2-Chlorodeoxyadenosine Induces Durable Remissions and Prolonged Suppression of CD4+ Lymphocyte Counts in Patients With Hairy Cell Leukemia

By John F. Seymour, Razelle Kurzrock, Emil J. Freireich, and Elihu H. Estey

A number of effective treatments are available for patients with hairy cell leukemia (HCL). 2-Chlorodeoxyadenosine (2-CdA) induces more than 80% complete responses, but is associated with profound suppression of CD4+ lymphocyte counts. However, the duration of each is uncertain. We have analyzed a previously reported cohort of 40 patients who had responded to 2-CdA. Eight patients (20%) have relapsed at a median of 16 months (range, 3 to 23 months). The remaining 32 patients were observed for a median of 30 months (range, 7 to 43 months). No patients have died. At 3 years, the actuarial disease-free survival rate is 77% (95% confidence interval, 70% to 84%). The median CD4+ lymphocyte count before therapy was 743/µL (range, 58 to 2,201/µL). The median CD4+ nadir after treatment was 139/µL (range, 25 to 580/µL). There was a single opportunistic infection and no second malignancies observed. Although there was evidence of some improvement in CD4+ lymphocyte counts on sequential testing, CD4+ counts remained significantly lower than baseline (P < .0001) at a median of 23 months after therapy (median, 237/µL; range, 25 to 514/µL), and were also lower than baseline (P < .002) in those patients with more than 1 year of follow-up (median, 27 months; range, 13 to 42 months). The median time to reach an absolute CD4+ lymphocyte count of 365/µL, the lower limit of the normal range, was 40 months. Although responses to 2-CdA are durable in the majority of patients with HCL, the uncertain long-term consequences of the observed CD4+ lymphocytopenia suggest caution in the broad application of this therapy.

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For the 90% of patients with hairy cell leukemia (HCL) who require treatment, a range of effective therapies are available. Although there are some disadvantages associated with both splenectomy and recombinant interferon-α (IFN-α), both have major clinical benefit and have been used for a sufficient period of time that the duration of responses and potential long-term complications are recognized.1

More recently there has been much enthusiasm for the use of two structurally related agents, deoxycoformycin (DCF) and 2-chlorodeoxyadenosine (2-CdA). Both of these agents produce higher rates of complete remission than IFN-α. 2-CdA is particularly attractive because of an overall greater than 80% complete remission rate with a single 7-day course of therapy, and the relative lack of short-term toxicity reported from major centers to date.2,3 Despite these definite advantages, the durability of response is still uncertain. The persistence of immunohistochemically detectable minimal residual disease suggests that these patients remain at risk of disease recurrence.6

Also, like other nucleoside analogues in clinical use, 2-CdA is potently toxic to normal lymphocytes. Most patients with HCL have normal T-cell numbers at diagnosis.7,10 Both CD4+ and CD8+ lymphocyte subset numbers are significantly lowered after 2-CdA therapy.2,20 Similar degrees of CD4+ lymphocytopenia are associated with a greatly enhanced risk of opportunistic infections15,13 and second malignancies, particularly non-Hodgkin’s lymphomas, in patients with the acquired immunodeficiency syndrome.14 Whether such associations also exist in patients with nucleoside-induced CD4+ lymphocytopenia is unknown.

The aims of this study were to update the response duration in a previously reported cohort of patients with HCL who had responded to treatment with 2-CdA at M.D. Anderson Cancer Center,2 and to assess the severity, duration, and clinical sequelae of drug-induced lymphocytopenia.

