Published OnlineFirst October 6, 2009; DOI: 10.1158/1078-0432.CCR-09-1339

Phase I Study of Ipilimumab, an Anti–CTLA-4 Monoclonal Antibody, in Patients with Relapsed and Refractory B-Cell Non–Hodgkin Lymphoma

Stephen M. Ansell,[1] Sara A. Hurvitz,[3] Patricia A. Koenig,[1] Betsy R. LaPlant,[1] Brian F. Kabat,[1] Donna Fernando,[3] Thomas M. Habermann,[1] David J. Inwards,[1] Meena Verma,[3] Reiko Yamada,[3] Charles Erlichman,[2] Israel Lowy,[4] and John M. Timmerman[3]

Abstract

Purpose: The growth of non–Hodgkin lymphomas can be influenced by tumor–immune system interactions. Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is a negative regulator of T-cell activation that serves to dampen antitumor immune responses. Blocking anti–CTLA-4 monoclonal antibodies improves host resistance to immunogenic tumors, and the anti–CTLA-4 antibody ipilimumab (MDX-010) has clinical activity against melanoma, prostate, and ovarian cancers.

Experimental Design: We did a phase I trial of ipilimumab in patients with relapsed/refractory B-cell lymphoma to evaluate safety, immunologic activity, and potential clinical efficacy. Treatment consisted of ipilimumab at 3 mg/kg and then monthly at 1 mg/kg × 3 months (dose level 1), with subsequent escalation to 3 mg/kg monthly × 4 months (dose level 2).

Results: Eighteen patients were treated, 12 at the lower dose level and 6 at the higher dose level. Ipilimumab was generally well tolerated, with common adverse events attributed to it, including diarrhea, headache, abdominal pain, anorexia, fatigue, neutropenia, and thrombocytopenia. Two patients had clinical responses; one patient with diffuse large B-cell lymphoma had an ongoing complete response (>31 months), and one with follicular lymphoma had a partial response lasting 19 months. In 5 of 16 cases tested (31%), T-cell proliferation to recall antigens was significantly increased (>2-fold) after ipilimumab therapy.

Conclusions: Blockade of CTLA-4 signaling with the use of ipilimumab is well tolerated at the doses used and has antitumor activity in patients with B-cell lymphoma. Further evaluation of ipilimumab alone or in combination with other agents in B-cell lymphoma patients is therefore warranted. (Clin Cancer Res 2009;15(20):6446–53)

B-cell non-Hodgkin lymphomas are malignancies in which cells other than tumor cells are typically present in the tumor microenvironment (1, 2). These cells include T lymphocytes that may be tumor antigen–specific but are unable to eradicate the malignant B cells, in part because of insufficient activation inhibited by infiltrating regulatory T cells or intrinsic negative signaling receptors. We postulated that promoting the activation of these infiltrating T cells might allow them to inhibit the malignant B cells, resulting in clinical benefit for patients with B-cell non–Hodgkin lymphoma.

Activation of T lymphocytes is thought to require at least two signals, one delivered by the T-cell receptor complex after antigen recognition and one provided on engagement of costimulatory receptors, such as CD28 (3). Opposing inhibitory signals, such as those delivered by cytotoxic T-lymphocyte antigen 4 (CTLA-4), modulate the immune response and increase the threshold for T-cell activation (4–6). CTLA-4 signaling has been implicated in tolerance induction *in vivo* and may also augment suppressor CD4+ T-cell activity, thereby downregulating the immune response (7–10). Blockade of CTLA-4 by administration of anti–CTLA-4 monoclonal antibodies (mAb) has been shown to enhance T-cell responses in a variety of settings and to enhance antitumor responses (11–16).

Ipilimumab is a fully human IgG1κ mAb specific for human CTLA-4 (formerly MDX-010; Medarex, Inc.) that has been

Authors' Affiliations: [1]Division of Hematology and [2]Department of Oncology, Mayo Clinic, Rochester, Minnesota; [3]Division of Hematology and Oncology, University of California, Los Angeles, California; and [4]Clinical Science, Medarex, Inc., Bloomsbury, New Jersey

Received 5/26/09; revised 7/22/09; accepted 8/3/09; published OnlineFirst 10/6/09.
Grant support: NIH grants R21 CA108182 and U01 CA69912 and Gabrielle's Angel Foundation for Cancer Research.
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.
Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).
Presented in part at the 48th Annual Meeting of the American Society of Hematology, December 9-12, 2006, Orlando, Florida.

Requests for reprints: Stephen M. Ansell, Division of Hematology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905. Phone: 507-284-0923; Fax: 507-266-4972; E-mail: ansell.stephen@mayo.edu.

doi:10.1158/1078-0432.CCR-09-1339

Published OnlineFirst October 6, 2009; DOI: 10.1158/1078-0432.CCR-09-1339

Translational Relevance

Non–Hodgkin B-cell lymphomas can be susceptible to host immune-mediated growth inhibition, but negative signals from tumor cells usually prevent the mounting of an effective antitumor immune response in the host. The regulatory molecule CTLA-4, expressed by host T cells, contributes to tumor tolerance but can be blocked by the anti–CTLA-4 monoclonal antibody ipilimumab. Our demonstration that ipilimumab can be safely given to lymphoma patients, and results in clinical responses and memory T-cell activation suggests that it may have a use in combination with other lymphoma immunotherapeutic agents, such as anti-CD20 antibodies.

developed for immunotherapy in humans. This agent has been evaluated in previous phase I/II clinical trials in patients with metastatic hormone-refractory prostate cancer, ovarian cancer, and advanced melanoma to determine the safety/tolerability, pharmacokinetics, immune effects, and clinical efficacy of the antibody (17–22). These trials not only show that administration of ipilimumab is safe but also provide evidence of its antitumor effects as a single agent. We therefore conducted a phase I clinical trial of ipilimumab in patients with relapsed or refractory B-cell non–Hodgkin lymphoma, primarily to determine the safety and potential efficacy of ipilimumab, and secondarily to determine whether treatment with ipilimumab boosts the activity of memory T cells to recall antigens.

