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Denileukin Diftitox in Combination with Rituximab for Previously Untreated Follicular B-cell Non-Hodgkin's Lymphoma

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Abstract

Follicular lymphoma exhibits intratumoral infiltration by non-malignant T lymphocytes including CD4+CD25+ regulatory T (T_{reg}) cells. We combined denileukin diftitox with rituximab in previously untreated, advanced-stage follicular lymphoma patients anticipating that denileukin diftitox would deplete CD25+ T_{reg} cells while rituximab would deplete malignant B-cells. Patients received rituximab 375 mg/m² weekly for 4 weeks and denileukin diftitox 18 mcg/kg/day for 5 days every 3 weeks for 4 cycles; neither agent was given as maintenance therapy. Between August 2008 and March 2010, 24 patients were enrolled. One patient died before treatment was given and was not included in the analysis. Eleven of 23 patients (48%; 95% CI: 27–69%) responded; 2 (9%) had complete responses and 9 (39%) had partial responses. The progression-free rate at 2 years was 55% (95% CI: 37–82%). Thirteen patients (57%) experienced grade 3 adverse events and 1 patient (4%) died. In correlative studies, soluble CD25 and the number of CD25+ T-cells decreased after treatment, however there was a compensatory increase in IL-15 and IP-10. We conclude that while the addition of denileukin diftitox to rituximab decreased the number of CD25+ T-cells, denileukin diftitox contributed to the toxicity of the combination without an improvement in response rate or time to progression.

Keywords

Follicular lymphoma; denileukin diftitox; rituximab

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Conflict of Interest Disclosures

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INTRODUCTION

Follicular B-cell non-Hodgkin lymphomas (NHL) are common lymphoid malignancies that exhibit significant intratumoral infiltration by T lymphocytes (ref 1). The pathophysiological significance of infiltrating T cells is poorly understood. We have previously shown that CD4+CD25+ regulatory T (T_{reg}) cells are involved in the regulation of anti-tumor immunity in lymphoma and are present in the biopsy specimens from patients with follicular B-cell NHL (ref 2–5). Intratumoral T_{reg} cells display the ability to suppress the proliferation and cytokine production of other tumor-infiltrating T cells and migrate to areas of follicular lymphoma in response to chemotactic signals provided by the malignant B-cells (ref 4,5).

Rituximab, an anti-CD20 chimeric antibody, is approved for the treatment of patients with relapsed follicular lymphoma and targets the CD20 antigen on the surface of normal and malignant B-cell lymphocytes. While the exact mechanism of cytotoxicity in humans is unclear (ref 6–8), it is felt to work predominantly through antibody dependent cellular cytotoxicity (ADCC) (ref 8). Rituximab has proven to be an effective biologic therapy in indolent B-cell lymphoma and the standard 4 weekly doses often followed by maintenance therapy has been found to be effective as frontline therapy for patients with follicular lymphoma (ref 9–11). In a previous clinical trial of rituximab as a single agent in newly diagnosed follicular lymphoma (ref 12), 37 patients received rituximab 375 mg/m² intravenous weekly for 4 doses without maintenance therapy. The overall response rate (ORR) was 72%, with 36% complete remissions (CR), and the median time to progression (TTP) was 2.2 years. To potentially improve on these results, we predicted that depletion of T_{reg} cells in conjunction depletion of malignant B-cells would be an effective strategy.

Denileukin diftitox, a chimeric immunotoxin composed of the modified cytotoxic domain of diphtheria toxin and human interleukin-2 (IL-2) protein, targets cells expressing CD25. It not only depletes CD25 expressing cells but also has proven efficacy in patients with relapsed B-cell lymphoma (ref 13). In a previous study, Dang et al treated 50 patients with B-cell lymphoma with denileukin diftitox 18mcg/kg/day for 5 days. Immunohistochemistry was done to assess CD25 expression on the malignant B-cells, but patients with both CD25+ and CD25- tumors were enrolled. Interestingly, denileukin diftitox produced a 25% response rate in B-cell NHL with a superior result in lymphomas that did not express CD25 on the malignant cells (29% versus 22%). Although this study did not specifically evaluate the effect of the agent on T_{reg} cells, the result in CD25 negative lymphomas suggested that the effect of the agent may be through inhibition of other CD25 expressing cells such as T_{reg} cells. Furthermore, denileukin diftitox has also been safely combined with rituximab in patients with relapsed B-cell lymphoma and a response rate of 65% was seen in previously treated follicular lymphoma patients (ref 14).

