IDEC-C2B8: Results of a Phase I Multiple-Dose Trial in Patients With Relapsed Non-Hodgkin's Lymphoma

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Purpose: To evaluate the safety, pharmacokinetics, and biologic effect of multiple doses of the chimeric anti-CD20 monoclonal antibody (mAb) IDEC-C2B8 in patients with relapsed B-cell lymphoma.

Patients and Methods: Twenty patients with relapsed low-grade (n = 15) or intermediate-/high-grade (n = 5) lymphoma received weekly infusions times four of 125 mg/m^2 (n = 3), 250 mg/m^2 (n = 7), or 375 mg/m^2 (n = 10) of IDEC-C2B8.

Results: Infusional side effects during the initial infusion were mainly grade I/II fever, asthenia, chills, nausea, rash, and urticaria. More serious events were rare. Peripheral-blood B cells were rapidly depleted and slowly recovered over 3 to 6 months. There was no change in mean immunoglobulin (Ig) levels. Antibody serum half-life (and maximum concentration [C_{max}]) generally increased between the first and fourth infusions (33.2 hours *v* 76.6 hours, respectively) following the 375-mg/m^2 doses. Six of 18 assessable patients had a partial remission (PR), with a median time to disease progression of 6.4 months (range, 3 to 21.7). Minor responses (MRs) were observed in five patients and progressive disease (PD) in seven. Tumor responses occurred in peripheral blood, bone marrow (BM), spleen, bulky lymph nodes, and extranodal sites, and in patients who had relapsed following high-dose myeloablative chemotherapy. Six of 14 patients (40%) with a low-grade histology responded. Four of six with bulky disease had a PR.

Conclusion: IDEC-C2B8 chimeric anti-CD20 mAb therapy is well tolerated and has clinical activity in patients with relapsed B-cell lymphoma. The 375-mg/m^2 dose has been selected for a phase II trial in patients with relapsed low-grade or follicular B-cell lymphoma.

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THE B-CELL–SPECIFIC CD20 antigen is an attractive target for monoclonal antibody (mAb) immunotherapy of B-cell non-Hodgkin's lymphoma (NHL).[1] More than 90% of B-cell NHLs express the CD20 antigen.[2] The antigen does not internalize,[3] is not shed from the cell surface,[3] and does not circulate as free protein.[4] Antigen expression occurs during the pre–B-cell stage of differentiation and persists through mature B-cell development, but is not expressed on early pre-B cells, stem cells, or antigen-presenting dendritic reticulum cells.[5] While the exact function of CD20 is not known, the antigen may regulate a step in the B-cell activation process required for cell-cycle initiation and differentiation[6-11] and may function as a calcium channel.[12]

Limited experience has been reported using unmodified murine anti-CD20 antibodies. A phase I trial that used the murine immunoglobulin G2a (IgG2a) anti-CD20 antibody 1F5 produced one partial remission (PR) in the treatment of four patients.[3] Radiolabeling murine anti-CD20 mAbs has been more widely evaluated. Treatment with marrow ablative doses of iodine 131–labeled 1F5 or B1[13,14] with autologous marrow rescue induced complete responses (CR) in most patients. In addition, therapy with lower doses of ^{131}I-B1 has demonstrated significant clinical activity,[15,16] including responses to the trace-labeled antibody. Finally, infusion of IDEC-Y2B8, a murine anti-CD20 antibody radiolabeled with yttrium 90 (20 to 50 mCi) resulted in four CRs and five PRs in a phase I study in 14 refractory B-cell lymphoma patients.[17] Autologous stem-cell rescue was required at the highest doses.

Most murine antibodies do not effectively activate human complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) effector mechanisms. In addition, repeated murine antibody infusions may result in the formation of a human antimouse antibody (HAMA) response that limits further clinical use. IDEC-C2B8 is an IgG1 kappa chimeric mAb that consists of variable regions from the heavy and light chains of the murine anti-CD20 antibody (IDEC-2B8) and human IgG1 and kappa constant regions.[18] This augments the lysis of target tumor cells using human CDC or ADCC mechanisms and decreases immunogenicity. High yields of this chimeric antibody are produced in suspension culture by Chinese hamster ovary cells.[18] In vitro, IDEC-C2B8 has specificity and affinity for the

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CD20 antigen, induces CDC and ADCC of antigen-positive cells,[18] induces apoptosis of some lymphoma cell lines, and increases sensitivity to the cytotoxic effect of chemotherapy/toxins in some resistant human lymphoma cell lines.[19] Preclinical data in cynomolgus monkeys showed B-lymphocyte depletion in peripheral blood, bone marrow (BM), and lymph nodes without dose-limiting toxicity.[18]

Clinical data from a phase I, single dose-escalating (10 to 500 mg/m^2) study in 15 patients with relapsed NHL demonstrated no dose-limiting toxicity and showed clinical activity (two PRs and four minor responses [MRs]).[20] Antibody infusion caused CD20$^+$ B-cell depletion in peripheral blood at 24 to 72 hours that persisted for 2 to 3 months in most patients. Flow cytometry evaluation of cell suspensions from lymph node biopsy specimens showed B-cell depletion and tumor cells coated with antibody 2 weeks posttreatment.