Patients and Methods

Patient eligibility. Eligible patients had relapsed or refractory B-cell non–Hodgkin lymphoma (WHO classification). The study was initially limited to patients with relapsed or refractory follicular lymphoma, but was later expanded to include all relapsed or refractory B-cell lymphomas with the exception of small lymphocytic lymphoma. Patients were required to have received at least one but not >3 prior chemotherapy regimens; antibody and vaccine therapies were not counted as chemotherapy regimens. All patients had measurable disease, an Eastern Cooperative Oncology Group performance status of 0 or 1, and life expectancy >24 wk. All patients had adequate hepatic, renal, and bone marrow function. Patients were excluded if they had previous treatment with ipilimumab, or with fludarabine or 2-chlorodeoxyadenosine within 12 mo of enrolment due to the immunosuppressive effect of this class of chemotherapy. Pregnant women, and patients with immunodeficiency, uncontrolled infection, cardiac disease, or central nervous system lymphoma were excluded. The use of concurrent antilymphoma therapy, immunosuppressive drugs, or corticosteroids was prohibited. Patients with active or recent clinically significant autoimmune disease were excluded due to the potential of ipilimumab to exacerbate the symptoms of these diseases. All patients were required to give informed consent, the Institutional Review Boards of the participating institutions approved the study, and the study was registered at ClinicalTrials.gov (Identifier: NCT00089076).

Study design and dose escalation. In this phase I dose escalation study done at the Mayo Clinic and the University of California Los Angeles, subjects received four monthly doses of ipilimumab i.v. Ipilimumab was provided by Medarex, Inc. through the Cancer Therapy Evaluation Program of the National Cancer Institute. Two dose levels of ipilimumab were planned. Patients treated at the lowest dose level would receive 3 mg/kg, followed by 1 mg/kg monthly for three further doses. If no significant dose-limiting toxicity were seen at this dose level, the dose would then be escalated to 3 mg/kg monthly for four doses. Enrolment was initially stratified into two cohorts, with half of the patients required to be previously treated with a lymphoma vaccine (idiotype or other), as it was hypothesized that previously vaccinated subjects were more likely to have antilymphoma T cells amenable to activation by an anti–CTLA-4 antibody. No differences were seen between patients treated at the first dose level who had had prior vaccine exposure and patients who had not, and so this requirement was removed for patients treated at the second dose level.

Dose escalation to the second dose level could occur if all six patients in each cohort finished at least the first three treatments with no more than one severe toxicity, and no more than two of the six patients with dose-limiting toxicity required discontinuation of the study treatment. If >1 patient had evidence of severe toxicity, or there were ≥3 patients out of a cohort of 6 patients who experienced dose-limiting toxicity requiring discontinuation of study treatment, the dose would not be escalated. Given the association of response with evidence of immune reactivity, a 50% incidence of autoimmune events without the incidence of severe unexpected toxicity was deemed acceptable. The goal of this study was to identify a biologically active dose of ipilimumab with an acceptable toxicity profile that could be used in combination with other agents in future studies. Although higher doses of ipilimumab have been given in other studies, doses of ipilimumab >3 mg/kg were not explored in this study due to the expectation that these higher dose levels would result in significantly increased toxicity and would limit the potential for combining ipilimumab with other agents.

Toxicity and response evaluation. Severe toxicity was considered to have occurred for any grade 4 toxicity; any long-lasting (>1 mo) grade ≥3 impairment of vital organ function; any grade 3 toxicity requiring prolonged steroid treatment or unresponsive to steroid treatment; or if additional interventions, such as additional immune suppressive treatment or surgery, were required. Dose-limiting toxicity was defined with the use of the Common Terminology Criteria for Adverse Events version 3 as any of the following as assessed by the investigator: any grade ≥3 adverse event suspected to be related to the study agent, any grade ≥2 allergic or autoimmune event that involved vital organ functions, or any other grade 3 allergic or autoimmune events that did not resolve to grade 1 toxicity before the next scheduled dose of antibody. Treatment responses or stable disease after participation in the study, as well as disease progression were determined based on the international workshop to standardize response criteria for non–Hodgkin lymphoma (23).

Flow cytometric analysis of peripheral blood T cells. Peripheral blood mononuclear cells were isolated from heparinized blood by Ficoll-Hypaque (GE Healthcare, Piscataway, NJ) density gradient separation and stored cryogenically in liquid nitrogen in human AB serum plus 10% DMSO until use. Cell surface markers were analyzed with the use of the following antibodies, all purchased from BD Biosciences (San Jose, CA): CD3 conjugated with FITC (clone HIT3a), CD4 allophycocyanin (clone RPA-T4), CD8 (phycoerythrin; clone RPA-T8), CD16 (phycoerythrin; clone 3G8), CD56 (allophycocyanin; clone B159), HLA-DR phycoerythrin (clone L243), CD25 phycoerythrin (clone MA251), and CD45RO phycoerythrin (clone UCHL1). Labeled isotype-matched antibodies were run in parallel as controls. Cryopreserved pretreatment and post-treatment peripheral blood mononuclear cells were tested in the same assay to eliminate interassay variability. Peripheral blood mononuclear cells were thawed quickly in a 37°C water bath, washed twice with warm complete RPMI, and counted with a hemacytometer. Cells (5×10^5) in 100 μL fluorescence-activated cell sorting buffer (1% bovine serum albumin, 0.1% sodium azide in 1× PBS) were stained for 25 min with the recommended concentrations of antibodies at 4°C, protected from light. Cells were then washed twice with fluorescence-activated cell sorting buffer; fluorescence was detected by a BD FACScan flow cytometer (BD Biosciences) and the data analyzed with the FCS Express software (De Novo Software, Los Angeles, CA).