In this study, we therefore combined denileukin diftitox with rituximab in a cohort of previously untreated, advanced-stage follicular lymphoma patients. We had previously shown that CD25+ intratumoral T_{reg} cells suppress the function of other tumor-infiltrating T cells and migrate to areas of follicular lymphoma in response to chemotactic signals provided by lymphoma B-cells (ref 4,5). Our hypothesis was therefore that denileukin diftitox would deplete the subset of highly suppressive CD25+ T_{reg} cells, thereby removing

the inhibition of the immune response, and rituximab would deplete the B-cells thereby preventing further recruitment of T_{reg} cells to the areas of lymphoma.

PATIENTS AND METHODS

Patient Eligibility

In this cooperative group study conducted by the North Central Cancer Treatment Group (NCCTG), patients were required to be 18 years of age or older with previously untreated follicular grade 1 or 2 B-cell lymphoma confirmed by central pathology review. Patients were required to have stage III or IV disease requiring treatment. All patients had measurable disease a lymph node or tumor mass that was >2 cm in at least one dimension by CT or MRI. Splenic enlargement could be used as a measurable parameter if the spleen was palpable 3 cm below the left costal margin. Patients were required to have adequate organ and marrow function defined as a white blood cell count (WBC) 3400×10^6 cells/L, platelet count $>100,000/\text{mm}^3$, hemoglobin 10 g/dl, total bilirubin $<1.5 \times$ upper limit of normal (ULN), AST $<3 \times$ ULN, serum creatinine $<1.5 \times$ ULN, and serum albumin of 3 g/dl. In addition, patients had to have a life expectancy of > 1 year and an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0, 1, or 2. Women of childbearing potential were required to have a negative pregnancy test done 7 days prior to registration.

Exclusion criteria included patients who were HIV positive; patients with central nervous system involvement; 5000 circulating tumor cells per microliter; previous investigational agents, corticosteroids, chemotherapy, immunotherapy, biologic therapy or radiation therapy for lymphoma; and known hypersensitivity to denileukin diftitox or any of its components: diphtheria toxin, interleukin-2, or excipients. Pregnant and nursing women were not eligible for the study. All patients were required to give informed consent and the Institutional Review Board of the Mayo Clinic approved the study. The study was registered with the National Cancer Institute (ClinicalTrials.gov Identifier: NCT00460109)

Study Design and Statistical Methods

Eligible patients received rituximab $375 \text{ mg}/\text{m}^2$ on days 1, 8, 15 and 22. Denileukin diftitox 18 mcg/kg/day was given on days 1–5 every 3 weeks for 4 cycles. Pre-medication recommendations for rituximab were diphenhydramine 25–50 mg intravenously or orally and acetaminophen 650 mg orally, and diphenhydramine 25–50 mg intravenously or orally, acetaminophen 650 mg orally and dexamethasone 2 mg intravenously or orally for denileukin diftitox. Denileukin diftitox was infused over 30 minutes on day 1 of the first cycle, and if well tolerated, given over an infusion time of at least 15 minutes for subsequent doses. Because denileukin diftitox has been associated with capillary leak syndrome, additional guidelines were included in the protocol. Denileukin diftitox was not given if the serum albumin decreased to $<3 \text{ g}/\text{dl}$. Five hundred ml of normal saline was given before and after the denileukin diftitox infusion. The patient's weight was monitored daily during each cycle until 72 hours after getting the fifth dose of denileukin diftitox. Patients were instructed to call their physician if they gained more than 5 pounds while receiving

denileukin diftitox or within the subsequent 72 hours. If weight gain was observed, patients were given furosemide 20mg daily orally until their weight decreased to baseline.