Here, we report results from a phase I dose-escalation trial that used multiple infusions of IDEC-C2B8 (125, 250, or 375 mg/m^2) in 20 patients with relapsed B-cell lymphoma. Primary study objectives included evaluation of safety and dose-limiting toxicities, determination of a biologically active tolerated dose (BATD), pharmacokinetic analysis, determination of the degree and duration of B-cell depletion, and analysis of relevant clinical activity.

PATIENTS AND METHODS

Protocol Design

Intravenous (IV) infusions weekly times four of IDEC-C2B8 at doses of 125, 250, or 375 mg/m^2 were given to patients with relapsed B-cell NHL. Evaluation of safety, dose-limiting toxicities, and clinical activity was performed, and circulating levels of IDEC-C2B8, HAMA, and human antichimeric antibody (HACA) levels were analyzed.

Patients

Adults with histologically confirmed, relapsed B-cell lymphoma that expressed the CD20 antigen were eligible. All patients met the following criteria: expected survival duration $\geq$ 3 months with no serious nonmalignant disease; prestudy performance status of 0, 1, or 2 on the World Health Organization (WHO) scale; negative for human immunodeficiency virus and hepatitis-B surface antigen; and serum IgG level $\geq$ 600 mg/dL, hemoglobin $\geq$ 8.0 g/dL, WBC count $\geq 3.0 \times 10^3/\mu L$, absolute granulocyte count $\geq 1.5 \times 10^3/\mu L$, platelet count $\geq 75 \times 10^3/\mu L$, bilirubin level less than 1.5 mg/dL, alkaline phosphatase level less than two times normal, AST less than two times normal, and serum creatinine concentration less than 2.0 mg/dL. All patients tested negative for HAMA. All patients signed an informed consent approved by the institutional review boards of Stanford University Medical Center, Palo Alto, CA, Sydney Kimmel Cancer Center, or Scripps Memorial Hospital, San Diego, CA.

IDEC-C2B8 Serum Analysis

Serum levels of IDEC-C2B8 were measured by enzyme-linked immunosorbent assay (ELISA). Microtiter plates were coated with purified polyclonal goat anti–IDEC-2B8–specific antibody. Patient serum was serially diluted across the plate and bound human IgG detected by goat antihuman IgG conjugated with horseradish peroxidase. Color was developed by adding 3-ethylbenzthiazoline sulfonic acid (ABTS) substrate. Known amounts of IDEC-C2B8 diluted into normal human serum were used to make a standard curve on each plate. Antibody concentration was determined by comparing the signal from the patients' sera to that from the standard curve.

Measurement of Host Anti–IDEC-C2B8 Antibody Response

Posttreatment sera from 1-, 2-, and 3-month evaluations were analyzed for the presence of a host antichimeric antibody immune response using a sandwich ELISA. Microtiter plates were coated with IDEC-C2B8. Dilutions of the patient's sera were added and, following washing, detected with biotin-labeled IDEC-C2B8 followed by avidin horseradish peroxidase (HRP) and the substrate ABTS. The minimum quantifiable level for these assays was 100 ng/mL (detectable to 5 ng/mL).

Flow Cytometry

Flow cytometry of peripheral blood was performed to determine the following lymphocyte subsets: CD3$^+$, CD4$^+$, and CD8$^+$ T cells; CD19$^+$ and CD20$^+$ B cells; and expression of surface Ig light chains. Samples were obtained before and 72 hours after the first infusion, at 1 and 3 months, and then every 3 months for 1 year. When available, tumor cells were obtained from excisional biopsies, fine-needle tumor aspirations, or bone marrow. Tumor-cell expression of CD20 was determined using fluorescein isothiocyanate (FITC)-conjugated mAb by flow cytometry or HRP-labeled L26 (DAKO, Carpinteria, CA) in paraffin-embedded tissue by immunohistochemistry. Tumor cells were analyzed for expression of surface Ig light chains using antihuman kappa- and antihuman lambda-conjugated antibodies.

