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Tremelimumab (CP-675,206), a Cytotoxic T Lymphocyte–Associated Antigen 4 Blocking Monoclonal Antibody in Clinical Development for Patients with Cancer

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Key Words. CTLA-4 • Melanoma • T cell • Antitumor

LEARNING OBJECTIVES
After completing the course, the reader will be able to:
1. Educate community oncologists about the promise of anti-CTLA-4 monoclonal antibodies for the treatment of advanced cancer.
2. Suggest that CTLA-4 blockade overcomes barriers to effective immunotherapy for cancer.
3. Describe the rational design and clinical development strategy taken with the CTLA-4 antagonist tremelimumab.

ABSTRACT
Tremelimumab (CP-675,206) is a fully human monoclonal antibody specific for human cytotoxic T lymphocyte–associated antigen 4 (CTLA-4, CD152) in clinical development for patients with cancer. Blocking the CTLA-4 negative costimulatory receptor with the antagonistic antibody tremelimumab results in immune activation. Administration of tremelimumab to patients with locally advanced and metastatic melanoma has resulted in a subset of patients with durable objective tumor regressions. Its IgG2 isotype minimizes the possibility of cytotoxic effects on activated T lymphocytes and cytokine release syndrome. Preclinical testing in vitro and in large animal models predicted the target concentrations of circulating antibody in humans necessary for a pharmacodynamic effect. Phase I clinical trials provided evidence of dose- or exposure-related effects consistent with the anticipated mechanism of action. Further clinical development has led to two ongoing registration trials in patients with metastatic melanoma: a phase III randomized trial of tremelimumab versus dacarbazine or temozolomide in previously untreated patients with advanced melanoma and a phase II trial of tremelimumab in previously treated patients with advanced melanoma. The Oncologist 2007;12:873–883

Disclosure of potential conflicts of interest is found at the end of this article.

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**INTRODUCTION**

**Rationale for Cytotoxic T Lymphocyte–Associated Antigen 4 Blockade to Treat Cancer**

In 1996, Leach et al. [1] demonstrated that systemic administration of antagonistic antibodies (Abs) to the negative costimulatory receptor cytotoxic T lymphocyte–associated antigen 4 (CTLA-4) induces tumor regressions in mice. Since then, preclinical models have confirmed that CTLA-4 blockade induces antitumor immune responses [2–8]. In mice bearing immunogenic tumors, administration of CTLA-4–blocking Abs alone induced rejection of established tumors [1, 9] and decreased relapses when given as adjuvant immunotherapy in a model of metastatic prostate cancer [3]. However, in poorly immunogenic tumor models, prior immunization with vaccines [2, 4, 5, 10–13] or prior depletion of CD25+ regulatory T (Treg) cells [7, 8] was required for CTLA-4 blockade to exert robust antitumor effects. In nonhuman primates, anti–CTLA-4 Abs enhanced T-cell and Ab responses to an infectious disease vaccine and a whole tumor cell vaccine [14]. After this experimental work, it became clear that the prior emphasis on activating immune system cells should be complemented with the modulation of immune system negative regulatory pathways. Other negative immune regulators amenable to pharmacologic modulation have been described, including programmed cell death 1, B and T lymphocyte attenuator, transforming growth factor beta, interleukin 10 (IL-10), and vascular endothelial growth factor [15–19]. CTLA-4 appears to be dominant in maintaining peripheral homeostasis to self, confirmed by the phenotype of CTLA-4 gene knockout mice that develop rapidly progressing, lethal, uncontrolled lymphoproliferation and autoimmunity [20, 21].

