Overcoming Immunologic Tolerance to Melanoma: Targeting CTLA-4 with Ipilimumab (MDX-010)

JEFFREY WEBER

Department of Interdisciplinary Oncology, Donald A. Adam Comprehensive Melanoma Research Center, and H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA

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Abstract
Targeted biologic therapies such as anti–cytotoxic T lymphocyte antigen (CTLA-4) monoclonal antibodies, either as monotherapy or in combination with chemotherapy or vaccines, have shown great promise in late-stage melanoma, which has a very poor prognosis. Melanoma is relatively resistant to both chemotherapy and radiotherapy. Blockade of CTLA-4, which inhibits T-cell proliferation, promotes stimulation of adaptive immunity and T-cell activation, resulting in eradication of tumor cells. Two human monoclonal antibodies are under investigation in melanoma. Phase II and III clinical trials are currently evaluating the efficacy and safety of ipilimumab (MDX-010, Medarex, Inc., Princeton, NJ, and Bristol-Myers Squibb, Princeton, NJ) and tremelimumab (CP-675,206; Pfizer Pharmaceuticals, New York) in melanoma. Data are available on ipilimumab, which has been explored as monotherapy and in combination with vaccines, other immunotherapies such as interleukin-2, and chemotherapies such as dacarbazine. Overall response rates range from 13% with ipilimumab plus vaccine in patients with stage IV disease to 17% and 22% with ipilimumab plus dacarbazine or interleukin-2, respectively, in patients with metastatic disease. Responses have been durable, and among those experiencing grade 3 or 4 autoimmune toxicities, even higher response rates have been seen—up to 36%. While the optimal dose of ipilimumab has yet to be established, studies also indicate that higher doses may be more effective. Importantly, the lack of an initial clinical response may not predict ultimate treatment failure, because the onset of a response may follow progressive disease or stable disease. Pending results from registration studies with ipilimumab and lessons learned from registration studies conducted with tremelimumab will help to define the role of anti–CTLA-4 blockade in the treatment of metastatic melanoma. The Oncologist 2008;13(suppl 4):16–25

Introduction
This year >100,000 Americans will be diagnosed with melanoma, including those with in situ tumors, and >8,000 will die as a result of the disease [1]. The risk for death is greatest for those with melanoma detected late in the course of the disease: the 5-year survival rate for melanoma detected and treated in its early stages is 89%–95%, but the 5-year survival rate is only 5%–10% in those diagnosed with stage IV disease [1–3]. While early diagnosis has reduced the mortality rate for melanoma, effective strategies for treating late-stage disease are desperately needed. The use of targeted biologic therapies such as the anti–cytotoxic
T lymphocyte antigen (CTLA)-4 abrogating antibodies, either as monotherapy or in combination with chemotherapy or melanoma vaccines, has shown great promise.

CTLA-4 is one of two homologous cell surface proteins that 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 secretion of interleukin (IL)-2, an important cytokine [4–7]. In contrast, its counterpart, CD28, is a costimulator of T-cell proliferation and the production of IL-2 [7, 8]. The idea of stimulating the immune system to destroy malignant tumors has long been a “holy grail” of cancer research, and it has been thought that the CD28–CTLA-4 axis might represent a viable therapeutic target. Inhibition of CTLA-4 would permit CD28 to function unopposed and might swing the balance in favor of immune stimulation, tolerance breakdown, and tumor eradication.

Melanoma is relatively resistant to both chemotherapy and radiotherapy, and although both therapies are used palliatively in the treatment of advanced disease, neither can effect a cure [9]. However, melanoma is an immunogenic tumor, which suggests that manipulation of the immune system with monoclonal antibody therapy that suppresses the CTLA-4 inhibitory function could produce a favorable clinical result. Currently, phase II and III clinical trials of anti-CTLA-4 monoclonal antibody therapy are being conducted in melanoma, and phase I and II trials are being conducted in other tumor types. Two human monoclonal antibodies are under investigation—ipilimumab (MDX-010; Bristol-Myers Squibb/Medarex, Princeton, NJ) and tremelimumab (CP-675,206; Pfizer Pharmaceuticals, New York). Both antibodies have a demonstrated ability to induce tumor regression and may prolong time to disease progression [10, 11], and ipilimumab has been shown to induce durable responses in melanoma, as well as in ovarian, prostate, and renal cell cancer [12–15]. Table 1 shows the major findings from phase I and II clinical trials of ipilimumab in patients with metastatic melanoma.

