INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease due to a unique gene rearrangement event occurring within a pluripotent stem cell. The main characteristic of this mutation is the formation of a fusion gene (BCR-ABL) with increased tyrosine kinase activity from which a transforming activity for myelopoiesis derives. Clinically, CML is characterized by an initial chronic phase, followed by inevitable progression to an accelerated phase, and then to a terminal blastic phase.

Allografting represents the only therapeutic procedure capable of curing the disease. Most centers currently use HLA-identical sibling transplants for patients less than 55 years of age. However, the majority of patients do not have an HLA-matched sibling donor. Matched or partially matched unrelated donor transplants are associated with a high transplant-related mortality within the first 100 days following the procedure. This approach should be offered to patients less than 50 years of age.

Conventional therapy for CML includes hydroxyurea, busulfan and interferon alpha (IFN-α). Conventional chemotherapy is unable to modify the natural history of the disease. On the contrary, recent randomized studies suggest that IFN-α produces cytogenetic conversions to Philadelphia (Ph)-negative in some cases and may prolong overall survival [1-5]. Considering that the majority of Ph-negative patients maintain evidence of BCR/ABL positivity after IFN-α therapy, it is unlikely that IFN-α can cure the disease. In upcoming years, we must evaluate whether the high cost of IFN-α, the poor tolerance of it in about one-fourth of treated patients, and the small chance of long-term benefit will be justified by a higher overall survival than with hydroxyurea.

Rationale for Autografting in CML

As recently stated [6], there are at least three possible related rationales for the use of autografting to prolong life for patients with CML: A) Progression from chronic phase to more advanced phases may depend in part on “random” events occurring in primitive BCR-ABL positive stem cells; therefore, any maneuver that reduced their number might delay transformation; B) There is some evidence that steps that temporarily reduce the numbers of both normal and leukemic stem cells to low levels provide a proliferative advantage of normal stem cell generation over that of leukemic cells; and C) Several groups have reported that significant but suppressed normal
populations of very primitive hematopoietic cells are contained in the marrow, at least in some newly diagnosed patients. Since it seems that the normal hematopoietic reservoir declines with time, it may be desirable to mobilize and collect peripheral stem cells in order to store Ph-negative progenitors as soon after diagnosis as possible.

Clinically, this observation has been confirmed after treatment with IFN-α [3-7], intensive chemotherapy [8-11], and mobilization of Ph-negative progenitor cells into the peripheral blood after intensive chemotherapy [12-15]. Cytogenetic and other clonality studies suggest but do not prove that these cells are part of the original “normal” population [16-20]. In order to obtain such cells, in vitro and in vivo approaches have been evaluated.

**In Vitro Manipulations**

The Vancouver group showed in a series of elegant experiments that Ph-positive cell numbers decline when put in culture, whereas Ph-negative cells not previously identifiable emerged in those cultures and showed better survival [21-24]. The basic mechanism of this behavior is still unclear; however, it is important to point out that some of the emerging Ph-negative cells show characteristics of very primitive hematopoietic cells. As a result of these findings, the Vancouver group devised a trial consisting of a 10-day culture of CML bone marrow and subsequent infusion into a conditioned patient previously selected on the basis of the ability of his bone marrow to produce an adequate number of normal long-term culture-initiating cells (LTC-ICs) in vitro. Over a five-year period, they evaluated 87 patients and selected 36 for the 10-day marrow culture, of whom 22 have been autografted. Sixteen patients remained alive up to 68 months post-autograft, and five were in complete or partial cytogenetic remission [25]. This technique requires considerable care and would not be suitable for many institutions.

The Minneapolis group has shown that CD34+ cells that are also DR- are predominantly or exclusively Ph-negative; in contrast, CD34+/DR+ cells are all Ph-positive [26]. It has been recently reported that in patients in early chronic phase, CD34+/DR- cells are BCR-ABL mRNA negative in 80% of patients [26]. Large-scale selection with a high-speed fluorescence-activated cell sorter, starting from a marrow harvest of 2-2.5 liters results in 1-3 x 10^7/kg CD34+/DR- cells [5]. The frequency of colony-forming cells (CFCs) and LTC-ICs ranged from 2.6%-8.6% and 0.187%-0.233%, respectively. Both CD34+/DR- and secondary CFCs were BCR-ABL mRNA negative. Therefore, this large-scale clinical grade selection of CD34+/DR- cells allows a highly purified autograft and represents a promising step toward further gene manipulation developments.

