survive, unlike CTLA4 knockout mice, suggesting that there would be less toxicity associated with its blockade in humans, and this appears to be true in human trials [2]. When over-expressed in mouse tumors, PDL1 promotes tumor-reactive T cell apoptosis and tumor cell proliferation. It has also been shown that human cancers such as lung, ovary, colon, and melanoma express increased PDL1 relative to their normal cell counterparts [14]. Therefore, PD-1 inhibition appears to be a viable strategy by which to augment antitumor response.

4 4-1BB/CD137

Positive, co-stimulatory regulators have also been shown to have potential as therapeutic targets. One such example is 4-1BB, also known as CD137, a 27 kDa member of the TNF receptor superfamily. 4-1BB signaling stimulates survival and proliferation signals and inhibits apoptosis. 4-1BB ligand activates 4-1BB. While CD28 co-stimulation causes expansion of CD4 T cells, CD8 T cells are activated preferentially by 4-1BB. In particular, beads linked to anti-CD28 stimulate CD4 but not CD8 T cells to expand in vitro, while the converse is seen with beads linked to anti-4-1BB antibodies. Prior to activation only a small proportion of resting naïve and memory CD8 T cells express 4-1BB. Furthermore it was found that 4-1BB signaling stimulated the growth and survival of CD8 memory cells while CD28 signaling allowed for proliferation of naïve CD8 cells. This suggests that temporally in CD8 T cell activation, CD28 signaling occurs first, which then leads to up-regulation of 4-1BB, allowing 4-1BB signaling which can then strengthen the positive co-stimulation. Potentially, 4-1BB signaling can be utilized to specifically increase antigen-specific memory CD8 T cells which can then be utilized in adoptive cell transfer to specifically allow proliferation of antigen-specific CD8 T cells [15]. 4-1BB, although mainly expressed on the surface of CD8 T cells, can also be found on CD4, NK, dendritic, and Treg cells. 4-1BB ligand also stimulates release of IL-12 and IL-8 by dendritic cells and macrophages [16].

Preclinical experiments studying 4-1BB show that the immune response is strengthened through increased 4-1BB signaling. 4-1BB can be activated in T cells by antibodies or by soluble 4-1BB ligand [17]. Mittler et al. showed that anti-4-1BB blocking antibodies decrease activation of T cells upon challenge with T cell-dependent antigens and increase the number of anergic CD4 T cells [18]. Similarly, Hong et al. showed that anti-4-1BB blocking antibodies suppress T cell reaction to ovalbumin in monkeys [19].

The first studies to show that 4-1BB antibody has anticancer activity were done using mouse models for sarcoma or mastocytoma. In mice treated with an agonistic anti-4-1BB antibody, cytotoxic T cell activity increased and the tumors regressed [20]; this effect required both CD4 and CD8 T cells [21]. Other experiments in mice have combined anti-4-1BB with other treatments to show anticancer effect; for example, treatment of melanoma cells with IL-12 gene transfer alone or with anti-4-1BB alone was not as effective as when these strategies were combined [2].

5 OX40

OX40 (CD134) is another co-stimulatory regulator present on the surface of activated T cells and like 4-1BB is also a member of the TNF receptor superfamily. OX40 is present mainly on CD4 T cells but is also found on CD8 T cells, DCs, PMNs, and Tregs. OX40 expression and up-regulation on the T cell surface membrane are

dependent on naïve T cell activation through CD28 [22]. OX40 is increased 48–72 h after the T cell is activated through CD28, and 120 h later it is down-regulated [23]. Binding of OX40 to its ligand OX40L (CD252), which is present mainly on APCs, stimulates proliferation, function, and survival of T cells [24]. It also stimulates the secretion of IL-2 and IL-2R [23]. Studies show that initial versus delayed OX40 signaling causes antibody class switching which allows different antibody subsets to be secreted. Initial OX40 binding causes interferon γ and IL-4 to be secreted as well as IgG2a and IgG1, while delayed signaling causes a stronger TH2 cell response than that seen in the initial response and facilitates IgG1 production.

Preclinical experiments demonstrated an increase in T cell proliferation with the use of OX40 agonists [24]. One of the initial experiments involved inoculating mice with the MCA 303 methylcholanthrene-induced sarcoma tumor cells and then injecting these mice with an OX40L:Ig fusion protein or saline control; tumor regression occurred in up to 60 % of OX40L:Ig-treated mice compared to controls in which no mice survived. In addition, the mice were resistant to re-challenge, suggesting development of antitumor memory. Similarly, in another experiment mice inoculated with the weakly immunogenic B16/F10 melanoma tumor cell line were treated with OX40L:Ig fusion protein, OX40 receptor agonist antibody, or PBS or IgG controls. Results showed that in both experimental groups 25 % of mice with increased OX40 signaling due to the applied therapy survived, whereas no mice in the control groups survived [25]. It was determined that the above result was dependent on both CD4 and CD8 T cells as their depletion allowed the tumors to grow [26].