Study Measurements

Infusion-related toxicity was evaluated using the National Cancer Institute's adult toxicity criteria. Measurements during treatment included analysis of hematologic, renal, and hepatic function; serum complement; and Igs. Tumor response was evaluated 1 and 3 months after treatment completion and every 3 months for a maximum of 1 year using tumor measurements obtained during physical examination and from radiologic imaging studies. The WHO/Eastern Cooperative Oncology Group (ECOG) guidelines for CR (complete resolution of all detectable disease), PR (> 50% reduction in measurable disease persisting for at least 28 days), MR (25% to 50% reduction in disease), and stable disease (no significant change in tumor measurements without progression over the period of observation) were used. Progressive disease (PD) was defined as a 25% increase in measurable disease or the appearance of any new lesion. Time to progression was measured from date of first study treatment to first date when PD was documented. All responses were confirmed through repeat evaluation $\geq$ 28 days after initial efficacy determination.

Table 1. Patient Characteristics

Characteristic	Dosing Group (mg/m²) 125 (n = 3)	250 (n = 7)	375 (n = 10)	Total (N = 20) No.	%
Age (years)					
Median	48.0	59.0	59.5	59.0	
Range	41-70	33-72	29-81	29-81	
Sex					
Female	1	3	6	10	50
Male	2	4	4	10	50
Race					
White	3	6	9	18	90
Asian	0	1	1	2	10
Histologic grade*					
Low	2	5	8	15	75
A	0	0	1	1	5
B	0	3	5	8	40
C	2	2	2	6	30
Intermediate	0	2	2	4	20
D	0	0	1	1	5
E	0	1	0	1	5
G	0	1	1	2	10
High	1	0	0	1	5
H	1	0	0	1	5
Stage†					
I	0	0	2	2	10
II	0	1	0	1	5
III	2	1	4	7	35
IV	1	5	4	10	50

*Based on the International Working Formulation.
†Stage at initial diagnosis, based on the stage grouping.

Table 2. Summary of Prior Therapy (N = 20)

Type of Prior Therapy	Prior Therapies No.	Median	Range	Time From Last Therapy* (months) Median	Range
Chemotherapy	20	2	1-4	6.6	0.9-67.2
Bioimmunotherapy	4	1	1-2	6.5	4.4-24.2
Radiotherapy	8	1	1-2	30.1	5.0-102.5
ABMT	4	1	1-1	13.5	5.0-31.4
All therapy	20	3	1-6	5.3	0.9-102.5

*Months to first IDEC-C2B8 infusion from stop date of last therapy.

Sample Size

A minimum of three patients and a maximum of nine assessable patients were entered at each dose level. Dose- and drug-related toxicity determinations were the primary study objectives, and no statistical analyses were performed.

Assessability Criteria and Concurrent Treatment Conditions

All patients who received one or more IDEC-C2B8 doses were considered assessable for safety. Patients were assessable for efficacy if they completed one full course of treatment (four doses), satisfied all prestudy criteria, had measurable disease, and met criteria for evaluation of response. No concomitant cancer chemotherapy, radiotherapy, hormonal therapy, or immunotherapy was allowed.

RESULTS

Demographics

Twenty patients received weekly IV infusions times four of IDEC-C2B8. Patient characteristics and prior lymphoma therapies are listed in Tables 1 and 2. Three patients (median age, 48.0 years) received 125 mg/m², seven patients (median age, 59.0 years) 250 mg/m², and 10 patients (median age, 59.5 years) 375 mg/m². The original diagnosis was low-grade NHL in 15 patients (75%) and intermediate- or high-grade disease in five (25%). Advanced-stage (III/IV) lymphoma was present at diagnosis in 17 patients. All patients required therapy due to disease progression and had failed to respond to prior chemotherapy (median of 2.0 prior regimens; Table 2). The median duration of response to last chemotherapy before IDEC-C2B8 treatment was 5.0 months. At baseline, three patients had lesions with measurements ≥ 7 cm and three had lesions that measured ≥ 10 cm. Marrow involvement was present in 50% of patients and 13 of 20 (65%) had extranodal disease. All patients, including those previously exposed to murine antibodies, were negative for HAMA. Four patients had progressed following autologous bone marrow transplantation (ABMT) and three patients had received a single infusion of IDEC-C2B8 in an earlier phase I single-dose trial.[20]

Infusion Data

Patients were treated primarily on an outpatient basis. Routine premedication was not given; however, patients received oral diphenhydramine and acetaminophen when appropriate. If the infusion was stopped due to significant infusion-related adverse events (AEs), it was restarted within 30 to 45 minutes at a lower rate (50 to 100 mg/h) and increased as tolerated to 200 mg/h. The mean infusion time across all infusions was 3.6, 3.8, and 4.5 hours in the 125-, 250-, and 375-mg/m² treatment groups, respectively. The overall mean infusion time was 4.5 hours (range, 2.5 to 7.1 hours). The mean total dose administered over the course of four infusions was approximately 947, 1,715, and 2,535 mg in the 125-, 250-, and 375-mg/m² treatment groups, respectively. The maximum total dose administered was 3,200 mg in two patients in the 375-mg/m² treatment group.

