Brief report

Very long-term eradication of minimal residual disease in patients with hairy cell leukemia after a single course of cladribine

Darren S. Sigal,[1] Robert Sharpe,[2] Carol Burian,[3] and Alan Saven[1]

Divisions of [1]Hematology/Oncology and [2]Hematopathology, Scripps Clinic Medical Group, La Jolla; and [3]Scripps Blood and Marrow Transplantation Program, Scripps Green Hospital, La Jolla, CA

Cladribine induces protracted remissions in patients with hairy cell leukemia (HCL). However, many long-term responders ultimately relapse. We sought to determine whether long-term complete responders subsequent to a single 7-day course of cladribine were without minimal residual disease (MRD) and potentially cured of HCL. From the 358-person Scripps Clinic cladribine database, we identified 19 patients in continuous and complete hematologic response (median age, 75 years; median time from diagnosis, 18 years; and median time from cladribine, 16 years). Nine of 19 (47%) patient samples had no evidence of residual disease; 7 of 19 (37%) samples had MRD; and 3 of 19 (16%) had morphologic evidence of HCL in hematoxylin and eosin–stained bone marrow sections. These results indicate that HCL is potentially curable after cladribine treatment. In addition, patients with MRD and even gross morphologic disease can live many years without manifesting hematologic relapses. (Blood. 2010;115:1893-1896)

Introduction

Hairy cell leukemia (HCL) is a rare, chronic lymphoproliferative disorder characterized by splenomegaly, pancytopenia, and the "hairy cell," a small- to medium-sized B lymphocyte with a typical serrated border.[1,2] At diagnosis, many patients require therapy for symptoms or cytopenias. The purine nucleoside analogs, pentostatin and cladribine, were major therapeutic advances over the less effective therapies of splenectomy and interferon.[3-6] Overall response and complete response (CR) rates for pentostatin were 96% and 81%, and for cladribine were 98% and 91%, respectively.[5,6] However, because of its single, 7-day treatment regimen and favorable toxicity profile, cladribine has generally been accepted as the preferred first-line treatment of HCL.

In 1990, Piro et al from Scripps Clinic, under the leadership of Dr Ernest Beutler, reported a 100% overall response (11 CRs and 1 partial response) for the first 12 HCL patients treated with a single 7-day course of cladribine.[7] Saven et al confirmed this dramatic response in an expanded cohort of 358 patients.[6] Long-term follow-up documented a median response duration of 98 months (range, 8-172 months), but without evidence of a plateau in the rate of relapse.[8] Other long-term follow-up studies have reported similar results.[5,9] Of HCL patients who received cladribine and achieved a CR by historical morphologic criteria, 20% to 50% were subsequently shown to have minimal residual disease (MRD) by immunohistochemical methods.[10,11] MRD in HCL has been associated with disease relapse.[12] With now more than 20 years of follow-up available in some patients, we sought to determine whether any patients were without MRD, and potentially cured of HCL, after a single 7-day course of cladribine.

Methods

We searched the Scripps Clinic cladribine HCL computer database to identify patients who were alive and in continuous complete hematologic remission after a single 7-day course of cladribine. This database includes all patients who were enrolled in phase 2 cladribine studies at Scripps Clinic between April 1986 and September 1996. Inclusion criteria have been previously reported.[6] Patients received cladribine as a 7-day continuous intravenous infusion at a dose of 0.085 to 0.1 mg/kg per day.[13] Of 358 HCL patients in the database, 143 met these criteria. Of these 143 patients, 21 were deemed eligible, willing to provide tissue samples, and enrolled; 19 were evaluable. The remainder of these patients declined (n = 38), could not be contacted (n = 69), relapsed (n = 13), or received chemotherapy for a secondary malignancy (n = 2). The Scripps Human Subjects Committee approved this study. All patients signed an informed consent before enrollment in accordance with the Declaration of Helsinki.

