

*Featured Article*

# Phase I Studies of Interleukin (IL)-2 and Rituximab in B-Cell Non-Hodgkin's Lymphoma: IL-2 Mediated Natural Killer Cell Expansion Correlations with Clinical Response

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**Abstract**

**Purpose:** Expansion and activation of natural killer (NK) cells with interleukin-2 (IL-2) may enhance antibody-dependent cellular cytotoxicity (ADCC), an important mechanism of rituximab activity. Two parallel Phase I studies evaluated combination therapy with rituximab and IL-2 in relapsed or refractory B-cell non-Hodgkin's lymphoma (NHL).

**Experimental Design:** Thirty-four patients with advanced NHL received rituximab (375 mg/m<sup>2</sup> i.v. weekly, weeks 1–4) and escalating doses of s.c. IL-2 [2–7.5 MIU daily (*n* = 19) or 4.5–14 million international units three times weekly (*n* = 15), weeks 2–5]. Safety, tolerability, clinical responses, NK cell counts, and ADCC activity were evaluated.

**Results:** Maximally tolerated doses (MTD) of IL-2 were 6 MIU daily and 14 million international units thrice weekly. The most common adverse events were fever, chills, and injection site reactions. Dose-limiting toxicities were fatigue and reversible liver enzyme test elevations. Of the 9 patients enrolled at the daily schedule MTD, 5 showed clinical response. On the thrice-weekly schedule at the MTD, 4 of 5 patients responded. Responders showed median time to progression of 14.9 and 16.1 months, respectively, for the two

studies. For the same total weekly dose, thrice-weekly IL-2 administration induced greater increases in NK cell counts than daily dosing, and NK cells correlated with clinical response on the thrice-weekly regimen. ADCC activity was increased and maintained after IL-2 therapy in responding and stable disease patients.

**Conclusions:** Addition of IL-2 to rituximab therapy is safe and, using thrice-weekly IL-2 dosing, results in NK cell expansion that correlates with response. This combination treatment regimen merits additional evaluation in a randomized clinical trial.

**Introduction**

Rituximab, a chimeric IgG1 monoclonal antibody directed against the CD20 antigen, is expressed on >90% of all B-cell lymphomas and is frequently used in the treatment of patients with follicular and other types of B-cell non-Hodgkin's lymphoma (NHL; Ref. 1). Approximately 50% of patients with relapsed or refractory low-grade B-cell lymphoma respond to their first treatment with single-agent rituximab (2, 3). As first line-therapy, rituximab produces responses in up to 75% of patients with follicular or low-grade NHL (4, 5). Whereas these data are encouraging, responses to single-agent rituximab are usually partial, with a complete response rate of only 6% after initial treatment, and responses are generally of limited duration (2). Furthermore, studies evaluating retreatment of patients responding previously to rituximab demonstrated an overall response rate (ORR) of only 38% (40% in evaluable patients; Ref. 6). ORR in patients with relapsed or refractory small cell lymphocytic lymphoma is only 12% (2), and intermediate-grade lymphoma patients who had received up to two prior chemotherapy regimens and no prior rituximab showed an ORR of only ~30% (7). Strategies to increase the clinical effectiveness of rituximab are, therefore, needed and are being explored, including alternative dosing regimens and combinations with chemotherapy.

The precise mechanisms by which rituximab exerts its effects are not fully understood, although accumulating data suggest that antibody-dependent cellular cytotoxicity (ADCC) may play a dominant role (8–10). Other important mechanisms implicated in the activity of rituximab include the direct induction of apoptosis and complement-dependent cytotoxicity (CDC; Refs. 8, 11, 12).

ADCC is mediated through immune effector cells, including natural killer (NK) cells, monocytes, and macrophages, which engage the Fc portion of IgG through specific receptors (FcγR; Refs. 13–18). NK cells account for approximately 10–15% of human lymphocytes and are important mediators of ADCC (19, 20). Two distinct subsets of human NK cells have been defined phenotypically by the reciprocal expression of the CD16 (FcγRIII) and CD56 receptors (20). Importantly, the

Received 7/28/03; revised 1/7/04; accepted 1/8/04.

**Grant support:** Research funding from Chiron Corporation (W. Gluck, A. Yuen, A. Levine, M. Dayton, J. Gockerman, and J. Lucas). 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.

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majority (~90%) of human NK cells are CD16+ CD56dim and express the intermediate affinity interleukin-2 (IL-2) receptor (IL-2R $\beta\gamma$ , kDa =  $10^{-9}$  M; Refs. 21, 22). Thus, intermediate doses of IL-2 are capable of expanding CD16+ (Fc $\gamma$ RIIIa) NK cells and activating cytotoxic effector functions, including ADCC activity (21–23). Through these mechanisms, IL-2 may enhance the cytotoxicity and clinical efficacy of antitumor monoclonal antibodies.

Preclinical data support this theory. In a human B-cell lymphoma xenograft model, the combination of IL-2 with an anti-CD20 monoclonal antibody resulted in substantially greater tumor growth inhibition than is observed with either agent alone, achieving complete and long-term eradication of tumor in some animals (24).

In clinical studies, IL-2 effectively expands NK cell populations through prolonged administration schedules at doses that are generally well tolerated (25–29). Increased NK cell cytotoxic activity, including ADCC activity, has also been demonstrated (27, 28).

On the basis of these preclinical and clinical data, which provide a strong rationale for adding IL-2 to rituximab therapy, we conducted two Phase I studies of s.c. recombinant IL-2, administered either daily (study NHL01) or thrice weekly (study NHL02), in combination with rituximab in patients with CD20+ B-cell NHL. This report summarizes the effects of combining recombinant IL-2 with rituximab on NK cell expansion and ADCC activity, correlates cellular responses with clinical response, and demonstrates the safety of combination rituximab and IL-2 therapy.

## Patients and Methods

**Patients.** Patients with stage III or IV histologically confirmed CD20+ B-cell NHL in relapse or refractory to prior therapy were eligible for enrollment. Patients with primary central nervous system lymphoma or lymphomatous meningitis were ineligible. Patients had to be at least 18 years of age, have a Karnofsky Performance Score of at least 70%, and provide written informed consent in accordance with Institutional Review Board guidelines. The patient or a personal assistant had to have been willing and able to administer IL-2 by s.c. injection in an outpatient setting.

Within 2 weeks before treatment initiation, patients were required to have laboratory values as follows: absolute neutrophil count of  $\geq 1,000/\mu\text{l}$ , platelet count of  $\geq 75,000/\mu\text{l}$ , lymphocyte count  $\leq 20,000/\mu\text{l}$ , serum creatinine  $\leq 1.5$  mg/dl, total bilirubin  $\leq 3$  mg/dl, and alanine aminotransferase and aspartate aminotransferase levels  $<2.5$  times institutional upper limits of normal. Women of childbearing age were required to have a negative pregnancy test within 2 weeks of treatment initiation, and all of the patients were to use appropriate contraception while on study.