6 GITR

The glucocorticoid-induced tumor necrosis factor receptor (GITR) is another co-stimulatory TNF superfamily member found on T cells. GITR binds to its ligand, GITR-L, which is expressed in low levels on antigen-presenting macrophages, B cells, and dendritic cells, where it is up-regulated when these cells are activated. Studies have shown that GITR provides a positive growth signal to CD4 and CD8 naïve T cells allowing for enhanced survival and function. GITR works by signaling through the NF-κB pathway causing up-regulation of IL-2R, IL-2, and interferon γ, and GITR knockout mice have decreased number and reduced survival of CD8 T cells. GITR is also expressed on NK T cells where signaling causes increased cytotoxicity, with increased production of interferon γ and other inflammatory cytokines [27]. GITR is expressed in high concentrations on Treg cells, and its activation decreases Treg function. GITR expression on Tregs is controlled by Foxp3, a potent transcriptional regulator. While GITR reduces Treg function, preventing their suppressive roles, GITR signaling also induces Treg proliferation. Therefore, once transient GITR signaling terminates, the Tregs will regain their suppressive function, and since there is now an expanded population, these suppressive activities are stronger than they were previously. Thus while the immediate function of GITR is to decrease Treg function, GITR stimulation causes a long-term strengthening of Treg suppressive abilities [28].

Studies have shown that agonistic anti-GITR antibodies help overcome self-tolerance and Treg suppression [29]. In other experiments using a mouse model of melanoma it was shown that GITR monoclonal antibody could be used as immunotherapy; the antibody induced suppression of Tregs and tumor challenge was rejected [30]. When dendritic cells engineered to express GITR-L-Fc fusion protein or GITR agonistic antibody were injected into a melanoma mouse model, they decreased tumor survival by about 60 %, compared to 100 % survival in controls [31]. Vaccines that targeted sarcoma antigens combined with GITRL also decreased tumor growth [32]. In vivo studies have shown that anti-GITR antibody causes tumor-infiltrating Tregs to lose suppressive properties because they lose expression of Foxp3—which may move T cells toward effector rather than regulatory function [30].

6.1 Clinical Trials

Table 1 provides a summary of the clinical trials discussed in this review.

7 Targeting CTLA4

One of the first clinical trials, led by Dranoff and co-workers, was a phase I study in which an antagonistic antibody against CTLA4 called MDX-CTLA4, a humanized monoclonal antibody, was injected into nine patients with different types of advanced cancer. MDX-CTLA4 caused tumor necrosis in three of the patients, each of whom had metastatic melanoma and had been previously injected with a GM-CSF-secreting tumor cell vaccine. In addition, anti-CTLA4 antibody treatment reduced or arrested the increase in CA-125 in two patients who had metastatic ovarian cancer that had also been previously treated with a GM-CSF-secreting tumor cell vaccine. However, there was no effect seen in four metastatic melanoma patients treated with a vaccine consisting of melanoma antigens. Several patients developed autoimmune side effects, as predicted by preclinical studies, including grade I reticular and erythematous rash, and T cells appeared to infiltrate into the area of the rash. Patients also had low levels of autoimmune antibodies, including antinuclear, antithyroglobulin, and rheumatoid factor; however, no additional symptoms were manifested. Therefore anti-CTLA4 treatment had an effect on malignancy with acceptable autoimmune side effects [33]. With this initial success, other trials were designed and a phase I trial in advanced stage IV melanoma patients was completed with 14 patients using the human monoclonal anti-CTLA4 antibody, MDX-010, also named ipilimumab. In this study by Seipp et al., ipilimumab was administered in conjunction with peptide vaccines derived from gp100 melanoma-associated antigen. The study achieved two complete remissions and one partial remission. Six of the 14 patients in this trial suffered severe grade 3 and 4 autoimmune side effects

