

Phase II Study of Rituximab in Combination With CHOP Chemotherapy in Patients With Previously Untreated, Aggressive Non-Hodgkin's Lymphoma

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Purpose: To determine the safety and efficacy of the combination of the chimeric anti-CD20 antibody Rituxan (rituximab, IDEC-C2B8; Genentech Inc, South San Francisco, CA) and cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy in patients with aggressive non-Hodgkin's lymphoma (NHL).

Patients and Methods: Thirty-three patients with previously untreated advanced aggressive B-cell NHL received six infusions of Rituxan (375 mg/m² per dose) on day 1 of each cycle in combination with six doses of CHOP chemotherapy given on day 3 of each cycle.

Results: The ORR by investigator assessment confirmed by the sponsor was 94% (31 of 33 patients). Twenty patients experienced a complete response (CR) (61%), 11 patients had a partial response (PR) (33%), and two patients were classified as having progressive disease. In the 18 patients with an International Prognostic Index (IPI) score ≥ 2 , the combination of Rituxan plus CHOP achieved an ORR of 89% and CR of 56%. The median duration of response and time to progression had not been reached after a median observation time of 26 months. Twenty-nine of 31 responding patients remained in remission during this follow-up period, including 15 of 16 patients with an IPI score ≥ 2 . The

most frequent adverse events attributed to Rituxan were fever and chills, primarily during the first infusion. Rituxan did not seem to compromise the ability of patients to tolerate CHOP; all patients completed the entire six courses of the combination. The *bcl-2* translocation of blood or bone marrow was positive at baseline in 13 patients; 11 patients had follow-up specimens obtained (eight CR, three PR), and all had a negative *bcl-2* status after therapy. Only one patient has reconverted to *bcl-2* positivity, and all patients remain in clinical remission.

Conclusion: This is the first report to demonstrate the safety and efficacy of the Rituxan chimeric anti-CD20 antibody in combination with standard-dose CHOP in the treatment of aggressive B-cell lymphoma. The clinical responses are at least comparable to those achieved with CHOP alone with no significant added toxicity. The presence or absence of the *bcl-2* translocation did not affect the ability of patients to achieve a CR with this regimen. The ability to achieve sustained remissions in patients with an IPI score ≥ 2 warrants further investigation with a randomized study.

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APPROXIMATELY 50% of patients with non-Hodgkin's lymphoma (NHL) have disease of aggressive histology as classified by the International Working Formulation (IWF) criteria, types E to H¹, and the Revised European-American Lymphoma Classification.² The majority of these cases can be classified as diffuse large-cell lymphoma.² Standard treatment for patients who do not have Burkitt's or lymphoblastic lymphoma is the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP).³ Treatment of aggressive lymphoma with CHOP has yielded overall response rates (ORRs) of 80% to 90%, with complete response (CR) rates of 45% to 55%. Long-term, 5-year survival is seen in 30% to 40% of patients. Attempts to improve these results by using more dose-intensive regimens have not resulted in a significant increase in the CR rate and have not translated into an improved disease-free survival or overall survival.⁴ Because fewer than 50% of all patients are cured, it is essential to develop new and improved therapeutic approaches for patients with advanced-stage, aggressive-histology NHL.⁵

Rituxan (rituximab, IDEC-C2B8; Genentech Inc, South San Francisco, CA) is a chimeric murine/human monoclonal antibody that reacts specifically with the B-cell antigen

CD20. In nonclinical studies, Rituxan was shown to affect both complement-mediated and antibody-dependent, cell-mediated lysis of CD20⁺ cells.⁶ Rituxan also induces apoptosis in vitro and sensitizes drug-resistant human B-cell lymphoma cell lines to the cytotoxic effects of some chemotherapeutic agents.⁷

Clinical trials to date have been conducted primarily in patients with relapsed or refractory low-grade NHL. Doses ranging from 10 mg/m² to 500 mg/m² have been well tolerated. A phase I, single-dose, dose-escalation trial showed rapid depletion of CD20⁺ B cells 24 to 72 hours

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after Rituxan administration; this depletion continued for 2 to 3 months.⁸ Subsequent studies evaluated four infusions of the monoclonal antibody administered at one dose each week over a total treatment course of 22 days. With this regimen, Rituxan induced durable remission in approximately 50% of patients with relapsed or chemoresistant low-grade or follicular NHL.^{9,10} The median duration of response was nearly 1 year, and activity was reported for patients with poor prognosis, including patients who were chemoresistant or unlikely to tolerate conventional chemotherapy.