Infusion-Related Toxicity

Eighteen patients received all four weekly infusions; two patients received only one infusion. Of 169 total AEs,

Table 3. Incidence of Most Frequent AEs by Dosing Group, Event, and Patient (N = 20)

Variable	Dosing Group (mg/m²) 125 No. of Patients	125 No. of Events	250 No. of Patients	250 No. of Events	375 No. of Patients	375 No. of Events	Total (N = 20) No. of Patients	Total (N = 20) %	Total (N = 20) No. of Events	Total (N = 20) %
Any AE	3	19	7	34	9	59	19	95.0	112	100.0
Body as a whole										
Fever	3	4	6	10	8	19	17	85.0	33	29.5
Chills	2	2	5	7	2	2	9	45.0	11	9.8
Asthenia	1	1	4	4	4	4	9	45.0	9	8.0
Night sweats	1	1	0	0	2	2	3	15.0	3	2.7
Digestive system										
Nausea	2	2	1	1	2	2	5	25.0	5	4.5
Vomiting	1	1	1	1	2	2	4	20.0	4	3.6
Dyspepsia	0	0	1	2	1	1	2	10.0	3	2.7
Blood or BM										
Thrombocytopenia	0	0	0	0	3	3	3	15.0	3	2.7
Coagulation disorder	0	0	0	0	2	2	2	10.0	2	1.8
Musculoskeletal system										
Arthralgia	0	0	1	1	1	1	2	10.0	2	1.8
Nervous system										
Vasodilation	0	0	0	0	1	3	1	5.0	3	2.7
Respiratory system										
Rhinitis	0	0	0	0	2	2	2	10.0	2	1.8
Skin and appendages										
Pruritus	0	0	1	1	1	2	2	10.0	3	2.7
Rash	0	0	0	0	2	2	2	10.0	2	1.8
Urticaria	1	1	0	0	1	2	2	10.0	3	2.7

NOTE. AEs probably or possibly related or of unknown relationship to treatment with frequency ≥1% of events.

112 were reported as related to study treatment (66%) and are listed in Table 3. AEs were most frequently infusion-related and resolved completely, usually within hours. Hematologic toxicity and infections were seen in a minority of patients and were usually mild. No clinically significant renal or hepatic toxicity was observed. No apparent relationship was noted between dose and severity of AEs. AEs occurred primarily at the first infusion and the number of AEs decreased dramatically during subsequent infusions. The most frequent infusion-related events were fever, asthenia, chills, nausea, vomiting, rash, and urticaria. Grade 2 hypotension, reported in one patient, resolved with treatment and did not require hospitalization. Non–infusion-related toxicities occurred in a minority of patients. Although most (91%) AEs were classified as grades 1 or 2, nine events that occurred in six patients were noted as severe (grade 3 or 4). Eight grade 3 events consisted of pain (two episodes), thrombocytopenia (two), fatigue, rigors, nausea, and bronchospasm (one each). Grade 4–related thrombocytopenia occurred within 24 hours of the first infusion in one patient (no. 015) with small lymphocytic histology and extensive BM involvement. This patient had previously experienced grade 3 thrombocytopenia with a single infusion of IDEC-C2B8. The platelet count declined from a baseline value of 93,000/μL to a nadir of 19,000/μL, lactate dehydrogenase (LDH) increased markedly from a baseline of 629 U/L to a peak of 2,660 U/L, and hemoglobin decreased from 12.6 g/dL to 7.8 g/dL. Uric acid peaked to 10.5 mg/dL within 24 to 48 hours. The patient was treated with fluids, allopurinol, corticosteroids, and a single platelet transfusion. Evaluation for disseminated intravascular coagulation was nondiagnostic, and the patient improved with normalization of LDH, platelet count, and hemoglobin level by day 31. This patient did not require hospitalization and received no further IDEC-C2B8 treatment.