Table 1 Summary of clinical trials

Antibody	Target	Phase	Diseases	Trial	Response	Reference
MDX-CTLA4	CTLA4	I	Melanoma, ovarian	Injected into 9 patients—3 melanoma and 2 ovarian patients previously treated with GM-CSF vaccine, and 4 melanoma patients treated previously with a vaccine consisting of melanoma antigens	Tumor necrosis in 3 melanoma patients treated with GM-CSF vaccine, reduced or stopped the increase of CA-125 in 2 ovarian patients, no response in a vaccine consisting of melanoma antigens	[33]
Ipilimumab	CTLA4	I	Melanoma	Ipilimumab was given to 14 patients with vaccinations of peptides derived from gp100 melanoma-associated antigen	2 CR, 1 PR	[34]
Ipilimumab	CTLA4	II	RCC	Ipilimumab was given to one group of 21 patients as a loading dose at 3 mg/kg and then subsequent dosing at 1 mg/kg every 3 weeks, and the second group of 40 patients was given ipilimumab at 3 mg/kg every 3 weeks	In the first group only 1 PR was seen, and the second group had 5 PRs	[35]
Ipilimumab	CTLA4	III	Melanoma	676 patients given either ipilimumab with or without GP100 and one group of patients given GP100 alone	10-month median survival vs. 6 months in the control group	[37]
Ipilimumab	CTLA4	III	Melanoma	502 patients received a combination of ipilimumab and dacarbazine or dacarbazine alone during weeks 1, 4, 7, and 10, followed by dacarbazine alone every 3 weeks until week 22	11-month median survival vs. 9 months in the control group	[38]
Tremelimumab	CTLA4	I	Melanoma	1-h infusions every 90 days up to four times, in 36 evaluable patients	4 PRs	[39]

(continued)

Table 1 (continued)

Antibody	Target	Phase	Diseases	Trial	Response	Reference
Tremelimumab	CTLA4	II	Melanoma	In this 246-patient study the test drug was given at 15 mg/kg every 90 days and repeated up to four times if the patient had tumor stabilization or response	Response rate of 6.6 %, with 16 partial responses. The duration of response lasted 9 to 30 months	[40]
Tremelimumab	CTLA4	III	Melanoma	655 treatment-naïve, unresectable stage III and IV melanoma patients received tremelimumab or standard chemotherapy	36-month duration of response vs. 14 months in the control group	[41]
MDX-1106	PD-1	I	Several refractory solid tumors including RCC, melanoma, colon cancer, NSCLC	Tested escalating doses of MDX-1106 to a maximum dose of 10 mg/kg in 39 patients with renal cell carcinoma, melanoma, prostate, colon cancer, or NSCLC	1 CR and 2 PRs	[43]
MDX-1106	PD-1	I	Melanoma, NSCLC, prostate cancer, RCC, colorectal cancer	296 patients received treatment with MDX-1106	Response rates were 18 % in NSCLC, 28 % in melanoma, and 27 % in RCC. Also, 20 of 31 patients followed for over a year had responses lasting over a year	[44]
MDX-1106	PD-1	I	Refractory metastatic RCC, prostate cancer, melanoma, NSCLC, and colorectal cancer	126 patients received 1, 3, or 10 mg/kg of the drug administered biweekly	16 RCC patients treated with 10 mg/kg had an overall response rate of 31.2 % with sustained disease of over 4 months in 6 of 16 patients. One of the 2 RCC patients treated with 1 mg/kg had a PR for 12 months, and one had sustained disease for 21 months. Also, one of the 15 evaluable patients with prostate cancer had a PR lasting over 2 months, and 3 of 15 had sustained disease for over 4 months	[46]

CT-011	PD-1	I	Hematologic malignancies	17 patients were given escalating doses of CT-011, to a maximum dose of 6 mg/kg, as a single IV infusion	33 % response rate and 1 CR	[47]
BMS-936559	PDL1	I	Melanoma, colon cancer, RCC pancreatic, gastric, breast, and NSCLC	207 patients were treated with 1-h infusions on days 1, 15, and 29 of 6-week cycles, and they received up to 16 cycles as long as they were able to tolerate the treatments	Overall response rate of 19 % in melanoma patients. An overall response rate of 10 % in NSCLC, 6 % in ovarian cancer, and 12 % of RCC	[48]
BMS-666513	4-1BB	I	Melanoma, renal cell carcinoma, prostate cancer, or ovarian cancer	83 patients studied. The drug, at several dose levels, was given IV every 3 weeks, and response was tested after the fourth dose and then every 2 doses thereafter	9 PRs in 54 melanoma patients tested	[49]
anti-OX40	OX40	I	Variety of refractory solid tumors	30-patient study in which the test drug was infused on days 1, 3, and 5 of each cycle at 0.1, 0.4, or 2 mg/kg, with 10 patients in each dose category	Being assessed	[50]
TRX518	GITR	I	Melanoma	Testing safety of TRX518	In process	[27]

