Chronic Myelogenous Leukemia

Collection of Philadelphia-negative stem cells using recombinant human granulocyte colony-stimulating factor in chronic myeloid leukemia patients treated with α-interferon

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Background and Objectives. Autologous stem cell transplantation is a therapeutic option for chronic myeloid leukemia (CML) patients who are not candidates for allogeneic transplant. To reduce the risk of post-autografting disease recurrence, different strategies of stem cell selection have been attempted. The results of using recombinant human granulocyte colony-stimulating factor (rHuG-CSF) for harvesting hematopoietic progenitors in CML patients treated with interferon-α (IFN) are reported.

Design and Methods. Twenty-one CML patients who received IFN for a median of 21 (8-68) months were mobilized with rHuG-CSF (10 µg/kg/day). Twelve were in complete (CCR) or major (MCR) cytogenetic response. Complete success was considered a sufficient harvest (> 1 x 10^6/kg CD34+ cells/kg) without Philadelphia (Ph)+ metaphases in at least one apheresis; a partial success was a sufficient harvest with 1-35% Ph+ cells.

Results. A total of 78 aphereses were performed. No patient had major side-effects. The median number (range) of mononuclear and CD34+ cells obtained was, respectively, 8.6 x 10^9/kg (0.9-22.6) and 3.3 x 10^6/kg (0.4-26.3) per patient. A sufficient cell yield was collected in all but three patients. A complete/partial success was achieved in seven CCR/MCR patients (63%) and in three (33%) with other responses. Four patients underwent successful autografting using the stem cells obtained after rHuG-CSF mobilization.

Interpretation and Conclusions. Mobilization of IFN-treated patients using rHuG-CSF is safe and provides a significant proportion of Ph-negative progenitors in CML patients in complete or major cytogenetic response. © 2002, Ferrata Storti Foundation

Key words: chronic myeloid leukemia, interferon, mobilization, rHuG-CSF, autografting.

To date, allogeneic stem cell transplantation is the only therapy with the potential to cure patients with chronic myeloid leukemia (CML). However, since less than 30 percent of patients are candidates for allogeneic transplantation because of lack of a donor or age limitations, alternative therapies are required. The rationale for the use of autologous stem cell transplantation (ASCT) is the coexistence of normal hematopoietic progenitors with their malignant counterparts in the marrow and blood of CML patients, such that these progenitor cells are temporarily able to restore a Ph-negative hematopoiesis following autograft.

Evidence of the role of Ph+ cells contaminating the graft in the recurrence of CML after autologous transplantation has led to the development of different strategies to improve stem cell selection. In vitro purging techniques have resulted in significant decreases in the proportion of Ph+ cells in the inoculum. In vivo purging is an alternative approach to obtaining Ph-negative cells. In this context, interferon-α (IFN) is able to induce 20-30% major cytogenetic responses in the early chronic phase of CML. However, given the difficulties in collecting a sufficient number of progenitors for autografting in IFN-treated patients, the use of recombinant human granulocyte colony-stimulating factor (rHuG-CSF) for stem cell mobi-
Second paragraph: The aim of the present study is to provide further information on the use of rHuG-CSF in harvesting hematopoietic progenitor cells in CML patients treated with IFN.

Table 1. Main clinicohematologic characteristics at time of mobilization with rHuG-CSF in 21 Ph-positive CML patients treated with IFN.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (range)</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs.</td>
<td>43 (16-61)</td>
<td>21</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>14/7</td>
<td></td>
</tr>
<tr>
<td>Previous treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Busulfan</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Busulfan and hydroxyurea</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ABMT*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Response to IFN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete cytogenetic response</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Major cytogenetic response</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Minor cytogenetic response</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Minimal cytogenetic response</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Hematologic response only</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Time lapse diagnosis-harvest, months</td>
<td>36 (13-102)</td>
<td>21</td>
</tr>
<tr>
<td>WBC count, ×10⁹/L</td>
<td>3.8 (2.8-9)</td>
<td>21</td>
</tr>
<tr>
<td>Platelet count, ×10⁹/L</td>
<td>126 (86-899)</td>
<td>21</td>
</tr>
</tbody>
</table>

* Autologous bone marrow transplantation.

