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Immunomodulatory Therapy for Melanoma: Ipilimumab and Beyond

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Abstract

In 2011, the FDA approved the first new therapy for melanoma in over a decade, ipilimumab (Yervoy). Ipilimumab is a novel antibody that blockscytotoxic T lymphocyte-associated antigen 4 (CTLA-4), a regulatory molecule expressed on activated T cells. Blockade of this important immune checkpoint can lead to durable tumor regression and Phase III studies showed an overall survival benefit for patients with advanced melanoma. During the clinical development of ipilimumab, several unique features of thisimmunotherapywere identified including the remarkable durability of responses and a distinct side-effects profile. Herein we review the preclinical and clinical development of CTLA-4 blocking antibodies, and describe current practices using ipilimumab for the treatment of advanced melanoma. Unique clinical issues related to ipilimumab will be summarized. Lastly, we will briefly previewcombination therapies that incorporate ipilimumab and new checkpoint targeting antibodies currently in clinical development.

Introduction

In the past year, the standard of care for the treatment of advanced melanoma has been transformed by the FDA approval of two new agents, ipilimumab and vemurafenib. Ipilimumab is a novel immunotherapy that works by blocking the engagement of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), a regulatory molecule expressed on activated T cells. Blockade of this important immune checkpoint can potentiate a robust antitumor immune response and lead to durable tumor regression. Ipilimumab was the first agent to demonstrate a benefit in overall survival for patients with metastatic melanoma.[1] During the clinical development of ipilimumab,several unique features of this ‘checkpoint blocking’ antibodywere identified including the remarkable durability of responses and a distinct side-effects profile. The success of ipilimumab offers a template for the development of the next generation of immunomodulatory antibodies. We shall review the preclinical and clinical development of CTLA-4 blocking antibodies, and describe current practices using ipilimumab for the treatment of advanced melanoma. Unique clinical issues related to

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ipilimumab will be summarized. Lastly, we will briefly previewcombination therapies that incorporate ipilimumab and new checkpoint targeting antibodies currently in clinical development.

Checkpoints that Regulate T cell Activation and Antitumor Immunity

Research into the fundamental mechanisms that regulate T cell activation informed the clinical development of CLTA-4 blocking antibodies (Figure 1). In 1970, Bretscher and Cohn proposed the “two signal” model of T cell activation.[2]In this model, antigen-specific T cell activation requires both T cell receptor (TCR) engagement (signal 1) and a co-stimulatory signal (signal 2).[2-5] In subsequent decades, this simple model was expanded to incorporate additional signals that fine-tune this process. A diversity of co-stimulatory and co-inhibitory molecules are required to both promote and regulate the complex orchestration of T cell activation (Figure 2).[8-14] CTLA-4 plays a pivotalrole as an inhibitory receptor, or checkpoint, during T cell activation.CTLA-4 was cloned in 1987,and its similarity to the costimulatory molecule CD28 was recognized.[15] Like CD28, CTLA-4 binds to B7-1 and B7-2, ligands expressed on antigen presenting cells,but with higher affinity.[16] Unlike CD28, engagement of CTLA-4 inhibits T cell activation.[17-19] CTLA-4 engagement on activated T cells inhibits cytokine synthesis and restricts cell proliferation.The characterization of CTLA-4 -/- knockout mice established the importance of CTLA-4-mediated regulation *in vivo*; these mice develop a lethal hyperproliferative lymphocyte expansion.[23-25]

Based upon the observation that CTLA-4 attenuates T cell activation, it was hypothesized that blockade of CTLA-4 could enhance antitumor immune responses.[26] This concept was initially validated using transplantable murine tumor lines of fibrosarcoma and colon carcinoma.[27] This finding has now been expanded to transplantable tumors of many types including prostate carcinoma, breast carcinoma, melanoma, ovarian carcinoma, lymphoma, and others.[27-31] For some poorly immunogenic tumors, such as the B16 melanoma, CTLA-4 monotherapy isinsufficient, but combinations of CTLA-4 blockade with vaccines are active.[32-34]Mice treated successfully with CLTA-4 blockade are protected from subsequent tumor challenge, consistent with the generation of protective antitumor immunity.

