editorial
Haematologica 1995; 80:199-205

RECOMBINANT HUMAN G-CSF: HOW WIDE IS THE FIELD OF CLINICAL APPLICABILITY?

Franco Locatelli*, Paolo Pedrazzoli°

**Clinica Pediatrica, Università di Pavia, IRCCS Policlinico San Matteo, Pavia; °Divisione di Oncologia Medica, IRCCS Fondazione Clinica del Lavoro, Pavia, Italy*

The proliferation, differentiation and survival of hematopoietic progenitor cells is sustained by a family of glycoproteins termed hematopoietic growth factors, which are also able to promote the survival and influence the function of mature cells. The effects of these cytokines are mediated by interaction with specific receptors present on the surface of their target cells and this extracellular event, in turn, leads to the activation of intracellular signaling pathways that modulate the transcription of genes involved in the control of cell proliferation and/or differentiation.[1,2] The cloning of the genes encoding for hematopoietic growth factors and the availability of recombinant proteins have allowed demonstration that recombinant human granulocyte colony-stimulating factor (rHuG-CSF) is both an early-acting cytokine able to trigger the cycling of dormant progenitors surviving in G0, and a late-acting factor that regulates proliferation, differentiation and function of neutrophils and their progenitors.[2,3] These biological effects of rHuG-CSF have represented the basis on which various trials have been planned in order to establish the role of this cytokine in clinical practice.

In the United States and the majority of European countries, including Italy, the clinical use of rHuG-CSF has been approved for the amelioration of chemotherapy-induced neutropenia and the restoration of neutrophil production after bone marrow transplantation.[4-7] However, in some countries rHuG-CSF has also been approved for various other conditions such as myelodysplastic syndromes (MDS),[8] aplastic anemia (AA)[9] and severe chronic neutropenia.[10] In the future, the clinical utility of this growth factor is likely to be further expanded.

In this issue of Haematologica, four papers[11-14] address the question of less consolidated or quite innovative clinical applications of rHuG-CSF, underlining how the administration of this colony-stimulating factor can significantly contribute to transforming the therapeutic approach to some clinical problems. Perugini *et al.*[11] describe an elderly patient affected by an aggressive variant of MDS that presented with a pancytopenic picture complicated by a potentially life-threatening infection. After treatment with rHuG-CSF, the patient experienced a resolution of the infectious complication and achieved hematological remission. rHuG-CSF has been used frequently in patients with MDS, where cytokine therapy represents one of the most attractive forms of treatment. The use of hematopoietic growth factors aims at reversing the defective proliferation and differentiation of hematopoietic precursors within MDS marrow and, consequently, at modifying the dominant cytopenias with their related morbidities. Both recombinant human granulocyte-macrophage colony-stimulating factor (rHuGM-CSF) and rHuG-CSF have proven to be effective in increasing granulocyte production in 75-90% of neutropenic MDS patients.[8,15-19] Studies on the clonality of hematopoiesis after treatment with cytokines suggest that GM-CSF and rHuG-CSF do not preferentially stimulate normal hematopoiesis, but rather they induce differentiation of the abnormal clone without being able to eradicate it completely.[19,20] Data on neutrophil function are less conclusive, even though Negrin *et al.*[15] demonstrated an improvement in *in vitro* chemotaxis and phagocytosis after therapy with rHuG-CSF. Authors have also reported a significant reduction in infectious risk in those

Correspondence: Dr. Franco Locatelli, Clinica Pediatrica, Università di Pavia, IRCCS Policlinico S. Matteo, 27100 Pavia, Italy. Fax. international +39.382.527976.