All patients underwent a bone marrow aspiration and biopsy. CD20 immunostaining (L26 antibody) was performed on all samples to identify cells characteristic of HCL and quantify residual HCL disease as a percentage ($< 5\%$, 5%-19%, or $> 20\%$) of marrow cellularity. DBA.44, TRAP, and annexin immunostains were performed in selected cases. Immunophenotypic analysis for markers typical of HCL (CD103, CD11c, and CD25) using multiparameter flow cytometry was performed on the bone marrow aspirates of 17 patients. Analysis for clonal immunoglobulin heavy chain gene rearrangements by consensus-primer polymerase chain reaction (IGH-PCR) was performed on 2 specimens that lacked immunophenotypic analysis of HCL.

Patients were classified as having morphologic evidence of disease, MRD, or no evidence of disease. Morphologic disease was defined as lymphoid infiltrates identifiable in hematoxylin and eosin–stained sections. MRD required the absence of lymphoid infiltrates in hematoxylin and eosin–stained sections, but either B cells characteristic of HCL on flow cytometry or a lymphoid infiltrate typical of HCL only on immunohistochemical staining (CD20, DBA.44, TRAP, and annexin$^+$). Specimens were deemed to have no evidence of residual HCL if there was (1) no lymphoid infiltrates on hematoxylin and eosin-stained sections, (2) less than 5% CD20$^+$ B lymphocytes, and (3) either no flow cytometric evidence of monoclonal B cells or no evidence of a clonal IGH-PCR.

Submitted October 29, 2009; accepted December 4, 2009. Prepublished online as *Blood* First Edition paper, January 7, 2010; DOI 10.1182/blood-2009-10-251645.

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Table 1. Patient characteristics

Patient no.	Age, y	Sex	Time since diagnosis, y	Time since cladribine, y	Treatment before cladribine	CBC before cladribine				Current CBC				Bone marrow studies		
						WBC, $\times 10^9$/L	ANC, $\times 10^9$/L	Hemoglobin, g/L	Platelets, $\times 10^9$/L	WBC, $\times 10^9$/L	ANC, $\times 10^9$/L	Hemoglobin, g/L	Platelets, 10^9/L	Bone marrow status	IHC, percentage	FC, percentage
1	77	Male	22	17	S	5.9	0.7	89	112	9.2	3.3	153	238	MRD^-	–	ND/PCR^-
2	70	Female	16	16	None	1.6	0.8	78	126	7.5	5.8	137	244	MRD^-	–	ND/PCR^-
3	78	Male	20	15	I	1.9	1.2	145	56	10.8	8.2	140	323	MRD^-	–	–
4	76	Male	25	16	None	2.5	1.4	116	87	4.6	2.8	124	162	MRD^-	–	–
5	79	Male	16	16	None	2	0.4	126	60	4.2	2.6	131	150	MRD^-	–	–
6	68	Male	23	17	S	2.5	0.4	101	112	6	3.2	150	293	MRD^-	–	–
7	56	Female	16	16	None	2.1	0.7	108	92	6	3.8	138	339	MRD^-	–	–
8	81	Female	19	16	None	8	0.2	102	80	8.6	7.3	113	125	MRD^-	–	–
9	75	Male	17	17	None	4.1	2.5	137	67	14.9	13.3	121	176	MRD^-	–	–
10	59	Female	16	15	I	1.8	0.8	123	80	5.9	2.7	142	246	MRD^+	2-3	0.30
11	71	Male	18	16	I	1.7	0.6	144	105	5.5	3.9	173	158	MRD^+	3-5	0.60
12	80	Male	20	17	None	1.4	0.6	150	68	3.6	2.3	149	136	MRD^+	3-5	3
13	61	Male	18	16	None	11.7	1.6	111	81	6.5	5.3	136	230	MRD^+	1	0.50
14	86	Male	12	11	None	2.9	1.1	138	90	6	3.9	142	281	MRD^+	3	0.50
15	81	Male	20	17	S	25.4	2	103	141	7.9	4.1	165	224	MRD^+	3-5	0.90
16	79	Male	16	16	None	1.7	0.3	108	51	7.4	6	131	240	MRD^+	2-3	+
17	57	Male	17	17	None	1.7	0.2	122	53	4.3	2.5	165	125	GM^+	20	0.50
18	67	Male	28	21	S	7.1	1	103	162	8.9	5.3	149	321	GM^+	35	–
19	67	Male	18	16	I, O	1.9	0.7	130	120	3.4	1.9	142	183	GM^+	50	3.00
Median	75		18	16		2.1	0.7	116	87	6	3.9	142	230			