Treatment responses or stable disease while on treatment as well as disease progression was determined using the International Workshop to Standardize Response Criteria for NHL (ref 15). This study was designed to assess the proportion of responses associated with rituximab given in combination with denileukin diftitox in previously untreated patients with follicular grade 1 and 2 B-cell non-Hodgkin lymphoma using a modified two-stage 3-outcome phase II study design (ref 16). The primary endpoint of this trial was the proportion of confirmed tumor responses. The treatment regimen would be considered ineffective in this population if the response rate was 70% or less. If the response rate was 85% or greater, subsequent studies would be warranted. The modified two-stage 3-outcome design (ref 16) used a minimum of 21 and a maximum of 48 evaluable patients to test the null hypothesis that the true success proportion in a given patient population was at most 70%. The following decision criteria were used for the evaluation of all 48 evaluable patients: a) "Not promising" - the regimen would be classified as not promising if at most 35 successes were observed in a total of 48 evaluable patients; b) "Inconclusive" - the results of this study would be classified as inconclusive with respect to this regimen demonstrating an improved success rate if 36, 37, or 38 successes were observed in 48 evaluable patients. In this case, we would formally consider other outcomes in determining whether or not this combination regimen was promising. In this scenario, we would evaluate the proportion of complete responses as well as the proportion of patients who are progression-free at 24 months, the toxicity profile, and changes in the immunologic markers of interest to make a final decision as to whether or not this treatment was considered promising and worthy of further study in this patient population; c) "Promising" - this regimen would be classified as promising with respect to increasing the success rate in this patient population if at least 39 successes were observed in 48 evaluable patients and subsequent studies would be recommended. An interim analysis would be conducted after the first 21 evaluable patients were accrued to the trial. If 15 or fewer successes were observed in the first 21 evaluable patients, we would consider this sufficient early evidence that the regimen was ineffective in this patient population and we would terminate accrual to this study.

The secondary endpoints included time to progression, duration of response, time to discontinuation of active treatment, and overall survival. Time to progression was defined as the time from registration to the date of progression. Patients who died without disease progression were censored at the date of their last evaluation. If a patient died without documentation of disease progression, the patient was considered to have had disease progression at the time of death unless there was sufficient documented evidence to conclude that progression did not occur before death. Duration of response was defined as the time from the date of documented response to the date of progression. Patients who went off treatment due to other reasons (eg, adverse reactions, refusal of further treatment) were censored at that time. Time to discontinuation of active treatment was defined as the time from registration to the date the decision was made to take the patient off active treatment. Patients who were still receiving treatment at the time of these analyses were censored at the date of their last evaluation. Overall survival was defined as the time from

registration to death resulting from any cause. The distributions of these time-to-event end points were each estimated using the Kaplan-Meier method (ref 17).

Toxicity Evaluation, Dose Modifications and Adverse Event Stopping Rules

As per the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3, toxicity was defined as adverse events that are classified as either possibly, probably, or definitely related to study treatment. There were no dose modifications for rituximab and rituximab was given even if denileukin diftitox was decreased or held for toxicity. Serum levels of albumin were checked prior to each cycle of therapy and denileukin diftitox was decreased to 9 mcg/kg/day if the serum albumin was between 3–3.4 g/dl. If the serum albumin was < 3g/dl, denileukin diftitox was held until it increased. If it did not recover within 3 weeks, denileukin diftitox was discontinued.

Administration of denileukin diftitox was also held if the absolute neutrophil count (ANC) was <1,000 or the platelet count was <50,000. Upon recovery to ANC 1,000 and platelets to 50,000, the dose of denileukin diftitox was decreased to 9 mcg/kg/day. If patients developed grade 3 or 4 edema or hypersensitivity, or retinopathy of any grade, denileukin diftitox was discontinued. The administration of denileukin diftitox was also held for any grade 3 or 4 non-hematologic adverse events and restarted at 9 mcg/kg/day once the toxicity had resolved to grade 1. Overall, if 3 out of the first 21 or if at any time 7 or more patients developed grade 4 non-hematologic toxicity (i.e., adverse events felt to be at least possibly related to treatment), then protocol accrual would be suspended. Infusion reactions related events to rituximab were not considered in the stopping rule.

Correlative Studies

To determine the effects of denileukin diftitox and rituximab on T_{reg} cells, CD3⁺, CD4⁺, CD8⁺ T cells, CD19⁺ B cells, CD14⁺ monocytes were isolated using positive selection with CD3, CD4, CD8, CD19 or CD14 microbeads. CD4⁺CD25⁻ or CD4⁺CD25⁺ T-cell subsets were purified by using CD4⁺CD25⁺ Regulatory T-cell Isolation Kit (Miltenyi Biotec, Auburn, CA) as previously described (ref 2,3). Purity was checked by FACS analysis and was typically greater than 95%. Cells were washed and subjected to fixation, permeabilization, stained with fluorochrome-conjugated antibodies against IL-2, IL-17, IFN- γ and analyzed by flow cytometry. Foxp3 expression was determined using flow-based intracellular staining following the manufacturer's instructions.

