when G-CSF is used as a single mobilizing agent the
mobilization procedures (e.g. cytokine-activated BM).
(e.g. G-CSF + stem cell factor) or alternative harvest-
protocol protocols using combinations of growth factors
attempts of mobilization or for experimental collec-
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istics associated with successful mobilizing and auto-
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Satellite DNA analysis shows stability of donor
hematopoiesis following allogeneic transplan-
tation of peripheral blood stem cells

We assessed the hematologic reconstitution and long
term stability of donor hematopoiesis after allogeneic
PBSC transplantation in 34 patients with different hema-
logic disorders. Their blood counts remained stable
during the observation period (1-4 years). Donor
chimerism, evaluated by satellite DNA, could be predic-
tive of a low risk of relapse.

Sir, After initial experiments in mice suggesting that cir-
culating stem cells (PBSC) provide marrow repopula-
tion after myeloablation, PBSC have been used in
humans to restore hematopoietic function after high
dose chemotherapy. Both in autologous and allogeneic
settings, it has been demonstrated that PBSC are able
to increase the speed of hematologic recovery.1-3 Recent
reports4-6 suggest that PBSC ensure not only a faster,
but also a stabler hematologic recovery following allo-
geneic transplantation (PBSCT). We present here the
analysis of engraftment and chimerism in 34 patients
who underwent PBSCCT for different hematologic dis-
ases. Donor mobilization, as well as cell characteri-
ization were performed following standard guidelines.10
In all patients the graft consisted of PBSC alone, with
a median content of 82.2×10^9/kg CFU-GM,
8.4×10^9/kg CD34+ cells. All patients engrafted but one
who died on day +7. The patients recovered >
0.5×10^9/L polymorphonuclear cells at a median of 14
days (range 11-20) and $>50 \times 10^9/L$ platelets at a median of 15.5 days (range 12-52). Twenty-one patients were evaluable for long-term graft performance. Their hematologic values at 1 (21 patients), 2 (17 patients), 3 (13 patients) and 4 years (6 patients) remained stable during the observation period and none of the patients experienced late graft failure.

The polymerase chain reaction (PCR) amplification of DNA mini and microsatellites was used to monitor both engraftment and chimeric status. This technique has been described elsewhere.\(^\text{10}\)

Twenty-eight patients were evaluable for chimerism assessment (Figure 1). They were studied 1 to 48 months post-transplantation. Eighteen patients (64.2%) showed full-donor chimerism at all times post-graft and are so far disease-free. Of the other 10 patients, one (#8) exhibited exclusively mixed chimerism, relapsed and died six months post-transplantation. Patients #19 and #27 showed a mixed chimerism on two occasions during the post-transplant course and relapsed with mixed chimerism 30 and 18 months respectively following the graft. One patient never showed recipient cells before his relapse 18 months post-graft (#20). Four patients (#4, 13, 15, 23) showed the presence of recipient cells at various times during their follow-up, but ultimately became full-donor chimeras at 18, 24, 9 and 3 months respectively and two patients (#11, 18) showed mixed chimerism only at the last follow-up at 36 months post-graft without any sign of disease.

The present work gives the molecular evidence of marrow donor chimerism over a long follow-up period after PBSCT, thus confirming that mobilized PBSC are not only able to ensure fast engraftment but also to sustain long-term hematopoiesis of donor origin after transplantation. Our data seem to suggest that the detection of complete donor chimerism could be predictive of a lower probability of relapse. In fact 18/19 of patients who achieved a complete and stable chimeric status remained disease-free. The possibility that a relationship between chimeric status and GVL effect of graft exists is an intriguing issue that needs to be further investigated.

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Key words
PBSC, allogeneic engraftment, stem cells, G-CSF.

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References
Unsuccessful allogeneic and autologous transplants after prolonged interferon-\(\alpha\) treatment in a pediatric patient with chronic myeloid leukemia

Recombinant or partially pure human leukocyte interferon-\(\alpha\) (IFN-\(\alpha\)) has shown promising activity in the treatment of chronic myeloid leukemia (CML).\(^4,5\) However, IFN-\(\alpha\) might inhibit self-renewal of the progenitor cells in CML\(^3\) and may result in irreversible alterations of the marrow microenvironment.\(^6\) This pediatric case report seems to confirm the negative impact of prolonged IFN-\(\alpha\) treatment on subsequent stem cell transplantation.\(^7\)

Sir,
A 13-year-old girl, diagnosed as having Ph1-positive CML at another hospital, had received hydroxyurea for three months and IFN-\(\alpha\) (5 MU/daily) for 27 months before she was referred to our hospital, still with chronic phase CML, for unrelated cord blood cells transplant with two incompatible loci (B, DR).

The number of cord blood mononuclear cells was 2.7 \times 10^7/kg and that of CD34+ cells 13.5 \times 10^4/kg. Conditioning consisted of busulfan 16 mg/kg over 4 days, cyclophosphamide 60 mg/kg/d for 2 days, antithymocyte globulin 15 mg/kg/d for 6 days and steroids 1 mg/kg/d for 6 days. The girl received 10 \mu g/kg G-CSF from day +40 to +46. On day +46 engraftment had not been achieved and a marrow biopsy showed complete aplasia.

On day +48 from the first hematopoietic progenitor cell transplant (PCT), previously harvested autologous bone marrow was infused (TNC 10.3 \times 10^7/kg, GFU-GM 13.8 \times 10^6/kg). On day +41 after the second PCT engraftment had not been achieved.

A third transplant with peripheral stem cell CD34+ selection from a sibling with two mismatched HLA loci (B, DR) was performed. Conditioning consisted of antithymocyte globulin and steroids at the same doses used for the unrelated cord blood cells transplant. The number of CD34+ cells infused was 1.5 \times 10^5/kg.

On day +32 after the third PCT without marrow engraftment, 5 \mu g/kg GM-CSF was administered for 12 days. The patient developed bilateral pneumonia; Candida albicans was isolated from her sputum and despite treatment with intravenous amphotericin B, she died 45 days after her third PCT, still with no evidence of marrow engraftment.

There are contradictory reports on the effects of prior IFN-\(\alpha\) therapy on the outcome of PCT for CML patients. Tomás et al.\(^8\) and Zuffa et al.\(^9\) found that previous IFN-\(\alpha\) exposure had no adverse effects on the outcome of HLA identical sibling donor PCT for adult patients with CML. However, the Essen group observed that IFN-\(\alpha\) therapy for more than one year can compromise PCT results, with a greater transplant-related mortality, more delay and graft failure, and lower survival. Graft failure was only observed in PCT with unrelated donors.\(^8\)

Prolonged IFN-\(\alpha\) treatment, together with the long interval between diagnosis and first transplant, and the HLA disparity of both grafts may explain the inability of stem cells to repopulate after the allogeneic transplants in our patient. Since children with Ph1- positive CML in the first chronic phase are initially all candidates for allogenic PCT from a related or unrelated donor, it would be wise to avoid the use of IFN-\(\alpha\) as front line cytoreductive therapy in these patients.\(^9,10\)

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Key words
Chronic myelogenous leukemia, interferon-\(\alpha\), hematopoietic progenitor cells transplant, graft failure.

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