Patients and Methods

As previously reported,2,46 patients received 2-CdA (4 mg/m²) by continuous intravenous infusion daily for 7 days. Responses were defined as follows: (1) complete remission (CR) is the absence of hairy cells in the bone marrow, or the presence of less than 1% atypical cells that, however, could not be definitely called hairy cells, together with the disappearance of all evidence of HCL on physical exam, and a platelet count of greater than 100,000/µL, a neutrophil count greater than 1,500/µL, and a hemoglobin of ≥12 g/dL; (2) for partial remission (PR), the marrow contained ≥1% to 84% hairy cells in the bone marrow, or the presence of fewer than 1% atypical cells that, however, could not be definitely called hairy cells, together with the disappearance of all evidence of HCL on physical exam, and a platelet count of greater than 100,000/µL, a neutrophil count greater than 1,500/µL, and a hemoglobin of ≥12 g/dL; (3) for minor response (MR), the marrow and physical findings were as described for CR or PR, and there was correction of greater than one cytopenia, as described above without worsening of any other blood count; (4) failure was defined as other responses. Forty-two patients responded to therapy (36 CR, 5 PR, 1 MR). Of these, 2 were lost to follow-up. The remaining 40 patients are the subject of this report. Sixteen of these patients had received some form of therapy before 2-CdA. One had received fludarabine and DCF. Both of these agents are potentially lymphocytotoxic. This patient had an absolute CD4+ count of 243/µL at commencement of 2-CdA therapy, and is excluded from analyses of lymphocyte counts, but is analyzed for response duration. Seven patients had undergone prior splenectomy, and 14 had undergone previous IFN therapy (including 6 patients who had also undergone splenectomy).

Patients in this study were referred from many areas within the United States and overseas, and follow-up intervals varied. All patients had a bone marrow examination at 2 to 3 months after 2-CdA to document response status. Overall, a bone marrow examination was performed at a median of 7 months of patient follow-up (range, 4 to 32 months).

Relapse was defined as the reappearance, or progression to greater...
follow-up of 2CdA-treated HCL patients

than 5% for those patients with an initial partial response, of marrow hairy cells, a reduction in peripheral blood counts less than those required for response, or the recurrence of clinical evidence of HCL. Duration of remission, was calculated from the date response was first documented until the first documentation of relapse, or the date of most recent follow-up, regardless of whether a bone marrow examination was performed on that date. At each follow-up clinic visit a thorough history was taken and a physical examination was performed. There were no specific screening tests undertaken to detect occult opportunistic infections or second malignancies. Patient status was analyzed as of October 23, 1993. Lymphocyte counts are dated from the day of commencement of 2-CdA therapy.

Statistical analysis. Correlations were tested using Spearman's rank correlation coefficient. Comparisons between groups for non-parametric data were made using the Mann-Whitney U-test, and for categorical data using the x2 test. Sequential studies in individual patients were compared using the Wilcoxon matched-pairs test. Response duration was calculated using the method of Kaplan and Meier and compared using the method of Gehan. Patients remaining free of relapse were considered censored at the date of last follow-up. All P values given are two-sided.

RESULTS

Response duration. Eight patients (20%) have developed evidence of recurrent HCL first documented at 3 to 23 months (median, 16 months). In 5 of the 8, recurrent disease was documented on routine follow-up marrow specimens while patients were asymptomatic with normal peripheral blood counts. In the other 3 patients, cytopenias and marrow involvement were noted simultaneously. The 32 patients who have not relapsed have been observed between 7 and 43 months (median, 30 months) from the documentation of response. No patients have died, and the actuarial relapse-free survival at 3 years is 77% (95% confidence interval [CI], 70% to 84%; Fig 1).

Of the relapsing patients, 2 have received a second course of 2-CdA. One patient is currently in ongoing PR at 44 months after the initial therapy, and 31 months after documentation of relapse. This patient had undergone splenectomy and received IFN-α, fludarabine, and DCF before 2CdA and, at most recent testing 1 year after the second course of 2-CdA, has an absolute CD4+ lymphocyte count of 62/μL. The second patient retreated with 2-CdA is also in ongoing PR 15 months after the second course. Three patients have begun treatment with IFN-α because of progressive cytopenias after relapse. One is in CR, 1 has attained an MR and is continuing on therapy, and the third is too early for assessment of response. The remaining 3 patients who have evidence of recurrent HCL have not required further therapy, although 2 have mild neutropenia.

Relapses were not found to be related to quality of response. Six of 34 CR patients and 2 of 6 PR/MR patients have relapsed (P > .1). Relapse is equally likely (P > .1) in patients who had, or had not, undergone prior splenectomy or therapy with IFN-α.