Serum cytokines were also measured from pre-treatment blood draws, with each cycle of denileukin diftitox, and at the end of therapy using a multiplex ELISA (Invitrogen, Camarillo, CA). Thirty cytokines were analyzed using the Luminex-100 system Version 1.7 (Luminex, Austin, TX). The following cytokines were tested EGF (epidermal growth factor), eotaxin, FGFb (basic fibroblast growth factor), GMCSF (granulocyte-macrophage colony-stimulating factor), GCSF (granulocyte colony stimulating factor), HGF (hepatocyte growth factor), IFN α (interferon alpha), IFN γ (interferon gamma), IL-2 (interleukin-2), IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, IL-17, IL-12p40, IL-2R (interleukin-2 receptor/CD25), IL-1RA (interleukin-1 receptor antagonist), IL-1 β (interleukin-1 beta), MCP-1 (monocyte chemotactic protein 1), IP-10 (inducible protein 10/CXCL10), MIG

(monokine induced by interferon γ /CXCL9), MIP-1 α (CCL3), MIP-1b (CCL4), RANTES (regulated on activation, normal T-cell expressed and secreted/CCL5), TNF α (tumor necrosis factor alpha) and VEGF (vascular endothelial growth factor). Data were acquired using STar Station software (Applied Cytometry, Dinnington, Sheffield, UK) and analysis was performed using the MasterPlex QT 1.0 system (MiraiBio, Alameda, CA).

RESULTS

Patient characteristics

Between August 2008 and March 2010, twenty-four patients with stage III and IV follicular grade 1 or 2 NHL were accrued to the study. One patient died of a myocardial infarction before treatment was given and is not included in the analysis. The median age of the 23 patients who received treatment and were eligible for the study was 60 years (range: 27–79). Twelve (52%) of the patients were male, 19 (83%) had a PS of 0 and 4 (17%) had a PS of 1. All patients had measurable disease at study entry. The patient baseline characteristics are outlined in Table 1. Based on the Follicular Lymphoma International Prognostic Index (FLIPI) (ref 18), 3 (13%) were low risk, 14 (61%) were intermediate risk and 6 (26%) were high risk.

Clinical Responses

Patients received rituximab 375 mg/m² on days 1, 8, and 15 of cycle 1 and day 1 of cycle 2. They also received denileukin diftitox 18 mcg/kg/day on days 1–5 of cycles 1–4. Each cycle was 3 weeks long. Patients were then observed without maintenance therapy. A median of 4 cycles of therapy was given (range: 1 – 4). The reasons for ending active treatment included: completed treatment per protocol (13/23; 57%), refused further treatment (2; 9%), adverse event (5; 22%), disease progression (1; 4%), other medical problems (1; 4%), and died on study (1; 4%).

As required by the protocol, an interim analysis was performed after 21 patients had been enrolled on the study; however, enrollment was not suspended while response data on these patients were collected. The study did not meet the efficacy requirements for continued accrual and the combination was deemed ineffective. The study was therefore terminated and the results of all patients accrued were summarized. Twenty-three patients were evaluable for response. Nine out of 23 (39%) patients had a response to therapy during the 4 cycles of treatment. Two patients had a subsequent response within 5 months after completing therapy and the overall response rate (ORR) was therefore 48% (11/23; 95% CI: 27–69%). Two patients (9%) had a complete response (CR) and 9 patients (39%) had a partial response (PR). Twenty-one patients (91%) remain alive with a median follow-up of 19.1 months (range: 9.2 –26.0). Ten (44%) patients have progressed and two (9%) have died. As shown in Figure 1, the progression-free rate at 12 months was 70.8% (95% CI: 54–94%) and 55% (95% CI: 37–82%) at 24 months. The median overall survival has not yet been reached.