No dose-limiting side effects were observed in the phase I/II studies. Adverse reactions were typically mild or moderate, with severe reactions occurring in 10% to 15% of patients, predominantly during the first infusion.^{10,11}

Rituxan has also demonstrated significant single-agent activity in patients with intermediate- or high-grade and mantle-cell lymphomas. A European trial performed by Coiffier et al¹² evaluated infusions of single-agent Rituxan administered once a week for eight weeks. Fifty-four patients with relapsed or refractory CD20⁺ NHL and elderly (aged > 60 years) patients with NHL who had not been treated previously were enrolled. In the intent-to-treat analysis, the ORR was 31%. The toxicity profile was similar to that seen with the low-grade/follicular patients. Of particular interest, hematologic toxicity was uncommon, and the lack of myelotoxicity indicated that Rituxan would be well suited for use in combination with chemotherapeutic agents.

A phase II open-label, single-arm study of Rituxan combined with a standard course of CHOP chemotherapy was conducted in patients with low-grade/follicular CD20⁺ lymphoma. Treatment consisted of six doses of the monoclonal antibody given throughout the six cycles of CHOP (two initial doses, one dose before cycles 3 and 5, and two doses after cycle 6).¹³

The rationale for this study design was to combine the independent activity and low toxicity of the monoclonal antibody with chemotherapy and to take advantage of possible synergy with chemotherapy. Forty patients (31 were previously untreated) with low-grade/follicular NHL were enrolled onto the study. On an intent-to-treat analysis, the objective response rate was 95% (38 of 40 patients). The response rate in the assessable patients was 100% (two patients withdrew before treatment). Tumor responses were observed in all treated patients; 22 patients experienced a CR and 16 patients had a partial response (PR). Of interest was the conversion from polymerase chain reaction (PCR) positive to negative for the *bcl-2/Ig* translocation in the peripheral blood and bone marrow from seven of eight patients.¹³ This conversion has rarely been reported using CHOP alone. The median duration of response has not been reached after more than 36 months of follow-up. The

toxicity of the treatment seemed to be comparable to that observed with Rituxan alone or CHOP alone. CHOP dose-intensity was not compromised by the addition of Rituxan, and patients on average completed more than 90% of the planned chemotherapy doses.

These clinical findings suggest that Rituxan might add therapeutic benefit to CHOP therapy without causing significant additional toxicity. These promising results led to a phase II open-label, single-arm, multicenter study designed to evaluate the clinical activity of the Rituxan antibody in combination with CHOP in first-line therapy for aggressive NHL.

PATIENTS AND METHODS

Eligibility

The patient population in this study consisted of newly diagnosed patients of at least 18 years of age with histologically documented aggressive lymphoma (IWF types D to H). Patients with mantle-cell, lymphoblastic, or Burkitt's lymphoma were excluded, because CHOP is not considered routine treatment in these patients. The stage of each patient's disease was assigned according to the Ann Arbor classification. Tumors were required to be CD20-positive. Patients were expected to have a survival of 6 months or more and a prestudy performance status of 0, 1, or 2 according to the World Health Organization scale.

The following exclusion criteria were applied: history of transformation from a low-grade lymphoma or history of a T-cell lymphoma, presence of CNS lymphoma, human immunodeficiency virus, human T-cell leukemia virus 1 or 2 positivity, prior anticancer therapy, significant organ function impairment as measured by serum creatinine level greater than 2.0 mg/dL, a total bilirubin level greater than 2.0 mg/dL, or an AST or alkaline phosphatase level more than two times normal, hemoglobin concentration less than 9 g/dL or absolute neutrophil count less than $1.5 \times 10^3/\mu\text{L}$, previous or concomitant malignancy other than basal cell or squamous cell carcinoma of the skin, and carcinoma-in-situ of the cervix. Patients with New York Heart Association class III or IV heart disease or myocardial infarction within the past 6 months were disqualified from entering onto the study. Pregnant or lactating women and patients of reproductive potential, unless using accepted birth control methods, were not allowed to enroll. Eligible patients signed a detailed written informed consent statement that met the requirements of the institutional review board of the participating institution. Institutional review board approval was given for this study at each participating center.

