Flow Cytometry of Blood and Bone Marrow Cells From Patients With Hairy Cell Leukemia: Phenotype of Hairy Cells and Lymphocyte Subsets After Treatment With 2-Chlorodeoxyadenosine

By Gunnar Juliusson, Rodica Lenkei, and Jan Lillemark

Hairy cell leukemia (HCL) is a rare B-cell disorder characterized by cytopenia, splenomegaly and infections. Hairy cells express Ig light chains and express the pan T-cell marker CD5, characteristically found in B-cell chronic lymphocytic leukemia, has also shown to associate with poor response to interferon.

Deoxycoformycin (dCF) is a tight-binding inhibitor of adenosine deaminase (ADA), leading to a spectrum of therapeutic activity similar to that of the purine analogues fludarabine and 2-chlorodeoxyadenosine (CdA), which are resistant to demethylation by ADA because of the halogen substitution at the 2-position. It was shown in 1984 that dCF may produce complete remissions in HCL. However, dCF induces a prolonged immunosuppression and patients with other diseases than HCL have developed late opportunistic infections. Treatment of HCL with CdA may result in an even higher complete remission rate from a single week of therapy, either as a continuous intravenous infusion or as daily subcutaneous injections.

Among patients with pretreatment anemia, treatment is frequently complicated by neutropenic fever, either during the treatment course, which might be caused by rapid tumor cell lysis, or during the following weeks, which may be caused by opportunistic infections.

To assess the phenotypic variability of hairy cells, and to evaluate the long-term effects of CdA on immune cells we used flow cytometry analyses with an extensive set of markers on blood and bone marrow cells in patients with active disease before and after CdA treatment.

PATIENTS AND METHODS

A total of 75 patients, 63 men and 12 women, with symptomatic HCL were studied, some of whom are previously reported. Diagnosis was made through morphology with cytochemistry on repeated bone marrow aspirations and biopsy specimens. Thirty-four patients were previously untreated, and 41 had received prior treatment, 1 of them (patient JJ in Juliusson and Lillemark) including deoxycoformycin. Their mean age was 53 years (range, 29 to 75), and sampling of blood and bone marrow was performed with a median of 10 months from diagnosis. All patients were subsequently treated with CdA for 7 days, either as a continuous infusion of 0.008 mg/kg or as daily subcutaneous injections of 3.4 mg/m². These treatment schedules give the same concentrations of CdA nucleotides in leukemic cells and (Lillemark and Juliusson, manuscript in preparation) and similar clinical results. Peripheral blood counts were in most cases evaluated once or twice weekly during the first months after CdA treatment, and such data were available from 70 patients. Blood and bone marrow sampling for flow cytometry was repeated 3 months (n = 46), 6 months (n = 50), 1 year (n = 39), and 2 years (n = 12) after CdA treatment. Thus, flow cytometry was performed on at least 4 occasions in most patients. In addition, blood for flow cytometry was sampled from some patients at lymphocyte nadir 1 week (n = 24) and 1 month from the start of CdA treatment (n = 7). Ten patients received a second course of CdA because of residual disease. Approval was obtained from the Institutional Review Board. Informed consent was provided according to the Declaration of Helsinki.

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FLOW CYTOMETRY IN CdA-TREATED HCL