CBC indicates complete blood count; WBC, white blood cell count; ANC, absolute neutrophil count; S, splenectomy; I, interferon; O, other; IHC, anti-CD20 antibody; FC, flow cytometry; ND, not done; GM^+, hairy cell morphology positive by hematoxylin and eosin stain; and –, negative.

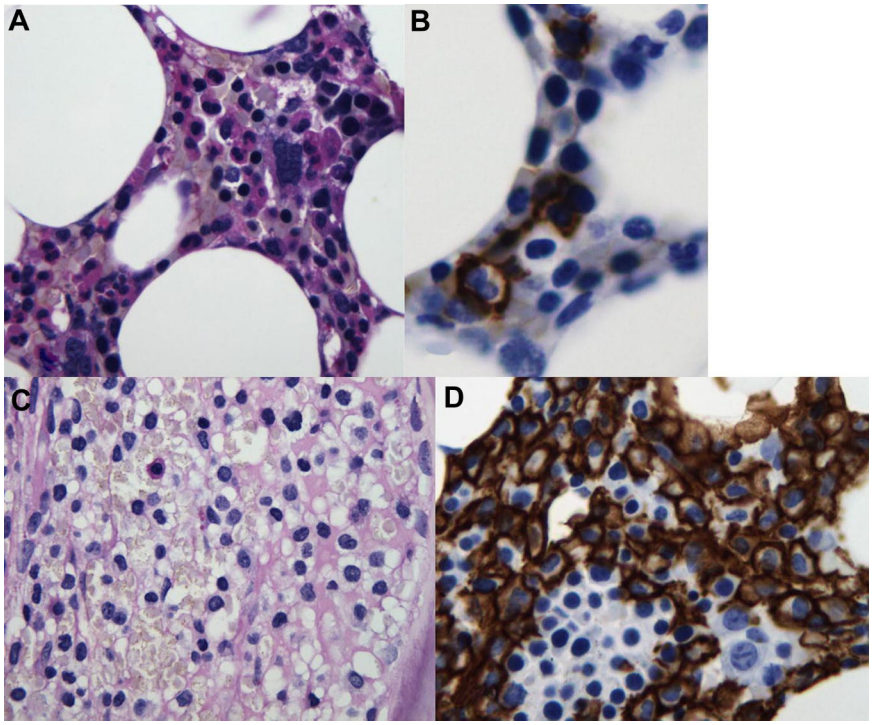

Figure 1. Examples of residual HCL in bone marrow specimens. (A) Bone marrow biopsy from patient 10 with MRD showing normocellular hematopoiesis with no morphologic evidence of an abnormal lymphoid infiltrate (periodic acid–Schiff, original magnification ×400). (B) CD20 immunostain of the bone marrow biopsy from patient 10 with MRD showing scattered abnormal B cells. The illustrated cell has a bilobed nucleus, abundant cytoplasm, and bright coarse CD20 positivity, all characteristic features of HCL (CD20 immunostain/hematoxylin, original magnification ×1000). (C) Bone marrow biopsy from patient 19 with gross morphologic disease showed multiple areas of lymphoid infiltrate. The infiltrate was loosely cellular with monotonous small lymphocytes with nuclear features and abundant cytoplasmic domains imparting a "fried egg" appearance characteristic of HCL (periodic acid–Schiff, original magnification ×400). (D) CD20 immunostain of the bone marrow biopsy from patient 19 with gross morphologic disease shows extensive involvement by a B-cell infiltrate with features of HCL. Both patients had their diagnosis confirmed by flow cytometry that showed a CD11c, CD25, and CD103$^+$ population of monoclonal B cells.

