Original Article

The Treatment of Recurrent/Refractory Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL) With Everolimus Results in Clinical Responses and Mobilization of CLL Cells Into the Circulation

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BACKGROUND: Patients with recurrent/refractory chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL) often have chemotherapy-resistant disease, resulting in poor prognosis. The aim of this study was to learn if inhibition of the mammalian target of rapamycin (mTOR) would produce tumor responses. **METHODS:** This was a phase 2 study of oral single-agent everolimus (10 mg/day) for recurrent/refractory indolent lymphoid malignancies including CLL. **RESULTS:** Four of 22 patients with CLL (18%; 95% confidence interval, 5%-40%) achieved a partial remission to therapy. An unanticipated finding in this study was an increase in absolute lymphocyte count (ALC) associated with a decrease in lymphadenopathy in 8 (36%) patients. ALC increased a median of 4.8-fold (range, 1.9- to 25.1-fold), and the clinically measurable lymphadenopathy decreased a median of 75.5% (range, 38%-93%) compared with baseline measurements. **CONCLUSIONS:** Everolimus has modest antitumor activity against CLL and can mobilize malignant cells from nodal masses into the peripheral circulation in a subset of CLL patients. Because CLL cells in lymphatic tissue and bone marrow can be more resistant to therapy than circulating CLL cells, the ability of everolimus to mobilize CLL cells into the circulation could be used in combination therapeutic regimens. ***Cancer*** **2010;116:2201–7.** © *2010 American Cancer Society.*

KEYWORDS: chronic lymphocytic leukemia/small lymphocytic lymphoma, mTOR, inhibitor, everolimus, rapamycins, mobilization.

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL) is one of the most common lymphoid malignancies in the United States, with an estimated incidence of 20,000 new diagnoses per year and a prevalence of >100,000.[1] There is now highly effective initial therapy for previously untreated, advanced stage CLL, with response rates of >90%, complete responses (CRs) in >40% of patients, and median response durations >3 years.[2,3] In contrast, outcome remains poor for patients with recurrent or treatment-refractory CLL.[4,5] More effective therapies for this group of patients are needed.

Rapamycin (sirolimus) is a highly specific inhibitor of the mammalian target of rapamycin (mTOR).[6] mTOR is a multifunctional signal transduction kinase with a critical role in the signal transduction pathway linking growth stimuli with cell cycle progression.[7] Rapamycin can inhibit cell growth and proliferation, induce apoptosis in some tumor cell lines, and inhibit tumor cell motility by impairing cell polarization and protrusion.[8] Rapamycin has been demonstrated to induce apoptosis in CLL cells in vitro, although the required concentration was higher than is achievable in vivo, and the clinical significance of this finding remains uncertain.[8] There are now 2 rapamycin analogs, temsirolimus and everolimus,

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which are currently approved by the US Food and Drug Administration for relapsed renal cancer. Temsirolimus also has demonstrated effectiveness in the treatment of relapsed/refractory mantle cell lymphoma.[9,10]

Although a major pathogenic mechanism in development of CLL is defective apoptosis, recent research has shown that CLL is a dynamic malignancy, with cellular turnover ranging between 0.1% and 1% per day.[11] The CLL tumor burden has 2 distinct cellular populations, a proliferative compartment morphologically characterized by larger cells in the proliferation centers of the lymphoid tissues and bone marrow, and the kinetically inactive circulating cells.[12,13] A targeted inhibitor of cell division such as everolimus could be effective in the treatment of CLL, especially in patients with recurrent/refractory disease, who often have more rapid cellular turnover.[14] This hypothesis is supported by data from in vitro studies that showed that rapamycin and everolimus induce cell cycle arrest in activated CLL cells without inducing apoptosis.[12,15,16] In addition, proliferation of CLL cells requires stromal support mediated through cytokines and adhesion molecules (eg, integrins),[17] and many of these supportive signals are transmitted by the PI3K and Akt pathways involving mTOR.[13] Thus, there is a sound biological rationale for testing everolimus as a treatment for CLL.

We have recently completed a phase 2 clinical trial using everolimus to treat patients with recurrent/refractory indolent lymphoid malignancies that included 22 patients with CLL. In this paper, we report the results of treatment of the CLL patients. The most striking finding was the increase in absolute lymphocyte count (ALC) and concomitant decrease in the lymph node size observed in 8 of these patients.