Ipilimumab as Monotherapy or in Combination with Vaccines
Ipilimumab has been investigated both as monotherapy and in combination with a vaccine or chemotherapy. An early, pilot trial of ipilimumab as monotherapy in 17 patients with progressive unresectable malignant melanoma explored the pharmacokinetics and safety of the drug [16]. The patients, who had progressed despite prior immunotherapy, radiation, or chemotherapy, received a single i.v. dose of ipilimumab of 3 mg/kg over 90 minutes. Plasma levels of the antibody persisted for 1–4 months. The drug was well tolerated, and other than a mild rash, there was no evidence of clinical autoimmunity. Two patients experienced a partial response (PR), including resolution of three soft tissue masses and a >50% reduction in a lung mass. The promising findings from that trial provided a basis for further investigation.

A second pilot trial examined the biologic activity of ipilimumab in seven patients with metastatic melanoma and two patients with ovarian carcinoma [14]. All nine patients had previously participated in vaccine studies for metastatic disease. The scientific rationale supporting the trial was the finding, in preclinical animal studies, that the combination of CTLA-4 blockade and cancer vaccination had synergistic effects, providing greater levels of antitumor activity than with either approach alone. A single i.v. dose of ipilimumab of 3 mg/kg was administered to all patients. Antitumor effects were noted in all five patients previously immunized with irradiated GM-CSF–secreting tumor cells, but only a minimal response was seen in four patients previously immunized with melanosomal antigens. Examination of tissue from metastases later resected revealed extensive ischemic necrosis in many of the lesions. CTLA-4 blockade also appeared to induce significant increases in circulating neutrophils, and neutrophilic infiltrates were associated with tumor necrosis. Although the development of a rash in patients with melanoma was observed, which suggested a partial disruption of self-tolerance, there was no evidence of autoimmune disease.

Because the optimal dosing of ipilimumab has yet to be established in an escalating dose cohort or randomized trial, several trials have explored the use of multiple dosing at different schedules. A trial with 14 patients with stage IV metastatic melanoma demonstrated that ipilimumab at a dose of 3 mg/kg given every 3 weeks, followed by a vaccine with a gp100 peptide emulsified with adjuvant Montanide ISA-51, had efficacy in late-stage disease [10]. Three patients experienced an objective response, two had a complete response (CR), and one had a PR. However, six patients developed grade 3 or 4 autoimmune side effects, including dermatitis, colitis/enterocolitis, hepatitis, and hypophysitis; all patients recovered and did not have a relapse of their side effects as their antibody levels subsided. Although these adverse events were successfully managed with corticosteroids and supportive care, the trial, which originally was to accrue 21 patients in the first stage, ceased accruing patients after 14 patients had enrolled. A subsequent trial was conducted in 56 patients with progressive stage IV melanoma, with 29 patients receiving ipilimumab at a dose of 3 mg/kg every 3 weeks, and 27 patients receiving 3 mg/kg as their initial dose, with subsequent doses reduced to 1 mg/kg every 3 weeks [12]. As in the prior trial, both cohorts...
received a gp100 peptide vaccine after each ipilimumab dose. The overall objective response rate was 13% (7 of 56), with two CRs and five PRs. Both CRs were ongoing at 31 and 30 months, and three of the PRs were ongoing at 34, 26, and 25 months. Two of the patients with a PR experienced disease recurrence—one at 6 months and one at 4 months.

Grade 3 or 4 autoimmune effects were noted in 25% of patients, including colitis, dermatitis, uveitis, enterocolitis, hepatitis, and hypophysitis. Among patients experiencing these effects, 36% had evidence of tumor regression, compared with only 5% of those who did not suffer grade 3 or 4 autoimmune toxicity \((p = .008)\).

