Background and Objectives. The potential role of autologous stem cell transplantation (ASCT) as an alternative therapeutic strategy in chronic myelogenous leukemia (CML) has been widely explored in pilot studies, but the clinical results in terms of survival have so far been evaluated only retrospectively and in heterogeneous groups of patients. The goal of our prospective study was to evaluate the feasibility and long-term efficacy of unmanipulated ASCT followed by low-dose interferon-α in a homogeneous group of patients affected by CML in a very early phase of disease.

Design and Methods. Twenty-six unselected consecutive patients with CML in chronic phase underwent stem cell collection at diagnosis, then received cytoreductive treatment with hydroxyurea and, subsequently, a busulphan-melphalan myeloablative regimen followed by unmanipulated stem cell graft within one year of diagnosis. Interferon was given a median of 6.5 months after transplant at escalating doses, starting from 0.5 × 10^6 IU 3 times/week, on the basis of the clinical and hematologic tolerance.

Results. Median chronic phase duration from diagnosis is 9 years. The ten-year projected probability of overall survival from diagnosis is 55% with a median follow-up of surviving patients of 9.5 years (8-10.5); median survival has not been reached after ten years.

Interpretation and Conclusions. Our experience suggests that high-dose therapy followed by unmanipulated peripheral blood stem cell transplantation and low-dose interferon-α is a feasible approach, which results in long-term survival in newly diagnosed CML patients. These data need to be confirmed in controlled trials comparing ASCT with other therapeutic approaches, such as the use of interferon-α alone or in combination with other agents.

Key words: chronic myelogenous leukemia, high-dose chemotherapy, autologous stem cell transplantation, interferon-α, long-term survival

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder characterized by a specific chromosomal translocation, t(9;22) (q34;q11), resulting in a BCR-ABL fusion gene which induces the production of a constitutively active dysregulated tyrosine kinase.1 At some stage of the disease, CML progresses from a chronic phase (CP) to a transformed phase. The moment at which this takes place is unpredictable but the median duration of the CP is 3–4 years. Death is usually due to the insidious or rapid onset of blast transformation (BC).

Currently accepted therapies for chronic phase CML range from relatively non-toxic oral medications to α interferon-based therapy or aggressive high-dose chemotherapy with allogeneic stem cell transplantation, which is the only potential curative strategy for CML. Unfortunately, allogeneic stem cell transplantation is limited to patients who can medically tolerate the procedure and have appropriate donors.2–7

Alternative approaches include conventional chemotherapy and investigational drugs such as homoharringtonine, a semi-synthetic plant alkaloid originally described in Chinese literature, and STI-571, which is a tyrosine kinase inhibitor with specific activity against ABL.8,9

Since the 1980s, autologous transplantation has been investigated in some studies trying to utilize the normal progenitor cells which co-exist with the malignant ones to reconstitute patients following myeloablative chemotherapy, but a definitive answer about the role of this procedure in CML is still lacking.10,11

We report here the ten-year follow-up of 26 Philadelphia chromosome positive (Ph+) CML patients autografted, in chronic phase early after diagnosis, with unmanipulated peripheral blood stem cells
(PBSC) and then given interferon-α (IFN) after the transplant. In this patient population, a 10-year projected probability of overall survival of 55% is documented after a minimum post-transplant follow-up of 8 years.

Design and Methods

Patients

Between 1987 and 1990, 26 patients referred to our Institution with a diagnosis of CML were autografted with unmanipulated PBSC within 1 year of diagnosis. Eligibility criteria were as follows: age ≤ 55 years, diagnosis of CML in chronic phase without an HLA compatible sibling, performance status <3 according to the World Health Organization (WHO) criteria, bilirubin less than 3 mg/dL, creatinine less than 2 mg/dL, left ventricular ejection fraction greater than 50% and no evidence of active infection at entry. Written informed consent was obtained from all patients. Their median age was 39 years (range 26-55) and 18 were males. All patients but two had Ph+ CML (100% bone marrow cells); the two Ph- patients showed a BCR/ABL rearrangement by Southern blot analysis. According to Sokal’s prognostic index, 20 cases were in the low-risk category, 5 in the intermediate-risk and 1 in the high-risk category.

