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FOCUSSED RESEARCH REVIEW

Ipilimumab: controversies in its development, utility and autoimmune adverse events

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Abstract A promising new class of anti-cancer drugs includes antibodies that mediate immune regulatory effects. It has become very clear over the last decade that different types of immune cells and different pathways serve to suppress anti-cancer immunity, particularly in the microenvironment of the tumor. The first examples of immune modulating antibodies are those directed against cytotoxic T lymphocyte antigen-4 (CTLA-4), a molecule present on activated T cells. Human antibodies that abrogate the function of CTLA-4 have been tested in the clinic and found to have clinical activity against melanoma. In this review, we discuss some of the controversies surrounding the potential clinical utility of one of those antibodies, ipilimumab, formerly MDX-010, from Medarex and Bristol Myers Squibb. The optimal dose and schedule of ipilimumab was derived in multiple clinical trials whose latest results are described below. Favorable survival in patients with stage IV melanoma were observed that appear to be associated with unique side effects of the drug called "immune-related adverse events". The management of these side effects is described, and the unusual kinetics of anti-tumor response with ipilimumab as well as a newly proposed schema for assessing anti-tumor responses in patients receiving biologic compounds like ipilimumab, which may supercede RECIST or WHO criteria, are addressed.

Keywords CTLA-4 · Melanoma · Antibody · Ipilimumab · T cell

Introduction

CTLA-4 is one of two homologous proteins present within T cells that are exported to the cell surface after immune cell activation and counterbalance each other in the stimulation and inhibition of T cell proliferation and activation. CTLA-4, which has a much greater binding affinity for the B7 surface molecules found on the antigen-presenting cell (APC) than CD28, effectively induces T cell anergy and inhibits cell proliferation and secretion of interleukin-2 (IL-2), an important cytokine [1–4]. In contrast, its counterpart, CD28, is a costimulator of T cell proliferation and the production of IL-2 [5, 6]. Stimulating the immune system to mediate regression of established malignant tumors has long been a goal of tumor immunologists, and it has been thought that the CD28/CTLA-4 axis might represent a viable therapeutic target. Abrogation of the function of CTLA-4 would permit CD28 to function unopposed and might swing the balance in favor of immune stimulation, tolerance breakdown and tumor eradication, as shown in Fig. 1.

Review of ipilimumab's clinical development path

Ipilimumab has not had a traditional linear and stepwise developmental pathway in registration trials. Although the

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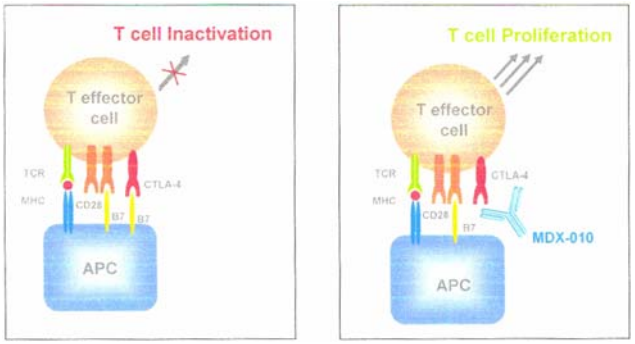

Fig. 1 CTLA-4 negatively regulates immune responses and CTLA-4 blockade potentiates antigen specific T effector cell responses

performance of a standard dose escalation trial with single or multiple dosing did not occur until after the initial few trials with the drug were performed, the evidence seems strong that an optimal dose and schedule for phase III registration trials were ultimately chosen. The initial studies with the drug included single fixed dose pilot protocols in small numbers of patients, followed by repetitive dosing trials, which included a peptide vaccine. In the earliest trials published by Hodi et al. [7], six patients were treated at a single dose of 3 mg/kg, and minimal side effects were observed. Clinical benefit was felt to be observed in three patients who had previously received a GM-CSF transduced cell vaccine and did not have tumor shrinkage, but had massive necrosis of large tumors that were subsequently resected. These "interesting" responses may have been beneficial since several patients had long-term freedom from progression after resection of large, necrotic tumor masses.

