

## Cladribine with Immediate Rituximab for the Treatment of Patients with Variant Hairy Cell Leukemia

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### Abstract

**Purpose:** In contrast with the classic form, variant hairy cell leukemia (HCLv) responds poorly to single-agent purine analogs, expresses unmutated BRAF, has shorter overall survival, and lacks effective standard therapy. No treatment has achieved a high complete remission (CR) rate even in small series, and of 39 reported cases from six studies, overall response rate after cladribine was 44% with 8% CRs. Rituximab has been found to increase the sensitivity of malignant cells to cladribine, suggesting that combination with cladribine might improve response in HCLv. To test this hypothesis, patients with HCLv were treated with simultaneous cladribine and rituximab.

**Experimental Design:** Patients with HCLv with 0 to 1 prior courses of cladribine received cladribine 0.15 mg/kg for days 1 to 5, with eight weekly doses of rituximab 375 mg/m<sup>2</sup> beginning day 1. Restaging was performed, and minimal residual disease (MRD) in blood and marrow was quantified using PCR, immunohistochemistry, and flow cytometry.

**Results:** By 6 months, 9 (90%) of 10 patients achieved CR, compared with 3 (8%) of 39 reported cases treated with cladribine alone ( $P < 0.0001$ ). Of the 9 CRs, 8 remain free of MRD at 12 to 48 (median 27) months of follow-up. No dose-limiting toxicities were observed when beginning cladribine and rituximab on the same day, although most patients required short-term steroids to prevent and treat rituximab infusion reactions. Cytopenias in CRs resolved in 7 to 211 (median 34) days without major infections.

**Conclusion:** Although cladribine alone lacks effectiveness for early or relapsed HCLv, cladribine with immediate rituximab achieves CRs without MRD and is feasible to administer. *Clin Cancer Res*; 19(24):6873–81. ©2013 AACR.

### Introduction

Hairy cell leukemia (HCL), a B-cell malignancy comprising about 2% of leukemias, was noted in 1980 to contain a variant form comprising 10% to 20% of patients, called variant HCL (HCLv; ref. 1). The World Health Organization (WHO) now recognizes HCLv as an entity distinct from HCL within the category "splenic lymphoma/leukemia, unclassifiable," resembling HCL immunophenotypically except lacking CD25, tartrate-resistant acid phosphatase (TRAP), and annexin A1 (2). Unlike classic HCL, which achieves high complete remission (CR) and overall response rates (ORR) with single-agent purine analogs cladribine or pentostatin (3–5), HCLv is primarily resistant. Among 39 patients with HCLv reported from six retrospective studies of 3 to 15 patients each (6–11), the CR rate with

cladribine was only 8%, and ORR 44%. Response was similarly poor with pentostatin (6). BRAF inhibitors such as Vemurafenib may be useful in classic HCL (12), but patients with HCLv have wild-type BRAF (13, 14) and hence would not be expected to benefit. Median overall survival from diagnosis is only about 9 years for HCLv compared with more than 25 years for classic HCL (6, 11). Thus, alternative treatment approaches are urgently needed for this disease.

In classic HCL, Ravandi and colleagues recently reported a 100% CR rate in 31 purine analog-naïve patients treated with cladribine followed by 8 weekly doses of rituximab begun 1 month after cladribine (15, 16). In addition, 5 patients with HCLv were treated, 2 of whom died of secondary malignancies and 1 relapsed at 6 months before dying of disease, but 2 remained in CR for 12 and 35 months (16). The status of minimal residual disease (MRD) in these 2 or the other 3 patients with HCLv was not reported. Rituximab is reported to sensitize malignant B cells to cladribine (17). To exploit such synergy in patients with HCLv, we prospectively treated patients with rituximab begun on the same day as cladribine. We report here clinical results in 10 consecutive patients with HCLv treated prospectively with cladribine and immediate rituximab.