Table 1. Monoclonal Antibodies

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>Name, Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>Leu 4</td>
<td>T-cell receptor</td>
</tr>
<tr>
<td>CD4</td>
<td>Leu 3</td>
<td>T4. Helper/inducer cells</td>
</tr>
<tr>
<td>CD5</td>
<td>Leu 1</td>
<td>Mature T-cells, thymocytes, B-cell subset, B-cell chronic lymphocytic leukemia cells</td>
</tr>
<tr>
<td>CD8</td>
<td>Leu 2</td>
<td>T8. Cytotoxic/suppressor cells</td>
</tr>
<tr>
<td>CD10</td>
<td>J5</td>
<td>CALLa. Early B and T precursors, common acute lymphoblastic leukemia, stem cells, renal cells</td>
</tr>
<tr>
<td>CD13</td>
<td>My 7</td>
<td>Myelomonocyte lineages, intestinal cells, renal cells, central nervous system cells</td>
</tr>
<tr>
<td>CD14</td>
<td>Leu M3</td>
<td>Mature monocytes</td>
</tr>
<tr>
<td>My 4</td>
<td>Leu 11</td>
<td>Myelomonocyte lineages, monoblasts, B-cell subset</td>
</tr>
<tr>
<td>CD15</td>
<td>Leu M1</td>
<td>Granulocytes, monocytes, embryonic cells</td>
</tr>
<tr>
<td>CD16</td>
<td>Leu 17</td>
<td>T10. Early or activated B and T cells, thymocytes, monocytes</td>
</tr>
<tr>
<td>CD19</td>
<td>Leu 12</td>
<td>B cells, B-cell precursors, follicular dendritic cells</td>
</tr>
<tr>
<td>CD20</td>
<td>Leu 16</td>
<td>B cells, pre-B cells</td>
</tr>
<tr>
<td>CD23</td>
<td>Leu 20</td>
<td>FcRII. Mature B cells, monocytes, macrophages, eosinophils, platelets, follicular dendritic cells</td>
</tr>
<tr>
<td>CD25</td>
<td>IL-2 R</td>
<td>IL-2 receptor alpha chain. Activated B-cells, T-cells, monocytes, thymocytes</td>
</tr>
<tr>
<td>CD33</td>
<td>My 9</td>
<td>Granulocyte and macrophage precursors, monocytes</td>
</tr>
<tr>
<td>CD38</td>
<td>Leu 17</td>
<td>T10. Early or activated B and T cells, thymocytes, pre-B cells, germinal centre B cells, Ig-secreting plasma cells</td>
</tr>
<tr>
<td>CD45RA</td>
<td>Leu 18</td>
<td>Virgin T cells, most B cells, monocytes, macrophages</td>
</tr>
<tr>
<td>CD45RO</td>
<td>UCHL1</td>
<td>Activated and memory T cells, B-cell subsets, monocytes</td>
</tr>
<tr>
<td>CD56</td>
<td>Leu 19</td>
<td>N-CAM, NK cells</td>
</tr>
<tr>
<td>CD57</td>
<td>Leu 7</td>
<td>NK cells, subsets of T cells, B cells</td>
</tr>
<tr>
<td>CD71</td>
<td>OKT9</td>
<td>T9. Transferrin receptor, all activated cells</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>MHC II</td>
<td>B cells, monocytes, macrophages, myeloid and erythroid precursors, dendritic cells, some epithelial cells</td>
</tr>
<tr>
<td>B-ly 7</td>
<td>TCR γδ</td>
<td>Hairy cells activated CD8+ cells, intestinal mucosa T cells</td>
</tr>
<tr>
<td>TCRA1</td>
<td></td>
<td>Minor peripheral T-cell subset</td>
</tr>
<tr>
<td>κ/λ</td>
<td></td>
<td>Ig light chains, B cells</td>
</tr>
</tbody>
</table>

Data partly from Barclay et al.27

Flow cytometry. Blood was collected in vacuum tubes with EDTA as anticoagulant. Bone marrow cells were aspirated from the dorsal iliac crest and immediately put into heparinized tubes. Flow cytometry was performed with a FACStar (Becton Dickinson, Mountain View, CA) and a large panel of antibodies (Table 1), including B-ly 7.24 Natural killer (NK) cells were defined as non-T cells (CD3+) expressing one or both of the markers CD16 and CD56.78 All fluorescence-activated cell sorter (FACS) analyses were performed by a single investigator (R.L.). Double staining with antibodies conjugated to fluorescein isothiocyanate (FITC) or phycoerythrin (PE) was routinely performed on whole blood as previously described.28 In 84% of the pre-CdA-treatment samples, the analyses were separately performed in gated cells containing (1) the small lymphocytes and (2) the hairy cells mixed with some monocytes (Fig 1). Normal values for the different markers were assessed with an identical technique in blood from 75 controls.

Statistical analysis. Parametric and nonparametric description of data, Student’s t-test for paired or independent samples, the Wilcoxon matched pairs test, and the Kolmogorov-Smirnov two-sample test were performed using the Statistica/Mac software (StatSoft, Tulsa, OK). Patients receiving 2 courses were censored at the date of the second course.

