A Pilot Trial of CTLA-4 Blockade with Human Anti-CTLA-4 in Patients with Hormone-Refractory Prostate Cancer

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Abstract

Purpose: Blockade of the T-cell inhibitory receptor CTL-associated antigen-4 (CTLA-4) augments and prolongs T-cell responses and is a strategy to elicit antitumor immunity. The objectives of this pilot study were to establish the pharmacokinetic and safety profile for a single dose of 3 mg/kg of the anti-CTLA-4 antibody Ipilimumab (MDX-010, BMS-734016) and to assess if this therapy resulted in prostate-specific antigen (PSA) modulation and the development of polyclonal T-cell activation and/or clinical autoimmunity in patients with hormone-refractory prostate cancer treated with Ipilimumab.

Experimental Design: Patients with metastatic hormone-refractory prostate cancer received a single 3 mg/kg i.v. dose of Ipilimumab. Serologic measures of autoimmunity were obtained, and T-cell activation was evaluated by flow cytometry. Pharmacokinetic sampling of plasma for MDX-CTLA-4, PSA measurement, and diagnostic imaging were also undertaken.

Results: Fourteen patients were treated: 12 patients received a single dose of Ipilimumab, and 2 patients were re-treated with a second dose upon PSA progression. Two patients showed PSA declines of $\geq$50%. Treatment was well tolerated with clinical autoimmunity limited to one patient who developed grade 3 rash/pruritis requiring systemic corticosteroids. The mean $\pm$ SD Ipilimumab terminal elimination half-life was 12.5 $\pm$ 5.3 days.

Conclusions: A single dose of 3 mg/kg Ipilimumab, an anti-CTLA-4 antibody, given to patients with prostate cancer is safe and does not result in significant clinical autoimmunity. PSA-modulating effects observed warrant further investigation.

Prostate cancer is the second leading cause of cancer death of men in the United States, with over 30,350 deaths estimated in 2005 (1). Virtually, all deaths are due to the development of progressive, metastatic hormone-refractory prostate cancer (HRPC). Although docetaxel provides modest prolongation of life, once HRPC develops, its course is uniformly fatal. Novel therapeutic approaches are warranted.

A variety of immunotherapeutic strategies have been tested in prostate cancer. Tolerance to tumor or tissue antigens can be broken, and both T- and B-cell responses can be induced, although it is not clear if immunologic responses translate into clinical benefit (2–5). T-cell activation depends on recognition by the T-cell receptor of specific antigenic peptides in the context of MHC molecules expressed by antigen-presenting cells such as dendritic cells. In addition to this interaction, additional antigen-independent costimulatory signals are required for the generation of a T-cell response. T cells can express two related receptors (CD28 and CTLA-4) on their cell surface, and both bind to the same ligands (CD80 and CD86, also known as B7-1 and B7-2) that are present on antigen-presenting cells. Whereas ligand engagement of CD28 activates T cells, interactions between CTLA-4 and these ligands inhibit T-cell stimulation (6). Preventing interactions between CTLA4 and its ligands by using a neutralizing or blocking antibody has been shown in preclinical models to sustain and potentiate immune responses (7). Thus, blockade of CTLA-4 represents an important mechanism of potentiating T-cell immunity and, potentially, antitumor T-cell responses.

In vivo CTLA-4 blockade has been shown effective in inducing tumor rejection in several murine tumor models (8–11). In the transgenic adenocarcinoma of mouse prostate model, the use of anti-CTLA-4 antibody resulted in a reduction in metastatic relapse after primary prostate tumor resection, and the combination of CTLA-4 blockade and antigen presentation produced significant antitumor immunity (12, 13).

No evidence of enhanced autoimmunity besides prostatitis and vitiligo has been observed in preclinical models employing CTLA-4 blockade. Similarly, studies in primates failed to identify any significant adverse clinical, immunologic, or histopathologic findings. Immunohistochemical studies in a

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broad spectrum of human tissues did not detect any unanticipated tissue cross-reactivity.