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### Translational Relevance

Although great progress has been achieved in treating classic hairy cell leukemia (HCL), with purine analogs, rituximab, immunotoxins, and BRAF inhibition, progress has been less for the more aggressive variant, HCLv, which lacks mutated BRAF, and no series has reported a high complete remission (CR) rate to any regimen. On the basis of the prior observation that rituximab increases malignant cell sensitivity to purine analogs, we used cladribine with simultaneous rituximab in either newly diagnosed or relapsed HCLv. We observed a CR rate of 90% of 10 patients and in 8 of 9 CRs, minimal residual disease became undetectable, without relapse after a median follow-up of 27 months. Soluble CD22 levels also resolved, evidence against persistent splenic disease. These results suggest that patients with HCLv should receive monoclonal antibody therapy in addition to purine analog. Further follow-up will be needed to determine if this strategy can eradicate the malignant clone.

## Materials and Methods

### Treatment protocol

Patients with HCLv were enrolled on a trial (NCT00923013) comparing immediate with delayed rituximab after cladribine for previously untreated or once-relapsed HCL. Patients with HCLv constituted a separate nonrandomized stratification receiving cladribine and immediate rituximab, with additional rituximab at least 6 months later if MRD is detected. Patients required therapy based on cytopenias, lymphocytosis, or symptomatic splenomegaly, and provided written informed consent approved by the NCI Institutional Review Board.

### Administration

Cladribine was administered in 5 daily doses at 0.15 mg/kg/d by 2-hour intravenous infusion. On day 1, 8 weekly doses of rituximab were begun at 375 mg/m<sup>2</sup>/dose. To prevent or treat rituximab infusion reactions, patients usually received diphenhydramine, acetaminophen, meperidine, and methylprednisolone.

### Response assessment

Disease was assessed by bone marrow studies before, 1 and 6 months after cladribine, yearly until 2.5 years, and then every 2 years. Blood studies were done more frequently, and flow cytometry and PCR for immunoglobulin rearrangements were performed on blood and bone marrow specimens as described in ref. (18). CR required absence of malignant cells in the blood or bone marrow by nonimmunologic stains and resolution of cytopenias and splenomegaly, as described in ref. (19). Normal blood counts required for CR included neutrophil count

of 1,500/mm<sup>3</sup>, platelets 100,000/mm<sup>3</sup>, and hemoglobin 11 g/dL. CR duration was defined from the date when all criteria of CR had been fulfilled, until the last assessment (either blood count or restaging), consistent with CR or the first assessment not consistent with CR. MRD in CR was defined as the presence of suspicious cells in the bone marrow biopsy by immunohistochemistry (IHC) as previously reported (20), or the presence of leukemic cells in the marrow aspirate or blood by flow cytometry or PCR. Statistical comparisons were performed using the Fisher exact test.

### Immunologic studies

IHC was performed on bone marrow biopsy sections using antibodies to CD20, CD79a, CD3, TRAP, mutated BRAF, and Annexin A1. For flow cytometry on bone marrow or peripheral blood, panels included antibodies to B-cell markers CD19, CD20, and CD22, HCL-associated antigens CD11c, and CD103, HCL-associated, and HCLv-nonassociated antigens CD123 and CD25, and nonassociated antigens CD5, CD10, and CD23, as described in ref. (21).

### Molecular studies

Immunoglobulin gene rearrangement studies targeting both the heavy chain and the  $\kappa$  light chain gene were performed as follows. For the immunoglobulin heavy chain (IGH) locus, two separate PCR reactions were performed, using consensus primers to the joining region and either framework region II or III (FRII-IGHJ and FRIII-IGHJ PCR), as described in ref. (22). For the  $\kappa$  light chain, two additional PCR reactions were performed using the Biomed II primer set described by van Dongen and colleagues (23). The PCR products were analyzed on an ABI 3130xl Genetic Analyzer. To characterize IGH variable gene (IGHV) sequences, total RNA was prepared from PAXgene tubes (PreAnalytiX) and the resulting first-strand cDNA was subjected to PCR using six IGHV FRI primers combined with one IGHJ consensus primer, as described in ref. (24).