Another phase II trial was conducted with ipilimumab at a dose of 3 mg/kg plus a multipeptide vaccine given every 8 weeks for 12 months in 25 patients with resected stage IIIIC/IV melanoma and no evidence of disease [17]. Dose-limiting autoimmune toxicities occurred in 20% of patients, and 48% had grade 2 or 3 immune-related adverse events (IRAEs), which were consistent with those previously reported. At a median of 22 months of follow-up, 10 of the 25 patients had relapsed, with four treated surgically and again rendered free of disease, four deaths, and three treated with biochemotherapy with response. At the time of writing of this report, 21 of the 25 patients remained alive and two had evidence of disease (Weber J et al., unpublished).

Among patients experiencing IRAEs, three experienced a relapse, compared with seven who had no autoimmunity. These results again suggest that response to treatment is associated with autoimmune events.

**Ipilimumab in Combination with Dacarbazine or IL-2**

Ipilimumab has also been investigated in other combination therapies—with immunotherapies, such as IL-2, or with chemotherapy, such as dacarbazine. In a phase II randomized trial involving dacarbazine, 72 chemotherapy- and vaccine-naïve patients with unresectable metastatic melanoma were randomized to receive either ipilimumab at 3 mg/kg monthly four times \((n = 37)\) or ipilimumab plus dacarbazine \((250 \text{ mg/m}^2)\) for 5 days per month four times \((n = 35)\) [18, 19]. Among those treated with ipilimumab alone, there were two PRs (overall response rate [ORR], 5%) and four patients with stable disease (SD), while in the combination ipilimumab plus dacarbazine group, there were two CRs and four PRs (ORR, 17%), as well as four cases of SD. These responses were durable, with the two PRs in the monotherapy group continuing at 11.9+ and 14+ months, and one patient with SD continuing at 18.2+ months. In the

<table>
<thead>
<tr>
<th>Ipilimumab dose</th>
<th>Complete response, % ((n)); duration</th>
<th>Partial response, % ((n)); duration</th>
<th>Stable disease, % ((n)); duration</th>
<th>Most common grade III/IV or serious IRAEs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipilimumab + vaccine (pretreated) [5]</td>
<td>3.6 (2/56); 30+ and 31+ mos</td>
<td>89 (5/56); 4, 6, 25+, 26+, and 34+ mos</td>
<td>NA</td>
<td>Colitis and dermatitis</td>
</tr>
<tr>
<td>Ipilimumab + vaccine (resected disease) [13]</td>
<td>6 of 25 patients had relapsed after a 12-mo follow-up (no deaths)</td>
<td>Colitis, rash, and hypophysitis</td>
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</tr>
<tr>
<td>Ipilimumab (first line) [16]</td>
<td>0</td>
<td>5.4 (2/37); 16+ and 18+ mos</td>
<td>10.8 (4/37); 3, 4, 9, and 14 mos</td>
<td>Rash, colitis, uveitis, and adrenal insufficiency</td>
</tr>
<tr>
<td>Ipilimumab + dacarbazine (first line) [16]</td>
<td>5.7 (2/35); 17+ and 20+ mos</td>
<td>11.4 (4/35); 3, 3, 4, and 21+ mos</td>
<td>11.4 (4/35); 4, 6, 7, and 8 mos</td>
<td></td>
</tr>
<tr>
<td>Ipilimumab + interleukin-2 [15]</td>
<td>8.3 (3/36); 13+, 13+, and 16+ mos</td>
<td>13.9 (5/36); 7, 11, 11+, 14+, and 19+ mos</td>
<td>NA</td>
<td>Enterocolitis</td>
</tr>
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Abbreviations: NA, not available or not reported.

combination group, the two CRs continued at 12.6+ and 16.3+ months, and one PR was ongoing at 16.7+ months. The median progression-free survival (PFS) time was 82 days in the ipilimumab monotherapy group and 99 days in the combination group.

Thirty-six patients with stage IV metastatic melanoma were treated with ipilimumab at doses in the range of 0.1–3 mg/kg every 3 weeks combined with IL-2 at 720,000 IU/kg every 8 hours to a maximum of 15 doses [20]. There was an ORR of 22%, with three patients having a CR and five patients having a PR. Five of the responses occurred among the 24 patients treated with ipilimumab at 3 mg/kg, and one each occurred in those treated with 0.3, 1, and 2 mg/kg. Six of the eight responders had ongoing responses at follow-up of 11–19 months. The 22% ORR reflects an additive rather than a synergistic effect for these two agents, although durable responses were demonstrated.