This laboratory and animal model research has been successfully translated into the clinic. Two fully human monoclonal antibodies (mAbs) with CTLA-4 antagonistic activity are in clinical development; ipilimumab (formerly MDX-010; developed by Medarex, Inc., Bloomsburg, NJ, and codeveloped with Bristol-Myers Squibb, Princeton, NJ) and tremelimumab (formerly CP-675,206; developed by Pfizer Pharmaceuticals, Inc., New York). This antibody was transiently named ticilimumab; the term was discontinued because the World Health Organization considered it too similar to the generic name tocilizumab, a humanized Ab against the IL-6 receptor. Several published studies attest to the biologic and clinical activity of ipilimumab and tremelimumab in patients with melanoma and other cancers [22–32], generating great enthusiasm for continued testing.

**Potential Mechanisms of Antitumor Activity of CTLA-4–Blocking Abs**

CTLA-4 is an activation-induced, type I transmembrane protein of the Ig superfamily, expressed by T lymphocytes as a covalent homodimer that functions as an inhibitory receptor for the costimulatory molecules B7.1 (CD80) and B7.2 (CD86) [9, 33–36]. Crosslinking of CTLA-4 by B7 in the context of T-cell antigen receptor (TCR) engagement inhibits T-cell activation, IL-2 gene transcription, and T-cell proliferation by directly inhibiting TCR signal transduction [1, 9]. CTLA-4 is also expressed by human monocytes [35], but its function is not clear in this cell type.

It has been hypothesized that CTLA-4 blockade using specific Abs may induce responses by sustaining the activation and proliferation of tumor-specific T cells [9]. Antigenic peptides from tumor debris can be crosspresented by host dendritic cells to activate tumor antigen-specific T cells [37]. However, these T cells are not efficient in preventing tumor growth, in part because of the dominant role of activation-induced CTLA-4 expression, which leads to T-cell anergy or tolerance. Blockade of the B7 costimulatory molecule–CTLA-4 interaction in the activated T cell prevents CTLA-4–mediated negative signaling, allowing for antitumor T-cell activation (Fig. 1) [9]. Consequently, CTLA-4 blockade with mAbs in murine models results in increased IL-2 and interferon-gamma production by lymphocytes, increased expression of major histocompatibility complex (MHC) class I molecules, and markedly increased tumor killing [38, 39].

Other potential mechanisms of action (MOAs) based on reverse signaling from CTLA-4 to B7 costimulatory molecules may have a role in the antitumor activity of CTLA-4 blockade [40–43] (Fig. 2). Some CD4+CD25+ Treg cells constitutively express CTLA-4 [44], which can induce reverse or back signaling through B7 costimulatory molecules on plasmacytoid dendritic cells. This back signaling increases expression of indoleamine 2,3-dioxygenase (IDO), a rate-limiting enzyme in tryptophan catabolism [40–42]. IDO-expressing plasmacytoid dendritic cells acquire potent and dominant T-cell suppressive properties because of reduced tryptophan in the microenvironment, resulting in inhibition of antigen-specific cytotoxic T cells [45]. Some Treg cells can also directly inhibit T cells through a cell-contact mechanism.
mediated by CTLA-4 on Treg cells and B7 costimulatory molecules on effector T cells [43].

Another potential MOA is mediated by changes in circulating antibodies induced by CTLA-4 blockade. Ab-based screening of tumor-derived cDNA expression libraries was performed on serum from ipilimumab-treated patients who had a durable response [31]. This screening identified high titers of Abs against soluble MICA, an MHC class I-like molecule shed by tumor cells that downregulates expression of the immune-activating receptor NKG2D in effector immune cells [46]. Anti-MICA Abs induced by CTLA-4 blockade may reverse soluble MICA-induced immune suppression [31]. Anti-CTLA-4 Abs may also directly affect tumor cells expressing CTLA-4 (e.g., melanoma and epithelial cancer cells) [47]. Despite the wealth of knowledge and hypotheses on mechanisms of antitumor activity induced by CTLA-4 blockade in animal models, the mechanisms that mediate tumor regression in cancer patients are currently not fully understood.