Investigators at the National Cancer Institute then chose a fixed dose of 3 mg/kg, calculated to achieve antibody levels in the serum of 10 μg/mL, administered with a multi-peptide vaccine derived from the melanoma antigen gp100 emulsified with the oil-based adjuvant Montanide ISA 51. In their first trial, they treated 14 patients and found a unique spectrum of dose limiting and severe colitis, rash and hypophysitis in 5 patients [8]. They subsequently treated a total of 56 patients who had failed prior IL-2 and other therapy with doses of ipilimumab between 1 and 3 mg/kg and achieved seven responses (2 CR, 5 PR, 13% RR) [9]. Five of the seven responders were sustained over 25 months, and 5/14 patients who had grade III or higher adverse events that seemed consistent with autoimmunity had a clinical response versus 2/42 without autoimmune side effects ($P = 0.008$). These side effects were called "immune-related adverse events," or irAEs and consisted of colitis, diarrhea, hypophysitis, hepatitis, nephritis with azotemia, rash and vitiligo; they were autoimmune or autoinflammatory. A dose-ranging trial of single administration of two different preparations of ipilimumab was then carried out at doses ranging from 2.8 to 20 mg/kg, followed by a phase II extension of the trial in which 23 stage IV melanoma patients received 10 mg/kg of ipilimumab four times every 3 weeks [10, 11]. Two patients in the phase II portion had an objective response, and an additional seven patients had stable disease with a disease control rate (DCR) of 39% and a median overall survival of 13.5 months. Both of the responses and the three patients with stable disease were ongoing at 24 months.

In a randomized phase II trial, 73 patients who were previously untreated received ipilimumab alone at 3 mg/kg four times every 4 weeks or combined with dacarbazine given over 5 days every 4 weeks [12, 13]. There was a 17% response rate with a 14.8 month median survival in the combination arm, compared with 9% and 11.2 months for the monotherapy arm. The favorable results of that small randomized phase II trial served to facilitate the conduct of a registration trial of ipilimumab plus DTIC versus DTIC alone in over 500 front-line melanoma patients that is ongoing.

At the National Cancer Institute, 139 patients were treated with multiple doses of ipilimumab, ranging from 3 to 9 mg/kg, with some intra-patient dose escalation, with or without a peptide vaccine [14, 15]. In that trial, the majority of patients received the vaccine, and a 17% objective response rate with a 15.7 month median survival was noted among patients who had predominantly failed IL-2 or chemotherapy. Based on those data and other data from the National Cancer Institute on ipilimumab with vaccine, 750 patients will be randomized in an ongoing second-line trial to receive ipilimumab at 3 mg/kg, a multi-peptide vaccine or a combination of ipilimumab and vaccine in another registration trial.

Several recent phase II studies of ipilimumab were presented in 2008 at the American Society for Clinical Oncology (ASCO) meeting. In one-three-arm randomized study, 221 previously treated melanoma patients received ipilimumab at either 0.3, 3 or 10 mg/kg four times, administered every 3 weeks [16]. Those patients with stable disease or any regression qualified to receive further "maintenance" therapy every 3 months until dose-limiting toxicity, progression or refusal. A clear dose response for objective response rate and for the onset of immune-related adverse events was observed in that trial, supporting the choice of the 10 mg/kg dose for subsequent registration trials. Another randomized phase II trial involved the use of budesonide, an oral non-absorbed steroid, with 115 first- and second-line melanoma patients who received ipilimumab at 10 mg/kg in a schedule similar to the above three-arm trial. They were randomly allocated to receive either budesonide as a preventive measure or placebo [17].

Table 1 Summary of clinical activity and immune-related adverse events in phase I/II trails of ipilimumab