Based on the initial experience with ipilimumab in combination with chemotherapy or IL-2, additive effects have been seen, as opposed to synergy. However, the results from these trials suggest that combination therapy with ipilimumab may play an important role in the treatment of metastatic melanoma; larger trials are required, including an ongoing phase III trial of ipilimumab plus dacarbazine compared with dacarbazine alone in frontline therapy, before further conclusions can be drawn.

**DOSING EXPERIENCE WITH IPILIMUMAB**

Other studies have explored escalating doses of ipilimumab to determine if titration of the antibody might improve response rates or change the tolerability of the drug. Nineteen patients with high-risk, resected stage III and IV melanoma were immunized with three tumor antigen epitope peptides from gp100, melanoma antigen recognized by T cells (MART)-1, and tyrosinase emulsified with adjuvant Montanide ISA-51 [21]. They were then treated with escalating doses of ipilimumab (0.3, 1, and 3 mg/kg) to determine the maximum-tolerated dose of vaccine plus monoclonal antibody. This regimen was administered every 4 weeks for 6 months, and then every 12 weeks for an additional 6 months. After 28.5 months of follow-up, 12 of the 19 patients had experienced disease relapse, and three patients had died. Relapse occurred in 82% of the 11 patients who had no evidence of an autoimmune response, and two of those patients had died. The median time to relapse was 18.3 months. Among the eight patients who showed signs of an autoimmune reaction, three (37%) had experienced a relapse of disease and one had died. In the highest dose cohort (3 mg/kg), all five patients demonstrated evidence of an autoimmune response. Although three of those patients have since experienced a relapse, four of the five patients were alive. In the two lower dose cohorts (0.3 mg/kg and 1 mg/kg), autoimmune effects were observed in only three of the 14 patients, nine patients had a disease relapse, and two have died.

In a study presented at the 2007 American Society of Clinical Oncology (ASCO) Annual Meeting, 88 patients with unresectable metastatic melanoma were treated with different ipilimumab preparations and regimens [22, 23]. Thirty-four patients received either 2.8 mg/kg or 5 mg/kg of transfectoma-derived or 3 mg/kg of hybridoma-derived ipilimumab preparations on days 1, 57, and 85. An additional 30 patients received single doses of 7.5 mg/kg, 10 mg/kg, 15 mg/kg, or 20 mg/kg of transfectoma-derived ipilimumab. When single doses of up to 20 mg/kg were found to be well tolerated, another 24 patients were treated with up to four doses of 10 mg/kg of ipilimumab on days 1, 22, 43, and 64.

Among the 88 treated patients, there were one CR, three PRs, and 10 cases of durable SD [22, 23]. The responses were durable for 29–39 weeks and ongoing in three patients at the end of the study. Importantly, several patients showed evidence of a delayed response. A PR that was observed in one patient after 18.5 weeks developed into an ongoing CR at 51 weeks, and another patient who had SD at 16 weeks had developed a PR by the 30th week. In four patients, there was ongoing SD, with a duration of 21–79+ weeks. Patients with an objective response or SD had IRAEs, and IRAEs were severe in 27 patients and considered to be related to ipilimumab. The finding that late-onset responses can occur sometimes after months of SD is important. For patients that achieved disease control (CR + PR + SD), there was an association with the development of grade 3–4 IRAEs (p = .04).