**PRECLINICAL DEVELOPMENT OF TREMELIMUMAB**

**Goals for the Development of CTLA-4–Blocking Abs**

The human CTLA-4 gene was cloned in 1988 [33, 48]. Pfizer’s preclinical immunology team focused on CTLA-4 as a potential target before it was clear whether it was a positive or negative costimulatory receptor [49]. Emerging data [1, 34, 50] provided strong evidence that blockade of CTLA-4 signaling enhanced T-cell activation in vitro and markedly enhanced antitumor responses in murine models. It was hypothesized that an enhanced T-cell immune response may result in tumor regression, leading to a focus on CTLA-4 as a potential drug target for cancer. Based on this rationale, a panel of CTLA-4–
blocking Abs was developed. It was essential that the CTLA-4–blocking Abs not kill activated T cells by fixing complement or triggering Fc receptor–mediated Ab-dependent cell-mediated cytotoxicity. Therefore, CTLA-4–blocking Abs of the human IgG2 subtype were chosen, because IgG2 induces minimal complement activation and Ab-dependent cell-mediated cytotoxicity [51] and decreases the possibility of cytokine release syndrome with Ab infusion into humans. To minimize potential development of immune responses to the CTLA-4 Abs, fully human CTLA-4–blocking Abs were generated at Abgenix, Inc. (Fremont, CA) in engineered XenoMice™ expressing transgenic human Ig genes.

Preclinical Tremelimumab Data

Extensive preclinical testing led to selection of tremelimumab for clinical development.

Tremelimumab is highly selective for CTLA-4, demonstrating over 500-fold greater selectivity than for CD28, a costimulatory TCR that binds B7 ligands with lower affinity than CTLA-4. Competition binding studies have shown that tremelimumab efficiently blocks binding of CTLA-4 to B7–1 and B7–2 with average 50% inhibition concentrations of 0.65 and 0.50 nM, respectively [52]. The lack of nonspecific cytokine release and binding to Fc receptors for the IgG2 isotype was confirmed by in vitro studies in human whole blood [52]. Flow cytometry demonstrated a high af-
finity of tremelimumab for CTLA-4 expressed on the surface of activated human and cynomolgus monkey T cells, but not T cells from mouse, rat, hamster, or rabbit. The affinity for binding to human CTLA-4 was subnanomolar (0.28 nM), and approximately fourfold higher than for monkey CTLA-4 (0.98 nM) [52]. The cynomolgus monkey was selected for preclinical toxicologic evaluation of tremelimumab.

Toxicology Studies in Cynomolgus Monkeys
Toxicity of tremelimumab was assessed in cynomolgus monkeys in single-dose and 1- and 6-month weekly dosing studies. Treatment-related effects observed in the 6-month study included skin rash and persistent loose stools with decreased appetite and weight loss at 50 mg/kg per week. These findings resulted in cessation of dosing and/or euthanasia of some monkeys at 50 mg/kg. Microscopically there was mononuclear cell inflammation in the skin, cecum, and/or colon. In concert with lymphoid hyperplasia in the lymphoid organs, a dose-dependent increase in the incidence and severity of mononuclear cell infiltration or inflammation was observed in most tissues with a spontaneous background incidence of mononuclear cell aggregates. With the exception of moderate to marked thyroid follicular atrophy, observed in association with alterations in thyroid hormones in two animals at >15 mg/kg per week, these changes appeared reversible. These findings in monkeys were generally consistent with tremelimumab predicted pharmacology and suggested that clinical use may affect the gastrointestinal tract, the skin, lymphoid and thyroid tissues, and the hematological system. Preclinical findings correlated with the dose-limiting toxicities (DLTs) (diarrhea and skin rash), as well as non-dose-limiting thyroid abnormalities, observed in clinical trials of tremelimumab [29, 53].