Reference no. No. of patients	Complete response % Duration	Partial response % Duration	Grade 3–4 immune-related AE, % Association with benefit	Median OS (months)
[9]	Ipi3→1 mg/kg + vaccine	5/56 = 89%	25%	n.d.
56	2/56 = 3.6%	4, 6, 25+, 26+, 34+	$P = 0.008$	
	30+, 31+			
[12], [13]	Ipi 3 mg/kg + DTIC	4/35 = 11.4%	20%	14.7
72	2/35 = 5.7%	3, 3, 4, 21+	No association	
	17+, 20+			
	Ipi 3 mg/kg alone	2/37 = 5.3%	12.8%	11.7
			No association	
[28]	Ipi 10 mg/kg	9/155 = 5.8%	21.9%	10.2
155	0/155		Association n.d.	
[17]	Ipi 10 mg/kg + budesonide	6/58 = 10.3%	41.4%	15.3
115	1/58 = 1.7%	1, 3+, 3+, 7, 8, 10	Association n.d.	
	4+			
	Ipi 10 mg/kg + placebo	9/57 = 15.7%	38.6%	17.1
	0/57	1, 1+, 2+, 2+, 3+, 3+, 7+, 8+	Association n.d.	
[16]	Ipi 0.3 mg/kg	0/73	0	8.5
217	0/73			
	Ipi 3 mg/kg	3/72 = 4.1%	7	8.6
	0/72			
	Ipi 10 mg/kg	6/72 = 8.2%	25%	14.5
	2/72 = 2.7%		Association n.d.	
[14]	Ipi 3→9 mg/kg ± vaccine	20/139 = 14.3%	36%	15.7
139	3/139 = 2.1%	4, 5, 6, 6, 10, 10, 11, 17+, 17+,	Association $P = 0.004$	
	29+, 52+, 53+	18+, 19, 22+, 28+, 30+, 31+,		
		43, 47+, 50+		
[11]	Ipi 10 mg/kg	1/23 = 4.3%	25%	13.5
23	1/23 = 4.3%	23+	Association $P = 0.05$	
	21+			

The endpoints of the trial were the rate of grade 2 or more diarrhea, and the overall response rate. Ironically, there was no impact of budesonide on diarrhea or any autoimmune side effect, but response rates were 12.1 and 15.0% in the budesonde and placebo arms, respectively. Overall survivals were excellent, at 15.3 and 17.1 months, respectively, and the rates of grade 3–4 irAEs were about 40% in either arm. Thus, in over 200 patients in the phase II trials, the use of ipilimumab at doses from 3 to 10 mg/kg with or without a peptide vaccine or chemotherapy given every 3–4 weeks resulted in median survivals at 13.5, 14.8, 15.3, 15.7 and 17.1 months. The clinical results of these trials are summarized in Table 1. Those favorable phase II data, if verified in a phase III setting would be superior to the results of any recent controlled randomized trial for metastatic melanoma. The data justify an ongoing registration trial of DTIC plus ipilimumab versus DTIC alone in 500 first-line patients, whose results will not be available for a number of months.

Autoimmune side effects of ipilimumab: is autoimmunity a clinical surrogate?

The earliest clinical experience with anti-CTLA-4 antibodies showed that a novel syndrome of autoimmune or autoinflammatory side effects appeared to be related to their use. The onset of these toxicities, which have been called "immune-related adverse events" or irAEs, is dose related, cumulative and schedule dependent [18–21]. A key issue that bears on the development strategy for CTLA-4 antibodies is whether the onset of these immune-related adverse events serve as the only known surrogate marker of benefit from the drug. The immune-related adverse events that have been

observed during treatment with ipilimumab have most importantly been enterocolitis of grades 3 and 4 in up to 16% of cases [18], hypophysitis or inflammation of the pituitary in approximately 5% of cases [19], hepatitis in less than 5% of cases [20], cutaneous manifestations with severe rash, associated with deep dermal and perivascular infiltrates of lymphocytes and itching in more than 50% of cases [20], and a variety of less common conditions like uveitis, pancreatitis and leukopenia, occurring in less than 1–2% of cases [15]. The onset of uveitis, generally of grades 1 and 2, has been associated with colitis [18]. Biochemical evidence of pancreatitis consisting of altered amylase and lipase has often been asymptomatic. The patients who have colitis may have other manifestations of enteritis, ranging from apthous mouth ulcers to erosive esophagitis to gastritis and jejunitis, although the principal pathology is colitis, often distally located with biopsies showing diffuse infiltrates of CD4+ T cells more than CD8 T cells, crypt abscesses and diffuse mucosal ulceration [18, 20].

