

# Minimal Residual Disease in Patients with Hairy Cell Leukemia in Complete Remission Treated with 2-Chlorodeoxyadenosine or 2'-Deoxycoformycin and Prediction of Early Relapse<sup>1</sup>

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## ABSTRACT

The purine nucleoside analogues 2-chlorodeoxyadenosine (2-CdA) and 2'-deoxycoformycin (2'-DCF) induce complete remission (CR) in the majority of patients with hairy cell leukemia. However, minimal residual disease (MRD) has been detected in bone marrow core biopsies using immunohistochemical techniques in patients achieving CR by conventional criteria. This study was designed to compare the prevalence of MRD with each agent in patients in CR by using conventional criteria and the relapse-free survival for patients with and without MRD.

Bone marrow biopsies from 39 patients treated with a single cycle of 2-CdA and 27 patients treated with multiple cycles of 2'-DCF were studied. The monoclonal antibodies anti-CD20, DBA.44, and anti-CD45RO were used to evaluate the paraffin-embedded bone marrow core biopsies for MRD.

Five of 39 patients (13%) treated with 2-CdA had MRD, as compared to 7 of 27 patients (26%) treated with 2'-DCF (two-tailed  $P = 0.21$ ). Relapse has occurred in two of the five patients with MRD after 2-CdA treatment and in four of the seven patients with MRD after 2'-DCF treatment. In total, 6 of the 12 patients (50%) with MRD have relapsed, whereas 3 of 54 patients (6%) without MRD have relapsed, and 2 patients have died without evidence of relapse. The estimated 4-year relapse-free survival among patients with MRD is 55% ( $\pm 15\%$ , SE), compared to 88% ( $\pm 5\%$ , SE) among patients without MRD (two-tailed  $P = 0.0023$ ).

The prevalence of MRD detected in a subset of patients in CR after either 2-CdA or 2'-DCF treatment did not differ significantly. However, the presence of MRD is associated with an increased risk of relapse.

## INTRODUCTION

The purine nucleoside analogues 2-CdA<sup>3</sup> and 2'-DCF induce CR in the majority of patients with HCL (1-8). A prospective, randomized trial comparing the efficacy of these two agents has not been conducted, nor is one likely to be carried out because of the extremely large sample size that would be required. However, it is clear that some patients treated with either agent will relapse and require subsequent treatment. Seymour *et al.* (9) have reported that 20% of patients treated with 2-CdA relapsed at a median of 16 months. In a series of patients treated at Northwestern University, 14% of patients treated with 2-CdA have relapsed at a median of 24 months (10). In a large series of patients treated with 2-CdA, Saven *et al.* (11) observed a relapse rate of 26% (90 of 349 patients) at a median of 29 months. The largest cohort of patients who have been treated with 2-CdA was reported by Cheson *et al.* (12). The 4-year DFS among 430 patients achieving CR was 84%. Kraut *et al.* (13) reported that among 23 patients treated with 2'-DCF, 48% relapsed at a median of 30 months. Golomb *et al.* (14) observed a much lower relapse rate of 14% among 78 patients treated with 2'-DCF. Catovsky *et al.* (15) reported that among 148 patients, 74% achieved CR, and 12 patients relapsed. An even lower relapse rate of 8.5% (11 of 117 patients) for 2'-DCF-treated patients was found by Grever *et al.* (16). Long-term follow-up of these patients shows that the 8-year RFS is 76% (17). Therefore, whereas high CR rates with both agents are now well established, a significant number of patients will relapse. If patients

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<sup>3</sup> The abbreviations used are: 2-CdA, 2-chlorodeoxyadenosine; 2'-DCF, 2'-deoxycoformycin; CR, complete remission; HCL, hairy cell leukemia; MRD, minimal residual disease; RFS, relapse-free survival; DFS, disease-free survival.

Table 1 Patient characteristics

	Patients treated with 2-CdA	Patients treated with 2'-DCF
Gender		
Male	35 (90%)	23 (85%)
Female	4 (10%)	4 (15%)
Age (yrs) Mean (range)	48 (34–85)	58 (35–74)
No. of purine analogue cycles		
Mean (range)	1	13 (5–25)
Disease duration before therapy (mo) Mean (range)	12 (1–199)	3.6 (0.033–39.7)
Prior therapy		
None	18	25
Splenectomy	5	0
IFN	11	2
Splenectomy, IFN	3	0
Splenectomy, IFN, 2'-DCF	2	0
% bone marrow HCs <sup>a</sup> pretherapy Mean (range)	69 (10–95)	77 (5.98)
WBC ( $\times 10^9$ /liter) Mean (range)	3.4 (0.6–22.2)	2.9 (0.2–8.3)
Platelets ( $\times 10^9$ /liter) Mean (range)	76 (11–466)	92 (29–302)
ANC ( $\times 10^9$ /liter) <sup>b</sup> Mean (range)	1.05 (0.092–4.712)	0.8 (0–2.1)

<sup>a</sup> HC, hairy cell.

