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Blockade of cytotoxic T-lymphocyte antigen-4 as a novel therapeutic approach for advanced melanoma

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Abstract

Introduction—The incidence of melanoma continues to rise and prognosis in patients with metastatic melanoma remains poor. The cytotoxic T-lymphocyte antigen-4 (CTLA-4) serves as one of the primary immune checkpoints and downregulates T cell activation pathways. Enhancing T cell activation by antibody blockade of the CTLA-4 provides a novel approach to overcome tumor-induced immune tolerance. Recently, anti-CTLA-4 therapy demonstrated significant clinical benefit in patients with metastatic melanoma, which led to the approval of ipilimumab by the Food and Drug Administration in early 2011.

Areas covered—The fundamental concepts underlying CTLA-4 blockade-potentiated immune activation, the scientific rationale for and the preclinical evidence supporting CTLA-4-targeted cancer immunotherapy are presented. We also provide an update on clinical trials with anti-CTLA-4 inhibitors and discuss the associated autoimmune toxicity.

Expert opinion—Given that overall survival is the only validated endpoint for the anti-CTLA-4 therapy, the clinical implications of the antigen or tumor-specific immunity in patients remain to be clarified. Additional research is necessary to elucidate the prognostic significance of immune-related side effects and significantly optimize the treatment regimens. An improved understanding of the mechanisms of action of CTLA-4 antibodies may also culminate in wide-ranging clinical applications of this novel therapy for other tumor types.

Keywords

cytotoxic T-lymphocyte-associated antigen; CTL-A blockade; T cell activation; tumor immunity; overall survival

1. Introduction

The incidence of malignant melanoma has been steadily increasing for the past 30 years in western countries [1]. The American Cancer Society estimates that about 68,130 new

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melanomas will be diagnosed in the United States during 2010 (about 38,870 in men and 29,260 in women) [2]. Despite an increasing understanding of the genetic and epigenetic causes of this malignancy, the death rate continues to increase faster than that for most other types of cancer [3]. Melanoma is staged 0–IV and the survival of patients with melanoma declines dramatically with increasing stage of disease [4]. The prognosis in melanoma patients with distant metastases is poor, and the 5-year survival rate is less than 10 %. Chemotherapy is often used for treatment of melanoma [5, 6]. Dacarbazine and hydroxyurea are approved by the Food and Drug Administration (FDA) for the treatment of metastatic melanoma. However, chemotherapy is of limited value in most patients with stage III or IV melanoma and a clear survival benefit for chemotherapy has yet to be demonstrated. Recently, the BRAF kinase inhibitor vemurafenib (PLX4032) was shown to dramatically improve progression-free and overall survival in patients with previously untreated melanoma with the BRAF V600E mutation [7]. The final estimate of survival outcomes and durability of the response after the discontinuation of vemurafenib remains to be determined. Currently, there is no standard of care for patients who have progressive disease. Due to the marginal benefits of conventional therapy, there has been considerable interest in the development of other strategies for melanoma treatment.

Most of our knowledge-base on human tumor immunology is derived from studies of melanoma. The identification of melanoma-associated antigens resulted in major progress in understanding of the antitumor immune responses [8–10]. Emerging evidence supports the concept of immunotherapy for the treatment of patients with malignant diseases [11, 12]. Indeed, a number of systemic adjuvant therapies using cytokines have been evaluated for melanoma patients with a high risk of disease recurrence following surgery [13, 14].

High-dose Interferon (IFN)-α2b (Intron A; Schering Corporation, Kenilworth, NJ) is the only agent approved by the FDA in the US for the adjuvant therapy of melanoma [15]. IFN-α has demonstrated therapeutic effects in the treatment of a variety of hematologic malignancies and solid tumors due to their immunoregulatory and antiproliferative properties [16]. The use of IFN-α as an adjuvant therapy for melanoma was based on multiple clinical trials showing a consistent improvement in relapse-free survival of patients with resectable, stage IIB/III melanoma. However, the effect of IFN-α therapy on OS remains unclear [15, 17, 18]. In addition, its high toxicity profile also affects the decision to use IFN-α as an adjuvant therapy for melanoma [19].

