Published in final edited form as:

Clin Cancer Res. 2009 October 15; 15(20): 6446–6453. doi:10.1158/1078-0432.CCR-09-1339.

Phase I Study of Ipilimumab (MDX-010), an anti-CTLA-4 monoclonal antibody, in patients with Relapsed and Refractory B-cell non-Hodgkin Lymphoma

Stephen M. Ansell¹, Sara A. Hurvitz², Patricia A. Koenig¹, Betsy R. LaPlant¹, Brian F. Kabat¹, Donna Fernando², Thomas M. Habermann¹, David J. Inwards¹, Meena Verma², Reiko Yamada², Charles Erlichman³, Israel Lowy⁴, and John M. Timmerman²

¹Division of Hematology, Mayo Clinic, Rochester, MN

²Division of Hematology & Oncology, University of California, Los Angeles, CA

³Department of Oncology, Mayo Clinic, Rochester, MN

⁴Clinical Science, Medarex, Inc. Bloomsbury, NJ

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 anti-tumor immune responses. Blocking anti-CTLA-4 monoclonal antibodies improve 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 performed 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, 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 and 6 at the higher dose level. Ipilimumab was generally well tolerated, with common adverse events attributed to ipilimumab including diarrhea, headache, abdominal pain, anorexia, fatigue, neutropenia and thrombocytopenia. Two patients had clinical responses, 1 patient with diffuse large B-cell lymphoma had an ongoing complete response (31+ months) and 1 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 using ipilimumab is well tolerated at the doses used, and has anti-tumor 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.

INTRODUCTION

B-cell non-Hodgkin lymphomas (NHL) 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

Address correspondence to: Stephen M. Ansell, M.D., Ph.D. Division of Hematology Mayo Clinic 200 First Street SW Rochester, Minnesota 55905 Tel: (507) 284-0923 Fax: (507) 266-4972 ansell.stephen@mayo.edu.

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 NHL.

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 co-stimulatory 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 down regulating the immune response (7–10). Blockade of CTLA-4 by administration of anti-CTLA-4 monoclonal antibodies has been shown to enhance T-cell responses in a variety of settings and to enhance anti-tumor responses (11–16).

Ipilimumab is a fully human IgG1 κ monoclonal antibody specific for human CTLA-4 (formerly MDX-010, Medarex, Inc.) that has been 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 demonstrate not only 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 NHL to primarily 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 NHL (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 1 prior but not more than 3 prior chemotherapy regimens; antibody and vaccine therapies were not counted as chemotherapy regimens. All patients had measurable disease; an ECOG performance status (PS) of 0 or 1; and life expectancy greater than 24 weeks. All patients had adequate hepatic, renal, and bone marrow function. Patients were excluded if they had previous treatment with ipilimumab; or previous treatment with fludarabine or 2-chlorodeoxyadenosine within 12 months of enrollment due to the immunosuppressive effect of this class of chemotherapy. Pregnant women or patients with immunodeficiency, uncontrolled infection, cardiac disease, or central nervous system lymphoma were excluded. The use of concurrent anti-lymphoma therapy, immunosuppressive drugs or corticosteroids was prohibited. Patients with active or recent clinically significant autoimmune disease were excluded due to the potential for 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, performed at the Mayo Clinic and the University of California Los Angeles, subjects received four monthly doses of ipilimumab intravenously. Ipilimumab was provided by Medarex, Inc., via the Cancer Therapy Evaluation Program (CTEP) 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 1mg/kg monthly for 3

further doses. If no significant dose-limiting toxicity was seen at this dose level, the dose would then be escalated to 3 mg/kg monthly for 4 doses. Enrollment was initially stratified into 2 cohorts, with half of 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 anti-lymphoma 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 after all 6 patients in each cohort finished at least the first three treatments with no more than one severe toxicity and no more than 2 out of the 6 patients with dose limiting toxicity requiring them to discontinue study treatment. If more than one patient had evidence of severe toxicity or there were 3 or more 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 administered in other studies, doses of ipilimumab above 3mg/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

A severe toxicity was considered to have occurred for any grade 4 toxicity; any long lasting (greater than 1 month) grade 3 or greater 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 using the Common Terminology Criteria for Adverse Events (CTCAE) Version 3 as any of the following as assessed by the investigator: any grade 3 or greater adverse event suspected to be related to the study agent, any grade 2 or greater 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 was determined using the International Workshop to Standardize Response Criteria for NHL (23).