<sup>b</sup> ANC, absolute neutrophil count.

at risk of relapse can be identified, they might be candidates for further treatment.

Recently, immunohistochemical techniques have been used to study bone marrow core biopsies from patients who were in CR after 2-CdA (18–20) treatment and 2'-DCF (20–23) treatment. A subset of these patients had MRD detected by immunohistochemistry that could not be identified by routine examination of H&E-stained bone marrow core biopsies and corresponding aspirates. Furthermore, an early study suggests that the presence of MRD detected by immunohistochemistry in the bone marrow core biopsy 3 months after a single cycle of 2-CdA may predict relapse (24).

In the present study, immunohistochemistry was used to evaluate paraffin-embedded bone marrow core biopsies from patients who met conventional morphological criteria for CR after therapy with either 2-CdA or 2'-DCF. The prevalence of MRD after achieving CR was compared between the two treatment groups. RFS was compared between patients with and without MRD.

## PATIENTS AND METHODS

**Patients Treated with 2-CdA.** Thirty-nine patients with previously treated or untreated HCL achieved CR after a single cycle of 2-CdA at a dose of 0.1 mg/kg/day for 7 days by continuous i.v. infusion between February 1991 and January 1995 at the Robert H. Lurie Cancer Center of Northwestern University (Table 1). All patients were required to have active disease as defined by one or more of the following parameters: (a) hemoglobin level  $\leq 12$  g/dl, absolute granulocyte count  $\leq 1,500/\mu\text{l}$ , or platelet count  $\leq 100,000/\mu\text{l}$ ; (b) recurrent infections requiring antibiotics; and (c) symptomatic splenomegaly. Patients were required to have normal organ function (including total bilirubin  $\leq 2.0$  mg/dl and creatinine  $\leq 2.0$  mg/dl). The median age was 48 years (range, 34–85 years). Eighteen patients were previously untreated. Of the 21 previously treated patients, 5 were treated with splenectomy, 11 were treated only with IFN, 3 were treated with splenectomy followed by IFN,

and 2 were treated with splenectomy, IFN, and then 2'-DCF. Complete blood counts were obtained every 6 months after the first 3-month evaluation; bone marrow aspirates and biopsies were done 3 months after treatment and then done annually. The interval between the start of treatment and the procurement of the bone marrow used to determine MRD status was 3–4 months in both the MRD-positive and the MRD-negative groups.

**Patients Treated with 2'-DCF.** The 2'-DCF group consisted of 27 patients with HCL treated with multiple courses of 2'-DCF (4 mg/m<sup>2</sup> every 2 weeks) as part of Intergroup Study 0073 (INT-0073), a prospective, multi-institutional, randomized trial comparing 2'-DCF to IFN- $\alpha$  as an initial induction therapy for HCL in unsplenectomized patients (16). INT-0073 was open to patients with active HCL as defined above, with minor modifications (hemoglobin level  $< 12$  g/dl, absolute granulocyte count  $< 1,500/\mu\text{l}$ , or, in addition, circulating hairy cell count  $> 20,000/\mu\text{l}$ ). The 27 patients in the present study were selected solely on the basis of the availability for this study of tissue blocks from remission bone marrow core biopsies. These patients, who were from 21 institutions, began treatment with 2'-DCF between September 1987 and January 1989 and received between 5 and 25 injections of 2'-DCF over periods ranging from 6–13 months. Twenty-five patients were randomized to 2'-DCF as their initial induction therapy, and the other two patients were crossed over to 2'-DCF after failing to respond to IFN- $\alpha$ . The median age of the patients was 58 years (range, 35–74 years; see Table 1). All 27 patients achieved CR. The study protocol called for disease status, including complete blood counts (CBCs), to be assessed at monthly intervals until the second anniversary of the start of 2'-DCF treatment and then assessed quarterly for 3 years and assessed annually thereafter. Bone marrow examinations were to be scheduled at 6-month intervals during and after completion of the treatment. The median number of days between successive bone marrow examinations (including that from the examination used for this study and the first follow-up examination) for the MRD-nega-

**Table 2** Number of 2'-DCF doses received on or after the bone marrow biopsy CR date for all 27 2'-DCF-treated patients

No. of injections	MRD-negative		MRD-positive	
	No.	%	No.	%
0	5	25	2	29
1	0	0	1	14
2	11	55	3	43
3	2	10	0	0
4	1	5	0	0
5	0	0	1	14
12	1	5	0	0
Total	20	100	7	100

tive patients was 184 days (range, 8–917 days), compared to 195 days (range, 30–465 days) for the MRD-positive patients.

