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Complete Remissions of Adult T-cell Leukemia with Anti-CD25 Recombinant Immunotoxin LMB-2 and Chemotherapy to Block Immunogenicity

Robert J. Kreitman[1], Maryalice Stetler-Stevenson[2], Elaine S. Jaffe[2], Kevin C. Conlon[3], Seth M. Steinberg[4], Wyndham Wilson[3], Thomas A. Waldmann[3], and Ira Pastan[1]

Abstract

Purpose: Adult T-cell leukemia (ATL) is usually CD25$^+$ and rapidly fatal. Anti-CD25 recombinant immunotoxin LMB-2 had phase I activity limited by immunogenicity and rapid growth. To prevent antidrug antibodies and leukemic progression between cycles, a phase II trial was performed with LMB-2 after cyclophosphamide and fludarabine.

Experimental Design: ATL patients received cyclophosphamide and fludarabine days 1 to 3 and 2 weeks later began up to 6 cycles at 3-week intervals of cyclophosphamide and fludarabine days 1 to 3 followed by LMB-2 30–40 μg/kg i.v. days 3, 5, and 7. Three different dose levels of cyclophosphamide and fludarabine were used, 20+200 ($n = 3$), 25+250 ($n = 12$), and 30+300 mg/m^2 ($n = 2$).

Results: Of 17 patients enrolled and treated with fludarabine and cyclophosphamide for cycle-1, 15 received subsequent cycle (s) containing LMB-2 and were therefore evaluable for response. Lack of antibody formation permitted retreatment in most patients. Of 10 evaluable leukemic patients receiving 25+250 or 30+300 mg/m^2 of fludarabine and cyclophosphamide, 6 (60%) achieved complete remission (CR) and 2 (20%) partial remission (PR), and all 5 with >25% leukemic cells achieved CR. No responses were achieved in 5 with lymphomatous ATL or lower fludarabine and cyclophosphamide doses. Median CR duration for the 6 CRs was 40 weeks. One is without detectable ATL at 47 months. Toxicity was mostly attributable to fludarabine and cyclophosphamide. Capillary leak from LMB-2 was non-dose limiting. One patient in CR died of a preexisting infection.

Conclusions: LMB-2, administered with fludarabine and cyclophosphamide to prevent antidrug antibodies and rapid intercycle progression, is highly effective in achieving CR in leukemia ATL. Fludarabine and cyclophosphamide dose/schedule is important for safety and efficacy in this high-risk population. *Clin Cancer Res; 22(2); 310–8. ©2015 AACR.*

Introduction

Adult T-cell leukemia is an aggressive CD4$^+$/CD25$^+$ T-cell lymphoproliferative disorder caused by human T-cell lymphotropic virus type I (HTLV-I; refs. 1, 2), with clusters of cases in southwestern Japan, the Caribbean basin, northeastern South America, central Africa, and the southeastern United States (3). It is estimated that 15 to 20 million people are infected with HTLV-1 worldwide (4). Seroprevalence rates are up to 10% in southwestern Japan, and up to 6% in the Caribbean basin, including Jamaica and Trinidad. Those infected have a cumulative lifetime risk of 1 to 5 percent for developing ATL (3, 5). Median survival from diagnosis was reported to be approximately 6, 10, and 24 months for acute, lymphoma, and chronic types, respectively, and longer for smoldering type (6). Treatment of aggressive (acute and lymphomatous) ATL includes multiagent cytotoxic and myelosuppressive chemotherapy, including VCAP-AMP-VECP or high-intensity (biweekly) CHOP (CHOP-14), with or without stem cell transplantation (7–9). Unfortunately, despite the complete remission (CR) rates of 40% and 25% for VCAP-AMP-VECP and CHOP-14, respectively, 3-year survival rate was only 24% and 13% and toxicity was high, with grade 4 neutropenia in 98% versus 83%. Thus, treatment options for aggressive ATL remain clearly suboptimal.

The HTLV-1 viral genome encodes the 42-kDa Tax transactivating transcription factor that activates the HTLV-I long terminal repeat (LTR), the expression of viral genes, and the transcription of cellular genes encoding multiple growth factors and their receptors, including IL2 receptor α (IL2Rα), also called CD25 (10). CD25 has low affinity for IL2 (11), and is expressed on ATL cells freshly obtained from patients at levels of 1,600 to 21,000 (median 8,800) sites/cells (12). To target CD25 on ATL, the anti-CD25 monoclonal antibody (mAb) anti-Tac was developed (13, 14), and in murine or humanized form displayed antitumor activity in patients with this disease (15–17). To direct a cytotoxic toxin to ATL cells via CD25, the variable domains of anti-Tac were fused together with a peptide linker and then connected to a truncated form of Pseudomonas exotoxin (18). The resulting recombinant immunotoxin anti-Tac(Fv)-PE40 and its slightly shorter derivative anti-Tac(Fv)-PE38 (called LMB-2) were selectively cytotoxic

[1]Laboratory of Molecular Biology, National Cancer Institute, NIH, Bethesda, Maryland. [2]Laboratory of Pathology, National Cancer Institute, NIH, Bethesda, Maryland. [3]Lymphoid Malignancies Branch, National Cancer Institute, NIH, Bethesda, Maryland. [4]Biostatistics and Data Management Section, Office of the Clinical Director, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland.