At diagnosis, hydroxyurea 40 mg/kg/day was utilized in 20/26 patients. After leukapheresis, 23/26 patients received hydroxyurea before admission for PBSC transplant. The median white blood cell (WBC) count at that time was 12.3×10⁹/L (range 3.8-49.3); mild splenomegaly (1-4 cm below the costal margin) was present in 5 cases. The median time from diagnosis to stem cell reinfusion was 6.5 months (range 2-12).

PBSC collection

Stem cell collection was performed when the WBC count ranged between 20 and 30×10⁹/L. Leukaphereses were carried out without any priming utilizing a continuous flow blood cell separator a median time of two months after diagnosis (range 0.5-9), in order to obtain a total number of nucleated cells of 1×10⁹/kg of body weight.

Transplant conditioning and supportive care

The preparative regimen consisted of busulphan (4 mg/kg/day orally, day -6 to -3) and melphalan (60 mg/m² i.v. on day -2), as reported in previous studies. No growth factors were administered after PBSC reinfusion. Patients were nursed in a double room in a general hematology ward; prophylactic oral quinolones were given routinely during aplasia and broad spectrum i.v. antibiotic therapy was promptly instituted if a fever > 38°C developed. Blood products were irradiated with 20 Gy before infusion in order to prevent graft-versus-host disease.

Cytogenetics and Southern blot analysis

Cytogenetic studies were carried out on bone marrow cells according to standard methods at diagnosis, on day +60 after ASCT, pre-IFN and, subsequently every 6-12 months. At least 20-25 metaphases were scored whenever possible; when less than 10 metaphases were available, the results of analyses were considered as not evaluable.

Molecular analyses included the identification of specific rearrangements within the major BCR (M-BCR) gene by Southern blotting as well as detection of the type of chimeric BCR/ABL mRNA junction by polymerase chain reaction (PCR). Southern blot procedures, probes used to explore the M-BCR region, oligoprimer and the PCR technique have been reported elsewhere.

Statistical methods

Actuarial curves were estimated according to the Kaplan and Meier method. Surviving patients were censored on the last day of follow-up.

Results

PBSC collection

In all patients the target of 1×10⁹/L nucleated cells was obtained with a single apheresis procedure. No cytogenetic analysis was performed on the apheresis product, nor are there any data available about the cellularity of the infused graft in terms of mononuclear cells, colony forming units-granulocyte/macrophage and CD34+ cells, which were not routinely evaluated in our Institution in 1987.

Engraftment

Hematologic recovery was documented after a median of 16 days (range 11-22) for neutrophils >0.5×10⁹/L and 23 days (range 11-210) for platelets >50×10⁹/L. One treatment-related death occurred 4 months after transplantation, as a result of interstitial pneumonitis of unknown origin.

Toxicity

The main side effect, which occurred in 14/26 cases, was a severe but transient mucositis. Fever occurred in 24/26 patients, but only in 5 was it associated with a microbiologically documented infection. In no case was empirical amphotericin B administered. Six out of 26 patients required transfusional support; in detail, a median of 2 packed red blood cells units (range 1-5) and 8 platelets units (range 7-12) were administered to these 6 patients. The median duration of hospitalization was 30 days (range 11-50).
Post-transplant therapy
Twenty-four of the 25 evaluable patients (1 early death in the original cohort of 26 patients) were submitted to treatment with interferon-α a median of 6.5 months (range 2-20) after transplant. In the first 9 patients IFN was started late, with the aim of evaluating the duration of the cytogenetic response; subsequently we decided to anticipate IFN treatment to the time when complete engraftment was achieved.

The starting dosage was 0.5\times10^6 IU/m^2 3 times/week, which was increased on the basis of clinical and hematologic tolerance up to at least 3\times10^6 IU/m^2 3 times/week. One patient did not receive interferon-α because of persistent autoimmune hemolytic anemia which developed immediately after PBSC transplant and required azathioprine treatment; in another patient, interferon-α therapy had to be stopped after the first dose because of the onset of a severe cutaneous reaction.