Treatment Design

This study consisted of a single treatment group. Patients were to receive a total of six intravenous infusions of Rituxan 375 mg/m² and six cycles of CHOP given every 21 days. Rituxan infusions were administered on day 1 before the CHOP cycle. Each CHOP cycle consisted of cyclophosphamide 750 mg/m², doxorubicin 50 mg/m², and vincristine 1.4 mg/m² (maximum dose, 2.0 mg/dose) given intravenously on day 3, and oral prednisone 100 mg on days 3 through 7.

Oral premedication with 650 mg of acetaminophen and 50 to 100 mg of diphenhydramine hydrochloride could be administered 30 to 60 minutes before each monoclonal antibody infusion. If toxicity occurred during the monoclonal antibody infusion, the infusion was to be slowed or temporarily discontinued and the patients were to be medicated as

Table 1. Response Criteria for NHL

Complete response
• Disappearance of the clinically detectable disease, no residual abnormality > 2.0 cm
• No new lesions
• Confirmed at \geq 28 days
Partial response
• \geq 50% SPD for all measurable lesions
• No new lesions or progression of any lesion
• Confirmed at \geq 28 days
Stable disease (no change)
• \geq 50% decrease in SPD has not been established
• \geq 25% increase in any lesion has not been established
• No new lesions
Progressive disease
• SPD increased \geq 25% in any measurable lesion
• New lesions

Abbreviation: SPD, sum of the products of the perpendicular diameters.

necessary with diphenhydramine (for rash, mucosal congestion, or other infusion-related reactions), and other medications were administered as needed. Once the adverse events abated, the antibody infusion could be resumed at 50% of the previous rate and then escalated as tolerated.

CHOP was to be administered according to standard preparation and procedures for each institution. Cyclophosphamide dose modification for hematologic toxicities was to be carried out according to an algorithm provided in the protocol. If grade 3 neurotoxicity occurred at any time during the treatment period, vincristine could be discontinued at the investigator's discretion. Hematopoietic growth factors (granulocyte or granulocyte-macrophage colony-stimulating factor) were administered per institutional guidelines. A patient whose treatment was interrupted for more than 3 weeks for either hematologic or nonhematologic toxicity was to be removed from the study. As stated in the informed consent, patients were allowed to withdraw from the study at any time. Furthermore, treatment was discontinued if disease progression was noted or if, in the opinion of the investigator, it was not in the patient's best interest to continue. If appropriate, consolidative radiotherapy was allowed after week 20 of the protocol.

Evaluation

Evaluation of the tumor burden and involved sites was performed at baseline, before the fourth cycle of Rituxan plus CHOP (week 10), and at the end of treatment (week 20). Evaluations from end of treatment indicating the onset of a PR or CR were followed by a confirmatory evaluation no sooner than 28 days later (week 24). All patients were evaluated for a total of 24 months after last study treatment.

Statistical Analysis

All patients were assessable for the intent-to-treat analysis of tumor response and toxicity. The primary efficacy measure was the CR rate of the lymphoma to treatment at week 24 (8 weeks after completion of six cycles of CHOP). Response was assessed by the investigator using World Health Organization criteria outlined in Table 1 and confirmed by the sponsor based on reported tumor dimensions at each assessment. Secondary efficacy variables were the ORR, time to disease progression, and survival at 2 years. Response rates were presented as the proportion meeting the response criteria assessed by the investigator

and confirmed by the sponsor. Ninety-five percent confidence intervals for the response rates were calculated.

Time to disease progression was defined as time from initiation of treatment to documented disease progression or death due to any cause, whichever occurred earlier. Survival time was defined as time from initiation of treatment to death due to any cause. Response duration was defined as time from response to the earlier of relapse or death. Time-to-event analyses were performed by the method of Kaplan and Meier.¹⁴ Response rates were presented by baseline risk factors as an exploratory analysis. No formal statistical comparisons between patient subsets were performed due to the limited number of patients enrolled onto the study.