Based on these observations, a pilot trial of a single dose of the human anti-CTLA-4 antibody Ipilimumab (Medarex, Inc., Bloomsbury and NJ/Bristol-Meyers Squibb, Wallingford, CT) was undertaken in metastatic HRPC. The objectives of this pilot study were to establish a safety and pharmacokinetic profile for a single dose of 3 mg/kg Ipilimumab, to assess if this therapy resulted in the development of clinical autoimmunity, and to record prostate-specific antigen (PSA) modulation and objective responses in HRPC patients. A protocol amendment allowed re-treatment with a single dose of 3 mg/kg Ipilimumab in patients who had a response followed by disease progression. An initial dose of 3.0 mg/kg was selected based on the observation that no adverse effects were seen in macaques receiving three bolus doses of 3.0 or 10 mg/kg. In macaques dosed at 3 mg/kg, peak plasma concentrations of the antibody were ~240 μg/mL. Based on these data and data from other clinical trials in humans with human antibodies, a dose of 3.0 mg/kg was expected to achieve peak plasma concentrations in humans in excess of 10.0 μg/mL. *In vitro* data suggest that CTLA-4/B7 interactions are efficiently blocked at concentrations of 1 to 10 μg/mL of anti-CTLA-4 antibody. Because these experiments were done with cells that overexpress CTLA-4 and B7, it was anticipated that similar antibody concentrations would be sufficient for saturation of CTLA-4 on the surface of activated circulating T cells, where CTLA-4 is expressed at relatively low levels, compared with the *in vitro* models.

Patients and Methods

Patients. Eligible patients had histologically confirmed adenocarcinoma of the prostate with evidence of metastatic spread on imaging studies and evidence of disease progression despite androgen deprivation (and if applicable, antiandrogen withdrawal) as defined by the PSA Consensus Criteria (14).

Other eligibility requirements included a Karnofsky performance status of ≥60% and an expected survival of at least 3 months. Required laboratory tests included adequate hematologic, renal, and hepatic function (WBC ≥1,500/mL, ANC ≥1,500/mL, platelets ≥150 × 10^3/mL, hematocrit ≥30 %, hemoglobin ≥10 g/dL, creatinine <1.25 × upper limit of normal, aspartate aminotransferase ≤1.25 × upper limit of normal, and bilirubin ≤1.0 × upper limit of normal) and serum testosterone <50 ng/mL. Patients with known autoimmune disorders were excluded, and a negative serum ANA test was required. Prior treatment with secondary hormonal therapies, chemotherapy, or investigational therapy was allowed, provided it was discontinued at least 1 month before treatment, the patient had recovered adequately, and further progressive disease as defined by the Consensus Criteria was shown. Per Consensus Criteria, an antiandrogen withdrawal response had to be excluded before enrollment. Prior radiation therapy had to have been completed at least 1 month before treatment, and radiopharmaceuticals could not have been administered within 2 months of treatment. Patients who required systemic corticosteroids for any indication were not eligible. Institutional review boards approved the trial at the two centers involved, and all patients provided written informed consent.

Treatment. Patients without prior orchiectomy continued gonadal suppression with a luteinizing hormone-releasing hormone agonist. Eligible patients underwent pretreatment physical examination, electrocardiogram, chest X-ray, bone scan, and computed tomography scan of abdomen and pelvis. Eligible patients were treated with a single i.v. dose of 3 mg/kg Ipilimumab. Patients with disease response were eligible for re-treatment, provided at least 6 months had elapsed following the first dose of Ipilimumab, eligibility criteria continued to be met, and plasma concentration levels of Ipilimumab were <2 μg/mL. Ipilimumab was administered i.v. as an infusion, using a volumetric pump. A test dose of 0.2 mg in 10 mL normal saline was administered over 10 min. Patients were observed for hypersensitivity reactions over the following 30 min. If a hypersensitivity reaction did not occur, the infusion was resumed so that the remaining dose, at a concentration of 2.5 mg/mL, was administered over a period of 90 min.