Corresponding Author: Robert J. Kreitman, National Cancer Institute, NIH, 9000 Rockville Pike, Building 37, Room 5124b, Bethesda, MD 20892. Phone: 301-648-7375; Fax: 301-451-5765; E-mail: kreitmar@mail.nih.gov

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Translational Relevance

Recombinant immunotoxins have shown clinical activity as single agents, but even patients with hematologic malignancies can make neutralizing antibodies to the bacterial toxin portion, limiting the number of treatments that may be given. One strategy to prevent antidrug antibodies and improve response is to coadminister chemotherapy, which would blunt the humoral immune response, and also reduce tumor burden. We used the highly aggressive adult T-cell leukemia (ATL) as a model for this approach and added fludarabine and cyclophosphamide chemotherapy to recombinant immunotoxin LMB-2. While LMB-2 alone previously resulted in transient responses, antidrug antibodies developed and disease rapidly progressed. In contrast, LMB-2 with fludarabine and cyclophosphamide produced durable partial and complete remissions. We believe that the approach of combining recombinant immunotoxin with chemotherapy, recently reported for mesothelioma, will prove effective in hematologic and other tumors.

toward $CD25^+$ malignant cells, including ATL-derived cell lines, and patient samples (19–23). In a phase I trial of LMB-2 in patients with hematologic malignancies, 2 patients with ATL were included (24), both of whom had rapid reductions of circulating malignant cells, but only one achieved partial response and outcome was compromised by both immunogenicity and rapid regrowth of ATL.

To address both immunogenicity and rapid regrowth after response of LMB-2 in ATL, we used fludarabine and cyclophosphamide with LMB-2. The choice of fludarabine and cyclophosphamide as the induction chemotherapy was based on the association of fludarabine with a lower incidence of human anti-mouse antibody (HAMA) after the mAb tositumomab (25), the utility of cyclophosphamide in the treatment of ATL, and the ability of fludarabine and cyclophosphamide to decrease T-cell augmentation (26, 27), which may be important for humoral immunity to LMB-2. We had also reported synergy when a nucleoside analogue, namely gemcitabine, was combined with LMB-2 in targeting murine xenografts displaying human CD25 (28). Patients were pretreated with fludarabine and cyclophosphamide alone, and after 2 weeks were treated with cycles of fludarabine and cyclophosphamide immediately preceding LMB-2. Because of the activity seen on this ongoing trial, we now report early promising results of safety, immunogenicity, and response to fludarabine and cyclophosphamide/LMB-2 in patients at different dose levels of fludarabine and cyclophosphamide.

Patients and Methods

Eligibility

LMB-2 and fludarabine and cyclophosphamide were administered by a phase II protocol approved by the Institutional review board of the NCI (Bethesda, MD). All patients received treatment at the NIH Clinical Center (Bethesda, MD). Patients required a diagnosis of HTLV-1–associated ATL and CD25 positivity. Acute, lymphomatous, and chronic subtypes are eligible, as well as smoldering ATL patients with symptomatic skin lesions. Patients required prior treatment unless they were ineligible for or refused other treatments. Baseline-neutralizing antibodies could not neutralize >75% of the activity of 200 ng/mL of LMB-2 (24). Performance status required ECOG 0–2, neutrophil count $\geq$ 1,000/μL, and platelets >50,000/μL. Exclusions were similar to those published (24) and included chemotherapy (except hydroxyurea) within 3 weeks.

Study design

The trial was designed with fludarabine and cyclophosphamide at a fixed dose level of 25+250 mg/m^2 on days 1, 2, and 3 of each cycle, and a dose escalation of LMB-2 from 30 to 40 μg/kg on days 3, 5, and 7 (QOD × 3) if 3 evaluable patients tolerated 30 μg/kg QOD × 3 without dose-limiting toxicity (DLT). Because of lack of toxicity and presence of anti-drug antibodies, the dose level of fludarabine and cyclophosphamide was raised by amendment with the 8th patient to 30 + 300 mg/m^2 but due to thrombocytopenia in patient CF09 returned to 25 + 250 mg/m^2. To explore whether a lower dose of fludarabine and cyclophosphamide might also be associated with major responses, 20 + 200 mg/m^2 was used for 3 patients (Table 2). The infusion times for fludarabine and cyclophosphamide were 30 and 60 minutes, respectively, each in 100 mL 0.9% saline, and fludarabine and cyclophosphamide was administered on days 1, 2, and 3 of each cycle, with cycle 1 containing only fludarabine and cyclophosphamide, and cycles 2 and 7 containing LMB-2 on days 3, 5, and 7. As shown in Fig. 1, cycle 2 began 2 weeks after day 1 of cycle 1, and subsequent cycles were 3 weeks apart.

Statistical trial design

The trial was designed using a Simon optimal two-stage design (29) to rule out a 35% response rate and target a 60% response rate. With α= 0.10 and β= 0.10 (the probabilities of accepting a poor treatment and rejecting a beneficial treatment, respectively), the trial was to have a first stage of 16 evaluable patients and if 7 or more patients would have response duration of 8 weeks or longer, then accrual would continue to a second stage. At the end of the second stage, 13 or more patients with responses of 8 weeks or longer out of a total of 27 patients would be considered promising. In this trial, the response endpoint for the first stage was met after achievement of response in the 13th patient, so this report includes early results from the trial for those initial 13 patients as well as 4 subsequently enrolled.

Drug administration

LMB-2, as previously reported (24), was given by intravenous infusion over 30 minutes. Per protocol, the LMB-2 dose level was 30 μg/kg every other day for 3 doses (QOD × 3) for the first 3 evaluable patients, and then subsequently 40 μg/kg QOD × 3. Hydration with 500 mL of 5% dextrose containing 0.45% saline was administered prior to and after fludarabine

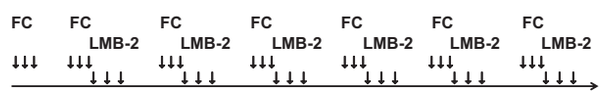

Figure 1.
Schema for the fludarabine and cyclophosphamide (FC)/LMB-2 trial. Cycle 1 contained only fludarabine and cyclophosphamide on days 1, 2, and 3. Cycles 2–7, which began 2 weeks after cycle 1, contained fludarabine and cyclophosphamide on days 1, 2, and 3, LMB-2 on days 3, 5 and 7, and were 3 weeks apart.

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and cyclophosphamide, 1 liter of the same fluid before and after LMB-2, but only 1 liter between fludarabine and cyclophosphamide and LMB-2 on day 3. Oral premedications to prevent allergic reaction of LMB-2 were as previously reported (24).