Lymphocyte subsets. The percentage of CD4+ and CD8+ lymphocytes were determined using standard flow-cytometric methods and the absolute CD4+ and CD8+ lymphocyte counts were then determined from simultaneous complete blood examinations with white blood cell differential counts. The normal ranges for absolute CD4+ and CD8+ lymphocytes at M.D. Anderson Cancer Center are 365 to 2,400/μL and 270 to 1,600/μL, respectively.

Eighteen patients had lymphocyte subset determinations performed less than 1 month before commencing 2-CdA (Table 1). Absolute CD4+ counts ranged from 58 to 2,201/μL (median, 743/μL), with 3 patients (17%) having counts less than the lower limit of normal, and only 1 (6%) less than 200/μL. Baseline absolute CD8+ counts ranged from 75 to 2,342/μL (median, 238/μL), with 11 patients (61%) having counts less than the lower limit of normal. At the time of initiation of the study, the effect of 2-CdA on normal lymphocyte counts was not recognized. Patients did not initially have lymphocyte subset determinations performed systematically during follow-up. After the recognition of the potential problem of lymphocytopenia, patients have generally had lymphocyte subsets analyzed at each follow-up visit.

Patients were generally reviewed at approximately 3 months after 2-CdA therapy to document remission status. Twenty-five patients had CD4+ and CD8+ lymphocyte

Table 1. Lymphocyte Subsets for Patients With HCL Responding to 2-CdA

<table>
<thead>
<tr>
<th>CD4+ lymphocyte counts/μL</th>
<th>Baseline (n = 18)</th>
<th>Nadir (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(normal range, 365 to 2,400/μL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>743</td>
<td>139</td>
</tr>
<tr>
<td>Range</td>
<td>58-2,201</td>
<td>25-580</td>
</tr>
<tr>
<td>No. ≤50</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>No. &gt;200</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>No. below normal</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>P &lt; baseline</td>
<td>—</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CD8+ lymphocyte counts/μL</th>
<th>Baseline (n = 18)</th>
<th>Nadir (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(normal range, 270 to 1,600/μL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>238</td>
<td>92</td>
</tr>
<tr>
<td>Range</td>
<td>75-2,342</td>
<td>26-870</td>
</tr>
<tr>
<td>No. below normal</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>P &lt; baseline</td>
<td>—</td>
<td>&lt;.005</td>
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</tbody>
</table>
counts determined within 4 months of 2-CdA therapy (median, 2.5 months) and these were considered to represent nadir levels (Table 1). The median nadir CD4+ count was 139/μL (range, 25 to 580/μL) and the median nadir CD8+ count was 92/μL (range, 26 to 879/μL). Both CD4+ and CD8+ counts were significantly lower than those obtained in patients before therapy (P < .001 and P < .005, respectively). Although few patients have relapsed, the nadir absolute CD4+ lymphocyte count was not related to subsequent relapse (P > .1). The median CD4+ counts were 124/μL (range, 47 to 165/μL) and 165/μL (range, 25 to 580/μL) for those who have (n = 6) and have not (n = 19) relapsed, respectively.

All 18 patients with lymphocyte subsets determined before therapy had at least one determination performed after 2-CdA, and 15 of these had multiple subsequent determinations (mean, 3.4; range, 2 to 8 times per patient). Using the most recent results in which more than one determination was made, testing in these 18 patients was performed at a median of 23 months (range, 2 to 42 months) after therapy. CD4+ lymphocyte counts remained significantly lower than those obtained in patients before therapy (P < .001 and P < .005, respectively). Although few patients have relapsed, the nadir absolute CD4+ lymphocyte count was not related to subsequent relapse (P > .1). The median CD4+ counts were 124/μL (range, 2,201/μL; median after, 237/μL; range after, 25 to 314/μL). Even when the analysis was limited to those 13 of 18 patients who were tested beyond 1 year after treatment (median, 27 months; range, 13 to 42 months), CD4+ lymphocyte counts remained significantly lower (P < .002) than before 2-CdA (median before, 749/μL; range before, 58 to 2,201/μL; median after, 237/μL; range after, 25 to 314/μL). Even when the analysis was limited to those 13 of 18 patients who were tested beyond 1 year after treatment (median, 27 months; range, 13 to 42 months), CD4+ lymphocyte counts remained significantly lower (P < .002) than before 2-CdA (median before, 749/μL; range before, 58 to 2,201/μL; median after, 237/μL; range after, 25 to 314/μL). In contrast, CD8+ lymphocyte counts in these 18 patients, at a median of 23 months after therapy, were not significantly different from baseline (P > .1; median before, 238/μL; range before, 75 to 2,342/μL; median after, 165/μL; range after, 26 to 495/μL).