PCR Assay for *bcl-2*

The assay for the detection of cells with the t(14:18) chromosomal translocation for *bcl-2* by PCR uses a nested primer amplification specifically for either the major breakpoint region or the minor cluster region. The assay was developed at the Laboratory of Molecular Diagnostics at IDEC Pharmaceuticals (San Diego, CA) in collaboration with John Gribben, MD. The PCR technique used in this study was essentially the same, with minor modifications, as the method used and described by Gribben et al.¹⁵ The assay will detect 1 in 10⁵ to 1 in 10⁶ t(14:18)-containing cells for either breakpoint region among a normal background and will discriminate all t(14:18) translocations that occur within these regions. Major breakpoint region- and minor cluster region-positive and -negative cell lines were used as controls. A separate PCR reaction was performed to amplify a region within the *bcl-2* gene to act as an internal control. Serial samples of blood and/or bone marrow for PCR analysis were obtained in those patients who tested *bcl-2* positive at baseline.

RESULTS

Patient Demographics and Disposition

The clinical features of the 33 patients enrolled onto this study are listed in Table 2. All 33 patients received all six infusions of Rituxan and CHOP. Two patients had disease progression before completing week 24 assessment, one died from late disease progression (18 months from study start), and one patient died from a stroke suffered at week 24 (which resulted in death 8.8 months from study start). As allowed per protocol, three patients who achieved PR at completion of treatment subsequently received local consolidative radiotherapy. All 33 patients were considered assessable for all safety and efficacy analyses.

Treatment Dose-Intensity

The Rituxan dose was not modified in 32 patients, but one patient had the Rituxan dose inadvertently modified for adjusted weight. Dose adjustments of one or more chemotherapy agents of the CHOP regimen were adjusted for toxicity in only four patients at some time during the course of the study. The mean dose-intensity of CHOP was 94% (range, 75% to 101%).

Table 2. Patient Demographics

Characteristic	No. of Patients	%
Age, years		
Median	52	
Range	20-79	
Age > 60 years	10	30
Sex		
Male	13	39
Female	20	61
Performance status*		
0	12	36
1	18	55
2	3	9
Histologic grade†		
Follicular large cell	7	21
Diffuse mixed cell	0	0
Diffuse large cell	22	67
Immunoblastic	2	6
Other‡	2	6
Stage		
I/II	9	27
III	7	21
IV	17	52
Lymphoma involvement in bone marrow	8	24
<i>bcl-2</i> status		
Positive	13	39
Negative	15	46
Not done	5	15
Single largest diameter \geq 10 cm	6	18
IPI score		
0	3	9
1	12	36
2	8	24
3	5	15
4	3	9
5	2	6

*World Health Organization.

†IWF classification.

‡Monocytoid (1), T-cell rich B cell (1).

Response to Therapy

The ORR to the combination of CHOP and Rituxan treatment at week 24 as confirmed by the sponsor was 94% (95% confidence interval, 86% to 100%) in the intent-to-treat population. Twenty (61%) of 33 patients (95% confidence interval, 44% to 77%) achieved a CR, and 11 patients (33%) had a PR. Only two patients by week 24 experienced disease progression, one of these patients was reported as a PR by the investigator, but by sponsor assessment of tumor dimensions, this patient met criteria for progressive disease. Response rates were also evaluated for patient subpopulations, using the following variables: International Prognostic Index (IPI) score (≥ 2 v < 2); age (≤ 60 years v > 60 years); lactate dehydrogenase level (normal v more than one times normal); performance status (0, 1, or 2); disease stage

(I or II v III or IV); number of extranodal sites (zero, one, or $>$ one); *bcl-2* status at baseline (positive or negative); bone marrow involvement (yes or no); IWF histologic classification; and single largest dimension ($>$ 10 cm, 5 to 10 cm, or $<$ 5 cm). The responses to therapy by subpopulation are listed in Table 3. In patients with an IPI score of less than 2, the Rituxan-CHOP combination achieved an ORR by sponsor assessment of 100% and CR rate of 67%, compared with an ORR of 89% and a CR of 56% in patients with an IPI score ≥ 2 . The above factors did not appreciably affect the ORR, but patients with a performance status of 1 or 2, lesions ≥ 5 cm, lactate dehydrogenase level greater than one times normal, stage I or II disease, marrow involvement, or IWF type G or H were somewhat less likely to achieve a CR by 24 weeks. Presence or absence of *bcl-2* and age did not seem to affect the ability of patients to achieve a CR with this combination.