Response evaluation

CR criteria included resolution of radiographic measurable disease to short axis ≤1.5 cm if >1.5 at baseline, or <1 cm if 1.1–1.5 cm at baseline, normalization of markers of response including LDH, regression in spleen size to nonpalpable if enlarged at baseline, negative bone marrow histology, and negative flow cytometry of blood for ATL cells. PET scans were used as a research tool but not to determine response. Partial response (PR) required a ≥50% reduction in circulating ATL count, ≥50% reduction in measurable lesions, and no growing or new lesions. Circulating ATL counts below 100 cells/mm^3 by flow cytometry were not considered high enough to prevent consideration of PR by other criteria. Progressive disease was defined as a ≥50% increase in tumor size or doubling of the circulating ATL count.

Assays

Neutralizing antibody and pharmacokinetic assays were performed as previously described (24). Plasma levels of LMB-2 were determined by testing dilutions of posttreatment plasma for cytotoxicity toward SP2/Tac and comparing with a standard cytotoxicity curve using purified LMB-2 (24). The soluble CD25 assay was performed either using the Human CD25/IL2 R alpha ELISA Kit from R&D Systems, or a lab ELISA assay with comparable results. For the latter, Nunc Maxi sorb plates (Sigma-Aldrich) were coated with daclizumab (humanized anti-Tac), 0.1 μg/100 μL/well, washed with TPBS (PBS containing 0.05% Tween 20 (Sigma-Aldrich)], blocked with 300 μL/well of 1% BSA in PBS, washed with TPBS, incubated with patient serum diluted at least 1:10 with PBS, washed with TPBS, incubated with murine mAb 7g7 in PBS, washed with TPBS, incubated with biotinylated goat anti-mouse mAb (Jackson ImmunoResearch), washed with TPBS, incubated with Avidine–HRP conjugate (Biosource) diluted 1:2,500 with PBS containing 1% BSA and 0.05% Tween 20, washed with TPBS followed by PBS, incubated 15 minutes with TMB-peroxide substrate (Sigma-Aldrich), stopped with 2N H_2SO_4 and read at 450 nm.

Statistical analyses

Duration of PR and CR were determined by the Kaplan–Meier method, beginning at the date the PR or CR was identified. Progression-free survival (PFS) was determined by the Kaplan–Meier method beginning at the on-study date and continuing until progression or last follow-up without progression. A log-rank test was used to determine the statistical significance of the difference between two Kaplan–Meier curves. Pharmacokinetic parameters were compared between two groups indicated using an exact Wilcoxon rank sum test, separately for cycle 2 and cycle 3. For these exploratory comparisons, results are presented without formal correction for multiple comparisons, but in the context of the large number of tests performed with varying degrees of independence and dependence, $P < 0.005$ could be considered a statistically significant difference while $0.005 < P < 0.05$ would be considered a strong trend. Comparison of the fraction of patients with respect to development of immunogenicity was done using the Fisher exact test. All P values are two tailed except where indicated.

Results

Patient characteristics

The characteristics of the patients are listed in Table 1. The 17 patients enrolled included 6 males and 11 females. Ages ranged from 22 to 69 (median 37). Four patients had lymphoma type disease with <1% malignant cells in the blood, while the other patients met criteria for acute leukemic ATL based on hypercalcemia and/or high LDH. Despite high LDH prior to enrollment, disease activity in one of the patients (CF05) was more consistent with smoldering ATL with symptomatic skin lesions. Only 1 patient was treatment naïve, 8 had prior CHOP, 2 had prior EPOCH with rituximab and siplizumab (EPOCH-RS), 3 had prior alemtuzumab with either EPOCH or CHOP, and one had ESHAP.

Table 1. Patient characteristics

Patient	Age	Sex	Type	Prior treatment	ATL cells/mm^3	ATL cells (%)	High Ca^{++}	High LDH	Extravascular sites	Max tumor size
CF01	28	M	Acute	CHOP	450	6.7	Y	Y	Bone, lung, skin	<1 cm
CF02	51	F	Lymphoma	EPOCH-C	0	0.0	N	Y	Thigh (skin)	9 cm
CF03	31	F	Acute	CHOP	408	11.8	Y	Y	Pelvis	5.6 cm
CF04	34	M	Acute	C, ESHAP, PTX, Gem	687	5.1	N	Y	Muscle	<1 cm
CF05	55	M	Acute	Medi507, Ontak, HAT	80	1.02	N	Y	Skin	<1 cm
CF06	43	F	Lymphoma	CHOP	0	0.0	N	Y	LN	1.9 cm
CF07	29	F	Acute	CHOP, C	246	12.5	N	Y	LN	2 cm
CF08	27	M	Acute	CHOP, C	5,604	48.1	Y	Y	Spleen, LN	20, 1.8 cm
CF09	69	F	Acute	EPOCH-RS	206	1.3	Y	Y	Subcutaneous	<1 cm
CF10	53	F	Acute	None	8,216	63.1	N	Y	None	<1 cm
CF11	37	F	Lymphoma	EPOCH-RS	1	0.1	N	Y	Subcutaneous	5.8 cm
CF12	45	F	Acute	CHOP	13,153	49.8	N	Y	Spleen, LN	18.5, 1.7 cm
CF13	27	F	Acute	Hydroxyurea	210,000	92.2	Y	Y	Spleen	17.5 cm
CF14	45	F	Acute	CHOP, Hydroxyurea	24,112	59.5	Y	Y	Spleen	17 cm
CF15	35	F	Acute	CHOP	7,737	39.0	N	Y	Spleen, LN	15, 2.5 cm
CF16	22	M	Lymphoma	LSG-15, radiation	0	0.0	N	Y	Bone, subcutaneous	6 cm
CF17	48	M	Acute	Hydroxyurea	213,000	95.6	Y	Y	LN	2.2 cm

NOTE: Prior treatments included cyclophosphamide, adriamycin, vincristine and prednisone (CHOP), these 4 agents with etoposide (EPOCH) and campath (C or alemtuzumab, together EPOCH-C), etoposide, methylprednisolone, cytarabine, and cisplatin (ESHAP), pralatrexate (PTX), gemcitabine (Gem), Siplizumab (Medi507), denileukin diftitox (Ontak), daclizumab or humanized anti-Tac (HAT), and EPOCH with rituximab and siplizumab (EPOCH-RS). The LSG-15 regimen includes prednisone, adriamycin, carmustine, etoposide, carboplatin, and vinorelbine. High Ca^{++} is a corrected calcium > 2.74, and high LDH is > 400 u/L. Extravascular sites include lymph nodes (LN) and the maximum size is listed, respectively, with the maximum spleen diameter.