The median duration of IFN treatment in the 23 evaluable patients is 60 months (range 3-113). So far 17 patients have stopped the treatment for the following reasons: disease progression (12), lack of hematologic response (2), intolerance (1), porphyria (1), and systemic lupus erythematosus (1).

Cytogenetics
Cytogenetic analysis routinely performed on day +60 and before IFN treatment, showed that 13 of the 24 (54%) Ph+ patients at diagnosis had a cytogenetic response; in particular, 2 complete (100% Ph- metaphases), 5 major (>65% Ph- metaphases) and 6 minor (>33% and ≤65% Ph- metaphases) responses were recorded. Of the 2 Ph+, BCR/ABL+ cases, one showed the disappearance of the molecular signal by Southern blot analysis, while the second was still positive. In the first case, the analysis subsequently performed by PCR was able to detect the presence of a BCR/ABL transcript.15

When interferon-α therapy was started, 9 of the 13 responding patients had lost their cytogenetic response. Sequential cytogenetic analyses performed at different times during the interferon-α treatment showed a recovery of the karyotypic conversion in 4 cases who had obtained a prior cytogenetic response; moreover, a major cytogenetic response was observed in 1 of the 11 patients with no karyotypic conversion following the autograft. A summary of the cytogenetic course and outcome of the patients is reported in Table 1; in any case, no correlation could be detected between the cytogenetic response after ASCT and after IFN treatment and outcome.

Table 1. Cytogenetic course and outcome.

<table>
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<tr>
<th>Case</th>
<th>Cytogen. before autograft</th>
<th>Best cytogen. response after autograft</th>
<th>Best cytogen. response after IFN</th>
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IFN, interferon; PCR, polymerase chain reaction; not eval, not evaluated; BC, blast crisis; CP, chronic phase; AP, accelerated phase; IP, interstitial pneumonitis.

Outcome
At the time of this analysis, 14 patients are alive a median of 9.5 years (range 8-10.5) after diagnosis, 12 are in first chronic phase, 1 in accelerated phase and 1 in blastic crisis. In detail, 5 of the 14 surviving patients belonged to the intermediate/high-risk category according to Sokal’s index. The median follow-up of these patients is not significantly different from that of the low-risk patients. A total of 12 patients have died (11 of disease progression and one of interstitial pneumonitis in chronic phase), a median of 5 years after diagnosis (range 0.6-10.5).

Overall survival from diagnosis is 73% at 5 years. The 10-year overall survival is 55% and the median survival has not yet been reached (Figure 1). The median chronic phase duration from diagnosis is 9 years (Figure 2). In our series the achievement of any degree of Ph-negative hematopoiesis at any time after autografting or IFN was not associated with a trend towards better survival.
Discussion

Following initial studies which showed that autografting CML patients with chronic phase cells reinjected after transformation was successful in reestablishing a short-term chronic phase,\textsuperscript{10,16} it became evident that autografting in chronic phase induced a sustained period of Ph-negative hematopoiesis in a limited group of patients who received unmanipulated autologous cells after intensive treatment.\textsuperscript{17,18} It is conceivable that the autograft acts either by resetting the balance between normal and neoplastic clones or by decreasing the number of stem cells which are stochastically available for a transforming hit.\textsuperscript{19} Many strategies have been proposed to improve the results of ASCT in CML, including chemical purging, long-term cultures, purging with gamma-interferon as well as in vivo selection of Ph- cells after chemotherapy, as reported by Carella et al. in Genoa.\textsuperscript{20-25} Despite these approaches having provided interesting information from a biological point of view and having suggested a potential role for high-dose chemotherapy in the therapeutic management of CML, no definitive evidence of their clinical relevance has so far been provided.\textsuperscript{26,27}

The survival of recipients of ASCT in chronic phase CML has been retrospectively evaluated in two series of patients: McGlave et al.\textsuperscript{28} analyzed data collected from 142 patients in 8 transplant centers in Europe and in the United States. Their results suggested a possible beneficial impact of this treatment on overall survival, by showing a plateau at 4 years after transplant and a 58% probability of survival after five years. The limits of this study were the selection of patients and the heterogeneity of treatments received and purging procedures, although in multivariate analysis survival was not influenced by either stem cell source or purging procedure.