Other approaches are the purging of Ph-positive marrow cells with interferon [27, 28] even if the previous reports were not completely confirmed, the incubation of harvested marrow with antisense oligodeoxynucleotides directed at the BCR-ABL junctional sequences [29, 30] or the upstream sequences of MYB [31, 32] CML patients. In a study conducted jointly by the Hammersmith Hospital in London and the University of Pennsylvania in Philadelphia autologous bone marrow cells were collected from patients with CML and subjected to an in vitro purging procedure using a 24mer phosphorothioate antisense oligomer directed against codons 2-7 of the human MYB gene [32]. To date, 10 patients have been recruited to this study and four have been rendered entirely or predominantly Ph-negative at the three-month post-autograft assessment. This Ph-negativity has been transient in all cases [31, 32]. In Rome, eight patients with advanced-phase CML have been treated with a 26mer phosphorothioate oligomer directed symmetrically at the BCR-ABL junction. In this study, in a similar manner to that of the MYB study, autologous stem cells were purged in vitro before subsequent return to the patient as an autograft [29]. After purging, 30%-100% of cells were Ph-negative, and in two patients, complete Ph-negativity was achieved post-autograft, albeit transiently.

Another concept for in vitro purging and in vivo usage is to employ an agent that would preferentially protect normal progenitors from the effects of chemotherapy. Macrophage inflammatory protein-1α is a candidate molecule that has an inhibitory effect on normal but not CML progenitors, and it may have clinical potential as a protective agent during chemotherapy or for chemotherapeutic purging of CML autograft material [33]. Finally, another novel approach involves incubation of marrow cells with ribozymes [34-36], catalytic RNA species that can be tailored to recognize and disrupt leukemia-specific mRNA molecules.

**In Vivo Manipulations**

Our team has now treated substantial numbers of patients, some resistant to IFN-α and some previously untreated, with more or less intensive chemotherapy (idarubicin, cytosine arabinoside, and etoposide) (ICE or mini-ICE protocols), followed by administration of G-CSF [13, 37, 38]. In most cases, it was possible to collect predominantly or exclusively Ph-negative myeloid progenitor cells; there is preliminary evidence that the Ph-negative progenitor cells...
were more readily collected in patients who had received only hydroxyurea and no previous interferon treatment.

The Swedish study has adopted an alternative approach to the in vivo purging technique [39]. CML patients are subjected to therapy of increasing intensity with the aim of achieving Ph-negativity in the bone marrow. Once Ph-negativity was achieved, patients proceeded to bone marrow harvest, which, despite a period on IFN, did not present any technical difficulties. A total of 194 patients have been recruited to this study, and only 4% of the 118 patients who have received IFN-α and hydroxyurea for six months became completely Ph-negative. Larger proportions became Ph-negative following successive cycles of chemotherapy. Overall, 47 patients (18% of total) achieved Ph-negativity, and 31 of these have undergone autografting with Ph-negative bone marrow. Of these 31 patients, 15 remain completely Ph-negative 35-65 months post-transplant. Sixty-eight percent of all patients entered into the study survive at six years. A proportion of all patients entered into the study have undergone allografting, which may significantly modify the survival data.

**Clinical Results with an In Vivo Technique Employed in Genoa**

One hundred sixty-two patients with Ph-positive CML in different phases of the disease entered our protocol (Table 1A). Thirty-eight patients were mobilized during their blastic phase, 28 patients in accelerated phase, and 96 patients in chronic phase. Thirty-eight patients in chronic phase were in the first year of disease and had not been pretreated with IFN (group A), 58 other patients had received previous IFN therapy: 27 patients for >12 months and 31 patients for <12 months (Table 1B).