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Table 2. Results of LMB-2 preceded by fludarabine and cyclophosphamide

Patient	Fludarabine (mg/m^2) d1, 2, 3	Cyclophosphamide (mg/m^2) d1, 2, 3	LMB-2[a] (μg/kg) d3, 5, 7	Total cycles given	Best response	Response duration (wk) CR	Response duration (wk) PR	Time (wk) to CR	Time (wk) to PR	LMB-2 immunogenicity
CF01	25	250	N/A	1	NE					NE
CF02	25	250	30	3	SD					N
CF03	25	250	30	5	CR	15	30	4.7	4.7	Y
CF04	25	250	30	3	SD					N
CF05	25	250	40	5	PR		96		14.0	Y
CF06	25	250	40	2	PD					N
CF07	25	250	40	3	SD					N
CF08[b]	30	300	40	4	CR	62	65	5.1	1.9	Y
CF09	30	300	40	3	PR		8		1.7	N
CF10	25	250	40	7	CR	203+	207+	4.7	0.6	N
CF11	25	250	40	2	PD					N
CF12[c]	25	250	40	7	CR	71	74	5.3	1.6	N
CF13	25	250	40	7	CR	19	108	10.6	1.7	Y
CF14[d]	25	250	40	5	CR	12	15	4.4	1.7	N
CF15	20	200	N/A	1	NE					NE
CF16	20	200	40	3	SD					N
CF17	20	200	40	2	PD					NE

[a]LMB-2 began with cycle 2.
[b]CF08 received fludarabine and cyclophosphamide but no LMB-2 for cycle 4.
[c]CF12 received cyclophosphamide 200 mg/m^2 for cycle 3 and no fludarabine and cyclophosphamide thereafter.
[d]CF14 received no fludarabine and cyclophosphamide after cycle 2.

Three patients had hydroxyurea, given to decrease rapidly progressive leukemic ATL and hypercalcemia prior to enrollment. While patients were restricted from prior chemotherapy or steroids within 3 weeks of enrollment, hydroxyurea could be used up until the day before enrollment providing it was not increased during the week prior to enrollment. As patients CF10, CF13, and CF17 refused standard chemotherapy and were ineligible for other protocols, they were eligible without further prior therapy. The 4 patients with lymphomatous ATL had solid masses 1.9 to 9 cm in maximal diameter. The other 13 patients had 80–213,000 (median 5604) ATL cells/mm^3 in the peripheral blood, comprising 1.02% to 95.6% (median 39%) of the circulating white blood cells. These levels were measured on or several days prior to the first dose of fludarabine and cyclophosphamide, except for patient CF09, who on the day of enrollment had <1% ATL cells because the white blood cell count contained 52% eosinophils due to ATL-associated eosinophilia. This patient had hypercalcemia, high LDH, and no lymphomatous masses consistent with acute ATL, and had >1% ATL cells in the blood 7.5 months prior to enrollment just before EPOCH-RS (Table 1). Seven (41%) of the 17 patients had hypercalcemia and all had high LDH consistent with acute or lymphomatous ATL. Extravascular sites included bone, lung, skin lesions and subcutaneous nodules, pelvis, muscle, lymph node, and enlarged spleen. Nodal or tumor masses, present in 10 patients, ranged from 1.7 to 9 cm (median 2.4 cm). Splenomegaly was present in 5 patients with a maximum spleen diameter of 15 to 20 cm (median 17.5 cm).

Response and dose levels

To determine whether fludarabine and cyclophosphamide chemotherapy would be associated with major response to LMB-2, patients received fludarabine and cyclophosphamide at 3 dose levels, 20 and 200, 25 and 250, and 30 and 300 mg/m^2, respectively, and LMB-2 at 2 different dose levels, 30 and 40 μg/kg QOD × 3. As shown in Table 2, 2 of 17 patients were nonevaluable for LMB-2 response due to inability to receive the second cycle, which was the first cycle containing LMB-2, in CF01 due to withdrawing and in CF15 due to progressing prior to cycle 2. Of the remaining 15 patients evaluable for LMB-2 response, there were 6 CRs and 2 PRs. All 8 major responses were in the 10 evaluable leukemic (nonlymphomatous) patients receiving at least 25 and 250 mg/m^2 of fludarabine and cyclophosphamide. In these 10 patients, the median PFS was 11.6 versus 1.1 months in the other 6 patients ($P < 0.0001$, Fig. 2A). In the 8 patients treated with 25 and 250 mg/m^2 of fludarabine and cyclophosphamide and LMB-2 40 μg/kg ×3, median PFS was 10.6 months (Fig. 2B). The duration of CR ranged from 12 weeks to still responding at 203 weeks (median 40.4). The patient with ongoing CR (CF10) originally presented with leukemic disease and both Cryptococcal meningitis and pulmonary abscess, requiring liposomal amphotericin and ventricular–peritoneal shunt. The patient returned to part-time work after cycle 5 and currently works full time. The duration of PR ranged from 8 weeks to still responding at 207 weeks (median 70). Among those with a PR, the time to PR was 0.6–14 (median 1.8) weeks, and among those with CR, the time to CR was 4.4–10.6 (median 4.9) weeks. Thus,