Prior and subsequent to Ipilimumab infusion, blood samples were taken for hematologic, biochemical, pharmacokinetic, and immune function assessments. Hematologic and biochemical variables were assessed at baseline and at 1, 2, 3, 14, 21, and 28 days after treatment and monthly thereafter. Pharmacokinetic sampling of plasma for Ipilimumab levels was undertaken just before infusion; at 15 and 30 min; at 1, 2, 4, 24, 48, and 72 h; at 7, 14, 21, and 28 days after infusion and monthly thereafter. Autoimmune and inflammatory markers were evaluated at baseline and monthly thereafter and included erythrocyte sedimentation rate and complement (C_3, C_4, and CH50). Peripheral blood mononuclear cells were isolated at the same time points as pharmacokinetic samples, and T-cell markers (CD3, CD4, CD8, CD16, CD19, CD25, CD56, HLA-DR, and CD45R0) were evaluated by flow cytometry. Anti-Ipilimumab antibodies (human anti-human antibody/HAHA) were assessed at baseline and monthly thereafter using an ELISA assay. After treatment, patients were followed monthly until disease progression with a physical examination and PSA measurement. Diagnostic imaging was undertaken at baseline, after 1 month of therapy, and as clinically indicated thereafter. Disease response and progression were assessed using the PSA Consensus Criteria (14).

Pharmacokinetics. The maximum concentration (C_{max}) and time to C_{max} (T_{max}) values were the observed values from the raw pharmacokinetic data. The Ipilimumab concentration-time data was analyzed using an open noncompartmental method (WinNonlin model 202). The terminal elimination rate constant (k_e) was determined by noncompartmental analysis using a linear regression of the terminal 4-8 points of the log plasma Ipilimumab concentration versus time plot, using a non-weighted paradigm. The terminal elimination half-life ($t_{1/2}$) was estimated from $0.693/k_e$. The area under the curve (AUC) to the last datum point was estimated using the linear-trapezoidal rule and extrapolated to infinity by adding the Wagner-Nelson correction (C_{last}/k_e). Total body clearance (CL) was calculated by dividing the dose/$AUC_{(0\text{-}\infty)}$. The apparent volume of distribution (Vd_z) was estimated from CL/k_e. The mean residence time (MRT) was estimated from AUMC/AUC. The apparent volume of distribution at steady state (Vd_{ss}) was estimated from the equation $Vd_{ss} = CL \times MRT$.

Toxicity assessment. Adverse events were graded by the Common Toxicity Criteria, version 2.0. In addition, patients were specifically evaluated for signs and symptoms of autoimmune phenomena, including wheezing, adenopathy, joint pain, spenomegaly, diarrhea, and rash. After disease progression, monthly telephone interviews were conducted until 6 months after treatment to collect information on adverse events that could be attributed to autoimmune phenomena.

Statistical considerations. An enrollment of 14 evaluable subjects was planned for this pilot study. The sample size of 14 was chosen to address the theoretical possibility that the treatment might trigger a serious adverse event. If no instances of a serious adverse event were observed in 14 subjects, the possibility that the underlying rate of the serious adverse event was ≥ 20% could be excluded with 95% confidence. If one serious adverse event was observed in the theoretical time frame in which this might occur (6 weeks), no further subjects would be enrolled, and the use of the treatment would be reassessed.

Results

Patient characteristics. Patient characteristics are summarized in Table 1. Median age was 70 years. All 14 patients had

metastases to bone, and 4 patients also had measurable soft tissue metastases. Seven patients (50%) had received prior chemotherapy. Two patients had received prior immunotherapy (allogeneic cellular vaccine in one and dendritic cell vaccine in one), and one patient had received a prior cytolytic adenovirus. Median PSA was 84.6 ng/mL; median alkaline phosphatase was 113.5 units/L; median lactate dehydrogenase was 165 units/L; and median hemoglobin was 12.65 g/dL. Karnofsky performance status was 70% in 3 patients (21%), 80% in 1 patient (7%), 90% in 9 patients (64%), and 100% in 1 patient (2%).