Previous treatment with rituximab was allowed provided it was completed at least 3 months before study entry. Patients were excluded if they had received prior radioimmunotherapy with ibritumomab tiuxetan or tositumomab, had received IL-2 previously, had a contraindication to receiving rituximab, had a history of type I hypersensitivity to murine proteins, had a previous or concurrent malignancy (except for inactive non-

melanoma skin cancer, cervical carcinoma *in situ*, or other solid tumor treated curatively and without evidence of recurrence for at least 2 years before study entry), had symptomatic thyroid disease requiring medical intervention other than replacement therapy for hypothyroidism, a history of autoimmune disease, human immunodeficiency virus, clinically significant cardiac, pulmonary, or hepatic disease, or serious active infection, had undergone organ transplantation, allogeneic bone marrow transplant, or had who required cyclosporine or other immunosuppressive medication. Patients could not have had chemotherapy, radiation therapy, or major surgery in the 30 days before treatment. During the study period, concomitant treatment with radiation therapy, any IFN, chemotherapy, or systemic corticosteroids was prohibited.

**Study Design.** Studies NHL01 and NHL02 were open-label Phase I trials evaluating escalating doses of IL-2 (aldesleukin; Proleukin), delivered either daily (NHL01) or thrice weekly (NHL02), in combination with a fixed dose of rituximab (Rituxan). The studies enrolled initial cohorts of 3 patients at each dose level, and it was required that the cohort complete the 5-week treatment course before opening accrual to the next higher dose level. Patients were assigned to treatment groups sequentially as they enrolled on the study, with no randomization for baseline characteristics. In the event a dose-limiting toxicity (DLT) occurred, an additional 3 patients were enrolled at that dose level. If  $\geq 2$  patients experienced a DLT at the same dose level, the preceding lower dose level would be declared the maximum tolerated dose (MTD). In study NHL01, an additional 5 patients were accrued to the MTD level to allow for additional data to be collected at this dose level.

Toxicities were graded according National Cancer Institute Common Toxicity Criteria. A DLT was defined as treatment-related adverse event of grade 4 for hematological or febrile events, or grade 3 or 4 for other adverse events. The design of these studies was reviewed and approved by the Institutional Review Boards of the investigation sites.

**Treatment.** In NHL01, patients received rituximab 375 mg/m<sup>2</sup> administered i.v. weekly for 4 consecutive weeks; starting at week 2, IL-2 was administered by s.c. injection daily for 4 consecutive weeks, at daily doses of 2, 4.5, 6, or 7.5 MIU, corresponding to cumulative weekly IL-2 doses 14, 31.5, 42, and 52.5 MIU per week, respectively. In NHL02, patients received rituximab 375 mg/m<sup>2</sup> administered i.v. weekly for 4 consecutive weeks; starting at week 2, IL-2 was administered by s.c. injection thrice weekly for 4 consecutive weeks, at doses of 4.5, 10, 14, or 18 MIU, corresponding to cumulative weekly IL-2 doses of 13.5, 30, 42, and 54 MIU per week, comparable with the cumulative weekly doses administered in NHL01. Treatment schemas for both studies appear in Fig. 1.

It was determined that the highest single doses of IL-2 to be administered would be 7.5 MIU daily in NHL01 and 18 MIU thrice weekly in NHL02, based on previous clinical experience. Dose modifications were not allowed except in those patients who experienced a DLT. For these patients, treatment was discontinued until resolution of the toxicity to baseline or grade 1, at which time treatment could be reinstated at the next lower dose level of IL-2.

**Toxicity and Response Evaluation.** Within 2 weeks before treatment initiation, a complete history and physical exam-

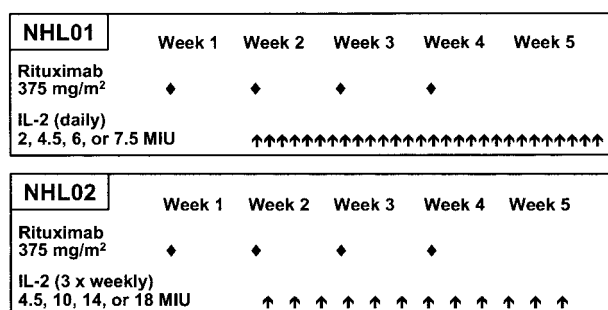


Fig. 1 Treatment schema, studies NHL01 and NHL02.

ination was performed, and a baseline complete blood count, platelet count, and serum chemistries were obtained for each patient. On study weeks 1–6, week 10, and week 14, physical examinations, complete blood counts, platelet counts, and serum chemistries were repeated, and toxicity evaluations performed and graded using National Cancer Institute Common Toxicity Criteria guidelines.

Baseline disease evaluation and appropriate radiological scans for tumor assessment were performed within 4 weeks preceding treatment initiation. Repeat tumor assessments were scheduled for weeks 6, 10, and 14. Tumor responses were defined according to International Workshop Response Criteria (30), and all of the responses had to be confirmed by a second evaluation at least 4 weeks after the initial indication of response. All of the subjects were followed for tumor status and survival until the last subject completed study week 14.

**Safety Evaluation.** Investigators classified adverse events by their relationship to study treatment and severity of the event (grade) according to the National Cancer Institute Common Toxicity Criteria.

**Lymphocyte Subset Analysis.** Lymphocyte subsets in the peripheral blood of patients were quantified using flow cytometry at a central laboratory (Covance Laboratories). Blood samples were obtained before commencement of rituximab treatment (week 1), before commencement of IL-2 treatment (week 2), and after 1, 2, and 9 weeks of IL-2 treatment (study days 15, 22, and 63, respectively). Lymphocyte subsets quantified included T cells (CD3, CD4, and CD8), B-cells (CD19 and CD20), and NK cells (CD16/CD56).

**ADCC Assays.** ADCC in patient blood samples was measured by examining the degree of lysis of a <sup>51</sup>Cr-labeled human B-lymphoma target cell line mediated by peripheral blood mononuclear cells (PBMCs) in the presence or absence of rituximab. Patient blood samples were collected before commencement of rituximab treatment (day 1), before commencement of IL-2 treatment (day 8), and after 1, 2, and 9 weeks of IL-2 treatment (study days 15, 22, and 63, respectively). Blood samples were collected in acid citrate dextrose and PBMCs isolated by Ficoll Hypaque density centrifugation. Freshly isolated PBMCs were tested at an E:T ratio range of 50:1 to 1.5:1 against <sup>51</sup>Cr-labeled Daudi cells (target cells) in the presence of a predetermined optimal concentration of rituximab (2 µg/ml) to evaluate ADCC activity. After a 4-h incubation period at 37°C, cells were pelleted by centrifugation and 50 µl of supernatant

harvested for quantification of <sup>51</sup>Cr release. Spontaneous and maximum <sup>51</sup>Cr release was determined by incubation of target cells alone in the presence of 2 µg/ml rituximab in culture media or in media containing 1% Triton X-100. The percentage of specific lysis, using the mean value of triplicate samples, was calculated as follows: % lysis = 100 × [(experimental cpm – spontaneous cpm)/(maximum cpm – spontaneous cpm)].

**Statistical Methods.** All of the patients receiving at least one dose of IL-2 were included in safety analysis. Clinical adverse event data were assigned preferred terms using the MedDRA thesaurus. The number and percentage of patients and events were calculated according to IL-2 dose level. If an individual event occurred more than once for a patient, the most severe grade was used to characterize this unified event.