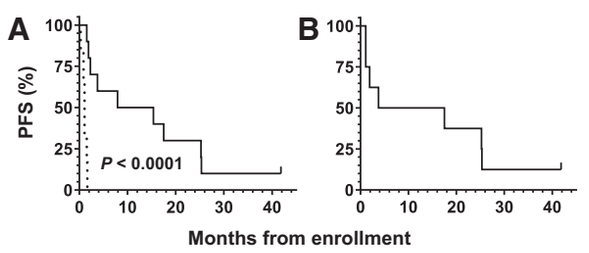

Figure 2.
PFS to fludarabine and cyclophosphamide /LMB-2. In A, 6 patients with lymphoma or those receiving fludarabine and cyclophosphamide at 20 + 200 mg/m^2 experienced a median PFS of 1.1 months versus 11.6 months in 10 patients with leukemia and 25 + 250 or 30 + 300 mg/m^2 of fludarabine and cyclophosphamide ($P < 0.0001$). B, 8 patients treated with fludarabine and cyclophosphamide 25 + 250 mg/m^2 and LMB-2 40 μg/kg had 10.6 month median PFS. Patient CF01 withdrew before cycle 2 and was nonevaluable for PFS.

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the majority of patients who responded did so prior to the second cycle. As the durations of the first and subsequent cycles were 2 and 3 weeks, respectively (Fig. 1), the majority of CRs were achieved prior to cycle 3, that is, as a result of the first cycle containing LMB-2. When comparing the soluble CD25 levels (ng/mL) between responders ($n = 8$) and nonresponders ($n = 8$), the median (range) pretreatment levels were 32 (3.2–190) and 13 (3.7–127), respectively ($P = 0.57$), while the median nadir/posttreatment levels were 1.1 (undetectable–6.0) and 29 (5.2–319), respectively ($P = 0.0006$). By protocol, patients with CR before cycle 2, that is, due to fludarabine and cyclophosphamide alone, would not be able to continue, but no patients had CR by that point. Thus, CR appeared facilitated by fludarabine and cyclophosphamide, but likely required at least the second cycle that contained LMB-2.

Neutralizing antibodies

To determine whether pretreatment with fludarabine and cyclophosphamide was associated with lack of neutralizing antibodies to LMB-2, we assessed antidrug antibodies by cytotoxicity assay prior to and after each cycle. To determine whether a serum sample contained neutralizing antibodies, the serum was incubated with LMB-2 at 37°C for 15 minutes, and then diluted with cell culture media and tested for cytotoxicity against $CD25^+$ cells. On the basis of previous results where 29 (60.4%) of 48 non-CLL patients historically produced antibodies neutralizing >25% of 1,000 ng/mL of LMB-2 when treated with it as a single agent, our goal was to avoid >25% neutralization of 1,000/mL. Patients with >75% neutralization of LMB-2 1,000 ng/mL could not receive additional cycles of LMB-2 as antitumor response was felt unlikely at this higher level of neutralization. CLL patients were not included in the historical control group because of their decreased ability to make antidrug antibodies to recombinant immunotoxins (24, 30). As shown in Table 2, 4 (29%) of 14 evaluable patients made high levels of neutralizing antibodies, and those patients received a total of 4, 5, 5, and 7 cycles. This rate of antidrug antibodies was substantially lower than that of our historical control group rate of 29 of 48 (60.4%, one-tailed $P = 0.036$, two-tailed $P = 0.066$), and thus was consistent with an effect of fludarabine and cyclophosphamide on immunogenicity. There was no evidence of less immunogenicity at higher doses of fludarabine and cyclophosphamide, with one of 2 patients at the highest dose level having neutralizing antibodies (Table 2), but the numbers of patients above and below $25 + 250$ mg/m^2 were few. To determine whether reductions of normal T and B cells were associated with immunogenicity of LMB-2, these cells were quantified by flow cytometry before and after the first cycle. Normal T cells were decreased by 27% to 96% (median 67%), and normal B-cells by 17% to 100% (median 96%), but the percent decreases were not associated with whether patients made high levels of neutralizing antibodies to LMB-2. Thus, fludarabine and cyclophosphamide was effective in decreasing normal T- and B-cells prior to exposure to LMB-2, and this decrease may have helped delay or prevent immunogenicity, but the amount of T- or B-cell reduction did not correlate with immunogenicity.

Discontinuation of therapy

As shown in Table 2, only 3 patients completed all 7 cycles. The most common reason for discontinuation was progressive disease (PD), which occurred before the first cycle of LMB-2 in 1 patient (CF15), after the first cycle of LMB-2 (cycle 2) in 3 patients (CF06, CF11, and CF17), and after cycle 3 in 5 patients (CF02, CF04, CF07, CF09, and CF16). Three patients stopped due to neutralizing antibodies (CF03, CF05, and CF08). One patient had grade 5 toxicity after cycle 5 (CF14), and one voluntarily withdrew before receiving LMB-2 (CF01). Thus, the most common cause of early discontinuation was not toxicity but rather progression in those not achieving CR early.