The Development of Human Reagents Ipilimumab and Tremelimumab

Based on the preclinical activity seen in mouse models, antibodies that blockCTLA-4 were subsequently developed forclinical use. Both ipilimumab (Yervoy™, Bristol Meyers Squibb, Princeton, NJ) and tremelimumab (formerly CP-675, 206 or ticilimumab, Pfizer, New York, NY) are fully human antagonist antibodiesrecognizing human CTLA-4.[35-37] Ipilimumab is an IgG1 antibody with a half-life of 12-14 days, whereas tremelimumab is an IgG2 antibody with a half-life of approximately 22 days. Both of these agents have been widely tested in patients with metastatic melanoma, where durable clinical responses have been well documented for both antibodies. Based on an overall survival benefit in phase III studies, the US Food and Drug Administration (FDA) approved ipilimumab for the treatment of patients with unresectable or metastatic melanoma in 2011;[1]however, aphase III study of tremelimumab was halted after an interim analysis failed to demonstrate an overall survival benefit compared to standard chemotherapy, though a follow up analysis did show a trend favoring tremelimumab.At present, it is unclear if differences in the dosing, schedule, clinical trial design/execution, or clinical activity explain the apparent shortcomings of tremelimumab in this study.

Ipilimumab in Clinical Trials

In 2002, the results of a pilot study of 17 patients with unresectable melanoma treated with a single dose of ipilimumab(3 mg/kg) were reported. There were 2 objective durable partial

responses (PR), and no serious toxicities were reported.[40]Subsequent early phase studies introduced a schedule of repeated dosing every 3 weeks.These studies demonstrated tolerability and clinical activity, and the dosing regimen of 3 mg/kg every 3 weeks for 4 doses was adopted in several subsequent studies.Unique toxicities were seen in these early studies with reports of colitis, dermatitis, hepatitis, hypophysitis, thyroiditis, and uveitis.This spectrum of toxicities was felt to be related to immune activation, latercategorized asimmune-related adverse events (irAEs). A dose-response relationship for ipilimumab was defined in a double-blind phase II study comparing doses of 0.3, 3, and 10 mg/kg every 3 weeks for 4 doses, followed by maintenance doses administered every 12 weeks. The highest dose cohort, 10 mg/kg, had the greatest response rate (11%), followed by 3 mg/kg (4.2%), and 0.3 mg/kg (0%). The irAEs followed a similar pattern.[46]

A randomized, double-blinded, phase III study examining 676 patients with advancedmelanoma demonstrated an improved median overall survival for patients receiving ipilimumab (10.1 vs. 6.4 months, P=0.003). [47] This three-armed study compared patients treated with ipilimumab at a dose of 3 mg/kg every 3 weeks for 4 doses versusthe gp100 peptide vaccine alone or gp 100 peptide vaccine plus ipilimumab. The survival rates for patients treated with ipilimumab alone were 45.6% at 1 year and 23.5% at the 2-years. Patients who initially achieved a confirmed partial or complete response or at least stable disease ≥24 weeks were eligible for re-induction within their original treatment arm ifthey subsequentlydeveloped disease progression.

A second randomized, placebo-controlled, phase III clinical trial compared dacarbazine plus ipilimumab versus dacarbazine plus placeboand accrued 502treatment naïve patients with metastatic melanoma. Patients received ipilimumab at a dose of 10 mg/kg every 3 weeks for 4 doses, followed by maintenance doses of ipilimumab given every 3 months. Again, a benefit in OS (11.2 vs. 9.1 months) was reported.[48] Survival rates for patients who received dacarbazine with ipilimumab were higher than patients who receiveddacarbazine alone at 1 year (47.3% vs. 36.3%), 2 years (28.5% vs. 17.9%), and 3 years (20.8% vs. 12.2%).