patients reaching more than 1.5×10^9/L neutrophils when compared with their pre-treatment clinical history. rHuG-CSF therapy can also contribute to the resolution of established infections in those patients who experience a substantial increase in their neutrophil count. rHuG-CSF alone did not improve platelet and reticulocyte production in the reported trials on MDS patients; however, recent studies have demonstrated *in vitro* synergy between rHuG-CSF and rHuEpo for normal and MDS erythropoiesis.[21,22] Leary *et al.*[3] showed that rHuG-CSF enhanced the development of early precursors into erythropoietin-responsive progenitor cells. Two clinical trials documented a synergistic *in vivo* effect of rHuG-CSF and rHuEpo on the anemia of patients with myelodysplasia, with a substantial percentage of subjects showing both erythroid and myeloid responses.[23,24] Response was more frequent in patients with less severe pancytopenia and lower endogenous erythropoietin levels, but the durability of these responses must still be clearly assessed. Concern about possible risks involved in stimulating the proliferation of the leukemic cells and the consequent evolution of myelodysplasia to acute myeloid leukemia was raised in the early study of GM-CSF.[17] However, since an increase in blasts is part of the natural history of MDS, no firm conclusion can be drawn about the impact of cytokine therapy on the transformation into acute leukemia. The long-term effects of both GM-CSF and rHuG-CSF on the natural history and survival of MDS patients remain to be established, and randomized controlled studies are awaited to determine whether colony-stimulating factors can really improve the duration and/or the quality of life of MDS patients. Moreover, since colony-stimulating factors should be administered for long periods, a careful evaluation of their cost must be taken into consideration.

Shwachman-Diamond syndrome is a rare inherited disorder characterized by pancreatic failure, short stature, metaphyseal chondrodysplasia and neutropenia that, together with an impairment of neutrophil chemotaxis, predisposes patients to developing infections.[25] The etiology of the Shwachman-Diamond syndrome remains elusive and patients have an increased risk of developing both AA and acute leukemia (20 and 5%, respectively). As recently demonstrated for patients with the Kostmann syndrome,[26] a specific defect in rHuG-CSF signal transduction that causes either defective myeloid proliferation or disturbed granulocyte maturation can be hypothesized. The case report described by Ventura and Dragovich in this issue of Haematologica[12] confirms that, as previously published by other authors,[27,28] rHuG-CSF may be useful in selected Shwachman-Diamond patients with more severe neutropenia and a high incidence of infectious complications. Pharmacological doses of rHuG-CSF can overcome the peculiar defect of these patients, driving their abnormal hematopoietic system to a more effective production of functionally active cells, with a consequent reduction of morbidity and mortality. As dramatically demonstrated in children with the Kostmann syndrome or cyclic neutropenia,[29,30] rHuG-CSF can substantially modify the natural history of the disease and the quality of life of children with the Shwachman-Diamond syndrome. However, since this therapy is palliative and not curative, its long-term effects must still be carefully assessed, with particular regard to the risk of stem-cell depletion or of cytokine-induced overstimulation of cells prone to neoplastic transformation.

Ippoliti *et al.*[13] report the case of a young male affected by AA who was successfully treated with a combination of cyclosporin-A (Cs-A) and rHuG-CSF. A number of studies over the last few years have documented the clinical utility of rHuG-CSF in patients with AA,[9,31,32] and there is no doubt that the natural history of AA is dramatically and rapidly changing; rHuG-CSF, through stimulation of granulopoiesis, may significantly contribute to the resolution of bacterial or fungal infections in patients with AA[9] and play a pivotal role in favoring the response to immunosuppressive treatment. Recently, the European Group for Blood and Marrow Transplantation (EBMT) Working Party on severe AA documented in a pilot study that the combined use of rHuG-CSF with immunosuppressive therapy (antilymphocyte