Management of irAEs from CTLA-4 antibodies

The irAEs can often have a quite rapid onset, with a normal-appearing colon on colonoscopy within a few days after a first dose of ipilimumab and diffuse colitis with crypt abscess formation seen on a colonoscopic biopsy 4 days later, after the onset of severe diarrhea [9, 18, 21]. All patients receiving anti-CTLA-4 antibodies need to be questioned closely about possible autoimmune side effects, and symptoms should be treated quickly with oral steroids for a prolonged period of time if they are dose limiting. Care should be taken to avoid tapering the steroids too rapidly. Grade III colitis, particularly if accompanied by diffuse ulceration on colonoscopy or bleeding, requires initial dosing with intravenous high-dose solumedrol [18]. A 30-day taper of prednisone starting at 60 mg per day is the minimum required schedule, 45–60 days being needed in some cases. A tapering schedule that is too rapid may lead to recurrence of symptoms, with consequent need for anti-TNF antibodies like infliximab, prolonged steroids and restriction on oral intake with a requirement for insertion of a central venous catheter and institution of total parenteral nutrition (TPN). Diarrhea should be treated early, the day it begins, with Lomotil and Imodium for grade I (two or fewer episodes in 24 h) diarrhea, performance of sigmoidoscopy and the addition of budesonide for grade II diarrhea (three to six episodes in 24 h), and immediate institution of oral steroids for grade III diarrhea (seven or more episodes in 24 h), and inpatient hospitalization for patients with symptomatic dehydration accompanied by bloody diarrhea and severe colitis observed on sigmoidoscopy. If diarrhea does not respond to intravenous steroids within 72 h, infliximab at 5 mg/kg should be infused intravenously; it may be repeated within 2 weeks, but is rarely needed more than once. Prolonged diarrhea in spite of steroids, bowel rest, TPN and infliximab is an indication for either a diverting ileostomy or partial/complete colectomy. The incidence of life-threatening perforation is quite rare, and has been 4 of 700 patients at doses of ipilimumab of 3 mg/kg or more.

Hepatitis has been uncommonly seen following treatment with ipilimumab and generally presents in asymptomatic patients as a rise in alanine aminotranferases (AST and ALT) with a lesser rise in bilirubin. Biopsies have revealed acute hepatic inflammation with ballooning degeneration and diffuse lymphocytic infiltrates. In most cases, if detected early, and ALT/AST are below five times normal, i.e., grade II, skipping a dose of ipilimumab and waiting 3 weeks or more until the next scheduled dose is acceptable if the liver functions decrease to grade I (up to twice normal) at that time. If the liver functions are above five times normal, which is grade III toxicity, ipilimumab should be discontinued and oral steroids given for a minimum 30-day course. The liver functions should be monitored closely every 48–72 h until they return to normal. Elevation of AST/ALT over eight times normal requires admission to the hospital, intravenous solumedrol, and if there is no decrease in the AST/ALT by 48 h, mycophenolic acid should be administered intravenously at 5 mg/kg. If that maneuver does not induce a decrease in the AST/ALT by 48 h, infliximab should be administered at 5 mg/kg intravenously.

Hypophysitis is another irAE, with documented loss of the cortisol axis, thyroid function or gonadal hormones, all three or any of the above [19]. Patients characteristically present with fatigue and/or headache, personality change, mood disorders or symptoms of hypothyroidism. Patients must have a endocrine panel drawn as soon as the symptoms become manifest, with ACTH, cortisol, T3, T4, TSH and testosterone for men, and the same panel with the addition of FSH and prolactin for women. An MRI scan of the brain with pituitary cuts should be performed. Treatment with gonadal hormone replacement, thyroid hormone replacement and a steroid taper are required, and replacement corticosteroids should be instituted with oral hydrocortisone as soon as prednisone has been tapered to 10 mg daily. Although some patients with hypopituitarism due to ipilimumab may have a return of their pituitary function, the majority will not and may be dependent on lifelong thyroid and/or steroid replacement. Recently, patients who had hypophysitis with the need for hormone replacement have been re-treated with ipilimumab without worsening side effects (Weber et al. unpublished data).