A large phase II trial of 139 patients treated at differing doses (3–9 mg/kg) of ipilimumab with or without a peptide vaccine was described for second-line treatment at a single institution [24]. The median overall survival time was 15.7 months, with a 17% ORR by the Response Evaluation Criteria in Solid Tumors (RECIST). The median time to progression in that trial was 2.9 months. There was no impact of receiving the peptide vaccine, or being treated with steroids, on response or overall survival (OS), suggesting that the immune suppression with steroids did not have a negative clinical impact. These studies suggested that higher doses of ipilimumab were associated with a higher response rate and favorable survival. Three dose levels of ipilimumab were subsequently administered as monotherapy in a randomized phase II trial in 217 patients who received 0.3, 3, and 10 mg/kg of antibody every 3 weeks four times. The results were presented at the 2008 ASCO Annual Meeting [25]. There was a dose–response relationship seen in that trial for response and for survival. The response rates
were 0%, 4%, and 11% in the 0.3, 3, and 10 mg/kg cohorts, respectively, and disease control rates (PR + CR + SD) of 13%, 26%, and 29% were achieved in those groups, respectively. The median OS times were 8 months, 8 months, and 14 months, respectively. The dose of 10 mg/kg administered every 3 weeks appeared to be optimal to achieve clinical benefit, and was chosen for further phase II and III testing.

Further phase II data of ipilimumab were discussed at the 2008 ASCO Annual Meeting. A randomized phase II trial of 115 patients with untreated or previously treated stage IV melanoma tested the idea that prophylactic budesonide, a nonabsorbed oral steroid, could decrease the incidence of grade ≥2 diarrhea when patients received ipilimumab at 10 mg/kg every 3 weeks four times. In that trial, if patients were stable or had a response and were without dose-limiting toxicity then they were able to receive maintenance ipilimumab at 10 mg/kg every 3 months until progression, dose-limiting toxicity, or refusal [26]. Patients received oral budesonide during their 12-week induction therapy, or placebo. There was no difference in the number of episodes of diarrhea, incidence of colitis, or overall IRAEs between the two arms, but the response rates, especially in previously untreated patients, and the estimated 1-year survival rates were quite favorable. The 1-year survival rate was 59% for all patients who received either budesonide or placebo. The 1-year survival rate for the untreated patients was 71% in the budesonide arm and 63% in the placebo arm. The disease control rate (SD + PR + CR) was associated with the development of IRAEs for all 115 patients ($p = .04$).

In a registration trial in patients who had failed frontline therapy, 155 patients with stage IV melanoma received the same dose and schedule of ipilimumab as in the trial just described above [27]. The primary endpoint was the ORR, with a 5.8% ORR, a 27.1% disease control rate (PR + CR + SD), and a median survival time of 10.3 months reported at the 2008 ASCO Annual Meeting. The 24-week PFS rate was 49%, and unfortunately this trial failed to meet its registration endpoint of a 15% ORR with a 10% lower response boundary for the 95% confidence interval.

**Correlation of Response with IRAEs**

Data from a number of clinical trials show a correlation between clinical response and IRAEs, such as dermatitis, uveitis, colitis/enterocolitis, hepatitis, and hypophysitis [12, 28, 29]. In a study by Attia and colleagues, ipilimumab-treated patients experiencing grade 3 or 4 autoimmune toxicity had a 36% clinical response rate, compared with a 5% rate in patients who did not have autoimmune toxicity [12]. As the organ systems targeted by the T cells in these reactions (other than skin) do not express gp100 antigen, it appears that CTLA-4 blockade resulting in disruption of self-tolerance, rather than the gp100 peptide vaccine, was the cause of the autoimmune toxicities. The incidence of grade 3 or 4 autoimmune toxicities in responders in this trial was 14% (Fig. 1).

The response rate was also significantly higher among ipilimumab-treated patients who developed IRAEs in a study conducted by Beck et al. [28]. While the ORR for the entire cohort was 14%, among those melanoma patients experiencing enterocolitis, the response rate was 36%, compared with 11% for patients with metastatic melanoma who did not develop enterocolitis. Overall, 21% of the patients were diagnosed with enterocolitis. Blansfield and colleagues reviewed 163 patients with advanced melanoma or renal cell cancer at their institution who had been treated with ipilimumab as of January 1, 2005 and found that eight (4.9%) had developed autoimmune hypophysitis [29]. All patients had received the drug i.v. every 3 weeks at doses of 3–9 mg/kg for four to nine doses. 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 [17]. Twenty-eight percent 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.