Safety of fludarabine and cyclophosphamide and LMB-2 in ATL

To determine toxicity of fludarabine and cyclophosphamide and LMB-2 in ATL, patients were monitored closely for adverse events due either to chemotherapy or LMB-2. Shown in Table 3 are adverse events attributed at least possibly to fludarabine and cyclophosphamide or LMB-2, including events of all grades occurring in at least 2 patients, and all grade ≥3 events. The most common events were fludarabine and cyclophosphamide related, including neutropenia, nausea/vomiting, and lymphopenia/leucopenia, thrombocytopenia, and anemia, encountered in 59% to 82% of the patients, and 35% to 71% experienced these toxicities at grade ≥3 level. Low CD4 count was attributed to fludarabine and cyclophosphamide and not to LMB-2, due to the low number of $CD4^+/CD25^+$ normal lymphocytes and their rapid renewal (31). The reduction of $CD4^+$ normal T cells was an intended effect of fludarabine and cyclophosphamide to reduce immunogenicity. Fever was observed as a result of LMB-2, and, like neutropenic fever, was partially related to fludarabine and cyclophosphamide. Transaminases elevations, usually AST and less commonly ALT, were LMB-2–related, usually < grade 3, and never dose limiting per protocol, consistent with previous clinical testing (24). Evidence of capillary leak syndrome (CLS) with LMB-2 included hypoalbuminemia, hypotension, proteinuria, weight gain, and edema. Grade ≥3 infections were reported in 3 patients, including 2 cases of reversible pneumonia. One patient with prior CHOP and hydroxyurea and preexisting infection with vancomycin-resistant enterococcus (VRE), pneumocystis, and cytomegalovirus, had grade 4 pneumonia with *Stenotrophomonas maltophilia* during cycles 2 and 3, and grade 5 (fatal) VRE sepsis after cycle 5. As shown in Table 3, the fludarabine and cyclophosphamide-related hematologic toxicities appear somewhat less common at the lowest dose of fludarabine and cyclophosphamide, but the numbers of patients treated above or below $25 + 250$ mg/m^2 were too small for formal comparison. Thus, severe infections were encountered in a minority of these already immunosuppressed patients (32), and interfered with outcome particularly when infection was present at baseline.

Pharmacokinetics

To determine the plasma levels of active LMB-2 that are available to bind to and kill the ATL cells, a cytotoxicity assay was performed in which dilutions of plasma were incubated with the $CD25^+$ cell line SP2/Tac, and the cytotoxicity compared with that of purified LMB-2 on a standard curve (24). Plasma samples were generally taken for doses 1 and 3 (days 1 and 5) of each cycle, just after the end of infusion and then 1, 2, 3, 4, 10, and 24 hours later. Pharmacokinetic parameters were calculated from best fit of mono- or biexponential decay curves based on Akaike's rule (24, 33). As shown in Table 4, peak plasma levels were 189–329 ng/mL (median 303 ng/mL) after the first dose of 30 μg/kg, versus 205–1,072 ng/mL (median 727 ng/mL) after the first dose of 40 μg/kg. As shown in Fig. 3, both peak levels and AUCs were higher

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Table 3. Toxicity of LMB-2/fludarabine and cyclophosphamide in patients with ATL

Toxicity in >1 patient	Fludarabine and cyclophosphamide dose levels (mg/m^2; all grade 3–5 toxicity)				Fludarabine and cyclophosphamide dose levels (mg/m^2)		
All grades	**20 + 200**	**25 + 250**	**30 + 300**		**20 + 200**	**25 + 250**	**30 + 300**
Patients treated	$n = 3$	$n = 12$	$n = 2$		$n = 3$	$n = 12$	$n = 2$
Neutropenia	2 (67%)	10 (83%)	2 (100%)	Neutropenia	1 (33%)	9 (75%)	2 (100%)
Nausea/vomiting/anorexia	2 (67%)	10 (83%)	2 (100%)	Leukopenia/lymphopenia	1 (33%)	9 (75%)	2 (100%)
Fever/chills	1 (33%)	10 (83%)	1 (50%)	Anemia	0 (0%)	8 (67%)	0 (0%)
Leukopenia/lymphopenia	1 (33%)	9 (75%)	2 (100%)	Transaminases	1 (33%)	4 (33%)	0 (0%)
Transaminases	3 (100%)	8 (67%)	1 (50%)	Thrombocytopenia	0 (0%)	5 (42%)	1 (50%)
Hypotension/tachycardia	1 (33%)	8 (67%)	2 (100%)	Fever/chills	0 (0%)	3 (25%)	1 (50%)
Thrombocytopenia	1 (33%)	8 (67%)	2 (100%)	Pneumonitis	0 (0%)	3 (25%)	0 (0%)
Anemia	0 (0%)	9 (75%)	1 (50%)	Low CD4 count	1 (33%)	1 (8%)	1 (50%)
Myalgia/headache	2 (67%)	7 (58%)	2 (100%)	Hypotension/tachycardia	0 (0%)	2 (17%)	0 (0%)
Hypoalbuminemia	2 (67%)	6 (50%)	1 (50%)	Rectal hemorrhage	0 (0%)	1 (8%)	0 (0%)
Hematuria	1 (33%)	5 (42%)	1 (50%)	Bilirubin	0 (0%)	1 (8%)	0 (0%)
Proteinuria	0 (0%)	7 (58%)	0 (0%)	Dysuria	0 (0%)	1 (8%)	0 (0%)
Fatigue/dizziness	1 (33%)	4 (33%)	2 (100%)	Bladder infection	0 (0%)	1 (8%)	0 (0%)
Edema	0 (0%)	6 (50%)	0 (0%)	Hypoxia	0 (0%)	1 (8%)	0 (0%)
Abdominal pain	0 (0%)	5 (42%)	0 (0%)	Proteinuria	0 (0%)	1 (8%)	0 (0%)
Diarrhea	1 (33%)	3 (25%)	0 (0%)	Sepsis	0 (0%)	1 (8%)[a]	0 (0%)
CPK	0 (0%)	4 (33%)	0 (0%)				
Mucositis	0 (0%)	3 (25%)	1 (50%)				
Hypomagnesemia	0 (0%)	3 (25%)	0 (0%)				
Bilirubin	1 (33%)	2 (17%)	0 (0%)				
Alkaline phosphatase/GGT	0 (0%)	3 (25%)	0 (0%)				
Pneumonitis	0 (0%)	3 (25%)	0 (0%)				
Low CD4 count	1 (33%)	1 (8%)	1 (50%)				
Lipase	0 (0%)	3 (25%)	0 (0%)				
Bladder infection	0 (0%)	2 (17%)	0 (0%)				
Dyspnea	0 (0%)	2 (17%)	0 (0%)				
Prothrombin time	1 (33%)	1 (8%)	0 (0%)				
Pruritus	1 (33%)	0 (0%)	1 (50%)				

[a]Grade 5 event.

at 40 than at 30 μg/kg QOD × 3, with the significance of the difference greater after the first (day 1) than after the third (day 5) dose.

