# Prognostic Factors Related to Clinical Response in Patients with Metastatic Melanoma Treated by CTL-Associated Antigen-4 Blockade

Stephanie G. Downey,[1] Jacob A. Klapper,[1] Franz O. Smith,[1] James C. Yang,[1] Richard M. Sherry,[1] Richard E. Royal,[1] Udai S. Kammula,[1] Marybeth S. Hughes,[1] Tamika E. Allen,[1] Catherine L. Levy,[1] Michael Yellin,[3] Geoffrey Nichol,[3] Donald E. White,[1] Seth M. Steinberg,[2] and Steven A. Rosenberg[1]

**Abstract**

**Purpose:** CTL-associated antigen 4 (CTLA-4) can inhibit T-cell activation and helps maintain peripheral self-tolerance. Previously, we showed immune-related adverse events (IRAE) and objective, durable clinical responses in patients with metastatic melanoma treated with CTLA-4 blockade. We have now treated 139 patients in two trials and have sufficient follow-up to examine factors associated with clinical response.

**Experimental Design:** A total of 139 patients with metastatic melanoma were treated: 54 patients received ipilimumab in conjunction with peptide vaccinations and 85 patients were treated with intra-patient dose escalation of ipilimumab and randomized to receive peptides in accordance with HLA-A*0201 status.

**Results:** Three patients achieved complete responses (CR; ongoing at 29+, 52+, and 53+ months); an additional 20 patients achieved partial responses (PR) for an overall objective response rate of 17%. The majority of patients (62%, 86 of 139) developed some form of IRAE, which was associated with a greater probability of objective antitumor response ($P = 0.0004$); all patients with CR had more severe IRAEs. Prior therapy with IFNα-2b was a negative prognostic factor, whereas prior high-dose interleukin-2 did not significantly affect the probability of response. There were no significant differences in the rate of clinical response or development of IRAEs between the two trials. The duration of tumor response was not affected by the use of high-dose steroids for abrogation of treatment-related toxicities ($P = 0.23$). There were no treatment-related deaths.

**Conclusion:** In patients with metastatic melanoma, ipilimumab can induce durable objective clinical responses, which are related to the induction of IRAEs.

Patients with metastatic melanoma experience a 5-year survival probability of <10%, with a median survival of ~7 months. Dacarbazine is the only chemotherapeutic agent approved for use in metastatic melanoma, with reported response rates of 6% to 20%, although few patients experience complete responses (CR) and even fewer are durable (1). High-dose interleukin-2 (IL-2) administration, the only other approved treatment for these patients in the United States, mediates objective responses in ~15% of patients, with 7% of patients experiencing complete durable responses (2).

We and others have recently reported that the administration of an anti–CTL antigen 4 (CTLA-4) monoclonal antibody (ipilimumab) can mediate objective tumor regression in ~15% of patients with metastatic melanoma (3–6). CTLA-4 expressed on lymphocytes can bind to B7-1 and B7-2 (CD80 and CD86) on the surface of an APC, suppress lymphocyte reactivity, and interfere with IL-2 secretion and IL-2 receptor expression (7–14). With the exception of T regulatory cells ($CD4^+CD25^+$, $foxp3^+$), resting lymphocytes do not constitutively express CTLA-4 on their surface (15, 16); however, expression is transiently up-regulated after binding of the T-cell receptor (17, 18). Preclinical murine models have shown that CTLA-4 blockade can enhance immune-mediated tumor rejection (19–22).

In this report, we present the results of 139 consecutive patients with metastatic melanoma treated with ipilimumab in the Surgery Branch, National Cancer Institute, evaluating objective cancer responses and toxicities and analyzing prognostic and treatment factors associated with these clinical events.

## Materials and Methods

***Patients and treatment.*** All patients had measurable stage IV melanoma, an Eastern Cooperative Oncology Group (ECOG) performance status ≤2, no clinical evidence or history of autoimmune disease,

**Authors' Affiliations:** [1]Surgery Branch and the [2]Biostatistics and Data Management Section, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland and [3]Medarex, Inc., Princeton, New Jersey

Received 1/24/07; revised 6/27/07; accepted 8/22/07.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**Requests for reprints:** Steven A. Rosenberg, Surgery Branch, National Cancer Institute, NIH, Room 3-3940, 10 Center Drive, Bethesda, MD 20892-1201. Phone: 301-496-4164; E-mail: sar@nih.gov.



doi:10.1158/1078-0432.CCR-07-0187

and were greater than 3 weeks from any prior systemic cancer therapy. After signing informed consent, patients were enrolled in one of two trials conducted in the Surgery Branch, National Cancer Institute (Bethesda, MD) between March 2002 and December 2005. These two trials were approved by the Institutional Review Board of the National Cancer Institute. Response to treatment and survival were assessed in all patients as of April 1, 2007 with median follow-up of 50 (range, 37-61) and 29 (range, 16-36) months, respectively, in the two trials.

Trial 1 consisted of two cohorts of patients. In cohort 1, 29 HLA-A*0201 patients received anti–CTLA-4 antibody (ipilimumab, Bristol-Myers Squibb/Medarex, Inc.) every 3 weeks at 3 mg/kg i.v. over 90 min in conjunction with the s.c. injection of 1 mg of gp100:209-217 (210M) peptide (IMDQVPFSV) emulsified in Montanide ISA-51 in one extremity and 1 mg of gp100:280-288(288V) peptide (YLEPGPVTV) similarly emulsified in another extremity. In cohort 2, 27 HLA-A*0201 patients received peptide vaccination in an identical fashion; however, an initial dose of 3 mg/kg ipilimumab was followed by subsequent doses every 3 weeks of 1 mg/kg. Ipilimumab was supplied by the manufacturer; peptides and Montanide ISA-51 were supplied by the Cancer Therapy Evaluation Program, National Cancer Institute.