Cytokine-based therapy with high dose Interleukin-2 (IL-2), a potent immunostimulatory molecule involved in T cell growth and expansion [20], is approved for patients with metastatic melanoma in the US [21]. Overall response rates were documented in 16% of patients, while 6% of patients achieved durable complete responses [22]. However, the serious and toxic side effects caused by high-dose IL-2 treatment represent significant limitations for its clinical applications [23]. Although the moderate success of agents such as IFN-α and IL-2 validated the principle of cancer immunotherapy, the overall limited efficacy of these agents as indicated by poor overall response rates and a lack of survival benefit for melanoma has prompted scientists to continue searching for novel therapeutic targets capable of potentiating immune activation.

Advances in defining the mechanisms involved in regulation of immune responses have provided new molecular targets for therapeutic intervention. Ipilimumab (Yervoy, Bristol-Myers Squibb, Princeton, NJ) is a humanized monoclonal antibody directed against cytotoxic T lymphocyte antigen 4 (CTLA-4), a co-inhibitory molecule expressed on activated T cells. It has been well documented that CTLA-4 contributes to tolerance to self-antigens by attenuating T cell response and proliferation [24, 25]. In a recent phase III trial, ipilimumab as a second-line therapy demonstrated a significantly improved OS in previously

treated unresectable stage III or stage IV melanoma patients [26]. The final FDA approval was granted for ipilimumab (3 mg/kg monotherapy) as a treatment of advanced melanoma in early 2011.

2. CTLA-4 is an immune checkpoint molecule

Activation of naïve T cells in response to a specific antigen requires multiple concerted signals. T cell activation required at least two signals, which includes recognition and binding of the T cell receptor to antigen-bound major histocompatibility complex (MHC) on the antigen-presenting cell (APC) as well as simultaneous co-stimulatory engagement of CD28 on the T cell by members of the B7 family (e.g., B7.1/CD80, B7.2/CD86) on the APC [27]. B7-CD28 interaction results in activation of the PI3K/AKT pathway, upregulating the transcription factor NF-κB, pro-survival signals (e.g., Bcl-2 and Bcl-X_L) as well as cytokine production and stimulation of subsequent cell expansion [28]. CTLA-4, also known as CD152, is a homologue of CD28 and functions as an inhibitory receptor for B7 costimulatory molecules expressed on APCs [29, 30]. CTLA-4 exhibits at least 20-fold higher binding avidity than CD28 for B7 molecules [31]. Upon T cell activation, CTLA-4 is upregulated and competes with CD28 for binding to B7, thereby transmitting a suppressive signal for T cell activation [31–33]. It has been shown that the blockade of CTLA-4 interaction with B7 enhances T cell activation *in vitro*, whereas antibodies that engage CTLA-4 signaling attenuate T cell activation [34, 35]. CTLA-4 as a negative regulator of T cell activation was supported by early studies using CTLA-4 knockout mice. These mice developed a massive lymphoproliferative disorder, which was lethal within 3 weeks of age [36]. Overexpression of full-length CTLA-4 *in vivo* completely rescued the lymphoproliferative disorders observed in CTLA-4 deficient mice [37]. Thus, CTLA-4 represents a critical checkpoint molecule that promotes T cell tolerance following an immune response.

Transcription and translation of CTLA-4 are upregulated upon T cell activation, and its cell surface expression is tightly regulated in a cyclical fashion [38]. CTLA-4 expression on the surface of human T cells is dynamically regulated by its transit between intracellular compartments and the cell surface in a phospholipase D- and ADP ribosylation factor-1-dependent manner [39], whereas the adapter protein- 1 (AP-1) targets CTLA-4 to the lysosomal compartment for degradation in murine cytotoxic T cells [40]. Interestingly, zeta-associated protein (ZAP)-70-dependent tyrosine phosphorylation of CTLA-4 in its cytoplasmic tail in Jurkat T cells is important for its cell surface retention but not for down-regulation of T cell activation [41]. Phosphorylation of CTLA-4 by the tyrosine kinases Lck, Fyn and resting lymphocyte kinase (RLK) prevents the binding of clathrin-associated adapter protein-2 (AP-2) to the cytoplasmic domain of CTLA-4 and limits the subsequent internalization of CTLA-4 in mouse T cells [42], resulting in increased levels of CTLA-4 on cell surface. Upon dephosphorylation of CTLA-4, AP-2 is then able to bind CTLA-4, triggering the endocytosis of the receptor [43].