Table 1A. Mobilization of Ph-negative progenitor cells in CML

<table>
<thead>
<tr>
<th>Patients</th>
<th>162</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median)</td>
<td>47 (range, 21-62)</td>
</tr>
<tr>
<td>Phase of disease</td>
<td></td>
</tr>
<tr>
<td>Blastic phase</td>
<td>38</td>
</tr>
<tr>
<td>Accelerated phase</td>
<td>28</td>
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<tr>
<td>Chronic phase</td>
<td>58</td>
</tr>
<tr>
<td>Early chronic phase*</td>
<td>38</td>
</tr>
<tr>
<td>Mobilization regimens</td>
<td>ICE, mini-ICE</td>
</tr>
<tr>
<td>Nonhematological toxicity</td>
<td>36 (22%) (ICE: 34)</td>
</tr>
<tr>
<td>Procedure-related deaths</td>
<td>8 (5%)</td>
</tr>
<tr>
<td>(BP: 5; AP: 2; CP: 1)</td>
<td></td>
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</tbody>
</table>

*<12 months from Dx not pretreated with IFN.

The treatment regimen for mobilization consisted of idarubicin 8 mg/m²/day on days 1-5, arabinosylcytosine 800 mg/m² by two-h infusion on days 1-5, and etoposide 150 mg/m²/day by two-h infusion on days 1-3 (ICE protocol). All the data concerning the patients pretreated with IFN-α have been recently updated [40]; we wish to focus here on patients in an early phase of disease and not previously pretreated with IFN. Seventeen patients received the ICE protocol, and, in the end, 21 patients were given the same drugs but for only three days (mini-ICE protocol). In all cases, G-CSF was given at 5 mg/kg from day 8 after chemotherapy. In Table 2, we have synthesized the patient characteristics at baseline before mobilization therapy. Leukaphereses were started the day the white blood cell (WBC) count exceeded 0.8 × 10⁹/l and at least 5-10 CD34+ cells/µl appearing in the peripheral blood. The procedure was performed until the total of CD34+ cells collected was at least 2 × 10⁶/kg (Fig. 1). All patients completed the mobilization protocol, and no patient died as a result of the procedure. Toxicity consisted mainly of modest alopecia, mild mucositis, and diarrhea in the majority of patients treated with the ICE protocol. In two patients, a grade 3 oral mucositis (WHO) and diarrhea occurred. In contrast, no patient treated with the mini-ICE protocol experienced any nonhematological toxicity. Cytogenetic analysis of collected peripheral blood...
progenitor cells (PBPCs) showed that disappearance of Ph chromosome was achieved in 24 patients (64%), and that there was a major cytogenetic response in seven patients (18%). A total cytogenic response (Ph-negative + MCyR) was achieved in 31/38 patients (ICE: 14/17; mini-ICE: 11/14) (Fig. 2). Comparison of these data with the results achieved in patients pretreated with IFN-α in late chronic phase revealed a higher rate of complete cytogenetic remission among patients mobilized early at diagnosis (Table 3). These results were supported by the significantly greater numbers of CD34+ cells, CFU-GMs, and LTC-ICs.

To date, 23 of 31 cytogenetic remitter patients on PBPCs underwent autografting (Ph1-negative: 18 patients; MCyR: five patients). High-dose therapy consisted of Busulfan (4 mg/kg/day × 4 days) (17 patients) or IVT (idarubicin, VP-16 and single-dose TBI) (six patients). No patient died, and all patients engrafted. After recovery, all patients were treated with low doses of interleukin 2 (2 M/U/daily for five days every nine weeks) and IFN (3 M/U/daily for eight weeks). After one week of rest, the cycle was repeated for a total of three courses. Subsequently, the patients were maintained with IFN alone. The median follow-up from autografting is 13 months (range, 2-63 months). Two patients evolved in blastic crisis at 18 and 29 months after autografting. The other 21 patients are in hematological remission, and 12 of them also in complete (six patients) or partial (six patients) cytogenetic remission in the marrow 2-35 months after autografting.

In conclusion, premobilization chemotherapy was able to provide preferential in vivo reduction of the Ph-positive stem cell population and to synergize the effects of G-CSF to stimulate the release of primitive Ph1-negative hematopoietic stem cells into the blood. According to these results, a European protocol under the aegis of EBMT has been proposed with the aim of finding answers to the following questions:

Which Patients Should Enter a Prospective Randomized Controlled Trial?