The results of trial 1 and the association seen between the induction of grade 3/4 immune-related adverse events (IRAE) and clinical response led to a more aggressive dosing strategy. Trial 2 was designed as an intra-patient dose-escalation study. HLA-A*0201–positive patients were randomized to receive ipilimumab alone or in conjunction with gp100:209-217(210M) and gp100:280-288(288V) peptides emulsified in Montanide ISA-51. HLA-A*0201–negative patients received ipilimumab alone. Ipilimumab treatment was started at 3 mg/kg. If after one course (two treatments), patients did not achieve an objective response or a dose-limiting toxicity, the dose was increased to 5 mg/kg. After another two treatments, patients could then be escalated to 9 mg/kg/dose. After initial enrollment of 38 patients, rapid disease progression limited the number of patients able to escalate as planned, and the trial was amended to start with a dose of 5 mg/kg and another 50 patients were accrued. Thus, a total of 144 patients were treated.

The majority of patients were treated until progression or unacceptable toxicity; a few patients, whose disease neither progressed nor responded, received the maximum number of cycles. No patients received concurrent systemic cancer therapy while enrolled on protocol.

Five patients were excluded from the final analysis; in two patients, a diagnosis of metastatic melanoma was presumed from clinical history and suspicious metastatic lesions, but resection of lesions after completion of therapy yielded a diagnosis of sarcoma and lung adenocarcinoma. Additionally, three patients were the only patients that had previously received non-myeloablative chemotherapy with adoptive cell transfer and remained highly immunosuppressed before ipilimumab. None of these five patients experienced an objective response. The remaining 139 patients are the subject of this analysis.

An additional trial conducted at the Surgery Branch studied combination therapy with ipilimumab and IL-2. Those patients are not included in this analysis of ipilimumab as single-agent i.v. therapy.

***Clinical evaluation of response.*** Each patient was evaluated radiographically, including, but not limited to, computed axial tomography of the chest, abdomen, and pelvis and magnetic resonance imaging of the brain within 4 weeks of initial therapy. Scheduled evaluations occurred after every two doses of therapy. Tumor assessments were done in accordance with Response Evaluation Criteria in Solid Tumors criteria (23). A partial response (PR) was defined as a ≥30% decrease in the sum of the longest diameters of target lesions lasting at least 1 month with no new tumors appearing. Complete responses (CR) required a total resolution of all lesions lasting at least 1 month. Response rates include only complete and partial responders. Patients not meeting these criteria were considered nonresponders.

***Autoimmunity screening and IRAE evaluation.*** All patients underwent baseline ophthalmologic evaluations, repeated at the end of each course or on development of visual symptoms. Patients were required to have negative baseline rheumatoid factor, antithyroglobulin, and anti–nuclear antibody at study entry. As clinical data accrued, additional mechanisms were instituted to rule out the appearance of IRAEs after the initiation of treatment, such as routine endocrine laboratory studies with each cycle of therapy, including adrenocorticotropic hormone, thyroid stimulating hormone, cortisol, and free T4. For the purposes of this study, the most severe grade of each IRAE type was recorded per patient. Clinical management of severe IRAEs, including use of high-dose steroids, evolved over time but became more standardized for later patients.

***Statistical analysis.*** Individual, dichotomous patient characteristics were compared between objective responders (CR + PR) and nonresponders using a $\chi^2$ test or Fisher's exact test, as appropriate. Age, number of cycles, and cumulative dose (mg/kg) were compared using a Wilcoxon rank sum test. A logistic regression model was used to identify factors that may jointly effect response.

Survival was calculated from the on-study date until the date of death or last follow-up (April 1, 2007), as appropriate. Progression-free survival was calculated from the on-study date until date of radiographically identified progression, or date of last known follow-up

**Table 1.** Patient demographics

| Protocol | Trial 1 | Trial 2 | Total |
|---|---|---|---|
| Total | 54 | 85 | 139 |
| Sex | | | |
| M | 36 | 56 | 92 (66%) |
| F | 18 | 29 | 47 (34%) |
| Age (y) | | | |
| Median (range) | 53 (21-67) | 49 (24-69) | 50 (21-69) |
| ECOG | | | |
| 0 | 43 | 54 | 97 (70%) |
| 1-2 | 11 | 31 | 42 (30%) |
| HLA | | | |
| A*0201 | 54 | 40 | 94 (68%) |
| Other | 0 | 45 | 45 (32%) |
| Prior therapy | | | |
| None | 15 | 5 | 20 (14%) |
| HD IL-2 | 13 | 31 | 44 (32%) |
| IFNα-2b* | 23 | 39 | 62 (45%) |
| Biochemotherapy | 10 | 19 | 29 (21%) |
| Chemotherapy | 6 | 27 | 33 (24%) |
| Other biological | 11 | 31 | 42 (30%) |
| Two or more | 18 | 45 | 63 (45%) |
| Stage of disease | | | |
| $M_{1a}$ (only s.c., lymph node) | 8 | 12 | 20 (14%) |
| $M_{1b}$ (lung sole site of visceral met) | 11 | 19 | 30 (22%) |
| $M_{1c}$ (visceral met, or elevated lactate dehydrogenase) | 35 | 54 | 89 (64%) |
| Treatment characteristics | | | |
| No. cycles | | | |
| Median (range) | 3.5 (1-12) | 4 (1-10) | 4 (1-12) |
| 1-2 | 23 | 25 | 48 (35%) |
| 3-4 | 20 | 25 | 45 (32%) |
| 5-6 | 5 | 23 | 28 (20%) |
| 7-8 | 3 | 7 | 10 (7%) |
| >8 | 3 | 5 | 8 (6%) |
| Total dose (mg/kg), median (range) | 6 (3-24) | 28 (3-70) | 12 (3-70) |
| Peptide administration | | | |
| Yes | 54 | 19 | 73 (53%) |
| No | 0 | 21 | 21 (15%) |
| Not eligible (non-A2) | 0 | 45 | 45 (32%) |

