

Phase 1 Study of Lumiliximab with Detailed Pharmacokinetic and Pharmacodynamic Measurements in Patients with Relapsed or Refractory Chronic Lymphocytic Leukemia

John C. Byrd,¹ Susan O'Brien,² Ian W. Flinn,³ Thomas J. Kipps,⁴ Mark Weiss,⁵ Kanti Rai,⁶ Thomas S. Lin,¹ James Woodworth,⁷ Dee Wynne,⁷ Jennifer Reid,⁷ Arturo Molina,⁷ Bryan Leigh,⁷ and Sarah Harris⁷

Abstract **Purpose:** Therapeutic antibodies have improved the outcome for patients with chronic lymphocytic leukemia (CLL). We conducted a phase 1, dose escalation and schedule optimization study of the primatized anti-CD23 antibody, lumiliximab, in patients with previously treated and refractory CLL. **Experimental Design:** Forty-six patients were assigned sequentially to cohorts 1 through 6 and received lumiliximab at 125, 250, or 375 mg/m² weekly for 4 weeks; 500 mg/m² weekly for 4 weeks [500(A)]; 500 mg/m² thrice during week 1 then 500 mg/m² weekly for the next 3 weeks [500(B)]; or 500 mg/m² thrice a week for 4 weeks [500(C)], respectively. **Results:** The median age was 62 years (range, 47-80), and the median number of prior regimens was four (range, 1-13). No partial or complete responses were observed. Toxicity was limited and unrelated to dose. The pharmacokinetics of lumiliximab was similar to other IgG₁ monoclonal antibodies with accumulation at doses ≥ 250 mg/m² and a median terminal half-life of 7 days. Pharmacodynamic studies showed dose-dependent increases in soluble CD23, but no down-regulation of CD23 antigen. Saturation of CD23 receptors occurred at 250 mg/m² and was maintained for ≥ 1 week following completion of therapy at ≥ 375 mg/m². **Conclusions:** Treatment with lumiliximab seemed to be well tolerated and to have clinical activity in patients with relapsed or refractory CLL.

Chronic lymphocytic leukemia (CLL) a common type of adult leukemia, is diagnosed on the basis of cells with lymphocytosis exhibiting the malignant B-cell immunophenotype of CD5⁺/CD19⁺/CD20⁺/HLA-DR⁺/CD23⁺/sIg^{dim} (1). Recent efforts have

generated a number of molecular and genetic markers to identify patients at high risk of early disease progression and short survival, including interphase cytogenetics [del(17p13.1) and del(11q22.3)], immunoglobulin heavy chain variable (IgV_H) gene mutational status, ZAP-70 expression, and p53 dysfunction (e.g., from p53 or ATM deletions with or without mutations; ref. 2).

Therapy is usually initiated only at the time symptoms develop because studies comparing early versus delayed chlorambucil (3) showed no survival benefit with immediate treatment. Although the use of purine nucleoside analogues has invigorated research in CLL (4, 5), and treatment outcome has improved with fludarabine (6-8) and fludarabine-based chemotherapy combinations (9, 10), most patients with active disease eventually relapse and become refractory to fludarabine. These findings emphasize the need for effective, new therapies for both symptomatic, untreated CLL and relapsed, fludarabine-refractory CLL.

Rituximab, a chimeric anti-CD20 antibody, showed efficacy in both untreated and previously treated patients with CLL (11-13). Studies combining rituximab with either fludarabine or fludarabine plus cyclophosphamide have noted complete responses and prolonged remissions in previously untreated patients (14, 15). Treatment with alemtuzumab, a humanized anti-CD52 antibody, had significant activity in fludarabine-refractory CLL, but was also associated with morbidity from infections and infusion toxicity (16, 17). The success of these monoclonal antibodies in CLL suggests that cell surface antigens are promising targets for the treatment of CLL. Agents that are selective for B cells would be ideal for this purpose to avoid immunosuppression.

Authors' Affiliations: ¹The Ohio State University Comprehensive Cancer Center, Columbus, Ohio; ²The University of Texas, M. D. Anderson Cancer Center, Houston, Texas; ³Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland; ⁴Moore's Cancer Center of the University of California, San Diego, La Jolla, California; ⁵Memorial Sloan-Kettering Cancer Center, New York, New York; ⁶Long Island Jewish Medical Center, New Hyde Park, New York; and ⁷Biogen Idec, Inc., San Diego, California. Received 6/15/06; revised 10/11/06; accepted 11/14/06.

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Requests for reprints: John C. Byrd, B302 Starling Loving Hall, The Ohio State University, 320 West 10th Avenue, Columbus, OH 43210. Phone: 614-293-7509; Fax: 614-293-7526; E-mail: John.Byrd@osumc.edu.

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Lumiliximab, a macaque-human primatized monoclonal antibody, targets the CD23 antigen, which has limited expression on activated B cells, including the majority of CLL cells. Preclinical studies have shown that lumiliximab induces the apoptosis of CD23-bearing lymphoid cell lines (18–22) and prolongs the survival of severe combined immunodeficiency mice inoculated with CD23-bearing lymphoblastic cell lines (18, 21, 23). A favorable safety profile for lumiliximab in patients with allergic disorders was shown, without significant infusion-related toxicities or immunosuppression typically observed with other therapeutic antibodies, e.g., rituximab and alemtuzumab (24). Based on the uniform expression of CD23 on primary CLL cells (25), preclinical studies suggesting that lumiliximab may have *in vitro* activity in primary CLL cells (18–23, 26), and the safety profile previously observed with lumiliximab (24), a phase 1 study incorporating detailed pharmacokinetic and pharmacodynamic measurements was done.