The median sum of the products dimensions among PR patients was 70.0 cm² (range, 15.2 to 161.8 cm²). The median tumor reduction among PR patients, calculated as maximum percentage of sum of the products dimensions compared with baseline, was 67% (range, -87% to -33%) at week 10 and 82% (range, 93% to 34%) by week 20. Seven (23%) of the 31 responding patients achieved their maximum response as early as week 10 (after three cycles), nine patients (29%), by week 20 (completion of six cycles), and two patients (7%), by week 24. Thus, 13 patients (42%) did not reach their maximum response until month 4 or later from completion of treatment. The CR rates at week 24 may underestimate the true response to the treatment because five of the 11 PR patients continued to regress after their 24-week staging (without additional therapy) and eventually achieved a radiographic CR.

Twenty-nine of 31 patients who achieved CR or PR are in continued remission after a median observation time of 26 months from entry of remission. Response duration among the 31 responders ranged from 6 to 35+ months. Of note is that of the 11 PR patients, five eventually converted to CR status (four by month 4 after completion of treatment with no further therapy and one by month 12 with consolidative radiotherapy). Three patients (9%) experienced disease progression or relapse. Two patients experienced disease progression by week 24; one of these patients died 12 months after study start and the other patient is alive after salvage chemotherapy. One CR patient relapsed at month 18 and died 30 months after study start. One CR patient died 8.8 months after study start after a cerebral vascular accident at week 24. Fifteen of 16 patients with an IPI score ≥ 2 who responded to therapy still have ongoing remissions. Therefore, 30 (91%) of 33 patients are alive and 29 (88%) of 33 patients are alive and progression-free after a

Table 3. Response to Therapy

Characteristic	No. of Patients	CR		PR		ORR	
		No. of Patients	%	No. of Patients	%	No. of Patients	%
Total	33	20	61	11	33	31	94
Age							
≤ 60 years	23	14	61	9	39	23	100
> 60 years	10	6	60	2	20	8	80
Performance status*							
0	12	10	83	2	17	12	100
1	18	9	50	7	39	16	89
2	3	1	33	2	67	3	100
Stage							
I/II	9	4	44	4	44	8	89
III/IV	24	16	67	7	29	23	96
Bone marrow involvement							
Yes	8	4	50	3	38	7	88
No	25	16	64	8	32	24	96
Single largest diameter							
< 5 cm	13	12	92	0		12	92
5-10 cm	16	8	50	7	44	15	94
> 10 cm	4	0		4	100	4	100
IPI							
0-1	15	10	67	5	33	15	100
≥ 2	18	10	56	6	33	16	89
Lactate dehydrogenase level							
Normal	14	11	79	3	21	14	100
> 1 × normal	19	9	47	8	42	17	89
Extranodal sites							
0	14	6	43	7	50	13	93
1	10	10	100	0		10	100
> 1	9	4	44	4	44	8	89
Histologic grade†							
Follicular large cell	7	7	100	0		7	100
Diffuse mixed cell	0	0	0	0		0	0
Diffuse large cell	22	11	50	10	46	21	96
Immunoblastic	2	1	50	0		1	50
Other	2	1	50	1	50	2	100
<i>bcl-2</i> positive							
Yes	13	8	62	3	23	11	85
No	15	10	67	5	33	15	100

NOTE. Responses were confirmed by sponsor.

*World Health Organization.

†IWF classification.

median observation time of 31 months from study start. The Kaplan-Meier curve for time to progression for all patients and the subsets of patients with an IPI score less than 2 or ≥ 2 are presented in Fig 1. The overall survival of the patient group is presented in Fig 2.

Thirteen (46%) of 28 patients were *bcl-2*-positive in either blood or bone marrow upon study entry (11 were *bcl-2*-positive at the major breakpoint, two at the minor breakpoint). Two patients whose disease progressed on study were *bcl-2*-positive and were not reassessed after progression. Eleven patients did convert to *bcl-2*-negative status, and of these, eight achieved CR and three achieved

PR. Ten of these 11 patients remain *bcl-2*-negative, and one patient reconverted to a positive *bcl-2* status at month 4 but remains in clinical CR at 24 months of follow-up.