An Ex Vivo Potency Assay for CTLA-4 Blockade
A potency assay was developed that detected enhanced cytokine production of superantigen-stimulated T cells. A plasma concentration ≥30 µg/ml was identified as the minimum effective in vitro concentration of tremelimumab that produced a significant increase in T-cell activation [52, 54]. Ex vivo studies using whole blood or peripheral blood mononuclear cells demonstrated that tremelimumab reproducibly enhanced superantigen-induced IL-2 production [54]. To assess the feasibility of the staphylococcal enterotoxin A assay for use in clinical trials, cynomolgus monkeys were given a single i.v. dose of 10 mg/kg tremelimumab and had blood samples collected for evaluation. Enhancement of IL-2 production was observed in ex vivo superantigen-stimulated blood samples from both animals, suggesting potential utility for the assay as a pharmacodynamic endpoint in clinical studies.

EARLY CLINICAL DEVELOPMENT OF CTLA-4–BLOCKING ABS
A lead prototype anti–CTLA-4 compound CP-642,570 was identified, but its clinical development was discontinued because of the induction of transient thrombocytopenia in the first-in-human (FIH) study. Tremelimumab was the second anti–CTLA-4 from Pfizer brought to the clinic (Table 1). Its early clinical development plan followed three treatment strategies: (a) single-agent activity in cancer, (b) combination with established anticancer therapies hypothesized to enhance release of endogenous tumor antigens as well as induce other favorable effects within the tumor, and (c) combination with a cancer vaccine as a source of exogenous tumor antigen. The first scenario has been pursued through pivotal studies in melanoma.

Single-Agent Clinical Development
Eligibility for the FIH phase I study of tremelimumab was broad, including patients with metastatic cancer with no restriction on histology and patients with resected cancer with a risk of relapse assumed to be ≥50%. Study investigators focused on enrolling patients with metastatic measurable melanoma, in which immunotherapy has a solid track record of inducing durable tumor regressions in subsets of patients [55–57]. Among 39 patients enrolled, 29 had metastatic melanoma and, among these patients, 82% had visceral metastasis. This phase I single-dose escalation trial tested doses from 0.01 to 15 mg/kg (n ≥3). A single dose of tremelimumab induced long-term remissions (36+ months) in some patients with metastatic melanoma starting at a dose of 3 mg/kg. Two of six patients treated at the highest dose tested (15 mg/kg) had objective tumor regressions with manageable toxicities [29]. Sites of responding metastases included skin, nodes, lung, liver, adrenal glands, and peritoneal carcinomatosis. However, at the 15 mg/kg dose level two patients had grade III toxicities (skin rash and diarrhea). Therefore, dose escalation was halted and the immediately lower dose level (10 mg/kg) was defined as the maximum-tolerated dose (MTD).

Pharmacokinetic analysis demonstrated that tremelimumab behaves exactly like endogenous human IgG2 [29]. At 10 and 15 mg/kg, plasma concentrations were >30 µg/ml in the majority of patients 4 and 8 weeks, respectively, after the initial dose [29]. Therefore, the minimal efficacious concentration of 10–30 µg/ml, defined by the preclinical studies, can be achieved in plasma for prolonged periods of time. These data cannot be taken as evidence of sustained CTLA-4 target saturation. Direct testing of target
<table>
<thead>
<tr>
<th>Approach</th>
<th>Trial name</th>
<th>Trial design</th>
<th>Eligibility</th>
<th>Timeline</th>
</tr>
</thead>
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<td>Single agent</td>
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<td>FIH phase I single dose</td>
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<td>Phase I multidose</td>
<td>Metastatic melanoma</td>
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<td>Phase II randomized</td>
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<td></td>
<td>A3671014</td>
<td>Phase II</td>
<td>Metastatic colon cancer</td>
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<tr>
<td></td>
<td>A3671015</td>
<td>Phase II</td>
<td>Locally advanced non-small cell lung cancer</td>
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<tr>
<td>Combination with immunotherapy</td>
<td>Study 0003 (NRA3670003)</td>
<td>Phase I with dendritic cell vaccines</td>
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<tr>
<td></td>
<td>A3671018</td>
<td>Phase I with PF-3512676</td>
<td>Metastatic melanoma</td>
<td></td>
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<tr>
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<td>Phase I with exemestane</td>
<td>Breast carcinoma</td>
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<tr>
<td></td>
<td>A3671025</td>
<td>Phase I with sunitinib malate</td>
<td>Renal cell carcinoma</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: FIH, first-in-human.
saturation is technically challenging, since the CTLA-4 target receptor is only transiently expressed on activated human T cells. However, it can be stated that these doses lead to toxicity and tumor response consistent with the anticipated pharmacodynamic effects of a CTLA-4–blocking mAb.