Although several mechanisms have been provided to explain how CTLA-4 may modulate T cell responses, the molecular details remain unclear. Due to its much higher affinity for binding of B7, CTLA-4 is thought to inhibit CD28-mediated costimulatory signal, leading to decreased T cell activation [38, 44]. Using *in vitro* migration assays and *in vivo* two-photon laser scanning microscopy, Schneider *et al.* showed that CTLA-4 increased T cell motility by interfering with the immune synapse formation between T cells and APCs, leading to decreased cytokine production and T cell proliferation. These findings support a reverse stop-signaling mechanism for modulating the threshold of T cell activation by CTLA-4 [45]. It has been reported that CTLA-4 inhibits the activation of transcription factors such as NF-κB, NF-AT and AP-1 [46] as well as Ca^{2+} mobilization and PLC-γ1 phosphorylation in

activated T cells *in vitro* [47]. CTLA-4 can also target activation of the type II serine/threonine phosphatase PP2A in human $CD4^+$ T cells [48]. Indeed, PP2A acts as a downstream effector of PI3K/Akt signaling pathways and has been shown to play a prominent role in mediating CTLA-4 suppression of human T cell activation [49]. Studies using T cells-derived from CTLA-4 deficient mice demonstrated that CTLA-4 also regulates expression of Casitas B cell lymphoma-b (Cbl-b), a negative intercellular adaptor protein that is critical for establishing the threshold for T cell activation [50]. Under culture conditions for mouse naïve T cell differentiation to T helper 1 (Th1) and Th2 cells, CTLA-4 engagement inhibits the IL-4/signal transducer and activator of transcription-6 (STAT6) pathway, leading to GATA-3 mRNA up-regulation and a tight control on Th2 cell differentiation [51]. In addition, another mechanism underlying CTLA-4-mediated T cell suppression may involve its ability to influence cell cycle progression. CTLA-4 ligation in purified mouse $CD4^+$ T cells blocks the activation of cell-cycle progression–associated proteins (Cdk-4, Cdk-6, and cyclin D3), resulting in delayed expression of the cell cycle inhibitor $p27^{kip1}$ and cell cycle arrest at the G_1 to S phase [52, 53].

In addition to its direct effects on T cell activation, CTLA-4 also regulates T cells by attenuating activation of APC. CTLA-4 engagement upregulates the expression of indoleamine 2,3-dioxygenase (IDO), a suppressor of dendritic cell (DC) function [54]. Induction of the tryptophan-degrading enzyme IDO in specific splenic DC subsets completely blocked clonal expansion of T cells [55]. It was proposed that modulation of tryptophan catabolism via IDO is a means by which CTLA-4 functions *in vivo* [56]. IDO activity induced by $CD4^+$ T cells were able to effectively inhibit proliferation of $CD8^+$ T cells *in vitro*, suggesting that IDO induction in DCs upon engagement by CTLA-4 on regulatory $CD4^+$ T cells may provide a negative feedback control of T cell responses [57, 58]. Additional evidence also suggests CTLA-4 directs proper functions of $FoxP3^+$ regulatory T cells (Tregs), which are critical in maintaining peripheral tolerance and preventing autoimmunity [59]. Qureshi *et al.* recently revealed a novel mechanism of CTLA-4 action in negatively regulating T cell immune responses. CTLA-4 captures and internalizes B7 molecules on the surface of APCs through trans-endocytosis, resulting in degradation and depletion of co-stimulatory molecules [60]. Interestingly, CTLA-4 is also expressed by human monocytes [61] and human monocyte-derived DCs [62, 63]. Ligation of CTLA-4 in the monocyte-like cell-line U937 with CTLA-4 antibodies partially inhibited the proliferation of cells and the upregulation of CD86, CD54, HLA-DR and HLA-DQ induced by IFN-γ [61]. CTLA-4 engagement on human mature DCs resulted in upregulation of IL-10, downregulation of IL-8 and IL-12, as well as reduced proliferation of autologous $CD4^+$ T cells [62]. Together, these findings establish an important role of CTLA-4 as a physiological checkpoint in T cell activation and immune homeostasis, which provides the immunological basis for enhancing the magnitude and duration of T cell activation by blocking the CTLA-4-B7 interaction.