## Results

### Patient demographics and prior therapy

At the time of treatment, patient ages ranged from 43 to 80 years, median 65 (Table 1), and the male to female ratio was 6:4. Patients were enrolled 3 to 148 (median 15) months after diagnosis, representing a wide variation in both progression of HCLv and the prior therapies used. Prior therapy included none in two cases, cladribine alone in five cases, rituximab alone in one case, and cladribine followed by rituximab and splenectomy in two cases. The 2 patients receiving cladribine and rituximab received the rituximab either 5 (RG08) or 2 (RG10) months after cladribine, with RG08 receiving only part of two doses of rituximab due to infusion reactions, and RG10 receiving four doses of rituximab. For those 8 patients with prior therapy, best response to prior therapy was judged on the basis of standard HCL response criteria (25, 26) using

**Table 1.** Clinical characteristics of patients with HCLv

Patient no.	Age (y)/sex	Months after diagnosis	Prior treatment (response)	Months after last therapy	HCL count (cells/mm <sup>3</sup> )	Spleen size (max, mm)
RG01	43/M	67	Cladribine (CR × 16 mo)	54	29,700	185
RG02	62/M	148	Cladribine (PR × 37 mo)	49	4	218
RG03	51/F	16	Cladribine (PR × 11 mo)	15	440	236
RG04	75/M	9	Cladribine (PD)	6	370	280
RG05	54/F	14	Cladribine (PR × 9 mo)	12	4,300	213
RG06	59/F	36	None	—	15,300	174
RG07	80/M	3	None	—	3,130	220
RG08	70/M	11	Cladribine (PR × 5 mo), rituximab (PD), splenectomy (PD)	1.5	15,000	0 <sup>a</sup>
RG09	68/M	5	Rituximab (PD)	2	28,000	370
RG10	75/F	50	Cladribine (PR × 2 mo), rituximab (SD), splenectomy (PD)	15	241,000	0 <sup>a</sup>

<sup>a</sup>Maximum (max) spleen diameter and weight, 271 mm, 6 kg (RG08); and 210 mm, 3.2 kg (RG10); before splenectomy.

available retrospective blood counts and spleen measurements. Five of the patients were considered primarily refractory to cladribine with either no response or response lasting less than 1 year, and 1 patient progressed on initial rituximab. One patient had CR after cladribine lasting 16 months followed by partial response (PR) lasting 7 months, and another patient had PR lasting 37 months (Table 1). Blood counts and tumor assessments were taken at intervals often exceeding a year, thus overestimating true response times to prior therapy in some patients. Thus, before enrollment, demographics and response to first-line purine analog were as expected for HCLv, with lower male–female ratio, higher age at diagnosis, and much lower durable response rate to single-agent purine analog therapy as compared with classic HCL (27).

#### Patient characteristics at the time of enrollment

Consistent with the relatively high lymphocyte count observed in HCLv, the circulating hairy cell leukemic count

was less than 350 cells/mm<sup>3</sup> in just 1 case, ranging from 4 to 241,000 with a median of 9,700 cells/mm<sup>3</sup>. Spleen size for the 8 patients without prior splenectomy, represented by maximum diameter, was 174 to 370 (median 215) mm. As shown in Table 2, all 10 patients met immunophenotypic criteria for HCLv based on the 2008 WHO definition (2), including negativity for CD25, TRAP, or annexin A1, and strong positivity for B-cell antigens (particularly CD20), and positivity for CD103 and CD11c. CD123 was negative as expected (18, 28–30) in all but one case. The positivity for CD103 in each case helped establish the diagnosis of HCLv in contradistinction to splenic marginal zone lymphoma (SMZL; refs. 18, 28–31). Malignant cells were abundant enough in 9 of 10 cases to perform molecular characterization. Three of these 9 patients expressed the IGHV4-34 immunoglobulin rearrangement, all unmutated between 99.58% and 100% homologous to germline. Three other patients expressed unmutated rearrangements, IGHV1-69, IGHV3-30, and VH4-4, with 99.17% to 100%