Immune-related Adverse Events

The potent ability of CTLA-4 blockade to activate the immune system can result in tissue specific inflammation characterized as immune-related adverse events (irAEs). Tissues that are most often involvedinclude the skin (dermatitis), gastrointestinal tract (enterocolitis), liver (hepatitis), and endocrine organs (hypophysitis, thyroiditis).In general, irAEs are transient and reversible; depending upon the severity of symptoms, interventions may include interruption of ipilimumab dosing, treatment with a course of steroids, or stronger immunosuppressants.

In particular, cases of enterocolitis can have serious consequences if appropriate treatment is not initiated promptly. In the phase III study reported by Hodi et al.,29% of patients treated with ipilimumab at a dose of 3 mg/kg developed gastrointestinal irAEs of any grade, and grade 3/4 colitis symptoms were reported in 8%.[1] Colitis typically resolves when treated with steroids. In cases refractory to steroids, treatment with tumor necrosis factor (TNF)-blocking antibodies such as infliximab may be helpful.[45] An algorithm for the management of colitis symptoms has been developed and adequate patient education and vigilance on the part of patient and physician are paramount. IrAEs involving the skin are common, but rarely serious and typically present as pruritus and/or a mild rash. Upon pathologic evaluation, findings of epidermal spongiosis and perivascular lymphocytic infiltrate with a predominance of eosinophils and CD4+ T cells have been described.Topical emollients, antihistamines, or topical steroids are often helpful to minimize dermatologic symptoms. Systemic steroids are rarely required. Other irAEs such as hepatitis or hypophysitis are seen

infrequently (typically <5%). Depending upon the extent of damage to the endocrine organ, supplemental hormones may be required in patients who develop hypophysitis, thyroiditis, or adrenal insufficiency. Additional irAEs including pancreatitis, uveitis, myopathy, neuropathy, arthritis, cytopenias, or pneumonitishave been described but are quite rare (1-2% or less).

Kinetics and Durability of Responses to Ipilimumab—Immune-related Response Criteria

While radiographic responses to ipilimumab are relatively infrequent, the durability of these responses can be measured in years rather than months. The remarkable stability of disease control was well represented in the phase III studies of ipilimumab, where a clear plateau in the 1-, 2- and 3-year survival rates were observed. Taking a closer look at the question of long-term responses to ipilimumab, 177 patients were treated on some of the earliest clinical trials. In the study population,15 patients achieved long-term durable complete responses (CR) and 14 of these are ongoing with the longest lasting 99+ months (median 83 months). Surprisingly, patients who achieved partial responses (PR) can also achieve long-term disease control. Nine patients who achieved PRs are alive many years after ipilimumab treatment, 3 without further treatment.

In addition to the remarkable durability of responses to ipilimumab, unusual patterns of radiographic responses were seen. Whereas responses to chemotherapy are usually seen within the first weeks or months of therapy, responses to ipilimumab can be quite delayed.[63] Furthermore, some ipilimumab treated patients will initially appear to have progressive disease with the development of new lesions but will ultimately go on to achieve a response. The distinct response patterns associated with ipilimumab were evaluated in a larger group of patients through a retrospective analysis of 487 patients treated across three multicenter phase II clinical trials.[64]Following this analysis, the immune-related response criteria (irRC) were proposed, to better characterize the response pattern. The irRC are based upon principles of the traditional modified World Health Organization (mWHO) criteria, but they differ in several important ways. According to the irRC, new lesions are included in the determination of the overall tumor burden and do not automatically indicate progressive disease. Additionally, evidence of disease progression requires confirmation with a subsequent radiographic assessment at least 4 weeks later. The irRC are being prospectively validated in ongoing studies of immunotherapeutic agents.