The patients were then analyzed to determine the duration of subnormal absolute CD4+ lymphocyte counts after 2-CdA therapy. Using the method of Kaplan and Meier, the median time to an absolute CD4+ lymphocyte count of 365/μL, the lower limit of the normal range, was 40 months (Fig 2). With the current duration of follow-up, 13 patients have absolute CD4+ lymphocyte counts greater than this value recorded after 2-CdA. It must be noted that recovery of CD4+ lymphocyte counts to within the "normal" range may not equate with recovery to pretherapy levels for that individual. Patients who had previously undergone splenectomy were more likely to attain an absolute CD4+ lymphocyte count of greater than 365/μL. Five of the 6 splenectomized patients reached this level, compared with 8 of the 32 patients without prior splenectomy (P = .03). Only two pretherapy and three nadir CD4+ lymphocyte counts are available from the splenectomized patients. It was not possible to determine if pretherapy CD4+ lymphocyte counts were higher in splenectomized patients.

To evaluate the recovery of CD4+ lymphocytopenia with time, those patients with sequential post-treatment counts were examined. Thirty-one patients had more than one lymphocyte subset determination performed after treatment. Where more than two determinations were made, the earliest and latest values were compared to maximize the inter-test interval (median, 22 months; range, 5 to 37 months). Using these sequential determinations there was an increase in both CD4+ (P < .0005) and CD8+ (P < .0005) lymphocyte counts. The median increment in absolute CD4+ lymphocyte count was +136/μL (range, −156 to +406/μL). The median increment in the absolute CD8+ lymphocyte count was +106/μL (range, −599 to +927/μL).

Overall, 38 patients had counts determined at least once after treatment (mean, 3.0; range, 1 to 8 times per patient). Hence, a total of 112 lymphocyte subset determinations were performed at a median of once every 10 months of patient follow-up (range, once every 5 to 38 months). The overall trend for CD4+ and CD8+ lymphocyte counts for these 38 patients are shown (Fig 3A and B). The 1 patient previously treated with DCF and fludarabine, and lymphocyte subset counts subsequent to the second course of 2-CdA in the relapsing patient who was retreated are excluded from this analysis. It should be borne in mind that not all eligible patients had counts performed during each time period shown. Thus, the previous analyses comparing lymphocyte counts in individual patients may provide more accurate comparisons.

No secondary malignancies have been reported by the patients or detected by history, physical examination, or imaging studies at any time during the period of follow-up. One patient had pre-existing prostate cancer at the time of entry into the study. The aggregate follow-up of the entire cohort is 105 person years after 2-CdA therapy. The only opportunistic infection observed has been one episode of dermato-ulcer herpes zoster that responded to conventional therapy with acyclovir.

**DISCUSSION**

The largest reported experience with 2-CdA in HCL is from the Scripps Clinic investigators.15-16 In a cohort of 148 responding patients observed between 8 and 68 months (median, 14 months), only 4 patients have relapsed (2.7%) at 12, 12, 36, and 48 months. Three of these patients had "minimal" hairy cell infiltrate and normal peripheral blood counts, and only 1 patient had progressive pancytopenia. In the cur-
current report, with a median follow-up of 30 months, 8 patients (20%) have evidence of recurrent disease. In 5 of these 8 patients, recurrent disease was found in bone marrow aspirate specimens at a time when peripheral counts were normal, although all but 1 have subsequently developed cytopenias. This suggests that the timing of detection of recurrent disease in reported series are likely to vary depending on the frequency of bone marrow examinations. The quality of response was not related to the risk of relapse. This suggests that it may not be prognostically meaningful to distinguish between complete and partial responses in HCL patients treated with 2-CdA using the current response criteria. Similarly, prior splenectomy or therapy with INF did not significantly influence remission duration (P > .1).