*Six of these 62 patients received IFNα-2b after resection of distant metastases.

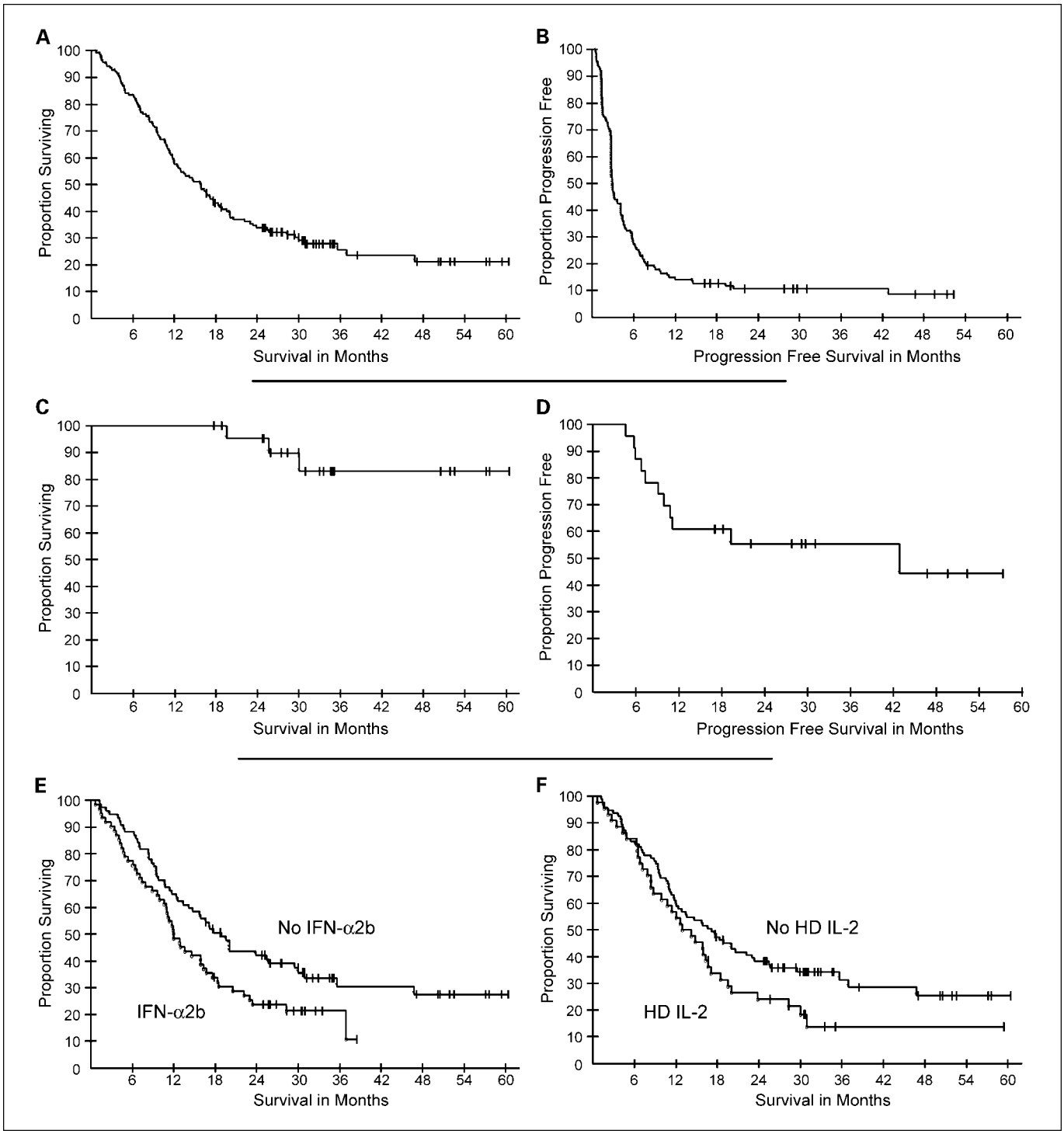


**Fig. 1.** *A* and *B,* clinical outcomes of 139 patients treated with CTLA-4 blockade. *A,* overall survival; median, 15.7 mo. *B,* progression-free survival; median, 2.9 mo. *C* and *D,* analysis of patients who responded to treatment with anti – CTLA4 antibody ($n = 23$). *C,* overall survival; three deaths noted to date among responders. *D,* duration of response from on-study date; median, 30.6 mo. *E* and *F,* effect of prior treatment regimens on overall and progression-free survival. *E,* patients previously treated with adjuvant IFNα-2b ($n = 62$) compared with those who had not received adjuvant IFN ($n = 77$). Survival durations were statistically different, with patients not previously treated with IFN surviving longer (median, 12.4 versus 18.2 mo; $P = 0.023$). *F,* patients previously treated with high-dose IL-2 ($n = 44$) compared with those who had not received high-dose IL-2 ($n = 95$). Survival was not statistically different (median, 12.9 versus 16.9 mo; $P = 0.07$).

without documented progression. Similarly, for responders, duration of response was calculated from the on-study date until the date of documented progression. For all patients, on-study date was the date of administration of the first dose of ipilimumab.