MATERIALS AND METHODS

This was a 2-stage, phase 2 study conducted to assess response in previously treated patients with lymphoid malignancies after treatment with single-agent everolimus. The study was conducted through the Mayo Clinic Cancer Center and approved by the Mayo Clinic Institutional Review Board according to the principles of the Helsinki Declaration, and all patients were provided written informed consent. Patients with CLL were eligible for this trial if they met the CLL diagnostic criteria defined by the National Cancer Institute (NCI) Working Group criteria of 1996[18] or the criteria for the small lymphocytic lymphoma (SLL) variant defined by the World Health Organization,[19] had previously received therapy for their lymphoid malignancy, and had recurrent disease or were refractory to their last treatment. The recurrence was required to be biopsy proven within 6 months before enrollment. There was no limit on the number of prior therapies. Patients were required to be $\geq$18 years old, and in addition to meeting diagnostic criteria, were also required to have pretreatment measurable disease by computed tomography or magnetic resonance imaging scanning, with at least 1 lesion that had a single greatest dimension of $>$2 cm, or an ALC $>5 \times 10^9$/L. Patients were to have a life expectancy of $\geq$3 months; Eastern Cooperative Oncology Group performance status of 0, 1, or 2; absolute neutrophil count (ANC) $\geq 1 \times 10^9$/L; platelet count $\geq 75 \times 10^9$/L; hemoglobin $\geq$8 g/dL; serum creatinine $\leq 2\times$ the upper normal limit (UNL); serum bilirubin $\leq 2\times$ UNL (if total bilirubin was $>2\times$ UNL, then a direct bilirubin of $<1.5\times$ UNL was acceptable); and aspartate aminotransferase $\leq 3\times$ ULN ($\leq 5\times$ ULN if liver involvement was present). Patients could not have known human immunodeficiency virus infection.

Patients were treated with 10 mg of everolimus by mouth in the fasting state every day, and 4 weeks of treatment was considered 1 cycle. A complete blood count was performed weekly during the first cycle and then before each subsequent cycle. If the platelet count was $\geq 40 \times 10^9$/L, ANC was $\geq 1 \times 10^9$/L, and there were no grade 3 or 4 nonhematological toxicities (NCI Common Toxicity Criteria version 3.0), the full dose of everolimus was prescribed for the next cycle. Patients who did not meet the retreatment criteria had treatment held until recovery followed by a stepwise dose modification to 5 mg daily, 5 mg every other day, and 5 mg every third day. Patients did not receive prophylactic white blood cell growth factors to maintain dosing, but could receive them at physician discretion if neutropenia developed. Erythropoietin treatment for anemia was also permitted at physician discretion.

Patients were restaged for tumor response after 2 and 6 cycles using the NCI Working Group 1996 criteria[18] for those with an ALC $>5 \times 10^9$/L and the International Workshop Criteria for patients who had never had an ALC $>5 \times 10^9$/L (SLL variant).[20] The maximum decrease in the clinically measurable lymphadenopathy was determined from the sum of the products of the largest lymph node dimensions on clinical examination of the largest cervical, axillary, and inguinal lymph nodes.[18] Patients who had disease progression or unacceptable toxicity at any time went off study. Patients with stable disease after 6 cycles continued treatment at their physician's discretion. Patients who had a CR after Cycle 6 were to receive 2 cycles past CR and then could discontinue

everolimus and be observed, or could continue on therapy at their physician's discretion. Patients with a partial response (PR) after 6 cycles of therapy continued on treatment until progression if tolerated.

Statistical Analysis

This phase 2 study used a 2-stage Simon design to assess the efficacy and tolerability of everolimus in patients with indolent lymphoid malignancies. Thirty-seven evaluable patients were required to test the null hypothesis that the true response rate for this regimen is at most 20% versus the alternative hypothesis that the true response rate is 40% or greater. The study had 90% power, with a 9% type I error rate. Patients were considered evaluable for response if they were eligible and received treatment. The response rate was estimated by the number of responses divided by the number of evaluable patients. A 95% binomial confidence interval for the true response rate was calculated. This report is limited to the 22 CLL patients enrolled in the study because of the unique characteristics of the response of these patients to treatment with everolimus.

Duration of response was defined for patients who achieved a response as the time from the date a response was first documented until the earliest date progression was documented. Progression-free survival (PFS) was defined for all patients as the time from the date of registration until the date of progression or death from any cause. Overall survival (OS) was defined as the time from the date of registration until the date of death from any cause. The distributions of time to event endpoints were estimated using the Kaplan-Meier method, and patients who were event-free were censored on the date of last follow-up.

Adverse events were graded using the NCI Common Toxicity Criteria (version 3.0). Toxicity was defined as an adverse event classified as being possibly, probably, or definitely related to study treatment.