globulin, Cs-A and steroids) significantly reduces the mortality risk and increases the chances of hematological response in patients with acquired severe AA.[32] In fact, in the past, treatment of AA patients with antilymphocyte globulin produced a hematological response in about half the subjects.[33,34] However, those with a low neutrophil count ($< 0.2\times10^9$/L) were exposed to a marked risk of early mortality within the first 100 days. In a cooperative report of the EBMT,[32] rHuG-CSF reduced early infectious complications, thus increasing the number of patients surviving long enough to achieve a hematological response. The efficacy of the combined treatment is clearly demonstrated by the fact that more than 80% of the patients enrolled in the study displayed a partial or complete hematological response and the actuarial survival at 3 years was 92%. rHuG-CSF determined not only an early increase in the neutrophil count, but also a mobilization of hematopoietic progenitors in the peripheral blood. This very interesting observation invites intriguing speculation on the possible role of rHuG-CSF in favoring a reseeding of primitive progenitors, as well as offering a chance to collect these hematopoietic stem cells in the case of patients with a disease relapse, or in a future, theoretically conceivable, scenario of autotransplantation. In our personal view, the only concern about using rHuG-CSF in AA patients is that it is currently unknown whether this growth factor can favor the development of myelodysplasia or acute leukemia. In fact, these two disorders, as well as paroxysmal nocturnal hemoglobinuria, frequently follow or are associated with AA, thus supporting the hypothetical model according to which AA represents the clinical expression of an attempt by the immune system to cure abnormal or even clonal stem cells.[35] As discussed above for the Shwachman-Diamond syndrome, the *in vivo* use of rHuG-CSF raises the question of whether it could stimulate the expansion of cells prone to neoplastic transformation. Further studies and longer observation should provide a conclusive answer to this crucial question.

The last paper in this issue of Haematologica addressing the question of the clinical use of rHuG-CSF is that by Majolino *et al.*[14] These authors analyze the role of rHuG-CSF for the mobilization of peripheral blood progenitor cells in healthy donors for allogeneic transplantation. Here, we are exquisitely in the field of innovative treatment and a novel, extremely intriguing, clinical application of hematopoietic growth factors. The use of circulating progenitor cells (CPCs) for allogeneic transplants has been proposed and made possible by previous experience and results obtained with infusion of autologous peripheral blood stem cell as hematological rescue after myeloablative chemo-radiotherapy. As a matter of fact, autotransfusion of CPCs is rapidly replacing autologous bone marrow transplantation after high-dose chemotherapy for lymphoma and solid tumors.[36] The main reason for the extraordinary success of this procedure lies in the capability of CPCs to determine a much faster recovery of all hematopoietic lineages than is possible with bone marrow.[37] Due to this property, high-dose chemotherapy regimens with CPC support are employed as the initial treatment of several chemosensitive tumors, and many authors believe that this procedure is going to change current therapeutic strategies in a variety of malignancies.[38-40]

In the setting of autologous transplantation, CPCs have been mobilized into peripheral blood and collected on a large scale by leukapheresis after treatment with hematopoietic growth factors administered as single agents or, more frequently, following myelosuppressive chemotherapy. Duhrsen *et al.*[41] were the first to demonstrate that rHuG-CSF administered at accepted therapeutic doses (10 μg/kg/day) during steady state hematopoiesis is able to mobilize circulating progenitors. Based on this observation, a number of clinical trials were carried out, and these studies clearly demonstrated that rHuG-CSF-primed progenitors can repopulate the bone marrow and sustain hematopoiesis after myeloablative chemo- or radiotherapy.[42-44] The yield of rHuG-CSF-primed progenitors is extremely variable among patients; previous myelotoxic therapy, bone marrow neoplastic involvement and some still unknown variables are the major factors influencing stem cell col-

lection. Accordingly, a variable number of aphereses (3 to 4 in most studies) are needed to harvest the optimal dose of CPCs. Collection after chemotherapy, either at conventional or high doses, followed by G-CSF or GM-CSF takes advantage of the additional mobilizing effect of myelotoxic drugs.[45,46] As a consequence, the collection yield is more predictable and fewer leukapheresis procedures are needed.

The number of autologous CPCs required for optimal hematological recovery is still controversial, a fact that partially reflects difficulties in standardizing the quantitative assay used. Roughly 2×10^6 $CD34^+$ cells/kg or 10×10^4 CFU-GM/kg is considered to be sufficient for complete hematopoietic reconstitution. However, a greater number of CFU-GM or $CD34^+$ cells reinfused results in more rapid engraftment, with consequent reduction in the duration of both neutropenia and thrombocytopenia.[47,48] In our Institutions, we consider optimal a dose of $6\text{-}8\times10^6$ $CD34^+$ cells/kg, which has been shown to determine a rapid neutrophil and platelet recovery.