including dermatitis, enterocolitis, hepatitis, and hypophysitis [34]. A phase II clinical trial in metastatic renal cell carcinoma (RCC) was performed by Rosenberg et al. In this study, one group of 21 patients was treated with a loading dose of ipilimumab at 3 mg/kg and then subsequent dosing at 1 mg/kg every 3 weeks, and the second group of 40 patients was treated with ipilimumab at 3 mg/kg every 3 weeks. In the first group only one partial response was seen. In addition, three of the patients suffered enterocolitis, and one of these three also developed a generalized rash and multiarticular arthritis. The second group who received ipilimumab only at 3 mg/kg had five partial responses. Seventeen patients suffered from immune-mediated autoimmune side effects. Thirteen had enteritis, one had hypophysitis, one had both enteritis and hypophysitis, one had primary adrenal insufficiency, and one had aseptic meningitis with cerebral spinal fluid lymphocytosis [35]. Interestingly, most of the patients who had antitumor responses also developed significant autoimmune side effects such that a response rate of 30 % was seen in those patients with autoimmune events and 0 % response rate seen in patients without autoimmune events ($P=0.009$) [36].

A phase III, randomized, double-blinded trial was carried out by Urba et al. and tested ipilimumab in advanced stage III and IV melanoma patients. In this trial ipilimumab was administered with or without GP100, a melanoma tumor antigen, and one group of patients received GP100 peptide alone. The results showed a 10-month median survival in the ipilimumab-administered groups whether or not GP100 was also given, and a 6.4-month median survival in patients only given GP100. Sixty percent of the 676 enrolled patients had severe immune-related side effects when given ipilimumab, including diarrhea, injection-site reactions, vitiligo, and colitis. Fourteen deaths occurred out of the 540 patients who received ipilimumab [37]. In another phase III trial, 502 patients with metastatic melanoma who were treatment naïve received a combination of ipilimumab and dacarbazine or dacarbazine alone during weeks 1, 4, 7, and 10, followed by dacarbazine alone every 3 weeks until week 22. After this time if patients had a response then they received dacarbazine or placebo every 12 weeks as maintenance regardless of their original treatment group. This randomized controlled trial led by Wolchok showed 20.8 % vs. 12.2 % survival, at 3 years in patients who received ipilimumab together with dacarbazine compared with those who received dacarbazine alone. Similar side effects were seen in this trial as in the earlier trial with GP100 randomization; however, no deaths and less severe gastrointestinal side effects were observed. Administering high-dose steroids appears to be effective in reducing grade III–IV diarrhea (colitis) and subsequent drug-induced mortality [38]. As a result, the FDA approved the use of ipilimumab in metastatic melanoma in 2010.

Another monoclonal anti-CTLA4 antibody was developed named tremelimumab, a human IgG2 monoclonal anti-CTLA4 antibody. A phase I trial was conducted by Gonzalez et al. in which stage 3 and 4 melanoma patients received tremelimumab in 1-h infusions every 90 days up to four times. Of 36 patients evaluable for response to therapy, 4 had a partial remission and there were no complete remissions, and the drug was well tolerated without major complications. The side effects included fatigue, diarrhea, and dehydration [39]. In addition, a phase II trial by

Bulanhagui et al. studied the tumor response of tremelimumab when administered to refractory melanoma patients. The drug was given at 15 mg/kg every 90 days. The infusion was repeated up to four times if the patient had tumor stabilization or response. This study involved 246 patients with a response rate of 6.6 %, with 16 partial responses. The duration of response ranged from 9–30 months [40]. The positive results of early trials led to the recent phase III trial by Hauschild et al. In this study 655 treatment-naïve, unresectable stage III and IV melanoma patients received tremelimumab or standard chemotherapy (either temozolomide or dacarbazine, investigators' choice). Results showed no difference between the groups in response rate, which was approximately 10 %; however, duration of response was longer in the tremelimumab group (36 months vs. 14 months, $P<0.0011$). Side effects included rash, pruritis, and diarrhea. In addition, there were 7 deaths due to the tremelimumab, which is 2 % of the 325 patients treated, versus 1 death of the 319 patients in the chemotherapy group, which is <1 % of the patients treated. The authors pointed out that 16 % of the control arm received ipilimumab as salvage therapy and that this may have impacted the difference in survival. Furthermore, this trial excluded patients with lactate dehydrogenase (LDH) more than twice the upper limit of normal, whereas ipilimumab trials did not. Thus, patients on the chemotherapy control arm may have done better than expected and this may have lessened the survival difference. This is suggested by analysis of the forest plot in which there is a trend toward better hazard ratio in patients with more advanced melanoma (e.g., higher LDH baseline levels). Thus, a number of factors may explain why the results of this trial were different from the results of the phase III ipilimumab trials [41]. Although these studies demonstrated little improvement compared to standard chemotherapy, additional clinical trials are ongoing [42].