Immune-monitoring, the search for biomarkers

Despite the OS improvement demonstrated in phase III trials for patients who receive ipilimumab, unfortunately only ~30% of patients appear to derive benefit.With the delayed response kinetics, identifying patients who will fall into this favorable category can be especially challenging. Ongoing efforts continue to evaluate biomarkers which may help guide clinical decisions and/or better inform our understanding about the mechanism(s) of activity for ipilumumab *in vivo*. Thus far, no clear predictive biomarker for clinical response has been identified in peripheral blood samples. Several biomarkers that appear reflect immune activation by ipilimumab during treatment and correlate with responses to ipilimumabhave been identified in retrospective studies and includeabsolute lymphocyte count (ALC) [6567], sustained upregulation of the T cell activation marker inducible co-stimulator (ICOS), [68-72]the development of a polyfunctional T cell response to the tumor antigen NY-ESO-1.[73]Prospective validation in larger studies will be necessary to determine the significance of these findings.

The tumor microenvironment may be a more relevant area to look for immunologic markers. ICOS upregulation on T cells after neoadjuvant ipilumumab treatment was first identified in the tumor microenvironment for bladder and prostate tumors.[68-72]More recently, the results of a prospective, double-blind phase II study exploring candidate biomarkers from the melanoma tumor microenvironment have ben reported.[74]Evaluation of tumor biopsies revealed significant associations between clinical benefitand high baseline expression of FoxP3 (P=0.014) and indoleamine 2,3-dioxygenase (P=0.012). Clinical activity also correlated with an increase in tumor-infiltrating lymphocytes (TILs) between baseline and after the second dose of ipilimumab (P=0.005). In a second study evaluating biopsy samples, Ji et al. reported on gene expression patterns in the tumor microenviroment. The investigatorsobserved that expression of inflammatory response genes at baseline predicts clinic benefit after ipilimumab treatment (P<0.01). [75] Several other small case series have described intratumoral changes after treatment with ipilimumab consistent with induction of a productive antitumor immune response.[76-78]

Ipilimumab in Clinical Use Today

Dosing and Schedule—The FDA has approved ipilimumab at a dose of 3 mg/kg to be administered once every three weeks for four doses, the schedule utilized by Hodi et al.[1] It is not clear, however,that this regimen reflects the optimalclinical activity for ipilimumab and several important questions are unanswered. First, what is the most effective dose of ipilimumab? Phase I studies did not identify a maximum tolerated dose. In a randomized, double-blinded phase II study comparing ipilimumab at three dose levels, 0.3, 3, and 10 mg/kg,dose-dependent antitumor activity was observed with response rates of 0%, 4.2% and 11.1% respectively. This must be balanced against an increased rate of Grade 3/4 irAEs (0%, 7 %, 25%). The activity of ipilimumab at the 10 mg/kg dose will be formally compared to the FDA-approved 3 mg/kg dose in an upcoming randomized, double- blind phase III study (NCT01515189). A second outstanding question relates to the appropriate duration of ipilimumab treatment. Some clinical trials have permitted additional, so-called "maintenance", doses of ipilimumab administered every three months after completion of the first 4 doses. Alternatively, some trials have permitted repeat dosing or "reinduction" therapy using the original four-dose induction schedule. The Hodi study provides some limited evidence that reinduction with ipilimumab may help some patients. In this study, 31 patients who initially benefitted from ipilimumab treatment and subsequently developed progressive disease were offered reinduction. After reinduction therapy, 1 patient achieved a CR, 5 patients achieved PRs, and 15 patients achieved SD.

BRAF mutant melanoma—For the approximately 50-60% of patients with advanced melanoma that harbor the BRAFV600E mutation, vemurafenib, a targeted inhibitor of mutated BRAF, has been approved by the FDA based upon an overall survival benefit.[79] Within this population, vemurafenib has a superior response rate (~50%) and faster kinetics of response when compared to ipilimumab.;however, unlike ipilimumab, responses to vemurafenib are rarely durable and progressionusually occurs within 6-8 months.[80]The presence or absence of a BRAF mutation does not appear to have any impact on the likelihood of response to ipilimumab.[81] The sequencing of these two agents in patients with BRAF mutant melanoma has not been clearly established. At present, our practice has been to treat patients with symptomatic BRAF mutant melanoma with vemurafenib upfront in the hope of achieving rapid palliative reduction in disease burden, given the slower kinetics of responses to ipilimumab. Otherwise, both vemurafenib and ipilimumab are reasonable in the first-line setting and the merits and liabilities of each should be balanced for the individual patient. A phase I study combining ipilimumab and vemurafenib has recently opened (NCT01400451).