Effects on Hematologic Parameters

Hematologic toxicity was usually mild and reversible and is listed in Table 4. The median nadirs calculated for WBC, granulocytes, hemoglobin, and hematocrit did not substantially deviate from baseline and did not change over dose groups. Three patients with trilineage hematologic effects recovered in ≤ 7 days, ≤ 19 days, and ≤ 35 days, respectively. Five of eight patients (63%) with clinically significant hematologic nadirs had BM involve-

ment. Five patients (one each at the lower dose levels and three at 375 mg/m^2) showed hemoglobin reductions (> 1.5 g/dL) and/or hematocrit reduction (> 5%). Platelet counts less than 100,000/μL occurred in three patients (375 mg/m^2) between days 1 and 8; counts recovered within 1 week in two patients and within 3 weeks in the other. The mean WBC count decreased to 2,200/μL in one patient (375 mg/m^2) and recovered within 9 days. Two patients with absolute granulocyte count nadirs less than 1,500/μL (1,200 and 500/μL, respectively) recovered within 1 week. One of these (no. 006), who had a circulating malignant lymphocyte clone of 50,000 to 70,000 μL, had a reduction in absolute granulocyte count to 500/μL on day 1 but recovered on day 8.

Effects on Peripheral-Blood Lymphocyte Subsets

Baseline CD20$^+$ cell counts were high in some patients due to the presence of circulating lymphoma cells. At baseline, two of 17 patients tested had CD20$^+$ monoclonal B-cell populations in peripheral blood and evidence of monoclonality was noted in BM from three of seven patients tested. The CD19$^+$ B-cell population decreased sharply from baseline by day 4 and remained below the lower limits of normal until 6 months posttreatment, at which time recovery began and continued thereafter. The mean percentages of CD3$^+$, CD4$^+$, and CD8$^+$ T cells did not vary significantly from baseline for all patients across all dosing groups.

Effects on Serum Igs and Complement

Mean serum Ig levels remained stable; however, individual patients experienced transient decreases in serum levels of unclear significance. The number of patients with a $\geq$ 20% decline from baseline and with absolute values below the normal range was two, three, and three for IgG, IgA, and IgM, respectively. One patient had decreases in IgG and IgA. Decreases were observed at a median of day 15 with recovery to baseline by study day 22 in five of seven patients. Mean serum complement levels fluctuated and reductions were noted in individual patients; however, no correlation with clinical response or toxicity was evident.

Table 4. Patients With Clinically Significant Hematologic Nadirs

Patient No.	Dosing Group (mg/m^2)	Marrow Involved	Clinically Significant Nadir				
			Hgb	Hct	Platelet	WBC	AGC
002	125	–	×	×			
006	250	+					×
008	250	+	×	×			
012	375	–	×	×		×	×
013	375	+			×		
014	375	–		×			
015	375	+	×	×	×		
016	375	+			×		

NOTE. Clinically significant defined as decrease in hemoglobin (in Hgb) > 1.5 g/dL; decrease in hematocrit (Hct) $\geq$ 5%; WBC count < 3,000/μL; absolute granulocyte count (AGC) < 1,500/μL; platelets < 100,000/μL.

Antibody Serum Pharmacokinetics

Serum levels of free antibody were measured by ELISA. Patients treated at all three dose levels exhibited detectable IDEC-C2B8 serum levels throughout the treatment period. Most patients analyzed showed an accumulation of antibody through the fourth infusion (Table 5). The maximum concentration (C_{max}) and serum half-life of IDEC-C2B8 increased between the first and fourth infusions for most patients, most likely due to the clearance of circulating B cells from the peripheral blood and saturation of CD20-binding sites after the first infusion. At the 375-mg/m^2 dose, the mean serum half-life after the first infusion was 33.2 hours (range, 11.1 to 53.1), while the mean serum half-life after the fourth infusion was 76.6 hours (range, 26.4 to 106.0). Wide interpatient variability was noted, both within and between dose groups, likely due to variable amounts of circulating tumor, overall tumor burden, and available CD20 antigen. No correlation was noted between any of the pharmacokinetic parameters and response to treatment.

Laboratory and Immune Response Tests

No clinically significant alterations in urinalysis, or in mean values for serum albumin, LDH, AST, ALT, uric acid, bilirubin, alkaline phosphatase, or creatinine were noted. No evidence of acute tumor lysis syndrome was noted using Hande's method.[21] No quantifiable HAMA immune response was noted, although one patient experienced a detectable but not quantifiable HACA response 7 months posttreatment that did not result in any clinical or laboratory abnormality.

Overall Clinical Response

Two patients were not assessable for efficacy; treatment was discontinued after one dose due to grade 4 thrombocytopenia in one and elevated liver enzymes later attributed to malignant lymphoma in the other. The 18 assessable patients were staged at baseline and restaged 1 and 3 months after treatment completion. Responders were reevaluated every 3 months until disease progression. Responses are listed in Table 6.