Association of irAEs with clinical response and benefit

In early clinical trials with CTLA-4 antibodies in metastatic melanoma, an association between anti-tumor response and irAEs, such as dermatitis, uveitis, enterocolitis with diarrhea, hepatitis and hypophysitis was found [9, 18]. In one study by Attia et al. [9], patients treated with ipilimumab who experienced grade 3 or 4 autoimmune GI irAEs had a 36% clinical response rate compared with 5% in patients who did not have autoimmune GI toxicity. Since the organs targeted by the T cells in these reactions other than skin, such as liver, gut or pancreas do not express any melanosomal antigen, CTLA-4 blockade may disrupt self-tolerance, causing autoimmune irAEs. The incidence of grade 3 or 4 autoimmune toxicities in responders in this trial was 14%.

The response rate was also significantly higher among ipilimumab-treated patients who developed irAEs in a study conducted by Beck et al. [18]. While the response rate was 14%, among those melanoma patients experiencing enterocolitis, the response rate was 36% as compared with 11% for patients with metastatic melanoma who did not develop enterocolitis. Overall, 21% of patients were diagnosed with enterocolitis. At their institution, Blansfield et al. [19] reviewed 163 patients, with advanced melanoma or renal cell cancer, who had been treated with ipilimumab as of January 1, 2005 and found that 8 (4.9%) had developed autoimmune hypophysitis. All patients had received the drug intravenously every 3 weeks at doses ranging from 3 to 9 mg/kg for doses between 4 and 9. Five of the eight patients (62.5%) had an objective tumor response to CTLA-4 blockade, including one patient with a CR. Five of the patients had also had previous IL-2 therapy. As in the other studies, tumor regression was associated with the development of autoimmunity. In a study discussed previously, which was conducted in 25 patients with resected stage III or IV melanoma treated with ipilimumab, 48% of the patients experienced grade 2 or 3 IRAEs, with 20% being dose-limiting. As much as 28% of the events involved gastrointestinal toxicity and 16% involved skin-related toxicity; one patient developed hypopituitarism. Although 16% of the patients relapsed, none of those experiencing grade 2 or 3 autoimmune toxicities did so. In contrast, in the study by Downey et al. [14], there was a high degree of any grade irAEs, but no clear association of autoimmunity with clinical response. Perhaps a better correlation would be that between irAEs and overall survival or disease control rate.

In general, even severe autoimmune toxicities have responded to high-dose steroids and/or supportive care, which interestingly did not appear to diminish the antitumor effect of CTLA-4 blockade. Among patients with steroid-refractory symptoms, infliximab was effective in most cases. In the Beck study, there was a 5% death rate among patients who developed autoimmune colitis [18]. Failure to recognize and promptly treat early symptoms, as well as poor compliance with steroid therapy, may have played roles in those patients who developed major complications associated with enterocolitis. As more experience has been gained with the drug, mortaliy rates have dropped, suggesting that while some of the toxicities can certainly be serious, proper education and established algorithms for side effect management could bring this therapy into community oncology practice. Among patients developing hypophysitis, all of them experienced resolution of symptoms with initiation of steroid, thyroid, testosterone replacement and cessation of CTLA-4 [19]. One patient could not be weaned off testosterone therapy 2 years after the initial event, while others experienced partial recovery of pituitary function. Nonetheless, hypophysitis is the one side effect that may not be reversible.

The incidence of irAEs with CTLA-4 blockade demonstrated a positive correlation with response, with the increasing severity of events, grade 2 or 3, being associated with greater benefit [18, 20]. For most of the studies with ipilimumab and even with tremelimumab, there was some association between irAEs and clinical benefit shown as anti-tumor response, disease control rate or time to relapse [6–8, 18, 20, 22]. The association, however, has not been absolute, since the rates of irAEs could be as high as 40% in some trials and response rates 5–17% for ipilimumab. In addition, some patients without evidence of autoimmunity have had clinical responses or long-term freedom from progression [20, 23]. However, the ipilimumab trials with high irAE rates have also been those with the longest overall survivals [15], again supporting the idea that there is a connection between irAEs and clinical outcome.

Unique kinetics of response with ipilimumab therapy: are traditional response criteria an adequate surrogate for benefit and survival?

As data accumulated from trials with ipilimumab, it became apparent that in some cases, clinical response was substantially delayed in patients with melanoma. Hamid et al. [24] undertook a review and analysis of five studies on 269 patients with stage III or IV melanoma to determine the kinetics and duration of response with the drug. Patients in the studies involved in the analysis received ipilimumab either alone or in combination with dacarbazine, IL-2 or gp100 peptide vaccine at doses ranging from 0.3 to 10 mg/kg in regimens involving single or multiple doses.