Individual subjects had to receive all 4 of the planned doses of rituximab, at least 70% of their planned total IL-2 doses, and to have not missed >2 consecutive doses of IL-2 to be considered as having received a potentially therapeutic dose and be evaluable for efficacy analysis. Response rate was the proportion of patients who showed a clinical response [complete response (CR) or partial response (PR)] at any time of the study. Response rate was calculated for all of the enrolled patients (intent to treat, ITT) as well as for all patients evaluable for efficacy. Time to progression was defined as the time from first rituximab infusion to first documented disease progression or death from all causes, whichever came first. Response rate and time to progression were summarized descriptively. The exact Wilcoxon rank sum test was used to test for differences in NK cell counts between responders and nonresponders, and two-sided P values < 0.05 were considered statistically significant.

**Results**

**Patient Characteristics**

In NHL01, a total of 19 subjects were enrolled across four study sites. NHL histological subtypes at enrollment included 5 diffuse large cell lymphoma, 9 follicular lymphoma (4 grade I, 2 grade II, and 3 grade III), 2 small lymphocytic lymphoma, and 1 each of mantle cell lymphoma, extranodal marginal zone lymphoma, and lymphoplasmacytoid lymphoma, respectively (Table 1). The median number of prior therapies was 4 (range, 1–10). Ten patients who participated in study NHL01 had been treated previously with a total of 12 courses of rituximab therapy, either alone (n = 8), with combination chemotherapy (n = 2), with IFN (n = 1), or with radiation therapy (n = 1). Two patients received 2 courses of rituximab: 1 had 2 courses of single agent rituximab and the other had 1 course of rituximab alone and 1 of rituximab combined with chemotherapy.

A total of 15 patients were enrolled in NHL02 across three study sites. NHL histological subtypes at enrollment included 7 follicular lymphoma (4 grade I and 3 grade II), 4 diffuse large cell lymphoma, 2 small cell lymphocytic lymphoma, 1 extranodal marginal zone lymphoma, and 1 mantle cell lymphoma (Table 1). The median number of prior therapies was 4 (range, 2–14). Ten patients who participated in study NHL02 had been treated previously with a total of 16 prior courses of rituximab therapy, alone (n = 13), with combination chemotherapy (n = 2), or with chemotherapy and transplantation (n = 1). Two patients had received 2 prior courses of therapy with rituximab

Table 1 Patient characteristics

Characteristic	NHL01 (n = 19)		NHL02 (n = 15)	
	No. of patients		No. of patients	
Median age, yr. (range)	63 (36–80)		58 (35–76)	
Sex				
Male	9		8	
Female	10		7	
Median Karnofsky performance status (range)	90 (70–100)		100 (80–100)	
NHL <sup>a</sup> histology at enrollment				
Follicular	9		7	
Diffuse large cell	5		4	
Small lymphocytic lymphoma	2		2	
Mantle cell	1		1	
Extranodal marginal zone B-cell lymphoplasmacytic	1		0	
Stage				
III	4		4	
IV	15		11	
Median time from initial NHL diagnosis, yrs. (range)	4.6 (0.4–24)		5.9 (0.9–17.3)	
Median no. of prior therapies (range)	4 (1–10)		4 (2–14)	
Prior NHL therapy				
Chemotherapy	19		15	
Chemotherapy + rituximab	2		3	
Rituximab alone	8		9	
Immunotherapy <sup>b</sup>	5		0	
High-dose chemotherapy	4		2	
Radiation therapy	10		8	

<sup>a</sup> NHL, non-Hodgkin's lymphoma.

<sup>b</sup> Immunotherapy: dendritic cell vaccine (1), idiotype vaccine (1), idiotype vaccine and dendritic vaccine (1), interleukin-4 (1), interferon with rituximab (1).

(1 in combination with high-dose chemotherapy and autologous transplant), and 2 patients had 3 prior courses of therapy with rituximab (1 in combination with chemotherapy).

### Toxicities and MTD

Seventeen of 19 (89%) enrolled patients in study NHL01 completed the planned study treatment, receiving all 4 of the doses of rituximab and at least 70% of the scheduled IL-2 doses. Two patients discontinued treatment early because of adverse events at study days 9 and 10, respectively. One patient in the 2 MIU cohort developed bacterial pneumonia that was considered not related to treatment, and 1 patient in the 6 MIU cohort had a grade 4 hypersensitivity reaction, which was considered to be a DLT. A DLT occurred in 2 of 3 patients treated in the 7.5 MIU

cohort. In this cohort, a transient grade 3 increase in aspartate aminotransferase and grade 3 fatigue occurred in 1 patient, and another patient experienced grade 3 fatigue (Table 2). The MTD for IL-2 in protocol NHL01 was determined to be 6 MIU administered on a daily basis. The most common treatment-related adverse effects were fever, chills, injection site reactions, and fatigue; these were predominantly grade 1 or 2 (Table 3).

In study NHL02, 13 of 15 (87%) enrolled patients completed all of the planned study treatment, receiving all 4 doses of rituximab and at least 70% of the scheduled IL-2 doses. Two patients discontinued the study prematurely; a patient receiving the 14 MIU dose chose to withdraw at study day 12 after experiencing grade 2 rigors, and a patient at the 18 MIU dose was discontinued because of a grade 3 hypersensitivity reaction

Table 2 Dose-limiting toxicities

Study	IL-2 <sup>a</sup> dose cohort	No. of patients enrolled	Patients experiencing a dose-limiting toxicity
NHL01 (IL-2-daily)	2 MIU	4	None
	4.5 MIU	3	None
	6 MIU	9	Hypersensitivity reaction (1)
	7.5 MIU	3	Elevated AST, fatigue (1), fatigue (1)
NHL02 (IL-2-3 × per week)	4.5 MIU	3	None
	10 MIU	3	None
	14 MIU	5	None
	18 MIU	4	Hypersensitivity reaction (1), elevated ALT (1)

<sup>a</sup> IL, interleukin; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 3 Most common treatment-related toxicities, study NHL01<sup>a</sup>

Toxicity	IL-2 <sup>b</sup> dose cohort									
	2 MIU (n = 4)		4.5 MIU (n = 3)		6 MIU (n = 9)		7.5 MIU (n = 3)		All patients (n = 19)	
	Grade 1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4
Nausea	–	–	1	–	2	–	2	–	5	–
Fatigue	2	–	1	1	5	–	–	2	8	3
Injection site erythema	1	–	–	–	2	–	–	–	3	–
Injection site reaction	2	–	3	–	4	–	3	–	12	–
Fever	–	–	2	–	6	–	2	1	10	1
Chills	–	–	2	–	5	–	3	–	10	–
Decreased weight	–	–	1	–	–	–	2	–	3	–
Anorexia	1	–	–	1	–	–	1	–	2	1
Decreased appetite	–	–	–	–	2	–	1	–	3	–
Myalgia	–	–	1	–	4	1	–	–	5	1
Headache	–	–	1	–	3	–	1	–	5	–
Insomnia	1	–	1	–	3	–	–	–	5	–
Dyspnea	–	–	1	–	1	–	1	–	3	–

<sup>a</sup> Toxicities of all grades occurring in  $\geq 3$  patients.