Materials and Methods

From September 2002 to January 2004, patients with symptomatic, previously treated CLL were enrolled if they gave written informed consent; were ≥ 18 years of age; had histologic confirmation of CD23⁺ CLL or small lymphocytic lymphoma by National Cancer Institute 96 (NCI 96) criteria (1); had progressive disease, defined by the NCI 96 criteria after ≥ 1 prior courses of chemotherapy (1); had a prestudy WHO performance status of ≤ 2 ; had an expected survival of ≥ 6 months; had a 4-week interval of no radiotherapy, radioimmunotherapy, biological therapy, or chemotherapy before study enrollment; and had adequate liver and renal function (bilirubin, ≤ 2.0 mg/dL; aspartate aminotransferase or alanine aminotransferase and serum creatinine, ≤ 1.5 institutional upper limit of normal). All patients had symptomatic, progressive CLL at the time of treatment. Patients were allowed to enroll in the study irrespective of the degree of disease progression that occurred between the time of screening and treatment, provided there was no new laboratory parameter that made them ineligible for treatment. There was no exclusion for prior rituximab treatment. Patients were not stratified in this phase 1 trial.

Pretreatment and serial laboratory assessments. Baseline laboratory assessments included complete blood count with differential, platelet count, and absolute lymphocyte count (ALC); serum chemistries, including liver functions; urinalysis; direct and indirect antibody tests; β_2 -microglobulin; interphase cytogenetics (27); and an electrocardio-

gram. Patient samples were collected weekly for complete blood count and serum chemistry measurements during the treatment period, every 3 months during posttreatment follow-up for 2 years in the absence of disease progression, and every 6 months during follow-up for 2 additional years.

Treatment. Patients were assigned sequentially to cohorts 1 through 6 and received lumiliximab at 125, 250, or 375 mg/m² weekly for 4 weeks; 500 mg/m² weekly for 4 weeks [500(A)], 500 mg/m² thrice during week 1 then 500 mg/m² weekly for the next 3 weeks [500(B)], or 500 mg/m² thrice a week for 4 weeks [500(C)], respectively (Table 1). The first dose of lumiliximab was divided, 20% and 80% on days 1 and 2, respectively, to minimize severe infusion reaction risks. Subsequent infusions were given over a 2-h period, unless infusion reactions necessitated a rate reduction. Granisetron hydrochloride (or equivalent) was provided for all patients before each of the first two doses of lumiliximab or as clinically indicated, and other supportive care was administered at the discretion of the treating physician. All patients received acetaminophen and diphenhydramine hydrochloride before each lumiliximab dose.

Toxicity assessment and dose-limiting toxicity. Toxicity assessments were determined using the NCI Common Toxicity Criteria version 2. Hematologic toxicity was assessed according to the NCI 96 criteria for CLL (1). Dose-limiting toxicity (DLT) was defined as any event with possible, probable, or unknown relationship to lumiliximab occurring up to day 28, including $\geq$ grade 3 nonhematologic toxicity, grade 2 acute allergic infusion reactions (urticaria and/or asymptomatic bronchospasm), and grade 4 hematologic toxicity persisting for ≥ 7 days. Follow-up safety visits occurred on days 29, 36, 43, and 50.

Criteria for dose escalation. To limit the number of patients exposed to potentially inadequate doses of lumiliximab, a standard phase 1, 3 + 3 dose escalation schema was followed until early evidence of clinical activity was observed (i.e., $\geq 50\%$ reduction in ALC by day 28) and no DLT by day 28. Enrollment into a cohort was to be expanded to 10 patients when early evidence of clinical activity was observed in at least 1 patient and either 0 of 3 or 1 of 6 patients experienced DLT; each 500 mg/m² dose cohort was expanded to 10 patients regardless of evidence of early clinical activity if no more than 1 of 6 patients experienced DLT. Escalation to the next dose cohort occurred as follows: when none of the first 3 patients experienced DLT and no early clinical activity was observed, when 1 of 6 patients experienced DLT and no early clinical activity was observed, or when < 3 of 10 patients experienced DLT.

Response assessments. The primary efficacy variable in this study was overall response rate, the percentage of subjects with response classified as complete response or partial response, using the NCI 96 criteria for CLL (1).

Table 1. Treatment groups

Cohort	Dose	Dose schedule	No. of patients enrolled
1	125 mg/m ² weekly $\times$ 4	25 mg/m ² on day 1, 100 mg/m ² on day 2, then 125 mg/m ² once weekly $\times$ 3	3
2	250 mg/m ² weekly $\times$ 4	50 mg/m ² on day 1, 200 mg/m ² on day 2, then 250 mg/m ² once weekly $\times$ 3	3
3	375 mg/m ² weekly $\times$ 4	75 mg/m ² on day 1, 300 mg/m ² on day 2, then 375 mg/m ² once weekly $\times$ 3	10
4	500 mg/m ² weekly $\times$ 4 [500(A)]	100 mg/m ² on day 1, 400 mg/m ² on day 2, then 500 mg/m ² once a week $\times$ 3 wks	10
5	500 mg/m ² 3 $\times$ first week, weekly $\times$ 3 [500(B)]	100 mg/m ² on day 1, 400 mg/m ² on day 2, 500 mg/m ² on days 3 and 5, then 500 mg/m ² weekly $\times$ 3	10
6	500 mg/m ² 3 $\times$ per week $\times$ 4 wks [500(C)]	100 mg/m ² on day 1, 400 mg/m ² on day 2, 500 mg/m ² on days 3 and 5, then 500 mg/m ² 3 $\times$ per week $\times$ 3 wks	10