The probability of survival, progression-free survival, and the probability of duration of response as a function of time were calculated using the Kaplan-Meier method, with the statistical significance of the difference between curves determined by the Mantel-Haenszel test. The

association between grade of IRAEs or stage of disease and response was determined with an exact Cochran-Armitage trend test (24). A Cox model using a time-varying covariate was used to determine the prognostic significance of development of any IRAE, which may occur anytime from the first dose of antibody. A time-varying covariate analysis was also used to determine the effect of steroid use on response duration.

All *P* values are two tailed and not adjusted for multiple comparisons.

## Results

***Patient characteristics.*** Patients ranged in age from 21 to 69 years (Table 1), and the distribution of ages was not statistically different between trial 1 and trial 2. Most patients had an ECOG performance status of 0. Eighty-six percent of patients had undergone one or more systemic therapies for metastatic melanoma; 45% (62 of 139) had received adjuvant therapy with IFNα-2b, 32% (44 of 139) had high dose IL-2, and 24% (33 of 139) had DTIC-based chemotherapy. Additionally, 21% (29 of 139) were treated with biochemotherapy regimens. A number of patients were heavily pretreated, with 17% (24 of 139) having undergone three or more therapies for metastatic melanoma. The majority of patients (109 of 139, 78%) had visceral metastases at the beginning of therapy; 10 patients had evidence of brain metastases at enrollment. Despite a more aggressive dosing strategy used in trial 2, the median number of cycles tolerated was similar between trials (Table 1). As expected from the study designs, the total dose of ipilimumab received was significantly higher among patients in trial 2.

***Clinical outcomes.*** There was no significant difference in response rates, survival, or progression-free survival among any of the individual cohorts or between trials 1 and 2 (data not shown), and thus the actuarial curves of survival and progression-free survival for the combined groups are presented in Fig. 1. The response rates and durations of response are presented in Table 2. Of 139 patients, 3 patients achieved a confirmed CR, including 1 who was enrolled with brain metastases, and 20 patients achieved a confirmed PR for an overall response rate of 17%. Four patients experienced a delayed response, not meeting response criteria for at least 12 weeks after their last dose of ipilimumab. Two patients had completed the intra-patient dose escalation and were noted to have response 15 and 24 weeks after cessation of therapy; the remaining two patients experienced high-grade IRAEs requiring steroid administration and termination of therapy, meeting response criteria at 27 and 33 weeks.

The median progression-free survival and overall survival of all patients were 2.9 and 15.7 months; for responding patients, median progression-free survival was 30.6 months and median overall survival has not been reached. This computation was made retrospectively after identifying those patients who had responded to therapy, the determination of which required up to 7 months after beginning treatment. Twelve patients, including all three complete responders, are ongoing responders at 17+ to 53+ months. Progression-free survival and overall survival of the 23 responding patients are shown in Fig. 1C and D; three responders have died.

***Prognostic factors associated with clinical outcome.*** Analysis of patient demographic data and treatment characteristics was done in an attempt to elicit factors that may predict response in patients being treated with ipilimumab (Table 3A and B). Only prior therapy with IFNα-2b was a negative prognostic factor, whereas no association with response was seen as a function of other prior treatment, sex, ECOG status, HLA type, and sites of disease. Although increased age was associated with response in univariate analysis, the prognostic value of age did not remain significant in a logistic regression analysis once IRAEs and prior IFN were included together in a model.

There was no statistically significant difference in response between patients who did or did not receive peptide vaccination, regardless of how the data were examined. For example, 40 patients were randomized to receive peptide ($n$ = 19) or not ($n$ = 21) as part of the design of trial 2. Looking at those 40 patients separately, as well as in conjunction with all 54 patients of trial 1, there were no significant differences in response rate according to peptide administration. However, this trial was not powered to detect small differences that may be due to peptide administration.

Because the dosing strategy was altered when designing the intra-patient dose escalation trial, only patients accrued to trial 2 were included in an analysis of whether the number of cycles and total doses administered were factors potentially associated with response. In univariate analyses, both total dose and number of cycles were significantly associated with response. The association between total dose and response, or number of cycles and response, likely was because of the inability to complete the dose escalation in patients with nonresponding rapidly progressive disease; thus, based on these data, we cannot draw any conclusions about the relationship of number of doses to the likelihood of achieving a response.

Patients who were previously treated with IFNα-2b had a decreased duration of survival when compared with those who did not receive adjuvant therapy with IFNα-2b (median, 12.4 versus 18.2 months; $P$ = 0.023; Fig. 1E). Previous treatment with high-dose IL-2 did not have a statistically significant effect on length of survival (median, 12.9 versus 16.9 months; $P$ = 0.07; Fig. 1F).

**Table 2.** Duration of responses

| | Total | PR | CR | PR + CR |
|---|---|---|---|---|
| All patients | 139 | 20 | 3 | 23 (17%) |
| Trial 1 | 54 | 5 | 2 | 7 (13%) |
| Duration (mo) | | 4, 5, 43, 47+, 50+ | 52+, 53+ | |
| Trial 2 | 85 | 15 | 1 | 16 (19%) |
| Duration (mo) | | 6, 6, 7, 9, 10, 10, 11, 17+, 17+, 18+, 19, 22+, 28+, 30+, 31+ | 29+ | |

NOTE: + indicates ongoing response.