<sup>b</sup> IL, interleukin.

temporally related to IL-2 administration on study day 15. The 18 MIU IL-2 dose was determined to be above the MTD, as DLTs occurred in 2 of 4 patients treated in this cohort. These included the grade 3 hypersensitivity reaction in 1 patient and, in the other patient, a grade 3 alanine aminotransferase elevation measured at a single time point. The alanine aminotransferase promptly returned to normal levels after discontinuation of concomitant atorvastatin (Lipitor) therapy without dose decrease or interruption of IL-2 therapy. The MTD for IL-2 in protocol NHL02 was determined to be 14 MIU administered thrice

weekly. As with the daily dosing schedule for IL-2, the most common adverse reactions observed with thrice-weekly IL-2 treatment were fever, chills, injection site reactions, and fatigue (Table 4). The majority of adverse reactions were grade 1 or 2, and no treatment-related grade 4 toxicities were observed.

#### Clinical Response

**NHL01.** Seventeen of 19 enrolled patients in study NHL01 were evaluable for response. Five patients responded to therapy, with 1 CR and 4 PRs, for an ORR of 5 of 19 (26.3%)

Table 4 Most common treatment-related toxicities, study NHL02<sup>a</sup>

Toxicity	IL-2 <sup>b</sup> dose cohort									
	4.5 MIU (n = 3)		10 MIU (n = 3)		14 MIU (n = 5)		18 MIU (n = 4)		All patients (n = 15)	
	Grade 1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4
Diarrhea	1	–	–	–	1	–	1	–	3	–
Nausea	–	–	1	–	3	–	2	–	6	–
Fatigue	2	–	2	–	4	–	4	–	12	–
Flu-like symptoms	1	–	–	–	1	–	1	–	3	–
Injection site erythema	1	–	1	–	1	–	2	–	5	–
Injection site pain	–	–	–	–	2	–	1	–	3	–
Injection site reaction	3	–	1	–	3	–	3	–	10	–
Fever	1	–	2	–	3	–	4	–	10	–
Chills	3	–	3	–	5	–	3	–	14	–
Pain	1	–	–	–	–	–	2	–	3	–
Edema	–	–	–	–	2	–	1	–	3	–
Elevated ALT	–	–	–	–	1	–	1	1	2	1
Tachycardia	–	–	–	–	–	–	3	–	3	–
Anorexia	–	–	1	–	2	–	2	–	5	–
Myalgia	1	–	1	–	2	–	2	–	6	–
Headache	–	–	1	–	2	–	2	–	5	–
Insomnia	–	–	1	–	2	–	2	–	5	–
Nasal congestion	1	–	–	–	–	–	2	–	3	–
Erythema	1	–	1	–	–	–	3	–	5	–
Rash	–	–	–	–	1	–	3	–	4	–

<sup>a</sup> Toxicities of all grades occurring in  $\geq 3$  patients.

<sup>b</sup> IL, interleukin; ALT, alanine aminotransferase.

Table 5 Response of patients to therapy in different IL-2<sup>a</sup> dose cohorts

A. NHL01 IL-2 daily					
IL-2 dose cohort (cumulative weekly IL-2 dose)					
Response	2 MIU (14 MIU)	4.5 MIU (31.5 MIU)	6.0 MIU (42 MIU)	7.5 MIU (52.5 MIU)	Total
CR	–	–	1	–	1
PR	–	–	4	–	4
SD	1	3	1	1	6
PD	2	–	2	2	6
NE	1	–	1	–	2
ORR-ITT	0/4 (0%)	0/3 (0%)	5/9 (55.6%)	0/3 (0%) <sup>b</sup>	5/19 (26.3%)
ORR-Eval	0/3 (0%)	0/3 (0%)	5/8 (62.5%)	0/3 (0%) <sup>b</sup>	5 (29.4%)

B. NHL02 IL-2 3×/wk					
Response	4.5 MIU (13.5 MIU)	10 MIU (30 MIU)	14 MIU (42 MIU)	18 MIU (54 MIU)	Total
CR	–	2	2	1	5
PR	–	–	2	1	3
SD	2	–	–	1	3
PD	1	1	–	–	2
NE	–	–	1	1	2
ORR-ITT	0/3 (0%)	2/3 (66.7%)	4/5 (80%)	2/4 (50%) <sup>c</sup>	8/15 (53.3%)
ORR-Eval	0/3 (0%)	2/3 (66.7%)	4/4 (100%)	2/3 (67%)	8/13 (61.5%)
Total	3	3	5	4	15

<sup>a</sup> IL, interleukin; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable for efficacy; ORR-ITT, overall response rate (CR+PR) for intent to treat population; ORR-eval, overall response rate (CR+PR) for patients evaluable for efficacy.

<sup>b</sup> 7.5 MIU/day was above the maximum tolerated dose; 2 patients required IL-2 dose delays and dose reductions secondary to toxicity.

<sup>c</sup> 18 MIU/3×/week was above the maximum tolerated dose; 1 patient developed dose-limiting toxicity and discontinued IL-2.

for all of the enrolled patients or 5 of 17 (29.4%) for evaluable patients (Table 5). None of the 7 patients treated with daily IL-2 doses <6.0 MIU/day achieved an objective response, whereas 5 of 9 (55.6%) patients or 5 of 8 (62.5%) evaluable patients who received daily doses of IL-2 of 6.0 MIU achieved a CR (1 patient) or PR (4 patients). The patient achieving complete response had marginal-zone B-cell lymphoma, whereas the partial responses occurred in 2 patients with follicular grade I lymphoma and 1 patient each with lymphoplasmacytic lymphoma and follicular grade III lymphoma, respectively. Two of the 5 (40%) responding patients had previously received rituximab and had achieved a CR ( $n = 1$ ) or PR ( $n = 1$ ) to prior therapy.

Comparison of responses to the current treatment with IL-2 and rituximab with patients' prior responses to rituximab alone or to rituximab combinations is provided in Table 6. Two of the responding patients in study NHL01 had shown the same responses (1 CR and 1 PR) to prior treatment with single agent rituximab in the past. Of the 3 patients with stable disease (SD) on the current study, 1 had shown a PR, and the other 2 had developed progressive disease (PD) after prior rituximab and rituximab combined with IFN, respectively. The patient who previously failed the IFN combination has continued with SD status after the IL-2 combination treatment through the last observation at 9.6 months after treatment. Two of 4 (50%) ITT and 2 of 3 (67%) evaluable patients treated previously with rituximab who received the recommended dose of IL-2, 6.0 MIU per day, achieved an objective response. In contrast, 0 of the 4 patients who had received rituximab previously and who received IL-2 at a dose <6.0 MIU/dose achieved an objective response (Table 6).

For all of the responding patients in the NHL01 study, the median time to best response was 6 weeks (range, 5–18 weeks). The median time to progression (TTP) in responding patients was 14.9 months (range, 8.9–18.9 months), overall. Four study subjects, 2 with SD as best response and 2 who achieved a PR, continue to be progression-free at 4.3, 8.9, 9.6, and 14.9 months, respectively.

