

Peripheral Blood Hematopoietic Progenitor Cells of Cytokine-Stimulated Healthy Donors as an Alternative for Allogeneic Transplantation

To the Editor:

Allogeneic transplantation in cancer patients is generally performed with bone marrow (BM) cells collected from the iliac crest of healthy donors under general anesthesia. In the autologous setting, peripheral blood stem cells (PBSCs) are increasingly used for transplantation.^{1,2} They are mobilized from the BM during recovery from chemotherapy or/and by treatment with recombinant human (rh) growth factors, and collected by at least one, but more commonly by several leukaphereses. In contrast, little is known about the quality and the potential use of PBSCs mobilized into the blood in healthy allogeneic transplant donors, as an alternative to BM stem cells.³⁻⁵

We have treated 17 healthy male volunteers aged between 20 and 30 years, with daily doses of 5 µg/kg body weight of either rh granulocyte colony-stimulating factor (rhG-CSF, Filgrastim, Hoffmann-La Roche, Basel, Switzerland; n = 9) or of granulocyte-macrophage colony-stimulating factor (rhGM-CSF, Molgramostim, Aesca, Traiskirchen, Austria; n = 8), for a period of 5 days. By day 5, the leukocytes per µL of blood had increased from a mean (±SD) of 6,500 (±790) to 33,000 (±5,400) in the G-CSF group and to 15,200 (±3,600) in the GM-CSF group. Compared with untreated controls (n = 5), the mononuclear cells (MNCs) collected after density separation of the blood samples had increased by 3.47-fold (±0.79) in the G-CSF cohort and by 2.6-fold (±0.85) in the GM-CSF cohort. The proportion of cells that expressed the CD34 antigen as determined by flow cytometric analysis rose from 0.14% (±0.07) to 1.7% (±1.3) and to 0.54% (±0.36) of the MNCs, respectively, thus resulting in an increase of absolute CD34⁺ cell numbers by 41.7-fold (±27.16, G-CSF) and by 13.82 fold (±4.89, GM-CSF). As confirmed in the clonogenic assay, the CD34⁺ MNC were hematopoietic progenitor cells, the majority of which gave rise to colonies derived from early colony-forming units (CFUs) (CFU-GM, erythroid burst-forming unit, CFU-mix) as previously described.⁶ Flow cytometric multiparameter analysis showed that they did not coexpress T- and B-cell antigens and that they were partially Thy-1⁺ and CD38⁻. In addition, their majority was found to be negative for CD45RA, c-kit (CD117), and CD33.

Two of the volunteers (one of each treatment group) agreed to undergo collection of PBSCs by leukapheresis in which their blood volume was processed twice. The leukapheresis products contained 3.7×10^{10} MNCs and 2.6×10^{10} MNCs, and the proportion of CD34⁺ progenitor cells was 0.35% of the MNC fraction in both cases. Multiparameter flow cytometric analysis and culture of the MNCs in the methylcellulose-based clonogenic assay showed that the collected stem cells were early myeloid progenitors of the red

and the white lineage that we expect to be required for successful engraftment.

These results are in agreement with another recent report⁷ and they show that, by a single leukapheresis, sufficient PBSCs can be collected from a cytokine-stimulated healthy donor to transplant greater than 3×10^8 MNCs and greater than 1×10^6 CD34⁺ MNCs per kg of an adult recipient.⁸

G. Fritsch
G. Fischmeister
O.A. Haas
C. Peters
H. Gadner
*Childrens' Cancer Research Institute
St. Anna Kinderspital
H. Strobl
Institute of Immunology
University of Vienna*
P. Höcker
M. Kurz
*Department of Transfusion Medicine
University of Vienna
Vienna, Austria*

REFERENCES

1. Kessinger A: Utilization of peripheral blood stem cells in auto-transplantation. *Hematol Oncol Clin North America* 7:435, 1993
2. Bensinger W, Singer J, Appelbaum F, Lilleby K, Longin K, Rowley S, Clarke E, Clift R, Hansen J, Shields T, Storb R, Weaver C, Weiden P, Buckner CD: Autologous transplantation with peripheral blood mononuclear cells collected after administration of recombinant granulocyte stimulating factor. *Blood* 81:3158, 1993
3. Kessinger A: Autologous and allogeneic transplantation with peripheral blood stem cells. *Prog Clin Biol Res* 377:603, 1992
4. Matsunaga T, Sakamaki S, Kohgo Y, Ohi S, Hirayama Y, Niitsu Y: Recombinant human granulocyte colony-stimulating factor can mobilize sufficient amounts of peripheral blood stem cells in healthy volunteers for allogeneic transplantation. *Bone Marrow Transplant* 11:103, 1993
5. Schwinger W, Mache C, Urban C, Beaufort F, Töglhofer W: Preliminary report: Single dose of Filgrastim (rhG-CSF) increases the number of hematopoietic progenitors in the peripheral blood of adult volunteers. *Bone Marrow Transplant* 11:489, 1993
6. Fritsch G, Buchinger P, Printz D, Fink FM, Mann G, Peters C, Wagner T, Adler A, Gadner H: Rapid discrimination of early

CD34⁺ myeloid progenitor cells using CD45-RA analysis. *Blood* 81:2301, 1993

7. Young DJ, Lane T, Terstappen LWMM, Ho AD: CD34⁺ subsets in growth factor mobilized apheresis products from normal subjects. *Ann Hematol* 68:A39, 1994 (suppl 1, abstr)

8. Shpall EJ, Jones RB, Bearman SI, Franklin WA, Archer PG,

Curiel T, Bitter M, Claman HN, Stemmer SM, Purdy M, Myers SE, Hami L, Taffs S, Heimfeld S, Hallagan J, Berenson RJ: Transplantation of enriched CD34-positive autologous marrow into breast cancer patients following high-dose chemotherapy: Influence of CD34-positive peripheral blood progenitors and growth factors on engraftment. *J Clin Oncol* 12:28, 1994