Toxicity. Overall, Ipilimumab was well tolerated. The most frequently reported adverse events included arthralgia (50%), malaise (43%), bone pain (43%), pallor (36%), back pain (36%), constipation (29%), fatigue (29%), and decreased appetite (19%; Table 2). The majority of these events were grades 1 and 2. Grade 3 toxicity consisted of asthenia in 1 patient (7.1%), limb pain in 1 patient (7.1%), and rash in 1 patient (7.1%). Fourteen serious adverse events were reported in six patients, but only two patients had events that were felt to be attributable to treatment: one patient had grade 3 fatigue, and the other had grade 3 rash and pruritis. The patient with grade 3 fatigue had no other clinical evidence of autoimmunity, although testing for hypophysitis (hypothyroidism/hypoadrenalism) was not undertaken. In the patient with rash and pruritis, after treatment on day 1 with Ipilimumab, moderate rash and pruritis were seen on day 12, which worsened by day 23 (grade 2 pruritis and grade 3 rash). The rash and pruritis were unresponsive to treatment with hydroxyzine, diphenhydramine, cimetidine, and ibuprofen. No diagnostic skin biopsy was undertaken. Treatment with prednisone 20 mg orally bid, starting on day 28, resulted in rapid resolution of the rash and pruritis, which did not re-occur after prednisone was discontinued. One patient had an episode of grade 3 diarrhea, which began on day 42 and lasted for 16 days and was attributed to a *Clostridium difficile* infection. No clinically significant changes in clinical laboratory values, physical examination results, electrocardiogram, or chest radiograph were observed in any patient, and no patient discontinued therapy due to toxicity. Two patients died of disease progression during the course of the study, on days 41 and 51 of the study.

Table 1. Patient characteristics

Age (y)	
Median	70.5
Range	56-79
Race	
Caucasian	12 (86%)
Hispanic	2 (14%)
Prostatic acid phosphatase (units/L)	
Median	2.7
Range	1-25
PSA (ng/mL)	
Median	84.6
Range	8-725
Alkaline phosphatase (units/L)	
Median	113.5
Range	53-840
Lactate dehydrogenase (units/L)	
Median	165
Range	113-441
Hemoglobin (g/dL)	
Median	12.65
Range	9.3-14.5
Karnofsky performance status, *n* (%)	
70	3 (21%)
80	1 (7%)
90	9 (64%)
100	1 (7%)
Extent of disease, *n* (%)	
Bone disease	14 (100%)
Bone and soft tissue	4 (28%)
Prior systemic therapy, *n* (%)	
Androgen deprivation	14 (100%)
Chemotherapy	7 (50%)
Immunotherapy	2 (14%)
Other investigational treatment	1 (7%)

Table 2. Adverse events associated with treatment

	Grade 1	Grade 2	Grade 3
Constipation	3 (21.4%)	1 (7.1%)	
Abdominal pain	3 (21.4%)	—	
Diarrhea	2 (14.3%)	1 (7.1%)	
Nausea	2 (14.3%)	—	
Decreased appetite	4 (28.6%)	2 (14.3%)	
Malaise	4 (28.6%)	2 (14.3%)	
Pallor	5 (35.7%)	—	
Worse fatigue	4 (28.6%)	—	
Asthenia	2 (14.3%)	—	1 (7.1%)
Gait abnormality	2 (14.3%)	—	
Rigors	2 (14.3%)	—	
Arthralgia	4 (28.6%)	3 (21.4%)	
Bone pain	5 (35.7%)	1 (7.1%)	
Back pain	3 (21.4%)	2 (14.3%)	
Limb pain	2 (14.3%)	1 (7.1%)	1 (7.1%)
Dizziness	2 (14.3%)	—	1 (7.1%)
Nasopharyngitis	2 (14.3%)	—	
Rash	—	2 (14.3%)	

Two responding patients received a second dose of Ipilimumab per protocol specifications. Single events of irregular heart rate, wheezing, pruritis, and rash (all grade 1) were assessed as possibly related to re-treatment with Ipilimumab. A single grade 3 toxicity (worsening dyspnea) was felt to be unrelated to treatment.

Immunologic effects. There were no significant changes in erythrocyte sedimentation rate, C3, C4, CH50, or ANA with therapy. Other than the two episodes of rash, there were no clinical events consistent with autoimmune events. Flow cytometric analysis of lymphocyte subpopulations indicated no significant change over time, with no significant change in CD3, CD4, CD8, CD4/CD8 ratio, CD19, and CD16+56. There was no depletion of activated lymphocyte subsets. There were no changes outside the 95% confidence interval for T-cell surface activation markers studied, specifically CD25, CD44, CD45RO, CD69, and MHC class II. However, a trend for an increasing percentage of CD4 T cells coexpressing MHC class II increased and remained increased up to day 28. A similar trend was not seen for CD8 T cells (Fig. 1).