The 2'-DCF treatment regimen called for two additional injections after patients met marrow, peripheral blood, and clinical criteria for CR. For some patients, the first remission marrow specimen was not available, and a later specimen was used. Other patients remained on treatment for various lengths of time after the remission marrow biopsy until all CR criteria were met. Consequently, 7 of the 27 patients were off treatment at the time of the remission marrow biopsy used for this study. Three of these patients were off treatment for less than 1 month, and three others were off treatment for between 3 and 7 months. The seventh patient was off treatment for 18 months. This patient received 2'-DCF for the maximum duration of 1 year. Upon completing treatment and at 6 and 12 months thereafter, his marrow studies indicated residual hairy cell leukemic infiltrates. At 18 months, he had his first remission marrow, which served as the specimen analyzed for MRD in this study. Marrow remission was confirmed at 24 months. Among the 20 patients who were still on treatment at the time of the remission marrow biopsy, 19 received between one and five doses (median, two doses) of 2'-DCF on or after the day of the marrow biopsy used for this study, over periods of up to 2 months (median, 17 days). The last patient received 12 doses over a period of 6 months after the biopsy used in this study because he was thought to be in partial remission. The number of 2'-DCF doses received on or after the bone marrow biopsy CR date for all 27 of the 2'-DCF-treated patients is shown in Table 2.

**Immunohistochemistry.** Bone marrow core biopsies obtained at Northwestern University from patients treated with 2-CdA were fixed in B5 for 90 min, decalcified in RDO (Apex Engineering Products Corp., Plainfield, IL), dehydrated in graded alcohols and xylene, and embedded in paraffin. Tissue blocks of paraffin-embedded bone marrow core biopsies or precut tissue sections from all other patients treated with 2-CdA were sent to Northwestern University for immunohistochemical processing and evaluation. Blocks from patients treated with 2'-DCF from Southwest Oncology Group institutions were sent to the University of New Mexico for immunohistochemical processing and evaluation. Immunohistochemical studies using monoclonal antibodies anti-CD20 (lineage-specific pan-B-cell marker), anti-CD45RO (pan-T-cell marker) and anti-DBA.44 (marks subset of B-cells, hairy cells, and some B-cell lymphomas) were performed at Northwestern University and the Uni-

**Table 3** Prevalence of MRD, frequency of relapse, and RFS

	MRD	No MRD
Prevalence <sup>a</sup>		
2-CdA	5/39 (13%)	34/39 (87%)
2'-DCF	7/27 (26%)	20/27 (74%)
Frequency of relapse		
2-CdA	2/5 (40%)	3/34 (9%)
2'-DCF	4/7 (57%)	0/20
Overall	6/12 (50%)	3/54 (6%)
RFS at 4 yrs <sup>b</sup>	55% (±15%, SE)	88% (±6%, SE)

<sup>a</sup>  $P = 0.21$  for comparison of MRD prevalence between 2-CdA and 2'-DCF.

<sup>b</sup>  $P = 0.0025$  for comparison of RFS for patients with or without MRD.

versity of New Mexico by the method described previously (18). Microscopic analysis of the bone marrow core biopsy slides from all patients were reviewed by two of the authors (S. W. and L. C. P.), and the slides from patients treated with 2'-DCF were also reviewed by a third author (K. F.). The entire core biopsy in each case was evaluated for the number, distribution, and morphological features of cells positive for CD20, DBA.44, and CD45RO. The morphological features that were identified as being consistent with hairy cells included moderate to abundant cytoplasm, distinct cytoplasmic projections that were best visualized when the cells were separate from one another, round to oval nuclei with occasional nuclear indentations, and indistinct nucleoli.

**Definition of CR.** The criteria for CR were similar in both treatment cohorts and included: (a) absolute granulocyte count  $\leq 1,500/\mu\text{l}$ , hemoglobin  $\leq 12$  g/dl, and platelet count  $\leq 100,000/\mu\text{l}$ ; (b) absence of adenopathy or hepatosplenomegaly; and (c) complete absence of hairy cells in the peripheral blood and bone marrow.