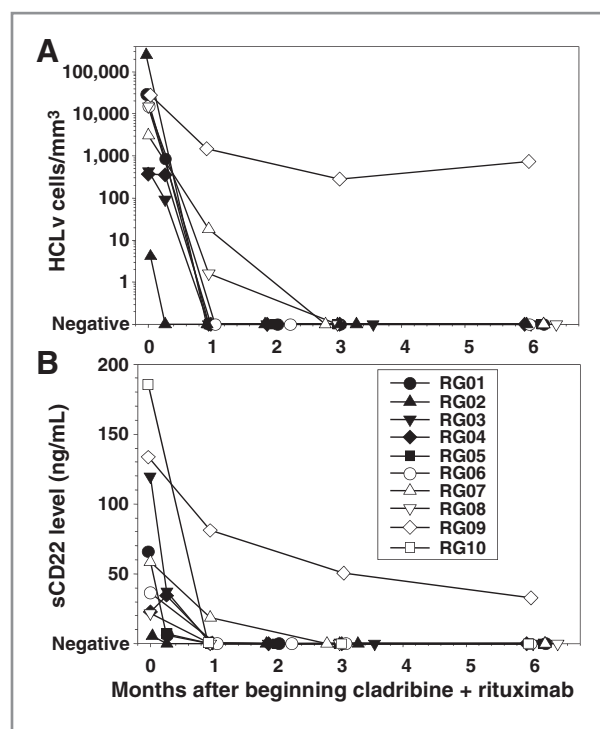
**Table 2.** Pretreatment phenotypic and molecular characteristics of patients

Patient no.	CD25	CD123	CD103	CD11c	CD20 sites/cell	IGHV	%homology	BRAF	Annexin A1
RG01	–	–	+	++	256,000	6–1	87.65%	Wild-type	–
RG02	–	–	+	+	98,000	Not done	Not done	Wild-type	–
RG03	–	–	+	++	195,000	3–9	94.45%	Wild-type	–
RG04	–	–	+	++	124,000	4–34	99.58%	Wild-type	–
RG05	–	–	+	++	127,000	4–34	100%	Wild-type	–
RG06	–	–	+	++	133,000	4–34	100%	Wild-type	–
RG07	–	–	+	++	185,000	3–30	100%	Wild-type	–
RG08	–	+	+	+	192,000	1–69	99.17%	Wild-type	–
RG09	–	–	+	+	189,000	4–4	99.17%	Wild-type	–
RG10	–	–	+	++	227,000	1–46	97.13%	Wild-type	–

homology to germline. The IGHV3-30 was an unproductive rearrangement containing a stop codon and out-of-frame junction in the third complementarity-determining region. All 10 patients expressed wild-type BRAF, as expected for HCLv (13, 14, 32). As shown in (Table 1), patient RG02 was unusual in that enrollment was 148 months after diagnosis and 49 months after his first and only therapy. Nevertheless, his initial presentation was consistent clinically with that of HCLv with an absolute lymphocyte count of 22,900 cells/mm<sup>3</sup>. His white blood cell count remained in the 20,000 to 50,000 cell/mm<sup>3</sup>-range during the years that he postponed therapy, until he presented with massive splenomegaly and renal failure. He had a PR to single-agent cladribine but relapsed with progressive splenomegaly and transfusion dependence 37 months later (Table 1). Bone marrow samples were examined before initial cladribine, and the leukemic infiltrate was negative as expected for annexin A1 and mutated BRAF, confirming the original diagnosis as HCLv. Thus, although HCLv can be difficult to differentiate from HCL and other splenic B-cell malignancies, including SMZL, the presence of B-cell markers, CD103, CD11c, and absence of CD25, TRAP, BRAFV600E, and annexin A1 in all cases firmly established the diagnosis of HCLv in these patients (2, 29, 31).

### Response

To determine response to cladribine with immediate rituximab, patients were evaluated before and at several time points after beginning five daily doses of cladribine and 8 weekly doses of rituximab, the rituximab starting the first day. Of the 10 patients, 9 (90%) achieved CR by 6 months after beginning cladribine, defined as absence of leukemic cells in the blood and bone marrow by non-immunologic stains, resolution of cytopenias, and resolution of splenomegaly. Bone marrow biopsies and aspirates were performed at 1 and 6 months after beginning cladribine, and were negative by morphology for HCL at the 1-month time point in all patients except RG07 and RG09, and RG07 was negative by 6 months. Besides RG08 and RG10, who had prior splenectomy, and RG09, who did not respond, splenomegaly decreased in the other 7 patients by 1 month and spleens were nonpalpable by 6 months. Of the 9 CRs, the median time to resolution of cytopenias and lymphocytosis to the level required for CR was 34 days, with RG07 requiring 211 days and the other 8 patients requiring 7 to 91 days. As shown in figure 1A, circulating leukemia cells quantified by flow cytometry decreased rapidly, remaining detectable in only 3 of 10 patients by 1 month and in 1 patient by 3 months. Because of the lack of CD25 expression in these patients with HCLv, soluble CD25, as expected, was not helpful as a tumor marker (data not shown). Therefore, soluble CD22, previously reported to correlate with HCL tumor burden and response (33), was followed instead. As shown in figure 1B, decreases in soluble CD22 paralleled those in circulating HCLv cells (Fig. 1A), with only 2 patients remaining elevated at 1 month and 1 at 2 or 3 months. One of the patients (RG04) had a slight decrease



