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Anti-CTLA4 Antibody Clinical Trials in Melanoma

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Abstract

The cytotoxic T lymphocyte-associated protein 4 (CTLA4) is a main negative regulator of the immune system, which inhibits the costimulatory signaling for T cells. Preclinical studies demonstrated that antibodies against CTLA4 induced regression of some murine tumors. Two CTLA4 blocking monoclonal antibodies have entered clinical development and are currently in pivotal clinical trial testing. Ipilimumab (formerly MDX010) is an IgG1 and tremelimumab (formerly CP-675,206 and transiently ticilimumab), is an IgG2, both being fully human monoclonal antibodies. Across several early clinical trials, including dose escalation, single dose, multi-dose, and in combination with a variety of other immune stimulants like peptide vaccines or interleukin-2, objective tumor responses in patients with metastatic melanoma have been observed in the in the range of 5 to 20%. A key feature is that some of these responses are extremely long-lived responses, lasting years. The early clinical testing also demonstrated that these CTLA4 blocking antibodies can lead to significant toxicities, most with an inflammatory or immune mediated mechanism of action. These include colitis and skin rash as the most common toxicities, and a variety of autoimmune and inflammatory processes against multiple organs. Some of these toxicities require immune suppressive therapy and may lead to permanent damage in occasional patients. In conclusion, two monoclonal antibodies blocking CTLA4 have demonstrated ability to break tolerance to self-tissues and result in long lasting objective cancer regressions, and have moved onto late stages of clinical development.

Introduction

Metastatic melanoma is notoriously resistant to standard forms of therapy, such as radiation therapy and chemotherapy, but occasionally undergoes spontaneous remission. There are two agents approved by the U. S. Food and Drug Administration (FDA) for the treatment stage IV melanoma, an old chemotherapy drug (DTIC or Dacarbazine) and the administration of high doses of the immune stimulant interleukin-2 (IL-2) (1). Both have response rates below 15%, and neither form of therapy has been shown to increase survival in a randomized trial. Adding more chemotherapy agents or combining chemotherapy with IL-2 or IFN (so called biochemotherapy regimens) has failed to improve survival in over 10 randomized clinical trials (1,2). Therefore, it is clear that standard cytotoxic drugs alone or in combination with immune stimulating cytokines will not provide a significant change in the natural history of metastatic melanoma.

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Immunotherapy for Melanoma

Genes that lead to immune recognition of melanoma cells have been cloned over the past 15 years, and the mechanisms that regulate antitumor responses have been thoroughly studied. Durable tumor responses in widely metastatic melanoma can now be achieved with a variety of immunotherapy strategies, including the FDA-approved cytokine IL-2, dendritic cell (DC) vaccines (3,4), cytotoxic T lymphocyte antigen-4 (CTLA4) blocking monoclonal antibodies (5–7), and the adoptive transfer of clonally-expanded antigen-specific T cells (8,9). These novel immunotherapy approaches lead to long-term remissions of metastatic melanoma in a small subset of patients, and clinical trial testing is actively pursued.

Among novel immunotherapy approaches, the use of antagonistic antibodies to CTLA4 has the potential to become a widely used approach since it is less toxic than high dose IL-2 and is an off-the-shelve immunotherapy reagent, as opposed to the personalized nature of DC vaccines and T cell adoptive transfer therapy.

CTLA4 Blockade for the Treatment of Melanoma

The cytotoxic T lymphocyte-associated protein 4 (CLTA4, CD152) is a dominant negative costimulatory receptor expressed on the surface of activated T cells. The phenotype of CTLA4 genetically knock-out mice highlights that CTLA4 has a critical role in maintaining lymphocyte homeostasis and the control of anti-self immune responses, since these mice dye of lymphoproliferation and autoimmunity early in life (10,11). Pioneering work by James Allison at Berkeley, CA provided evidence that CTLA4 antagonistic antibodies could induce regression of established tumors in mice (12). Wider testing in murine models demonstrated that only immunogenic tumors responded to single agent CTLA4 blocking antibodies, while less immunogenic tumors required the addition of other interventions, like tumor vaccines, depletion of T regulatory (Treg) cells or chemotherapy (13–19).