Published OnlineFirst October 6, 2009; DOI: 10.1158/1078-0432.CCR-09-1339

Table 1. Patient characteristics

	n = 18
Gender	
Male	12
Female	6
Performance status	
0	11
1	7
Age (y), median (range)	56 (37-79)
Disease histology	
Follicular grade 1 lymphoma	9
Follicular grade 2 lymphoma	5
Diffuse large B-cell lymphoma	3
Mantle cell lymphoma	1
Stage of disease-	
I/II	2
III/IV	16
No. of prior treatments, median (range)	2 (1-4)
Prior therapy	
Idiotype vaccine	6
Rituximab	7
Radioimmunotherapy	4
Chemotherapy	15
Dose level	
3 mg/kg first dose, then 1 mg/kg monthly × 3 doses	12
3 mg/kg monthly × 4 doses	6

T-cell proliferation was measured at baseline, and at months 1 and 4 by incubation of peripheral blood mononuclear cells with keyhole limpet hemocyanin (KLH), tetanus toxoid, or media alone at various concentrations, and ^{3}H-thymidine pulsing on day 4. As previously described (24), cryopreserved pretreatment and post-treatment peripheral blood mononuclear cells (10^5 cells per well) were seeded in quadruplicate in 96-well U-bottom plates (Nunc, Rochester, NY) in complete RPMI. Graded concentrations of KLH (Pierce, Rockford, IL) at 0, 10, or 100 μg/mL, or tetanus toxoid (Sanofi Pasteur, Swiftwater, PA) at 2 or 10 μg/mL were added to the wells for a final volume of 200 μL and incubated at 37°C in a 5% CO_2 humidified incubator for 4 d. Cells were then pulsed with 1 μCi/well ^{3}H-thymidine (MP Biomedicals, Solon, OH) and harvested 16 h later. Incorporated radioactivity (counts per minute) was measured with the use of a β-liquid scintillation analyzer (Perkin-Elmer, Waltham, MA). The arithmetic mean result from quadruplicate cultures at each condition was divided by that of the media-alone control to give a stimulation index.

Statistical methods. The primary objectives of this phase I study were to characterize the safety and tolerability of ipilimumab, and to determine the optimal biological dose and dose-limiting toxicity of ipilimumab when given on a monthly dosing schedule. The safety variables included adverse events, vital sign measurements, clinical laboratory tests, physical examinations, and diagnostic tests (including chest X-rays and computed tomography scans). Hematologic toxicity measures of thrombocytopenia, neutropenia, and leukopenia were assessed with the use of the continuous variables as outcome measures (primarily nadir and percent change from baseline values) as well as categorization through the Common Terminology Criteria version 3 standard for toxicity grading. Nonhematologic toxicities were evaluated only through the ordinal Common Terminology Criteria version 3 standard for toxicity grading. Frequency distributions and other descriptive measures formed the basis of the analysis of these variables. The secondary objective was to characterize the immunologic effects of ipilimumab. The immunologic results were summarized with the use of descriptive statistics. Clinical responses were summarized by simple descriptive summary statistics delineating complete and partial responses, as well as stable and progressive disease.

Results

Patient characteristics. Between August 2004 and September 2007, 18 patients with relapsed or refractory B-cell lymphoma were enrolled in this study. Fourteen patients had follicular lymphoma, three had diffuse large B-cell lymphoma, and one had mantle-cell lymphoma. Eleven patients (61%) had Eastern Cooperative Oncology Group performance status 0; the remaining seven patients had Eastern Cooperative Oncology Group performance status 1. All patients were pretreated (median number of previous treatments was 2; range, 1-4), and six patients had previously received a tumor-specific idiotype-KLH vaccine plus granulocyte macrophage colony-stimulating factor. One patient received KLH alone plus granulocyte macrophage colony-stimulating factor in the control arm of a randomized idiotype vaccine trial (25). Twelve patients received ipilimumab at a dose of 3 mg/kg for the first dose and then monthly at 1 mg/kg × 3, with subsequent escalation to 3 mg/kg monthly × 4 months for the next six patients. Patient characteristics are shown in Table 1.

Dose escalation and adverse events. Twelve patients were treated at the first dose level of the ipilimumab therapy; six patients had received prior lymphoma vaccine therapy, and six patients had not. Two dose-limiting toxicities were seen at the first dose level in the previous vaccine cohort. Both were grade 3 diarrhea and were attributed to therapy. One dose-limiting toxicity of a related grade 3 diarrhea was seen at the first dose level in the cohort of patients who had not received a previous lymphoma vaccine. No severe toxicities were seen in either cohort at the first dose level. The study was reviewed before escalating to the second dose level. Accrual to the study had been significantly limited by the requirement of a cohort that had previously received a lymphoma vaccine. Because there was no obvious difference in response in the vaccinated group, the requirement of two separate cohorts was eliminated for the second dose level. Six patients were treated at the second dose level, and

Table 2. Adverse events possibly related to ipilimumab

Event	Grade 1	Grade 2	Grade 3	*n* =18 (%)
Anemia	3	1	0	4 (22)
Neutropenia	3	0	0	3 (17)
Thrombocytopenia	4	1	0	5 (28)
Increased AST	4	0	0	4 (22)
Edema: limb	1	0	0	1 (6)
Hyperglycemia	2	0	0	2 (11)
Hyperkalemia	2	0	0	2 (11)
Abdominal pain	3	2	0	5 (28)
Headache	4	0	0	4 (22)
Myalgia	1	2	0	3 (17)
Dyspnea	1	0	0	1 (6)
Fatigue	7	2	1	10 (56)
Weight loss	0	3	0	3 (17)
Rash	3	0	0	3 (17)
Pruritis	2	0	0	2 (11)
Anorexia	2	2	0	4 (22)
Diarrhea	5	0	5	10 (56)
Nausea	3	0	0	3 (17)
Vomiting	2	0	0	2 (11)

Abbreviation: AST, aspartate aminotransferase.