Patients with CNS metastases—For patients with advanced melanoma who develop CNS metastases, prognosis is especially poor. While the data are limited, it appears thatipilimumab may provide some benefit for this population.[82] Initial observations supporting this notion came from two case reports of patients treated with ipilimumab who had either regression or stabilization of CNS disease.In a retrospective analysis of patients treated on a phase II study of ipilimumab, 12 patients with stable brain metastases before starting treatment were identified.[85] In this group, 2/12 achieved a partial response and 3/12 had stable disease, with one patient developing grade 3 cerebral edema responsive to treatment with steroids. Lastly, a study prospectively evaluating the activity of ipilimumab in patients with brain metastases was reported in abstract form at ASCO in 2010.[86]In 51 patients with brain metastasis not requiring steroids whowere treated with ipilimumab, 4/51 achieved a systemic PR and 5/51 achieved SD for an overall disease control rate of 9/51 (18%). In evaluating CNS disease alone, 5 patients had a PR and 6 had SD at 12 weeks. Thus, ipilimumab appears to have similar activity for brain metastases as for non-CNS disease. The activity of ipilimumab in the setting of CNS metastases requiring steroids has not been reported. Aphase II study (NIBIT-M1)evaluating the combination of fotemustine with ipilimumab in patients with metastatic melanoma with or without asymptomatic brain metastases is underway.

Ipilimumab in the Adjuvant Setting—At present, the indications for ipilimumab restrict its use to patients with either stage IV or unresectable stage III melanoma, as the benefit of ipilimumab for earlier stage disease has not been established. Ipilimumab in the adjuvant setting is being evaluated in two ongoing phase III trials (NCT00636168 and NCT01274338). In NCT00636168, ipilimumab is being compared to placebo after resection of high risk stage III melanoma with recurrence-free survival as the primary endpoint. Accrual has been completed and results are anticipated. Ipilimumab is also being compared to high-dose recombinant interferon-alpha-2b (NCT01274338).

Beyond Ipilimumab Monotherapy

Combination Therapy—Combining ipilimumab with traditional or experimental therapies may improve upon response rates and expand the durable benefits of ipilimumab. Preclinical evidence from mouse models offers support for combinations with conventional cancer therapies including surgery [87], radiation, chemotherapy [90] cryoablation[91], and radiofrequency ablation.[92] CTLA-4 has also been combined successfully with a diversity of immunotherapies including tumor vaccines and immunomodulatory antibodies in the preclinical setting.Lastly, limited evidence supports the combination of CTLA-4 blockade with molecularly targeted agents, an area likely to enjoy increased attention.[103]

A number of combination strategies have been explored in clinical trials to date. Combinations of ipilimumab with tumor vaccines have been the most common, including peptide vaccines, cellular vaccines[105], and DNA/RNA vaccines[106]. The combination of ipilimumab with a peptide vaccine against gp100 was tested in a randomized phase III study but failed to show superior activity to ipilimumab alone.[1] Alternative vaccination strategies may be more successful in combination with ipilimumab but have not yet been tested in larger, randomized studies. A regimen combining ipilimumab and IL-2 was tested in a single arm phase I/II study.[43] The combination proved tolerable and responses were seen in 22% of patients, but it is unclear if this regimen is superior to monotherapy.