RESULTS

Patient Characteristics

Twenty-two patients with CLL (10 with the SLL variant) were enrolled in this study between January 2006 and July 2008 (Table 1). All patients were eligible and evaluable for response. The median time from diagnosis of CLL to registration was 7 years (range, 2-22 years).

Response to Treatment

Four patients (18%; 95% confidence interval [CI], 5%-40%) achieved a PR to therapy at 1.8, 6.4, 9.1, and 14.1 months of treatment and maintained a response for 2.2, 11.0, 19.8, and 8.9+ months, respectively. Seventeen patients had disease progression, and 15 patients have died. Causes of death were disease progression (n = 11), infection possibly attributable to treatment as detailed below (n = 2), infection unrelated to treatment (n = 1), and unknown (n = 1). The median follow-up for patients still alive was 17 months (range, 6-30 months), the median OS was 10.5 months (95% CI, 4.9-20.7), and the median PFS was 5.1 months (95% CI, 2.3-8.3).

Table 1. Patient Characteristics

Characteristic	No.
Median age, y (range)	74 (46-85)
Sex (%)	
Men	16 (73)
Women	6 (27)
Median pretreatment blood count values (range)	
Lymphocyte count, $\times 10^9$/L	3.0 (0.4-292)
Neutrophil count, $\times 10^9$/L	2.8 (1.0-8.6)
Hemoglobin, g/dL	11.9 (9.6-16.4)
Platelet count, $\times 10^9$/L	147 (78-269)
Absolute lymphocyte count $>5 \times 10^9$/L at baseline (%)	
Yes	9 (41)
No	13 (59)
Lymph nodes measuring $\geq$5 cm at baseline (%)	
Yes	13 (59)
No	9 (41)
ECOG PS at baseline (%)	
0	11 (50)
1	9 (41)
2	2 (9)
Median No. of prior treatments (range)	6 (1-10)

ECOG PS indicates Eastern Cooperative Oncology Group performance status.

Increasing ALC Associated With Decreasing Lymph Node Size

An unanticipated and potentially important finding of this study was the increase in ALC associated with a decrease in lymphadenopathy in 8 (36%) patients (Fig. 1). This lymphocyte mobilization was observed in 6 (66%) of the 9 patients with an ALC $>5 \times 10^9$/L at baseline and 2 (15%) of the 13 patients with an ALC $\leq 5 \times 10^9$/L at baseline. Among the 8 patients in whom this phenomenon was observed, all had measurable lymphadenopathy at baseline, and in 6 (75%) at least 1 lymph node was $\geq$5 cm in its greatest dimension. ALC increased from

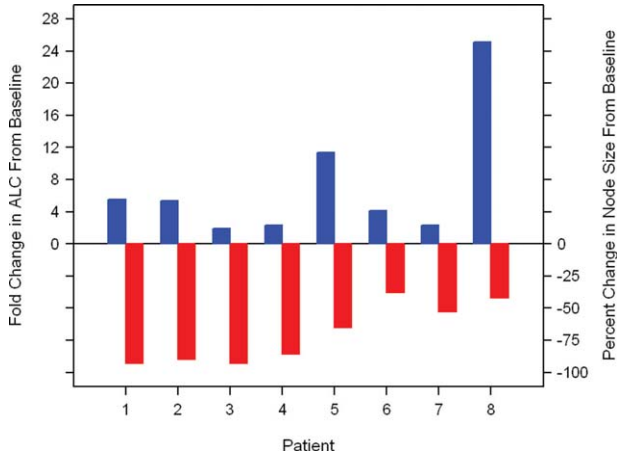

Figure 1. Everolimus treatment resulted in an increase in absolute lymphocyte count (ALC) and a decrease in lymphadenopathy in 8 patients. The maximum decrease in the clinically measurable lymphadenopathy (lymph node size) is indicated by the red bars and the associated increase in ALC by the blue bars.

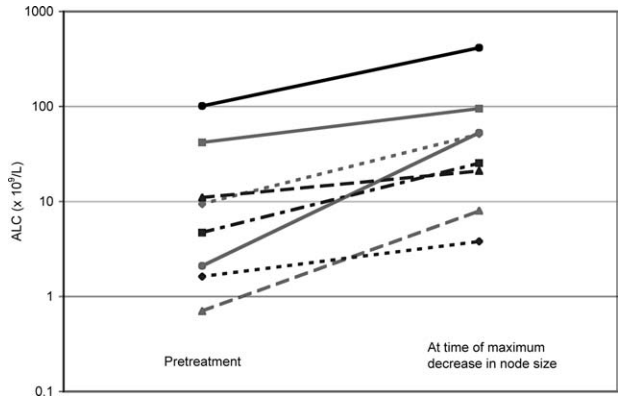

Figure 2. The increase in absolute lymphocyte count (ALC) from the pretreatment level to the time of maximum decrease in lymphadenopathy is shown.