Pharmacokinetics. Inspection of the log plasma MDX-CTLA4-01 concentration versus time plots showed that the plasma concentration decay over time was monoexponential in four subjects and biexponential in 10 subjects. The observed T_{max} occurred at, or after, the end of the infusion in all subjects, with a median value of 1.9 h (range, 1.5-71.6 h). A T_{max} of

71.6 h in one subject may be due to an error in sample labeling, handling, or measurement as the C_p at this time point is the C_{max} for this subject; this is most likely an aberrant observation. The observed mean ± SD MDX-CTLA4-01 C_{max} was 155.94 ± 64.5 μg/mL. The mean ± SD MDX-CTLA4-01 terminal elimination half-life was 299.4 ± 126.9 h (12.475 days). The mean ± SD apparent volume of distribution at steady state (Vd_{ss}) was 4.07 ± 1.3 liters, which is relatively low and in keeping with the antibody being distributed mainly within the intravascular space. The Vd_z and Vd_{ss} variable estimates were very similar, with mean values of 4.02 and 4.07 liters, respectively. The mean ± SD total body clearance of MDX-CTLA4-01 was low at 0.01 ± 0.004 L/h and ranged from 0.005 to 0.017 L/h (Table 3). Plasma levels of MDX-010 above 10 μg/mL were maintained for 60 days with a single MDX-010 infusion (Fig. 2). There was considerable intersubject variability in the pharmacokinetic variable estimates (coefficient of variation varies from 30-43%).

Responses to treatment. Two of 14 patients had a decline in PSA of >50%, which lasted 135 and 60 days, respectively (Fig. 3). Neither patient had bidimensionally measurable disease. One of the two patients had developed grade 2 pruritis and grade 3 rash that ultimately required treatment with steroids. This patient's PSA fell from a baseline value of 36 ng/mL on three separate occasions on days 7, 14, and 28 to a value of 22 ng/mL on day 28. Prednisone 20 mg orally bid was initiated on day 28. PSA continued to decrease to a nadir of 15 on day 56, which lasted for 60 days. Both patients chose to be retreated with Ipilimumab upon relapse but did not have a subsequent PSA decline ≥50% upon re-treatment. An additional eight patients had a decline in PSA that was <50%. Four patients had bidimensionally measurable disease at baseline and of these, only two patients had scans repeated. Using the Response Evaluation Criteria in Solid Tumors, neither patient showed an objective response.

Table 3. Mean pharmacokinetic variables

Characteristics	Mean (SD)	% Coefficient of variation
C_{max} (μg/ML)		
T_{max} (h)	155.44 (64.81)	41.7
$t_{1/2}$ (h)	3.24 (1.40)	43.3
$AUC_{0-\infty}$ (h μg/mL)	317 (123)	38.7
V_z (L)	223926 (9237)	38.6
V_{ss} (L)	4.10 (1.14)	34.5
CL (L/h)	4.40 (1.84)	41.9
Mean residence time (h)	0.0095 (0.0033)	34.6
	474 (164)	34.5

Discussion

This report is the first to document the safety and immune effects of an anti-CTLA-4 antibody (Ipilimumab) in patients with prostate cancer. Ipilimumab is a fully human anti-CTLA-4 monoclonal antibody (IgG) isolated from transgenic mice and produced from a hybridoma clone. The putative mechanism of action for Ipilimumab is hindrance of the interaction of CTLA-4 with B7 on antigen-presenting cells, with subsequent blockade of the inhibitory modulation of T-cell activation promoted by the CTLA-4/B7 interaction. Subsequent trials have shown that repetitive dosing of Ipilimumab seems to be fairly well