Table 2. Patient demographics

	Total (N = 46)
Age, median (range)	62 (47-80)
n (%) ≥ 65 y	17 (37)
Female, n (%)	13 (28)
Weight (kg), median (range)	82 (56-116)
Rai stage at study entry, n (%)	
I/II	24 (52)
III/IV	22 (48)
WHO Performance Status, n (%)	
0	9 (20)
1	36 (78)
2	1 (2)
Organomegaly	
n (%) with splenomegaly	16 (35)
n (%) with hepatomegaly	5 (11)
n (%) with lymphadenopathy	44 (96)
Hematologic, median (range)	
WBC ($\times 10^3$ cells/mm ³)	21.80 (2.1-307.6)
Hgb (g/dL)	11.90 (7.5-16)
Platelets ($\times 10^3$ plts/mm ³)	113.5 (39-297)
β 2-Microglobulin (μ g/mL), median (range)	3.55 (1.6-12.6)
Interphase cytogenetic abnormalities	
n (%) with del(13q14.3)	27 (59)
n (%) with del(11q22.3)	10 (22)
n (%) with del(17p13.1)	8 (17)
n (%) with Trisomy 12	4 (9)
Treatment history	
Prior therapies, median (range)	4 (1-13)
n (%) alkylator-refractory	14 (30)
n (%) fludarabine-refractory	25 (54)
Months since most recent relapse, median (range)	2.80 (0.1-27.3)

Pharmacokinetics. Blood samples were collected preinfusion and 10 min postinfusion on all infusion days with additional samples collected on days 1 and 2 (cohorts 1-4) and day 8 (all cohorts) at 1, 2, and 24 h postinfusion (optional samples at 4, 6, and 48 h). Sampling continued weekly for all cohorts up to day 50 and then every 3 months from months 3 to 12.

Serum concentrations of total lumiliximab antibody were determined using a validated ELISA developed by Biogen Idec, in which a monoclonal antilumiliximab antibody was used as the capture reagent, followed by a blocking step and incubation with standards, controls, and patient samples. Lumiliximab in patient samples was detected by the addition of antihuman IgG-horseradish peroxidase (Southern Biotech), color was developed with tetramethylbenzidine substrate, and lumiliximab concentrations were calculated by extrapolation from a four-parameter standard curve. The assay was validated according to International Conference on Harmonization guidelines, and has a lower limit of quantitation of 450 ng/mL.

Serum concentrations of free lumiliximab were determined by a three-part process. First, CD23/lumiliximab complexes were removed from patient serum by incubation with anti-CD23-coated magnetic beads (anti-CD23 clone M-L233; BD Biosciences) for at least 1 h, followed by separation with the Promega Magnabot 96 Magnetic Separation Device. Next, removal of all complexes was verified by evaluating samples that had been incubated with the beads in the CD23/lumiliximab complex assay described below. Finally, the verified serum samples were tested in the lumiliximab ELISA described above.

Lumiliximab serum concentrations versus time data were analyzed with the program NONMEM (Globomax). Multiple models were evaluated and the best model was determined by minimization of the minimum objective function. Models were constructed using clearance model variables. For the one-compartment model, clearance (Cl) and volume of distribution (V) were the primary model-fitting variables,

with the elimination rate constant (K) related to both Cl and V. A two-compartment model was also attempted using Cl, V in the central compartment (V₁), V in the peripheral compartment (V₂), and intercompartmental Cl (Q) as the primary model variable. Michaelis-Menten models for both the one-compartment and two-compartment models were also tested. The Michaelis-Menten models were described by V, the maximum metabolic rate (V_{max}), and the Michaelis rate constant (K_m), which equals one-half the concentration needed to attain V_{max}.

Other variables measured included the maximum concentration (C_{max}), time to achieve the maximum concentration (t_{max}), the area-under-the-serum-concentration-time curve from 0 to infinity (AUC), and half-life. AUC was estimated from the calculated clearance through the following relationship: AUC = (total dose) / Cl, where the total dose reflects all doses given during the course of therapy received by the patients.

Pharmacodynamic studies. Serum concentrations of soluble CD23 (sCD23) using a validated ELISA were determined at the same time points as specified for lumiliximab concentrations. Antihuman CD23 (clone M-L233, BD Biosciences) was used as a capture reagent. After overnight incubation, plates were blocked and incubated with standards, controls, and patient serum samples. CD23 was detected by the addition of a monoclonal anti-CD23 antibody (clone EBVCS-5, BD Biosciences) labeled with biotin, streptavidin-horseradish peroxidase, and tetramethylbenzidine as the substrate. CD23 concentrations were calculated by extrapolation from a four-parameter standard curve. The assay was validated according to International Conference on Harmonization guidelines and had a lower limit of quantitation of 1,600 pg/mL (1.6 ng/mL).

Serum concentrations of CD23/lumiliximab complexes were determined in baseline and on-study samples using a semiquantitative, validated ELISA developed by Biogen Idec. Briefly, anti-human CD23 monoclonal antibody (clone M-L233, BD Biosciences) was used as the capture reagent. After overnight incubation, plates were blocked, and patient samples, standards, and controls were added. An Anti-human IgG-horseradish peroxidase (Southern Biotech) and tetramethylbenzidine were used as the detection reagent and substrate, respectively. CD23/lumiliximab concentrations were determined by extrapolation from a four-parameter standard curve. The assay was validated according to International Conference on Harmonization guidelines.

Flow cytometry assessment. Flow cytometric analyses were conducted to assess changes in lymphocyte subsets and to study both CD23 expression and binding of lumiliximab during and following treatment. Blood samples for flow cytometry were obtained at baseline and at protocol-specified visits. Cohorts 1 to 4 had samples obtained on days 2 and 8 [preinfusion and 10 min, 1, 24, and 48 h (optional) postinfusion]. Cohorts 5 and 6 had samples obtained on days 2, 3, and 5 (preinfusion and 10 min, 1 and 24 h postinfusion), and days 8, 10 (cohort 6 only), 15, and 22 (preinfusion and 10 min postinfusion). Sampling continued weekly for all cohorts up to day 50, then every 3 months from months 3 to 12 if the patient was still on the study.