**Definition of MRD.** The criteria for MRD used in this study have been reported previously (15). They require: (a) the absence of HCL by routine morphology of peripheral blood, aspirate, and H&E-stained bone marrow core sections; (b) the presence of CD20- or DBA.44-positive cells in numbers that are equal to or greater than the number of CD45RO-positive cells; and (c) the presence of  $>50\%$  of the CD20- or DBA.44-positive cells exhibiting morphological features consistent with hairy cells. The latter value was determined by performing a differential count on 200 L26- or DBA.44-positive cells and calculating the percentage of B cells with the morphology of hairy cells. Using these criteria, there was agreement between the two reviewers regarding the presence or absence of MRD in the bone marrow biopsies from all of the patients treated with 2-CdA. The three reviewers agreed on the results of 25 of the 27 biopsies from patients treated with 2'-DCF; consensus was reached for the other 2 patients after a re-review of the slides by the three investigators.

**Definition of Relapse.** Relapse in both treatment cohorts was diagnosed when HCL was present on routine evaluation of H&E-stained bone marrow core biopsies, with or without identifiable hairy cells on peripheral blood and bone marrow aspirate. The biopsies were reviewed without knowledge of the results of the prior biopsies. All relapses were diagnosed without

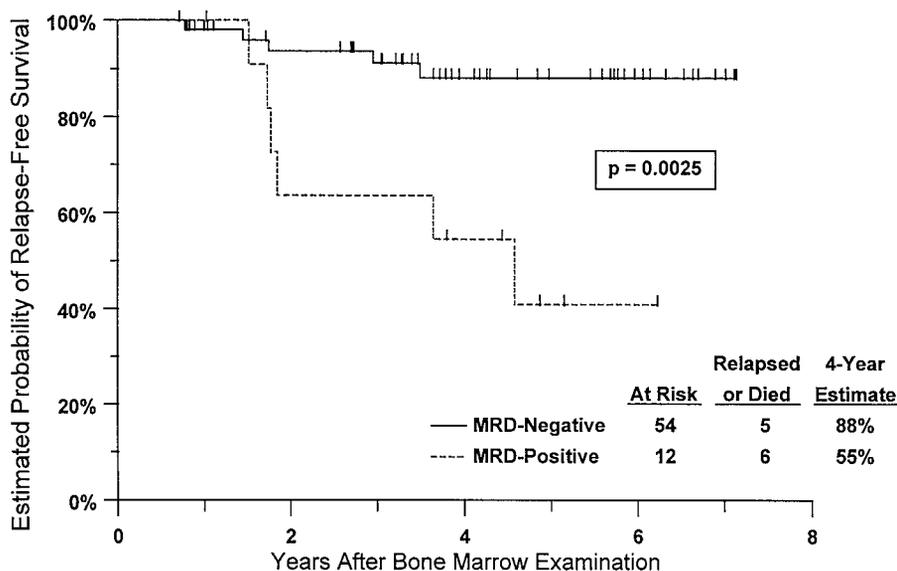


Fig. 1 Kaplan-Meier estimates of RFS by MRD status for 2'-DCF- and 2-CdA-treated patients combined. Tick marks indicate patients last known to be alive without report of relapse.

knowledge of whether or not the patients had MRD previously. The diagnosis of relapse was based on routinely stained bone marrow aspirates and core biopsy sections without immunostaining.

**Statistical Analysis.** Prevalence of MRD was compared between 2-CdA- and 2'-DCF-treated patients using Fisher's exact test (25). RFS was defined as the number of days from the collection of the bone marrow specimen examined for MRD until relapse or death from any cause; observation was censored for patients last known to be alive without report of relapse. Distributions of RFS for patients with and without MRD were estimated using the method of Kaplan and Meier (26) and Greenwood's formula (27) and compared using the log-rank tests stratified by treatment group (28). Statistical significance of all comparisons was expressed by two-tailed *P*s calculated directly using the commercially available StatXact Turbo program to avoid large sample approximations (29). Predictive powers and corresponding sensitivity and specificity were not estimated because these measures are best defined for dichotomous outcomes rather than time-to-event outcomes, because the number of relapses is likely to change with additional follow-up. The closest analogue to predictive power is the RFS at a given time.