An objective response was observed in 41 patients (15%). Some patients had a late onset CR or PR, occurring

at 10–106 weeks and 5–62 weeks after treatment initiation, respectively. In 28 patients, onset of response occurred after more than 12 weeks of treatment, and in 4 patients, PD preceded a response without additional therapy. In some patients, PD was followed by SD, and ultimately, PR. The duration of response has been considerable as well, with the overall response duration ranging from 6 to 187+ weeks. At the time of the analysis response was ongoing in 25 patients. Late-onset response was not associated with dose, regimen or concomitant therapy.

The findings from this study demonstrate that response can be late in onset or occur after disease progression with ipilimumab, in contrast to the brief responses often seen rapidly after traditional chemotherapy regimens for melanoma. Furthermore, the responses seen with this agent appear to be more durable than traditional chemotherapy in this population of patients with advanced stage melanoma. The implications of this are that continued treatment and observation might be beneficial in patients experiencing SD or even PD that does not reduce performance status or compromise the major organs with ipilimumab therapy. The observations made in this analysis, as well as in multiple trials of ipilimumab have led to the idea that a new criterion for response that would serve as an accurate surrogate for clinical benefit might be postulated, called irRC, or immune related response criteria [25–27]. In this scenario, response would be defined by measuring the diameters of all lesions at a specific point in time after initiation of therapy in spite of new lesions arising, as long as the comparator was the total disease burden as measured at the initiation of therapy. Compared to RECIST or WHO, the baseline measurement would be the same, but the first disease assessment might be at week 12 instead of week 6 or 8, as often utilized in chemotherapy trials with rapid kinetics of tumor regression. Also, the onset of new disease would not automatically constitute progression, as long as the patient did not have significant disease-related decrease in performance status or a total increase in measured disease beyond the predefined endpoint of 30% compared to baseline. Based on these new criteria, overall survival was very well described compared to the use of WHO in the study presented by Hodi [25], validating the concept.

The majority of patients who respond, by traditional RECIST or WHO criteria, to ipilimumab may have sustained and long-term freedom from the progression of disease, which has been accepted as strong evidence for the clinical benefit of the drug [23, 28]. The proportion of patients with response based on the standard definition to ipilimumab therapy has been generally less than 15%, which may not be consistent with the prolongation of the median on the survival curve. However, the median overall survivals of four trials cited above with over 200 patients were 13.5, 14.8, 15.7 and 17.1 months [10–14, 17], highly favorable for patients with unresectable stage IV melanoma. If one assumes that patients with stable disease and those with a response based on the immune-related response criteria above also have sustained freedom from progression of disease, then 30–40% of those who receive ipilimumab may derive clinical benefit, increasing the likelihood that there will be a meaningful and significant impact on the survival median. While response based on WHO or RECIST is an important parameter, particularly in trials of cytotoxic agents, it is possible that the patients with a irRC who may not have had a standard WHO or RECIST response may also benefit from ipilimumab and also be represented in the groups with prolonged survival, as indicated in the data described by Hodi. In fact, in a recent small pilot study of 11 melanoma patients who had received a GM-CSF transduced cell vaccine and then ipilimumab within several months, three objective responders all had evidence of progression followed by regression, highlighting the hypothesis that there is a potential for benefit in patients who did not have a traditional objective response [29]. The outcome of the randomized first-line registration trial of ipilimumab plus DTIC compared with DTIC alone will determine if that hypothesis is correct, and that outcome is eagerly awaited.