Published OnlineFirst October 6, 2009; DOI: 10.1158/1078-0432.CCR-09-1339

Table 3. Grade 3 adverse events regardless of attribution

Event	Prior vaccine	No prior vaccine	*n* = 18 (%)
Fatigue	1	0	1 (6)
Glucose intolerance	1	0	1 (6)
Diarrhea	3	2	5 (28)
Neutropenia	0	1	1 (6)

no dose-limiting or severe toxicities were seen in these patients. Because the goal of the study was to define a dose of ipilimumab that was biologically active but sufficiently well tolerated so that it could potentially be combined with other agents, the dose of ipilimumab was not increased further.

Ten of the 18 patients discontinued treatment early, and the median number of doses received was 3 (range, 1-4). Of the 10 patients who did not receive all four planned doses, 7 discontinued due to disease progression, 2 refused further therapy, and 1 discontinued due to progression of a newly diagnosed chondrosarcoma that required therapy. The most common adverse events attributable to therapy seen in the study at both dose levels were fatigue, diarrhea, abdominal pain, and thrombocytopenia. The most common adverse events possibly related to ipilimumab are summarized in Table 2. There were no grade 4/5 adverse events seen, and the grade 3 adverse events, regardless of attribution, are listed in Table 3. One patient treated at the lowest dose level had a history of type 2 diabetes mellitus, and after three doses of ipilimumab, developed grade 3 hyperglycemia in association with upper respiratory tract infection, anemia, anorexia, weight loss, and erectile dysfunction. Endocrine evaluation revealed low serum testosterone, low-normal gonadotropin levels, and a normal magnetic resonance imaging scan of the pituitary. The findings were felt to be possibly consistent with an early, mild case of hypophysitis.

Memory T-cell responses. Systemic CTLA-4 blockade with ipilimumab can result in polyclonal T-cell activation and relative expansion of T cells bearing the memory T-cell–associated marker CD45RO (17). We measured the expression of activation markers in T-cell subsets in the peripheral blood during therapy with ipilimumab (Table 4). In contrast to earlier trials with the use of more intensive dosing of ipilimumab (17), we did not observe consistent increases in T cells expressing the activation marker HLA-DR. Enhanced T-cell expression of HLA-DR

Table 4. Flow cytometric analysis of T-cell surface markers before and 1 mo after initiation therapy

Patient	HLA-DR+		CD45RO+	
	Pre	Post	Pre	Post
	% of CD3+CD4+		% of CD3+CD4+	
3	5.7	21.8	64.2	74.2
4	10.9	11.6	NA	NA
5	3.9	7	59.7	69.5
6	19.2	20.4	66	81.2
7	6.5	8.9	88.7	89.5
9	4.9	7.2	85.1	88.6
10	27.9	27.1	67.3	67.7
12	3.1	4.4	97	97
13	8	5.3	91.9	94.2
14	16.1	13.7	66.2	74.6
15	6.4	6.7	1.1	2.2
16	25.3	24.5	88.7	88
17	4.7	8.3	54.6	66.2
18	8.3	9.8	82.4	85.4
Mean Δ ± SE	+1.8 ± 1.2 (P = 0.15)		+5.0 ± 1.5 (P = 0.005)	
	% of CD3+CD4-		% of CD3+CD4-	
3	5.3	10.6	13.7	21.1
4	5.9	9.4	NA	NA
5	2	4.4	15	18.6
6	15.5	9.2	9.1	17.6
7	6.9	7	28	26.6
9	4.4	8	22.6	29.6
10	25.7	36.5	25.1	28
12	1.8	3.6	24.2	24.9
13	9.2	30.6	33.7	35.6
14	20.3	15.7	16.3	18.6
15	7.4	7.1	2	2.3
16	23.3	15.1	5.3	6.9
17	2.6	2.5	5.8	8.3
18	26.3	23.4	13.1	12.7
Mean Δ ± SE	+1.9 ± 2.0 (P = 0.36)		+2.8 ± 0.9 (P = 0.006)	

NOTE: Values compared with the use of paired two-tailed t test.
Abbreviation: NA, not available.