Phase I testing of multiple monthly doses (3–10 mg/kg) of tremelimumab (Study 1002) was initiated [53]. Fourteen patients with measurable metastatic melanoma were enrolled. Patients in the 3 and 6 mg/kg monthly cohorts experienced rapid tumor progression. One of eight patients entered at 10 mg/kg monthly had a pathologically defined partial response (PR) of in-transit metastasis [53]. The phase I monthly redosing study was followed by a phase II redosing trial (Study 1002 randomized phase II study) comparing two dose levels and schedules (10 mg/kg monthly and 15 mg/kg quarterly). Final results from this study are not yet available; accrual was completed in November of 2005. Interim analysis indicated similar antitumor activity between the two regimens; three (8%) patients treated with 10 mg/kg monthly (n = 40) had an objective response (1 complete response [CR] and 2 PRs) and three (7%) patients treated with 15 mg/kg (n = 42) had a response (1 CR and 2 PRs) [58]. Both regimens were well tolerated, but compared with 10 mg/kg monthly, 15 mg/kg quarterly was associated with a lower incidence of serious adverse events (AEs) (9% versus 14%, respectively) and grade 3 or 4 AEs (11% versus 18%, respectively) [58].

Collectively, these three studies support the notion that tremelimumab can break peripheral tolerance to self tissues, including cancer, based on the development of immune-related and autoimmune phenomena (e.g., colitis, vitiligo, thyroiditis with elevated antithyroid antibodies) and objective tumor regressions in patients with melanoma [29, 53]. Given this body of evidence, the single-agent strategy of tremelimumab was taken to registration trials in locally advanced and metastatic melanoma (Table 2).

Combination with Established Therapy
Some standard cytotoxic therapies have been hypothesized to release tumor antigens that are crosspresented by host antigen-presenting cells and may trigger immune responses to cancer cells. However, combinations of cytotoxic chemotherapy and immunomodulatory drugs have failed to live up to expectations, exemplified by the nine negative randomized trials with biochemotherapy [56, 59], perhaps because of the myelosuppressive effects of chemotherapy. Therefore, a study population and design were sought that would allow (a) testing of this hypothesis with an anticancer agent without myelosuppressive effects and (b) access to tumor specimens for assessing pathologic response. Study 1004 (Table 1) was proposed as a randomized phase II trial of tremelimumab with neoadjuvant hormonal therapy in patients with high-risk prostate cancer. Randomization allowed the pathologic assessment of specimens from patients treated with hormonal therapy only. Dose-limiting diarrhea occurred in two patients treated at 6 mg/kg, suggesting a potential pharmacodynamic interaction between hormonal therapy and tremelimumab. Initial challenges with procurement of the whole prostatectomy specimen and slow enrollment led to the early termination of the study. Exploratory analysis of tumor specimens is ongoing. Two ongoing phase I clinical trials are testing the concept of the combination of tremelimumab with standard therapy for cancer. One of these clinical trials is in combination with exemestane for patients with metastatic breast carcinoma, and one is in combination with sunitinib malate for patients with metastatic renal cell carcinoma (Table 1).

