Mobilization/transplantation of Ph\textsuperscript{-} negative blood progenitor cells in chronic myelogenous leukaemia


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Introduction

Chronic myelogenous leukaemia (CML) is a clonal myeloproliferative disease which originates from primitive stem cells and is characterized by a reciprocal translocation between chromosomes 9 and 22. This results in generation of a chimeric gene (BCR/ABL) with increased tyrosine kinase activity. Such modifications cause CML-like myelopoiesis as has been recently demonstrated in mice [1]. Probably due to adhesion receptor abnormalities, CML progenitor cells display a defective adherence to normal bone marrow stroma and to purified fibronectin whereas Philadelphia-negative progenitors retain their ability to adhere to stroma [2, 3].

CML is characterized by an initial chronic phase inevitably followed by progression to the fatal blastic phase. Conventional therapy for CML includes hydroxyurea, busulphan and interferon alpha (IFN-\alpha). Although chemotherapy is unable to modify the natural history of this disease, recent reports appear to demonstrate that treatment with IFN-\alpha leads to a complete cytogenetic remission in 5% to 10% of patients and might prolong their overall survival. In a randomized study, the Italian Co-Operative Study Group demonstrated that IFN-\alpha induced more cytogenetic responses (complete/major/minor) than hydroxyurea (30% vs. 5%) and was associated with better overall survival (median 72 vs. 56 months) [4]. These data were confirmed in a U.K. MRC randomized multicentre trial [5]. However, a randomized study of the German Co-Operative Group did not demonstrate a survival advantage of IFN-\alpha versus hydroxyurea (median, 66 months vs. 57 months), although there was better survival with hydroxyurea than with busulphan. Patients with a complete cytogenetic remission following IFN-\alpha did not improve their survival compared with non-responders [6].

Allografting

Allografting could cure more than 60% of CML patients with positive results more evident in young patients receiving transplantation early in their disease [7]. Unfortunately approximately 75% of presenting patients are unsuitable for allografting either due to lack of an appropriate donor or to their relatively advanced age. In this circumstance, search for an HLA-unrelated donor has become a treatment option although this procedure is associated with a high rate of early mortality and morbidity resulting from acute and chronic graft versus host disease (GvHD) [8]. This mortality is reduced by T-cell depletion of donor marrow; however, an increase in the rate of CML relapse occurs. Recent data is also available on donor leukocyte infusions, alone or combined with IFN-2b, in patients relapsing after allografting [9, 10]. Future efforts will be addressed to identification of the effector cell populations (alloreactive CD8+ T-cells?) in patients relapsing after allografting, the specific cell able to develop only graft versus leukaemia (GvL) effect thus avoiding GvHD.

Autografting

Autografting could be an alternative approach for these elderly patients. Most studies of autologous peripheral progenitor cell transplants for CML in chronic phase indicate employment of blood progenitor cells which are collected at diagnosis [11-13]. Although significant decreases in the percentage of Ph\textsuperscript{-}positive marrow metaphases post-transplant were reported, only a minority of patients showed less than 25% Ph\textsuperscript{-}positive cells and up to a year only rare cases were completely Ph\textsuperscript{-}negative [11, 12]. Several authors suggest, based on historic controls, that there might be a survival advantage in autografting patients, particularly those who exhibit some Ph\textsuperscript{-}negative cells after grafting [11-13]. However, it is also likely that a significant percentage of
such patients will require repeat autografts because of early or late graft failure [11].

There is limited experience in the use of Ph'-negative cells in an attempt to increase the curative potential of autografting in CML patients. Differing approaches have been evaluated to obtain such cells: repeated courses of intensive chemotherapy [14], in vitro purging [15–17] and short-term culture techniques [18]. These promising studies have, to date, been limited to selected patients and in two studies only 25% of candidates were eligible for a transplant [14, 18].

Mobilization of Ph'-negative blood progenitor cells

That blood progenitor cells might be as successful as, or preferable to, marrow when used as an autograft for CML was suggested by Goldman et al. in 1974. He found that CFU in blood was greatly increased in patients with untreated CML, and was proportionally greater than in their marrow [19]. It was subsequently reported that Ph'-negative cells are detectable in newly diagnosed patients [20, 21], and that long term culture initiating cells (LTC-ICs) are found in high numbers [22]. Korbling reported that Ph'-negative blood progenitor cells collected from a single patient after recovery from cyclophosphamide-induced aplasia could reconstitute haematopoiesis in a myeloablated host [23]. Our team investigated this approach using acute leukaemia-type intensive therapy in patients previously treated with interferon. We observed that Ph'-negative collections were more likely to be harvested from patients in the chronic phase than the accelerated phase and within two years of diagnosis [24]. This was recently confirmed also in Ph'-positive acute lymphoblastic leukaemia patients [25]. Following this experience, the same harvesting procedure has been employed in patients within the first year of diagnosis and without prior exposure to interferon.

Mobilization/transplantation of Ph'-negative blood progenitor cells: The Genoa experience

Between May 1993 and July 1995, 50 patients with Ph'-positive CML in chronic phase entered our protocol. Sixteen patients had not been pre-treated with IFN-α (group A) and 34 had received previous IFN-α therapy (16 patients <12 months, 18 patients >12 months) (group B). Of the 16 patients in group A, 7 were female and 9 male with a median age of 47 years (range 21–59). Of the 34 patients in group B, 17 were female and 17 male with a median age of 41 years (range 20–59). Twenty-three patients in the trial were subsequently autografted.