Three- and four-color immunophenotyping was done to determine the relative mean fluorescence intensity of CD23⁺, CD38⁺, CD55⁺, and CD59⁺ cells and the absolute numbers of total T cells (CD3⁺), T helper (T_H) cells (CD3⁺, CD4⁺), T suppressor (T_S) cells (CD3⁺, CD8⁺), natural killer cells (CD3⁺, CD16⁺, CD56⁺), total B cells (CD19⁺), and CLL cells (CD5⁺ and CD19⁺), % CD38⁺ CLL cells (CD5⁺, CD19⁺, CD38⁺). To determine the proportion of lumiliximab-coated CLL cells at serial time points, a labeled CD23 antibody (clone EBVCS-5, BD Biosciences/PharMingen) and a lumiliximab antibody that bind to different epitopes of CD23 were used.

Antilumiliximab antibodies. A validated antilumiliximab ELISA developed by Biogen Idec was used to determine the concentration of human antibody to lumiliximab in serum. Samples were tested at baseline and at 3-month intervals during the posttreatment follow-up period. The lower limit of quantitation for this assay was 400 ng/mL of antilumiliximab.

Results

Patient demographics. Forty-six patients gave consent and were treated at seven clinical sites. Most patients were Caucasian (93%) and male (72%), with a median age of 62 years (47-80 years) at baseline (Table 2). The median time from diagnosis to study entry was 7 years (1-22 years), with a median of 3 months from the most recent relapse (0-27 months). The majority of patients presented with a WHO Performance Status of 1 (78%), had Rai disease stage of I/II (52%), and had a median sCD23 serum concentration of 0.2235 $\mu\text{g/mL}$ (0.030-2.400 $\mu\text{g/mL}$). The median number of prior CLL regimens was four (1-13). The most common prior CLL agents included fludarabine (98% of patients), rituximab (78%), and cyclophosphamide (63%); 54% of patients were fludarabine-refractory, as previously defined (16).

Toxicity assessments. Infusion toxicity with lumiliximab was modest. The duration of each infusion was a median of 2 h (1.2-5.6 h). Transient grade 1 and 2 fatigue (22%), nausea (13%), hypotension (13%), headache (13%), myalgia (9%), and rigors (7%) occurred during the infusion. One patient withdrew from therapy on day 1 due to a grade 4 headache, without symptoms of aseptic meningitis, which resolved spontaneously by day 2.

Thirty-three infections were reported in 19 of 46 patients (41%); 26 were grade 1 or 2 infections, treated on an outpatient basis; 6 were grade 3; and 1 was grade 4. The incidence rate for grade 3 or 4 infections prior to subsequent CLL therapy was 0.03 per patient month (multiple infections per subject were counted). The majority of infections were upper respiratory ($n = 8$) or pneumonia ($n = 7$). One case of systemic parainfluenza was diagnosed; however, no other opportunistic infections were observed during the follow-up period before

Table 3. Incidence of most common ($\geq 5\%$) and all grades 3 and 4 study-related adverse events by system organ class, by preferred term, and by grade

	Grade*					Total N = 46 (%)
	1	2	3	4	Unknown	
Any adverse event	26 (57)	8 (17)	4 (9)	3 (7)	0 (0)	41 (89)
Blood and lymphatic system disorders	2 (4)	1 (2)	3 (7)	1 (2)	0 (0)	7 (15)
Anemia NOS	4 (9)	0 (0)	0 (0)	1 (2)	0 (0)	5 (11)
Neutropenia	0 (0)	0 (0)	1 (2)	1 (2)	0 (0)	2 (4)
Autoimmune hemolytic anemia NOS	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)
Hemolytic anemia NOS	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)
Leukopenia NOS	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)
Thrombocytopenia	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	1 (2)
Gastrointestinal disorders	21 (46)	3 (7)	0 (0)	0 (0)	0 (0)	24 (52)
Constipation	8 (17)	1 (2)	0 (0)	0 (0)	0 (0)	9 (20)
Nausea	9 (20)	0 (0)	0 (0)	0 (0)	0 (0)	9 (20)
Abdominal pain NOS	2 (4)	2 (4)	0 (0)	0 (0)	0 (0)	4 (9)
Abdominal distension	3 (7)	0 (0)	0 (0)	0 (0)	0 (0)	3 (7)
Abdominal pain, upper	3 (7)	0 (0)	0 (0)	0 (0)	0 (0)	3 (7)
General disorders and administration site conditions	9 (20)	6 (13)	1 (2)	0 (0)	0 (0)	16 (35)
Fatigue	4 (9)	2 (4)	0 (0)	0 (0)	0 (0)	6 (13)
Fatigue, aggravated	4 (9)	0 (0)	1 (2)	0 (0)	0 (0)	5 (11)
Rigors	1 (2)	3 (7)	0 (0)	0 (0)	0 (0)	4 (9)
Pyrexia	1 (2)	2 (4)	0 (0)	0 (0)	0 (0)	3 (7)
Infections and infestations	3 (7)	2 (4)	2 (4)	0 (0)	0 (0)	7 (15)
Pneumonia NOS	0 (0)	0 (0)	2 (4)	0 (0)	0 (0)	2 (4)
Parainfluenzae virus infection NOS	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)
Musculoskeletal and connective tissue disorders	10 (22)	2 (4)	0 (0)	0 (0)	0 (0)	12 (26)
Myalgia	4 (9)	0 (0)	0 (0)	0 (0)	0 (0)	4 (9)
Nervous system disorders	10 (22)	0 (0)	0 (0)	1 (2)	0 (0)	11 (24)
Headache NOS	8 (17)	0 (0)	0 (0)	1 (2)	0 (0)	9 (20)
Respiratory, thoracic and mediastinal disorders	12 (26)	1 (2)	1 (2)	1 (2)	0 (0)	15 (33)
Cough	6 (13)	2 (4)	0 (0)	0 (0)	0 (0)	8 (17)
Dyspnea NOS	3 (7)	0 (0)	1 (2)	1 (2)	0 (0)	5 (11)
Nasal congestion	3 (7)	0 (0)	0 (0)	0 (0)	0 (0)	3 (7)
Hypoxia	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)
Skin and s.c. tissue disorders	7 (15)	4 (9)	1 (2)	0 (0)	0 (0)	12 (26)
Sweating increased	3 (7)	2 (4)	0 (0)	0 (0)	0 (0)	5 (11)
Night sweats	3 (7)	0 (0)	0 (0)	0 (0)	0 (0)	3 (7)
Rash NOS	2 (4)	1 (2)	0 (0)	0 (0)	0 (0)	3 (7)
Dermatitis allergic	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)
Vascular disorders	5 (11)	3 (7)	0 (0)	0 (0)	0 (0)	8 (17)
Hypotension NOS	2 (4)	3 (7)	0 (0)	0 (0)	0 (0)	5 (11)