Concerns have been raised about the capability of CPCs to sustain life-long hematopoiesis. This issue was recently addressed in an elegant paper by Siena and colleagues.[49] The authors demonstrated that the behavior of a hematopoietic system reconstituted by autografting solely with mobilized CPCs is the same as that of one autografted with bone marrow. After a median follow-up of three years, no secondary irreversible graft failure was observed in 34 patients treated with myeloablative total body irradiation.

The results summarized above have made it possible to consider, as previously mentioned, the use of CPCs as an alternative to bone marrow for allogeneic transplantation, and some recently published reports have produced encouraging data in this field.[50,51] For obvious reasons, in the case of allogeneic transplantation only hematopoietic growth factors can be used ethically for the release of large numbers of progenitors in the circulation of healthy donors. Already at present and probably even more so in the future, the use of circulating hematopoietic stem cells mobilized with rHuG-CSF has the potential to deeply modify the traditional approach to donation for allogeneic transplantation. In fact, at present, donors undergo traumatic harvesting by means of multiple bone punctures, with the associated (albeit minimal) anesthesiological risk.

Therefore from the strictest ethical point of view, the practice of bone marrow transplantation poses, especially for young donors, a conceptual dilemma between *primum non nocere* (above all, do no harm) and *primum adiuvare* (above all, help) which is experienced with some discomfort.[52] It is evident that the use of CPCs mobilized by means of hematopoietic growth factors has the great advantage of saving the donor from both general anesthesia and the back pain associated with bone marrow harvesting. Moreover, preliminary studies highlight the possibility that the technique of transplanting peripheral blood hematopoietic stem cells may hasten the hematological recovery of patients and permit, through an effect of stem cell competition, achievement of marrow engraftment in clinical situations where the risk of graft failure is particularly high (namely allogeneic transplants using HLA-partially matched family donors or HLA-matched unrelated volunteers).[53,54]

Major concerns about the wide diffusion of this technique, at least today, for the donor are represented by the unknown long-term consequences of administering a hematopoietic growth factor, capable of stimulating the proliferation of both normal and malignant stem cells to a healthy subject, and for the recipient by the theoretical risk of increasing the incidence and/or severity of graft-versus-host disease (GVHD). In fact, patients transplanted with rHuG-CSF-primed progenitors receive a massive number of T-lymphocytes, whose subpopulations may differ significantly from those present in the bone marrow. Since the incidence of GVHD correlates with the number of donor clonable T-cells administered to the recipient,[55] it cannot be excluded that this kind of transplant could lead to an increased risk of immunological complications. However, it would not be completely surprising if the use of peripheral blood hematopoietic stem cells were associated

with a similar or even lower probability of GVHD than bone marrow transplantation. In fact, the percentage of cytotoxic T-lymphocytes, responsible for tissue damage in GVHD, is higher in bone marrow than in peripheral blood, where T-lymphocytes with helper/inducer activity are prevalent. Moreover, since bacterial and viral infections are known to act as a trigger for GVHD,[56] accelerating the hematological recovery after transplantation and consequently reducing the risk of infectious complications through the use of peripheral blood progenitors could lower the risk of this complication. Clinical trials on larger numbers of patients are urgently awaited in order to provide definitive answers to these problems.

Many other important questions remain concerning the use of CPCs in allogeneic transplantation. What is the optimal schedule of administration and the dosage of rHuG-CSF? When is the best time to collect peripheral blood progenitors? What is the minimun number of circulating stem cells that should be infused in order to achieve an optimal and durable engraftment of donor hematopoiesis? The study by Majolino *et al.*[14] confirms that treatment of normal donors with rHuG-CSF daily leads to collection of large amounts of circulating progenitor cells.[53,54,57] rHuG-CSF was given at 16 μg/kg/day subcutaneously for 4 days and leukapheresis was performed on days 4 and 5. $CD34^+$ cells peaked on day 4, with a median increase of 65.3 times over baseline values; a median of 754×10^6 $CD34^+$ cells were collected. As was shown in the autologous setting, there is also a wide variability among donors in the yield of CPCs for allogeneic transplants. Because the peak of peripheral blood progenitors varies in different patients, daily monitoring of $CD34^+$ cells is recommended in order to achieve the best results.