3. Preclinical studies of CTLA-4 blockade in murine tumor models

Given the negative regulatory effect of CTLA-4 in T cell response, which is essential to cancer immunotherapy, it was proposed that blockade of CTLA-4 might overcome the CTLA-4-mediated suppression and enhance preexisting or induced immune responses to cancer. In a vaccination setting, CTLA-4 blockade during the $CD8^+$ T cell priming phase led to increased expansion and effector function of antigen-specific memory $CD8^+$ T cells [64, 65], suggesting the potential use of CTLA-4-blocking antibodies during vaccination to augment immune memory formation and maintenance. *In vivo* administration of CTLA-4-blocking antibodies has been shown to result in the rejection of pre-established tumors in mice, including fibrosarcoma, ovarian and colon carcinoma [66, 67]. However, anti-CTLA-4 monotherapy failed to induce regression of poorly immunogenic B16 melanoma, suggesting

that the degree of response is shown to correlate directly with the immunogenicity of the tumor. Based on the immune-attenuating activities of CTLA-4, disabling CTLA-4 was expected to potentiate the therapeutic efficacy of other immune-based cancer treatment. Indeed, CTLA-4 blockade in combination with a whole tumor cell vaccine engineered to secrete GM-CSF not only synergistically enhanced efficacy, but also induced loss of fur pigmentation (vitiligo) in some animals, indicative of loss of tolerance to melanocyte differentiation antigens[68, 69]. CTLA-4 blockade has also been evaluated in combination therapy with melanoma cell vaccines expressing Flt3 ligand (Flt3L) [70], DNA vaccine targeting melanoma-associated antigen [71], anti-4-1BB antibody [72], CD40 stimulation [73], PD-1 blockade [74], and CpG administration [75].

CTLA-4 blocking antibodies have been shown to exhibit antitumor effects though several mechanisms. CTLA-4 blockade may induce antitumor responses by sustaining the activation of tumor-specific T cells [76]. In addition, CTLA-4 inhibition may enhance antitumor response by depletion of $CD4^+CD25^+$ Tregs or modulation of their suppressive functions [56–58]. However, the fact that CTLA-4 blockade and depletion of $CD25^+$ Tregs can act synergistically in inhibition of tumor growth suggests that CTLA-4 blockade does not function entirely through regulation of Treg [77, 78]. Alternatively, $CD25^+$ Tregs and CTLA-4 signaling may represent two alternative pathways for suppression of autoreactive T cell immunity [77]. Therefore, the effects of CTLA-4 blocking antibodies on Treg appear to be complex and have been suggested to involve the balance of effector T cells (Teff) and Treg [79] which appears to be crucial for effective anti-tumor immmunotherapy. Recent studies showed that the combination of CTLA-4 blockade with a GM-CSF-transduced melanoma cell vaccine induced a greater intratumoral ratio of Teff to Treg, which directly correlated with tumor rejection [80]. Similar observations were also made in CTLA-4 and PD-1 combination blockade-treated melanoma-bearing mice, which displayed high ratios of Teff to Treg as well as Teff to myeloid-derived suppressor cells (MDSCs) within the tumor compartment [74]. The increased Teff infiltration may be able to override the suppression of regulatory immune cells in the tumor site, thereby promoting overall immunotherapeutic efficacy. Analysis of the immune responses in tumor-bearing mice treated with GM-CSF-secreting tumor cell vaccine in combination with CTLA-4 blockade revealed the induction of $CD8^+$ T cells specific for a melanocyte differentiation antigen tyrosinase-related protein 2 (TRP-2) [69]. In addition, this combination therapy elicited a humoral immune response against multiple angiogenic cytokines, such as angiopoietin-1, angiopoietin-2 and macrophage inhibitory factor (MIF), and inhibit the angiogenic network in the tumor microenvironment [81]. Taken together, these preclinical studies not only validate the proof-of-concept for improving cancer immunotherapy by inhibiting CTLA-4-mediated negative signaling, but also provide important insights into the mechanisms underlying the antitumor activities of CTLA-4 blockade.

4. Clinical efficacy of anti-CTLA-4 therapy for human melanoma

Two human antibodies directed against CTLA-4 have been developed by two pharmaceutical companies and evaluated in patients with metastatic melanoma. Ipilimumab (formerly MDX010, Bristol-Myers Squibb) is an IgG1 antibody with a plasma half-life of approximately 14 days [82]. Tremelimumab (formerly CP745-206, Pfizer, New York, NY) is an IgG2 antibody with a plasma half-life of 22 days [83]. Initial clinical studies indicated that both ipilimumab [84] or tremelimumab [83, 85] displayed biological activities and induced objective tumor responses in patients with metastatic melanoma. However, tremelimumab in a phase III trial involving patients with unresectable stage IIIC or stage IV melanoma (15 mg/kg every 90 days) did not improve OS compared with standard chemotherapy using dacarbazine or temozolomide [86]. At the interim analysis, the trial was closed for futility, as the tremelimumab arm did not demonstrate a survival advantage. At

the time of closure, patients in the tremelimumab arm had a median OS of 11.8 months, compared to 10.7 months in the control arm.