RESULTS

Phenotype of hairy cells. The hairy cell phenotype could be assessed in 68 patients, either by analysis of the peripheral blood, bone marrow cells, or both (Table 2). The median percentage of hairy cells in the large cell gate (Fig 1), as assessed by the CD20 positivity, was 90% (Fig 2), and contained less than 5% monocytes. In three quarters of the patients there were more than 80% hairy cells in the gate, enabling the identification of aberrant expression of cell markers without double fluorescent staining, which, however, confirmed expression of certain cellular markers in many patients.

Almost all hairy cells expressed CD19, CD20, CD25, HLA-DR, and CD45RA (Fig 2). A median of two-thirds of the cells expressed B-ly 7, and 8% of the patients had less than 20% B-ly 7-positive cells in the large cell gate. However, unusual expression of one or another marker was found in a majority of the cases (Table 3). CD38, CD45RO, CD15, CD4, CD10, CD5 (Fig 1b, plot C), and/or CD23 were occasionally expressed on a majority of the hairy cells (Fig 2). Also, CD25 and B-ly 7 were expressed only on a minority of the hairy cells in some patients.

Total lymphoid cell counts after CdA treatment. Lymphoid cell counts from the start of CdA treatment are shown in Fig 3. The cell populations included hairy cells in a majority of the patients at start of CdA treatment, whereas only 2 patients, both previously reported,18 had residual circulating hairy cells more than 2 weeks from treatment. Patients receiving two CdA courses were censored at time of the second course. The mean blood lymphocyte count returned to 1,000/μL at 4 months from start of treatment.

Initial changes after treatment in defined lymphocyte subsets. The lymphocyte gate of all samples contained less than 3% monocytes. Through the flow cytometry data achieved at lymphocyte count nadir we were able to determine the early kinetics of the CdA effects on different cell populations. Figure 4 shows the median percentage of cell subsets for 24 patients with nadir data available. The variation of the percentages in between patients was great, with less change in between measurements on different time points in individual patients (Fig 4); however, the changes were in the same direction in most patients. CD20+, CD8+ cells and NK cells were found in lower percentages at nadir than pretreatment, whereas the percentage of CD4+ cells were higher at nadir (differences significant when analyzed with either the t-test for paired samples or the Wilcoxon matched pairs test, Fig 4). This indicates a slower decrease of CD4+ cells after CdA treatment than of the other cell populations. Correspondingly, in these 24 cases, the CD4/CD8 ratio was significantly higher at nadir as compared with
the pretreatment value (median, 1.81 and 1.27, respectively; 
\( P = 0.037 \), t-test for paired values). On the other hand, a
significantly higher proportion of NK cells and CD8 cells
were found at subsequent time points as compared with na-
dir. Thus, the recovery from nadir was more rapid for NK
cells and CD8+ cells than for CD4+ cells and B cells, leading
to a slowly but progressively decreasing CD4/CD8 ratio.

The absolute cell counts for various lymphoid cell subsets
in relation to treatment are shown in Fig 5 and Table 4. The
median CD4+ cell count was close to the normal range 1
year after the start of CdA treatment, whereas the CD8+
cells normalized within 3 months. Of interest was that sev-
eral specific B- and T-cell subsets had a quicker recovery
than the overall B- and T-cell populations (Fig 5 and Table
4). The results were not different whether the patients had
received CdA intravenously or subcutaneously (Kolmogor-
rov Smirnov nonparametric two-sample test, Table 5).

**T-cell receptor \( \gamma \delta \) positive lymphocytes.** The percentage
of T-cell receptor \( \gamma \delta \) lymphocytes decreased initially from
a median of 2.2% to 1.4% (normal, 1% to 10%). At the 3-
month evaluation, the median value was 6%, one-third had
more than 10%, and a few patients had an overshoot to 40%
or more. Subsequently, both relative and absolute values
normalized.