Published OnlineFirst October 6, 2009; DOI: 10.1158/1078-0432.CCR-09-1339

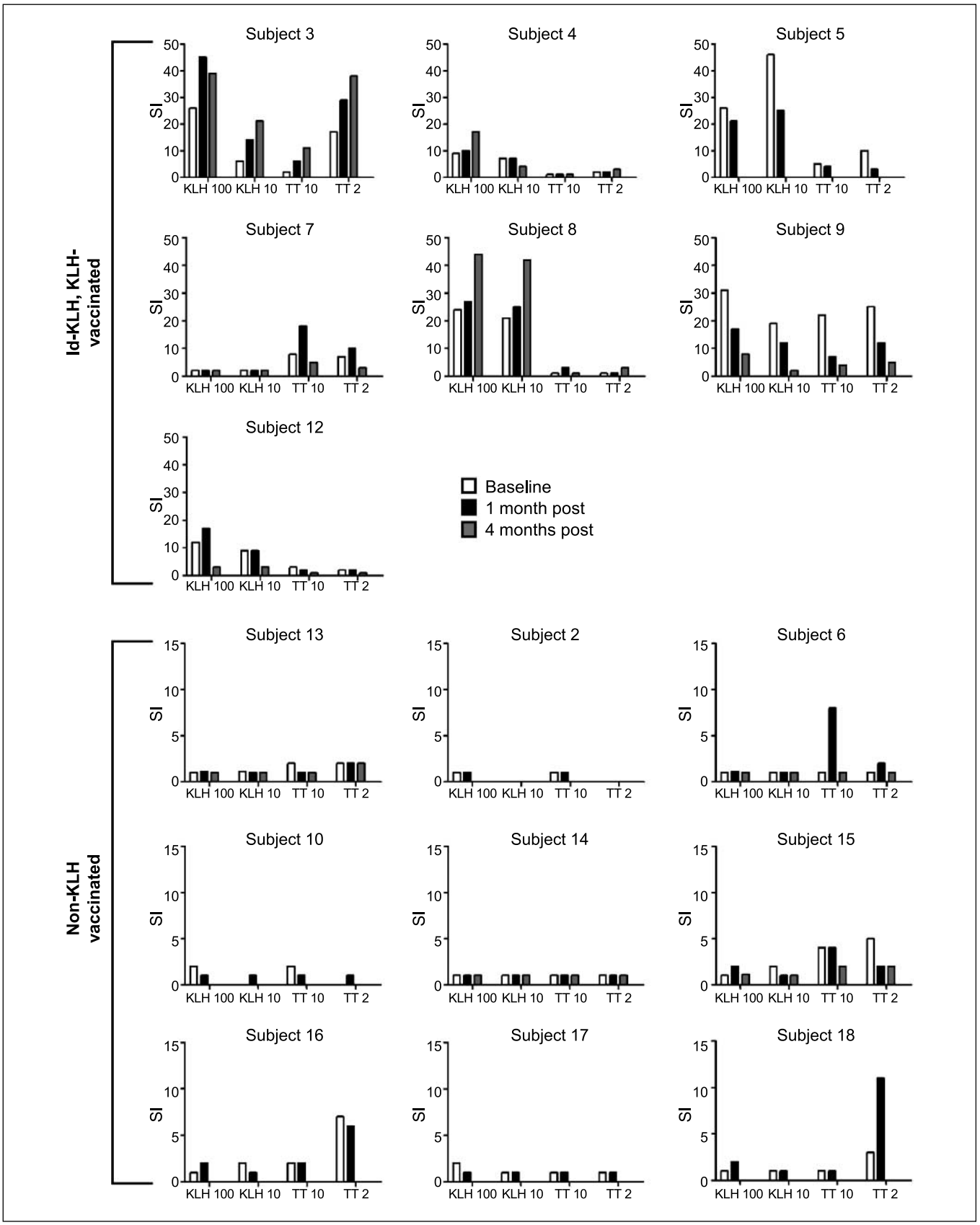

Fig. 1. Individual patient T-cell proliferation to recall antigens, KLH, or tetanus toxoid during treatment. Samples were collected at baseline, 1 mo, and 4 mo after initiation of treatment. Data are represented as stimulation index. Subjects are divided into those with prior idiotype-KLH or KLH vaccination (*top*) and those without (*bottom*). TT, tetanus toxoid.

Published OnlineFirst October 6, 2009; DOI: 10.1158/1078-0432.CCR-09-1339

during therapy was seen in only one patient (subject 3), who also experienced partial tumor regression (see below). When all patients were considered, there was a modest increase in the proportion of $CD4^+$ and $CD4^-$ T cells expressing CD45RO ($P = 0.005$ and $P = 0.006$, respectively), but there were no consistent changes in the total number of CD4+ or CD8+ T cells, or CD16+CD56+ natural killer cells in the peripheral blood between baseline and 1 month after therapy. Interestingly, however, patient 13, who had a complete response to therapy, did have a 10.9% absolute increase in CD3-CD16+CD56+ natural killer cells, as well as a 17.6% increase in CD8+ T cells and a corresponding 17.3% decrease in CD4+ T cells. Other serologic markers of immunity, including antinuclear antibody and rheumatoid factor titers, were measured monthly while patients were on treatment and did not change.

The antigenic specificity of potentially tumor-reactive T cells in B-cell lymphoma patients is unknown. To evaluate the potential capacity of ipilimumab to potentiate memory T-cell responses, we measured the activation of T cells toward recall antigens before and after therapy. T-cell proliferative responses to KLH (in idiotype-KLH and KLH-vaccinated cases) were measured pre-ipilimumab and post-ipilimumab, and are shown in Fig. 1. In 5 of 16 cases tested (31%), including the follicular lymphoma patient who had a clinical response, T-cell proliferation to KLH and/or tetanus was significantly increased (>2-fold) at 1 or 4 months after initiation of ipilimumab, implying expansion and/or activation of the memory T-cell pool. However, these increases were rarely sustained during therapy (subjects 3 and 8 only). Furthermore, recall antigen responses decreased during therapy in several instances, often in association with anorexia and weight loss (subjects 5, 9, and 12), suggesting that nutrition and other constitutional factors may also affect responsiveness.

Clinical responses. A 44-year-old female (patient 3) with follicular grade 1 non–Hodgkin lymphoma stage IVA treated at dose level 1 had partial response to therapy. The patient had intra-abdominal lymphadenopathy and bone marrow involvement, and had previously received two chemotherapy regimens and treatment with anti-idiotype vaccine. The patient's partial response was sustained for 19 months (Fig. 2A). Interestingly, this patient was the only subject showing significant activation of peripheral blood T cells (Table 4) and enhanced reactivity to recall antigens (Fig. 1) during therapy. The toxicities experienced by this patient included transient grade 3 diarrhea, grade 1 fatigue, and grade 2 pleuritic chest wall pain, all of which resolved spontaneously. Additionally, a 79-year-old male (patient 13) with diffuse large B-cell lymphoma stage IVA from the dose level 2 group had complete response. The patient had previously failed three chemotherapy regimens, and had intra-abdominal lymphadenopathy and liver lesions.