A single fixed dose (3 mg/kg) or in multiple dosing schedules, with or without gp100 vaccine, was evaluated in early clinical studies of ipilimumab [84, 87]. Subsequently, a randomized phase II dose escalation study was conducted in previously treated patients with advanced melanoma (0.3, 3, 10 mg/kg every 3 weeks × 4) [88]. Response rates correlated with dose, with a best overall response rate of 11.1% in patients who received the highest dose. Two additional dose-ranging phase II studies used ipilimumab at 10 mg/kg every 3 weeks for a total of four doses, followed by ipilimumab treatment every 12 weeks beginning at week 24 [89, 90]. Ipilimumab in all these three phase II trials achieved significant clinical benefits and 24-month survival rates for patients with metastatic melanoma were shown to be in the range of 19.1–41.1% [88–90], which indicates the therapeutic potential of ipilimumab in the treatment of advanced melanoma.

Recent results of a randomized, double-blind, phase III trial demonstrated the first OS benefit in patients with metastatic melanoma who were treated with ipilimumab, with or without the gp100 vaccine [26]. Patients with unresectable stage III or IV melanoma who had received previous treatment were randomized and received ipilimumab (3 mg/kg) plus gp100, ipilimumab alone or gp100 alone. Patients receiving ipilimumab plus gp100 had a significantly longer median OS compared to the gp100 alone group (10 vs 6.4 months, $p < 0.001$). A survival benefit was also shown in the ipilimumab monotherapy group and there was no significant difference in OS between the two groups that received ipilimumab, indicating that the use of the gp100 vaccine did not affect the efficacy of ipilimumab. Notably, a significant proportion of patients on the ipilimumab arms survived at least 1–2 years. Survival rates for ipilimumab treated patients were 45.6% at 1 year and 23.5% at the 2-year mark. Given the OS advantage demonstrated in this phase III trial, the FDA approved the use of ipilimumab in the 3 mg/kg every 3-week regimen.

A phase II study of ipilimumab in combination with chemotherapy, dacarbazine, in patients with unresectable, metastatic melanoma showed durable objective responses and longterm survival [91]. The objective response rate was 14.3% with ipilimumab plus dacarbazine and was 5.4% with ipilimumab alone. A multicenter, double-blind, randomized, phase III trial of ipilimumab (10 mg/kg) plus dacarbazine (850 mg/m^2) compared with dacarbazine and placebo as a first-line-therapy for unresectable stage II or metastatic melanoma was recently completed [92]. OS was significantly prolonged in the group receiving ipilimumab plus dacarbazine than in the group receiving dacarbazine plus placebo (11.2 months vs. 9.1 months). The survival rates in the ipilimumab–dacarbazine group and control group at 3 years was 20.8% vs 12.2%, respectively [92]. Clinical trials including ipilimumab with other chemotherapy drugs, including melphalan, dactinomycin, temozolomide and bevacizumab, in the treatment of advanced unresectable melanoma are currently recruiting patients (ClinicalTrials.gov Identifier: NCT01323517, NCT01119508 and NCT00790010).

During the clinical trials evaluating ipilimumab treatment of metastatic melanoma, it was observed that patients may have a slow response to the anti-CTLA-4 therapy, as indicated by a delayed tumor regression [82]. There are several variable response patterns [93, 94], including shrinkage in baseline lesions; stable disease followed by a slow, steady decline in total tumor burden; progressive disease followed by a clinical response; and an decrease in total tumor burden accompanied by the appearance of other new lesions. These findings indicate that traditional response criteria may not be adequate for evaluating clinical response to CTLA-4-targeted biological therapy (i.e., ipilimumab). Conventional response criteria including the WHO and RECIST guidelines were initially developed to monitor patients treated with cytotoxic chemotherapies. In light of the unique features of responses

to anti-CTLA-4 therapy, new guidelines for the evaluation of immunotherapeutic activity in solid tumors have been developed to better classify clinical activity [93].