Mobilization procedure
rHuG-CSF was given at a dose of 10 µg/kg/day s.c. for six consecutive days unless the WBC count increased up to 70 × 10⁹/L or significant side-effects appeared. Prior to 1997, the policy was to discontinue IFN administration either 14 days earlier (n = 8) or the day before (n = 2) starting rHuG-CSF. From 1997, IFN was maintained during the mobilization (n = 11). Apheresis sessions were started on day 3 (until 1997) or 4 (thereafter) of rHuG-CSF administration and performed with a Fenwald CS3000-plus blood-cell separator (Baxter, Deerfield, Ill, USA) for four consecutive days; 10 liters of blood were processed daily, at flow rates of 50-60 mL/min using antecubital veins. To evaluate the results, a complete success was considered when the sum of the patient's collections reached the target cell dose (> 1 × 10⁶ CD34+ cells/kg) without detectable Ph+ metaphases, whereas a partial success was considered when the target cell dose was collected but with 1-35% Ph+ cells; other situations were regarded as a failure. In the latter cases, an additional harvest was considered using rHuG-CSF at a dose of 24 µg/kg/day s.c. three months later, while the patient had discontinued IFN treatment. Successful harvests were cryopreserved in DMSO without further manipulation.

Cytogenetic studies
Prior to mobilization, cytogenetic studies were carried out on both the patients’ marrow and each of the apheresis products. Conventional G-banding analysis was performed on a minimum of 10 metaphases using short (24h) cultures without addition of mitogens. Complete cytogenetic response (CCR) was defined as no detectable Ph-positive marrow metaphases, major cytogenetic response (MCR) as 1-34% Ph+ metaphases, minor cytogenetic response (mCR) as 35-64% Ph+ metaphases, minimal cytogenetic response as 65-99% Ph+ metaphases, and hematologic response as normalization of the blood cell counts without a decrease in the Ph+ marrow cells. Fluorescence in situ hybridization (FISH) studies for the BCR-ABL rearrangement were carried out on interphase cells using the LSI bcr spectrum green/abl spectrum orange probe (Vysis, Downers Grove, USA). The technique was performed as described in the proceedings supplied by the manufacturer, with a total of 100 evaluable interphase nuclei being analyzed per sample. In order to rule out false-positive cases due to the coincidental co-localization of the two signals, at least 10 interphase nuclei showing the BCR-ABL fusion gene were required to consider a result as positive.

Statistical methods
Correlations were studied by the Spearman rank and Mann-Whitney rank sum tests. Fisher’s exact probability test and Student’s t-test were used to compare categorical and continuous variables, respectively. p values were considered statistically significant if < 0.05. All computations were performed using Statistica software (Statsoft Inc., Tulsa, OK, USA).
Results

Mobilization and harvest

The apheresis procedures were well tolerated, except in a patient in whom rHuG-CSF had to be discontinued due to severe bone pain; in two additional cases rHuG-CSF was also prematurely stopped because of high WBC counts (> 70 × 10⁹/L). Overall, a total of 78 aphereses were performed, with a median of four procedures per patient (range 1-4). Fewer than 4 aphereses were carried out in 3 patients at the physician’s discretion; this decision was made following collection of a sufficient cell yield with a single apheresis (n = 1) or, conversely, in view of very poor initial collections (n = 2). As shown in Table 2, the median total number of mononuclear cells (MNC) and CD34+ cells of the harvest were 8.6 × 10⁸/kg (range, 0.9-22.6) and 3.3 × 10⁶/kg (range, 0.4-26.3) per patient, respectively. A sufficient number of progenitors (> 1 × 10⁶/kg CD34+ cells) was collected in all but 3 patients; in one of these patients an adequate cell dose was achieved following a second mobilization three months later.