## RESULTS

**Prevalence of MRD in Remission Marrows.** Five of the 39 patients (13%) treated with 2-CdA had MRD detected in the CR marrow examined for this study (Table 3). This compares to 7 of 27 patients (26%) treated with 2'-DCF. This difference is not statistically significant ( $P = 0.21$ ).

**Frequency of Relapse among Patients with MRD.** Two of the five patients (40%) with MRD after 2-CdA treatment have relapsed at 24 and 25 months after CR, and four of the seven patients (57%) with MRD after 2'-DCF treatment have relapsed at 18, 21, 44, and 59 months after CR. In total, 6 of the 12 patients (50%) with MRD have relapsed. In contrast, 3 of the 54 patients (6%) without MRD have relapsed, and 2 others have

died without evidence of relapse (Table 3). One of the five relapses among the 2'-DCF-treated patients was established from examination of the peripheral blood because the patient refused a bone marrow examination. The two nonrelapse deaths occurred in a patient treated with 2'-DCF who died of melanoma and in an 89-year-old patient treated with 2-CdA who died of a ruptured aortic aneurysm.

**Detection of Relapse.** All relapsed patients had clinically meaningful decreases in peripheral blood counts requiring therapy within 8 months of the detection of relapse. The percentages of hairy cells in the bone marrows of the patients in the MRD-positive group at relapse ranged from 40–90%, compared to 12.5–75% in the MRD-negative group.

**RFS of Patients Treated with 2-CdA and 2'-DCF Combined.** RFS differs significantly between patients with and without MRD ( $P = 0.0023$ ; Fig. 1). The estimated RFS at 4 years after the remission marrow examined for this study is 55% ( $\pm 15\%$ , SE) among patients with MRD. The corresponding estimate for patients without MRD is 88% ( $\pm 5\%$ , SE).

## DISCUSSION

This study demonstrates several findings that may be important in the treatment of patients with HCL with purine analogues: (a) a subgroup of patients treated with either 2-CdA or 2'-DCF who achieve CR by conventional criteria have MRD that can be detected by immunohistochemical techniques; (b) there was no statistically significant difference in the prevalence of MRD between patients treated with one purine analogue and patients treated with the other; and (c) the presence of MRD after successful purine analogue therapy is associated with a significantly shorter RFS.

MRD has been identified in the bone marrow of patients with HCL in CR by conventional criteria, using a variety of techniques (18–23). Thaler *et al.* (21) have identified residual disease by immunostains in four patients treated with 2'-DCF and achieving CR routine morphology. Using the PCR tech-

nique and clonogenic probes derived from the hypervariable region of the immunoglobulin heavy chain genes, Filleul *et al.* (30) detected MRD in each of seven patients in apparent CR after 2-CdA treatment.

Immunohistochemical techniques have been used to detect small numbers of occult residual hairy cells after successful therapy with 2-CdA (18–20). However, the prevalence of MRD detected in several series has varied because the criteria to define MRD have not been uniform. The criteria in this study required not only the presence of CD20- or DBA.44 (B-cell)-positive cells in equal or greater numbers than CD45RO (T-cell)-positive cells but also required the presence of >50% of the CD20- or DBA.44-positive cells exhibiting morphological features of hairy cells (24). These criteria are deliberately conservative to avoid overestimating the number of neoplastic cells, given that CD20 and DBA.44 are not specific for hairy cells and positive cells, including rare cells with morphology resembling hairy cells, were present in control biopsies (24, 31). These criteria appear to detect a threshold of MRD that is clinically meaningful because the presence of MRD in such patients appears to predict relapse (24). In the study reported here, there was no statistically significant difference in the percentage of patients with MRD detected after 2-CdA or 2'-DCF treatment; however, the number of patients was too small to detect even large differences. One difference between the patients treated with 2-CdA and those given 2'-DCF is that most of the latter received additional cycles of therapy after the marrow examination that was used for this study. The impact of this additional therapy on RFS cannot be evaluated in this study due to the limited number of patients. However, approximately one-fourth of both MRD-negative and MRD-positive patients received no 2'-DCF on or after the CR date used for this study. Similarly, approximately 55% of patients in both groups received only one or two doses on or after the CR date. Therefore, among the 2'-DCF-treated patients, the MRD-negative and MRD-positive patients are similar with respect to the amount of treatment received after achieving CR. The impact of additional treatment with 2-CdA on outcome once CR is achieved is not known. In contrast to results reported here, Matutes *et al.* (23) found no statistical difference in DFS among patients with or without MRD, as determined by a panel of antibodies including CD11c, CD25, CD103, and HC2. However, there was a trend toward a higher probability of relapse among MRD-positive patients.