Safety

The most frequently experienced adverse events in this trial were alopecia (26 patients), asthenia (23 patients), neutropenia (24 patients), nausea (19 patients), and fever (18 patients). Fourteen patients had at least one serious adverse event. Serious adverse events reported in more than one patient were sepsis (three patients), fever (three patients), neutropenia (four patients) or leukopenia (three

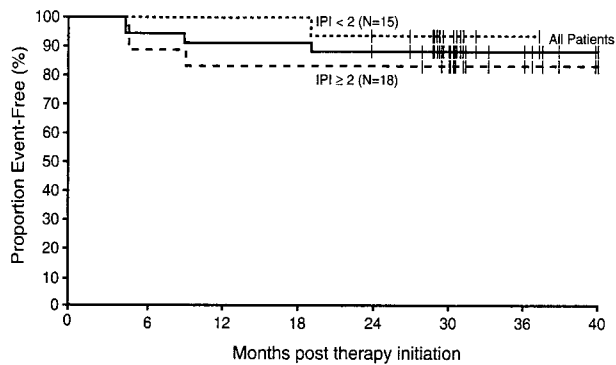


Fig 1. Kaplan-Meier plot of time to disease progression or death for all patients (N = 33) and for the subsets with an IPI score < 2 (n = 15) or ≥ 2 (n = 18). Three patients experienced disease progression at 4.3, 4.5, and 18.3 months after the initiation of therapy. One patient died, of an unrelated cause, 8.8 months after start of therapy.

patients), and dehydration (two patients). There was one grade 4 intestinal perforation and one grade 3 bowel obstruction. The treating physicians attributed most of the adverse events to CHOP chemotherapy. The most frequent adverse events attributed to Rituxan treatments alone were infusion-related events: grade 1 or 2 fever (11 patients) and chills (10 patients). The only grade 3 or 4 event attributed to Rituxan alone was in one patient who was hospitalized for an allergic reaction/anaphylaxis during the first infusion. That patient recovered and subsequently received all planned therapy.

Hematologic toxicity was primarily neutropenia in 24 patients (73%) or leukopenia in 11 patients (33%). There was a 58% grade 4 neutropenia rate with the combination. Eighteen patients (55%) required at least one course of granulocyte colony-stimulating factor during their treatment course. Grade 3 anemia (four patients) and grade 3 thrombocytopenia (one patient) were rare; there were no grade 4

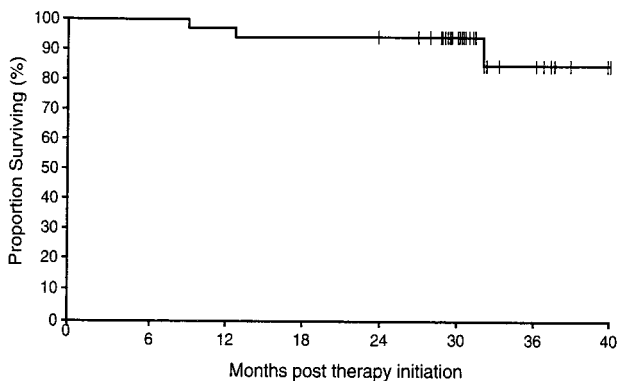


Fig 2. Kaplan-Meier plot of overall survival for all patients (N = 33).

Table 4. Hematologic Adverse Events by Grade (incidence by patient) (N = 33)

	Grade				Total	
	1	2	3	4	No. of Patients	%
Anemia	2	4	4	0	10	30.3
Neutropenia	0	0	5	19	24	72.7
Leukopenia	1	3	3	4	11	33.3
Thrombocytopenia	1	0	1	0	2	6.1

NOTE. Thirty of 33 patients experienced either neutropenia or leukopenia.

anemia or thrombocytopenia events reported (Table 4). Only five patients (15%) required blood transfusions and no patient required platelet transfusion. Infectious events were generally mild or moderate and related to the respiratory system. The most common infectious events were pharyngitis (six patients), sinusitis (five patients), rhinitis (three patients), and oral thrush (three patients). Grade 3 or 4 infectious events included sepsis (two patients), cellulitis (one patient), oral thrush (one patient), sialadenitis (one patient), and infection not specified (one patient).