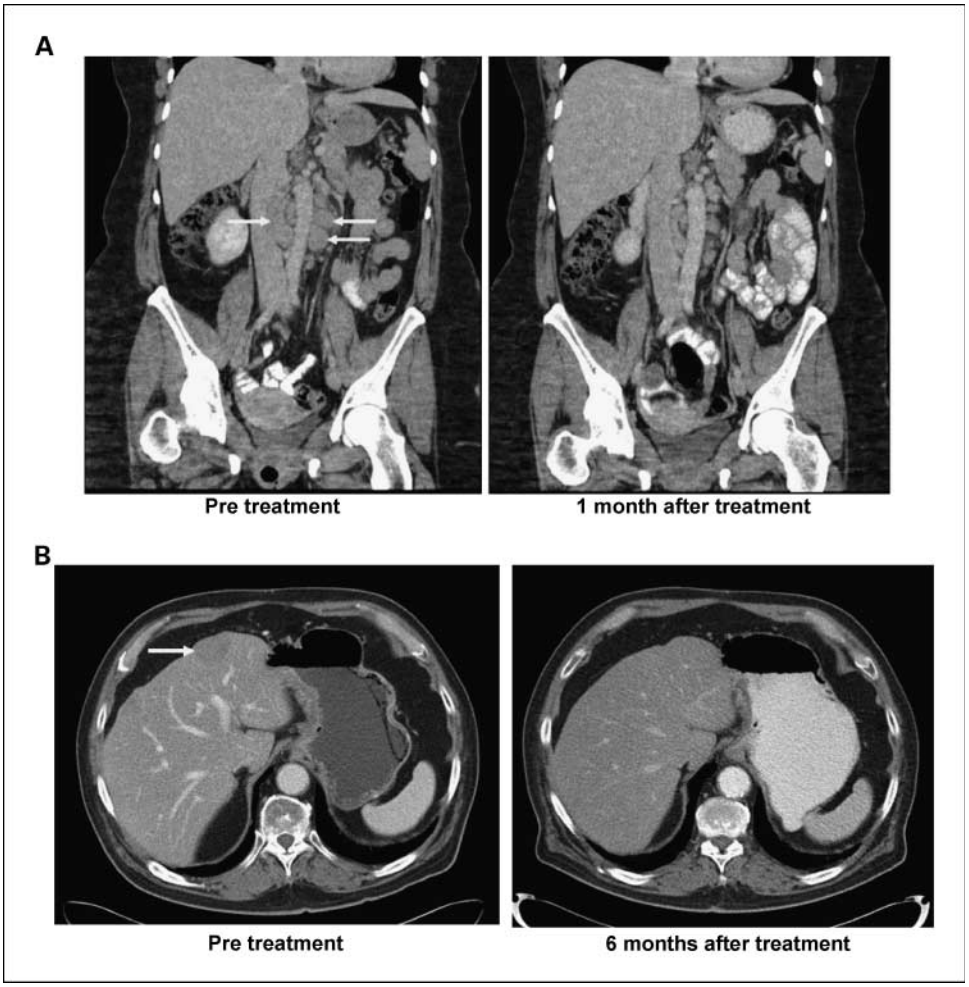

Fig. 2. Clinical responses to ipilimumab. *A,* partial response in periaortic lymph nodes in a patient with follicular lymphoma (subject 3) 1 mo after completion of treatment. *B,* complete regression of a liver lesion in a patient with diffuse large cell lymphoma (subject 13) 6 mo after completion of treatment.

Published OnlineFirst October 6, 2009; DOI: 10.1158/1078-0432.CCR-09-1339

The patient remains progression-free at >31 months (Fig. 2B). This patient had no significant side effects from treatment. Supplementary Table S1 displays the relationship between clinical responses and *in vitro* T-cell response data.

Discussion

Insights into the molecular machinery governing T-cell activation suggest opportunities for cancer immunotherapy. The optimal activation of naïve T cells requires not only ligation of the T-cell antigen receptor by peptide/MHC complexes but also confirmatory "costimulatory" signals mediated by engagement of CD28 on T cells by B7 molecules expressed on the surface of antigen-presenting cells (3). These CD28-B7 interactions are critical to the induction of T-cell proliferation, cytokine secretion, and other effector functions. A counter-regulatory circuit also exists to dampen T-cell activation. CTLA-4 (CD152) is a molecule that is upregulated on the T-cell surface after activation, and binds to CD80 and CD86 with higher avidity than CD28. In doing so, CTLA-4 delivers a negative regulatory signal to T cells. CD28 and CTLA-4 have opposite effects in the fine-tuning of immune responses, with CD28 decreasing and CTLA-4 increasing the threshold for T-cell activation (4, 5). CTLA-4 has also been implicated in tolerance induction *in vivo* and has been shown to regulate suppressive $CD4^+CD25^+$ T cells, thereby downregulating the immune response (8–10). The function of CTLA-4 as "brakes" for T-cell activation has led to its targeting as a means to augment protective host immunity. Administration of blocking anti-CTLA4 mAbs has been shown to enhance antitumor immunity and promote tumor eradication in a variety of mouse tumor model systems, including prostate, breast, melanoma, and lymphomas (11–16).

mAbs specific for human CTLA-4, such as ipilimumab and tremelimumab, have been developed for immunotherapy in humans and represent the first among a growing list of immunomodulatory antibodies for the treatment of cancer (26). Ipilimumab has been evaluated in phase I/II clinical trials in patients with metastatic hormone-refractory prostate cancer, metastatic melanoma, or other advanced malignancies, as well as in patients treated with allogeneic stem cell transplantation (17–22, 27). Multiple doses of up to 10 mg/kg have been given. Ipilimumab was well tolerated, and no evidence of generalized T-cell activation was observed. The most significant adverse events were asthenia, rash, myalgias, arthritis, anorexia, pneumonitis, diarrhea, and hyperthyroidism. The clinical activity of ipilimumab was observed predominantly in melanoma patients and in lymphoma patients post-transplant (20, 27). Similar adverse events were seen with tremelimumab, with a 10% overall response rate in patients with metastatic melanoma (28). In a recently completed large (n = 210), randomized, double-blind dose-ranging study (22), the safety and activity of 0.3, 3.0, and 10.0 mg/kg of ipilimumab were compared in previously treated patients with melanoma. Four doses were given every 3 weeks as induction, followed by a maintenance dose given every 12 weeks beginning at week 24. The overall incidence of adverse events was almost identical between 3 mg and 10 mg. The 10 mg/kg dose did have more serious or severe (grade ≥3) adverse events (24% versus 8%) than the 3 mg/kg dose, but the implementation of detailed treatment algorithms (typically based on a course of high-dose steroids with a month-long taper) resulted in timely resolution of the higher-grade adverse events and maintenance of clinical responses when these occurred. There was a clear 3-fold greater efficacy associated with 10 mg/kg compared with the 3 mg/kg dose. In a pharmacokinetic analysis of the correlation between antibody levels and the expected saturation of available CTLA-4 receptors (29) at a dose of 3 mg/kg, only one third of the patients have enough drug left at trough to prevent the reestablishment of CTLA-4–medicated tolerance, but at 10 mg/kg, 95% of the patients remain above the threshold. These data indicate that the CTLA-4 blockade dose intensity is critical to achieving optimal antitumor effects.