**Table 3.** Prognostic factors vs response

**(A) Pretreatment factors**

| | Total | PR | CR | PR + CR | *P* |
|---|---|---|---|---|---|
| Sex | | | | | |
| M | 92 | 15 | 2 | 17 (18%) | |
| F | 47 | 5 | 1 | 6 (13%) | 0.39 |
| Age (y) | | | | | |
| Median (range) | 50 (21-69) | | | 54 (35-67) | 0.03* |
| ECOG | | | | | |
| 0 | 97 | 16 | 3 | 19 (20%) | 0.14 |
| 1-2 | 42 | 4 | 0 | 4 (9%) | |
| HLA | | | | | |
| A201 | 94 | 14 | 3 | 17 (18%) | 0.63 |
| Non-A2 | 45 | 6 | 0 | 6 (13%) | |
| Prior therapy | | | | | |
| None | 20 | 3 | 2 | 5 (25%) | 0.33 |
| HD IL-2 | 44 | 5 | 0 | 5 (11%) | 0.26 |
| IFN$\alpha$-2b | 62 | 5 | 0 | 5 (8%) | 0.016 |
| Biochemotherapy | 29 | 3 | 1 | 4 (14%) | 0.78 |
| Chemotherapy | 33 | 6 | 0 | 6 (18%) | 0.77 |
| Other biological | 42 | 10 | 0 | 10 (24%) | 0.13 |
| Two or more | 63 | 7 | 0 | 7 (11%) | 0.12 |
| Sites of disease | | | | | |
| No evidence of visceral disease | 30 | 6 | 0 | 6 (20%) | 0.58 |
| Presence of visceral disease | 109 | 14 | 3 | 17 (16%) | |
| No evidence of brain metastases | 129 | 18 | 2 | 20 (15%) | 0.37 |
| Presence of brain metastases | 10 | 2 | 1 | 3 (30%) | |
| Stage of disease | | | | | |
| $M_{1a}$ | 20 | 5 | 0 | 5 (25%) | 0.35 |
| $M_{1b}$ | 30 | 5 | 0 | 5 (17%) | |
| $M_{1c}$ | 89 | 10 | 3 | 13 (15%) | |

**(B) Treatment factors**

| | All | NR | PR + CR | *P* |
|---|---|---|---|---|
| Protocol | | | | |
| Trial 1 | 54 | 47 | 7 (13%) | 0.36 |
| Trial 2 | 85 | 69 | 16 (19%) | |
| Peptide† | | | | |
| Yes | 73 | 60 | 13 (18%) | 1.00‡ |
| No | 21 | 17 | 4 (19%) | |
| Non–HLA-A*0201 | 45 | 39 | 6 (13%) | |
| No. cycles§ | | | | |
| Median (range) | 4 (1-10) | 4 (1-10) | 6.5 (3-10) | <0.0001 |
| 1-2 | 25 | 25 | 0 (0%) | |
| 3-4 | 25 | 23 | 2 (8%) | |
| 5-6 | 23 | 17 | 6 (26%) | |
| 7-8 | 7 | 3 | 4 (57%) | |
| >8 | 5 | 1 | 4 (80%) | |
| Total dose§ (mg/kg) | | | | |
| Median (range) | 28 (3-70) | 23 (3-70) | 35.5 (19-70) | 0.0005 |

*$P$ > 0.05 in logistic regression analysis after adjusting for any IRAE and prior IFN.
† Within trial 2, 6 of 19 (32%) patients receiving peptides were responders ($P$ = 0.18).
‡ Non–HLA-A*0201 patients were excluded.
§ Due to different dosing strategies, number of cycles and total dose were compared only for patients in trial 2 ($n$ = 85).

***IRAEs.*** The majority of patients (86 of 139, 62%) experienced some form of IRAE (Table 4). The most common was grade 1/2 dermatitis (30%) usually accompanied by pruritis (27%). Enterocolitis and hypophysitis were the most common clinically significant grade 3/4 IRAEs and were treated as previously reported (25, 26). The relationship between development of an IRAE and clinical response is shown in Table 5. Of the 50 patients who developed grade 3/4 IRAEs, 14 (28%) experienced an objective response, with a median duration of response of 34 months. All three complete responders came from the subset of patients who experienced high-grade IRAEs. Of 36 patients experiencing grade 1/2 IRAEs only, 8 (22%) experienced an objective response, all partial, with a median duration of response of 11 months. For the 86 patients experiencing any grade of IRAE, 22 (26%) were objective responders, compared with 2% (1 of 53) of patients who did not have any IRAE. Development of an IRAE was significantly associated with likelihood of response ($P$ = 0.0004). There were no treatment-related deaths in this study.