a median baseline level of 7.1 $\times$ 10^9/L (range, 0.7-101.2 $\times$ 10^9/L) to 18.3 $\times$ 10^9/L (range, 1.2-415.3 $\times$ 10^9/L) at the first post-treatment ALC (median interval from start of therapy, 29 days; range, 7-49 days) (Fig. 2). Patients achieved a median 76% (range, 38%-93%) decrease in clinically measurable lymph node size compared with baseline. This was associated with a median 4.8-fold (range, 1.9- to 25.1-fold) increase in ALC compared with baseline (median, 38.4 $\times$ 10^9/L; range, 3.8-415.3 $\times$ 10^9/L), which occurred at a median interval of 43 days (range, 29-242 days) from the start of therapy. Of the 8 patients with lymphocyte mobilization, 2 achieved a clinical PR (both had SLL), 4 had clinically stable disease, 1 had disease progression, and 1 was removed from the study because of an adverse event before clinical response could be evaluated.

Lymphocyte mobilization was not an anticipated finding in this study, and the mechanism of this response was not studied. In 1 patient with lymphocyte mobilization, a bone marrow (BM) examination was performed on Day 6 of everolimus treatment because of concern about the patient's clinical status. Although the results of the BM biopsy showed minimal change compared with baseline, the majority of the BM and circulating lymphocytes had the same morphological appearance as CLL cells. This finding suggests that the increase in circulating lymphocytes was caused by an increase in CLL cells. No confirmatory immunophenotyping studies were done on the circulating lymphocytes.

Toxicity and Tolerability

Patients received a median of 2 months (range, 1 week to 29 months) and a median of 2 cycles (range, 1-18 cycles) of treatment. Twenty patients have gone off treatment, 13 because of disease progression, 3 because of toxicity (1 patient with grade 2 pancreatitis, 1 with grade 3 fatigue, 1 with cytopenia), 2 died on-study (1 patient because of sepsis possibly related to treatment, 1 from disease progression), and 2 refused further therapy without response or progression. Nine patients experienced dose delays on 12 cycles of therapy because of hematological adverse events (10 cycles), hospitalization (1 cycle), and a delay in getting laboratory results (1 cycle). Nine patients experienced dose reductions on 11 cycles because of hematological adverse events (9 cycles), mucositis (1 cycle), and infection (1 cycle).

Fifteen patients experienced a grade 3 or higher hematologic adverse event (7 grade 3, 8 grade 4), and 9 patients experienced a grade 3 or higher nonhematologic adverse event (5 grade 3, 1 grade 4, 3 grade 5). Focusing on adverse events at least possibly attributable to the study therapy (Table 2), 14 patients experienced a grade 3 or higher hematologic toxicity (6 grade 3, 8 grade 4), and 7 patients experienced a grade 3 or higher nonhematologic toxicity (5 grade 3, 2 grade 5). Grade 3 or 4 anemia, neutropenia, and thrombocytopenia occurred in 23%, 32%, and 50% of patients, respectively. The most common grade 3 nonhematologic toxicities were pneumonia (n = 2) and hypertriglyceridemia (n = 2). One patient, who died of infection with positive blood cultures, had grade 4 adult respiratory distress syndrome complicating sepsis and grade 3 neutropenia; both complications were

Table 2. Treatment Toxicity: Grade 3+ Adverse Events Possibly, Probably, or Definitely Related to Treatment With Everolimus[a]

Toxicity	Grade					
	3		4		5	
	No.	%	No.	%	No.	%
Hematology						
Anemia	5	23				
Leukopenia	3	14				
Neutropenia	5	23	2	9		
Thrombocytopenia	5	23	6	27		
Infection/febrile neutropenia						
Pneumonia	2	9				
Sepsis					2	9
Cellulitis	1	5				
Metabolic/laboratory						
Hypertriglyceridemia	2	9				
Pulmonary						
ARDS (secondary to infection)			1	5		
Constitutional symptoms						
Fatigue	1	5				
Gastrointestinal						
Diarrhea	1	5				

ARDS indicates adult respiratory distress syndrome.
[a]Adverse events were graded using the National Cancer Institute Common Toxicity Criteria (version 3.0).

considered to be probably related to treatment. Another patient with grade 2 neutropenia died of sepsis possibly related to treatment.