Adverse Events

All 23 treated patients were evaluable for toxicity. The addition of denileukin diftitox to rituximab significantly increased the frequency of adverse events compared to the adverse events typically seen with rituximab alone (see Table 2). Thirteen patients (57%) experienced grade 3 or greater adverse events and in 12 of these patients (52%) the adverse events were felt to at least possibly be associated with the study drugs. Seven patients (30%) had grade 4 or 5 adverse events. Six patients (26%) had symptoms of capillary leak syndrome. Two patients had capillary leak syndrome with associated hypotension 1 (4%) was grade 4 and 1 (4%) grade 5. The patient who died with capillary leak syndrome developed symptoms of fluid retention during the first cycle of denileukin diftitox. He died due to refractory cardiogenic shock despite discontinuation of the drug and admission to the intensive care unit. One patient (4%) had a cardiac ischemic event confirmed by increased cardiac enzymes and complicated by ventricular tachycardia, dyspnea, hypotension, hypoxia and decreased level of consciousness. One patient with pre-existing valvular heart disease had rupture of the chordae tendineae of the mitral valve that required surgical repair. One patient (4%) had grade 4 myositis with increased CPK levels and myalgia, 1 patient (4%) had grade 4 thrombosis and 1 (4%) had grade 4 neutropenia. The high rate of adverse events seen in the study further contributed to closure of the study at the time of the interim analysis.

Correlative Studies

To determine whether treatment with rituximab and denileukin diftitox depleted naturally occurring T_{reg} cells, we measured the number of CD4+CD25+ T-cells in the peripheral blood by flow cytometry (Figure 2). We found that the number of CD25+ T-cells decreased after treatment when compared to pretreatment numbers. We measured CD4+CD25+ T-cells as a percentage of the total number of CD4+ T-cells and found that they decreased from a mean of 8.2% ($\pm 1.6\%$) to 4.4% ($\pm 1.2\%$). We also measured serum IL-2R (CD25) before and during treatment and showed a significant decrease in the serum levels of sIL-2R (Figure 3A). However, the serum levels of other cytokines known to promote T_{reg} cells, including IL-2 (Figure 3B) and IL-12 (data not shown), did not change. Also, serum levels of IL-15 (Figure 3C) and IP-10 (CXCL10, Figure 3D) increased as sIL-2R decreased. IL-15 also promotes T_{reg} cell survival and with IL-2 redundantly governs T_{reg} cell development. Furthermore, a decrease in CD4+CD25+ cells has been associated with an increase in IP-10, but IP-10 has been associated with a poor clinical outcome in lymphoma patients due in part to the suppressive environment generated by the inflammatory response. These changes suggested that the use of denileukin diftitox did not effectively suppress T-cells with regulatory function.

DISCUSSION

Rituximab is commonly used in the treatment of follicular non-Hodgkin lymphomas (ref 9–12). While the antibody has been shown to directly induce apoptosis (ref 6), the Fc domain has also been shown to recruit immune effector functions to mediate lysis of the B-cell (ref 8). Although apoptosis and complement activation play a role, ADCC is felt to be the major mechanism of action in NHL. ADCC relies on immunological competent effector cells, but

these cells are often suppressed by intratumoral T_{reg} cells (ref 4,5). In this study, we focused on patients with untreated follicular lymphoma who typically have an intact immune system and predicted that depletion of T_{reg} cells would optimize ADCC induced by rituximab. We used denileukin diftitox that targets CD25 expressed on naturally occurring T_{reg} cells to deplete this subset of T-cells with suppressive regulatory function and thereby promote the activity of immune effector cells. Because T_{reg} cells are recruited to tumor sites by malignant B-cells, we hypothesized that concomitant depletion of B-cells with the use of rituximab would further inhibit T_{reg} cells in the tumor microenvironment by preventing their recruitment.

Rituximab is commonly administered as a single agent in follicular lymphoma patients with a relatively low burden of disease. In this setting, rituximab alone is associated with overall response rates of 50–80% and durations of response of 18–28 months (ref 9–11). In a previous clinical trial of 4 doses of rituximab as a single agent in newly diagnosed follicular lymphoma conducted by NCCTG (ref 12), the ORR was 72%, with 36% CR. The median TTP was 2.2 years. For the current study in which denileukin diftitox was added to rituximab, we used identical eligibility criteria as the previous NCCTG study of rituximab alone and anticipated an increase in the ORR to approximately 85%. In contrast to what was expected, the ORR seen in this study was significantly lower than anticipated. We observed an ORR of 48% with a CR rate of 9%. Furthermore, the percentage of patients in this study that remain progression-free was also fewer than expected. In addition, denileukin diftitox significantly increased the rate of adverse events. In the previous NCCTG study of rituximab alone, grade 3 or 4 adverse events were observed in 14% of the patients. These included neutropenia, rash, hemorrhage, infection and colitis. In this study, more than half of the patients had grade 3 or greater adverse events and 1 patient died on study from treatment-related complications. The severe adverse events seen with the combination of denileukin diftitox and rituximab included capillary leak syndrome, cardiac ischemic, mitral valve rupture, ventricular tachycardia, dyspnea, hypoxia, thrombosis, myositis and hypotension. The lack of efficacy and substantial toxicity seen with this combination led to the early closure of the study.