It is now apparent that some patients treated with 2-CdA or 2'-DCF will relapse and require subsequent treatment. Seymour *et al.* (9) reported that 8 of 40 patients (20%) with HCL treated with 2-CdA have relapsed at a median of 16 months (range, 3–30 months), and the actuarial DFS rate is 77% at 3 years after treatment. In our series of 50 patients treated with 2-CdA, 7 of 50 patients (14%) have relapsed at a median of 24 months (range, 6–45 months), and the progression-free survival rate at 4 years is 72% (10). Similarly, Kraut *et al.* (13) reported 11 of 23 patients (48%) treated with 2'-DCF relapsed at a median of 30 months (range, 7–80 months). Golomb *et al.* (14) reported that among 78 patients with good performance status (performance status 0–2) who failed initial therapy with IFN and were treated with 2'-DCF, 12 have relapsed, and the remission duration rate at 36 months is 84% (median follow-up, 44 months). Grever *et al.*

(16) reported that 10 of 117 patients (8.5%) who achieved CR with 2'-DCF relapsed between 13 and 44 months. The estimated RFS at 4 years in that study was 88% ( $\pm$  3% SE).<sup>4</sup> It is quite clear that a rational therapeutic strategy will need to be developed for patients who relapse after purine analogue therapy.

Whether postremission therapy is beneficial for patients who achieve CR by conventional criteria with a purine analogue but have MRD is unknown. Patients with MRD after 2-CdA treatment may benefit from a second cycle; however, the potential additive or synergistic myelosuppressive and immunosuppressive effects of two cycles in this population is not known. Indeed, patients treated with a single cycle of 2-CdA may sustain prolonged suppression of CD4-positive cells (9). Although an increased rate of opportunistic infections has generally not been reported in patients with HCL after successful therapy with 2-CdA, second malignancies, generally solid tumors, have been reported (4, 9–12). However, opportunistic infections have been observed in patients given multiple cycles of 2-CdA for chronic lymphocytic leukemia and lymphoma (32). Among 53 patients given a second cycle of 2-CdA at first relapse after a first cycle, 62% achieved a second CR (11).

To avoid prolonged immunosuppression that may be induced with a second cycle of 2-CdA or 2'-DCF, Seymour *et al.* (33) have explored the benefits of IFN- $\alpha$  in relapsed patients and found that two of three patients achieved CR, and one patient achieved partial remission. It is possible that an alternative dose or schedule of 2-CdA may be equally effective with fewer long-term consequences. Indeed, treatment of patients at a lower dose (2 mg/m<sup>2</sup>/day) than usually given appears to result in a similar remission rate and less lymphopenia than treatment with the standard dose (3–4 mg/m<sup>2</sup>/day; Ref. 34). Similarly, it is not clear how to treat patients with MRD after 2'-DCF. These patients have already had multiple cycles, both before and after CR. It would seem unlikely that additional cycles will be useful in this setting, and an alternative approach may be needed.

Regardless of which purine analogue is given, it is not clear whether early retreatment at the time of MRD will lead to a more favorable long-term outcome than treatment at relapse. A prospective randomized trial addressing this issue may be an important direction to pursue to improve the cure rate of patients with HCL.

## REFERENCES

- Piro, L. D., Carrera, C. J., Carson, D. A., and Beutler, E. Lasting remissions in hairy cell leukemia induced by a single infusion of 2-chlorodeoxyadenosine. *N. Engl. J. Med.*, 322: 1117–1121, 1990.
- Estey, E. H., Kurzrock, R., Kantarjian, H. M., O'Brien, S. M., McCredie, K. B., Beran, M., Koller, C., Keating, M. J., Hirsch-Ginsberg, C., Huh, Y. O., Stass, S., and Freireich, E. Treatment of hairy cell leukemia with 2-chlorodeoxyadenosine (2-CdA). *Blood*, 79: 882–887, 1992.
- Tallman, M. S., Hakimian, D., Variakojis, D., Koslow, D., Sisney, G. A., Rademaker, W., Rose, E., and Kaul, K. A single cycle of 2-chlorodeoxyadenosine results in complete remission in the majority of patients with hairy cell leukemia. *Blood*, 80: 2203–2209, 1992.

<sup>4</sup> Unpublished results.