Results and discussion

Nineteen patients had evaluable bone marrow tissue specimens: 17 with corresponding flow cytometry and 2 with IGH-PCR. Sixteen patients were male and 3 were female. The median age was 75 years (range, 56-86 years). The median age at diagnosis was 54 years (range, 39-74 years). The median interval from diagnosis was 18 years (range, 12-28 years). The median time elapsed from receiving one 7-day course of cladribine was 16 years (range, 11-21 years). All patients had normal peripheral complete blood counts and were without any other clinical manifestations of HCL, as required for study enrollment (Table 1).

The small number of patients in this study speaks to the difficulty in conducting very long-term follow-up studies in rare diseases. In addition, we purposely limited patient selection to patients most probably cured, including only those in continuous and complete hematologic remissions. Our use of immunohistochemical stains for the determination of MRD was consistent with prior studies.[11,12] Flow cytometry and IGH-PCR added increased stringency in identifying patients without any residual disease, with flow cytometry being able to detect hairy cells constituting less than 1% of lymphocytes in a specimen.[14,15]

Of 19 patient samples, 9 (47%) had no evidence of residual disease. Seven of these patients had a negative flow cytometric study and 2 patients had no evidence of residual hairy cells by IGH-PCR (Table 1). Although not as sensitive as clone-specific PCR, the negative assays of 2 tests that are still highly sensitive coupled with very extended follow-up (median, 16 years) of these patients raises the possibility that a single course of cladribine can potentially cure HCL.[16] However, declaring cures in low-grade lymphoproliferative disorders must always be done cautiously, as very late relapses are known to occur.[17]

Residual disease was identified in 10 patients (53%), 7 (37%) with MRD and 3 (16%) with morphologic disease. Median follow-up was 16 and 17 years for patients with MRD and morphologic disease, respectively, a duration equivalent to patients without residual disease. A clear difference in clonal B-cell bone marrow involvement existed between patients with MRD and those with morphologic disease. All patients with MRD had less than a 5% clonal B-cell population, whereas the 3 patients with morphologic disease had clonal B-cell populations of 20%, 35%, and 50%. It is important to emphasize that patients with MRD and morphologic disease were in a continuous and complete hematologic remission and had peripheral blood counts indistinguishable from patients without any residual disease (Table 1; Figure 1).

Two key conclusions can now be made about the long-term activity of cladribine in HCL. First, HCL is a potentially curable disease after a single 7-day course of cladribine. Second, patients with MRD or even gross morphologic disease can live many years without manifesting a hematologic relapse. This is not to conclude that MRD is irrelevant in the natural history of HCL. Our study only included patients who were in a continuous and complete hematologic remission. However, other studies have reported a statistically significant association between MRD and HCL relapse.[12,18] This has led some to combine cladribine and rituximab as first-line therapies for HCL, eliminating MRD in more than 90% of patients.[19] Long-term follow-up of these strategies is necessary to determine whether eliminating MRD affects relapse rates. Our results demonstrate that some patients can experience a very protracted interval of hematologic CRs with variable degrees of residual disease.

Authorship

Contribution: D.S.S. and A.S. conceived of research, analyzed and interpreted data, and wrote the manuscript; D.S.S. and C.B. collected data; R.S. prepared hematopathology sections; and R.S. and C.B. reviewed the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Alan Saven, Division of Hematology/Oncology, Scripps Clinic Medical Group, 10666 N. Torrey Pines Rd, MS217, La Jolla, CA 92037; e-mail: saven.alan@scrippshealth.org.

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