**NHL02.** In study NHL02, 8 patients responded to therapy on the thrice weekly schedule, with 5 CRs and 3 PRs (Table 5). Thirteen of 15 enrolled patients were evaluable for response. The ORR was 8 of 15 (53.3%) for all of the enrolled patients or 8 of 13 (61.5%) for evaluable patients (Table 5). None of the 3 (0%) patients treated with thrice weekly IL-2 doses of 4.5 MIU achieved an objective response, 2 of 3 (66.7%) patients treated with doses of 10 MIU achieved an objective response (both CR), and 4 of 5 (80%) ITT patients or 4 of 4 (100%) of evaluable patients treated with doses of 14 MIU achieved an objective response (2 CR and 2 PR). Complete responses occurred in 1 patient with diffuse large cell lymphoma, 1 patient with follicular grade I lymphoma, 2 patients with follicular grade II lymphoma, and 1 patient with extranodal marginal-zone lymphoma. PRs occurred in 1 patient each with small cell lymphocytic lymphoma, diffuse large cell, and mantle cell lymphoma, respectively.

Three responding patients on study NHL02 had responded previously to single agent rituximab (Table 6). In addition, 1 patient (ID 2–3045) who had developed PD after single agent rituximab achieved a PR lasting 12.4 months after IL-2/rituximab treatment. The current therapy was this individual's fifth treatment course for diffuse large cell NHL, after treatment that included high-dose chemotherapy and autologous transplant

Table 6 Response to treatment in subjects who received prior rituximab

IL-2 dose	Patient ID	Time since last prior rituximab (R) or rituximab combination (type/s of rituximab regimen)	Response to prior rituximab regimen	Response to IL-2 + rituximab	Overall response rate (CR+PR) to IL-2 + rituximab Number/total (%) Patients for each dose level	
					All patients	Evaluable
A. Study NHL01 (IL-2 <sup>a</sup> daily)						
2.0 MIU	1-1001	>12 mo (R + chemotherapy)	SD (comb)	NE	0/2 (0%)	0/1 (0%)
2.0 MIU	1-2003	>12 mo	SD	PD		
4.5 MIU	1-2042	4–8 mo	PR	SD	0/2 (0%)	0/2 (0%)
4.5 MIU	1-2043	>12 mo (R + interferon)	PD (comb)	SD		
6.0 MIU	1-3061	4–8 mo	PR, PR	NE	2/4 (50%)	2/3 (67%)
6.0 MIU	1-3062	>12 mo	CR	CR		
6.0 MIU	1-2063	>12 mo	PR	PR		
6.0 MIU	1-2065	>12 mo ago (R + chemotherapy)	CR, PD (comb)	PD		
7.5 MIU <sup>b</sup>	1-2081	4–8 mo (R + radiation)	PR (comb)	PD	0/2 (0%) <sup>b</sup>	0/2 (0%) <sup>b</sup>
7.5 MIU <sup>b</sup>	1-1082	>12 mo	PD	SD		
B. Study NHL02 (IL-2 thrice weekly)						
4.5 MIU	2-2001	9–12 mo (R)	PR	SD	0/2 (0%)	0/2 (0%)
4.5 MIU	2-2002	>12 mo (R,R)	SD, SD	SD		
10 MIU	2-1021	>12 mo (R)	PR	CR	1 / 1	1 / 1
14 MIU	2-1041	>12 mo (R)	PR	PR	2/3 (67%)	2/2 (100%)
14 MIU	2-2042	>12 mo (R)	CR	NE		
14 MIU	2-3045	4–8 mo (R, R + chemotherapy/transplant)	PD, PR (comb)	PR		
18 MIU	2-2061	>12 mo (R + chemotherapy)	CR (comb)	CR	2/4 (50%) <sup>c</sup>	2/3 (67%) <sup>c</sup>
18 MIU	2-2062	>12 mo (R)	CR	PR		
18 MIU <sup>c</sup>	2-2063	>12 mo (R, R, R + chemotherapy)	PR, PR, PR (comb)	SD		
18 MIU	2-2064	>12 mo (R, R, R)	CR, SD, CR	NE		

<sup>a</sup> IL, interleukin; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable for tumor response due to premature study withdrawal; Comb, indicates response was to a combination therapy of rituximab plus chemotherapy, radiation (RT), or interferon.

<sup>b</sup> IL-2 dose of 7.5 MIU/day was above the maximum tolerated dose.

<sup>c</sup> IL-2 dose of 18 MIU 3×/week was above the maximum tolerated dose.

with rituximab, 2 other chemotherapy regimens, and radiation therapy. Another patient (2–2061), with a CR lasting 17.8 months after IL-2 and rituximab, had previously achieved a CR after combination treatment with rituximab plus Fludarabine/Mitozantrone for treatment of extranodal marginal zone NHL. The ORR in the patients on study NHL02 treated previously with rituximab was 5 of 10 (50%) for ITT patients and 5 of 8 (62.5%) for evaluable patients. At the recommended dose of IL-2 (14 MIU three times a week) 2 of 3 (67%) ITT patients and 2 of 2 (100%) evaluable patients treated previously with rituximab achieved an objective response *versus* only 1 of 3 (33%) patients who received IL-2 at a dose <14 MIU three times per week (Table 6).

For all of the responding patients in the NHL02 study, the median time to best response was 5 weeks (range, 5–36 weeks). The median time to disease progression in all of the responding patients was 16.1 months (range, 3.0–24.6 months). The time to disease progression for the 5 patients achieving a complete response was 3.2, 17.8, 20.0, 21.7, and 24.6 months, respectively. The subject with disease progression at 3.2 months had

mantle cell lymphoma. Two subjects, 1 with follicular grade I and 1 with follicular grade II histology, remain in complete remission after 21.7 and 24.6 months, respectively.

### Changes in NK Cell Counts and Clinical Response

Median NK cell counts throughout the study period for patients receiving daily IL-2 (NHL01) and thrice-weekly IL-2 (NHL02) are shown in Fig. 2. Median NK counts at baseline were somewhat higher on the NHL01 study than on the NHL02 study for three of the four dose levels, particularly at the 6.0 MIU daily dose. As expected, NK cell counts increased during the period of IL-2 administration (study weeks 2–5) in both studies. The effects were more pronounced for all of the dose levels with the thrice-weekly dosing schedule (NHL02). At the two highest dose levels, the thrice weekly IL-2 dosing was associated at week 4 with increases in median NK cells of ~7 times the baseline value; in contrast, smaller increases of ~2 times baseline were associated with the daily IL-2 dosing. In both studies, higher NK cell counts were associated with higher doses of IL-2. At week 10,