8 Targeting PD-1

Following the success of CTLA4 monoclonal antibodies, anti-PD-1 therapies have also been developed. There are three PD-1 antagonistic antibodies and one fusion protein currently being tested in the clinic. These are known as MDX-1106, CT-011, MK-3475, and AMP-224, respectively. The first three are monoclonal anti-PD-1 antagonistic antibodies, and the fourth is a B7-DC/IgG1 fusion protein [8]. In addition, trials of antibodies targeting PDL1 are also being conducted, and BMS-936559 is one such antagonistic anti-PDL1 antibody.

9 MDX-1106

A phase I trial conducted by Topalian et al. tested MDX-1106, a human monoclonal IgG4 antagonistic antibody also known as BMS-936558. It was given to patients with several types of metastatic refractory solid tumors including RCC, melanoma,

prostate, colon cancer, and non-small-cell lung cancer (NSCLC). The study was designed to test escalating doses to a maximum dose of 10 mg/kg of MDX-1106 and included 39 patients. Results include one complete remission in colon cancer and two partial responses in melanoma and NSCLC. The study also demonstrated durability of the drug's effect and showed that the drug was well tolerated [43].

Another phase I trial reported by Sznol involved patients with advanced melanoma, NSCLC, castration-resistant prostate cancer, RCC, or colorectal cancer and treatment with MDX-1106. Complete or partial responses were seen in NSCLC, melanoma, or RCC. Of the 296 patients included in this study, response rates were 18 % in 76 patients with NSCLC, 28 % in 94 patients with melanoma, and 27 % in 33 patients with RCC. These responses were often durable, with 20 of 31 patient responses lasting over a year in patients that had at least a year of follow-up. Thirty-two of the total 296 patients had severe drug-related events, including pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis. Intriguingly, PDL1 expression on tumors appears to correlate with response [44]. Also, effective reinduction therapy with anti-PD-1 antibody has been reported [45]. A recent phase I trial by Wigginton et al. tested MDX-1106 in refractory metastatic RCC, prostate cancer, melanoma, NSCLC, and colorectal cancer. The study tested 1, 3, or 10 mg/kg of the drug administered biweekly in 126 patients. Side effects included rash, pruritus, diarrhea, and fatigue. This study included 16 RCC patients treated with 10 mg/kg, and at this dose an overall response rate of 31.2 % (5 of 16 patients) was observed with sustained disease of over 4 months observed in 6 of 16 patients. One of the two RCC patients treated with 1 mg/kg had a partial remission lasting over 12 months, and one had sustained disease lasting over 21 months. One of the 15 evaluable patients with prostate cancer had a partial response lasting over 2 months, and 3 of 15 had stable disease for over 4 months [46].

CT-011 is a humanized monoclonal IgG1 antagonistic antibody against PD-1. CT-011 has also been investigated in a phase I trial by Nagler et al. to identify the maximum tolerated dose. In this study 17 patients were given escalating doses of CT-011, to a maximum dose of 6 mg/kg, as a single IV infusion. The drug was well tolerated with diarrhea being the main side effect, and no maximal tolerated dose was determined. CT-011 showed preliminary antitumor efficacy, with 33 % of patients having a response and one patient having a complete remission. This trial tested the drug in hematologic malignancies including acute myeloid leukemia (AML), chronic lymphocytic leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma, or multiple myeloma [47].

A phase I anti-PDL1 antibody trial has also been conducted. BMS-936559 is a humanized IgG4 monoclonal antibody, which prevents PDL1 binding to PD-1. BMS-936559 was tested in patients with several cancers including melanoma, colon, pancreatic, gastric, and breast cancer, RCC, and NSCLC. Patients were treated with 1-h infusions on days 1, 15, and 29 of 6-week cycles, and they received up to 16 cycles as long as they were able to tolerate the treatments. This study, conducted by Wigginton et al., showed durable tumor regression, with an overall response rate of 19 % in melanoma patients with response seen in 9 of 52 patients.