Combination with a Cancer Vaccine
Preclinical studies suggest that a cancer vaccine may be needed for CTLA-4–blocking mAbs to induce regression in poorly immunogenic experimental tumor models ([2, 4–6, 9–13]. One of the best examples is the B16 murine melanoma model, where tumor response to CTLA-4 blockade is only evident if the anti-CTLA-4 Abs are given with a GM-CSF B16 tumor engineered vaccine [4], peptide pulsed dendritic cell vaccines [6], or depletion of Treg cells [7]. Because most human cancers are believed to be poorly immunogenic, combination with a cancer vaccine was pursued in the clinical setting. A small pilot trial was pursued combining tremelimumab with a vaccine that had demonstrated antitumor activity against metastatic melanoma and for which there was anecdotal evidence of enhanced activity with sequential CTLA-4 blockade [60, 61]. Study 0003 combines an autologous dendritic cell vaccine pulsed with the MART-126–36 immunodominant peptide within HLA-A*0201 with a dose escalation of tremelimumab administered at monthly intervals. This study completed accrual of 16 patients in February 2007. The accrual rate was limited because of the lengthy interval between patients and cohorts to detect potential delayed autoimmune phenomena. Analysis of immunologic and clinical endpoints is ongoing. A new phase I clinical trial combining tremelimumab with the oligodeoxynucleotide PF-3512676 (formerly known as CPG 7909, Table 1) is currently open to accrual.

Choice of Tremelimumab Dose and Schedule for Registration Trial Testing
Preclinical studies demonstrated a concentration-dependent effect of tremelimumab on activated human T cells [52, 54, 62], and the dose-escalation Phase I studies allowed
verification of these findings. In both phase I tremelimumab clinical trials, increasing doses resulted in longer durations of exposure, with a greater frequency of responses and a higher incidence of clinical adverse effects (Table 3). In Study 1001 [29], grade I–II toxicities attributable to tremelimumab first developed at the 1 mg/kg dose, with occasional diarrhea. Grade III–IV diarrhea was noted in one patient at 10 mg/kg and two patients at 15 mg/kg after a single infusion [29]. The most commonly reported toxicities thought to be at least possibly related to tremelimumab administration were diarrhea, dermatitis, pruritus, and fatigue [58]. Antitumor activity also increased with higher doses and longer duration of systemic exposure [29, 53]. Most patients who experienced antitumor activity had plasma concentrations of tremelimumab above the target of 30 µg/ml for ≥4 weeks [29]. Therefore, the preclinical studies accurately predicted concentrations of tremelimumab needed to break tolerance to self and induce antitumor responses.

The protocol-specified DLTs that led to selection of 10 mg/kg as the MTD in the phase I clinical trials (Studies 1001 and 1002) were non–life-threatening toxicities, like skin rash or diarrhea managed without maximal medical intervention [29, 53]. The favorable clinical outcome of the 15 mg/kg cohort within Study 1001 and the observation that the DLTs (three of six patients at 15 mg/kg developed dose-limiting dermatitis and diarrhea) resolved within 3 months without immune-suppressive therapies (e.g., corticosteroids) provided the rationale for re-exploring the safety of the 15 mg/kg dose level with redosing every 3 months in the Study 1002 phase II randomized trial. Interim data reviewed at the time of expansion of Study 1002 supported the 15 mg/kg quarterly regimen based on an antitumor activity identical to the 10 mg/kg monthly regimen with an apparent better safety profile, suggesting a superior therapeutic index [58].

