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ABSTRACT

Background and Objective. Allogeneic bone marrow transplantation remains the only potentially curative treatment for CML, but more than 70% of patients will be ineligible for allogeneic marrow transplant either because they do not have a suitable HLA-matched related or unrelated donor or because they are more than 50 years old. Several experimental and clinical findings support a role for autologous stem cell transplantation (ASCT) in CML. It has been suggested that in the early phase following autografting the Ph-negative clone has a proliferative advantage over the Ph-positive clone. We hypothesized that post-transplant GM-CSF administration could reactivate the functional activity of quiescent normal progenitors and prolong the duration of the post-transplant proliferative advantage of Ph-negative over Ph-positive progenitors. In order to evaluate the effect of post-transplant GM-CSF administration, a pilot clinical study was performed in which CML patients resistant to IFN-α therapy were autografted with unmanipulated marrow or blood cells and given prolonged GM-CSF therapy post-transplant.

Methods. Five adult CML patients conditioned with the BAVC regimen were reinfused with either marrow (n = 2) or blood (n = 3) cells and given granulocyte-macrophage colony-stimulating factor (GM-CSF). Recombinant GM-CSF was initially administered at standard dosage (5 µg/kg/day) until a white blood cell count ≥ 2 × 10^9/L was achieved on two consecutive examinations, and thereafter at a low dose (1 µg/kg/day) for 5 to 9 months. On a weekly basis, GM-CSF was discontinued and hydroxyurea (1,000 mg/d) was given for two days.

Results. Evidence of trilineage engraftment was observed in all cases. At autografting, 3 out of the 5 patients revealed 8-9% Ph-negative metaphases. During the initial phase of hematopoietic regeneration, direct cytogenetic analysis revealed 81% and 100% Ph-negative metaphases in two cases; nonleukemic hematopoiesis progressively decreased and was no longer detectable at +9 months. One patient showed cyclic Ph-negative hematopoiesis that appeared 3 months following autografting and peaked at +4 and +8 months. The fourth patient showed a low percentage (20%) of Ph-negative metaphases 1 month after ASCT, followed by a significant expansion of nonleukemic hematopoiesis, which could be detected up to month +13. No evidence of Ph-negative hematopoiesis could be detected in one patient. Three patients are in chronic phase 28, 30 and 31 months after autografting, respectively, and two patients evolved into blast crisis.

Interpretation and Conclusions. This pilot study demonstrates that combined GM-CSF and hydroxyurea therapy seems to be effective in inducing and/or prolonging a transient period of Ph-negative hematopoiesis. The late appearance of Ph-negative hematopoiesis detected in two patients suggests an antileukemic activity of the combined GM-CSF/hydroxyurea therapy rather than an antileukemic effect of the conditioning regimen.

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key words: autologous stem cell transplantation, chronic myelogenous leukemia, granulocyte-macrophage colony-stimulating factor, hydroxyurea

USE OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF) IN COMBINATION WITH HYDROXYUREA AS POST-TRANSPLANT THERAPY IN CHRONIC MYELOGENOUS LEUKEMIA PATIENTS AUTOGRAPHED WITH UNMANIPULATED HEMATOPOIETIC CELLS

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder due to an acquired abnormality in a pluripotential hematopoietic stem cell. CML is cytogenetically marked by the Philadelphia (Ph) chromosome, which originates from a reciprocal translocation between chromosomes 9 and 22, and molecularly marked by a chimeric BCR/ABL gene, resulting...
from the juxtaposition of the ABL proto-oncogene on chromosome 9 with the BCR gene normally located on chromosome 22. The chimeric BCR/ABL gene expresses an 8.5-kb hybrid messenger RNA transcript that gives rise to a 210-kD fusion protein (p210BCR/ABL) with increased tyrosine kinase activity, transforming activity for hematopoietic cells, and the ability to cause CML-like myelopoesis in mice.