**CD10+ cells in bone marrow after CdA treatment.** The
mean percentage ± SD CD10+ cells from bone marrow in the
lymphocyte gate was 1.30% ± 5.92% before treatment.
The corresponding figures at 3 months, 6 months, 1 year, and
2 years after CdA treatment were 3.53% ± 8.01%, 5.43% ±
6.73, 3.56% ± 4.24%, and 1.33% ± 1.5%, respectively.
Table 2. Percentage of Positive Cells in Hairy Cell Gate

<table>
<thead>
<tr>
<th>Marker</th>
<th>No. of Patients</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45RA</td>
<td>54</td>
<td>83.3</td>
<td>19.3</td>
</tr>
<tr>
<td>CD19</td>
<td>54</td>
<td>61.2</td>
<td>24.9</td>
</tr>
<tr>
<td>CD38</td>
<td>60</td>
<td>14.8</td>
<td>15.2</td>
</tr>
<tr>
<td>CD45RO</td>
<td>62</td>
<td>6.4</td>
<td>8.7</td>
</tr>
<tr>
<td>CD71</td>
<td>58</td>
<td>89.4</td>
<td>10.7</td>
</tr>
<tr>
<td>Percentage of positive cells among CD19' cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD25</td>
<td>58</td>
<td>68.5</td>
<td>27.5</td>
</tr>
<tr>
<td>CD23</td>
<td>39</td>
<td>11.5</td>
<td>14.5</td>
</tr>
<tr>
<td>CD15</td>
<td>55</td>
<td>11.3</td>
<td>14.4</td>
</tr>
<tr>
<td>CD5</td>
<td>63</td>
<td>8.7</td>
<td>16.4</td>
</tr>
<tr>
<td>CD10</td>
<td>62</td>
<td>4.4</td>
<td>15.4</td>
</tr>
</tbody>
</table>

These values were significantly different from the baseline value by nonparametric statistics at 3, 6, and 12 months (Wilcoxon matched pairs test: P = .036, P = .0007, and P = .0017, respectively). Sixteen of 36 evaluable patients (44%) with less than 2% CD10' cells of the pretreatment bone marrow lymphoid cells showed an increase to 5% or more at follow-up, with the kinetics shown in Fig 6.

DISCUSSION

By flow cytometry with an extensive set of antibodies and double fluorescent staining, we have analyzed the phenotype of hairy cells from 68 patients with symptomatic disease. Furthermore, by frequent samplings from 50 patients after CdA treatment, we were able to determine the selective toxicity of this agent towards specific lymphoid cell subsets and their subsequent recovery.

Fig 2. The percentage of cells staining positive with specified markers in the hairy cell gate. Data on CD25, CD15, CD23, CD5, and CD25 indicate the percentage of positive cells among CD19' cells as assessed by double-fluorescence staining. Shaded areas represent the four quartiles.

Table 3. Number of Patients With Aberrant Phenotype Expression

<table>
<thead>
<tr>
<th>Marker</th>
<th>Limit (%)</th>
<th>With Aberrant Expression/Evaluable (%)</th>
<th>Double Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD38</td>
<td>&gt;20</td>
<td>24/55 (44)</td>
<td>No</td>
</tr>
<tr>
<td>CD15</td>
<td>&gt;5</td>
<td>32/55 (58)</td>
<td>CD19</td>
</tr>
<tr>
<td>CD23</td>
<td>&gt;20</td>
<td>10/55 (18)</td>
<td>CD19</td>
</tr>
<tr>
<td>CD20</td>
<td>&gt;5</td>
<td>20/39 (51)</td>
<td>CD19</td>
</tr>
<tr>
<td>CD22</td>
<td>&gt;20</td>
<td>8/39 (21)</td>
<td>CD19</td>
</tr>
<tr>
<td>CD25</td>
<td>&gt;20</td>
<td>24/63 (38)</td>
<td>CD20</td>
</tr>
<tr>
<td>CD10</td>
<td>&gt;5</td>
<td>8/62 (13)</td>
<td>CD19*</td>
</tr>
<tr>
<td>CD15</td>
<td>&gt;20</td>
<td>3/62 (5)</td>
<td>No†</td>
</tr>
<tr>
<td>Bly 7/CD19</td>
<td>&lt;20</td>
<td>4/58 (7)</td>
<td>CD19</td>
</tr>
</tbody>
</table>

* Double staining with CD19 in 4 cases, otherwise defined as more than 20% CD4' in the hairy cell gate and percentage of CD19 + percentage of CD4 greater than 110.
† Ratio between percentage of B-ly 7' and percentage of CD19 in different tubes.