NOTE: Study-related adverse events of probable, possible, or unknown relationship to lumiliximab. Incidence, $\geq 5\%$ of total patients evaluated and all grade 3 and 4 events.

Abbreviation: NOS, not otherwise specified.

*Each patient counted only under worst grade experienced.

initiation of subsequent CLL therapy. Examination of T_h ($CD3^+/CD4^+$), T_s ($CD3^+/CD8^+$), and natural killer cell ($CD3^+/CD16^+/CD56^+$) subsets before, during, and at completion of therapy showed no meaningful change. For all patients enrolled, no substantial changes in CD4, CD8, or CD56 counts were observed from baseline to day 29. The median pretreatment CD4 count was 476 cells/mm³ (range, 0-4475) and did not change substantially with lumiliximab treatment as evidenced by the posttreatment (day 29) median value of 514 cells/mm³ (range, 0-5859). Similar results were observed for CD8: pretreatment median count of 498 cells/mm³ (range, 63-4475) versus posttreatment median count of 658 cells/mm³ (range, 0-5859), and CD56: pretreatment median count of 232 cells/mm³ (range, 0-4475) versus posttreatment count of 311 cells/mm³ (range, 0-7,226).

Forty-one of 46 patients (89%) experienced adverse events classified as related (probably, possibly, or of unknown relationship) to lumiliximab (Table 3). Toxicities observed in this study were not related to lumiliximab treatment dose or fludarabine-refractory status. Most patients had adverse events of short duration, with a maximum severity of grade 1 ($n = 26$, 57%) or grade 2 ($n = 8$, 17%). Seven patients experienced grade 3 ($n = 4$) or grade 4 ($n = 3$) related adverse events. The most common related adverse events were fatigue (24%), constipation (20%), headache (20%), nausea (20%), cough (17%), and anemia (15%). Grade 3 and 4 events classified as related included autoimmune hemolytic anemia ($n = 2$), neutropenia ($n = 2$), pneumonia ($n = 2$), anemia/thrombocytopenia ($n = 1$), dyspnea/hypoxemia/fatigue ($n = 1$), dyspnea ($n = 1$), headache ($n = 1$), leukopenia ($n = 1$), and parainfluenza virus infection ($n = 1$). The autoimmune hemolytic anemia observed was responsive to immunosuppressive therapy. A patient with autoimmune hemolytic anemia had earlier developed symptomatic grade 3 pruritic dermatitis on day 85, which eventually progressed to an atypical possible paraneoplastic pemphigus in the setting of active disease. It is possible that this was related to therapy. Immunosuppressive therapy administered to treat the presumed paraneoplastic pemphigus resulted in a secondary infection on day 198 that ultimately resulted in the patient's death. Two DLTs were observed (grade 4 headache on day 1, probably related to treatment, and grade 4 neutropenia on day 15, possibly related to treatment; both resolved), but did not prohibit further dose escalation.

The incidence of grade 3 and 4 adverse events increased with baseline Rai stage; the observed incidence was 20% (3/15), 22% (2/9), 25% (1/4), and 39% (7/18) for subjects with a baseline Rai stage of 1, 2, 3, and 4, respectively. The incidence of grade 3 and 4 adverse events was lower in subjects who were refractory to fludarabine at baseline. The incidence was 20% (5/25) for fludarabine-refractory subjects compared with 38% (8/21) for subjects who were not fludarabine-refractory at baseline. Subjects who experienced a grade 3 or 4 adverse events had lower baseline ALC measurements than those who did not experience a grade 3 or 4 adverse events. No apparent toxicities occurred as a consequence of immunocomplexes.

Response assessments. A confirmed complete response or partial response by NCI 96 criteria at the 3-month response evaluation was not observed in this trial. One patient in the 375 mg/m² treatment group had stable disease at the 6-month follow-up visit and progressed after 28 months. This patient previously had stable disease for 24 months after fludarabine

treatment. The median time to progression was 2.6 months (0.5-28 months). The median TTNT was 3.3 months (0.8-28 months) with 42 patients undergoing subsequent CLL therapy. Of the four patients who did not receive additional therapy, two patients had disease progression, but did not receive subsequent CLL therapy prior to their deaths; one patient did not have disease progression or receive subsequent CLL therapy and died 21 months posttreatment; and one patient did not have disease progression or receive subsequent CLL therapy and remains alive. All deaths were related to CLL disease progression.