![Figure 1. Response rate to ipilimumab among patients who experienced grade 3 or 4 immune-related adverse events (IRAEs) versus those who did not [12, 19, 22].](http://theoncologist.alphamedpress.org/Downloaded from)
In general, even severe autoimmune toxicities have responded to high-dose steroids and/or supportive care, which did not appear to diminish the antitumor effect of CTLA-4 blockade [19, 28]. Among patients with steroid-refractory symptoms, infliximab was effective in virtually all cases [28]. In the Beck et al. [28] study, there was a 5% death rate among patients who developed autoimmune colitis. Failure to recognize and promptly treat early symptoms as well as poor compliance with steroid therapy played roles in those patients who developed major complications associated with enterocolitis. As more experience has been gained with the drug, mortality rates have been approximately 1%, suggesting that, while some of the toxicities can certainly be serious, proper education and established algorithms for management could bring this therapy into community oncology practice. Among patients developing hypophysitis, all experienced resolution of symptoms with the initiation of steroid, thyroid, and testosterone replacement and cessation of CTLA-4 blockade [29]. 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 a better response. Although these IRAEs can be serious, they can be managed when recognized, and are generally reversible when promptly treated.

**Unique Kinetics of Response with Ipilimumab Therapy**

As data accumulated from trials with ipilimumab, it became apparent that, in some cases, clinical response was substantially delayed in patients with melanoma [30]. Hamid et al. [31] undertook a review and analysis of five studies in 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 of 0.3–10 mg/kg in regimens involving single or multiple doses. An objective response occurred in 41 patients (15%) at the time of the analysis [31]. Some patients had a late onset CR or PR, occurring 10–106 weeks and 5–62 weeks after treatment initiation, respectively. In 28 patients, the onset of response occurred after >12 weeks of treatment, and in four patients, progressive disease (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 in the range of 6–187+ weeks. At the time of the analysis, an objective response was ongoing in 25 patients. Late-onset response was not associated with dose, regimen, or concomitant therapy. The findings from this study are extremely interesting, because they demonstrate that response can be late in onset or occur after disease progression with ipilimumab, in contrast to traditional chemotherapy [30]. Furthermore, the responses seen with this agent appear to be more durable than those with 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 (Figs. 2–4). Based on the above data, Hodi et al. [32], at the 2008 ASCO Annual Meeting, promulgated a new set of response criteria to supplement or even replace the World Health Organization (WHO) criteria or RECIST, called the

![Figure 2. Evolution of partial response to complete response](http://theoncologist.alphamedpress.org/)

**Figure 2.** Evolution of partial response to complete response [21]. Prior treatment included surgery, interferon-α, GM-CSF, bacille Calmette-Guerin, interleukin-2, and an unspecified biologic. Immune-related adverse effects included grade 1 diarrhea and grade 1 rash.

**Figure 3.** Durable partial response (PR) [21]. Prior treatment included surgery, interferon, and interleukin-2. Immune-related adverse effects included grade 1 rash and grade 2 colitis.
immune-related response criteria (irRC). New lesions are allowed with these criteria and the sum of the perpendicular diameters of all lesions, new and baseline, is used to calculate the burden of disease that determines response. PD by the modified WHO criteria or even the RECIST may not properly predict treatment failure and the lack of clinical benefit in patients receiving ipilimumab. The new criteria evaluate the total tumor burden, not just changes in baseline lesions, and might more accurately reflect OS. In the ASCO presentation, the use of irRC accurately reflected OS in 227 patients with stage IV melanoma treated with ipilimumab at 10 mg/kg [32].

**Phase III Ipilimumab Registration Studies**

Two registration studies that would support approval of ipilimumab as second-line therapy for metastatic melanoma have been conducted, and one is still recruiting patients. One phase III study began in September 2004 and is being conducted in patients with previously treated, unresectable stage III or IV melanoma [33]. That study compares the safety and efficacy of ipilimumab in combination with MDX-1379, a melanoma peptide vaccine, with those of either agent alone. The primary outcome measure is the best objective response rate, with secondary measures of OS, major durable response rate, duration of response, PFS, and time to disease progression. The total projected enrollment is 750 patients.