B-cell lymphomas, particularly those of the common follicular subtype, are felt to be the most "immune responsive" of all human cancers (25). This is supported by their capacity to undergo spontaneous regression (30, 31), their occasional responsiveness to nonspecific immune activators, such as bacillus Calmette-Guerin and interleukin 2, and a high rate of response to B-cell–specific mAbs and tumor-specific vaccines (24, 25, 32–35). Thus, tumor–host immune system interactions have the potential to profoundly influence lymphoma growth, and B-cell lymphomas offer an attractive testing ground for immunologic interventions aimed at potentiating antitumor immunity. As such, B-cell lymphomas may be uniquely susceptible to the immunologic effects of CTLA-4 blockade. We have previously shown that an increase in the percentage of $CD4^+$ cells in the biopsies of patients with B-cell lymphomas significantly correlates with favorable patient outcome (1). The tumor-infiltrating T cells displayed a surface immunophenotype of activated memory T cells ($CD3^+$, $HLA\text{-}DR^+$, $CD45RO^+$, and $CD62L^{low}$). Those patients with increased numbers of $CD4^+$ cells in their pretreatment biopsy specimens had a significantly longer duration of complete response as well as a significantly better 5-year overall survival. These data suggest that increased numbers of activated T cells infiltrating B-cell lymphomas are indicative of a more effective immune response to the malignancy and contribute to improved survival. Despite this recruitment of potential T-cell effectors, however, tumor tolerogenic mechanisms may limit antitumor immunity in most lymphoma-bearing hosts (36). In biopsy specimens from patients with follicular lymphoma, we have recently found that ~10% to 15% (range, 8%-33%) of infiltrating T cells express CTLA-4 and have a regulatory T-cell phenotype (2). We hypothesized that ipilimumab might reverse the hyporesponsiveness of these and other potentially tumor-reactive T cells, thereby favoring the development of a clinically significant host antitumor response.

In this study, ipilimumab displayed clinical activity, with 2 of 18 (11%) patients showing a measurable response, which in both cases were durable. The responses did not seem to be dose dependent because a response was seen at both dose levels, although the more durable and complete response occurred at the higher dose. The overall response rate seems similar to that seen in phase II clinical trials of anti–CTLA-4 antibodies in metastatic melanoma (20, 28). The evidence for the clinical activity of ipilimumab in non–Hodgkin lymphoma is further supported by a study of CTLA-4 blockade in patients with various cancers progressing after therapeutic tumor antigen vaccination (19). In this study, among the four patients with B-cell lymphoma previously treated with idiotype-KLH vaccines, two experienced tumor regression, including one with a partial response of 14-month duration. Given the evolving information

Published OnlineFirst October 6, 2009; DOI: 10.1158/1078-0432.CCR-09-1339

about optimal active doses of ipilimumab, however, it is possible that enhanced activity may be seen at the higher dose of 10 mg/kg. The most common adverse events seen in the study were fatigue, diarrhea, and abdominal pain; and this side effect profile also seems similar to that seen in other studies. Because the frequency and activity of tumor-reactive T cells may be greater in lymphoma patients who have received therapeutic lymphoma vaccines (37), we theorized that vaccinated patients may have a greater pool of potentially reactive T cells susceptible to activation by CTLA-4 blockade and, therefore, a higher response rate. In the small cohort of previously vaccinated patients in the current trial, there was no obvious difference in response rate.

In this study, we have found that blockade of CTLA-4 signaling with the use of ipilimumab is well tolerated at the doses used. We have also found that ipilimumab has antitumor activity in patients with B-cell lymphoma, resulting in durable responses in a minority of patients. However, consistent activation of memory T cells to recall antigens was observed infrequently, albeit strongly in one patient experiencing tumor regression. Although we have shown that ipilimumab can have efficacy against B-cell lymphomas as a single agent, a likely ultimate strategy will be to give this agent in combination with other available lymphoma immunotherapies, such as anti-CD20 mAbs. This phase I trial has shown that ipilimumab at 3 mg/kg monthly for 4 months can be given safely and is a dose that can be used for future combination studies. Dosing at 10 mg/kg remains to be explored. Further evaluation of the efficacy of ipilimumab alone or in combination with other agents in B-cell lymphoma patients is warranted.