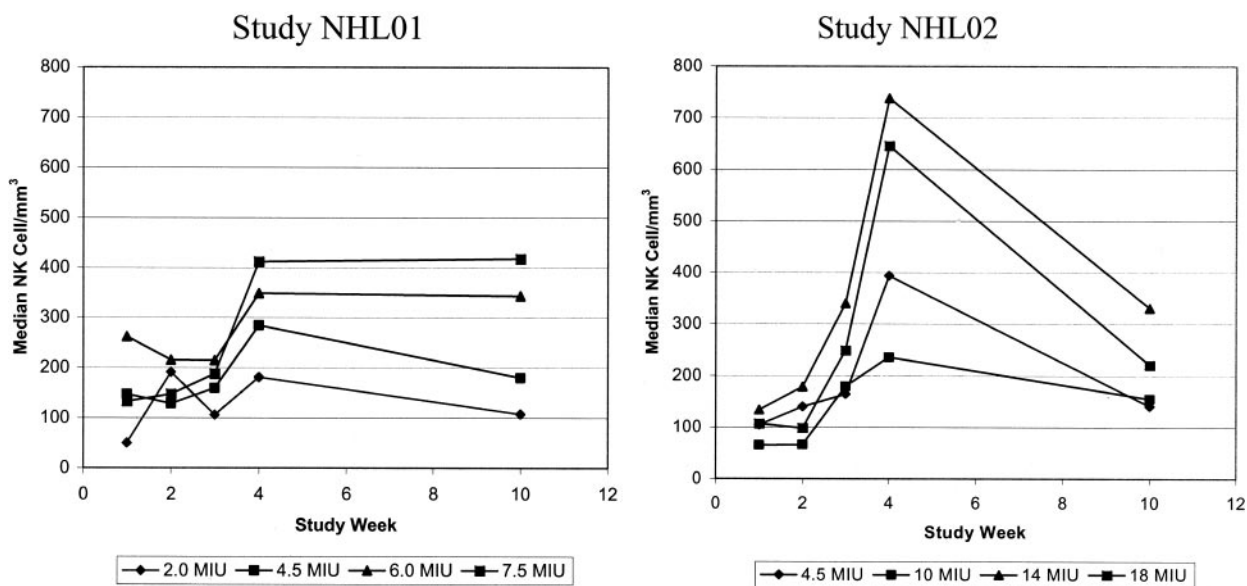


Fig. 2 Median natural killer (NK) cell counts associated with each interleukin (IL)-2 dose by week on study for study NHL01 and study NHL02. Median NK cell counts increased progressively throughout the period of IL-2 administration (study weeks 2–5). In both studies, higher NK cell counts were associated with higher doses of IL-2, although the effect was much more pronounced with the thrice-weekly IL-2 dosing regimen in study NHL02. At week 10, mean NK cell counts remained generally higher than baseline counts, especially for the two highest dose levels.

median NK cell counts remained higher than baseline counts for all of the dosing levels.

For patients on the NHL01 and NHL02 studies, the relationship between the NK cell count at week 10 and the subsequent clinical response at week 14 is shown in Fig. 3. In both studies, there was a trend toward higher absolute NK cell counts at week 10 in responders compared with nonresponders. Patients who achieved CR or PR had the highest median NK cell counts at week 10, and patients who had PD at week 14 had the lowest median NK cell counts at week 10. In NHL02, patients who achieved SD at week 14 had intermediate median NK cell count at week 10.

In NHL01, NK cell counts at baseline were not significantly higher for those who ultimately responded (CR and PR) at week 14 than for those who did not (SD and PD), with medians 262.5  $\text{mm}^3$  and 148.0  $\text{cells}/\text{mm}^3$ , respectively ( $P = 0.35$ ). Median NK cell counts at week 4 for the two groups were 544 and 339.0  $\text{cells}/\text{mm}^3$  ( $P = 0.61$ ), and at week 10 were 525.5  $\text{cells}/\text{mm}^3$  and 181.5  $\text{cells}/\text{mm}^3$ , respectively ( $P = 0.37$ ). Thus, in NHL01, there was no significant difference in NK cell count between responders and nonresponders at any time point, although there was a trend toward higher NK cell counts in responding patients.

In study NHL02, NK cell counts at baseline were similar for patients who ultimately responded (CR and PR) at week 14 and those who did not (SD and PD), with medians 127.0  $\text{cells}/\text{mm}^3$  and 99.5  $\text{cells}/\text{mm}^3$ , respectively ( $P = 0.52$ ). At week 4, the NK cell counts for the two groups were also not statistically different, with medians 631.0  $\text{cells}/\text{mm}^3$  and 361.5  $\text{cells}/\text{mm}^3$ , respectively ( $P = 0.22$ ). However, at week 10, NK cell counts were significantly greater for patients who ultimately responded (CR and PR) at week 14 than for those who did not

(SD and PD), with medians 280  $\text{cells}/\text{mm}^3$  versus 155.0  $\text{cells}/\text{mm}^3$ , respectively ( $P = 0.03$ ). All of the  $P$ s were based on a two-sided exact Wilcoxon test. Thus, in NHL02, the week 10 NK cell count was correlated with clinical response at week 14, suggesting that NK cell response may be a predictive factor for clinical response associated with the thrice weekly dosing schedule. Of interest, the outlier patient in Fig. 3 with the highest NK cell count and PD in study NHL02 was an individual with mantle cell lymphoma who had a complete response at week 10 but developed a new lesion by the week 14 evaluation.

#### Changes in Other Lymphocyte Subsets

After rituximab administration, B cells (CD19) declined to very low levels within a week and remained very low throughout the 10-week observation period, as would be expected. There was an increase in T cells (CD4 and CD8) after administration of IL-2, but these increases did not correlate with clinical response. In study NHL02, 7 patients had CD4 counts  $<200$   $\text{cells}/\text{mm}^3$  at baseline. By day 15, CD4 counts rose above 200  $\text{cells}/\text{mm}^3$  in 5 of these subjects. At week 10, CD4 counts remained above 200  $\text{cells}/\text{mm}^3$  in 1 subject and returned to  $<200$   $\text{cells}/\text{mm}^3$  in 5 subjects.

#### Correlation of ADCC Activity with Response

ADCC was measured by the lytic activity of PBMCs from patients treated on study against the human Daudi B-lymphoma cell line in the presence of an optimal concentration of rituximab. In study NHL02, ADCC activity increased with IL-2 administration and was maintained throughout the study period in the majority of patients with a



clinical response or stable disease (Fig. 4). However, in patients with disease progression, ADCC activity was not maintained after IL-2 administration (Fig. 5). A similar association between profiles of ADCC activity and clinical responses was also observed in NHL01 for 6 patients treated in the 6 MIU cohort, for whom ADCC assays were conducted using freshly isolated PBMC (data not shown).

When activity was adjusted for the number of NK cells in the PBMC specimens, the percentage lysis was directly related to NK cell number. This indicates that that increased ADCC was due to the increased number of NK cells rather than due to enhanced activity per cell (data not shown).