Recent studies on the mode of action of CTLA-4 abrogating antibodies

Significant speculation and interest has centered around the anti-tumor mechanism of action of CTLA-4 antibodies, both ipilimumab (Bristol Myers Squibb and Medarex) the topic of this review, and Tremelimumab from Pfizer. High levels of CTLA-4 are found within most T cells and is exported to the surface at high levels both on activated helper and cytolytic T cells [30]. It is particularly found at high levels on T regulatory cells [31]. One hypothesis was that CTLA-4 abrogation overcame the activity of T regulatory cells or rendered effector cells resistant to their inhibitory effects. This has been found not to be the case, and testing of sorted T regulatory cells using in vitro treatment or from patients treated with CTLA-4 abrogating antibodies showed that T regulatory function was unimpaired [32, 33]. Elevation of CD4+ DR+ T cells was the only reproducible variable in the peripheral blood that appears to occur with CTLA-4 abrogating antibodies, and the altered ratio of effector to regulatory cells may be the key to their activity [33]. In tumors from patients treated with ipilimumab, elevations of ICOS+ CD4+ cells have been particularly marked [34], and within the tumor microenvironment, changes in ratios of T effector and T regulatory cells appeared to correlate with tumor necrosis in one melanoma study [29]. Genetic polymorphism

studies have shown that certain CTLA-4 single nucleotide polymorphisms may be statistically associated with a worse outcome after treatment with ipilimumab [35], although the alteration of CTLA-4 levels that occurs with the unfavorable polymorphism is actually positively associated with autoimmunity, a factor that appears to correlate with a better outcome with CTLA-4 abrogation. Extensive microarray analyses of tumors pre- and post-therapy, of serum samples and of sorted T cells from patients receiving ipilimumab are underway and may provide answers to how microenvironmental and peripheral immunologic factors impact on the clinical effects of CTLA-4 abrogation.

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References

1. Chambers CA, Allison JP (1999) CTLA, the costimulatory molecule that doesn't: regulation of T-cell responses by inhibition. Cold Spring Harb Symp Quant Biol 64:303–312
2. Chambers CA, Kuhns MS, Egen JG, Allison JP (2001) CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. Annu Rev Immunol 19:565–594
3. Leach DR, Krummel MF, Allison JP (1996) Enhancement of antitumor immunity by CTLA-4 blockade. Science 271:1734–1736
4. Pentcheva-Hoang T, Egen JG, Wojnoonski K et al (2004) B7-1 and B7-2 selectively recruit CTLA-4 and CD28 to the immunological synapse. Immunity 21:401–413
5. Krummel MF, Allison JP (1995) CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med 182:459–465
6. Newton Bishop JA (1997) Molecular pathology of melanoma. Cancer Metastasis Rev 16:141–154
7. Hodi S, Mihm MC, Soiffer RJ et al (2003) Biologic activity of cytotoxic T lymphocyte antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci USA 100(8):4712–4717
8. Phan GQ, Yang JC, Sherry RM et al (2003) Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci USA 100:8372–8377
9. Attia P, Phan GQ, Maker AV et al (2005) Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. J Clin Oncol 23:6043–6053
10. Weber JS, Hersh EM, Yellin M et al (2007) The efficacy and safety of ipilimumab (MDX-010) in patients with unresectable stage III or stage IV malignant melanoma. J Clin Oncol suppl 25: abstr 8523
11. Weber J, O'Day S, Urba W (2008) Phase I/II study of ipilimumab for patients with metastatic melanoma. J Clin Oncol 26(36): 5950–5956
12. Fischkoff SA, Hersh E, Weber J et al (2005) Durable responses and long-term progression-free survival observed in a phase II study of MDX-010 alone or in combination with dacarbazine (DTIC) in metastatic melanoma. J Clin Oncol, ASCO annual meeting proceedings 23 June, suppl 1: 7525
13. Hersh EM, Weber JS, Powderly JD et al (2008) Disease control and long-term survival in chemotherapy-naïve patients with advanced melanoma treated with ipilimumab (MDX-010) with or without dacarbazine. J Clin Oncol suppl 26: abstr 9022
14. Downey SG, Klapper JA, Smith FO et al (2007) Prognostic factors related to clinical response in patients with metastatic melanoma treated with CTL-associated 4 blockade. Clin Cancer Res 13:6681–6688
15. Maker AV, Yang JC, Sherry RM et al (2006) Intrapatient dose escalation of anti-CTLA-4 antibody in patients with metastatic melanoma. J Immunother 29:455–463
16. Hamid O, Chin K, Li J et al (2008) Dose effect of ipilimumab in patients with advanced melanoma: results from a phase II, randomized, dose-ranging study. J Clin Oncol suppl 26: abstr 9025
17. Weber JS, Berman D, Siegel J et al (2008) Safety and efficacy of ipilimumab with or without prophylactic budesonide in treatment-naive and previously treated patients with advanced melanoma. J Clin Oncol ASCO annual meeting proceedings, Part I, 26 May suppl 20: abstr 9010
18. Beck KE, Blansfield JA, Tran KQ et al (2006) Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. J Clin Oncol 24:2283–2289
19. Blansfield JA, Beck KA, Tran K et al (2005) Cytotoxic T-lymphocyte-associated antigen-4 blockage can induce autoimmune hypophysitis in patients with metastatic melanoma and renal cancer. J Immunother 28:593–598
20. Sanderson K, Scotland R, Lee P et al (2005) Autoimmunity in a phase I trial of a fully human anti-cytotoxic T-lymphocyte antigen-4 monoclonal antibody with multiple melanoma peptides and Montanide ISA 51 for patients with resected stages III and IV melanoma. J Clin Oncol 23:741–750
21. Weber J (2007) Review: anti-CTLA-4 antibody ipilimumab: case studies of clinical response and immune-related adverse events. Oncologist 12:864–872
22. Weber JS, Targan S, Scotland R et al (2006) Phase II trial of extended dose anti-CTLA-4 antibody ipilimumab (formerly MDX-010) with a multipeptide vaccine for resected stages IIIC and IV melanoma. J Clin Oncol, ASCO annual meeting proceedings, Part I, 24 June 20, suppl 2510
23. Urba WJ, Weber JS, O'Day SJ et al (2008) Long-term survival of patients with advanced melanoma who received ipilimumab administered at 10 mg/kg every 3 weeks for 4 doses (induction dosing). J Clin Oncol suppl 26: abstr 3018
24. Hamid O, Urba WJ, Yellin M et al (2007) Kinetics of response to ipilimumab (MDX-010) in patients with stage III/IV melanoma. J Clin Oncol suppl 25: abstr 8525
25. Hodi FS, Hoos A, Ibrahim R et al (2008) Novel efficacy criteria for antitumor activity to immunotherapy using the example of ipilimumab, an anti-CTLA-4 monoclonal antibody. J Clin Oncol suppl 26: abstr 3008
26. Saenger YM, Wolchok JD (2008) The heterogeneity of the kinetics of response to ipilimumab in metastatic melanoma: patient cases. Cancer Immun 8:1
27. Wolchok JD, Ibrahim R, DePril V et al (2008) Antitumor response and new lesions in advanced melanoma patients on ipilimumab treatment. J Clin Oncol suppl 26: abstr 3020
28. O'Day SJ, Ibrahim R, DePril V et al (2008) Efficacy and safety of ipilimumab induction and maintenance dosing in patients with advanced melanoma who progressed on one or more prior therapies. J Clin Oncol suppl 26: abstr 9021
29. Hodi FS, Butler M, Oble DA et al (2008) Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. Proc Natl Acad Sci USA 105(8):3005–3010