Flow cytometric analysis of peripheral blood T cells

Peripheral blood mononuclear cells (PBMC) 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% dimethyl sulfoxide (DMSO) until use. Cell surface markers were analyzed using the following antibodies, all purchased from BD Biosciences (San Jose, CA): CD3 conjugated with fluorescein isothiocyanate (FITC)(clone HIT3a), CD4 allophycocyanin (APC)(clone RPA-T4), CD8 (PE)(clone RPA-T8), CD16 (PE) (clone 3G8), CD56 (APC)(clone B159), HLA-DR phycoerythrin (PE)(clone L243), CD25 PE (clone MA251), and CD45RO PE (clone UCHL1). Labeled isotype-matched antibodies were run in parallel as controls. Cryopreserved pre- and post-treatment PBMC were tested in the same assay to eliminate inter-assay variability. PBMC were thawed quickly in a 37°C water bath and washed twice with warm RPMI complete medium and counted with a hemacytometer. 5×10^5 cells in 100µl FACS Buffer (1% BSA, 0.1% Sodium Azide in 1X PBS) were stained for 25 minutes with the recommended concentrations of antibodies at 4°C, protected from light. Cells were then washed twice with FACS Buffer and fluorescence was detected by BD

FACScan flow cytometer (BD Biosciences), and data analyzed with FCS Express software (De Novo Software, Los Angeles, CA).

T cell proliferation was measured at baseline and at months 1 and 4 by incubation of PBMC with KLH, tetanus toxoid, or media alone at various concentrations, and ^3H -thymidine pulsing on day 4. As previously described (24), cryopreserved pre- and post-treatment PBMC (10^5 cells/well) were seeded in quadruplicate in 96-well U-bottom plates (Nunc, Rochester, NY) in RPMI complete medium. Graded concentrations of KLH (Pierce, Rockford, IL) at 0, 10, 100 $\mu\text{g}/\text{ml}$ or tetanus toxoid (Sanofi Pasteur, Swiftwater, PA) at 2 or 10 $\mu\text{g}/\text{ml}$ 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 days. Cells were then pulsed with 1 $\mu\text{Ci}/\text{well}$ ^3H -thymidine (MP Biomedicals, Solon, OH) and harvested 16 h later. Incorporated radioactivity (counts per minute, cpm) was measured using a β -liquid scintillation analyzer (PerkinElmer, 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 administered on a monthly dosing schedule. The safety parameters included adverse events, vital sign measurements, clinical laboratory tests, physical examinations, and diagnostic tests (including chest X-rays and CT scans). Hematologic toxicity measures of thrombocytopenia, neutropenia and leukopenia were assessed using the continuous variables as the outcome measures (primarily nadir and percent change from baseline values) as well as categorization via CTC Version 3 standard toxicity grading. Non-hematologic toxicities were evaluated via the ordinal CTC Version 3 standard toxicity grading only. Frequency distributions and other descriptive measures formed the basis of the analysis of these variables. The secondary objective was to characterize the immunological effects of ipilimumab. The immunological results were summarized using 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, 3 had diffuse large B-cell lymphoma and 1 had mantle-cell lymphoma. Eleven patients (61%) were ECOG PS 0; the remaining 7 patients were ECOG PS 1. All patients were pretreated (median number of previous treatments was 2, range 1–4) and 6 patients had previously received a tumor-specific idiotype-KLH vaccine plus GM-CSF. One patient received KLH alone plus GM-CSF 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 then monthly at 1 mg/kg $\times$ 3; with a subsequent escalation to 3 mg/kg monthly $\times$ 4 months for the next 6 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 – 6 patients had received prior lymphoma vaccine therapy and 6 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 both 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 prior to escalating to the second dose level. Accrual to the study had been significantly limited by the requirement for a cohort who had previously received a lymphoma vaccine. As there was no obvious difference in response in the vaccinated group, the requirement for two separate cohorts was eliminated for the second dose level. Six patients were treated at the second dose level and 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 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 4 planned doses, 7 discontinued due to disease progression, 2 patients refused further therapy and 1 patient 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 3 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 MRI 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 utilizing 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 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⁺ NK cells in the peripheral blood between baseline and one 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⁺ NK cells, as well as a 17.6% rise in CD8⁺ T cells and a corresponding 17.3% decrease in CD4⁺ T cells. Other serological 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 towards recall antigens before and after therapy. T-cell proliferative responses to KLH (in idiotype-KLH and KLH-vaccinated cases) were measured pre- and post-ipilimumab, and are shown in Figure 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, 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 NHL stage IVA treated at dose level one had a partial response to therapy. The patient had intra-abdominal lymphadenopathy and bone marrow involvement, and had previously received 2 chemotherapy regimens and treatment with anti-idiotypic vaccine. The patient's partial response was sustained for 19 months (Figure 2A). Interestingly, this patient was the only subject showing significant activation of peripheral blood T cells (Table 4) and enhanced reactivity to recall antigens (Figure 1) during therapy. 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 a complete response. The patient had previously failed 3 chemotherapy regimens and had intra-abdominal lymphadenopathy and liver lesions. The patient remains progression free at 31+ months (Figure 2B). This patient had no significant side effects from treatment. Supplemental Table 1 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 T cell antigen receptor by peptide/major histocompatibility complex (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. Cytotoxic T lymphocyte antigen 4 (CTLA-4, CD152) is a molecule which is up-regulated on the T cell surface following 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 monoclonal antibodies has been shown to enhance anti-tumor immunity and promote tumor eradication in a variety of mouse tumor model systems, including prostate, breast, melanoma, and lymphomas (11–16).