Naturally occurring CD4+CD25+ T_{reg} cells (nT_{reg}), which specifically express the forkhead family transcription factor Foxp3, are essential for the maintenance of immunological self-tolerance and immune homeostasis (ref 19,20). These cells are present in increased numbers in tumors including lymph nodes involved by B-cell NHL and suppress intratumoral CD4+ and CD8+ T-cells (ref 4,5,21,22). Other CD4+ T-cells however express FoxP3 but lack CD25 and also have suppressive function. These cells are induced to express FoxP3 and gain suppressive function when they come into contact with malignant B-cells and are called inducible T_{reg} cells (iT_{reg}) (ref 2). Treatments such as denileukin diftitox that target CD25 expressing cells therefore deplete nT_{reg} but not iT_{reg}. In the correlative studies performed in this trial, CD4+CD25+ T-cells were depleted by the treatment, as were the serum levels of soluble CD25 (IL-2R). However, depletion of nT_{reg} resulted in increases in IL-15 and IP-10 and had no effect on other cytokines such as IL-2. IL-15, IP-10 and IL-2 promote the function and survival of all T_{reg} cells, and increased levels of IL-15 and IP-10 therefore would potentially stimulate residual iT_{reg} cells after nT_{reg} cell depletion (ref 23,24).

Therefore this compensatory increase in cytokines may possibly account for the poor response rate and duration of response seen in this study.

We therefore conclude that while the addition of denileukin diftitox to rituximab decreased the numbers of CD25+ T_{reg} cells, denileukin diftitox contributed significantly to the toxicity of the combination. The frequency of adverse events seen in this cohort of previously untreated patients was significantly greater than what was seen using the same combination in patients who had been previously treated. This suggests that patients with a relatively intact immune system, who may be more likely to benefit from immune manipulation, are also potentially at greater risk of toxicity from these therapies due to more robust response from the immune system. Furthermore, the ORR and TTP seen in this study were disappointingly low and clearly no better than what would be expected in follicular lymphoma patients treated with rituximab alone, suggesting that depleting nT_{reg} alone does not result in improved clinical outcomes in follicular lymphoma. Future studies exploring methods to deplete both malignant B-cells and intratumoral T_{reg} cells will need to utilize more effective mechanisms to deplete all T_{reg} cells or inhibit the function of all regulatory cells.