Discussion

To determine whether chemotherapy pretreatment could facilitate the efficacy of anti-CD25 recombinant immunotoxin LMB-2 in ATL by preventing both antidrug antibodies and rapid progression between cycles, patients with ATL received cycles of fludarabine and cyclophosphamide prior to LMB-2. We found that fludarabine and cyclophosphamide/LMB-2 was highly effective in inducing CR, particularly in ATL patients with leukemia rather than lymphoma, and in patients receiving at least 25 and 250 mg/m^2 of fludarabine and cyclophosphamide, respectively. The endpoint of reducing the immunogenicity of LMB-2 to below

Table 4. Pharmacokinetics of LMB-2 in ATL

Dose (μg/kg)	Time point	*n*		Peak (ng/mL)	C_{max} (ng/mL)	$t_{1/2}$ (min)	AUC (μg-min/mL)	V_d (L)	Clearance (mL/min)
30	C2 D1	3	Range	189–329	212–379	91–370	50–113	5.3–11.3	21–40
			Median	303	356	104	54	5.6	37
30	C2 D5	3	Range	204–306	88–182	208–581	66–152	8.9–22.1	16–30
			Median	302	103	433	66	13.4	30
30	C3–4 D1	4	Range	265–566	251–428	144–332	67–155	4.7–71.4	17–274
			Median	374	346	176	90	32.4	143
30	C3–4 D5	4	Range	354–916	229–582	136–411	105–214	3.6–8.4	10–18
			Median	581	458	286	159	5.9	15
40	C2 D1	12	Range	205–1,072	97–1,062	154–549	130–265	3.3–313	10.9–191
			Median	727	243	365	167	73.6	115
40	C2 D5	12	Range	166–932	119–1,629	173–530	111–406	2.2–193	5–223
			Median	580	256	361	171	32.6	72
40	C3–7 D1	23	Range	201–1,566	83–1,577	69–541	14–433	2.4–253	9–1,274
			Median	609	479	178	147	20.0	69
40	C3–7 D5	23	Range	453–1,905	242–1,677	70–509	50–565	2.3–73	0.2–355
			Median	610	469	220	163	10.6	26

NOTE: Cycles of LMB-2 given but not evaluable due to neutralizing antibodies include cycle 5 for patients CF03 and CF05, and cycle 7 for patient CF13.

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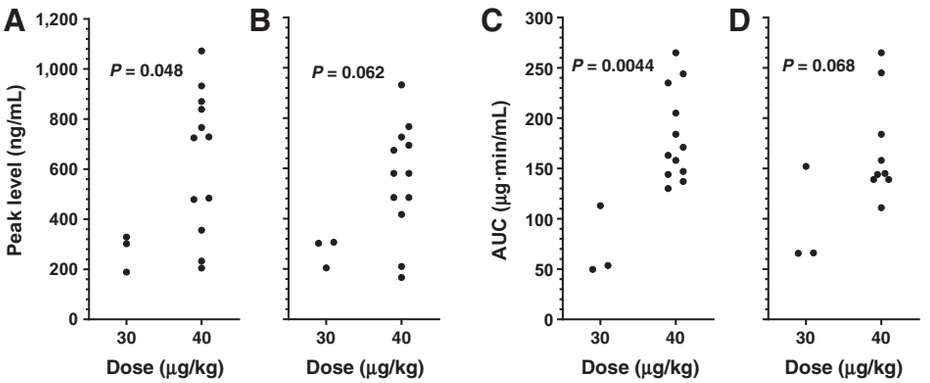

Figure 3.
Pharmacokinetics of LMB-2 in patients treated with fludarabine and cyclophosphamide/LMB-2. Peak levels are shown in A and B after the first and third doses, respectively. AUCs are shown in C and D after the first and third doses, respectively. All results are from cycle 2, the first cycle containing LMB-2.

the 60% incidence noted historically in non-CLL was reached at this point in the trial with a 29% rate of immunogenicity (one-tailed $P = 0.035$; two-tailed $P = 0.066$). The first-stage response endpoint of at least 7 responses out of up to 16 evaluable was also met. One patient remains in CR without minimal residual ATL for nearly 4 years. Additional patients with leukemic ATL on the second stage of this trial will receive fludarabine and cyclophosphamide at 25 and 250 mg/m^2 and LMB-2 at 40 μg/kg QOD × 3; this combination was associated with a PFS of 10.6 months in leukemic ATL (Fig. 2B) and this LMB-2 dose was without DLT. No advantage was observed with a higher or lower dose level of fludarabine and cyclophosphamide.

Established and investigational treatments for ATL

Effective treatment of acute and lymphomatous ATL, and even less aggressive subtypes, including chronic ATL, has remained suboptimal at best. The most recommended first-line treatment for aggressive ATL is the multiagent sequential chemotherapy regimen VCAP–AMP–VECP, composed of vincristine, cyclophosphamide, doxorubicin, prednisolone (VCAP), doxorubicin, ranimustine, prednisolone (AMP), vindesine, etoposide, carboplatin, and prednisolone (VECP; refs. 7–9). A randomized trial showed that 3-year overall survival after first-line VCAP–AMP–VECP was higher than that of CHOP-14 (24% vs. 13%), but more toxic (8). Patients who can undergo allogeneic stem cell transplantation have better outcomes than those who cannot (34, 35). For relapsed ATL, which most of our patients had, there is no effective standard therapy (7). Options recently under clinical testing include the anti-CCR4 mAb mogamulizumab, brentuximab vedotin, and lenalidomide, bortezomib, forodesine, pralatrexate, panobinostat, alisertib, alemtuzumab, interferon, retroviral agents, and denileukin diftitox (7, 36). Clinical trials of denileukin diftitox, containing IL2 and truncated diphtheria toxin, have not been reported for ATL, and evidence of clinical activity is limited to several case reports, one of which required aggressive chemotherapy with hyper-CVAD (37, 38).