More recently, the EBMT Registry reported on 49 patients autografted over a 3-year period. These patients received transplants because they did not respond to IFN or had unfavorable prognostic factors at diagnosis; they showed a probability of survival at 3 years of about 75%.\textsuperscript{29} However, this study was retrospective and based on a cohort of patients selected for negative prognostic factors; furthermore, the relatively brief follow-up (3 years) does not allow definitive conclusions to be drawn.

We treated 26 patients with a homogeneous approach and followed them up for a median of 9.5 years over an 8-10.5 year time period. Our results show a 10-year projected probability of overall survival of 55% and appear extremely encouraging. Despite the limitation represented by the small number of cases reported herein, our study has some intrinsic features which strengthen it. In fact, our patient population, which was included in the study over a three-year period (1987-1990), received a homogeneous pre-transplant conditioning regimen, was grafted within one year of diagnosis with unpurged autologous blood progenitors and received post-transplant interferon therapy. The first patients enrolled in the study started IFN treatment with some delay because at that time we were trying to estimate the response to PBSCT, and concomitantly we were afraid of creating excessive toxicity by starting the IFN too early.

One major criticism of our work could concern the skewed distribution of the Sokal score in the study population, which may suggest either a selection bias in referrals or some other biasing process. A careful retrospective analysis of all CML patients who were referred to our institute between 1987 and 1990 ruled out the possibility of a selection bias in the patients’ enrollment. In fact patients with blasts in their peripheral blood and patients aged more than 55 years were considered ineligible for the transplant procedure, as were those with an HLA-compatible sibling, and it is these features which probably influ-

\[\text{Figure 1. Overall survival from diagnosis.}\]

\[\text{Figure 2. Time to blastic transformation from diagnosis.}\]
enced the Sokal distribution. In any case, of the 48 patients eligible for the transplant procedure, 26 were actually enrolled, 10 refused the transplant and 12 were included in a concomitant multicenter trial investigating the role of ASCT in IFN-α-responsive patients, and interestingly, the Sokal distribution in the 22 patients not included in the present study was skewed in a similar manner to that observed in the study population. Of course the question is: how would these patients have fared if they had been treated with interferon alone? We cannot make any comparison with results from other studies published in the literature, we can simply restate the results of a multicenter study from the Italian Study Group on Chronic Myeloid Leukemia which reported a ten-year probability of survival of 47% in low-risk patients treated with interferon alone.30

In conclusion, autologous transplantation followed by IFN-α as first-line therapy for newly presenting chronic phase CML patients lacking an HLA-matched donor, could represent a valid therapeutic strategy, also considering that the procedure need not be complicated by chronic debility; in fact most autograft recipients report normal or near normal activity levels following transplantation and low-dose IFN-α therapy. Of course the efficacy of this strategy needs to be conclusively confirmed in controlled, randomized trials comparing ASCT with other approaches, such as the use of IFN alone or in combination with other chemotherapeutic agents.

Contributions and Acknowledgments

GA was responsible for the cytogenetic analyses; FM and GM supervised the study over a twelve-year period; SC, PDF, MV, EM were responsible for data collection, analysis and medical care. The manuscript was prepared by GM and SC and reviewed by all the authors.

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Disclosures

Conflict of interest: none.

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Manuscript processing

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Potential implications for clinical practice

- High-dose chemotherapy followed by unmanipulated autologous PBSC reinfusion can be delivered, with low toxicity, to CML patients.
- Autograft followed by low-dose interferon-α treatment can result in long-term survival, but controlled studies are needed to evaluate the value of this combined approach compared to other treatment modalities.32-36

References