Although no patient attained a complete response or confirmed partial response, some clinical benefit was observed. Seventeen of 33 patients (52%) evaluated for lymphadenopathy at day 29 had a decrease in lymph node bulk; 8 patients achieved a reduction of $\geq 50\%$ as assessed by CT scan and physical examination (data not shown). Similarly, 42 of 46 patients (91%) had some reduction in lymphocytosis, with a median maximum decrease in ALC of 32% (5.2-84.3%) by day 29 which was most predominant in patients with a low lymphocyte count. The decline in lymphadenopathy and lymphocytosis was transient, with most patients progressing by 2 months posttherapy. Lymph node reduction or decreased lymphocytosis showed no relationship with each other or with the treatment dose or schedule.

Other measures of possible clinical benefit included performance status improvement in 11 of 37 patients (WHO Performance Status 1 or 2); unexplained, persistent, or recurrent fever ($\geq 100.5^\circ\text{F}$) at baseline resolved in 1 of 1 patient; baseline night sweats resolved ($n = 3$) or improved ($n = 2$) in 5 of 12 patients; and baseline unexplained weight loss ($\geq 10\%$) resolved ($n = 1$) or improved ($n = 1$) in 2 of 2 patients.

Pharmacokinetics. Forty (87%) of 46 patients had measurable concentrations that permitted the calculation of pharmacokinetic variables. Six of these 40 patients had concentrations for which only $C_{\max}$ and $t_{\max}$ could be reported; 2 had concentrations for which all pharmacokinetic variables could be calculated, except $C_{\max}$ and $t_{\max}$.

Median total lumiliximab serum concentration over time for all patients is shown by treatment group in Fig. 1. Samples from all patients were used in the pharmacokinetic modeling. A two-compartment Michaelis-Menten model best described the data, suggesting a saturable component to the drug's disposition. Population mean values of 114 mg/d, 167 $\mu\text{g/mL}$, 3.21 L/m², 1.90 L/m², and 0.522 L/h were determined for $V_{\max}$, K_m , V_1 , V_2 , and Q , respectively.

Concentrations of lumiliximab were above the mean population K_m value at doses of 375 mg/m² and above; the half-life of lumiliximab at these doses was ~ 7 to 10 days. The preference for a Michaelis-Menten model still suggests a saturable pathway and may be related to the saturation of CD23 receptors. The absence of consistently measurable concentrations from the lowest two doses suggests that saturation did occur and seemed to be complete following the 375 mg/m² dose. The relative dose linearity was examined by plotting AUC and $C_{\max}$ separately versus the total dose administered, suggesting dose linearity was present with escalating total doses of lumiliximab above 375 mg/m².

Pharmacodynamic measurements. The concentrations of sCD23, surface-bound lumiliximab, and surface CD23 were assessed serially during and after treatment to determine the ideal dosing of lumiliximab (Fig. 2). Median total sCD23

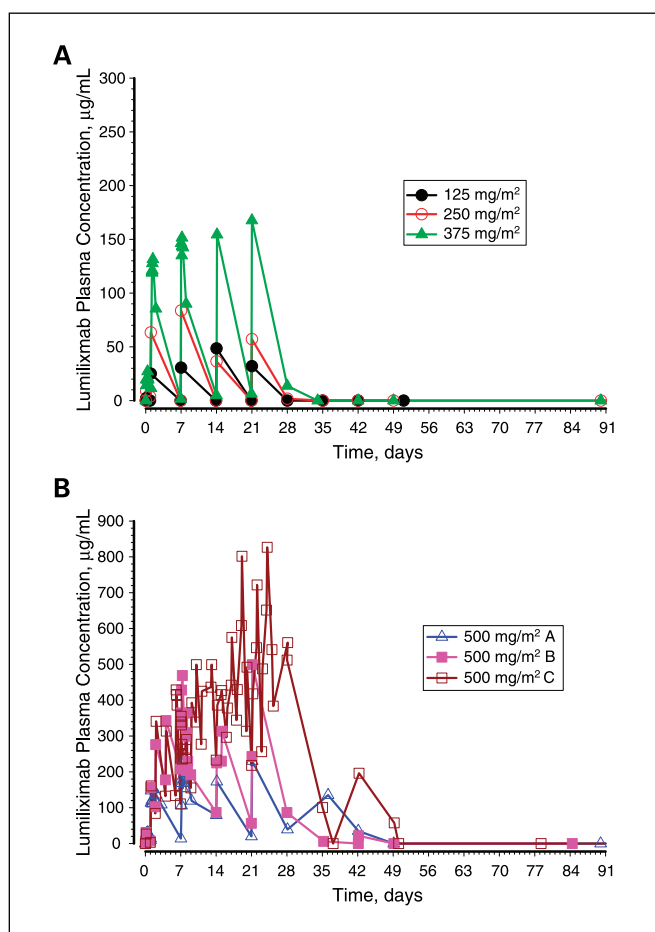


Fig. 1. A, median lumiliximab serum concentrations over time by treatment group. Concentrations represent doses of 125 (●), 250 (○), and 375 mg/m² (▲). B, median lumiliximab serum concentrations over time by treatment group. Concentrations represent the 500(A) (△), 500(B) (■), and 500(C) mg/m² treatments (□). Note the different Y-axis scales.

concentration increased over time with increasing total dose of lumiliximab and then decreased after treatment. A positive correlation ($r = 0.55$) was shown between the maximum sCD23 concentration observed 1 h after the infusion on day 22 and the dose group. The etiology of increasing sCD23 during therapy is currently under investigation and may represent circulating membrane fragments of dead CLL cells or enhanced shedding of CD23 antigen induced by treatment.

There seemed to be little or no association between sCD23 concentration at baseline and the percentage of reduction in ALC. This was assessed both graphically and using Pearson's correlation coefficient.