Fludarabine and cyclophosphamide/LMB-2 compared with primary chemotherapy

It is difficult to compare the results of fludarabine and cyclophosphamide/LMB-2 in this small study of mostly relapsed/refractory patients to those of first-line chemotherapy. As LMB-2 was without significant myelosuppressive toxicities, the incidence of grade 4 neutropenia and thrombocytopenia were 41% and 35%, respectively, compared with 98% and 74% reported for the VCAP–AMP–VECP regimen. Improved safety of fludarabine and cyclophosphamide might be obtained by longer delays for patients with fludarabine and cyclophosphamide–related toxicity. It is possible that fludarabine and cyclophosphamide decreased the ability of LMB-2 to cause CLS, as CLS-related events seemed milder and more infrequent than on the phase I single-agent trial. Of the 10 patients with nonlymphomatous ATL receiving LMB-2 with at least 25 and 250 mg/m^2 of fludarabine and cyclophosphamide, the median OS was 18.5 months, and OS was 42, 53, and 56 months in 3 patients, 2 of whom are still alive. Thus, fludarabine and cyclophosphamide/LMB-2 containing 40 μg/kg QOD × 3 of LMB-2 and 25 and 250 mg/m^2 of fludarabine and cyclophosphamide has activity and safety profile appropriate for further clinical testing in patients with ATL, particularly those with relapsed disease and limited alternatives. This protocol could be attempted as a bridge to allogeneic transplantation, which might improve the outcome in patients who completely respond but then relapse. However, that was not tested in this trial, and the 2 patients who went to allogeneic transplantation were either fully relapsed from CR (CF03) or had not responded to fludarabine and cyclophosphamide/LMB-2 (CF16).

Response to both fludarabine and cyclophosphamide and LMB-2

The fludarabine and cyclophosphamide regimen was chosen not only to prevent immunogenicity, but also for its potential cytotoxic effect against ATL. As fludarabine and cyclophosphamide was not possible to model in animals due to its pharmacokinetics in mice, we studied the pyrimidine analogue gemcitabine in CD25$^+$ tumor bearing mice. We found that a single dose of gemcitabine was sufficient to decrease the intratumoral sCD25 in the mice, which prior to treatment was several orders of magnitude higher than the level of serum sCD25 (28). This antitumor activity of gemcitabine was associated with synergism when combined with LMB-2 (28). We have recently reported that targeted antigen shedding, while potentially helpful to the delivery of antimesothelin recombinant immunotoxin SS1P to tumor cells, may prevent delivery of LMB-2 to CD25$^+$ tumor cells (39). Thus, depletion of intratumoral sCD25 by chemotherapy may allow LMB-2 to reach tumor cells *in vivo*, and allow complete remissions to occur. Chemotherapy might not only cause better delivery of LMB-2 to ATL tumor masses by reducing intratumoral

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sCD25, but also by decreasing circulating tumor cells and plasma sCD25, allowing longer exposure to higher plasma levels of LMB-2. We did not see complete remissions of ATL by fludarabine and cyclophosphamide alone after cycle 1, which would have made patients ineligible to begin LMB-2 with cycle 2. In fact, we often saw evidence of tumor progression prior to beginning cycle 2. Thus, while we cannot rule out that fludarabine and cyclophosphamide alone for repeated cycles could have achieved CRs, we believe this is unlikely due to the consistent presence of disease prior to LMB-2 and the known rapidity of progression of ATL between treatments. One unanswered question is whether fludarabine and cyclophosphamide is necessary for every cycle of LMB-2. During design of the protocol, fludarabine and cyclophosphamide was felt to be necessary on every cycle to prevent antidrug antibody formation to repeated doses of LMB-2. However, several patients, including CF12 and CF14, received multiple cycles of LMB-2 without fludarabine and cyclophosphamide due to its toxicity on prior cycles, without resulting in antidrug antibodies. Thus, it is possible that some patients might be able to receive repeated doses LMB-2 without antibodies after just a few cycles of fludarabine and cyclophosphamide.

Chemotherapy to reduce the immunogenicity of truncated pseudomonas exotoxin

We have reported high immunogenicity of the same truncated form of pseudomonas exotoxin (PE38), fused to an antimesothelin Fv. The resulting recombinant immunotoxin SS1P, either by continuous infusion or bolus dosing, had limited clinical activity at least partly due to inability to deliver multiple cycles (40, 41). To prevent immunogenicity of SS1P in mesothelioma and in potentially other solid tumors, it was administered combined with pentostatin and cyclophosphamide, which markedly delayed the onset of neutralizing antibodies (42). This led to major tumor regressions in several patients despite lack of response to prior chemotherapy. The pentostatin was particularly successful in lowering normal B and T cells without prolonged neutropenia or other dose-limiting myelosuppression. An important difference between the mesothelioma and ATL trials is that while chemotherapy was given to reduce antidrug antibodies in both, in the ATL trial it also was given to increase antitumor activity. Prevention of immunogenicity with purine analogues, including pentostatin and fludarabine, may be of use in the future for highly cytotoxic recombinant toxins, which may be limited by immunogenicity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R.J. Kreitman, S.M. Steinberg, W. Wilson, T.A. Waldmann
Development of methodology: R.J. Kreitman
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R.J. Kreitman, M. Stetler-Stevenson, E.S. Jaffe, T.A. Waldmann
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R.J. Kreitman, M. Stetler-Stevenson, S.M. Steinberg, I. Pastan
Writing, review, and/or revision of the manuscript: R.J. Kreitman, M. Stetler-Stevenson, E.S. Jaffe, K.C. Conlon, S.M. Steinberg, W. Wilson, T.A. Waldmann, I. Pastan
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R.J. Kreitman, W. Wilson, T.A. Waldmann
Study supervision: R.J. Kreitman
Other (care for patients treated on the protocol): K.C. Conlon

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Robert J. Kreitman, Maryalice Stetler-Stevenson, Elaine S. Jaffe, et al.

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