The minimum number of CPCs to be infused for allogeneic transplants has not yet been established. A minimum of 2×10^6 $CD34^+$ cells/kg of recipient body weight was suggested for bone marrow transplantation,[58] but this amount may not be enough when rHuG-CSF-mobilized peripheral blood stem cells are used.[57] It is evident that if engraftment of donor hematopoiesis is really a dynamic phenomenon depending on competition between both immunocompetent and hematopoietic stem cells of donor and recipient, the use of rHuG-CSF-mobilized peripheral blood progenitors offers a unique possibility for enormously increasing the magnitude of primitive donor progenitor cells infused. As previously mentioned, this technique could therefore also represent a way of optimizing the chances of hematopoietic engraftment in situations where the risk of graft rejection is markedly increased, either due to the intrinsic nature of the original disorder (namely severe AA) or due to a genetic disparity between donor and recipient (i.e. transplants using HLA-partially matched family donors or HLA-matched unrelated donors).[53]

Finally, it has been shown that CPCs obtained in large quantity by mobilizing procedures are ideal targets for transplantation-based gene therapy applications in AIDS, hematological genetic diseases and cancer.[59,60] As an example of such foreseeable applications, the transfection of drug resistance genes in neoplastic patients may render their stem cell compartment insensitive to the toxic effects of chemotherapy.[61] On the one hand, this will permit to increase the therapeutic index of cancer patients, and on the other hand, it will avoid the need for rHuG-CSF to hasten granulocyte recovery after chemotherapy, which at present is the main indication for the clinical use of this growth factor.

References

1. Metcalf D. The molecular control of cell division, differentiation, commitment and maturation in hemopoietic cells. Nature 1989; 339:27-30.
2. Ogawa M. Differentiation and proliferation of hematopoietic stem cells. Blood 1993; 81:2844-53.
3. Leary AG, Zeng HQ, Clark SC, Ogawa M. Growth factor requirements for survival in G0 and entry into the cell cycle of primitive human hematopoietic progenitors. Proc Natl Acad Sci USA 1992; 89:4013-7.
4. Morstyn G, Campbell L, Souza L, et al. Effect of granulocyte colony-stimulating factor on neutropenia induced by cytotoxic chemotherapy. Lancet 1988; i:667-72.
5. Crawford J, Ozer H, Stoller R, et al. Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small-cell lung carcinoma. N Engl J Med 1991; 325:164-70.
6. Sheridan WP, Morstyn G, Wolf M, et al. Granulocyte colony-stimulating factor and neutrophil recovery after high-dose chemotherapy and autologous bone marrow transplantation.