Monoclonal antibodies 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 10mg/kg have been administered. 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. 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 administered every 3 weeks as induction, followed by a maintenance dose administered every 12 weeks beginning at week 24. The overall incidence of adverse events was almost identical between 3mg, and 10 mg. The 10 mg/kg dose did have more serious or severe ($\geq$ Grade 3) adverse events (24% vs. 8%) than the 3 mg/kg dose, but the implementation of detailed treatment algorithms (typically based upon 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 those occurred. There was a clear three-fold greater efficacy associated with 10 mg/kg compared to the 3 mg/kg dose. In a pharmacokinetic analysis of the correlation of antibody levels and expected saturation of available CTLA-4 receptors (29) at a dose of 3 mg/kg, only 1/3 of the patients have enough drug left at trough to prevent the re-establishment of CTLA4 mediated tolerance but at 10 mg/kg, 95% of the patients remain above the threshold. These data indicate that CTLA-4 blockade dose-intensity is critical to achieving optimal anti-tumor 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 non-specific immune activators such as bacillus Calmette-Guerin and interleukin-2, and a high rate of response to B-cell specific monoclonal antibodies 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 anti-tumor 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-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 anti-tumor immunity in most lymphoma-bearing hosts (36). In biopsy specimens from patients with follicular lymphoma, we have recently found that approximately 10–15% (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 anti-tumor response.

In this study, ipilimumab displayed clinical activity, with 2 of 18 (11%) patients demonstrating a measurable response, both of which were durable. The responses did not appear to be dose dependent as a response was seen at both dose levels, although the more durable and complete response occurred at the higher dose. The overall response rate appears 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 NHL 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 4 patients with B-cell lymphoma previously treated with idiotype-KLH vaccines, 2 experienced tumor regression, including one with partial response of 14 months duration. Given the evolving information regarding 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 appears 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 using ipilimumab is well tolerated at the doses used. We have also found that ipilimumab has anti-tumor 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. While we have shown that ipilimumab can have efficacy against B-cell lymphomas as a single agent, a likely ultimate strategy will be to administer this agent in combination with other available lymphoma immunotherapies such as anti-CD20 monoclonal antibodies. This phase I trial has shown that ipilimumab 3mg/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.

STATEMENT OF TRANSLATIONAL RELEVANCE

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

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported in part by grants R21 CA108182 and U01 CA69912 from the National Institutes of Health.

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Figure 1. Individual patient T cell proliferation to recall antigens keyhole limpet hemocyanin (KLH) or tetanus toxoid (TT) during treatment

Samples were collected at baseline, 1 month and 4 months after initiation of treatment. Data are represented as stimulation index. Subjects are divided according to those with prior idiotype-KLH or KLH vaccination (top panels) and those without (lower panels).

Pre treatment**1 month after treatment** **Pre treatment****6 months after treatment****Figure 2. Clinical responses to ipilimumab**

A) Partial response in peri-aortic lymph nodes in a patient with follicular lymphoma (subject 3) one month after completion of treatment. B) Complete regression of a liver lesion in a patient with diffuse large cell lymphoma (subject 13) six months after completion of treatment.

Table 1

Patient Characteristics

	n=18
Gender -	
Male	12
Female	6
Performance status -	
0	11
1	7
Age (years), 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 -	
Idiotypic 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

Table 2

Adverse Events Possibly Related to Ipilimumab

Event	Grade 1	Grade 2	Grade 3	Total 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)

Table 3

Grade 3 Adverse Events (Regardless of attribution)

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

Table 4

Flow cytometric analysis of T cell surface markers before and 1 month 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	n.a.	n.a.
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 $\Delta \pm$ SEM	$+1.8 \pm 1.2$ ($p = 0.15$)		$+5.0 \pm 1.5$ ($p = 0.005$)	
	% of CD3+CD4-		% of CD3+CD4-	
3	5.3	10.6	13.7	21.1
4	5.9	9.4	n.a.	n.a.
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 $\Delta \pm$ SEM	$+1.9 \pm 2.0$ ($p = 0.36$)		$+2.8 \pm 0.9$ ($p = 0.006$)	

Values compared by using paired 2 tailed *t* test

n.a. = not available