Conventional chemotherapy has not altered the natural history of CML. Busulfan or hydroxyurea may control clinical symptoms but neither is able to delay blast transformation, although hydroxyurea may prolong survival by one year. Recently, recombinant interferon-α (IFN-α) has been shown to induce hematological remission in 70 to 80% of CML patients, with a complete cytogenetic remission and polyclonal hematopoiesis being detectable in 10 to 20% of patients, and delay blast transformation. Allogeneic bone marrow transplantation remains the only potentially curative treatment for CML. However, more than 70% of patients will be ineligible for allogeneic marrow transplant either because they do not have a suitable HLA-matched related or unrelated donor or because they are more than 50 years old.

Several experimental and clinical findings support a role for autologous stem cell transplantation (ASCT) in CML. Current clinical results using either unmanipulated or purged autologous stem cells show that, despite a transient phase of Ph-negative hematopoiesis, ASCT leads to 5% long-term cytogenetic remission, with leukemic relapse being the main cause of treatment failure. Although cure of the disease has not yet been achieved using autologous transplantation, long-term survival is possible and indeed approaches the survival rate for allogeneic related donor transplant recipients. ASCT should be performed early in the clinical course of the disease, results in advanced CML being completely disappointing. To improve the therapeutic index of ASCT, it is likely that either in vitro purging of the leukemic stem cells or in vivo post-transplant manipulation of the lymphohematopoietic system will be required.

Colony-stimulating factors (CSFs) are a family of glycoproteins able to control the survival, proliferation, differentiation and functional activation of hematopoietic cells both in vitro and in vivo. Randomized trials demonstrated that recombinant granulocyte-macrophage-CSF (GM-CSF) and recombinant granulocyte-CSF (G-CSF) can be safely administered to patients undergoing ASCT for lymphoid or myeloid malignancies as well as for solid tumors in order to accelerate post-transplant hematopoietic reconstitution. Despite the fact that CSFs have the potential to stimulate the in vitro growth of leukemic cells, several randomized trials conducted in patients with acute mylogenous leukemia (AML) have shown that GM-CSF administered following remission induction therapy does not stimulate the regrowth of AML cells and does not induce increased relapse rates, whereas it is capable of preferentially stimulating the proliferation of residual normal stem cells, as suggested by a significant reduction in chemotherapy-induced neutropenia. The potential of CSFs to directly or indirectly activate proliferative mechanisms by selectively stimulating residual normal hematopoietic cells has also been shown in patients with leukemia or myelodysplasia who relapsed following allogeneic marrow transplantation and were given G-CSF. In addition to its effects on hematopoietic cell proliferation, GM-CSF has been shown to have the capacity to activate immune defense mechanisms which mediate tumor cell killing.

Based on in vivo as well as in vitro evidence, including defective self-maintenance and deregulated cycling control of primitive leukemic progenitors capable of initiating and sustaining hematopoiesis in long-term culture (LTC-IC), it has been suggested that the early phase following autografting of the Ph-negative clone has a proliferative advantage over the Ph-positive clone. We hypothesized that post-transplant GM-CSF administration could reactivate the functional activity of quiescent normal progenitors and prolong the duration of the post-transplant proliferative advantage of Ph-negative over Ph-positive progenitors.

In order to evaluate the effect of post-transplant GM-CSF administration, a pilot clinical study was carried out in which CML patients resistant to IFN-α therapy were autografted with unmanipulated marrow or blood cells and given prolonged GM-CSF therapy post-transplant.

**Materials and Methods**

**Patient population**

Five adult (age range: 30 to 44 years) patients with a diagnosis of Ph-positive CML were treated with high-dose chemotherapy followed by ASCT with either marrow (n = 2) or blood (n = 3) cells. The clinical characteristics of the patients are reported in Table 1. All patients fulfilled the following criteria set for entry into the study: 1) Ph-positive CML; 2) ≤ 60 years old; 3) ineligible for allogeneic or unrelated marrow transplant; 4) resistant to IFN-α therapy. At the time of transplant, 4 patients were in chronic phase and one in blastic phase. The interval between diagnosis and ASCT ranged from 18 to 54 months.