Lancet 1989; ii:891-5.
7. Taylor K, Jagannath S, Spitzer G, et al. Recombinant human granulocyte colony-stimulating factor hastens granulocyte recovery after high-dose chemotherapy and autologous bone marrow transplantation in Hodgkin's disease. J Clin Oncol 1989; 7:1791-9.
8. Negrin RS, Haeuber DH, Nagler A, et al. Treatment of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor. Ann Intern Med 1992; 110:976-84.
9. Kojima S, Fukuda M, Miyajima Y, Matsuyama T, Horibe K. Treatment of aplastic anemia in children with recombinant human granulocyte colony-stimulating factor. Blood 1991; 77:937-41.
10. Bonilla MA, Gillio AP, Ruggiero M, et al. Effects of recombinant human granulocyte colony-stimulating factor on neutropenia in patients with congenital agranulocytosis. N Engl J Med 1989; 320:1574-80.
11. Perugini O, Montanari G, Nalli G. Trilineage response to granulocyte colony-stimulating factor administration in a patient with myelodysplastic syndrome. Haematologica 1995; 80:234-7.
12. Ventura A, Dragovich D. Recombinant human granulocyte colony stimulating factor (rHuG-CSF) for treatment of neutropenia in Shwachman syndrome. Haematologica 1995; 80:227-29.
13. Ippoliti G, Invernizzi R, Negri M, Incardona S, Ascari E. Response to cyclosporin A and rhG-CSF in a case of aplastic anemia. Haematologica 1995; 80:230-3.
14. Majolino I, Buscemi F, Scimè R, et al. Treatment of normal donors with rhG-CSF 16 μg/kg for mobilization of peripheral blood stem cells and their apheretic collection for allogeneic transplantation. Haematologica 1995; 80:219-26.
15. Negrin RS, Nagler A, Kobayashi Y, et al. Maintenance treatment of patients with myelodysplastic syndromes using recombinant granulocyte colony-stimulating factor. Blood 1992; 78:36-43.
16. Vadhan-Raj S, Keating M, LeMaistre A, et al. Effects of recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes. N Engl J Med 1987; 317:1545-52.
17. Ganser A, Volkers B, Greher J, et al. Recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes. A phase I/II trial. Blood 1989; 73:31-7.
18. Herrmann F, Lindemann A, Klein H, et al. Effect of recombinant granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndrome with excess of blasts. Leukemia 1989; 3:335-8.
19. Greenberg PL. Treatment of MDS with hemopoietic growth factors. Semin Oncol 1992; 19:106-14.
20. Janssen JWG, Buschle M, Layton M, et al. Clonal analysis of myelodysplastic syndromes: evidence of multipotent stem cell origin. Blood 1989; 73:248-54.
21. Greenberg PL, Negrin R, Ginzton N. G-CSF synergizes with erythropoietin for enhancing erythroid colony formation in myelodysplastic syndromes. Blood 1991; 78:38a.
22. Souza LM, Boone TC, Gabrilove J, et al. Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. Science 1986; 232:61-7.
23. Negrin RS, Stein R, Doherty K, et al. Treatment of the anemias of MDS using recombinant human granulocyte colony-stimulating factor in combination with erythropoietin. Blood 1993; 82:737-43.
24. Hellstrom-Lindberg E, Birgegard G, Carlsson M, et al. A combination of G-CSF and erythropoietin may synergistically improve the anemia in patients with myelodysplastic syndromes. Leuk Lymphoma 1993; 11:221-8.
25. Alter BP, Young N. The bone marrow failure syndromes. In: Nathan DG, Oski FA, eds. Hematology of infancy and childhood. Philadelphia:Saunders Co. 1993:252-4.
26. Dong F, Hoefsloot LH, Schelen AM, et al. Identification of a nonsense mutation in the granulocyte colony-stimulating factor receptor in severe congenital neutropenia. Proc Natl Acad Sci USA 1994; 91:4480-4.
27. Bonilla MA, Gilman B, Gillio AP, et al. In vivo administration of recombinant human G-CSF corrects the neutropenia associated with Shwachman-Diamond syndrome. Blood 1989; 74:324a.
28. Adachi N, Tsuchiya H, Nunoi H, Higuchi S, Alcabochi I, Chikazawa S. RHG-CSF for Shwachman's syndrome. Lancet 1990; 336:1136.
29. Dale DC, Bonilla MA, Davis MS, et al. A randomized, controlled phase III trial of recombinant human granulocyte colony-stimulating factor for treatment of severe chronic neutropenia. Blood 1993; 10:2496-502.
30. Hammond WP, Price TH, Souza LM, Dale DC. Treatment of cyclic neutropenia with granulocyte colony-stimulating factor. N Engl J Med 1989; 320:1306-11.
31. Bessho M, Toyoda A, Itoh Y, et al. Trilineage recovery by combination therapy with recombinant human granulocyte colony-stimulating factor (rhG-CSF) and erythropoietin (rhEpo) in severe aplastic anemia. Br J Haematol 1992; 80:409-11.
32. Bacigalupo A, Broccia G, Corda G, et al. Antilymphocyte globulin, cyclosporine and granulocyte colony-stimulating factor in patients with acquired severe aplastic anemia (SAA): a pilot study of the EBMT SAA working party. Blood 1995; 85:1348-53.
33. Speck B, Gluckman E, Haak HL, Van Rood JJ. Treatment of aplastic anemia by antilymphocyte globulin with and without allogeneic bone marrow infusions. Lancet 1977; 2:1145-7.
34. Young NS, Alter BP. Definitive treatment of acquired aplastic anemia. In: Young NS, Alter BP, eds. Aplastic anemia: acquired and inherited. Philadelphia:Saunders Co. 1993:159.
35. Nissen-Druey C. The pathophysiology of aplastic anemia. Baillière's Clin Hematol 1989; ii:37-49.
36. Gianni AM. Where do we stand with peripheral blood progenitor cells. Ann Oncol 1994; 5:781-4.
37. Siena S, Bregni M, Bonsi L, et al. Clinical implications of the heterogeneity of hematopoietic progenitors elicited in peripheral blood by anticancer therapy with cyclophosphamide and cytokine(s). Stem Cells 1993; 11(suppl 2):72-5.
38. Gianni AM, Bregni M, Siena S, et al. Rapid and complete hematopoietic reconstitution following combined transplantation of autologous and bone marrow cells. A changing role of high dose chemo-radiotherapy? Hematol Oncol 1989; 7:139-48.
39. Eaves CJ. Peripheral blood stem cells reach new heights. Blood 1993; 82:1957-9.
40. Holyoake TL, Franklin LM. Bone marrow transplant from peripheral blood. Br Med J 1994; 309:4-5.
41. Duhrsen U, Villeval JL, Boyd J, et al. Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients. Blood 1988; 72:2074-81.
42. Sheridan WP, Begley CG, Juttner CA, et al. Effect of peripheral blood progenitor cells mobilized by filgrastim (G-CSF) on platelet recovery after high dose chemotherapy. Lancet 1992; 339:640-4.
43. Chao NJ, Schriber JR, Grimes K, et al. Granulocyte colony-stimulating factor "mobilized" peripheral blood progenitor cells accelerate granulocyte and platelet recovery after high-dose chemotherapy. Blood 1993; 81:2031-5.
44. Bensinger W, Singer J, Appelbaum F, et al. Autologous transplantation with peripheral blood mononuclear cells collected