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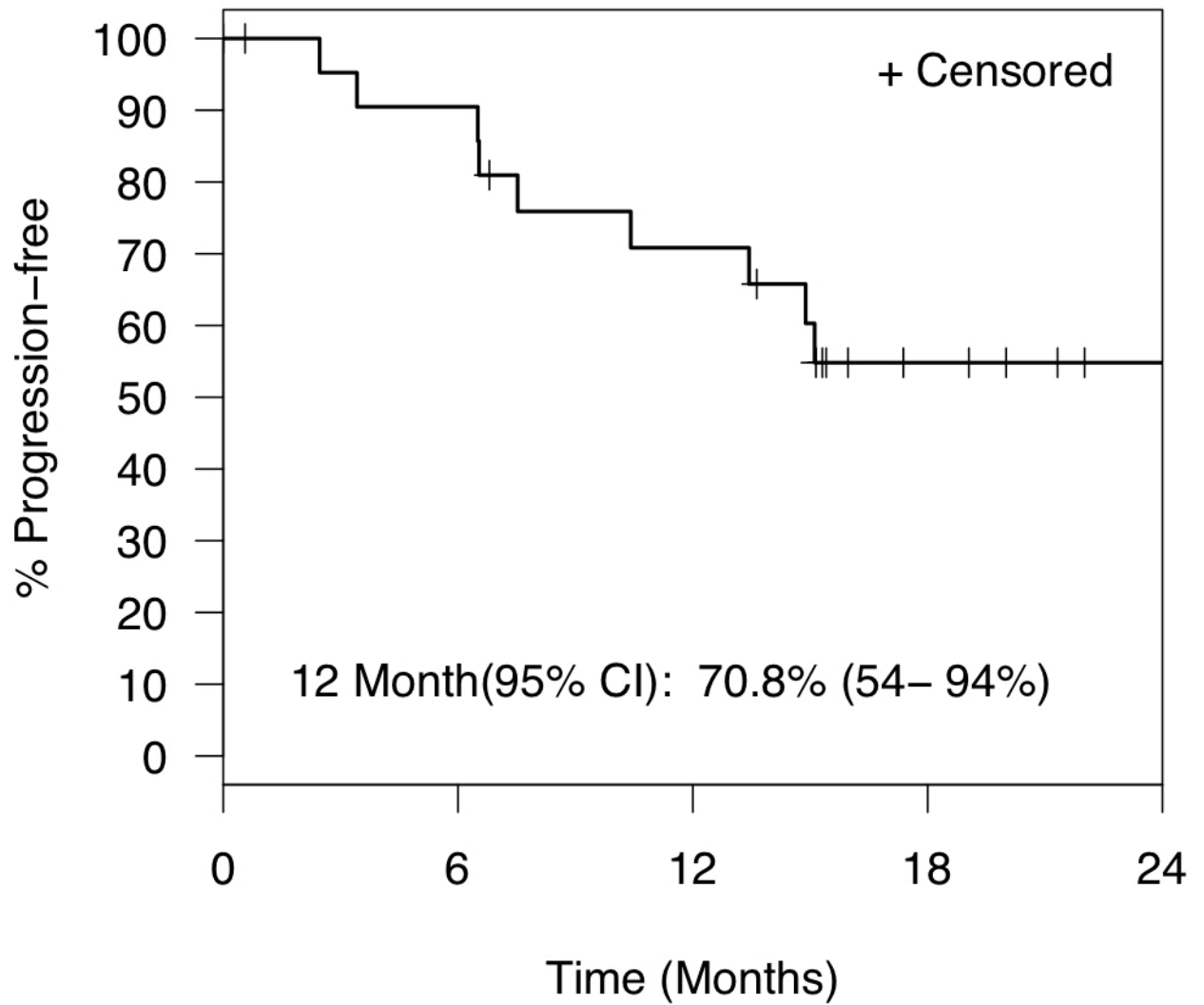


Figure 1.
Time to Progression (TTP) for patients with follicular grade 1 and 2 non-Hodgkin lymphoma treated with rituximab and denileukin diftitox

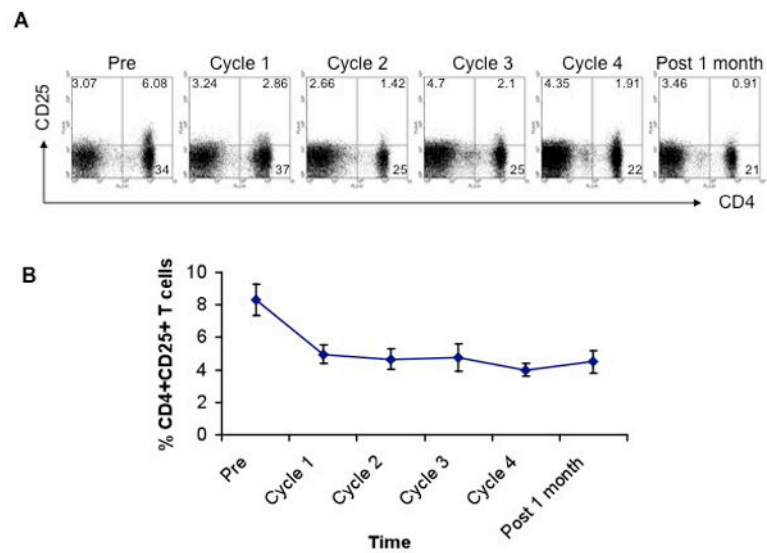


Figure 2. Percentage of CD4+CD25+ T-cells in the peripheral blood after treatment with rituximab and denileukin difitox

A) Flow cytometry on peripheral blood mononuclear cells from a representative patient showing a decrease in CD4+ T-cells expressing CD25. B) CD4+CD25+ T-cells as a percent of all CD4+ cells in the peripheral blood during treatment with rituximab and denileukin difitox (n=13).