**Table 4.** Frequency of IRAEs

| | Grade 1/2 | | | Grade 3/4 | | |
|---|---|---|---|---|---|---|
| | All *n* = 139 | Peptide *n* = 73 | No peptide *n* = 66 | All *n* = 139 | Peptide *n* = 73 | No peptide *n* = 66 |
| None | 76 (55%) | 37 (51%) | 39 (59%) | 89 (64%) | 48 (66%) | 41 (62%) |
| One or more | 63 (45%) | 36 (49%) | 27 (41%) | 50 (36%) | 25 (34%) | 25 (38%) |
| Alveolitis | 1 (1%) | 1 (1%) | 0 | 1 (1%) | 0 | 1 (2%) |
| Arthralgia | 11 (8%) | 9 (12%) | 2 (3%) | 3 (2%) | 1 (1%) | 2 (3%) |
| Conjunctivitis | 2 (1%) | 0 | 2 (3%) | 0 | 0 | 0 |
| Dermatitis | 40 (29%) | 22 (30%) | 18 (27%) | 8 (6%) | 7 (10%) | 1 (2%) |
| Enterocolitis | 4 (3%) | 2 (3%) | 2 (3%) | 24 (17%) | 14 (19%) | 10 (15%) |
| Hepatitis | 0 | 0 | 0 | 2 (1%) | 2 (3%) | 0 |
| Hypophysitis | 0 | 0 | 0 | 13 (9%) | 1 (1%) | 12 (18%) |
| Hypothyroidism | 3 (2%) | 1 (1%) | 2 (3%) | 0 | 0 | 0 |
| Episcleritis | 0 | 0 | 0 | 1 (1%) | 1 (1%) | 0 |
| Nephritis | 0 | 0 | 0 | 1 (1%) | 0 | 1 (2%) |
| Pruritis | 35 (25%) | 19 (26%) | 16 (24%) | 0 | 0 | 0 |
| Uveitis | 1 (1%) | 0 | 1 (2%) | 3 (2%) | 2 (3%) | 1 (2%) |
| Death | 0 (0%) | 0 | 0 | 0 (0%) | 0 | 0 |

Furthermore, as indicated previously, the development of any autoimmune side effect was found to have a significant effect on clinical response when incorporated in a logistic model based on all 139 patients, which included previous IFN as well. No other factors besides autoimmune toxicity and prior IFN were jointly associated with response. In addition, by the Cox model including development of any autoimmune toxicity as a time-varying covariate, both prior IFN status ($P = 0.013$) and any autoimmune toxicity ($P < 0.0001$) were conditions associated with an increasing probability of survival.

***Steroid administration.*** Treatment of the most severe of the high-grade IRAEs required administration of high-dose systemic steroids. A time-varying covariate analysis of the subset of 23 responding patients revealed that steroid administration had no significant effect on the duration of clinical response ($P = 0.23$). Twelve patients were treated with steroids with a median duration of response of 19.3 months (Table 6), which was somewhat less than the median response for all responders of 30.6 months. The 11 responders not treated with steroids have not yet reached a median duration of response. These results require careful interpretation, however, as they were retrospectively determined because the initiation of steroids may take place up to 2 years after beginning CTLA-4 blockade treatment.

Twenty-six of the 116 nonresponding patients required steroid administration; this was not associated with a survival difference ($P = 0.99$) when compared with the 90 nonresponding patients not requiring steroid treatment.

## Discussion

Completion of two trials treating patients with metastatic melanoma by blockade of CTLA-4 signaling provided the opportunity to evaluate prognostic factors related to response. The novel finding of this analysis was that prior therapy with IFNα-2b diminished the likelihood of response. The link between tumor regression and IRAEs was again shown. No other patient or treatment characteristic meaningfully affected likelihood of response.

CTLA-4 blockade is thought to mediate its antitumor and IRAE-inducing effects by reducing peripheral tolerance to self-antigens and increased T-cell activation, rather than by depletion of $CD4^+CD25^+$ T regulatory cells (27–30). Administration of anti–CTLA-4 monoclonal antibody had no effect on levels of *foxp3* expression or on numbers of circulating $CD4^+CD25^+$ cells in peripheral blood; however, HLA-DR expression on $CD4^+$ and $CD8^+$ cells was increased *in vivo*, accompanied by increased expression of CD45RO on $CD4^+$ cells. These changes were seen in the circulating lymphocytes of both clinical responders and nonresponders (27).

The mechanism behind the CTLA-4 blockade–induced lymphocyte activation in murine models is dependent on a mixed population of $CD4^+$ and $CD8^+$ lymphocytes (31, 32). In a murine model crossing gp100-specific TCR transgenic mice (pmel-1) with CTLA-$4^{-/-}$ mice, the pmel-1 CTLA-$4^{-/-}$ mice developed profound autoimmune vitiligo, which was abrogated in CTLA-$4^{-/-}$, Rag-$1^{-/-}$ pmel mice. Cotransfer of CTLA-$4^{-/-}$ $CD4^+$ cells with CTLA-4 wild-type $CD8^+$ cells could not induce

**Table 5.** Relationship between IRAEs and response

| | All | NR | PR + CR | *P* | Duration of response (mo), median (range) |
|---|---|---|---|---|---|
| IRAE | | | | | |
| None | 53 | 52 | 1 (2%) | 0.0004 | 18+ |
| Only grade 1/2 | 36 | 28 | 8 (22%) | | 11 (4-30+) |
| Grade 3/4 | 50 | 36 | 14 (28%) | | 35 (7-53+) |

**Table 6.** Duration of response in patients requiring steroid administration

| | No. patients | Duration of response | Median (mo) | *P* |
|---|---|---|---|---|
| All responders | 23 | | 30.6 | |
| Requiring steroids | 12 | 6, 7, 9, 10, 11, 19, 28+, 29+, 31+, 43, 47+, 52+ | 19.3 | 0.23* |
| Not requiring steroids | 11 | 4, 5, 6, 10, 17+, 17+, 18+, 22+, 30+, 50+, 53+ | Not reached | |

*By time-varying covariate analysis.

vitiligo. When both CD4 and CD8 populations lacked CTLA-4, CD8 cells exhibited increased markers of activation; in the absence of CD4 cells, the CD8 population expressed a more naïve phenotype. Dysregulation of both populations thus seems to be necessary to induce tumor regression and autoimmunity (33, 34).