Twelve patients required hospitalization during the study period; reasons are listed in Table 5. Some patients were hospitalized with more than one adverse event. The most common reasons for hospitalization were neutropenia (three patients) or leukopenia (three patients) and fever (two patients) or sepsis (two patients).

Twenty-nine patients were tested for quantifiable immune response to the chimeric antibody; human anti-chimeric antibody was not detected in any patient (limit of detection

Table 5. Clinical Outcomes (N = 33)

	No. of Patients	%
Hospitalized	12	36
Hospitalization reasons*		
Leukopenia	3	9
Neutropenia	3	9
Fever	2	6
Sepsis	2	6
Allergic reaction	1	3
Anaphylaxis	1	3
Cerebral vascular accident	1	3
Cholecystitis	1	3
Dehydration	1	3
Heart failure right	1	3
Injury accident	1	3
Intestinal perforation	1	3
Obstruction intestine	1	3
Received G-CSF	18	55
Received blood transfusions	5	15
Received antimicrobials	26	79

Abbreviation: G-CSF, granulocyte colony-stimulating factor.

*Some patients were hospitalized for more than one reason.

7 ng/mL). Baseline immunoglobulins (Igs) were available for 31 patients; eight patients had baseline levels below normal in one or more Ig subsets (seven of eight below normal for IgM). Although the mean serum Ig levels of IgG, IgA, and IgM decreased throughout week 20, levels dropped to below normal in only eight patients with normal or high levels at baseline.

Baseline CD19 and CD20 counts were available for 29 patients; in 29 of 29 patients and in 27 of 29 patients, CD20 and CD19 counts were undetectable by week 10. B-cell recovery, as measured by CD19 levels in patients observed for at least 1 year, was available in 21 patients; 15 (71%) of 21 patients recovered to 100% of baseline by 24 months after completion of treatment.

DISCUSSION

Therapy for advanced-stage, aggressive NHL (IWF, types D to H) is combination chemotherapy. During the 1970s and 1980s, a series of single-institution and third-generation chemotherapy regimens seemed to demonstrate a near doubling of the CR rate and overall survival compared with the first-generation combination chemotherapy studies that produced CR rates of 45% to 53% with 30% to 37% long-term survivors.¹⁶⁻¹⁹ The Southwest Oncology Group (SWOG) conducted a randomized trial that compared standard therapy (CHOP) with third-generation regimens (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone [m-BACOD]; prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide, cytarabine, bleomycin, and vincristine [ProMACE-CytaBOM]; and methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin [MACOP-B]).⁴ After 6 years, there is still no difference in CR rate, time to treatment failure, or overall survival between CHOP and the third-generation regimens. CHOP remains the best available standard of care.

From the SWOG four-arm randomized study, the ORR with eight cycles of CHOP was 80%, with 44% of patients achieving a CR. It should be noted that in this study the CHOP response data were determined at a time when computed tomographic (CT) scanning and magnetic resonance imaging were evolving. In the SWOG randomized trial comparing standard therapy with the third-generation chemotherapy regimens, the rate of CR was estimated conservatively, no peripheral disease could be present, and any abnormalities detected on abdominal or chest radiography had to be less than 2.5 cm in diameter. In the current study, the assessment for CR by sponsor required lymph nodes to regress to ≤ 2 cm in any dimension. The CR rates at week 24 may underestimate the true response to the treatment, because five of the 11 PR patients continued to

regress after their 24-week staging (without additional therapy) and eventually achieved a radiographic CR. This pattern of continued tumor regression after therapy has been completed has also been noted with single-agent Rituxan in indolent lymphoma.²⁰

Residual lymphadenopathy based on CT findings always creates a difficult clinical dilemma. Although in some circumstances this represents remaining disease, it can also represent fibrotic tissue that will not necessarily lead to relapse. Alternatives for evaluating such patients include the use of single-photon emission computed tomography gallium scans, positron emission tomographic scans, biopsy of the residual mass, or repeated CT scans over time. If there is concern about persistent disease, a biopsy should be pursued to direct the next therapeutic option.