4. Hoffman, M. A., Janson, D., Rose, E., and Rai, K. R. Treatment of hairy cell leukemia with cladribine: response, toxicity and long-term follow-up. *J. Clin. Oncol.*, *15*: 1138–1142, 1997.
5. Cassileth, P. A., Chevart, B., Spiers, A. S. D., Harrington, D. P., Cummings, F. J., Neiman, R. S., Bennett, J. M., and O'Connell, M. J. Pentostatin induces durable remissions in hairy cell leukemia. *J. Clin. Oncol.*, *9*: 243–246, 1991.
6. Kraut, E. H., Bouroncle, B. A., and Grever, M. R. Pentostatin in the treatment of advanced hairy cell leukemia. *J. Clin. Oncol.*, *7*: 168–172, 1989.
7. Johnston, J. B., Eisenhauer, E., Corbett, W. E. N., Scott, J. G., and Zaentz, S. D. Efficacy of 2'-deoxycoformycin in hairy-cell leukemia: a study of the National Cancer Institute of Canada Clinical Trials Group. *J. Natl. Cancer Inst.*, *80*: 765–769, 1988.
8. Annino, L., Ferrari, A., Giona, F., Cimino, G., Crescenzi, S., Cava, C. M., Pacchiarotti, A., and Mandelli, F. Deoxycoformycin induces long-lasting remissions in hairy-cell leukemia: clinical and biological results of two different regimens. *Leuk. Lymphoma*, *14*: 115–119, 1994.
9. Seymour, J. F., Kurzrock, R., Freireich, E. J., and Estey, E. H. 2-Chlorodeoxyadenosine induces durable remissions and prolonged suppression of CD4+ lymphocyte counts in patients with hairy cell leukemia. *Blood*, *83*: 2906–2911, 1994.
10. Tallman, M. S., Hakimian, D., Rademaker, A. W., Zanzig, C., Wollins, E., Rose, E., and Peterson, L. A. Relapse of hairy cell leukemia after 2-chlorodeoxyadenosine: long-term follow-up of the Northwestern University experience. *Blood*, *88*: 1954–1959, 1996.
11. Saven, A., Burián, C., Koziol, J. A., and Piro, L. D. Long-term follow-up of patients with hairy-cell leukemia after cladribine treatment. *Blood*, *92*: 1918–1926, 1998.
12. Cheson, B. D., Suresen, J. M., Vena, D. A., Montello, M. J., Barrett, J. A., Damasio, E., Tallman, M., Annino, L., Connors, J., Coiffier, B., and Lauria, F. Treatment hairy cell leukemia with 2-chlorodeoxyadenosine via the Group C Protocol Mechanism of the National Cancer Institute: a report of 979 patients. *J. Clin. Oncol.*, *16*: 3007–3015, 1998.
13. Kraut, E. H., Grever, M. R., and Bouroncle, B. A. Long-term follow-up of patients with hairy cell leukemia after treatment with 2'-deoxycoformycin. *Blood*, *84*: 4061–4063, 1994.
14. Golomb, H. M., Dodge, R., Mick, R., Budman, D., Hutchison, R., Horning, S. J., and Schiffer, C. A. Pentostatin treatment of hairy cell leukemia patients who fail initial therapy with recombinant  $\alpha$ -interferon: a report of CALGB Study 8515. *Leukemia*, *8*: 2037–2040, 1994.
15. Catovsky, D., Matutes, E., Talavera, J. G., O'Connor, N. T. J., Johnson, S. A. N., Emmett, E., Corbett, L., and Swansbury, J. Long-term results with 2'-deoxycoformycin in hairy cell leukemia. *Leuk. Lymphoma*, *14*: 109–113, 1994.
16. Grever, M., Kopecky, K., Foucar, M. K., Head, D., Bennett, J. M., Hutchison, R. E., Corbett, W. E. N., Cassileth, P. A., Habermann, T., Golomb, H., Rai, K., Eisenhauer, E., Appelbaum, F., and Cheson, B. D. Randomized comparison of pentostatin *versus*  $\alpha$ -interferon in previously untreated patients with hairy cell leukemia: an intergroup study. *J. Clin. Oncol.*, *13*: 974–982, 1995.
17. Flinn, I. W., Kopecky, K. J., Foucar, M. K., Head, D., Bennett, J. M., Hutchinson, R. E., Corbett, W. E. N., Cassileth, P. A., Habermann, T., Golomb, H., Rai, K., Eisenhauer, E., Appelbaum, F. R., Cheson, B. D., and Grever, M. R. Long-term results in hairy cell leukemia (HCL) treated with pentostatin. *Blood*, *90*: 2575a, 1997.