Based on this body of work, CTLA4 blocking monoclonal antibodies are being tested as therapy for patients with a variety of cancers, but have been most extensively studied in patients with advanced malignant melanoma. Two fully human CTLA4 blocking monoclonal antibodies are currently in clinical development, ipilimumab (formerly known as MDX010) from Medarex Inc. in joint clinical development with Bristol-Myers-Squib, and tremelimumab (formerly CP-675,206 and transiently known as ticilimumab) from Pfizer Inc. Early clinical data suggests that a subset of patients with metastatic melanoma respond to these CTLA4 blocking antibodies, and pivotal trials have been started with both agents (20,21)

Potential Mechanisms of Antitumor Activity of CTLA4 Blocking Monoclonal Antibodies

Preclinical data has provided evidence for several potential mechanisms of action of antitumor activity with the administration of CTLA4 blocking monoclonal antibodies:

1. CTLA4 blocking monoclonal antibodies have been shown to activate antitumor T cells by blocking this major negative regulator of T cell function. CTLA4 is an immunoglobulin superfamily surface receptor that is expressed on the surface of T cells upon activation. It efficiently competes with CD28, the constitutive positive receptor for the costimulatory molecules CD80 and CD86. Engagement of CTLA4 on the surface of activated T cells by costimulatory molecules inhibits IL-2 and IFN-γ production upon TCR engagement (12,22–24). Blockade of this negative signaling with CTLA4 blocking monoclonal antibodies may result in further activation and expansion of activated T cells leading to antitumor activity (12).

2. CTLA4 expressed by activated T cells also has a dynamic effect. Presence of surface CTLA4 on T cells results in restless cells that engage in shorter interactions with cells expressing their cognate antigen (25,26). The suspected effect is a suboptimal triggering of TCR signaling. Stable interactions are required to trigger stronger signaling able to activate T cell effector functions like cytokine production or cytotoxic granule release. (27). Blocking CTLA4 with antagonistic antibodies may allow longer interactions between activated T cells and cancer cells, lowering the threshold of TCR signaling and inducing cytotoxic effects on cancer cells.

3. Blockade of CTLA4 signaling may decrease the functional activity of Treg cells or may inhibit indolamine 2,3 dioxygenase (IDO) on plasmacytoid dendritic cells (pDC). Treg are dominant suppressor cells with a critical role in controlling autoimmune reactions in peripheral tissues (28). These cells have constitutive expression of CTLA4, which provides reverse signaling to cells that express B7 costimulatory molecules and induces the expression of the immune suppressive enzyme IDO in pDC (29–31). Anti-CTLA4 antibodies could directly deplete or inhibit the function Treg, or block the negative CTLA-4-mediated reverse signaling to pDC and inhibit IDO expression.

4. A direct effect of CTLA4 engaging reagents on CTLA4 positive melanoma cells has been suggested, leading to cancer cell apoptotic death (32). Treatment of CTLA4 positive melanoma cells with recombinant forms of the CTLA4 ligands CD80 and CD86 resulted in triggering the cellular pro-apoptotic machinery, leading to direct killing of cancer cells (32). The main difference with the currently available anti-CTLA4 antibodies in clinical development is that these antibodies have been selected based on their blocking activity upon binding to the CTLA4 molecule, while these recombinant B7 costimulatory molecules were designed to activate CTLA4 signaling. Therefore, even if functional levels of CTLA4 are expressed on the surface of melanoma cells in vivo, the option of direct tumor killing may not be realistic with CTLA4 blocking monoclonal antibodies.

CTLA4 Blocking Monoclonal Antibodies in Clinical Trials

The two CTLA4 blocking antibodies in clinical development were generated in transgenic mice with human immunoglobulin genes knocked-in, therefore being fully human antibodies. They were both raised against the same antigen, but they are unlikely to be targeted to the same epitope. It is quite clear that different antibodies raised to the same molecule can have dramatically different effects on that target, ranging from activating to depleting to blocking effects (26). Ipilimumab is an immunoglobulin 1 (IgG1) antibody, and tremelimumab is an IgG2 antibody (20). The immunoglobulin subtype may result in differences in their biological activities in vivo. As a group, IgG1s are far better at inducing antibody-dependent cellular cytotoxicity (ADCC) and fixing complement than IgG2s. The induction of either one would be an unwanted effect of administering CTLA4 blocking antibodies, since they are intended to activate the cellular target they bind to. However, preclinical data suggests that ipilimumab does not induce ADCC against cells it binds to (33). Overall, it is reasonable to think that the biologic activity of these two anti-CTLA4 antibodies in humans may be slightly different, which may not be detected during early clinical development.