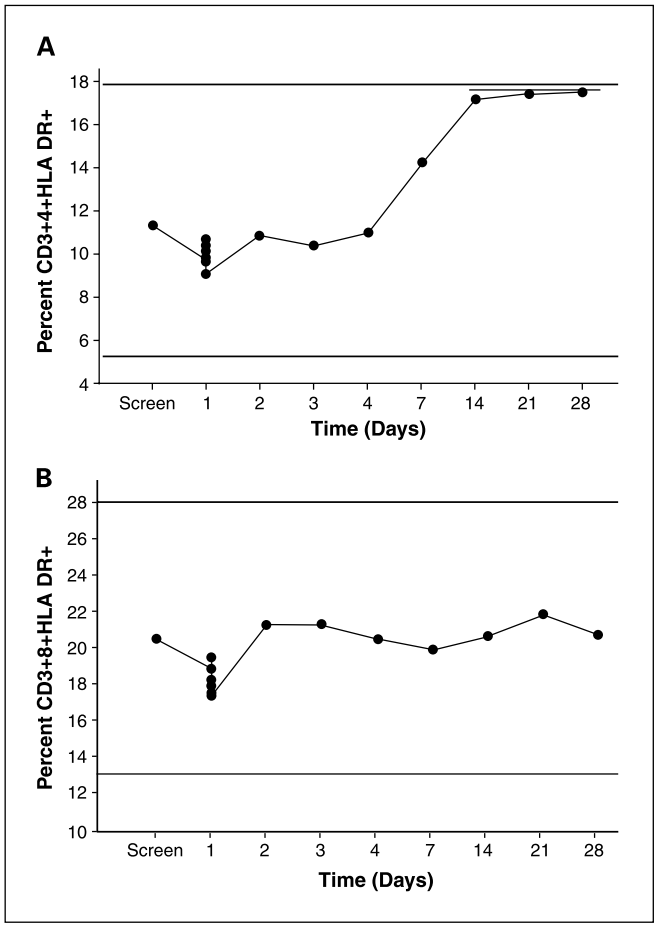

Fig. 1. Changes over time in CD4 Tcells (*A*) and CD8 Tcells (*B*) coexpressing the MHC class II activation marker HLA-DR. Peripheral blood mononuclear cells were isolated from each patient at baseline; at 1, 2, 4, 24, 48, and 72 h after treatment; and at 7, 14, 21, and 28 d after Ipilimumab treatment. Points, mean of the percentage of CD4 (*A*) or CD8 (*B*) Tcells coexpressing HLA-DR. The horizontal lines represent the 95% confidence intervals of the screening value.

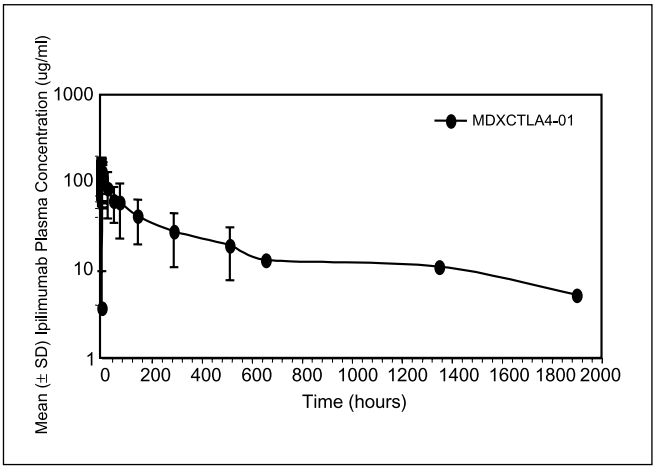

Fig. 2. Pharmacokinetics of Ipilimumab plasma concentration (μg/mL) was measured at baseline; at 1, 2, 24, 48, and 72 h after treatment; and at 7, 14, 21, and 28 d after Ipilimumab treatment. Point, mean Ipilimumab plasma level.

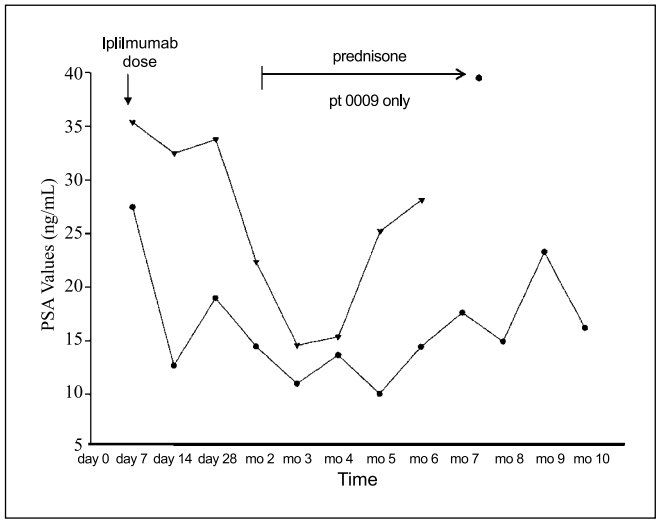

Fig. 3. PSA versus time plots of two patients who had a ≥50% decline in PSA after treatment with Ipilimumab.

tolerated, and that dosing in combination with peptides or autologous vaccination in melanoma and ovarian carcinoma patients has antitumor activity (15–18).