No patients have died, and the actuarial relapse-free survival at 3 years is 77% (95% CI, 70% to 84%). Thus, 2-CdA is certainly an effective agent with durable antileukemic activity in HCL.

Of major concern was the significant and prolonged lymphocytopenia observed after treatment. Patients previously treated with lymphocytotoxic agents, or who have been retreated with such agents after a single course of 2-CdA were excluded from analysis. Thus, the effects reported are certainly related to the use of 2-CdA. Before treatment, 17% of patients had CD4+ counts less than the lower limit of normal, but only 1 patient (6%) had an absolute CD4+ count less than 200/μL. This contrasts with previous reports in which CD4+ counts were normal at presentation in 17 patients with HCL. However, Urba et al described CD4+ counts less than 300/μL in 3 of 13 patients (23%), and in 1 of these patients the count was less than 100/μL.

After 2-CdA treatment, patients' CD4+ counts decreased to a median nadir of 139/μL. This degree of early CD4+ suppression after 2-CdA has previously been reported.

Although absolute CD4+ counts were not provided, a previous study in 11 patients suggested restoration of T-cell subsets to normal beyond 6 months posttherapy in most patients. The current report does not confirm these findings.
but demonstrates failure of CD4+ lymphocyte counts to return to baseline levels even beyond 1 year after a single course of 2-CdA. However, there is evidence of moderate improvement in CD4+ counts with time. The median time to achieve an absolute CD4+ lymphocyte count of 365/μL, the lower limit of the normal range at M.D. Anderson Cancer Center, was 40 months. Conversely, beyond 3 years after a single course of 2-CdA, approximately half of the patients with HCL have CD4+ lymphocyte levels persisting less than the lower limit of normal. More frequent assessment will define the time course of CD4+ lymphocyte counts more accurately. In contrast, CD8+ lymphocyte counts were indistinguishable from pretherapy levels at a median of 23 months after treatment. It appears that 2-CdA is selectively lymphotoxic, having a more prolonged effect on CD4+ than on CD8+ T-cell numbers.

The suggestion of higher CD4+ counts in splenectomized patients is intriguing, but requires confirmation in a larger patient group before any firm conclusions can be drawn. Increases in CD8+ T-cell numbers.

The clinical significance of this CD4+ lymphocytopenia is unclear. In patients with the acquired immunodeficiency syndrome, the risk of opportunistic infections increases greatly with diminishing absolute CD4+ counts. None of the patients in the current series or in the Scripps Clinic series have reported major infectious episodes. This supports the findings of previous studies that did not note increased risk of opportunistic infections, except for dermatomal herpes zoster, in HCL patients in remission after DCF treatment, despite severe suppression of CD4+ counts. This is consistent with observations in patients with the syndrome of "idiopathic" CD4+ T-lymphocytopenia. In this condition, some patients, despite very low CD4+ counts, do not suffer from opportunistic infections.

Although non-Hodgkin's lymphomas have been reported concurrently, and after various therapies for HCL, patients treated with 2-CdA in this series or the literature have been reported to develop this complication. The potential influence of prolonged CD4+ lymphocytopenia on the development of secondary non-Hodgkin's lymphomas is unknown.

Although a significant proportion of our patients have developed recurrent HCL after treatment, 2-CdA is certainly an effective agent with durable antileukemic activity. However, before this therapy is broadly applied, the duration and consequences of the profound 2-CdA–induced CD4+ suppression must be carefully evaluated in the large cohort of patients already treated.

REFERENCES

2-chlorodeoxyadenosine induces durable remissions and prolonged suppression of CD4+ lymphocyte counts in patients with hairy cell leukemia

JF Seymour, R Kurzrock, EJ Freireich and EH Estey