Antitumor Activity of CTLA4 Blocking Monoclonal Antibodies in Clinical Trials

Several manuscripts and presentations at major oncology meetings attest to the biological and clinical activities of CTLA4 blocking monoclonal antibodies. The overall response rate is low, between 5 and 22% of patients with measurable melanoma in experiences reported to date

(Table 1). Despite the low response rate, these studies provide a proof-of-concept that the observations in mice of modulating an immune negative regulatory pathway (12) translates into objective benefit to a subset of patients with cancer.

i. Ipilimumab

The first report of the use of a CTLA4 blocking monoclonal antibodies in humans was an abstract describing the single dose infusion of ipilimumab (at that time MDX010) to patients with melanoma (34). This early experience already provided evidence of durable tumor regression in two our of 17 patients with metastatic melanoma treated with 3 mg/kg of ipilimumab. The first clinical trial reported in a full-length manuscript described the single agent, single dose infusion of ipilimumab to patients with a variety of tumors at the Dana Farber Cancer Institute (35). No objective tumor responses were observed among 7 patients with melanoma using a single dose of 3 mg/kg of ipilimumab. A series of reports from the Surgery Branch of the National Cancer Institute (NCI) demonstrated that 7 out of 56 (12.5%) HLA-A*0201 positive patients had objective responses to repeated dosing with ipilimumab, administered at 3 mg/kg every 3 weeks together with two gp100 peptide vaccines (5,36). These results have been pursued into an ongoing 3-arm phase 3 randomized clinical trial comparing ipilimumab alone, ipilimumab with gp100 peptides, or gp100 peptides alone in HLA-A*0201 positive patients with previously treated metastatic melanoma.

One study from the University of Southern California (USC) administered ipilimumab at 0.3, 1 or 3 mg/kg every 4 weeks to different cohorts of patients, together with gp100, MART-1 and tyrosinase peptide vaccines. In this study the combination of ipilimumab and peptide vaccines was administered in the adjuvant setting to HLA-A*0201 positive patients with completely resected metastatic melanoma (6). Since it was administered to patients without measurable disease in small cohorts of patients, no information on antitumor activity can be derived from this trial. Peptide-specific T cell responses were monitored in these two clinical trials administering ipilimumab together with HLA-A*0201-restricted melanoma peptides (5,6,36). These responses, analyzed by ELISA, MHC tetramer and ELISPOT assays, did not convincingly demonstrated that the peptide vaccines together with the CTLA4 blocking monoclonal antibodies resulted in the expansion of peptide-specific T cells detectable in peripheral blood.

Ipilimumab has also been combined with DTIC chemotherapy and reported in abstract form (37). This was a multicenter randomized phase II clinical trial of ipilimumab administered at 3 mg/kg monthly with or without concomitant DTIC. Responses in the combination arm seemed to be higher than in the single agent ipilimumab arm (17% compared to 5%), at the cost of higher toxicities (28% compared to 18%) (37). However, the 95% confidence intervals for all these endpoints were overlapping. The combination of ipilimumab with DTIC was chosen for further testing in an ongoing phase 3 randomzied clinical trial comparing DTIC alone with DTIC plus ipilimumab in patients with previously untreated metastatic melanoma.

The early clinical data with ipilimumab suggested that patients with toxicity were more likely to have clinical benefit (6,36). Following this lead, two clinical trials have been reported where ipilimumab alone or in combination with high doses of interleukin-2 (IL-2) has been dosed to maximum allowable toxicity. Ipilimumab at a dose escalation of 0.1 to 3 mg/kg every 3 weeks was administered in conjunction with high dose IL-2. There were 8 objective responders out of 36 patients (22%). The authors concluded that this data did not seem to support a synergistic effect of IL-2 and anti-CTLA4 antibodies, since treatment with either agent alone could obtain the observed response rate, or it could be an additive effect (38). Of note, ipilimumab did not seem to worsen the expected toxicities from high dose IL-2. A study of single agent ipilimumab with intra-patient dose escalation every 2 cycles of therapy also resulted in increased toxicity with no improvement in response rates (39). In this study, patients were initially dosed at 3

mg/kg every 3 weeks for 2 doses. If there was no objective response or grade 3 or higher autoimmune toxicity, the dose was increased to 5 mg/kg for 2 doses and then to 9 mg/kg for 2 doses. In this study, 5 out of 46 patients (11%) achieved an objective clinical response at the expense of 35% of patients having grade 3 or 4 toxicities. The authors concluded that increasing doses of ipilimumab to increase autoimmune toxicities did not seem to increase the antitumor activity (39). It is interesting to note that in this study there was no correlation between patients with toxicities and objective tumor response.

