CFU-GM/mL did have a predictive value on collection (r^2= 0.51, p=0.0001 for PB CD34$^+$ cell count as shown in Figure 1; r^2= 0.57, p=0.0001 for CFU-GM, data not shown).

A large number of studies proved that the quantification of CD34$^+$ cells mobilized in PB is the most reliably factor predicting an adequate harvest.[4,5] Only a few authors investigated baseline parameters able to reflect mobilization capacities in single patients. Fruehauf *et al.*[6] in 1995 found that steady state PBPC counts allow estimation of the yield of mobilization when G-CSF is used in association with chemotherapy; the same authors recently confirmed this finding in a larger group of patients.[7] Paralleling the results of Husson *et al.*[8] and Haug *et al.*[9] our data prove that also when G-CSF is used as a single mobilizing agent the baseline PBPC count has a predictive value.

The amount of steady-state circulating CD34$^+$ cells before mobilization probably reflects the BM reserves which can depend on some individual characteristics and/or of therapy-induced microenvironment damage. The simple and fast flow-cytometric evaluation of circulating PBPC can help to recognize poor mobilizers,[10] identifying patients eligible for second attempts of mobilization or for experimental collection protocols using combinations of growth factors (e.g. G-CSF + stem cell factor) or alternative harvesting procedures (e.g. cytokine-activated BM).

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Satellite DNA analysis shows stability of donor hematopoiesis following allogeneic transplantation 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 hematologic disorders. Their blood counts remained stable during the observation period (1-4 years). Donor chimerism, evaluated by satellite DNA, could be predictive of a low risk of relapse.

Sir,

After initial experiments in mice[1] suggesting that circulating stem cells (PBSC) provide marrow repopulation after myeloablation, PBSC have been used in humans to restore hemopoietic 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.[2,3] Recent reports[4-9] suggest that PBSC ensure not only a faster, but also a stabler hematologic recovery following allogeneic transplantation (PBSCT). We present here the analysis of engraftment and chimerism in 34 patients who underwent PBSCT for different hematologic diseases. Donor mobilization, as well as cell characterization were performed following standard guidelines.[10] In all patients the graft consisted of PBSC alone, with a median content of 82.2×10^4/kg CFU-GM, 8.4×10^6/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

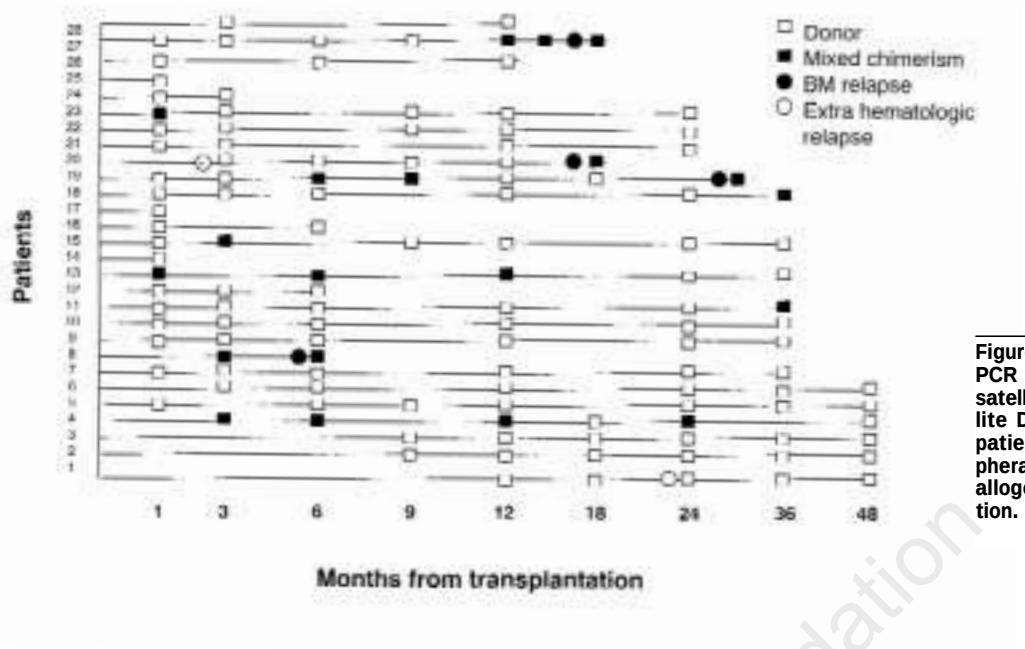

Figure 1. Results of PCR analysis for minisatellite and microsatellite DNA pattern in 28 patients receiving peripheral blood stem cell allogeneic transplantation.

days (range 11-20) and >50×10⁹/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.[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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Unsuccessful allogeneic and autologous transplants after prolonged interferon-α treatment in a pediatric patient with chronic myeloid leukemia

Recombinant or partially pure human leukocyte interferon-α (IFN-α) has shown promising activity in the treatment of chronic myeloid leukemia (CML).[1,2] However, IFN-α might inhibit self-renewal of the progenitor cells in CML3 and may result in irreversible alterations of the marrow microenvironment.[4,5] This pediatric case report seems to confirm the negative impact of prolonged IFN-α treatment on subsequent stem cell transplantation.

Sir,

A 13-year old girl, diagnosed as having Ph1-positive CML at another hospital, had received hydroxyurea for three months and IFN-α (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 2.7×10^7/kg and that of $CD34^+$ cells 13.5×10^6/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 µg/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 10.3×10^7/kg, GFU-GM 13.8×10^4/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 1.5×10^5/kg.

On day +32 after the third PCT without marrow engraftment, 5 µg/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-α therapy on the outcome of PCT for CML patients. Tomás *et al*.[6] and Zuffa *et al*.[7] found that previous IFN-α 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-α 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-α 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 allogeneic PCT from a related or unrelated donor, it would be wise to avoid the use of IFN-α as front line cytoreductive therapy in these patients.[9,10]

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