This trial showed that a single 3.0 mg/kg dose of Ipilimumab in patients with progressive, metastatic HRPC is well tolerated. Possible autoimmune adverse events were limited to a single patient with pruritis and rash. Events such as rash, pruritis, enterocolitis, and hypophysitis resulting in panhypopituitarism, which are consistent with an inflammatory or immunologic basis and occur within several weeks of Ipilimumab administration, have been termed immune breakthrough events. In other diseases such as melanoma and renal cell carcinoma, immune breakthrough events have been associated with clinical responses (16–19). In this series, the patient with an immune breakthrough event (rash) went on to develop a PSA decline of >50%, which lasted for 60 days, suggesting that immune breakthrough events may occur in prostate cancer patients as well. The absence of colitis or hypophysitis in this series may simply reflect the small sample size or the consequences of using a single dose of Ipilimumab, although some patients with melanoma and renal cell carcinoma did develop colitis after just one dose of Ipilimumab. One patient had grade 3 diarrhea associated with *C. difficile* colitis. A second patient had grade 3 fatigue that was not attributed to an autoimmune event, although there was not a heightened awareness of these phenomena at the time, and adrenal and thyroid function was not tested. Whether different patterns of immune breakthrough events will occur in different tumor types is unknown. There was no change in lymphocyte subpopulations over time or depletion of activated lymphocyte subsets. The trend towards an increase in CD4 and CD8 cells coexpressing HLA-DR, which was used as a marker of T-cell activation, was the only trend observed over time, did not seem to be clinically significant, but suggests the possibility of T-cell activation and warrants further investigation.

The pharmacokinetic profile of this antibody is consistent with that seen with other clinical antibodies tested in humans. The prolonged terminal half-life (12.5 days) ensures that a single dose of 3 mg/kg MDX-CTLA-4 will result in antibody levels that can be sustained for 60 days at over 10 μg/mL, a concentration thought to be necessary to saturate CTLA-4. This pharmacokinetic profile should permit repetitive dosing at 3- to 4-week intervals. The relatively small sample size precludes comparison with the plasma half-life of Ipilimumab reported elsewhere, or the plasma half-life of another anti-CTLA-4 antibody, CP-675,206.

Although a decline in PSA cannot be considered a marker for clinical benefit, there is nevertheless a consensus that a PSA decline of ≥50% in patients with metastatic HRPC is a reasonable screen for activity (20). Although this study was not designed to evaluate efficacy, 2 of 14 patients had a PSA decline of >50%. Although one of the patients with a PSA decline received prednisone, an agent that can lower PSA in 10% to 20% of patients, this patient's PSA had already declined from 36 to 22 ng/mL before receiving steroids. This patient's PSA continued to decrease while on prednisone to a nadir value of 15 on day 56. The relative contributions of CTLA-4 blockade and steroid treatment to this PSA decline are not known. Recent reports suggest that treatment with CTLA-4 blockade can result in humoral responses to circulating proteins; thus, the induction of anti-PSA antibodies leading to clearing of circulating PSA is another potential explanation for these results and warrants further investigation. Nevertheless, these results were unexpected because preclinical reports suggest that, except in the case of highly immunogenic tumors, CTLA-4 blockade in the absence of specific antigen presentation has been largely ineffective in promoting tumor rejection. The basis for the development of apparent antitumor activity with CTLA-4 blockade monotherapy in this trial is not clear. It is possible that chronic antigen presentation occurs at a low level in HRPC patients, as small populations of prostate cancer cells undergo apoptosis. The therapeutic efficacy of CTLA-4 blockade may therefore be significantly enhanced by simultaneously providing increased levels of antigen presentation. Future research will explore the combination of CTLA-4 blockade with concurrent antigen presentation strategies such as vaccination, treatment with immunomodulatory cytokines, androgen deprivation, or chemotherapy.

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Clinical Cancer Research

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