The utility of combining ipilimumab with chemotherapy in advanced melanoma is unclear, perhaps reflecting the limited activity of standard chemotherapies like dacarbazine in this disease. In an open label, randomized phase II study, Hersh et al. reported a non-significant trend favoring ipilimumab combined with dacarbazine compared to ipilimumab alone, with

disease control rates of 37.1% vs. 21.6%, respectively. In a phase III study, Robert et al. evaluated a similar combination of ipilimumab and dacarbazine and observed a response rate of 15% with over 40% of patients experiencing grade 3/4 toxicity. While there was no comparator arm of ipilimumab alone, it seems unlikely that this combination is superior given the historical response rates for ipilimumab. Lastly, in a case report, radiation therapy has been identified as an attractive partner for combination with ipilimumab and formal studies of this combination are underway (NCT01449279, NCT01497808). [107]

On the horizon, combinations of ipilimumab with novel immunotherapies or molecularly targeted therapies are likely to be promising based upon preclinical studies. At present, ipilimumab is being tested in combination with MDX-1106, a programmed death-1 (PD-1) blocking antibody, in the phase I setting (NCT01024231). And, a first-in-human trial combining ipilimumab with vemurafenibhas recently opened (NCT01400451).

Expanding the Repertoire of Checkpoint Blocking Antibodies—Ipilimumab has clearly expanded and re-established the important role for immunotherapy in the treatment of melanoma and has demonstrated the robust clinical activity of a checkpoint blocking antibody. CTLA-4 is the first on a growing list of immunological checkpoints that now includes PD-1, LAG-3 (Lymphocyte-activation gene 3), TIM-3 (T cell immunoglobulin mucin-3), BTLA (B- and T-lymphocyte attenuator), and others (Figure 2). The development and clinical testing of antibodies for these newer checkpoint molecules are in various stages of pre-clinical or clinical development.[108-111] Several antibodies targeting PD-1 or its ligand PD-L1 have been developed for clinical use including BMS-936558/MDX-1106, BMS-936559/MDX-1105 (both from Bristol-Myers Squibb), MK-3475 (Merck), MPDL3280A/RG7446 (Genentech), and CT-011 (Cure Tech); an anti-PD-1 fusion protein, AMP-224 (Amplimmune) is in development as well. BMS-936558 is a fully human IgG4 antibody, which has a serum half-life of 20 days at the highest doses tested.[114] A first-in-human, Phase I, single-dose dose-escalation study of BMS-936558 showed activity and was followed by a second Phase I study investigating a schedule of bi-weekly dosing.On a biweekly schedule, BMS-936558 had an objective response rate of 37.5% (6/16) including 5 PRs (melanoma, renal-cell carcinoma, non-small cell lung cancer) and one CR (renal-cell carcinoma).

The remaining checkpoint molecules are in earlier stages of pre-clinical or clinical development. The relative contribution of each checkpoint in fostering a protected tumor environment are beginning to be unraveled and will likely be unique for eachtumor. Ultimately, assays that determine the most relevant checkpoints to target in an individual tumor may guide clinical decisions. Building upon the success of ipilimumab, the cannon of clinically available checkpoint blocking antibodies will likely expand over the next decade.

Conclusion

The CTLA-4 blocking antibody, ipilimumab, is the first in new class of checkpoint blocking antibodies. With a demonstrated survival benefit in two randomized phase III studies, ipilimumab has been recently approved by the FDA for the treatment of advanced melanoma. Unique clinical features of ipilimumab were identified during its clinical development including delayed response kinetics and a distinct profile of side effects. As ipilimumab is incorporated into the standard of care of advanced melanoma, patient and physician education is paramount to the successful and safe use of this promising new therapy. The success of ipilimumab as a monotherapy, opens the door for the development of new checkpoint blocking antibodies and new combinations of ipilimumab with standard and experimental therapies.