Repeated doses of ipilimumab beyond the maximum tolerable dose of 3 mg/kg defined in dose escalation clinical trials have been further tested. An abstract reported a cohort of 24 patients dosed at 10 mg/kg every 4 weeks, with a response rate of 8% and grade 3 or 4 toxicities attributed to the study drug in 10% of patients (a rate much lower than all other studies with this antibody). This dose and regimen has been taken into pivotal clinical trials with ipilimumab, with a completed single agent phase II clinical trial in patients with previously treated metastatic melanoma.

ii. Tremelimumab

Results from two phase I clinical trials with another anti-CTLA4 antagonistic antibody in clinical development, tremelimumab, have been reported (7,40). The first-in-human (FIH) single-dose phase I trial of tremelimumab (at that time CP-675,206) was conducted at the University of California Los Angeles (UCLA) and the M.D. Anderson Cancer Center. A single antibody infusion at doses ranging from 0.01 mg/kg to 15 mg/kg was tested in seven cohorts of patients (7). There were 5 objective responses among 29 patients with measurable melanoma, which were evident at doses of 3 mg/kg and above. The majority of responses were noted in patients that achieved sustained plasma levels of tremelimumab beyond 30 μg/ml at one month (7), which was the target plasma level predicting a biological effect of CTLA4 blockade in preclinical models (41,42).

The doses of 10 mg/kg administered every month and 15 mg/kg administered every 3 months have been studied further in a phase II randomized clinical trial. A manuscript reported the single institution results at M.D. Anderson using these two regimens, with an overall response rate of 16% and a strong trend towards a correlation between toxicity and response (43). However, results of the multi-institutional data presented in abstract form suggested that response rates in patients with previously treated metastatic melanoma with both regimens were comparable (7% and 10%), but toxicity was doubled when dosing more frequently with the 10 mg/kg monthly regimen (27% compared with 13% at 15 mg/kg every 3 months, although the differences were not statistically significant) (44). Based on these data, single agent tremelimumab at 15 mg/kg every 3 months was chosen to be been taken into pivotal trial testing, including a single arm phase II clinical trial in patients with previously treated metastatic melanoma, and a phase III randomized clinical trial comparing the survivals of patients treated in first line with tremelimumab or chemotherapy with DTIC or temozolomide.

Toxicity of CTLA4 Blocking Monoclonal Antibodies in Clinical Trials

CTLA4 blocking monoclonal antibodies have resulted in toxicities most consistent with breaking peripheral tolerance to self-tissues or induction of organ-specific inflammatory processes. The largest reported series dosing patients with ipilimumab at 3 mg/kg every 3 weeks reported a frequency of 25% (14 out of 56 patients) of grade 3 or 4 toxicities (36). The rate of grade 3 and 4 toxicities with tremelimumab is related to the dosing regimen. In a two-arm phase II clinical trial, grade 3 or 4 toxicities were 13% at the pivotal regimen of 15 mg/kg every 90 days, while it was doubled when dosed at 10 mg/kg every 30 days (44). The most commonly observed toxicities with both antibodies have included skin rash, colitis, hypophysitis, thyroiditis, uveitis, pneumonitis and hepatitis (5–7,35–37,45,46).

Three clinical trials with dose-escalation design have provided clear evidence of a monoclonal antibodies dose (or duration of systemic exposure)-effect in the development of toxicities (6, 7,40). Therefore, higher doses or longer exposures to circulating antibody result in higher toxicity and higher likelihood of antitumor responses. A critical question is if both effects are correlated, where patients with autoimmune toxicities are more likely to have antitumor responses. Statistical analysis two from studies with ipilimumab does suggest that this may be the case (36,38). However, as described before, two subsequent clinical trials failed to maintain this correlation (38,39). Therefore, it is currently unclear if toxicity is a requirement for response, as opposed to higher levels of circulating monoclonal antibodies resulting in higher systemic exposure correlating independently with both toxicities and response.

The exact mechanism of the anti-CTLA4-induced toxicities has not been thoroughly studied. It would require repetitive biopsies of normal organs before and after these toxicities, which is feasible only in sites like the intestinal tract or the skin. The suspicion of immune-mediated mechanism of action of some toxicities in remote organs, like the hypophysis, has relied and will probably continue to rely on the symptoms and imaging results, and response to high doses of corticosteroids (46). A manuscript provided pathological evaluation of cases of colitis induced after the administration of ipilimumab (47). There were three histological patterns of colitis: neutrophilic inflammation only, lymphocytic inflammation only and combined neutrophilic and lymphocytic inflammation. It is unclear at this time if these three patterns represent a spectrum of different mechanisms of action, are due to different timing of biopsies in an ongoing colitis, or are dependent on the site of biopsy. Overall, it seems like the histological changes in sites of toxicity after anti-CTLA4 antibody administration have inflammatory as well as autoimmune features.