**Discussion**

Rituximab is often an effective treatment option for patients with low-grade B-cell NHL. However, a substantial number of patients will fail to respond to therapy or develop disease progression within a year (2). The mechanisms of action of

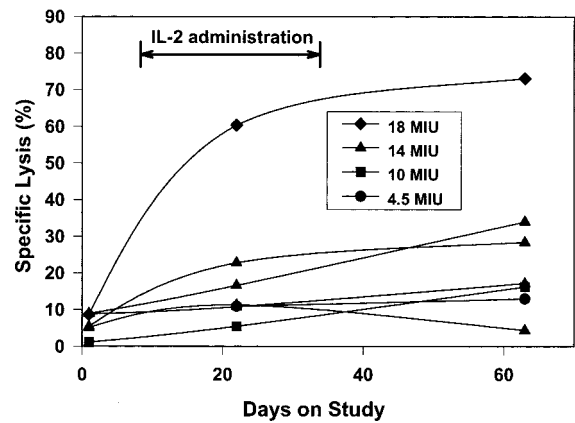


Fig. 4 Antibody-dependent cellular cytotoxicity activity in patients achieving complete or partial response or stable disease in study NHL02. Antibody-dependent cellular cytotoxicity activity was increased and generally maintained after interleukin (IL)-2 administration in patients with clinical response or stable disease at study week 14.

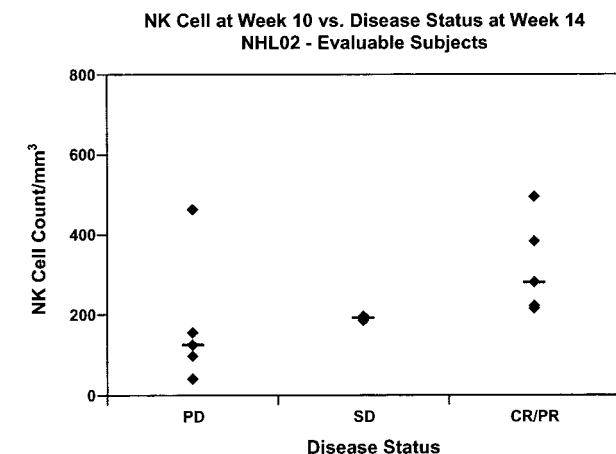
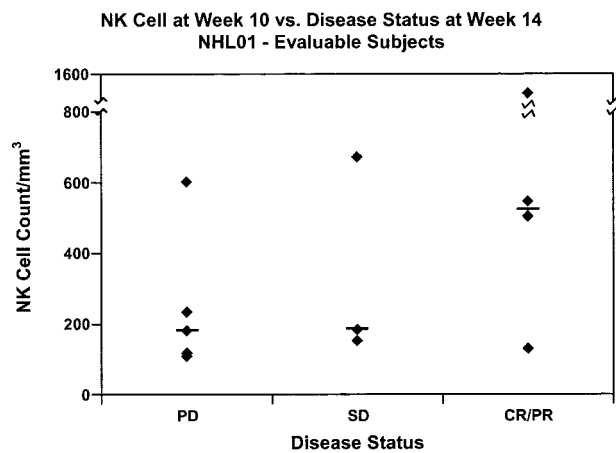


Fig. 3 Clinical response at week 14 versus natural killer (NK) cell count at week 10 for patients treated on study NHL01 and NHL02. Median NK cell counts were highest in patients who ultimately achieved complete or partial clinical responses at week 14, intermediate in patients whose week 14 outcome was stable disease (in study NHL02), and were lowest in patients whose week 14 outcome was disease progression.

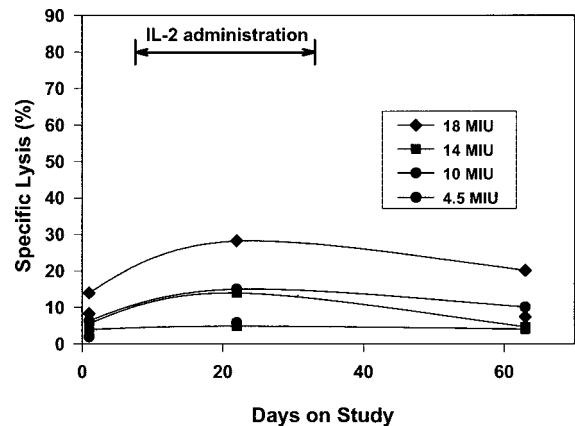


Fig. 5 Antibody-dependent cellular cytotoxicity activity in patients with disease progression in study NHL02. Antibody-dependent cellular cytotoxicity activity was not maintained after interleukin (IL)-2 administration in patients with disease progression at study week 14.

rituximab are not yet fully understood, although accumulating data suggest that ADCC is likely important (8–10). ADCC is mediated through immune effector cells, including NK cells, monocytes, and macrophages, which engage the Fc portion of IgG through specific receptors (FcγR). Preclinical studies in murine tumor FcR knockout models demonstrated that engagement of activating FcγR on effector cells plays an important role in mediating antitumor efficacy of rituximab *in vivo*, which is counterbalanced by inhibitory FcR regulatory events (13). Moreover, recent evaluation of FcR polymorphisms in rituximab-treated NHL patients has demonstrated a significantly higher clinical response rate in subjects expressing particular receptor allotypes that exhibit a higher affinity for IgG1 and increased ADCC (14–18). Thus, strong preclinical and clinical data support the investigation of approaches to augment ADCC activity through cytokine-mediated expansion and activation of NK cells.

With the goal of exploiting the stimulatory effects of IL-2 on NK cells to augment rituximab-mediated ADCC, these two Phase I studies evaluated the feasibility of IL-2 and rituximab combination therapy. IL-2 was administered by s.c. injection either daily (NHL01) or thrice weekly (NHL02), in combination with standard weekly doses of rituximab in patients with relapsed or refractory CD20+ B-cell NHL. The two studies used approximately the same total weekly dose of IL-2 at four escalating dose levels. The MTDs were determined to be 6 MIU of IL-2 administered daily and 14 MIU of IL-2 administered thrice weekly, both of which yielded a cumulative weekly IL-2 dose of 42 MIU. In almost all of the cases, the injections were self-administered on an outpatient basis, and treatment was generally well tolerated. The most common adverse effects were mild to moderate fatigue, fever, chills, and injection-site reactions. Serious adverse events occurred infrequently, with reversible liver function test elevations, hypersensitivity reactions, and fatigue found to be dose-limiting events. There was no apparent increase in rituximab-related infusional adverse events with the coadministration of IL-2.

In both studies, all of the patients had advanced-stage disease and were heavily pretreated, having had a median of four prior therapies. In the daily IL-2 dosing NHL01 study, responses occurred in 5 of 17 (29%) evaluable patients across all of the dose levels combined, or in 5 of all 19 enrolled patients (26.3%). However, in patients treated at the MTD, responses occurred in 5 of 8 evaluable patients, for an overall response rate of 62.5% in this cohort or in 5 of 9 ITT patients (55.6%). With thrice weekly IL-2 administration, responses occurred in 8 of 13 (61.5%) evaluable patients across all of the dose levels combined, or in 8 of 15 ITT patients (53.3%). In patients treated at the MTD, responses occurred in 4 of the 4 evaluable patients (100%) or 4 of the 5 ITT patients (80%), an encouraging sign of clinical activity. Moreover, a response was seen in 1 patient with diffuse large cell NHL on the NHL02 study who had not responded to prior single agent rituximab, but achieved a PR with the rituximab/IL-2 combination, lasting 12.4 months. Objective responses were observed in other patients with intermediate-grade as well as those with low-grade and follicular types of NHL.