18. Hakimian, D., Tallman, M. S., Kiley, C., and Peterson, L. Detection of minimal residual disease by immunostaining of bone marrow biopsies after 2-chlorodeoxyadenosine for hairy cell leukemia. *Blood*, *82*: 1798–1802, 1993.
19. Ellison, D. J., Sharpe, R. W., Robbins, B. A., Spinosa, J. C., Leopard, J. D., Saven, A., and Piro, L. D. Immunomorphologic evaluation of post-therapy bone marrows in patients treated with 2-CdA for HCL. *Blood*, *84*: 4310–4315, 1994.
20. Konwalinka, G., Schirmer, M., Hilbe, W., Fend, F., Geisen, F., Knobloch, A., Petzer, A., and Thaler, J. Minimal residual disease in hairy cell leukemia after treatment with 2-chlorodeoxyadenosine. *Blood Cells Mol. Dis.*, *21*: 142–151, 1995.
21. Thaler, J., Denz, H., Dietze, O., Gastl, G., Ho, A. D., Gattringer, C., Greil, R., Lechleitner, M., Huber, C., and Huber, H. Immunohistologic assessment of bone marrow biopsies from patients with hairy cell leukemia: changes following treatment with  $\alpha$ -2-interferon and deoxycoformycin. *Leuk. Res.*, *13*: 377–383, 1989.
22. Thaler, J., Grunewald, K., Gattringer, C., Ho, A. D., Weyrer, K., Dietz, O., Stauder, R., Fluckinger, T., Lang, A., and Huber, H. Long-term follow-up of patients with hairy cell leukemia treated with pentostatin: lymphocyte subpopulations and residual bone marrow infiltration. *Br. J. Haematol.*, *84*: 75–82, 1993.
23. Matutes, E., Meeus, P., McLennan, K., and Catovsky, D. The significance of minimal residual disease in hairy cell leukemia treated with deoxycoformycin: a long-term follow-up study. *Br. J. Haematol.*, *98*: 375–383, 1997.
24. Wheaton, S., Tallman, M. S., Hakimian, D., and Peterson, L. A. Minimal residual disease may predict bone marrow relapse in patients with hairy cell leukemia treated with 2-chlorodeoxyadenosine. *Blood*, *87*: 1556–1560, 1996.
25. Cox, D. R. *Analysis of Binary Data*. London: Chapman and Hall, 1970.
26. Kaplan, E. L., and Meier, P. Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.*, *53*: 457–481, 1958.
27. Kalbfleisch, J. D., and Prentice, R. L. *The Statistical Analysis of Failure Time Data*. New York: John Wiley and Sons, 1980.
28. Mantel, N. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother. Rep. Part 1*, *50*: 163–170, 1966.
29. Cytel Software Corporation. *StatXact Turbo User Manual*. Cambridge, MA: Cytel Software Corp., 1992.
30. Filleul, B., Delannoy, A., Ferrant, A., Zenebergh, A., Van Daele, S., Bosly, A., Doyen, C., Mineur, P., Glorieux, P., Driesschaert, P., Sokal, G., Martiat, P., and Michaux, J. L. A single course of 2'-chlorodeoxyadenosine does not eradicate leukemic cells in hairy cell leukemia patients in complete remission. *Leukemia (Baltimore)*, *8*: 1153–1156, 1994.
31. O'Donnell, L. R., Alder, S. L., Balis, U. J., Perkins, S. L., and Kjeldsberg, C. R. Immunohistochemical reference ranges for B lymphocytes in bone marrow biopsy paraffin sections. *Am. J. Clin. Pathol.*, *104*: 517–523, 1995.
32. Betticher, D. C., Fey, M. F., von Rohr, A., Tobler, A., Jenzer, H., Gratwohl, A., Lohri, A., Pugin, P., Hess, U., and Pagani, O. High incidence of infections after 2-chlorodeoxyadenosine (2-CdA) therapy in patients with malignant lymphomas and chronic and acute leukemias. *Ann. Oncol.*, *5*: 57–64, 1994.
33. Seymour, J. F., Estey, E. H., Keating, M. J., and Kurzrock, R. Response to interferon- $\alpha$  in patients with hairy cell leukemia relapsing after treatment with 2-chlorodeoxyadenosine. *Leukemia (Baltimore)*, *9*: 929–932, 1995.
34. Juliusson, G., Lenkei, R., Tjonnfjord, G., Heldal, D., and Liliemark, J. Low-dose cladribine for symptomatic hairy cell leukaemia. *Br. J. Haematol.*, *89*: 637–639, 1995.

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