Preclinical murine models showed that blockade of CTLA-4 binding in conjunction with antitumor vaccination could lead to tumor regression. Our initial clinical studies with fully human anti–CTLA-4 antibody in combination with a peptide vaccine described objective clinical responses in patients with metastatic melanoma, accompanied by a range of IRAEs, the presence of which were highly related to response (25, 26, 35). We now update that experience and report additional patients treated with a more aggressive dosing strategy (trial 2) designed to increase both the dose and duration of treatment as well as treat patients in the absence of an antitumor vaccine (6). Despite more aggressive dosing and higher total doses (in mg/kg) per patient, overall, no significant difference in response rates was achieved nor were there significantly more IRAEs noted (data not shown). However, in study 2, response rate was correlated with total dose (mg/kg) and the number of cycles of ipilimumab administered. This apparent dose-response effect is likely due to rapidly progressing non-responding patients withdrawing from therapy during the dose-escalation phase. Given these observations, however, coupled with a degree of latency in the onset of response, with considerable delay in some patients, a strategy of initiating treatment with higher doses of ipilimumab (in the 10 mg/kg range), rather than dose escalation from lower doses, should be evaluated.

Administration of a peptide vaccine had no effect on clinical response rates in either a randomized or nonrandomized fashion. Although we saw no added effect, the numbers of patients were small and larger studies are necessary to evaluate the combined administration of ipilimumab and peptide vaccination.

Analysis of the larger number of patients presented herein continued to support the strong association between clinical response and the induction of IRAEs ($P = 0.0004$), thus emphasizing the close relationship between self-tolerance and tolerance to cancer antigens. It is of interest to note that the median duration of response was longer in patients experiencing high-grade IRAEs (11 versus 34 months); however, this was determined retrospectively and requires careful interpretation.

As our experience with the administration of ipilimumab increased, the recognition and treatment of severe IRAEs, particularly hypophysitis and colitis, became more standardized (25, 26). No patient in these trials suffered a lethal toxicity; all symptoms, except hypophysitis with corticosteroid insufficiency, were reversible with administration of high-dose steroids. Patients with enterocolitis typically presented with onset of watery diarrhea ~11 days after receiving a dose of ipilimumab; most were admitted for observation and kept on restricted oral intake. If the diarrhea did not resolve, endoscopy was done, and patients with severe symptoms or histologic evidence of acute or chronic enteritis were started on i.v. dexamethasone. Symptoms in four patients were refractory to steroids but responded to infliximab treatment (25). Often, the first sign of hypophysitis was a swelling of the pituitary gland as noted on magnetic resonance imaging of the brain. Some patients developed clinical hypophysitis with headache and fatigue. Both subclinical and clinical presentations were treated with i.v. steroids. Early treatment of subclinical hypophysitis did not obviate the need for eventual hormone replacement. Although many patients recovered thyroid and testosterone/estrogen production, all but one have required continued corticosteroid replacement (26). Long-term steroid usage more closely approximates physiologic replacement doses and is not associated with steroid sequelae. Despite administration of high-dose steroids for treatment of severe IRAEs, the subset of responding patients maintained an antitumor effect.

An analysis of prognostic factors related to response showed no statistically significant effect of sex, age, performance status (0 to 2), HLA type (A*0201 or non-A*0201), sites of disease, or concomitant vaccine administration. Prior therapy with IFNα-2b, but not with IL-2 or other therapies, was associated with a lower response rate. It is difficult to understand why IFN therapy, but not other immunologic therapies, negatively affected response. In a trial of adjuvant IFN therapy, the appearance of clinical manifestations of autoimmunity or the development of autoantibodies seemed to be associated with improvements in progression-free survival and overall survival (36). Thus, prior treatment with IFN may have affected the tumor to select cells less responsive to CTLA-4 blockade.

Whereas there was no difference in response rates between IL-2 naïve and IL-2–treated patients, there was a statistically significantly higher incidence of bowel perforations in patients receiving high-dose IL-2 after anti–CTLA-4 therapy (3 of 22, 14%; ref. 37). In addition, concurrent use of steroids as necessary for management of IRAEs is a contraindication to IL-2, suggesting that, if the patient is a suitable candidate, IL-2 therapy should be initiated before ipilimumab.