5. Clinical evidence of immune augmentation by anti-CTLA-4 therapy

In addition to demonstrating the therapeutic efficacy against advanced melanoma, the clinical studies of anti-CTLA-4 antibodies have provided insights into their antitumor mechanisms. Treatment with ipilimumab resulted in an increase in activated ($HLA\text{-}DR^+$) $CD4^+$ cells, antigen specific granzyme B^+CD8^+ T cells, IFN-γ expressing $ICOS^{high}CD4^+$ T cells, and the ratio of Teff to Treg in cancer patients [87, 95–97]. Melan-A-specific $CD8^+$ T cells were shown to be associated with tumor rejection following ipilimumab administration [96]. Tumor *in situ* monitoring is important to understand the immunological response in patients treated with anti-CTLA-4 therapy. It was reported that tumor necrosis in patients treated with vaccination followed by anti-CTLA-4 therapy was related to the ratio of intratumoral $CD8^+$ Teff to $FoxP3^+$ Treg in posttreatment biopsies [87]. A similar observation was made in patients with metastatic melanoma receiving the anti-CTLA4 antibody tremelimumab [98]. There was a highly significant increase in intratumoral infiltration by $CD8^+$ cells after tremelimumab treatment. However, there was no difference between clinical responders and patients with non-responding lesions [98].

Although Treg cells are known to express high levels of CTLA-4 [59], anti-CTLA-4 therapy with ipilimumab did not appear to affect the levels of Tregs [99, 100]. Similar results were also found in melanoma patients treated with tremelimumab [101]. Tremelimumab therapy induced a substantial tumor infiltration by $CD8^+$ CTLs, but only had varying effects on intratumoral infiltrates of $CD4^+FoxP3^+$ Tregs or expression of IDO at the tumor site [101]. The study by Menard *et al.* reported that anti-CTLA-4 treatment with tremelimumab promoted memory T cell resistance to Treg in patients with metastatic melanoma, suggesting that CTLA-4 blockade may reverse Treg-mediated suppression [102], most likely through enhancing the functional activation of Teffs [101].

Interestingly, a higher frequency of T helper (Th) 17 inducible cells appeared to be positively associated with freedom from relapse in patients receiving the ipilimumab [103]. Both naturally occurring Th17 cells and Tregs have been shown to be present in the tumor microenvironment and tumor-draining lymph nodes in both human and mice tumors [104]. Given recent studies in animal models implicating the involvement of Th17 cells and IL-17 in T cell recruitment, priming $CD8^+$ T cells and antitumor immunity [105, 106], more work is required to define the contribution of Th17 cells in the tumor compartment to the therapeutic effects of anti-CTLA-4 therapy.

Although no significant difference in OS was seen in the recent phase III trial between the ipilimumab alone group and the ipilimumab plus gp100 vaccine group [26], the immunostimulatory actions of CTLA-4 blockade, especially on Teff, provide an immunological basis for incorporating anti-CTLA-4 therapy into other active immunotherapies including vaccines. CTLA-4 blockade enhanced NY-ESO-1 antigen-specific B cell and T cell immune responses in the ipilimumab-treated patients with durable objective clinical responses and stable disease [107], further supporting the immunotherapeutic designs that combine antigen-specific vaccination with CTLA-4 blockade. Indeed, autologous DCs pulsed with $MART\text{-}1_{26–35}$ peptide administered with tremelimumab resulted in enhanced T cell responses as well as objective and durable tumor responses at the higher range of the expected response rate with either agent alone [108]. Administration of anti-CTLA-4 antibodies after vaccination with irradiated, autologous tumor cells engineered to secrete GM-CSF (GVAX) generated clinically meaningful anti-tumor immunity in a majority of metastatic melanoma patients [87]. Furthermore, several

recent studies demonstrated that ipilimumab enhanced T cell immunity induced by antigen-specific vaccines or adoptive transferred tumor antigen-specific T cells [109, 110]. Clinical trials evaluating different combinations of ipilimumab with vaccines are planned or ongoing in the adjuvant and metastatic setting for treatment of different types of cancer, such as melanoma, prostate cancer and pancreatic cancer (ClinicalTrials.gov Identifier: NCT01302496, NCT00124670, NCT00836407). These trials are expected to shed more light on the feasibility and potential of combining ipilimumab with vaccine therapy. Adoptive immunotherapy using tumor-infiltrating lymphocytes represents a promising and effective cancer treatment for patients with metastatic melanoma [111, 112]. Phase I/II study of cellular adoptive immunotherapy using autologous $CD8^+$ NY-ESO-1-specific T cells and anti-CTLA-4 antibodies for patients with metastatic melanoma is currently recruiting patients (ClinicalTrials.gov Identifier: NCT00871481). The clinical trial of adjuvant therapies with high-dose IFN-α2b and tremelimumab for patients with recurrent stage III or stage IV melanoma is ongoing (ClinicalTrials.gov Identifier: NCT00610857).