A near-linear relationship was present between free and total lumiliximab, with free drug concentrations similar to total drug concentration (slope = 0.979, $r = 0.98$). The percentage of difference between free and total lumiliximab was <20% in patients evaluated from the 500(C) dose group (Fig. 3A). Figure 3B indicates that the released sCD23 binds to lumiliximab forming immunocomplexes in the circulation; however, the significance of these circulating complexes is unknown.

Flow cytometry. Assessment of both CD5⁺/CD19⁺ cells expressing CD23 antigen and CD23⁺ mean fluorescence

intensity measurements using an anti-CD23 antibody that is not blocked by lumiliximab showed no discernible change in CD23⁺ density on CLL cells for any treatment group, suggesting that there was no dose-related up-regulation or down-regulation of CD23 receptors on CLL cells. In contrast, there was a reduction in the ratio of median lumiliximab-FITC-positive to CD23⁺ cell counts with treatment that became greater with increasing dose of lumiliximab administered (Fig. 4). This reduction suggests that the CD23 receptors on CLL cells were being saturated with lumiliximab, thus blocking lumiliximab-FITC binding to the cells. No reduction was present at the 125 mg/m² dose, whereas full reduction was observed with doses at and above 250 mg/m², both immediately after the first dose and during the entire treatment period, suggesting receptor saturation at the higher doses. At 250 mg/m², the reduction of CD23⁺ cells was not maintained during the weekly observation period following treatment completion, whereas the duration of reduction lasted at least 2 weeks beyond the last dose for doses ≥ 375 mg/m².

There seemed to be little or no association between CD23 mean fluorescence intensity at baseline and the percentage of reduction in ALC. This was assessed both graphically and using Pearson's correlation coefficient.

Antilumiliximab antibodies. Of 46 treated patients, 13 had evaluations of serum antilumiliximab antibodies measured at both baseline and at the end of the study. All measured values were negative. Two additional patients had samples evaluated at the end of the study only; both exit samples were negative.

Discussion

This is the first comprehensive phase 1 dose escalation study of lumiliximab in patients with relapsed or refractory CLL. In this study, infusions of lumiliximab at doses up to 500 mg/m² thrice a week for 4 weeks were well tolerated. No objective partial or complete responses, as defined by the NCI 96 criteria were observed (1). The incidence and severity of adverse events or infections were not related to the dose of lumiliximab administered. There was no evidence of significant myelosuppression or cellular immune suppression. As a consequence, infectious morbidity was quite low relative to other phase 2

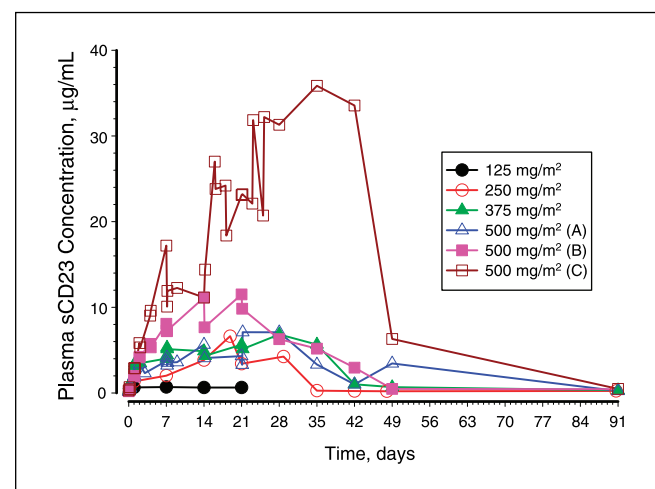


Fig. 2. Median sCD23⁺ concentrations over time by treatment group. Symbols for treatments are identical to those in Fig. 1.

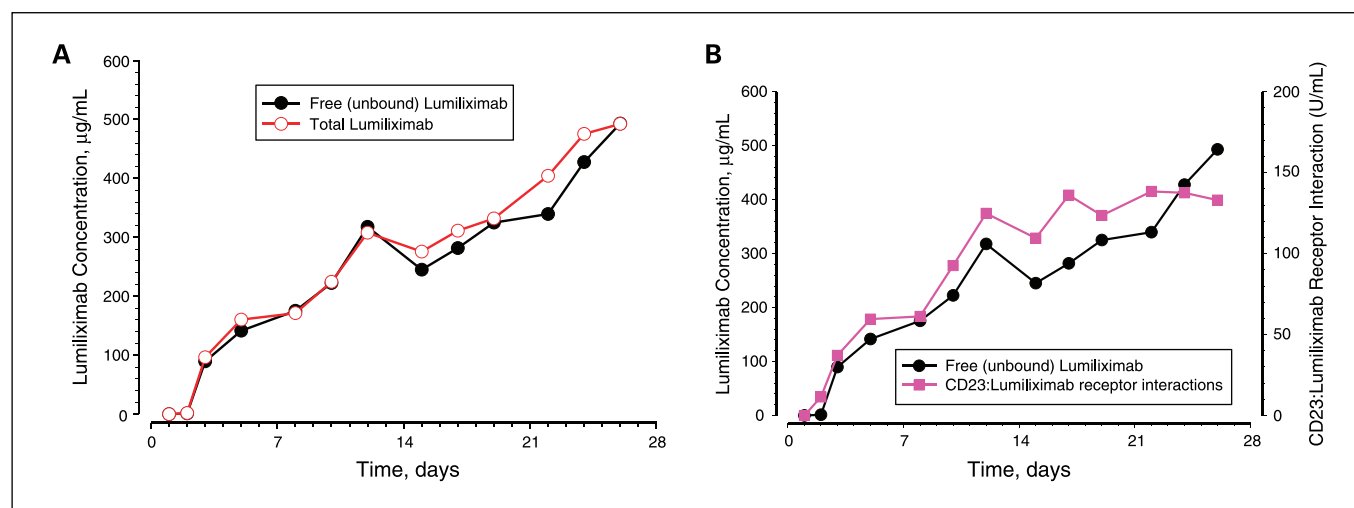


Fig. 3. A, median free and total lumiliximab concentrations in serum over time from treatment group 500(C). Free and total lumiliximab concentrations are nearly identical. B, median free lumiliximab concentrations and CD23/lumiliximab complex concentrations in serum over time from treatment group 500(C).

studies examining new therapeutic antibodies or alternative treatment combinations for this patient population; lumiliximab was not immunosuppressive (16). Using classic phase 1 definitions, the maximally tolerated dose of lumiliximab was not reached. No lumiliximab antibody formation was seen in this population.