**Figure 1.** Time course of response after cladribine plus rituximab. For each of the indicated patients, tumor burden is quantified either by flow cytometry (A) or by soluble CD22 level (B).

in circulating hairy cells and a slight increase in soluble CD22 during the first week of treatment, but by 1 month both parameters were undetectable. Patient RG09 had a slight increase in circulating HCLv cells but continued decrease in sCD22, consistent with significant response in the spleen, the major source of HCLv generating sCD22, despite beginning to relapse in the peripheral blood. Thus 9 (90%) of 10 patients achieved CR with cladribine and immediate rituximab, compared with only 8% of 39 historic patients with HCLv reported from six retrospective studies using cladribine alone (refs. 6–11;  $P < 0.0001$ ). These retrospective reports of cladribine alone in HCLv included four PRs out of eight (6), two PRs out of six (7), one CR and two PRs out of four (8), one PR out of three (9), one CR and two PRs out of three (10), and one CR and three PRs out of 15 (11) patients. In the present study with cladribine combined with immediate rituximab, all 9 CRs maintained evidence of CR by restaging studies at 12 to 48 (median 30) months of follow-up.

### MRD

Because patients with HCL achieving CR with cladribine commonly have leukemic cells detectable in the marrow presenting a risk factor for early relapse (20, 34–37), MRD after cladribine and rituximab was followed in the patients with HCLv. Flow cytometry of the blood became negative in 9 (90%) of 10 patients by 3 months (Figs. 1A and 2A). Bone marrow IHC became

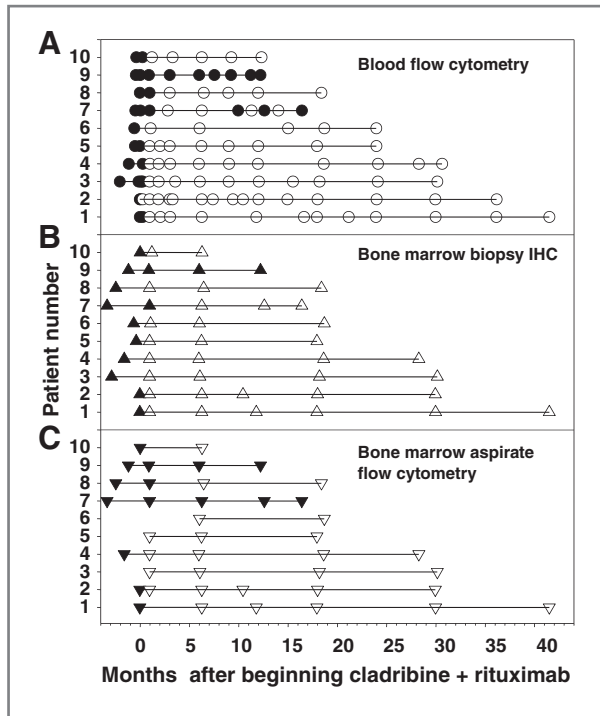


Figure 2. MRD before and after cladribine plus rituximab. Patients with HCLv, identified as in Figure 1, were tested for flow cytometry of blood (A), immunohistochemistry of bone marrow biopsy (B), and flow cytometry of bone marrow aspirate (C), with positive results indicated by solid and negative results by open markers.