## References


1. Balch CM, Atkins MB, Sober AJ. Malignant melanoma: cutaneous melanoma. In: DeVita VT, Hellman S, Rosenberg SA, editors. Cancer: principles and practice of oncology. 7th ed. Philadelphia: Lippincott-Raven Publishers; 2005. p. 1754–808.
2. Rosenberg SA, Yang JC, White DE, Steinberg SM. Durability of complete responses in patients with metastatic cancer treated with high-dose interleukin-2. Ann Surg 1998;228:307–19.
3. Phan GQ, Yang JC, Sherry RM, et al. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci U S A 2003;100:8372–7.
4. Sanderson K, Scotland R, Lee P, et al. 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 stage III and IV melanoma. J Clin Oncol 2005;23:741–50.
5. Attia P, Phan GQ, Maker AV, et al. Autoimmunity correlates with tumor regression in Patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte Antigen-4. J Clin Oncol 2005;25:6043–53.
6. Maker A, Yang JC, Sherry RM, et al. Intrapatient dose escalation of anti-CTLA-4 antibody in patients with metastatic melanoma. J Immunother 2006;29:455–63.
7. Linsley PS, Brady W, Grosmaire L, Aruffo A, Damle NK, Ledbetter JA. Binding of the B cell activation antigen B7 to CD28 costimulates T-cell proliferation and interleukin 2 mRNA accumulation. J Exp Med 1991;173:721–30.
8. Koulova L, Clark EA, Shu G, Dupont B. The CD28 ligand B7/BB1 provides costimulatory signal for alloactivation of CD4+ T cells. J Exp Med 1991;173:759–61.
9. Linsley PS, Brady W, Urnes M, Grosmair LS, Damle NK, Ledbetter JA. CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med 1991;174:561–9.
10. Alegre ML, Frauwirth KA, Thompson CB. T-cell regulation by CD28 and CTLA-4. Nat Rev Immunol 2002;1:220–8.
11. Walunas TL, Bakker CY, Bluestone JA. CTLA-4 ligation blocks CD28-dependent T-cell activation. J Exp Med 1996;183:2541–50.
12. Krummel MF, Allison JP. CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells. J Exp Med 1996;183:2533–40.
13. Brunner MC, Chambers CA, Chan FK, Hanke J, Winoto A, Allison JP. CTLA-4-mediated inhibition of early events of T-cell proliferation. J Immunol 1999;162:5813–20.
14. Greenwald RJ, Oosterwegel MA, van der Woude D, et al. CTLA-4 regulates cell cycle progression during a primary immune response. Eur J Immunol 2002;32:366–73.
15. Shevach E. CD4+CD25+ suppressor T cells: More questions than answers. Nat Rev Immunol 2002;2:389–400.
16. Hori S, Nomura T, Sakaguchi S. Control of regulatory T-cell development by the transcription factor, foxp3. Science 2003;299:1057–61.
17. Lindsten T, Lee KP, Harris ES, et al. Characterization of CTLA-4 structure and expression on human T cells. J Immunol 1993;151:3489–99.
18. Walunas TL, Lenschow DJ, Bakker CY, et al. CTLA-4 can function as a negative regulator of cell activation. Immunity 1994;1:405–13.
19. Egen JG, Kuhns MS, Allison JP. CTLA-4: New insights into its biological function and use in tumor immunotherapy. Nat Immunol 2002;3:611–8.
20. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science 1996;271:1734–6.
21. Hurwitz AA, Yu TF, Leach DR, Allison JP. CTLA-4 blockade synergizes with tumor-derived granulocyte-macrophage colony-stimulating factor for treatment of an experimental mammary carcinoma. Proc Natl Acad Sci U S A 1998;95:10067–71.
22. van Elsas A, Hurwitz AA, Allison JP. Combination immunotherapy of B16 melanoma using anti-cytoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. J Exp Med 1999;190:355–66.
23. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205–16.
24. Agresti A. Categorical Data Analysis. New York: John Wiley and Sons, Inc.; 1990. p. 79–129.
25. Beck KE, Blansfield JA, Tran KQ, et al. Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. J Clin Oncol 2006;24:2283–9.
26. Blansfield JA, Beck KE, Tran KQ, et al. Cytotoxic T-lymphocyte-associated antigen-4 blockage can induce autoimmune hypophysitis in patients with metastatic melanoma and renal cancer. J Immunother 2005;28:593–8.
27. Maker AV, Attia P, Rosenberg SA. Analysis of the cellular mechanism of antitumor responses and autoimmunity in patients treated with CTLA-4 blockade. J Immunol 2005;175:7746–54.
28. Vasu C, Prabhakar BS, Holterman MJ. Targeted CTLA-4 engagement induces $CD4^+$ $CD25^+$ CTLA-4high T regulatory cells with target (allo)antigen specificity. J Immunol 2004;173:2866–76.
29. Takahashi T, Tagami T, Yamazaki S, et al. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med 2000;192:303–10.
30. Taylor PA, Noelle RJ, Blazar BR. CD4(+) CD25(+) immune regulatory cells are required for induction of tolerance to alloantigen via co-stimulatory blockade. J Exp Med 2001;193:1311–8.
31. Waterhouse P, Penninger JM, Timms E, et al. Lymphoproliferative disorders with early lethality in mice deficient in CTLA-4. Science 1995;270:985–8.
32. Chambers CA, Sullivan TJ, Allison JP. Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4+ T cells. Immunity 1997;7:885–95.
33. Gattinoni L, Ranganathan A, Surman DR, et al. CTLA-4 dysregulation of self/tumor-reactive $CD8^+$ T-cell function is $CD4^+$ T cell-dependent. Blood 2006;108:3818–23.
34. Chambers CA, Kuhns MS, Egen JG, Allison JP. CTLA-4-mediated inhibition in regulation of T-cell responses: Mechanisms and manipulation in tumor immunotherapy. Annu Rev Immunol 2001;19:565–94.
35. Robinson MR, Chan CC, Yang JC, et al. CTL-associated antigen 4 blockade in patients with metastatic melanoma: A new cause of uveitis. J Immunother 2004;27:478–9.
36. Gogas H, Ioannovich J, Dafni U, et al. Prognostic significance of autoimmunity during treatment of melanoma with interferon. N Engl J Med 2006;354:709–18.
37. Smith FO, Goff SL, Klapper JA, et al. Risk of bowel perforation in patients receiving Interleukin-2 after therapy with anti-CTLA-4 monoclonal antibody. J Immunother 2007;20:130.