30. Chambers CA, Sullivan TJ, Allison JP (1997) Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4+ T cells. Immunity 7:885–895
31. Wing K, Onishi Y, Prieto-Martin P et al (2008) CTLA-4 control over Foxp3+ regulatory T cell function. Science 322(5899):271–275
32. Maker AV, Attia P, Rosenberg SA (2005) Analysis of the cellular mechanism of antitumor responses and autoimmunity in patients treated with CTLA-4 blockade. J Immunol 175(11):7746–7754
33. Ménard C, Ghiringhelli F, Roux S et al (2008) CTLA-4 blockade confers lymphocyte resistance to regulatory T-cells in advanced melanoma: surrogate marker of efficacy of tremelimumab? Clin Cancer Res 14(16):5242–5249
34. Liakou CI, Kamat A, Tang DN et al (2008) CTLA-4 blockade increases IFNgamma-producing CD4+ ICOShi cells to shift the ratio of effector to regulatory T cells in cancer patients. Proc Natl Acad Sci USA 105(39):14987–14992
35. Breunis WB, Tarazona-Santos E, Chen R et al (2008) Influence of cytotoxic T lymphocyte-associated antigen 4 (CTLA4) common polymorphisms on outcome in treatment of melanoma patients with CTLA-4 blockade. J Immunother 31(6):586–590