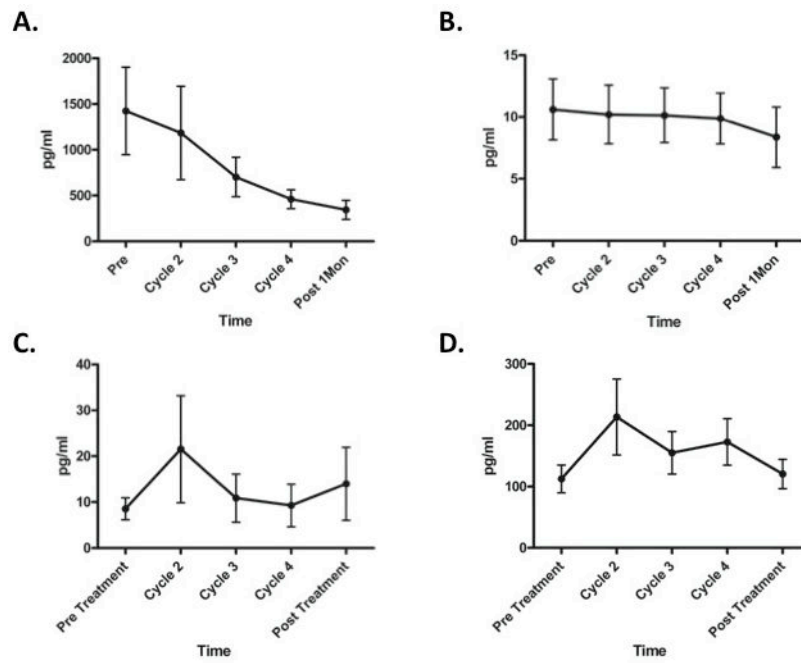


Figure 3. Serum cytokines and soluble receptors after treatment with rituximab and denileukin diftitox

Serum levels of A) IL-2R (soluble CD25), B) IL-2, C) IL-15, and D) IP-10 measured by multiplex ELISA after treatment with rituximab and denileukin diftitox (n=13). While IL-2R decreased with therapy, serum levels of IL-2 remained essentially unchanged and a compensatory increase in IL-15 and IP-10 was seen.

Table 1

Patient Baseline Characteristics

	Total (N=23)
Age	
N	23
Median	60.0
Range	(27.0–79.0)
Gender	
female	11 (47.8%)
male	12 (52.2%)
Performance Score	
0	19 (82.6%)
1	4 (17.4%)
FLIPI Score	
Low Risk	3 (13%)
Intermediate Risk	14 (60.9%)
Poor Risk	6 (26.1%)
Age Group	
≤60	14 (60.9%)
>60	9 (39.1%)
Clinical Stage	
III	7 (30.4%)
IV	16 (69.6%)
Hemoglobin	
≥12g/dL	22 (95.7%)
<12g/dL	1 (4.3%)
Number Of Nodal Sites	
≤4	7 (30.4%)
>4	16 (69.6%)

Table 2

Grade 3/4 Adverse Events by organ site - regardless of attribution.

Toxicity	Grade											
	3		4		5		3		4		5	
	N	%	N	%	N	%	N	%	N	%	N	%
Hematology	Lymphopenia	2	9									
	Neutropenia			1	4							
	Increased ALT	3	13									
Hepatic	Increased AST	3	13									
	Hypoalbuminemia	1	4									
	Catheter related infection	1	4									
Infection	Wound infection	1	4									
	Limb edema	2	9									
	Hyperglycemia	2	9									
Metabolic	Hypophosphatemia	1	4									
	Hypokalemia	1	4									
	Hyperkalemia	1	4									
	Hyponatremia	4	17									
	Muscle weakness	2	9									
	Myositis			2	9							
Neurology	Decreased level of consciousness			1	4							
	Syncope	1	4									
	Chest pain	1	4									
Pain	Headache	1	4									
	Angina pectoris	1	4									
	Body pain	1	4									
	Dyspnea			1	4							
Pulmonary	Hypoxia			1	4							
	Hypersensitivity	2	9									
Cardiovascular	Capillary leak syndrome	4	17	1	4	1	4	1	4	1	4	

Toxicity	Grade					
	3		4		5	
	N	%	N	%	N	%
Hypotension Cardiac ischemia Thrombosis Ventricular tachycardia Left ventricular failure Valvular heart disease			2	9		
			1	4		
			2	9		
			1	4		
	1	4				
			1	4		
Coagulation						
Prolonged aPTT	1	4				
Constitutional symptoms	1	4	1	4		
Fatigue						
Rash	1	4				
Dermatology/skin						
Gastrointestinal	1	4				
Dehydration	1	4				
Enteritis	1	4				
Small bowel obstruction	1	4				

ALT - alanine aminotransferase, AST - aspartate aminotransferase, aPTT activated partial prothrombin time