An overall response rate was observed in 10 % or 5 of 49 patients with NSCLC as well as in 6 % or 1 of 17 patients with ovarian cancer and in 12 % of 207 patients with RCC. These data are overall average responses seen in patients treated with 1, 3, or 10 mg/kg BMS-936559; and the highest doses gave the highest response rates when analyzed alone. Immune side effects related to the drug occurred in 81 of the 207 patients and included rash, hypothyroidism, hepatitis, sarcoidosis, endophthalmitis, diabetes, and myasthenia gravis [48].

10 Anti-4-IBB Therapy

There are several anti-4-1BB agonist antibodies under study in the clinical setting. BMS-666513 is a human monoclonal antibody that has been tested in a phase I trial by Logan et al. The study was conducted in 83 patients with melanoma, RCC, prostate cancer, or ovarian cancer. The drug, at several dose levels, was given intravenously every 3 weeks, and response was tested after the fourth dose and then every two doses thereafter. There were partial responses in 9 of 54 melanoma patients. The therapy was well tolerated with a 6–15 % side effect rate including neutropenia, increased liver function tests, fatigue, rash, pruritis, diarrhea, and fever [49].

11 Anti-OX40 Therapy

A phase I trial of anti-OX40 led by Weinberg and Curti using a mouse monoclonal agonist antibody. To determine a safe and effective dose the antibody was infused on days 1, 3, and 5 of each cycle at 0.1, 0.4, or 2 mg/kg. The study involved 30 patients with a variety of refractory solid tumors, with 10 patients in each dose category. It was thought that since there are few T cells that express OX40 the immune response would be efficacious without having the same high side effect profile as agents targeting CTLA4. This turned out to be the case, with mild fatigue and lymphopenia being most common adverse effects. The efficacy of the OX40 antibody in this trial is still being assessed [50], and anti-OX40 antibodies are also being used in other ongoing trials.

12 GITR-Targeted Therapy

Phase I trials targeting GITR are ongoing. One is a trial in melanoma testing the safety of GITR agonistic antibody TRX518. Another is a phase I trial in melanoma patients testing dendritic cells alone or dendritic cells expressing GITRL, anti-CTLA4, or both together [27].

13 Conclusion

There are many other receptor and ligand co-stimulatory or inhibitory pairs that affect T cell activity that are being studied in the laboratory and in the clinic. As we gain knowledge regarding the normal functions of the different T cell co-regulatory receptors, we will be able to better manipulate their functions in hopes of further improving immunotherapy as a standard treatment option for cancer patients, to be used alone or in conjunction with other treatment modalities such as chemotherapy or radiation therapy. Manipulation of co-regulatory receptor signaling has already demonstrated early efficacy and will increasingly be incorporated in combination with additional immune therapy strategies in a variety of human tumors.

References

1. Janeway Jr CA, Travers P, Walport M, Shlomchik MJ. Immunobiology; the immune system in health and disease. 6th ed. New York, NY: Garland Science; 2005. p. 26–31. 327-328.
2. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:254–62.
3. Sansom DM. CD28, CTLA4, and their ligands: who does what and to whom? Immunology. 2000;101:169–77.
4. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996;271:1734–6.
5. 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;19: 355–66.
6. Capece D, Verzella D, Fischietti M, Zazzeroni F, Alesse E. Targeting costimulatory molecules to improve antitumor immunity. J Biomed Biotechnol. 2012;2011:1–17.
7. Poirier N, Blancho G, Vanhove B. CD28-Specific immunomodulating antibodies: what can be learned from experimental models. Am J Transplant. 2012;7:1682–90.
8. Topalian SL, Drake CG, Pardoll DM. Targetting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. Curr Opin Immmunol. 2012;24:207–12.
9. Sharpe AH, Wherry JE, Ahmed R, Freeman GJ. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. Nat Immunol. 2007;8:239–45.
10. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006;439: 682–7.
11. Titanji K, Velu V, Chennareddi L, Vijay-Kumar M, Gewirtz AT, Freeman GJ, Amara RR. Acute depletion of activated memory B cells involves the PD-1 pathway in rapidly progressing SIV-infected macaques. J Clin Invest. 2010;120:3878–90.
12. Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, Sharpe AH. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med. 2009;206:3015–29.
13. 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.

14. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, Roche PC, Lu J, Zhu G, Tamada K, Lennon VA, Celis E, Chen L. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med. 2002;8:793–800.
15. Zhang H, Snyder KM, Suhoski MM, Maus MV, Kapoor V, June CH, Mackall CL. 4-1BB Is superior to CD28 costimulation for generating $CD8^+$ cytotoxic lymphocytes for adoptive immunotherapy. J Immunol. 2007;179:4910–8.
16. Vinay DS, Kwon BS. Immunotherapy of cancer with 4-1BB. Mol Cancer Ther. 2012;11: 1062–70.
17. Croft M. Co-stimulatory members of the TNFR family: keys to effective T-cell immunity. Nat Rev Immunol. 2003;3:609–20.
18. Mittler RS, Bailey TS, Klussman K, Trailsmith MD, Hoffmann MK. Anti-4-1BB monoclonal antibodies abrogate T cell-dependent humoral immune responses in vivo through the induction of helper T cell anergy. J Exp Med. 1999;190:1535–40.
19. Hong HJ, Lee JW, Park SS, Kang YJ, Chang SY, Kim KM, Kim JO, Murthy KK, Payne JS, Yoon SK, Park MJ, Kim IC, Kim JG, Kang CY. A humanized anti-4-1BB monoclonal antibody suppresses antigen-induced humoral immune response in nonhuman primates. J Immunother. 2000;23:613–21.
20. Melero I, Shuford WW, Newby SA, Aruffo A, Ledbetter JA, Hellstrom KE, Mittler RS, Chen L. Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors. Nat Med. 1997;3:682–5.
21. Kim JA, Averbook BJ, Chambers K, Rothchild K, Kjaergaard J, Papay R, Shu S. Divergent effects of 4-1BB antibodies on antitumor immunity and on tumor-reactive T-cell generation. Cancer Res. 2001;61:2031–7.
22. Lane P. Role of OX40 signals in coordinating CD4 T cell selection, migration, and cytokine differentiation in T helper Th1 and Th2 cells. J Exp Med. 2000;2:201–6.
23. Redmond WL, Ruby CE, Weinberg AD. The role of OX40-mediated Co-stimulation T cell activation and survival. Crit Rev Immunol. 2009;29:187–201.
24. Weinberg WD, Morris NP, Kovacsovics-Bankowski M, Urba WJ, Curti BD. Science gone translational: the OX40 agonist story. Immunol Rev. 2011;244:218–31.
25. Weinberg AD, Rivera MM, Prell R, Morris A, Ramstad T, Vetto JT, Urba WJ, Alvord G, Bunce C, Shields J. Engagement of the OX-40 receptor in vivo enhances antitumor immunity. J Immunol. 2000;164:2160–9.
26. Kjaergaard J, Tanaka J, Kim JA, Rothchild K, Weinberg A, Shu S. Therapeutic efficacy of OX-40 receptor antibody depends on tumor immunogenicity and anatomic site of tumor growth. Cancer Res. 2000;60:5514–21.
27. Schaer DA, Murphy JT, Wolchok JD. Modulation of GITR for cancer immunotherapy. Curr Opin Immunol. 2012;24:217–24.
28. Nocentini G, Ronchetti S, Cuzzocrea S, Riccardi C. GITR/GITRL: more than an effector T cell co-stimulatory system. Eur J Immunol. 2007;37:1165–9.
29. Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S. Stimulation of CD25(+)CD4(+) regulatory T cells through GITR breaks immunological self-tolerance. Nat Immunol. 2002;3:135–42.
30. Cohen AD, Schaer DA, Liu C, Li Y, Hirschhorn-Cymmerman D, Kim SC, Diab A, Rizzuto G, Duan F, Perales MA, Merghoub T, Houghton AN, Wolchok JD. Agonist anti-GITR monoclonal antibody induces melanoma tumor immunity in mice by altering regulatory T cell stability and intra-tumor accumulation. PLoS One. 2010;5:1–12.
31. Boczkowski D, Lee J, Pruitt S, Nair S. Dendritic cells engineered to secrete anti-GITR antibodies are effective adjuvants to dendritic cell-based immunotherapy. Cancer Gene Ther. 2009;16:900–11.
32. Nishikawa H, Kato T, Hirayama M, Orito Y, Sato E, Harada N, Gnjatic S, Old LJ, Shiku H. Regulatory T cell-resistant CD8+ T cells induced by glucocorticoid-induced tumor necrosis factor receptor signaling. Cancer Res. 2008;68:5948–54.
33. Hodi FS, Mihm MC, Soiffer RJ, Haluska FG, Butler M, Seiden MV, Davis T, Henry-Spires R, MacRae S, William A, Padera R, Jaklitsch MT, Shanker S, Chen TC, Korman A, Allison JP,