DISCUSSION

Treatment of patients with recurrent/refractory CLL using single-agent everolimus showed biological activity, with 18% of patients achieving a PR. An additional finding was that 36% of patients had an unexpected and marked increase in ALC with a concomitant decease in lymph node size. These results show that everolimus can be clinically active in some patients with recurrent/refractory CLL, and suggest that mTOR inhibition can mobilize CLL cells from tissue sites into the circulation. Both of these findings could have important implications for the treatment of CLL.

Patients with recurrent/refractory CLL, especially those with purine analogue refractory disease or loss of p53 function because of gene deletions or mutations, have a poor prognosis.[4] The 18% PR rate achieved with everolimus therapy in this study, although modest, warrants further study in light of the heavily pretreated nature of the patient population, the fact that this study used single-agent everolimus, and that tumor cell mobilization was observed. In addition, the modest response rate needs to be interpreted in the context of the NCI Working Group 1996 criteria that requires a minimum 50% decrease in ALC to qualify for a response to treatment. The increase in ALC observed in 6 of the patients with CLL who had a decrease in adenopathy prevents them from being considered responsive to treatment. This could have resulted in an underestimation of the efficacy of single-agent everolimus therapy in this group of patients.

The mobilization effect observed could be especially useful for selecting drugs to be tested in combination with everolimus. In the only previously published report of the treatment of patients with CLL using everolimus,[21] the pilot trial was stopped after treatment of only 7 patients because of infectious complications. Of the treated patients, 1 achieved a PR, and 3 had stable disease. Although not commented on by the authors, review of the published data shows that 6 of the 7 patients appear to have had progressive lymphocytosis in response to initiation of therapy with everolimus at a dose of 5 mg/day; there was no information on the effect of everolimus on lymphadenopathy.

The stromal microenvironment of the lymphoid tissues and bone marrow provides critical support for CLL cell proliferation, survival, and resistance to therapeutic drugs.[22] Preclinical data suggest that disruption of the

interaction between CLL cells and stroma can decrease drug resistance in CLL cells.[17] In addition, the therapeutic unconjugated monoclonal antibodies alemtuzumab and rituximab, which are highly effective against circulating CLL cells, have limited efficacy against bulky adenopathy and splenomegaly.[23,24] Mobilization of CLL cells from tissue into the circulation is likely to remove these CLL cells from the protective stromal microenvironment and enhance the cytotoxicity of drugs such as alemtuzumab, which have the additional advantage of being effective in patients with purine analogue-refractory CLL with defective p53 function.[25,26] Combinations of everolimus and drugs such as alemtuzumab could potentially be synergistic in patients with bulky adenopathy, resulting in improved treatment outcomes.

Everolimus is an immunosuppressive drug that can also be myelosuppressive. Thus, it was not surprising that infections were observed in this study of CLL patients already immunocompromised by their underlying disease and prior therapy. Indeed, infection resulted in 2 deaths. Neutropenia could have contributed to the risk of infection, but thrombocytopenia was not associated with bleeding complications. The other grade 3-4 toxicities responded well to appropriate management. These data suggest that CLL patients treated with everolimus in the future will likely benefit from prophylaxis and surveillance for opportunistic infections, and that combination therapies including everolimus should be designed to minimize marrow toxicity in this patient population.

mTOR has been shown to be an integral component of important signaling pathways in CLL cells, and inhibition of these pathways by everolimus could be expected to decrease cell growth and be cytotoxic.[12,15,16] In contrast, the mechanism by which everolimus causes CLL cell mobilization into the circulation is unknown, and will need to be investigated to develop interventions to optimize this effect. These investigations were not performed in this study, because the finding was unexpected. The extent of mobilization and the concomitant decrease in the clinically detectable lymphadenopathy was only fully appreciated at the time of analysis of the study results. The study was designed to evaluate ALC each cycle and measurable lymph nodes after Cycles 2 and 6 and every 3 cycles thereafter. The study was not designed to measure or record ALC more frequently; therefore, the kinetics and magnitude of the CLL cell mobilization could actually have been underestimated in this study population. Future studies should be designed to more frequently measure the mobilization effect of everolimus in CLL patients, study the cell surface immunophenotype of the mobilized cells, and determine whether the CLL cells mobilized into the circulation are as sensitive to monoclonal antibody-mediated cytotoxicity as cells collected before treatment with everolimus.

In conclusion, this study shows that everolimus could be a valuable drug for use in combination therapy for CLL patients. Further understanding of the mechanism of action of everolimus in CLL will be very useful in designing trials to improve therapy for this incurable disease.

CONFLICT OF INTEREST DISCLOSURES

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