6. Immune-related adverse events associated with anti-CTLA-4 therapy

The adverse events associated with anti-CTLA-4 antibodies, designated as immune-related adverse events (irAEs), are consistent with the proposed role of CTLA-4 in maintenance of immune tolerance [113]. The common side effects occurring during anti-CTLA-4 treatment were the development of inflammatory conditions occurring mainly in the skin (e.g., rash) and the gastrointestinal tract (e.g., diarrhea and colitis) [114, 115]. Anti-CTLA-4 therapy also causes serious injury to the liver [116], spleen [117], kidney [118] and endocrine system [119, 120]. Rare deaths have been associated with bowel perforation. Endocrinopathies, including thyroiditis and hypophysitis, as well as hepatitis have also been reported in clinical trials, but have significantly lower incidence.

It appears that most of the irAEs were due to inflammatory cell infiltration, as evidenced by perivascular lymphoid aggregates of both $CD4^+$ and $CD8^+$ T cells as well as neutrophils and macrophages [101, 121]. The tremelimumab therapy was reported to increase Th17 cells in peripheral blood of patients with metastatic melanoma [122], which may have contributed to anti-CTLA-4 therapy-induced autoimmune toxicity. In addition, the increased number of Tregs in the gastrointestinal mucosa has also been suggested to play a role in the gastrointestinal pathogenicity or toxicity associated with anti-CTLA-4 therapy [123]. Other reported irAEs in patients receiving ipilimumab include red cell aplasia and neutropenia [124, 125], Graves ophthalmopathy like syndrome [126], and Guillain–Barré syndrome [127]. The standard of care for treating irAEs is systemic administration of corticosteroids [26, 88, 90]. Additional therapies with immunosuppressants (e.g., infliximab, mycophenolate) may also be given [26, 90, 128]. Furthermore, other treatments using hormone replacement for hypophysitis and intravenous immunoglobulin for immune-mediated blood disorders have been used [26, 124, 125]. Thus, the adverse events can be managed by these measures in the vast majority of cases.

A few studies have indicated a possible correlation between irAEs and clinical benefit or therapeutic efficacy of anti-CTLA-4 treatment [121, 129, 130]. In one study of patients with stage IV disease receiving ipilimumab (10 mg/kg), the clinical response rate (36%) was significantly higher in ipilimumab-treated patients who developed grade III or IV irAEs compared with the patients who did not have autoimmune toxicity (5%) [129]. Another study of patients treated with ipilimumab also showed that the durable objective clinical responses were significantly associated with the development of irAEs [130]. These results suggest that autoimmunity may be considered a surrogate maker of host response to cancer immunotherapy, including anti-CTLA-4 therapy. However, high-grade irAEs are not required for clinical response. Moreover, it has been recognized that concomitant tumor

immunity and autoimmunity can be uncoupled even though similar mechanisms of action are involved [131, 132]. With the increasing use of anti-CTLA-4 antibodies in the clinic, further studies are needed to confirm the potential association between side effects and antitumor responses, and to elucidate these mechanisms in melanoma patients for future optimization of the therapies. The biomarkers and factors that may be used to predict or determine the antitumor or autoimmune responses in response to anti-CTLA-4 therapy are an area of active investigation.

Conclusion

Advances in deciphering immune homeostasis have led to a new age of melanoma immunotherapy. The role of CTLA-4 in the inhibition of T cell activation and maintenance of T cell peripheral tolerance has been well established. Blockade of CTLA-4 represents a rational strategy to potentiate activation of Teff and to induce antitumor immunity. Clinical studies in patients with advanced melanoma have demonstrated that treatment with CTLA-4 blocking antibodies provided clinical benefits, which led to the recent approval of ipilimumab by the FDA for therapy of unresectable or metastatic melanoma. The results of two Phase III trials have demonstrated that ipilimumab improved survival in patients with metastatic melanoma in both first and second-line treatment settings.