The overall response rate was 33% (95% confidence

Table 5. Pharmacokinetic Parameter Summary: 375-mg/m² Dose

Patient No.	Model*	First Infusion $T_{1/2}$ (hours)	First Infusion C_{max}† (μg/mL)	First Infusion Cl‡ (L/h)	Fourth Infusion $T_{1/2}$ (hours)	Fourth Infusion C_{max}† (μg/mL)	Fourth Infusion Cl‡ (L/h)	Clinical Response
014	1	35.4	413.7	0.0196	106.0	663.9	0.0045	PD
016§	1	11.1	118.9	0.2820	26.4	131.3	0.1188	PD
017	1	53.1	230.9	0.0441	97.5	504.8	0.0114	PR
Mean		33.2	254.5	0.1152	76.6	433.3	0.0449	
SD		21.1	148.8	0.1449	43.7	273.4	0.0641	

NOTE. Data from the first and fourth infusions analyzed independently.

Abbreviation: $T_{1/2}$, half-life.

*One- or 2-compartment IV infusion model.

†C_{max} is the maximum observed concentration of IDEC-C2B8 attained during the sampling period.

‡Clearance for the first and fourth infusion was determined over comparable time intervals using the equation clearance (Cl) = dose/area under the concentration-time curve.

§The immediate post-fourth infusion sample for patient no. 016 was not available for analysis.

interval, 11% to 55%); PRs were noted in six of 18 patients (one at 125, two at 250, and three at 375 mg/m²). The median time to onset of response for the these six patients was 35.5 days (range, 7 to 64) and the median time to disease progression was 6.4 months (range, 3 to 21.7). MRs were observed in five patients, and seven patients had PD. The mean reduction in measured lesions was 79% (range, 50% to 100%) in responders. At baseline, 13 of 18 assessable patients (72%) had extranodal disease, and three of these 13 had a PR (23%; 50% of responders). Two of 10 patients (20%) with baseline BM invasion responded with a PR. Six of 15 patients (40%) with low-grade histology responded with a PR. Four patients who had undergone prior high-dose therapy with ABMT had the following responses: one PR that lasted 21 months, one MR (30% shrinkage) that lasted 2.6 months, one PD, and one nonassessable patient. Of six patients with bulky disease, four responded with a PR and two had PD.

An example of the clinical response (patient no. 017) following four infusions of 375 mg/m² IDEC-C2B8 is shown in Fig 1. This patient had follicular mixed small- and large-cell (FML) NHL previously treated with aggressive chemotherapy. Relapsed disease was treated with high-dose chemotherapy and total-body irradiation with autologous bone marrow support. PD was noted less than 1 year following high-dose therapy and was treated with chemotherapy. The disease again progressed rapidly and the patient was treated with IDEC-C2B8. Pretreatment computed tomographic images (Fig 1A) demonstrated a large abdominal mass. Following the fourth mAb infusion, all peripheral disease resolved and the abdominal mass progressively decreased in size as shown on the 3-, 6-, and 9-month images (Fig 1B, C, and D, respectively). The patient remained in PR for 22 months and then had disease progression at a distant site. Biopsy confirmed continued FML histology expressing CD20 and the patient was re-treated at the same dose level and has had an additional ongoing response.

Table 6. Dose-Response Relationship (N = 18)

Response	Dose Group (mg/m²) 125	250	375
CR	0	0	0
PR	1	2	3
SD	1	2	3
PD	1	2	3
OR	1/3 (33%)	2/6 (33%)	3/9 (33%)

NOTE. Two patients were not assessable. Time to progression for PR: 125 dose, 3 months; 250 dose, 6.2+ months to 21 months; 375 dose, 21.7, 4.4, and 10.1 months.

Abbreviations: OR, overall response; SD, stable disease (neither PR/CR or PD).

Three patients had previously received a single infusion of IDEC-C2B8 (50, 100, and 500 mg/m² with no response, mixed response and MR, respectively) in an earlier trial.[20] Upon disease progression, these patients continued to express the CD20 antigen on tumor biopsy with intensity similar to baseline. In the current trial, two responded with a PR and the other was not assessable. Two patients with intermediate-grade bulky disease died 2 and 4 months following treatment due to progressive lymphoma.