Another 150-patient phase II single-arm registration study of ipilimumab at 10 mg/kg every 3 weeks times four has been conducted, to which all patients have been accrued. The primary outcome measure was the best objective response rate. The primary response rate endpoint of 10% at the lower boundary of the 95% confidence interval was not reached in that study, with a 5.8% objective response rate achieved. The median OS duration for patients in that study was 10.3 months, although survival at 1 and 2 years was unknown at the time the data were presented at the 2008 ASCO Annual Meeting [27].

A second phase III registration study began in June 2006 in frontline patients. It compares combination therapy with ipilimumab (10 mg/kg) and dacarbazine versus dacarbazine and placebo for patients with untreated, unresectable stage III or IV melanoma. The overall PFS rate at 6 months is the primary outcome measure, and secondary measures are the PFS rate at week 12, ORR, duration of response, disease control rate, time to response, health-related quality of life, OS duration, survival rate at 1 year, safety profile, and population pharmacokinetics. The projected enrollment for this trial is 500 patients.

**Promise of Other Approaches to Immune Stimulation and Abrogation of Inhibition in Melanoma**

Although results with ipilimumab in advanced-stage melanoma have been very promising, other therapies are in development. The anti–CTLA-4 monoclonal antibody tremelimumab (CP-675,206) was explored in phase I and II trials in patients with metastatic melanoma. In an escalating-dose phase I trial, 34 patients were treated with single doses of tremelimumab at doses of 0.03–15 mg/kg. There were two CRs (maintained for 34+ months and 25+ months), two PRs (maintained for 26+ months and 25+ months), and four patients with SD [11]. Dose-limiting toxicities included rash, colitis, and diarrhea, similar to the IRAEs observed with ipilimumab. In a subsequent phase II study, 20 patients received tremelimumab at 10 mg/kg every month and 10 patients received the drug at 15 mg/kg every 3 months [34]. Four antitumor responses were observed in those who developed an IRAE, compared with only one response in those without an IRAE. Patients receiving 10 mg/kg every month had more grade 3 IRAEs. These results suggest that, as with ipilimumab, IRAEs may be associated with clinical benefit in those patients who receive tremelimumab. In a follow-up randomized phase II study, 84 patients received one of the same regimens used in the above phase II trial, with an objective response rate of 10% and median survival time of 10.2 months for the 10-mg/kg cohort, and 7% response rate and median survival of 11.5 months for the 15 mg/kg cohort [35]. The results of those trials of tremelimumab provoked a phase II registration trial of the antibody at a dose of 15 mg/kg every 3 months for stage IV melanoma patients who had failed at least one prior chemotherapy. The endpoint for registration was the achievement of at least a 15% ORR, with a 10% lower bound on the 95% confidence interval for response required for success.
The initial results were reported at the 2008 ASCO Annual Meeting, showing that, for the 258 patients accrued, the ORR was 8.3% [36]. The median survival time in that trial was 10.0 months, with a 22% disease control rate and two treatment-related deaths noted. A large, randomized, phase III frontline trial has also been finished in which tremelimumab at 15 mg/kg every 3 months was compared with a chemotherapy regimen of dacarbazine or temozolomide in 550 patients. That trial was halted at its second interim analysis, and the results were presented at the 2008 ASCO Annual Meeting [37]. The median OS duration for patients in the chemotherapy arm was 10.7 months, and for the tremelimumab arm it was 11.7 months. The hazard ratio for OS was 1.05, with a p-value of .45 between the two cohorts, and with the two survival curves overlapping over the first year of follow-up. Neither tremelimumab registration trial met its endpoint.

Another compound under investigation for use in metastatic melanoma is STA-4783, which has been found to dramatically enhance the antitumor activity of paclitaxel without increasing host toxicity [38]. STA-4783 is a bis-thiobenzoylhydrazide compound and an inducer of heat shock proteins. In a randomized, double-blind phase II study, STA-4783 combined with paclitaxel was more effective than paclitaxel alone in a total of 81 patients with metastatic melanoma [39]. The median PFS time for the STA-4783 plus paclitaxel group was 3.68 months, compared with 1.84 months for paclitaxel alone. In chemotherapy-naïve patients, the combination therapy had a PFS time of 8.28 months, compared with 2.40 months for paclitaxel alone. Furthermore, a few patients benefited from crossing over to combination therapy following failure on single-agent paclitaxel.