Expert opinion

An increased understanding of immunoregulatory functions of CTLA-4 in T cell tolerance has prompted efforts to develop CTLA-4 blocking antibodies for potentiating immune responses to cancer. Clinical evaluation of CTLA-4 blockade therapy has demonstrated durable tumor responses and OS benefits in patients with advanced melanoma. Results of the phase III studies of ipilimumab in combination with dacarbazine chemotherapy in patients with previously untreated metastatic melanoma are very encouraging. The results of clinical trials of combination therapy with vemurafenib and ipilimumab (ClinicalTrials.gov Identifier: NCT01400451) in advanced melanoma are also eagerly awaited. In addition to advanced melanoma, ongoing studies of CTLA-4 blockade for the treatment of colorectal carcinoma, prostate cancer, and nonsmall cell lung cancer will determine the potential clinical benefits of anti-CTLA-4 therapy in patients with other tumor types. Moreover, the success of CTLA-4 blockade in the clinic has set a precedent for immunotherapeutic approaches targeting several other immune modulators with distinct roles in immune regulation (e.g., PD-1 and CD137/4-1BB), which are being clinically tested (ClinicalTrials.gov Identifier: NCT01176461, NCT01176474 and NCT00612664).

The autoimmune toxicity associated with anti-CTLA-4 therapy appears to be a major obstacle for broad clinical applications. However, treatment related irAEs can be managed in the clinical setting. Notably, systemic steroid treatment of autoimmune symptoms does not appear to compromise the clinical effects of CTLA-4 blockade. Nonetheless, anti-CTLA-4 antibodies should be used with caution in patients with a history of autoimmune disease. Further studies are needed to better clarify the mechanisms involved in anti-CTLA-4 antibody-mediated autoimmune toxicity and to examine whether the clinical outcomes and the concomitant irAEs may be separated.

It still remains unclear whether genetic variation in CTLA-4 could influence the antitumor response or autoimmunity to CTLA-4 blockade therapy in metastatic melanoma patients [133, 134]. CTLA-4 single nucleotide polymorphisms (SNPs) have been described in various autoimmune diseases [135] and certain CTLA-4 SNPs may be associated with a poor clinical outcome after treatment with ipilimumab [133]. Therefore, the impact of polymorphisms in the CTLA-4 gene and patient genotype on the clinical response should

also be evaluated further. In addition, future efforts should also be placed on identification and validation of diagnostic biomarkers in response to anti-CTLA-4 treatment. Obtaining this critical information on the predictors of immune and clinical responsiveness and defining the risk of toxicity to the treatment will greatly facilitate the clinical development of CTLA-4 blockade therapy.

Progress in tumor immunology has led to the recognition of tumor immune invasion mechanisms [136]. The host immune response in patients with early disease has been shown to differ from that in patients with more advanced disease [137]. The less pronounced immune tolerance or suppression status in the former patients may enable these patients to be more responsive to immunological interventions such as anti-CTLA-4 therapy. Therefore, clinical trials testing CTLA-4 blockade in the neoadjuvant setting should help better define the role of anti-CTLA-4 therapy in the treatment of melanoma. Moreover, the clinical trials should not only determine clinical response but also provide more insights into the interactions between the CTLA-4 inhibitors and the host's immune system. The increase of the immune suppressive cells including MDSC and Treg following treatment with anti-CTLA-4 antibodies is expected to limit T cell-mediated antitumor efficacy. Multiple strategies are needed to continue building upon this platform, including combination with other agents or approaches capable of enhancing antitumor immunity synergistically should lead to further improved clinical outcomes.

Article highlights

- CTLA-4 counterbalances a co-stimulatory signal required for T cell activation and is an essential participant in maintenance of immune tolerance. Antagonizing CTLA-4 with blocking antibodies results in amplified T cell activation and antitumor immunity.
- CTLA-4 blockade therapy is a promising approach to potentiate a patient’s immune system to induce antitumor responses and represents a significant advance in the fields of immunotherapy and oncology. Ipilimumab is the first agent ever proven to improve overall survival in patients with advanced melanoma.
- Unique patterns of tumor responses are shown with this class of agents. Some patients treated with ipilimumab developed a clinical response after disease progression (increase in tumor volume or emergence of new lesions), indicating a clinical response pattern induced by CTLA-4 blockade that is distinct from cytotoxic chemotherapy.
- Appearance of autoimmune toxicities such as colitis, dermatitis and hypophysitis in patients receiving anti-CTLA-4 therapy is consistent with the immune-suppressive functions of CTLA-4. Immune-related adverse events associated with ipilimumab are manageable in the majority of cases, and might also reflect positive treatment responses.
- Understanding the mechanisms of action of CTLA-4 blockade and identifying prognostic immunological correlates of clinical endpoints are important for guiding the future clinical applications of anti-CTLA-4 therapy either alone or in combination with other treatment modalities.

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