In prospectively designing this trial, we sought to examine several different doses and schedules of lumiliximab previously used with the anti-CD20 antibody, rituximab, in CLL. Unlike studies with rituximab, no obvious benefit of dose escalation was observed relative to therapeutic efficacy. In the absence of such clinical benefit, pharmacology and pharmacodynamic studies provided evidence that the recommended phase 2 dose for combination studies might be below the maximal dose or dosing schedule. Lumiliximab exhibited pharmacokinetic qualities similar to other IgG₁ antibodies, including a volume of distribution similar to plasma volume, relatively low Cl, and a terminal half-life of approximately 1 week. A two-compartment Michaelis-Menten model best described the data, and saturation at which antibody accumulation occurred during treatment seemed to be related to CD23 binding. Examination of the vascular compartment of tumor cells showed no change in surface CD23 expression throughout treatment; however, lumiliximab CD23 receptor coating was complete at doses of 250 mg/m² and greater. Doses ≥ 375 mg/m² were needed to maintain binding of lumiliximab to the CLL cells for at least 7 days after cessation of dosing. Given the more sustained lumiliximab binding at higher doses, e.g., beyond 2 weeks at 500 mg/m² weekly, but no further benefit with more frequent dosing, future clinical trials should consider a weekly dosing schedule of 500 mg/m².

One limitation to this study is that the heavily pretreated study population may have precluded the observation of clinical benefit. Future efforts with this antibody should include the investigation of lumiliximab in less heavily pretreated patients given the acceptable toxicity profile or those with minimal residual disease.

Although CD23 expression varies considerably among CLL patients, we allowed all CD23⁺ patients to enroll in this study.

This was allowed because preclinical work showed that apoptosis occurred at low and high surface concentrations of CD23 (data not shown).

A rationale for the future development of lumiliximab in CLL can be based on the results of preclinical studies with this antibody and phase 1 clinical results derived from this work. Although evidence of lumiliximab antibody binding to the CLL cells was observed, clinical activity was not effectively noted as measured by a reduction in disease volume. This finding is consistent with preclinical studies showing that antibody-dependent cellular cytotoxicity does not occur with lumiliximab against primary CLL cells (data not shown). In contrast, preclinical studies in CLL cells and CD23-bearing lymphoblastic cell lines showed that lumiliximab induced apoptosis (18–22, 26) and antitumor activity was observed in lymphoblastic lymphoma xenograft models (18, 21, 23). Subsequent combination studies with lumiliximab and fludarabine or rituximab in preclinical *in vitro* and *in vivo*

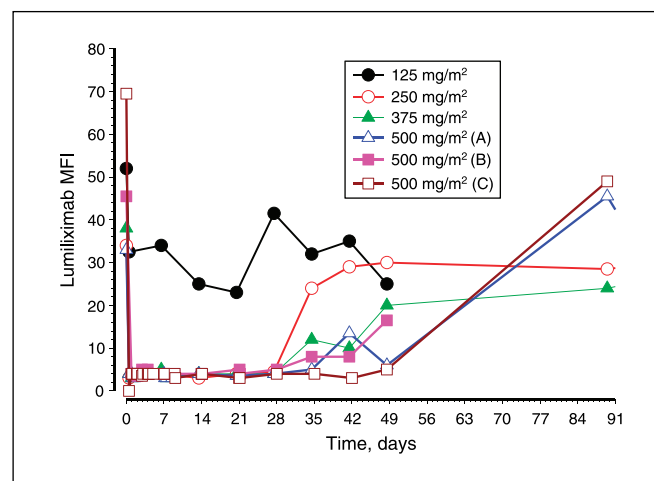


Fig. 4. Median lumiliximab-positive, CD23⁺ cell counts over time by treatment group. Symbols for treatments are identical to those in Fig. 1.

models have been conducted (21, 23, 26). These preclinical findings, combined with the remarkable safety profile of lumiliximab (relative to lack of immune suppression and lower than expected infection frequency compared with historical controls; refs. 28, 29) and with alemtuzumab treatment (30), provide justification for the pursuit of combination strategies with other effective CLL therapies (21, 23, 26). Strategies combining lumiliximab with fludarabine, rituximab, and cyclophosphamide (FCR) are supported by the promising data seen with both FR and FCR in symptomatic untreated and previously treated CLL (14, 15). Such a clinical trial of combining lumiliximab with FCR in previously treated CLL patients is ongoing, and promising preliminary results have been presented (31). In addition, the favorable toxicity profile and the potential synergy of

rituximab and lumiliximab would make this combination therapy an attractive alternative strategy to be pursued.

In summary, treatment with the anti-CD23 monoclonal antibody, lumiliximab, was well tolerated in patients with relapsed and refractory CLL. The pharmacokinetic and pharmacodynamic data provided in this study sets the foundation for the pursuit of phase 2 studies on the activity of lumiliximab either alone in patients with limited or early stage disease or in combination with other antileukemia agents.

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