**Stem cell collection**

Patients #1 and 2 were autografted with marrow cells harvested 26 and 53 months after diagnosis, respectively. Marrow was harvested in first chronic phase (case #1) or at transplant (case #2) from the posterior iliac crest using routine methods. Harvested marrow was suspended in TC199 medium containing 1000 U/dl heparin. The marrow cell suspension was passed through steel mesh filters, centrifuged (2,500 rpm, 20 min) and the buffy-coat was cryopreserved in 10% dimethyl sulfoxide, 55% autologous plasma and TC199 medium. Patients #3-5 were autografted with peripheral blood cells harvested and cryopreserved at diagnosis. No mobilization regimen was used before collection of peripheral blood cells.
When platelet counts were < 10^9/L, platelet transfusions were administered subcutaneously at a dose of 5 µg/kg/day, starting within 24 hours of completion of the autologous stem cell infusion and continuing until a white blood cell (WBC) count ≥ 2 × 10^9/L was achieved on two consecutive examinations. An absolute neutrophil count of 0.5 × 10^9/L was achieved by days +18 and +25, in patients reinfused with autologous marrow, and days +13, +14, and +15 in those reinfused with autologous blood. A platelet count of 50 x 10^9/L was achieved by days +20 and +48 (marrow reinfusion) and +14, +16, and +14 (blood reinfusion).

**Cytogenetic analysis**

Table 2 and Figure 1 summarize the results of cytogenetic analysis. At stem cell harvest, cytogenetic analysis revealed 100% Ph-positive metaphases in all patients, while at autografting 3 out of 5 patients revealed 9% Ph-negative metaphases (Table 2). During the initial phase of hematopoietic regeneration, direct cytogenetic analysis revealed 100%, 81%, and 20% Ph-negative marrow metaphases in cases #1, 2, and 4, whereas no evidence of functional activity of a nonleukemic clone was detected in cases #3 and 5 (Table 2). In patient #1, sequential cytogenetic analysis revealed a decrease of Ph-negative hematopoiesis which was no longer detectable at month +9 post-ASCT when the patient evolved into blast crisis (Figure 1). A similar decrease over time of nonleukemic hematopoiesis was evident in case #2. The behavior of Ph-negative hematopoiesis was different in cases #3 and 4. Case #3 displayed cyclic Ph-negative hematopoiesis that peaked at months +4 and +8. Patient #4 showed a low percentage (20%) of Ph-negative metaphases 1 month after ASCT, followed by a significant expansion of nonleukemic hematopoiesis, which could be detected up to month +13. No evidence of Ph-negative hematopoiesis could be detected in patient #5.

### Clinical course

No serious reactions occurred during the infusion of unmanipulated stem cells. As shown in Table 2, GM-CSF therapy was regularly administered in all patients for 5 to 9 months following ASCT without significant evidence of leukocytosis. Patients #1, 2, and 5 failed to show the side effects usually reported in patients receiving GM-CSF. By contrast, in the other two patients the appearance of allergic phe-
Table 2. Results of cytogenetic analysis before and after autografting.

<table>
<thead>
<tr>
<th>Case</th>
<th>Harvest</th>
<th>Autograft After autograft (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 19 25 34</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0 40 33 17 0 8 14 17 0 0 0 7 12</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>8 71 75 80 50 44 33 21 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0 9 21 32 15 0 11 25 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>9 20 51 42 20 36 21 42 37 38 35 33 25 20 0 0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>8 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
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*Percentage of Ph-negative bone marrow metaphases obtained after routine cytogenetic analysis of freshly aspirated bone marrow.