Clinical trials with anti–CTLA-4 Abs indicate a potential association with autoimmune phenomena, but there are limited long-term data. Autoimmune phenomena observed in Study 1001 included diarrhea, dermatitis, vitiligo, pan-hypopituitarism, and hyperthyroidism [29]. Among HLA-A*0201+ patients with stage IV melanoma (n = 56) treated with 3 mg/kg ipilimumab with or without gp100 peptide vaccine, 14 (25%) patients developed grade 3 or 4 autoimmune phenomena (e.g., colitis, dermatitis, uveitis, enterocolitis, hepatitis, and hypophysitis) [25]. At this time, it is not known whether the appearance of autoimmunity is associated with response or prolonged survival. Based on these data, pivotal clinical trials have been initiated with single-agent tremelimumab given at 15 mg/kg every 3 months to patients with metastatic melanoma (Table 2). A single-arm phase II clinical trial in patients with previously treated disease (Study 1008) completed accrual in October 2006. A phase III randomized clinical trial in previously untreated patients with metastatic melanoma is currently open to accrual. This dose and schedule are also being tested in phase II clinical trials in patients with metastatic colorectal carcinoma and non-small cell lung cancer (Table 1).

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Indication</th>
<th>Trial design</th>
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<th>Primary endpoint</th>
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<td>Overall survival</td>
<td>Safety, response rate, duration of response, progression-free survival</td>
</tr>
</tbody>
</table>

**Table 2. Tremelimumab registration program in melanoma**
based peptide vaccine clinical trials [23, 25–27]. In contrast, Sanderson et al. [24] concluded that T-cell activation to immunodominant peptides from MART-1, gp100, and tyrosinase administered with ipilimumab in the adjuvant setting may be higher than that seen with other adjuvants (e.g., GM-CSF, IL-12). In addition, ipilimumab also induces nontumor antigen-specific T cell activation as detected by increased expression of the T cell activation marker HLA-DR on the surface of peripheral CD4+ and CD8+ T cells [23, 25].

**MODULATION OF TREG CELLS AFTER ADMINISTRATION OF CTLA-4–BLOCKING mABS**

CTLA-4 blockade may induce antitumor immune responses by blocking negative signaling provided by CTLA-4 expressed on Treg cells, which may either stimulate IDO production in dendritic cells and have tolerizing rather than immunogenic effects on T cells [40, 42] or directly interact with B7 costimulatory molecules on T cells [43]. Studies were conducted in a subset of patients within the Study 1002 phase II study to determine the potential role of modulating Treg cells on antitumor activity [66]. It was reported that patients with objective response or disease stabilization had a decrease in CD4+/CD25+ Treg cells and in constitutive IL-10 secretion compared with patients with progressive disease. However, functional assays were not reported [66]. In contrast, CTLA-4 blockade with ipilimumab did not alter the number or function of circulating Treg cells when tested by functional assays of suppressor activity (the gold standard for Treg cell quantitation) [44], mRNA levels of the Treg-specific transcription factor Foxp3 [67], and flow cytometric analysis of surface markers on Treg cells [26].

**CONCLUSIONS**

Fully human anti–CTLA-4 mAbs appear to break tolerance to self and tumor antigens, resulting in antitumor activity in some patients. The preclinical development of tremelimumab guided the early clinical development. In particular, ex vivo assays suggested that exposure to achievable concentrations of circulating anti–CTLA-4 Ab would result in immune activation. Clinical dose-escalation trials suggested that the clinical and biologic effects of anti–CTLA-4 Abs are dose dependent, the predicted target plasma concentrations providing a reasonable model of the anticipated clinical effects of CTLA-4 blockade in humans. Combined preclinical and clinical data have allowed the rational choice of dosing and schedule for ongoing clinical trials in patients with malignant melanoma.

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**Table 3. Safety profile and antitumor activity associated with tremelimumab in phase I trial in patients with melanoma**

<table>
<thead>
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<th>Dose level (mg/kg)</th>
<th>n of patients dosed*</th>
<th>Total n of events</th>
<th>Grade 1 or 2</th>
<th>Grade 3</th>
<th>Objective response</th>
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<td>1</td>
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*Includes five patients redosed at a higher level.

Abbreviations: CR, complete response; PR, partial response.

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST
J.G.-N., R.M., D.J.G., P.C.C., and D.C.H. own stock in and have received support from Pfizer. A.R. has acted as a consultant for and performed contract work for Pfizer. D.A.N. is an employee of Pfizer.

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