Exploration of the Mechanism of Action of CTLA4 Blocking Monoclonal Antibodies in Clinical Trials

A potential mechanism of action to induce tumor regressions by CTLA4 blocking antibodies is the expansion of tumor antigen-specific cytotoxic T cells (CTL). However, studies to date have failed to provide evidence for the expansion of tumor antigen-specific T cells detectable in peripheral blood using modern immunological assays (48–50). Clinical trials administering ipilimumab in conjunction with melanoma peptide vaccines have not provided clear evidence that the CTLA4 blocking antibody results in enhancement of circulating CD8+ T cell responses to the immunizing peptides (5,6,36). In addition, administration of tremelimumab as single agent similarly did not result in a clear trend in the expansion of T cell responses to melanoma antigens nor to infectious disease antigens (49). Conversely, biopsies of regressing tumor lesions have demonstrated dense infiltration with immune cell subsets, most commonly CD8 + CTL (51). Therefore, it seems like the relevant antitumor effects will need to be studied inside tumors and not in peripheral blood.

The role of Treg has been explored most frequently using surface phenotype analysis in peripheral blood, which is unable to adequately define cells with functional Treg properties. There was no clear change in the levels of circulating Treg cells after administration of ipilimumab, tested both by surface expression of Treg markers and by the expression of the Treg specific transcription factor FoxP3 (48). In a subset of patients treated with tremelimumab at the M.D. Anderson Cancer Center, it was concluded that patients with a clinical benefit had a decrease in T cells with Treg markers (52). However, these same markers are expressed on the surface of activated T cells (53), and therefore it is unclear if tremelimumab does deplete Treg or changes the number of circulating, recently activated, T cells.

Finally, the potential effect of modulating the function of IDO competent pDC by anti-CTLA4 antibodies has been explored in a subset of patients that underwent tumor biopsies while

receiving tremelimumab. There was no evidence that CTLA4 blockade with therapeutic levels of this antibody resulted in inhibition of IDO (51).

Conclusions

Advances in the understanding of the regulatory mechanisms of immune system are being translated into new treatment options tested in patients with cancer. The clinical experience to date provides proof-of-concept that blocking negative immune regulatory pathways can lead to objective tumor responses. The most encouraging data is the very long-lived objective tumor regressions after administration of CTLA4 blocking monoclonal antibodies to patients with advanced melanoma. An improved understanding on the mechanisms that lead to toxicity and response may allow better defining the study populations and providing adjuvant treatments to modulate response or toxicity.

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Table 1

Summary of full-length reports describing antitumor activity of fully human CTLA4 blocking monoclonal antibodies in patients with measurable metastatic melanoma.

Reference	Antibody	Combination	Antibody dose	Schedule	Number of patients with measurable melanoma	Number of patients with objective tumor response
(34)	Ipilimumab	None	3 mg/kg	Single dose	17	2
(35)	Ipilimumab	None	3 mg/kg	Single dose	7	0
(5,36)	Ipilimumab	gp100 peptides	3 mg/kg	Q3 weeks	56	7
(6)	Ipilimumab	gp100, tyrosinase, MART-1 peptides	0.3–3 mg/kg	Q4 weeks	0	0
(38)	Ipilimumab	IL-2	0.1–3 mg/kg	Q3 weeks	36	8
(39)	Ipilimumab	None	3–9 mg/kg	Q3 weeks	46	5
(7)	Tremelimumab	None	0.01–15 mg/kg	Single dose	29	5
(43)	Tremelimumab	None	10–15 mg/kg	Q1–3 months	30	5

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Table 2

Correlation of toxicity grade with objective tumor responses.

First Author	Attia		Maker		Maker		Reuben	
Treatment	Ipilimumab 3 mg/kg + gp100		Ipilimumab 3 mg/kg + IL-2		Ipilimumab 3–9 mg/kg		Tremelimumab 10–15 mg/kg	
Toxicity Grade	0–II	III–IV	0–II	III–IV	0–II	III–IV	0–I	II–IV
No Response	40	9	25	3	28	13	17	8
Response	2	5	6	2	2	3	1	4
P Value	P = 0.008*		P = 0.3*		P = 0.32*		P = 0.045**	

*Fisher exact test

**Chi-square