Of importance, responses were durable, with median TTP for responding patients of 14.9 and 16.1 months for the daily and thrice weekly IL-2 dosing regimens, respectively. Both of these TTP durations are longer than has been observed for initial treatment with single agent rituximab, approximately 11–12 months, (2) but similar to the value of 17.8 months reported after retreatment with rituximab in patients with low-grade or follicular NHL who had previously responded to rituximab therapy (6). As the patients in the current studies had been heavily pretreated, such long TTP might not have been expected. Of note, it has been suggested that IFN- $\alpha$ -2a, another immune modulator, may also extend TTP when combined with rituximab therapy (31). Additional studies will be needed to determine whether IL-2, when added to rituximab therapy, leads to a significantly longer time to progression than would be observed with rituximab therapy alone.

The current Phase I population includes small numbers of patients with different tumor types and past treatment histories. Moreover, the two studies were conducted independently with

overlapping, but not identical, sites and with no randomization for patient characteristics, including baseline NK cell count. Despite the heterogeneity, however, the MTD was determined on each study to be the same weekly dose of 42 MIU/week (6.0 MIU/day in NHL01 and 14 MIU thrice weekly in NHL02, respectively). Moreover, the majority of responses on each study were observed at the MTD, and no responses were observed at the lowest doses, a consistency between the two studies that strengthens the impression of a dose response and provides evidence that IL-2 may enhance the biological activity of rituximab.

The ORR in patients who had been treated previously with rituximab was of particular interest. In NHL01, at the MTD IL-2 dose of 6.0 MIU/day, the ORR in all of the patients treated previously with rituximab was 2 of 4 (50%) among ITT patients and 2 of 3 (66.7%) among evaluable patients. In NHL02, at the MTD IL-2 dose of 14 MIU thrice weekly, the ORR in all patients treated previously with rituximab was 2 of 3 (66.7%) among ITT patients and 2 of 2 (100%) among evaluable patients. In a combined analysis of patients on both studies treated at the MTD, the ORR was 4 of 7 (57.1%) ITT patients and 4 or 5 (80%) evaluable patients. All of these ORR percentages are greater than expected response to rituximab retreatment of 38% (40% in evaluable patients) reported previously in a study restricted to patients who had responded previously to rituximab and had low-grade or follicular NHL (6), a population expected to show optimal results, because follicular histology is associated with the highest response to rituximab therapy. In contrast, on the current studies, the 7 patients at the MTD who had received prior rituximab had diffuse large cell ( $n = 2$ ), small lymphocytic ( $n = 1$ ), and extranodal marginal zone, as well as follicular ( $n = 3$ ) types of NHL, and might have been expected to show a <40% response rate to rituximab retreatment. Moreover, the patients in the current study had advanced disease and were heavily pretreated, also factors that lower expectations for favorable responses. Thus, the high ORRs observed at the MTD for IL-2 in the current study are intriguing.

The magnitude of NK cell count increased in a dose-related manner with increasing doses of IL-2. The effect was seen in both IL-2 dosing regimens, but was more pronounced with the thrice-weekly IL-2 dosing regimen used in NHL02. The absolute number of NK cells observed at week 10 after IL-2 administration correlated with the clinical response at week 14, with higher median NK cell counts observed in responding patients than in those with either SD or disease progression.

Although not providing definitive proof, these observations are intriguing and suggest that treatment with IL-2 led to increases in the NK cell count that could have contributed to or been responsible for achievement of objective clinical responses. Corresponding to the changes in NK cell number, ADCC activity was generally maintained after IL-2 administration in clinically responding patients and those with SD, whereas ADCC activity was not maintained in patients with disease progression, consistent with the effect of IL-2 on NK cell numbers

These findings extend those of a recent study conducted by Friedberg *et al.* (32) who administered IL-2 in a dose of 1.2 MIU daily for 56 days in combination with rituximab in patients with relapsed or follicular NHL. These investigators also found

the combination to be well tolerated. Significant increases in NK cell counts were observed in that study as well, but the magnitude of the increases was much smaller than in the current studies, probably due to the use of lower IL-2 doses and a daily administration schedule.

Increases in NK cells were much less prominent in the NHL01 daily dose IL-2 regimen than in the NHL02 thrice-weekly dosing regimen. These data suggest that NK cells were not as effectively stimulated with daily dosing. Although patient numbers are small, it is intriguing that response rates were also consistently higher in the thrice-weekly IL-2 dosing regimen than observed with the daily dosing IL-2 regimen, supporting the conclusion that increases in NK cells, as a result of IL-2 therapy, are important and may have contributed to the higher response rates observed with the thrice weekly IL-2 treatment regimen. Responses were seen as well at the dose below the MTD when IL-2 was administered three times weekly but were not seen at lower doses with daily administration. The correlation of responses with NK cell numbers in the NHL02 study ( $P = 0.03$ ) supports the hypothesis that NK response was critical to maximizing efficacy of antibody treatment. In contrast, the NHL01 study using daily dosing, showed less robust NK cell responses and no correlation of NK cell number with response ( $P = 0.37$ ), as well as a lower response rate and fewer complete responses.

These factors, in addition to the increased convenience and tolerability of thrice weekly dosing, strongly suggest that the thrice-weekly IL-2 dosing regimen is superior to the daily IL-2 dosing regimen. The reasons for the apparent increased effectiveness of the intermittent dosing schedule are currently being investigated in ongoing clinical studies that will provide pharmacokinetic analysis of IL-2 and IL-2 soluble receptor levels, as well as additional investigation of a possible predictive relationship between NK cell changes and clinical response.

On the basis of the encouraging response results and safety of combined IL-2/rituximab therapy observed in these Phase I studies, especially using the thrice weekly dosing regimen, we believe Phase II trials are warranted. Combined immunotherapy with IL-2 and rituximab is a feasible and rational clinical strategy for enhancing monoclonal antibody efficacy mediated by ADCC. The thrice-weekly IL-2 administration schedule appears preferable to a daily schedule based on tolerability, degree of NK cell expansion, and clinical response. This schedule is being used in ongoing trials in different NHL subpopulations as well as a randomized Phase II trial planned to start in 2004. If this treatment approach proves beneficial, the combination regimen could offer a nonmyelosuppressive alternative to patients with relapsed lymphoma who would otherwise require additional courses of chemotherapy or radioimmunoconjugates.

In conclusion, addition of s.c. IL-2 to standard rituximab therapy is safe and induces CD16/56+ NK cell expansion that correlated with response when IL-2 was administered on the preferred thrice weekly schedule. This treatment regimen may provide an important new alternative treatment option for patients with relapsed or refractory NHL and merits additional evaluation in a randomized trial.

## Acknowledgments

We thank the research nurses and study coordinators who assisted with patient care and data collection: Elizabeth Anderson (Duke University Medical Center), Jaime Chang (California Cancer Care), Susanne Cox (Stanford University Medical Center.), Byron Espina (University of Southern California), Kim Williams (Cancer Center of the Carolinas), and Janice Yager (Louisiana State University Medical Center). We also thank Richard Schwartz and Christine Gutheil for editorial assistance.

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