Clearly, all newly untreated patients, without HLA-compatible donors could reasonably be entered into an autografting study. Our proposal is different from a recent EBMT study and an MRC/ECOG study which have adopted somewhat different approaches. In the EBMT study, all patients are first treated with IFN for three months; the patients who achieve complete hematological response during this time are excluded from the autograft trial [41]. The main problem with this study is the high number of patients achieving complete hematological remission (~40%) after three months who are excluded from the autograft trial. Conversely, the MRC/ECOG study excludes only patients who achieve a good cytogenetic response after treatment with IFN, but, in this case also, three months is too short a time to achieve a major cytogenetic remission. We think that it would be better to start a randomized protocol early—at

![Figure 2. Flow chart of mobilization/autografting results on patients with CML early after diagnosis.](image-url)
diagnosis—in order to evaluate the role of IFN-α alone versus an intensive approach and autografting followed by IFN-α when the reduction of Ph1-positive clones is achieved.

When Should the PBPCs be Collected?

There is no doubt that the best collection should be free of disease and contain a high number of progenitors. The collection of these cells in patients who become Ph-negative after IFN could only be applied to a very small proportion of patients; on the contrary, the idea of collecting Ph-negative or prevalently Ph-negative PBPCs in an early phase of CML after chemotherapy would be attractive. In previous studies, we demonstrated that PBPCs collected from CML patients during early recovery after chemotherapy-induced aplasia contain Ph-negative cells which are able to sustain long-term Ph-negative polyclonal hemopoiesis after autografting [42]. In these studies, a high variability in the content of LTC-ICs was observed from patient to patient and among different collections. We have also found that LTC-ICs are more likely to be present in the Ph-negative collections [43]. Cytogenetic analysis of colonies derived from LTC-ICs showed there to be normal diploid elements. In patients with a long interval between diagnosis and mobilization, we could detect LTC-ICs in Ph-negative collections as well as in the absence of committed progenitor cell growth. Moreover, this suggests that this mobilizing protocol is able to produce an overshoot of probably quiescent (G0) normal cells. In order to quantify the normal hemopoietic reservoir in CML patients and to assess whether the duration of the disease reduces the normal hemopoietic stem cell pool, we compared the cumulative content of mononuclear cells, CD34+, CFU-GMs, and LTC-ICs of Ph1-negative blood from patients not pretreated with IFN-α who were mobilized in the first few weeks after diagnosis versus those pretreated with IFN-α one year or later after diagnosis. Moreover, we compared these data with those obtained when peripheral blood was collected from normal donors after five days of G-CSF. There was a significant difference between harvests in patients mobilized at diagnosis versus patients receiving IFN-α for > one year (Table 1B); therefore, the possibility of collecting a high number of Ph-negative progenitors decreases during the course of the disease [38].

CONCLUSIONS

Residual normal hematopoiesis is present at diagnosis in some patients. During their chronic phase, the leukemic cells can be destroyed with relative ease by therapy. Various markers exist whereby leukemia cells can readily be recognized and quantitated and may thus be exploited for therapeutic purposes. Recently, we confirmed that it was possible in some cases to mobilize “diploid” PBPCs in other hematological diseases with cytogenetic markers such as Ph-positive acute lymphoblastic leukemia and myelodysplastic syndromes [44, 45]. Thus, it is likely that attention in upcoming years will focus on this aspect, giving more impact to the role of autografting. From a therapeutic point of view, there seems to be general agreement that a patient less than 55 years with an HLA-identical sibling should receive an allograft. This procedure should also be offered to younger patients who are able to find a volunteer unrelated donor (VUD). Since it has been clearly demonstrated that the normal hematopoiesis reservoir declines with time, it may be desirable to mobilize and collect PBPCs in order to store Ph-negative or prevalently Ph-negative blood as soon as diagnosis as possible when the WBC count is being controlled by hydroxyurea while the search for a VUD proceeds. After six to eight months, if a donor is not found, the patient could undergo autografting with the previously stored Ph-negative progenitors followed by IFN-α therapy. In Figure 3, we have synthesized a therapeutic algorithm for newly diagnosed patients with CML.

Figure 3. Suggested therapeutic algorithm for newly diagnosed patients with CML.
REFERENCES


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