The phenotype of hairy cells is known to be CD19', CD20', CD22', CD25', B-ly 7', CD5', and CD11c'. We also found that HLA-DR and CD45RA were consistently expressed. In addition, hairy cells from a large proportion of the patients express other markers. CD11c is a monocyte marker that is typically found on hairy cells, but we also frequently found expression of CD15, another monocyte marker. Expression of CD4, the T-helper/inducer cell marker; CD10, the common acute lymphoid leukemia antigen indicating early B-cell differentiation; and CD5, the T-cell marker typically found on B-cell chronic lymphocytic leukemia, were also verified by double fluorescent staining in significant numbers of patients. Thus, hairy cells commonly have aberrant phenotypes, with simultaneous expression of divergent markers of differentiation and cell lineage.
Hairy cells from some patients also lacked expression of characteristic markers, such as CD25 and B-ly 7, at least in a proportion of the leukemic cells. The identification of minor subpopulations of hairy cells with aberrant phenotype mostly requires double fluorescent staining using the relevant combination of markers. Because far from all combinations were possible to determine, it is likely that aberrant expression is more common than we have been able to document. Cells from the disease entity called HCL variant lack CD25, but were recently found to express IL-2 receptor β chains. Our phenotype data rather indicate a spectrum of HCL variants than one typical variant entity.

We did not find any correlation between the phenotype and the response to treatment (data not shown). CdA gives a much higher complete remission rate in HCL than interferon, and thus divergent response to CdA treatment according to any marker is difficult to assess.

There is limited previous data available on the immune response to CdA treatment. Day 1 is the baseline. Patients receiving two courses are censored at the time of the second course. Median values; (-) quartiles, 5% and 95% values; and (···) extreme values. The number of patients is indicated in the graph.
An early decrease in both CD4+ and CD8+ cells after treatment is found, and a slow recovery during the subsequent year was indicated from sporadic observations. From the combined data collected at the Scripp's clinic and the MD Anderson Cancer Center, it was concluded that the mean CD4 count nadir was 272/μL and occurred 4 to 6 months from treatment. This discrepancy to our data probably reflects the lack of systematic phenotype analyses in the early posttreatment phase in the US studies.

In the present study, we have characterized the degree and the kinetics of both the decrease and the recovery as regards several of the lymphocyte subsets. During Cda treatment, there is a rapid decrease of most of the studied lymphocyte subsets, but CD8, CD20, and NK-cell subsets decreased significantly more than CD4 cells. The median CD8 cell count at nadir was well below 100/μL, whereas the corresponding CD4 count was 128/μL. The recovery from nadir was quicker for CD8 cells, NK cells, and T-cell receptor γδT cells, and the median blood counts of these subsets were all normal within 3 months, whereas CD4+ cells required 1 year or more for normalization, leading to a slowly but progres-
CDA-treated HCL patients are at great risk for neutropenic fever if they have pretreatment pancytopenia, especially anemia. However, no infections present later than 1 month from CDA treatment, despite continuing low levels of several lymphocyte subsets during 6 to 12 months. The activated CD38⁺ and HLA-DR⁺ T-cell subsets, the CD5⁺ B cells, and the NK cells that rapidly normalize after CDA-treatment might thus, in combination with the recovering neutrophils, be an adequate first-line defense.

As regards the B cells, the CD23⁺ B cells also recovered promptly from 3 to 6 months, whereas the total CD20⁺ cell population required 1 year for normalization of the cell counts. Increased relative numbers of B-cell progenitors, ie, CD10⁺ cells, appeared in the bone marrow after treatment in 16 of 36 patients (Fig 6). We have seen the similar phenomenon during successful CDA treatment of chronic lymphocytic leukemia. It is unlikely that these CD10⁺ B cells represented leukemia cells, because they were seen primarily in patients with leukemia cell clones lacking CD10, and in those achieving complete remission. There was a normalization of the percentage of CD10⁺ cells with longer follow-up without further treatment. This could not be caused by a selective depletion of bone marrow subsets other than CD10⁺ cells, according to bone marrow cellularity, morphology, and flow cytometry data (not shown). It is more likely that we observed a regeneration of normal B cells, enabled by an effective and brief cytotoxic treatment. Such B-cell regeneration might be a common but hitherto unknown phenomenon after successful chemotherapy, because CD10⁺ cells were very rare in the peripheral blood, and systematic phenotype studies on bone marrow cells have not been performed previously. However, CD10⁺ bone marrow cells are described to appear after autologous bone marrow transplantation. B-cell regeneration is probably also mirrored by the recent finding that CDA and fludarabine, in contrast to conventional chemotherapy, may increase polyclonal gammaglobulin levels in patients with chronic lymphocytic leukemia. However, we cannot exclude the possibility that CDA has a direct stimulatory effect on normal B-cell progenitors, which, if it would occur with a lower dose range than the cytotoxic effect, could be exploited in B-cell dysfunction states.