Data on the cell yield obtained in the successive aphereses are presented in Table 3. The proportion of patients reaching the cumulative target CD34+ cell dose was 33%, 71%, 81%, and 86% after apheresis #1, #2, #3, and #4, respectively. No significant difference was observed between the cell yield (MNC and CD34+ cells) obtained in each of the 4 aphereses. With regard to the factors influencing CD34+ cell harvest, a significantly higher...
harvest was obtained in patients who discontinued IFN prior to the mobilization as compared to the remaining ones (median CD34+ cells 8.4 × 10⁶/kg, range: 1.1-26.3 vs. 1.8 × 10⁶/kg, range: 0.4-4.7, respectively; p = 0.006).

Cyto genetic studies
Conventional cytogenetic studies were informative in 62 of the 78 apheresis (Table 4). FISH studies were carried out on 43 of the 78 apheresis products, with a total of 36 samples being assessed by both methods. The median number of metaphases and interphase nuclei analyzed were 14 (range, 10-73) and 100 (range, 50-100) per sample, respectively. Before mobilization, the median percentage of Ph+ metaphases in the patients’ bone marrow was 29% (range, 0-100), while it was 52% (range, 0-100), 30% (range, 0-100), 18% (range, 0-100) and 0% (range, 0-95) in apheresis #1, #2, #3 and #4, respectively. The median percentage of Ph+ metaphases per apheresis in CCR/MCR patients was 1.5 (range, 0-100) vs 70.5 (range, 0-100) in the remaining situations (p < 0.001). No detectable Ph+ cells were observed in 20 of 62 (32%) collections. However, in 13 of 18 such cases (72%), residual (> 10%) BCR-ABL positive cells (range, 12-28) were detected by FISH analysis.

Final assessment
Adequate information was available for all but one patient who had insufficient metaphases for analysis in all apheresis samples (#8). A completely or partially successful collection was achieved in 7 patients (63%) who were in CCR/MCR prior to mobilization and in only 3 (33%) with other pre-harvest cytogenetic responses (p = NS). Actually, patient #8, considered not evaluable, could be considered to have had a partially successful mobilization if the results obtained by FISH analysis of his apheresis products are used for evaluation. In only 2 cases (#6 and #11) was the degree of cytogenetic response in the harvest worse than the one observed in the bone marrow. Four patients (#2, #3, #8, and #12) were subsequently autografted using the stem cells from the harvest obtained with rHuG-CSF mobilization.

Discussion
Early studies showed that cytogenetic responses could be obtained with ASCT using unmanipulated autologous marrow or blood stem cell progenitors harvested in chronic-phase CML. Moreover, autografted patients, particularly those attaining a cytogenetic response, survived longer than age-matched controls treated with conventional che-
In summary, mobilization of IFN-treated CML patients using rHuG-CSF is a feasible and safe strategy for obtaining Ph-negative progenitors for autotransplant purposes. This approach allows a substantial proportion of completely Ph-negative apheresis products to be obtained in patients with a good pre-harvest response to IFN.

Contributions and Acknowledgments

JC-HB analyzed the data and wrote the paper. EC designed the protocol. FC was responsible for the clinical management of the patients and collaborated in writing the paper. PM performed the mobilization procedures and stem cell cryopreservation. E-AR, MR and AO collaborated in the clinical management of the patients. FS, EL and BE performed the cytogenetic studies and EM gave some ideas for the production of the paper and contributed to the final writing.

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Disclosures

Conflict of interest: none.

Redundant publications: no overlapping with previous papers.

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PEER REVIEW OUTCOMES

What is already known on this topic
IFN induces cytogenetic remissions in a minority of patients with CML. Philadelphia-negative PBSC can be harvested from IFN responders.

What this study adds
Confirmation of the role of IFN as in vivo purging agent. Feasibility of autologous SCT using such cells.

Manuscript processing
This manuscript was peer-reviewed by two external referees and by Prof. Eduardo Olavarria, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Prof. Olavarria and the Editors. Manuscript received July 30, 2001; accepted November 14, 2001.

Potential implications for clinical practice
Development of new strategies combining IFN with tyrosine kinase inhibitors to achieve long-term cytogenetic remissions in CML.

Eduardo Olavarria, Associate Editor