The treatment regimen for mobilization consisted of idarubicin 8 mg/m²/day on days 1–5, arabinosycytosine 800 mg/m² given by 2 hour infusion on days 1–5 and etoposide 150 mg/m²/day given by 2 hour infusion on days 1–3 (ICE protocol). Recombinant G-CSF was given at 5 μg/kg or 150 μg/m² from day +8 until the total neutrophil count was greater than 0.8 × 10⁹/l for three consecutive days. Leukapheresis was begun when the WBC exceeded 0.8 × 10⁹/l and was performed daily until the WBC exceeded 3.0 × 10⁹/l. Intravenous antibiotic and antimycotic prophylaxis was given.

All patients of group A tolerated chemotherapy well and completed therapy. In group B, two patients did not complete the mobilization procedure: one patient died of gram-negative infection as prolonged aplasia and the other was found to have evolved to blast crisis. The median time to leukapheresis was 17 days (range 12–28) in group A and 20 days (range 11–69) in group B. In group A cytogenetic analysis of collected blood progenitor cells showed an absence of Ph'-chromosome in 12 patients (75%), a major response (<34% of Ph'-positive metaphases) in one patient. In group B the disappearance of Ph'-chromosome was documented in 12 patients (36%) and major responses in 10 patients. The comparison of the two groups' data revealed a higher rate of complete cytogenetic remission among patients in group A and these results were supported by the significantly greater number of mononuclear cells (5.3 vs. 0.5 × 10⁹/kg, P = 0.04), CD34⁺ DR⁻ cells (1.5 vs. 0.5 × 10⁹/kg, P = 0.0001) and CFU-GMs (10.8 vs. 0.5 × 10⁹/kg, P = 0.0001) collected from patients in group A.

Twenty-three of 50 mobilized patients underwent blood progenitor cell autografting (group A: 10 patients, group B: 13 patients). The high dose therapy consisted of busulphan or chemotherapy plus total body radiation. In group A all are alive and in haematological remission; five maintain a complete cytogenetic remission; five display cytogenetic relapse at 12 to 40 months. Median survival was 10 months from autografting (range 3–40). In group B, only two patients are still Ph'-negative at 3+ and 45+ months. Overall, 30% of the 23 patients who were autografted maintain Ph'-negative haematopoiesis after transplantation. The causes of death after transplantation in group B were: infection due to long lasting aplasia (2 patients) and blast crisis (2 patients).

Conclusions and biological considerations

In previous studies we have demonstrated that leukaphereses collected from CML patients during early recovery after chemotherapy-induced aplasia contain Ph'-negative cells which, autografted after high-dose chemotherapy ± radiotherapy, are able to sustain long-term Ph'-negative polyclonal haemopoiesis [26]. In those studies a high variability in the content of LTC-ICs was observed between patients and among different collections. It is unknown whether their values are related to the number of normal residual haemopoietic
cells. We have also found that LTC-ICs are more likely to be present in Ph\(^1\)-negative aphereses; subsequently, cytogenetic analysis of colonies derived from LTC-ICs identified normal diploid elements. In those patients with a long interval from diagnosis we could detect LTC-ICs in Ph\(^1\)-negative collections in absence of progenitor cell growth. This identifies a subset of patients in whom normal haemopoiesis is unlikely and normal Ph\(^1\)-negative haemopoietic reconstitution starts from very primitive cells. Moreover, it suggests that this mobilizing protocol is able to produce an excess of quiescent (G\(_\text{0}\)) normal cells. In another subset of patients, those mobilized shortly after diagnosis, LTC-ICs were detected at the same time as progenitor cells. Since the latter originate from continuous proliferation and differentiation of more primitive haemopoietic cells, these findings indicate the presence of actively proliferating normal Ph\(^1\)-negative haemopoiesis though overwhelmed by the expansion of Ph\(^1\)-positive population.

In order to quantify a normal haemopoietic reservoir in CML patients and to assess whether the duration of the disease reduces the normal haemopoietic stem cell pool, we have compared the cumulative content of Ph-negative aphereses in MNC, CD34\(^+\), GM-CFC and LTC-IC from patients not pre-treated with IFN-\(\alpha\), mobilized in the first few weeks from diagnosis, versus those pre-treated with IFN-\(\alpha\) over one year from diagnosis. Moreover, we have compared these two groups with collections obtained from peripheral blood of normal donors after five days of G-CSF.

A significant difference is shown between harvests from patients mobilized at diagnosis versus patients receiving IFN-\(\alpha\) for more than one year; therefore the possibility of collecting higher number of Ph\(^1\)-negative progenitors decreases with the course of the disease [27]. In patients not pre-treated with IFN-\(\alpha\) and mobilized at diagnosis, a correlation between CD34\(^+/\)Thy1\(^+\)/Lin\(^-\) and LTC-ICs was found only in those patients with entirely Ph-negative collections. In six of seven Ph\(^1\)-negative collections tested, there were more than 2 \(\times 10^5\) cells/kg CD34\(^+/\)Thy1\(^+\)/Lin\(^-\) cells found, but in only one of four Ph\(^1\)-positive collections were cells at these levels found. Additionally, in 10 of 11 Ph\(^1\)-negative collections, more than 3 \(\times 10^2\) cells/kg LTC-ICs were found but in only three of 12 Ph\(^1\)-positive collections were any LTC-ICs found and only at low levels (Carella et al., Genova).

In conclusion, in patients not pre-treated with IFN-\(\alpha\) and mobilized during the first weeks from diagnosis, the collection of blood progenitor cells during haemopoietic recovery after chemotherapy produces a yield of precursor cells (committed and LTC-ICs) largely sufficient for performing autografts associated with rapid haemopoietic recovery. This allows restoration of Ph\(^1\)-negative haemopoiesis in many patients. Further studies need to prove the negative influence of interferon treatment on mobilization and to determine whether, and for how long, Ph\(^1\)-negative status after autografting can be maintained.

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References


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