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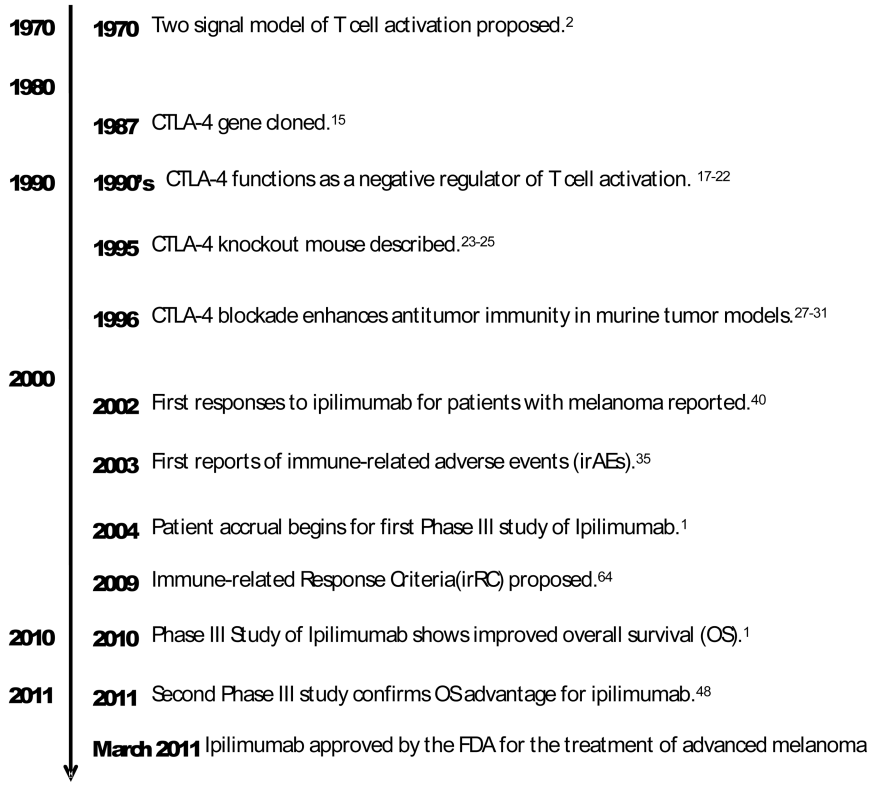

Figure 1.
Pre-Clinical and Clinical Development of CTLA-4 blocking Antibodies.

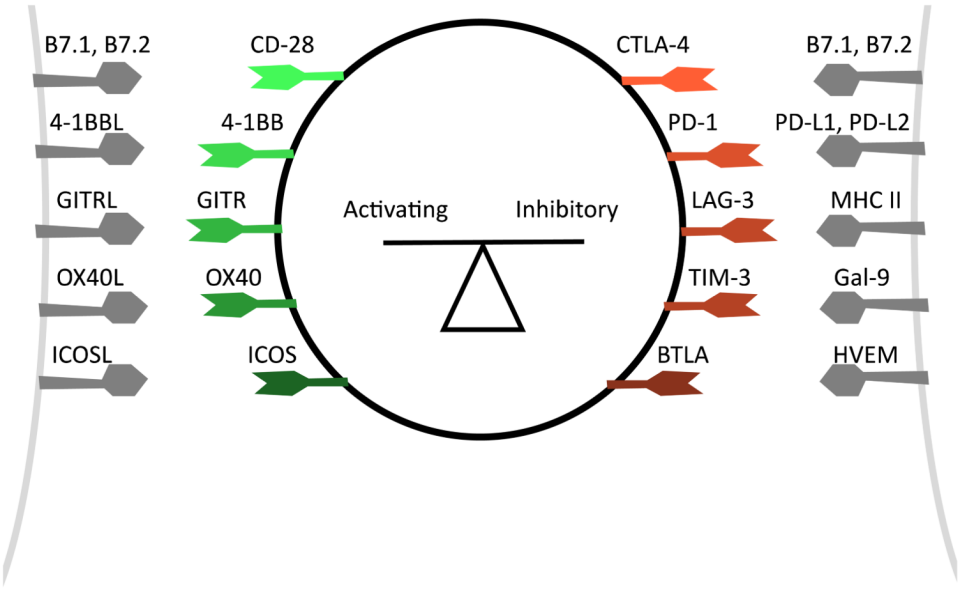

Figure 2. Co-stimulatory and Co-inhibitory Molecules Regulate T cell Activation

A diversity of activating and inhibitory signals are integrated to modulate the process of T cell activation. CTLA-4 is one of many inhibitory checkpoint molecules that regulate T cell activation.