A compound that targets CD40, a member of the tumor necrosis receptor superfamily that is important in CD4+ T-cell activation, has demonstrated considerable antitumor potential [40, 41]. The anti-CD40 monoclonal antibody CP-870,893 showed antitumor activity in a small study, inducing objective PRs in 27% (4 of 19) of melanoma patients [42].

The identification of other potential therapeutic targets and strategies that may increase the efficacy of standard treatments also holds promise. B7-H1, a member of the B7 family of molecules often expressed on human cancers, has been identified as a promoter of the apoptotic death of activated tumor antigen-specific human T cells. Recognition of this mechanism of immune system evasion in tumors points a potential therapeutic target [43]. Another potential target may be the cell surface molecule programmed death (PD)-1, which modulates T- and B-cell activation and is involved in maintaining peripheral tolerance. In vitro studies have shown that PD-1 blockade increases the percentage of CTLs recognizing melanoma targets [43]. Data suggest that the combination of CTLA-4 blockade with anti–4-1BB antibodies may increase cancer immunity while simultaneously suppressing autoimmune side effects and increasing antibody responses [44].

Finally, lymphodepleting chemotherapy followed by adoptive transfer of antigen-specific T cells with IL-2 may be a useful strategy in patients with refractory metastatic melanoma. The removal of cytokine-responsive endogenous T cells and APCs that may serve as cytokine sinks has been explored, and the results suggest that restricted availability of cytokines may contribute to peripheral tolerance and may limit the effectiveness of tumor-specific T cells [45]. A trial of lymphodepletion was conducted with cyclophosphamide and fludarabine followed by cell infusion with autologous tumor-reactive rapidly expanded infiltrating lymphocyte culture and high-dose IL-2 therapy in 35 patients with metastatic melanoma [46]. In all but one patient, the disease was refractory to IL-2 therapy. After treatment, 18 (51%) of the patients had an objective clinical response—three CRs and 15 PRs, with a mean duration of 11.5 months. The results are very impressive in this heavily pretreated population with IL-2–refractory disease.

**Conclusions**

Late-stage melanoma is a disease with a very poor prognosis. Research in preclinical models has suggested that the CD28–CTLA-4 axis presents a promising target for cancer therapy. The CTLA-4 molecule contributes to peripheral tolerance that impedes tumor rejection by the immune system. Phase II and III clinical trials are being conducted with the anti–CTLA-4 monoclonal antibody ipilimumab. Durable objective response rates have been in the range of 7%–15% of patients with metastatic melanoma, with a further increment of patients with long-term SD, or progression followed by response, or stability followed by response. The response rate in patients experiencing grade 3 or 4 autoimmune toxicities is higher however—approximately 36% respond as compared with only 5%–11% of patients experiencing no autoimmune effects. Ipilimumab has been found to have clinical activity when combined with GM-CSF and melanoma peptide vaccines in early-stage trials; preliminary results suggest that excellent survival times have also been observed in small trials when ipilimumab is combined with dacarbazine or IL-2. These unique response scenarios provide a new challenge to the treating physician in that the patient may potentially experience a period of disease progression before he or she experiences a very good antitumor response. Education of the oncologist is the key to the appropriate management of the patient on CTLA-4 therapy.
Ongoing registration studies will better define the place of ipilimumab in the treatment of metastatic melanoma. Other trials are being conducted with new and innovative compounds in melanoma. STA-4783 is a heat shock protein inducer that is being explored as combination therapy with paclitaxel, and CP-870,893, an anti-CD40 monoclonal antibody, is being investigated as monotherapy and in combination treatments for melanoma. Future potential targets for blockade include B7-H1 and PD-1, and the addition of anti–4–1BB to CTLA-4 blockade may improve efficacy and decrease toxicity. Finally, lymphodepleting chemotherapy followed by infusion of tumor-reactive lymphocytes and high-dose IL-2 may improve the outcome in heavily pretreated patients with refractory metastatic melanoma. The immune system offers many potential therapeutic targets, but further research is needed before we can optimally harness its power in the fight against cancer.

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