negative in 8 (80%) of 10 patients at 1 month, and in 9 (90%) of 10 patients by 6 months (Fig. 2B). Of 8 patients with sufficient follow-up, all were still negative at 16 to 18 months and negativity at 28 to 30 months was documented in 4 patients. Finally, as shown in figure 2C, bone marrow aspirate flow cytometry became negative in 4 (57%) of 7 evaluable (aspirable) patients by 1 month. By 6 months, all marrows were aspirable and 8 (80%) of 10 patients were negative by bone marrow aspirate flow cytometry. RG07 remained positive (0.1% of the marrow cells) at 6 months but was negative by flow cytometry of blood and IHC of the bone marrow biopsy. At 9 months, this patient had detectable leukemic cells by flow cytometry of blood, still met criteria for CR, and per protocol began a second 8-week course of rituximab, resulting in continued CR with negative marrow IHC (Fig. 2B). Thus 9 (90%) of the patients achieved a negative bone marrow biopsy by IHC. Although consensus PCR was previously reported to be less sensitive than flow cytometry (21), in the 10 patients with HCLv, PCR and flow cytometry agreed (data not shown) when comparing tests on the same patient, time point, and tissue (bone marrow aspirate or blood). Thus cladribine with early rituximab achieved CRs in 9 (90%) of the 10 patients with HCLv, and was associated in 8 (89%) of 9 CRs with absence of MRD. As shown in figure 2, 8 patients remain MRD-free at 12 to 48 (median 27) months of follow-up.

### Toxicity of rituximab immediately following cladribine

Cladribine followed immediately by rituximab was tolerated well, with the first 2 patients reporting minimal infusional side effects without steroid prophylaxis. Because of infusional side effects observed with the third patient, prophylactic methylprednisolone was instituted for the last 5 patients to prevent infusion reactions. Maximum toxicity grades in each of the patients for each toxicity experienced are listed in Table 3. On the basis of NCI Common Toxicity Criteria Version 3.0, the adverse events occurring in more than 30% of patients were lymphopenia (100%), leukopenia (70%), neutropenia, chills, hypoalbuminemia, and nausea (60% each), thrombocytopenia and aspartate aminotransferase (50% each), and arthralgia and cough (40% each). The grade 3 to 4 events seen in more than 1 patient included lymphopenia (70%), neutropenia (40%), thrombocytopenia (30%), and leukopenia (20%). Patient RG10 had the highest circulating count before enrollment ( $241,000/\text{mm}^3$ ) and had transient laboratory evidence of mild tumor lysis syndrome including potassium 5.3 mmol/L, uric acid 14.1 mg/dL, and phosphorus 7.2 mg/dL, but without associated clinical symptoms or evidence of renal insufficiency. Lymphopenia and leukopenia reflected decreases in both normal and malignant cells and in most cases did not represent true adverse events. Only 1 patient had febrile neutropenia, which was limited to a temperature of  $38.3^\circ\text{C}$ , neutrophil count of 0.4, and resolution on intravenous antibiotics without evidence of serious infection. Thus, cladribine followed immediately by cladribine had an acceptable safety profile, and the lack of infections despite combination therapy may be related to the lower rate of cytopenias in HCLv as compared with classic HCL (6).

### Thrombocytopenia immediately following cladribine and rituximab

When beginning rituximab the same day as cladribine, a previously unreported transient decrease in platelets was observed, shown in figure 3, which was noted immediately after beginning cladribine and rituximab. Platelets decreased by 7 to 136 (median 49) cells/L  $\times 10^{-9}$  to platelet nadirs of 14 to 382 (median 50) cells/L  $\times 10^{-9}$  with a percentage decrease of 9 to 91 (median 42%), and resolved more rapidly than expected of chemotherapy-induced marrow toxicity. In 50% of cases, the platelet count nadir was not worse than the baseline grade. Patient RG08 was one of 2 asplenic patients and as expected had a higher platelet count than other patients before treatment. Thus cladribine and immediate rituximab had an acceptable safety profile and the rapid thrombocytopenia observed was not significant enough to lead to bleeding or platelet transfusions.