DISCUSSION

IDEC-C2B8 is a practical outpatient treatment given over a brief, 3-week course. Dose-limiting toxicities were

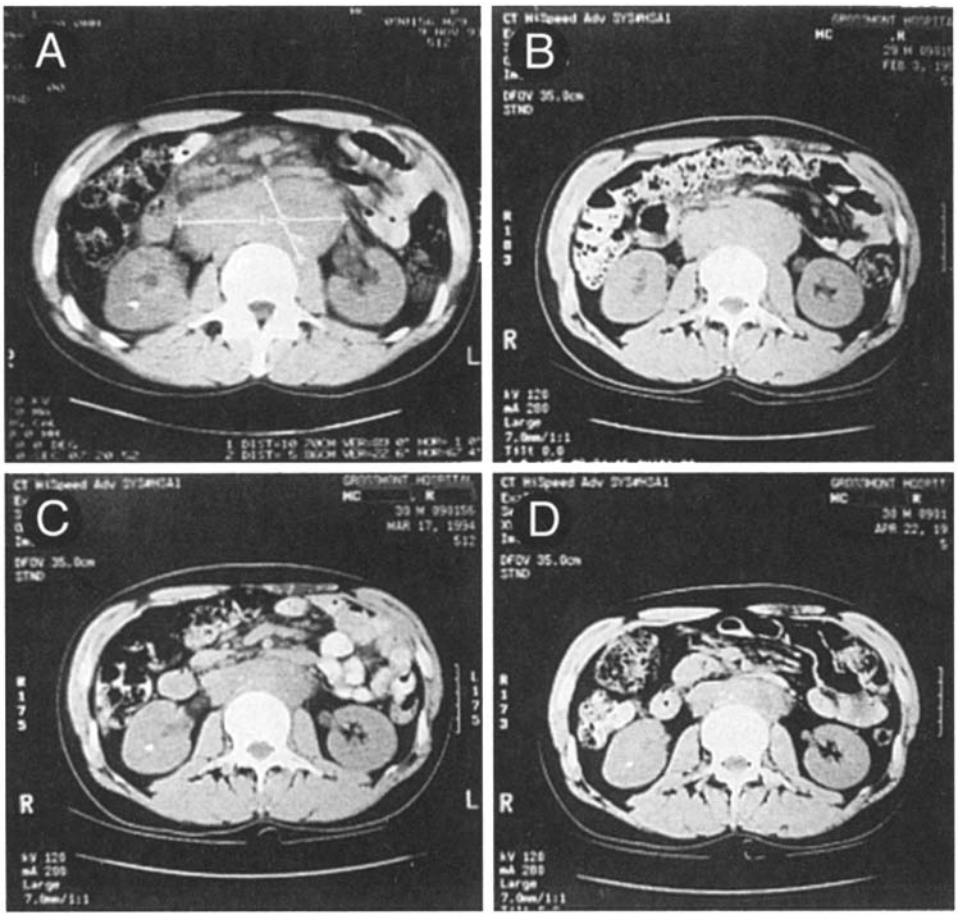

Fig 1. Response to antibody therapy. Images of abdominal mass of patient no. 017 who had progressed following ABMT. (A) Pre-antibody infusion. (B) One month, (C) 3 months, and (D) 6 months posttreatment with 4 infusions of 375 mg/m^2 IDEC-C2B8 chimeric anti-CD20 mAb.

not identified in the highest dose group and a maximum-tolerated dose (MTD) was not reached. No HAMA responses were identified and one detectable, but not quantifiable, HACA response noted 7 months posttreatment produced no clinical or laboratory abnormalities. Although some infusions were slowed or stopped temporarily, all infusions initiated were completed.

All AEs were reversible and all but one was infusion-related. AEs, classified as primarily grade 1 or 2, were limited to the length of infusion or shortly thereafter. Incidence was highest with the first infusion that coincided with rapid and specific B-cell depletion and subsequent infusions resulted in markedly fewer events. Similar types of AEs have occurred with administration of other biologic agents such as IV gamma globulin. These symptoms seen with the initial infusion may represent biologic effects that result from B-cell depletion. Although the in vivo mechanism of B-cell depletion is unknown, B cells coated in vitro with IDEC-C2B8 are lysed through complement-mediated cytolysis and ADCC using human complement or effector cells.[18] Antibody-coated cells may be removed via Fc-receptor binding and phagocytosis by the reticuloendothelial system. B-cell lysis or binding of immune complexes to Fc receptors on phagocytic cells may lead to cell activation and release of mediators/cytokines that could produce the observed infusion-related symptoms. The paucity of AEs observed in the second through fourth infusions may be explained partially by the depletion of circulating B cells following the first infusion and by the persistence and accumulation of circulating antibody observed following the first infusion. Other possibilities include tachyphylaxis to secreted cytokines.