Disclosure of Potential Conflicts of Interest

I. Lowy is employed by Medarex.

References

1. Ansell SM, Stenson M, Habermann TM, Jelinek DF, Witzig TE. CD4+ T-cell immune response to large B-cell non-Hodgkin's lymphoma predicts patient outcome. J Clin Oncol 2001;19:720–6.
2. Yang ZZ, Novak AJ, Stenson MJ, Witzig TE, Ansell SM. Intratumoral CD4+CD25+ regulatory T-cell-mediated suppression of infiltrating CD4+ T cells in B-cell non-Hodgkin lymphoma. Blood 2006;107:3639–46.
3. Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 system of T cell costimulation. Annu Rev Immunol 1996;14:233–58.
4. Walunas TL, Lenschow DJ, Bakker CY, et al. CTLA-4 can function as a negative regulator of T cell activation. Immunity 1994;1:405–13.
5. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med 1995;182:459–65.
6. Riley JL, June CH. The CD28 family: a T-cell rheostat for therapeutic control of T-cell activation. Blood 2005;105:13–21.
7. Chambers CA, Krummel MF, Boitel B, et al. The role of CTLA-4 in the regulation and initiation of T-cell responses. Immunol Rev 1996;153:27–46.
8. Greenwald RJ, Boussiotis VA, Lorsbach RB, Abbas AK, Sharpe AH. CTLA-4 regulates induction of anergy *in vivo*. Immunity 2001;14:145–55.
9. Perez VL, Van Parijs L, Biuckians A, Zheng XX, Strom TB, Abbas AK. Induction of peripheral T cell tolerance *in vivo* requires CTLA-4 engagement. Immunity 1997;6:411–7.
10. Eagar TN, Karandikar NJ, Bluestone JA, Miller SD. The role of CTLA-4 in induction and maintenance of peripheral T cell tolerance. Eur J Immunol 2002;32:972–81.
11. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science 1996;271:1734–6.
12. 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.
13. Yang YF, Zou JP, Mu J, et al. Enhanced induction of antitumor T-cell responses by cytotoxic T lymphocyte-associated molecule-4 blockade: the effect is manifested only at the restricted tumor-bearing stages. Cancer Res 1997;57:4036–41.
14. Shrikant P, Khoruts A, Mescher MF. CTLA-4 blockade reverses CD8+ T cell tolerance to tumor by a CD4+ T cell-and IL-2-dependent mechanism. Immunity 1999;11:483–93.
15. Sotomayor EM, Borrello I, Tubb E, Allison JP, Levitsky HI. *In vivo* blockade of CTLA-4 enhances the priming of responsive T cells but fails to prevent the induction of tumor antigen-specific tolerance. Proc Natl Acad Sci U S A 1999;96:11476–81.
16. Kwon ED, Hurwitz AA, Foster BA, et al. Manipulation of T cell costimulatory and inhibitory signals for immunotherapy of prostate cancer. Proc Natl Acad Sci U S A 1997;94:8099–103.
17. 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.
18. Small EJ, Tchekmedyian NS, Rini BI, Fong L, Lowy I, Allison JP. A pilot trial of CTLA-4 blockade with human anti-CTLA-4 in patients hormone-refractory prostate cancer. Clin Cancer Res 2007;13:1810–5.
19. O'Mahony D, Morris JC, Quinn C, et al. A pilot study of CTLA-4 blockade after cancer vaccine failure in patients with advanced malignancy. Clin Cancer Res 2007;13:958–64.
20. Weber JS, O'Day S, Urba W, et al. Phase I/II study of ipilimumab for patients with metastatic melanoma. J Clin Oncol 2008;26:5950–6.
21. Hodi FS, Butler M, Oble DA, et al. Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. Proc Natl Acad Sci U S A 2008;105:3005–10.
22. Hamid O, Chin K, Li J, et al. Dose effect of ipilimumab in patients with advanced melanoma: results from a phase II, randomized, dose-ranging study. J Clin Oncol 2008;26 suppl:abstr 9025.
23. Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol 1999;17:1244–53.
24. Timmerman JM, Czerwinski DK, Davis TA, et al. Idiotype-pulsed dendritic cell vaccination for B-cell lymphoma: clinical and immune responses in 35 patients. Blood 2002;99:1517–26.
25. Hurvitz SA, Timmerman JM. Current status of therapeutic vaccines for non-Hodgkin's lymphoma. Curr Opin Oncol 2005;17:432–40.
26. Fong L, Small EJ. Anti-cytotoxic T-lymphocyte antigen-4 antibody: the first in an emerging class of immunomodulatory antibodies for cancer treatment. J Clin Oncol 2008;26:5275–83.
27. Bashey A, Medina B, Corringham S, et al. CTLA4 blockade with ipilimumab to treat relapse of malignancy after allogeneic hematopoietic cell transplantation. Blood 2009;113:1581–8.
28. Camacho LH, Antonia S, Sosman J, et al. Phase I/II trial of tremelimumab in patients with metastatic melanoma. J Clin Oncol 2009;27:1075–81.
29. Dai D, Wu C, Parker SM, et al. Model-based evaluation of ipilimumab dosage regimen in patients with advanced melanoma. J Clin Oncol 2008;26 suppl:abstr 9073.
30. Horning SJ, Rosenberg SA. The natural history of initially untreated low-grade non-Hodgkin's lymphomas. N Engl J Med 1984;311:1471–5.
31. Krikorian JG, Portlock CS, Cooney P, Rosenberg SA. Spontaneous regression of non-Hodgkin's lymphoma: a report of nine cases. Cancer 1980;46:2093–9.
32. Kwak LW, Campbell MJ, Czerwinski DK, Hart S, Miller RA, Levy R. Induction of immune responses in patients with B-cell lymphoma against the surface-immunoglobulin idiotype expressed by their tumors. N Engl J Med 1992;327:1209–15.
33. Bendandi M, Gocke CD, Kobrin CB, et al. Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma. Nat Med 1999;5:1171–7.
34. Davis TA, Maloney DG, Czerwinski DK, Liles TM, Levy R. Anti-idiotype antibodies can induce long-term complete remissions in non-Hodgkin's lymphoma without eradicating the malignant clone. Blood 1998;92:1184–90.
35. McLaughlin P, Grillo-López AJ, Link BK, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998;16:2825–33.
36. Timmerman JM. Immunotherapy for lymphomas. Int J Hematol 2003;77:444–55.
37. Hsu FJ, Caspar CB, Czerwinski D, et al. Tumor-specific idiotype vaccines in the treatment of patients with B-cell lymphoma-long-term results of a clinical trial. Blood 1997;89:3129–35.

AACR American Association for Cancer Research

Clinical Cancer Research

Phase I Study of Ipilimumab, an Anti–CTLA-4 Monoclonal Antibody, in Patients with Relapsed and Refractory B-Cell Non–Hodgkin Lymphoma

Stephen M. Ansell, Sara A. Hurvitz, Patricia A. Koenig, et al.

Clin Cancer Res 2009;15:6446-6453. Published OnlineFirst October 6, 2009.