Duration of GM-CSF therapy.
between leukemic and normal stem cells could be driven in favor of the latter by means of in vivo agents which enhance the proliferative activity of the normal clones or trigger antileukemic immune effects.13

In vitro evidence demonstrating defective self-maintenance of Ph-positive stem cells associated with a deregulated cycling control of leukemic LTC-IC supports the concept that in the early phase following autografting the Ph-negative clone has a proliferative advantage over the leukemic clone.25-28 We hypothesized that prolonged post-transplant GM-CSF administration could further enhance the proliferative advantage of Ph-negative over Ph-positive progenitors. We report that the combined post-transplant administration of GM-CSF and hydroxyurea was able to induce a transient phase of partial Ph-negative hematopoiesis in four out of the five CML patients autografted with unmanipulated cells in chronic phase (3 cases) or blast transformation (1 case). In the present study, the percentage of CML patients achieving a transient phase of Ph-negative hematopoiesis was significantly higher than in previous reports using unmanipulated hematopoietic cells,31-33 and similar to those detected using in vitro purged hematopoietic cells.34,35 The two patients (#1 and 2) who were autografted with bone marrow showed Ph-negative hematopoiesis that appeared immediately after transplant and progressively declined. In contrast, in cases #3 and 4 Ph-negative hematopoiesis appeared 3 and 1 months after ASCT and was evident up to 8 and 13 months post transplant, respectively. The late appearance of Ph-negative hematopoiesis detected in patients #3 and 4 cannot be viewed as a direct consequence of the antileukemic effect of the conditioning regimen; it is likely to be due to the antileukemic activity of the combined GM-CSF/hydroxyurea therapy.

The mechanism(s) by which GM-CSF administration could induce and/or prolong Ph-negative hematopoiesis remain speculative. In a murine model of spontaneous B-cell leukemia, it has been shown that GM-CSF has the potential to directly or indirectly activate proliferative mechanisms favoring the proliferation of normal hematopoietic cells.36 It is well known that glycosaminoglycan-mediated adherence of hematopoietic cells to stromal cells plays a major role in the compartmentalization of GM-CSF and in hematopoietic cell proliferation and differentiation.37,38 Therefore one could hypothesize that GM-CSF administration might preferentially stimulate the proliferation of stroma-adherent progenitors and increase their rate of commitment towards terminal differentiation.39 Since we have previously shown that the stroma-adherent progenitor cell compartment is enriched in Ph-negative progenitors,40 one could argue that post-transplant GM-CSF therapy may have preferentially stimulated the proliferation of otherwise quiescent stroma-adherent Ph-negative progenitors that are not capable of expressing their functional activity during chronic phase.

Although we did not study the immune effects triggered by GM-CSF therapy, the theoretical possibility exists that in vivo changes in the immunological reactivity of our CML patients were involved in the achievement of post-transplant Ph-negative hematopoiesis. GM-CSF has been shown to have the capacity to activate immune defense mechanisms which mediate tumor cell killing.25-27 In vitro studies have demonstrated that GM-CSF can increase the functional activities of effector cells, such as antibody-dependent and antibody-independent cytotoxicity, through a tumor necrosis factor-dependent mechanism.41-44 An effect of GM-CSF in combination with interleukin-2 on lymphokine-activated killer cell induction and function was recently reported.45,46 In patients with non-Hodgkin lymphoma who underwent ASCT, we have demonstrated that low-dose, post-transplant administration of GM-CSF results in a significant increase in natural killer cell activity.47 Similarly, GM-CSF has been shown to enhance activated killer cell function following ASCT in AML patients.48

In conclusion, the results of the present pilot study demonstrate that combined GM-CSF and hydroxyurea therapy in CML patients seems to be effective in inducing and/or prolonging a transient period of Ph-negative hematopoiesis. The therapeutic efficacy of this combination might be related either to preferential stimulation of nonleukemic progenitors or to activation of immune mechanisms which down-modulate the proliferation of leukemic progenitors. However, studies on a larger group of patients with disease in the first chronic phase and molecular monitoring of the CML clone49 will be required to evaluate the potential of this combination therapy. Finally, post-transplant manipulation of the immune-hematopoietic system could be associated with in vitro purging techniques which have already been shown to be able to reduce the size of the neoplastic clone.

References