### Discussion

Our goal was to determine whether cladribine followed by rituximab would be effective for treating patients with HCLv who were either newly diagnosed or had one prior

**Table 3.** Maximum toxicity grade for each patient

Toxicity	RG01	RG02	RG03	RG04	RG05	RG06	RG07	RG08	RG09	RG10	%Grade 1-4	%Grade 3-4
Lymphopenia	1	4	3	3	4	3	3	3	1	2	100%	70%
Leukopenia	2		2		4	2	2		2	3	70%	20%
Neutropenia	3				4	3	2		2	4	60%	40%
Chills	2		1	2			1		2	2	60%	0%
Hypoalbuminemia	1	1	1		1				1	2	60%	0%
Nausea	1		1	1	1	1				1	60%	0%
Platelets			3	2	3				2	4	50%	30%
AST	1		2		1			2		2	50%	0%
Arthralgia			1				1		1	1	40%	0%
Cough		1						1	1	1	40%	0%
Fatigue					2	1			2		30%	0%
Rash			1	1		2					30%	0%
GGT	1					1	1				30%	0%
Rhinitis				1	1			1			30%	0%
Vomiting			1	1						1	30%	0%
Hyperuricemia							1			4	20%	10%
Myalgia						2		2			20%	0%
Fever				2						2	20%	0%
Bronchospasm									2	2	20%	0%
Flushing				2		1					20%	0%
Dyspnea						1				2	20%	0%
ALT					1	1					20%	0%
Dizziness		1			1						20%	0%
Hematuria	1		1								20%	0%
Hypotension			1							1	20%	0%
Febrile neutropenia					3						10%	10%
Haptoglobin							3				10%	10%
Anemia		3									10%	10%
Hypophosphatemia	3										10%	10%
Tumor lysis										3	10%	10%
Bilirubin							2				10%	0%
Bone pain						2					10%	0%
Flu syndrome						2					10%	0%
Sweating					2						10%	0%
Back pain										2	10%	0%
Alkaline phosphatase						1					10%	0%
Headache			1								10%	0%
Pruritus			1								10%	0%
Throat Pain		1									10%	0%
Diarrhea									1		10%	0%
Oral mucositis									1		10%	0%
Systolic hypertension										1	10%	0%

Abbreviation: AST, aspartate aminotransferase.

course of cladribine. Our approach was to use the regimen of rituximab following cladribine, with which Ravandi and colleagues, using rituximab delayed by 1 month, reported excellent results in early classic HCL (15, 16), but durable (more than 1 year) CR in only 2 of 5 patients with HCLv (16). Because of the aggressiveness of HCLv, and preclinical data demonstrating the ability of rituximab to sensitize leukemia and lymphoma cells to cladribine (17), we

began rituximab the same day as cladribine, rather than wait 1 month (16). A regimen almost identical to this, in which the first dose of rituximab began the day before cladribine, was reported by Forconi and colleagues to achieve an MRD-free CR in a patient with multiply relapsed classic HCL (38). We found that of 10 patients with HCLv enrolled and staged 6 months after treatment, 9 (90%) had CR, and 8 (80%) had CR without MRD. Tumor burden

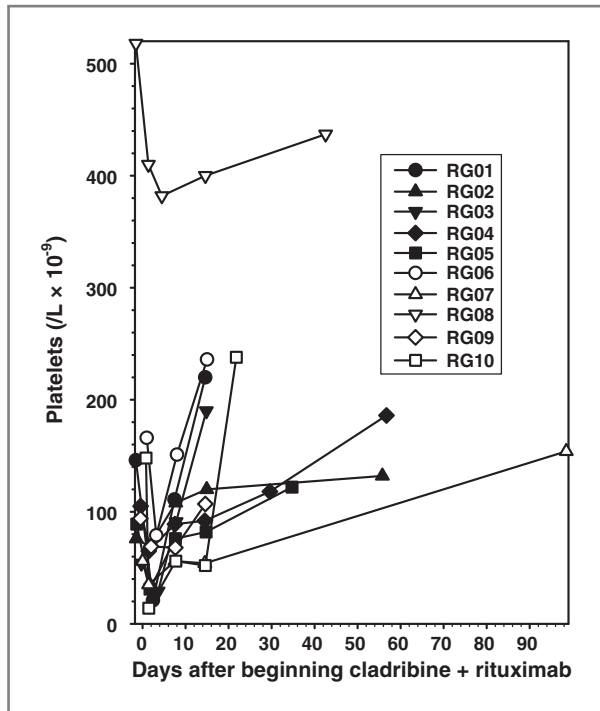


Figure 3. Thrombocytopenia after cladribine plus rituximab. Patients identified as in Figure 1. The beginning of treatment is considered day 1.

responded rapidly to combination treatment and no patient relapsed from CR at 12 to 48 (median 30) months of follow-up.