The IPI has been used to develop a predictive model of outcome for aggressive NHL. The following five pretreatment characteristics were found to be independently statistically significant: age (≤ 60 v > 60 years), disease stage (I or II [localized] v III or IV [advanced]), number of extranodal sites of involvement (\leq one v $>$ one), Eastern Cooperative Oncology Group performance status (0 or 1 v > 2), and serum lactate dehydrogenase level (normal or elevated). The IPI established risk on the basis of the number of adverse factors as follows: low = 0 or 1, low intermediate = 2, high intermediate = 3, and high risk = 4 or 5. Shipp et al²¹ analyzed more than 2000 patients by risk factors and reported that patients have a different outcome with regard to CR rate, relapse-free survival, and overall survival. Response rates were evaluated for patient subpopulations, and the IPI was used. In this study, the combination Rituxan and CHOP achieved an ORR of 100% in patients with IPI scores less than 2 (15 of 15) and a CR rate of 67%, compared with an ORR of 89% (16 of 18) and a CR of 56% in patients with an IPI score ≥ 2 . Only one of the 31 patients who achieved remission has relapsed (one CR patient with an IPI score of 1 at 18 months after study start). Historically, the majority of relapses from aggressive lymphoma occur within the first 2 years after treatment. There were 16 patients with an IPI score ≥ 2 in this study who responded to treatment. Fifteen of these patients have sustained remissions for at least 2 years, but one patient died of a cerebral vascular accident while still in remission.

In addition to the clinical prognostic factors, biologic parameters have also been investigated as prognostic factors in NHL lymphoma. *Bcl-2*, a member of a family of antiapoptotic genes, is the most widely studied.²² Expression of the Bcl-2 protein in B-cell large-cell lymphoma has been described as an important prognostic factor, independent of the clinical parameters of the IPI.²³ The proportion of positive cases based on percentage of tumor cells staining

for Bcl-2 protein has been reported to range from 45% to 58%; however, it should be noted that there is no correlation between *bcl-2* gene rearrangement (t14:18) and *bcl-2* gene expression.²³ Expression of the Bcl-2 protein correlated with inferior disease-free survival and with inferior overall survival but had no significant impact on the CR rate.^{24,25} Detection of *bcl-2* by gene rearrangement in blood and marrow, but not expression, was monitored in this study to assess clearing of minimal residual disease. Thirteen patients enrolled onto this study were *bcl-2*-positive by PCR (11 by major breakpoint, two by minor breakpoint). Eleven of 13 patients did convert to *bcl-2*-negative status; eight of these patients were assessed to be in CR, and three in PR at week 24. All remain in clinical remission with 2 years of follow-up after treatment. It is notable that the three PR patients eventually converted to radiographic CR (two at 4 months and one at 12 months). One patient has reconverted to a positive *bcl-2* status but without clinical progression. Larger studies will be required to determine whether Rituxan will have an impact on different biologic subtypes as defined by molecular markers, but the ability to achieve and maintain a molecular CR in a non-bone marrow transplant setting. However, it should be pointed out that the clearance of *bcl-2* in the blood and bone marrow can be seen with

Rituxan in the presence of adenopathy in other locations, the so-called compartment syndrome.²⁶

The clinical findings from this pilot trial demonstrate the feasibility and safety of the addition of Rituxan to CHOP and show that Rituxan does not interfere with the chemotherapy delivery. Ongoing phase III randomized trials are clearly indicated and, together with the intriguing response data, will establish whether there is clinical benefit with the combination. In the United States, a large cooperative group study by the Eastern Cooperative Oncology Group, Cancer and Leukemia Group B, and SWOG has an ongoing trial randomizing previously untreated elderly patients with aggressive NHL to either CHOP alone or the Rituxan-CHOP combination. A similar study in Europe is being conducted by the Groupe d'Etudes des Lymphomes de l'Adulte and has recently completed accrual. A large international randomized multicenter study has also begun of this combination in patients with previously untreated intermediate- and high-grade NHL for all patients 18 years and older with an IPI score ≥ 2 . It is hoped that we can not only demonstrate an improved long-term disease-free survival in the newly diagnosed patients at risk for relapse but, by pairing biologic analyses with these studies, also further our understanding of how Rituxan and monoclonal antibodies can contribute to that improvement.

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