Importantly, only limited myelosuppression with few significant hematologic changes was observed. Thrombocytopenia, the only AE that lasted significantly beyond the infusion time, occurred in three patients (one of whom had significant thrombocytopenia previously and all of whom had marrow involvement pretreatment). Platelet levels recovered to greater than 100,000/μL in 3, 7, and

21 days. Platelets do not express the CD20 antigen or react with IDEC-C2B8, but their Fc receptors can bind to immune complexes, which leads to platelet activation and perhaps removal. This "innocent bystander" effect may account for transient thrombocytopenia following only the first infusion when a large number of circulating B cells were present in the peripheral blood. Since this antibody does not appear to impair marrow reserves, it could possibly be used in patients who are myelosuppressed due to recent chemotherapy or following high-dose chemotherapy with ABMT or peripheral stem-cell rescue. Some alterations in IG levels were noted, but did not result in an increased incidence of infections.

The described AE pattern differs from that found with chemotherapy, radiotherapy, or with other antibodies such as the CAMPATH antibodies, where both incidence and severity of AEs increase with each successive treatment. Most patients in this trial experienced minimal side effects and two patients experienced no AEs.

A pharmacokinetic analysis of IDEC-C2B8 serum levels showed that the C_{max} for both the first and fourth infusions increased with increasing dose. In addition, the C_{max} and serum half-life increased between the first and fourth infusions for most patients. It is known that a majority of normal and malignant circulating B cells are cleared after the first infusion, which has a pronounced effect on the half-life of the antibody. In addition, circulating free antibody is cleared from the serum by antigen present on lymphoma cells. The saturation of these antigenic sites by IDEC-C2B8 during the early portion of the treatment course could also contribute to the differences in the pharmacokinetic profile of the antibody between the first and fourth infusions.

Responses were observed in a variety of clinical situations. Three of 13 patients with extranodal disease had a PR. Responses occurred in patients heavily pretreated with chemotherapy, including aggressive regimens and ABMT, and in patients with bulky disease. Responding sites included BM, lymph nodes, extranodal masses, and spleen.

The antibody appears most active in patients with low-grade or follicular histologies, although the number of patients with aggressive histologies was limited. In the previously published single-dose trial, one PR was documented at each of the 100- and 500-mg/m^2 levels.[20] During this multiple-dose phase I trial, confirmed PRs occurred at all three dose levels: one at 125 mg/m^2, two at 250 mg/m^2, and three at 375 mg/m^2. Response rates were as follows: (1) overall response rate, 30% (six of 20); (2) response rate in assessable patients, 33% (six of 18); and (3) response rate in assessable, low-grade or follicular patients, 37.5% (six of 16). The 375-mg/m^2 dose has been selected for phase II clinical trial evaluation to determine the response rate in patients with follicular or low-grade, relapsed B-cell lymphoma.

Several mechanisms may account for the clinical antitumor activity of this mAb. The construction of a chimeric antibody that contains the human IgG1 heavy-chain constant region greatly augments its capacity to lyse CD20$^+$ B-cell lymphoma targets in vitro using human complement and human effector cells capable of ADCC.[18] In addition, studies have demonstrated that anti-CD20 antibodies may have direct antiproliferative activity on B-lymphoma cell lines, including in some cases the induction of apoptosis.[19,22] However, it is also clear that not all CD20$^+$ cell lines are sensitive to this direct growth inhibition. The CD20 molecule appears to function as a calcium channel and to be involved with progression through the cell cycle.[11] Antibodies that bind to CD20 have been shown to induce cellular protein phosphorylation.[23] Unfortunately, it has been difficult to grow low-grade B-cell lymphoma cells directly from the patient and, thus, it is not known to what extent direct antiproliferative signals mediated through CD20 may contribute to observed clinical activity. Studies to explore these issues are underway in several laboratories.

Multiple therapeutic treatment alternatives are available to patients with relapsed low-grade or follicular lymphoma and range from observation to chemotherapy with single-agent or high-dose protocols that require stem-cell support. In general, a continuous pattern of relapse with mounting cumulative BM toxicity has been observed with these approaches. IDEC-C2B8 represents a novel form of therapy with a unique, minimal toxicity profile. Using the schedule described in this study, the antibody is infused during a brief outpatient treatment and therapy is completed in 22 days. The lack of measurable antiglobulin immune responses makes re-treatment possible. Finally, the lack of substantial short- or long-term toxicity suggests that use of this antibody will not preclude subsequent use of traditional chemotherapy. Potentially, the agent could be used singly, in combination with standard chemotherapy, or following standard chemotherapy in an attempt to decrease minimal residual lymphoma and extend the duration of remission. Preliminary results to explore the combination of this antibody with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy have

been encouraging.[24] Additional potential applications include in vivo B-cell depletion before harvesting peripheral-blood or BM stem cells, as well as possible treatment of patients with autoimmune diseases caused by autoreactive antibodies.

In summary, the IDEC-C2B8 anti-CD20 antibody offers safe, nonmyelosuppressive, well-tolerated therapy with significant activity in patients with relapsed, low-grade B-cell lymphoma.

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