Dranoff G. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci U S A. 2003;100:4712–7.
34. Phan GQ, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, Restifo NP, Hawarth LR, Seipp CA, Freezer LJ, Morton KE, Mavroukakis SA, Duray PH, Steinberg SM, Allison JP, Davis TA, Rosenberg SA. 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.
35. Yang JC, Hughes M, Kammula U, Royal R, Sherry R, Topalian SL, Suri KB, Levy C, Allen T, Mavroukakis S, Lowy I, White DE, Rosenberg SA. Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. J Immunother. 2007;30:825–30.
36. Escudier B. Emerging immunotherapies for renal cell carcinoma. Ann Oncol. 2012;23 Suppl 8:viii35–40.
37. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbe C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363:711–23.
38. Robert C, Thomas L, Bondarenko I, O'Day S, M D JW, Garbe C, Lebbe C, Baurain JF, Testori A, Grob JJ, Davidson N, Richards J, Maio M, Hauschild A, Miller Jr WH, Gascon P, Lotem M, Harmankaya K, Ibrahim R, Francis S, Chen TT, Humphrey R, Hoos A, Wolchok JD. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364:2517–26.
39. Ribas A, Chesney JA, Gordon MS, Abernethy AP, Logan TF, Lawson DH, Chmielowski B, Glaspy JA, Lewis K, Huang B, Wang E, Hsyu PH, Gomez-Navarro J, Gerhardt D, Marshall MA, Gonzalez R. Safety profile and pharmacokinetic analyses of the anti-CTLA4 antibody tremelimumab administered as a one hour infusion. J Transl Med. 2012;10:1–6.
40. Kirkwood JM, Lorigan P, Hauschild A, Robert C, McDermott D, Marshall MA, Gomez-Navarro J, Liang JQ, Bulanhagui CA. Phase II trial of tremelimumab (CP-675,206) in patients with advanced refractory or relapsed melenoma. Clin Cancer Res. 2010;16:1042–8.
41. Ribas A, Kefford R, Marshall MA, Punt CJ, Haanen JB, Marmol M, Garbe C, Gogas H, Schachter J, Linette G, Lorigan P, Kendra KL, Maio M, Trefzer U, Smylie M, McArthur GA, Dreno B, Nathan PD, Mackiewicz J, Kirkwood JM, Gomez-Navarro J, Huang B, Pavlov D, Hauschild A. Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. J Clin Oncol. 2013;31(5):616–22.
42. Dolgin E. FDA okays potential blockbuster melanoma drug. Http://blogs.nature.com. N.p., 25 Mar. 2011. Web. 10 Feb. 2013.
43. Brahmer JR, Drake CG, Wollner I, Powderly J, Picus J, Sharfman W, Stankevich E, Pons A, Salay TM, McMiller TL, Gilson MM, Wang C, Selby M, Taube JM, Anders R, Chen L, Korman AJ, Pardoll DM, Lowry I, Topalian SL. 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.
44. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366:2443–54.
45. Lipson EJ, Sharfman WH, Drake CG, Wollner I, Taube JM, Anders RA, Xu H, Yao S, Pons A, Chen L, Pardoll DM, Brahmer JR, Topalian SL. Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. Clin Cancer Res. 2013;19:462–8.
46. McDermott D, Drake CG, Sznol M, Sosman JA, Smith DC, Powderly JD, Feltquate DM, Kollia G, Gupta KA, Wigginton J. A Phase I study to evaluate safety and antitumor activity of

biweekly BMS-936558 (Anti-PD-1, MDX-1106/ONO-4538) in Patients with RCC and other advanced refractory malignancies. J Clin Oncol 2011;29:7(Suppl): Abstract 331.
47. Berger R, Rotem-Yehudar R, Slama G, Landes S, Kneller A, Leiba M, Koren-Michowitz M, Shimoni A, Nagler A. Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. Clin Cancer Res. 2008;14:3044–51.
48. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366:2455–65.
49. Sznol M, Hodi FS, Margolin K, McDermott DF, Ernstoff MS, Kirkwood JM, Wojtaszek C, Feltquate D, Logan T. Phase I Study of BMS-663513, a Fully Human Anti-CD137 Agonist Monoclonal Antibody, in Patients with Advanced Cancer (CA). J Clin Oncol 26: 2008 (May 20 suppl; abstr 3007).
50. Jensen SM, Maston LD, Gough MJ, Ruby CE, Redmond WL, Crittenden M, Li Y, Puri S, Poehlein CH, Morris N, Kovacsovics-Bankowski M, Moudgil T, Twitty C, Walker EB, Hu HM, Urba WJ, Weinberg AD, Curti B, Fox BA. Signaling through OX40 enhances anti-tumor immunity. Semin Oncol. 2010;37:524–32.