As yet, no studies have attempted to optimize CDA scheduling. Monitoring of the immune cells by flow cytometry and the time to recovery from cytopenias after various treatment schedules might facilitate the establishment of an optimal regime without the need for large-scale clinical trials. The immunosuppressive effects of CDA may also be possible to use in nonmalignant disorders, such as rheumatoid arthritis and multiple sclerosis. Flow cytometry monitoring might then again prove useful when establishing adequate dosing and scheduling of the treatment.

Cytopenias and infections are the major side effects for most agents used for treatment of malignancy, and have to be considered when selecting treatment. DCF is highly effective therapy for hairy cell leukemia, but has to be administered in repeated doses. A long-standing suppression of CD4⁺ cells has, in disorders other than HCL, been associated sively decreasing CD4/CD8 ratio. Of specific interest was that the activated HLA-DR⁺ subset of CD4⁺ cells was fully normalized already at 3 months after CDA treatment, as were the NK cells, the CD5⁺ B cells, and the CD45RA/CD45RO cells (Fig 5 and Table 4). Furthermore, the absolute numbers of HLA-DR⁺ subset of CD8⁺ cells decreased within the normal range, and often recovered with an overshoot. We have previously documented post-CDA-treatment overshoot of other leucocytes, such as monocytes and eosinophils. However, CD4⁺/CD45RA⁺ cell counts had a late nadir, almost within the normal range.

Fig 5. (Cont’d).
with severe opportunistic infections, such as candida pneumonitis, central nervous system infections with Listeria and Aspergillus, and disseminated herpes zoster. In Steis et al's study of dCF in HCL, the median nadir of CD4+ cell counts was 54/μL, and in other studies the median CD4 nadir was between 70 and 155/μL. Normalization of CD4 counts and CD8 counts may occur a median of 23 and 24 months after the start of dCF treatment, respectively. Be-

*Fig 6. Percentage of CD10+ cells in the lymphocyte gate of bone

marrow cells from individual patients according to time from start

dCF and CdA, and the lowest effective dose for HCL has not been established for either of the drugs.

Fludarabine is not recommendable for HCL, but it is an important nucleoside analog for the treatment of other lymphoid neoplasms and is structurally closely related to CdA. Fludarabine also produces a severe depletion of CD4+ cells when administered in repeated courses to patients with chronic lymphocytic leukemia and lymphoma.

Interferon, the established first-line treatment of HCL, does not induce T-cell depletion to a degree similar to that of the nucleoside analogues. However, HCL by itself causes immunologic impairment, the complete response rate to in-

terferon is considerably lower than for CdA, and the im-

provement is also slower. Thus, patients may die from opportunistic infections during induction treatment with interferon.

In conclusion, CdA is highly cytotoxic towards all lymphoid subsets during treatment. These short-term effects cause a significant acute morbidity through neutropenic in-

*Fig 6. Percentage of CD10+ cells in the lymphocyte gate of bone

marrow cells from 16 individual patients according to time from start of CdA treatment.*
fections. Thus, the evaluation of other dose schedules, facilitated by the use of subcutaneous and flow cytometry monitoring, should be performed. Prolonged immunosuppression from CdA is not a significant clinical problem in HCL. Partial regeneration of several normal subsets occurs rapidly, whereas the full recovery may be delayed, although this recovery is quicker than after dCF treatment. Drug-induced immune suppression will be a major concern when choosing between the available treatment options for lymphoproliferative disorders, and this has to be considered in comparative trials in the near future.

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Flow cytometry of blood and bone marrow cells from patients with hairy cell leukemia: phenotype of hairy cells and lymphocyte subsets after treatment with 2-chlorodeoxyadenosine

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