Up until now, a consistently effective treatment strategy for HCLv is unknown. Cladribine alone achieves CR in less than 10% and overall response in less than 50% (6–11) and CRs to rituximab in HCLv have been reported only anecdotally (39–41). The 90% CR rate in the present series of 10 patients with HCLv is clearly superior to 3 out of 39 historic patients (6–11) completely responding to cladribine alone ( $P < 0.0001$ ). In fact, ORR, which is independent of bone marrow status and therefore more accurately compared historically, was also higher with cladribine-rituximab than cladribine alone (90% vs 44%,  $P = 0.012$ ). However, it is not possible to determine if rituximab is significantly more effective if begun immediately with cladribine or 1 month later, as Ravandi and colleagues reported that 2 of the 5 patients with HCLv treated died of secondary malignancies and only 1 died of rapidly recurrent HCLv (16). Theoretically, immediate rituximab might be advantageous for enhancing synergy between rituximab and cladribine, and 1-month delayed rituximab might be advantageous for avoiding infusion reactions in high-risk, medically fragile patients. Regardless, we believe that HCLv should be treated with combined therapy with purine analog (cladribine or pentostatin) and monoclonal antibody therapy, such as rituximab.

In the classic or typical form of HCL, CR, overall response, and progression-free survival are excellent with purine analog alone, so that any incremental improvement

achieved by addition of rituximab would require very long follow-up to assess its benefit. Nevertheless, recent data reporting median relapse-free survivals of 16, 11, and 6.5 years after first-, second-, and third-line purine analogs, respectively (5), argue that even patients with classic HCL are likely to become treatment refractory if diagnosed at young age and if treatment is restricted to purine analog as single agent. To examine the benefit of rituximab in newly diagnosed or once-relapsed classic HCL, using MRD 6 months after cladribine as a surrogate endpoint, a randomized trial is underway in which patients with classic HCL receive cladribine with rituximab begun either immediately or delayed at least 6 months. In patients with classic HCL, unlike those with the more rapidly progressive HCLv, delayed rituximab may be as effective as immediate rituximab in eliminating MRD and preventing or at least delaying relapse.

It has been reported that HCLv is often difficult to differentiate from SMZL, each having similar B-cell antigens and HCL scores (42, 43), but this differentiation is important as SMZL is more indolent than HCLv and responds better to treatment (44). It is unlikely that our patients had SMZL rather than HCLv not only based on their morphology, but also their expression of CD103 in all 10 cases, an antigen expressed in less than 10% of cases of SMZL (28), including none of 43 patients from three studies (29, 45, 46). In our 2 patients who had splenectomy, splenic pathology was consistent with HCLv in both cases. Thus, although the ability to differentiate SMZL from HCLv is not 100% reliable, it is unlikely that our efficacy data with cladribine and rituximab are significantly affected by the sensitivity of SMZL to this regimen.

Although classic HCL is associated with the BRAF V600E mutation in a high percentage of cases (14, 32), HCLv, as well as IGHV4-34–expressing classic HCL cells, lack this mutation (13). IGHV4-34–expressing patients with HCL have been shown to have a poor prognosis with single-agent cladribine as initial therapy, and poor overall survival, whether they are classified as classic HCL or HCLv immunophenotypically (11). These patients, like the others in this study, would not be appropriate for therapy with BRAF inhibition (12). It is notable that of the 3 patients in this study expressing IGHV4-34 (Table 2), all achieved CR with no MRD (Fig. 2). Two of our patients (RG07 and RG08) were noted to have a 17p13.1 abnormality resulting in p53 deletion, which as previously noted is also a poor prognostic factor (44, 47). Although RG07 was the 1 patient with MRD detectable in CR, RG08 achieved elimination of MRD by the cladribine–rituximab combination. Patient RG09, the only one without response, had normal p53 but had loss of 14q32, expressing IgH, by FISH. It will be important to determine whether the addition of rituximab or possibly other antibody-based therapies to purine analog will improve the outcome of both poor-risk and good-risk HCL.

#### Disclosure of Potential Conflicts of Interest

R.J. Kreitman received other commercial research support from Genentech. No potential conflicts of interest were disclosed by the other authors.

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