after administration of recombinant granulocyte colony-stimulating factor. Blood 1993; 81:3158-63.
45. Siena S, Bregni M, Brando B, et al. Circulation of CD34+ hematopoietic stem cells in the peripheral blood of high-dose cyclophosphamide-treated patients. Enhancement by intravenous recombinant human granulocyte-macrophage colony-stimulating factor. Blood 1989; 74:1905-14.
46. Kanz L, Brugger W, Merteslmann R. The use of hematopoietic growth factors for high dose chemotherapy and peripheral blood progenitor cell transplantation. Semin Oncol 1994; 21 (suppl. 16):10-8.
47. Kawano Y, Takaue Y, Watanabe T, et al. Effects of progenitor cell dose and preleukapheresis use of granulocyte colony-stimulating factor on the recovery of hematopoiesis after blood stem cell autografting. Exp Hematol 1993; 21:103-8.
48. Van der Wall E, Richel DJ, Holtkamp MJ, et al. Bone marrow reconstitution after high-dose chemotherapy and autologous peripheral blood progenitor cell tranplantation. Effect of graft size. Ann Oncol 1994; 5:795-802.
49. Siena S, Bregni M, Di Nicola M, et al. Durability of hemopoiesis following autografting with peripheral blood hematopoietic progenitors. Ann Oncol 1994; 5:935-41.
50. Russel NH, Hunter A, Rogers S, et al. Peripheral blood stem cells as an alternative to marrow for allogeneic transplantation (letter). Lancet 1993; 341:1482.
51. Gratwohl A, Hermans J. Bone marrow transplantation activity in Europe 1992: report from the European Group for Bone Marrow Transplantation (EBMT). Bone Marrow Transpl 1994; 13:5-10.
52. Burgio GR, Nespoli L, Locatelli F. Bone marrow transplantation in children: between *primum non nocere* (above all, do no harm) and *primum adiuvare* (above all, help). In: Burgio GR, Lantos JD, eds. *Primum non nocere* today. A symposium on pediatric bioethics. Amsterdam:Elsevier, 1994:77-93.
53. Aversa F, Tabilio A, Terenzi A, et al. Successful engraftment of T-cell-depleted haploidentical "three-loci" incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum. Blood 1994; 84:3948-55.
54. Lane TA, Law P, Maruyama M, et al. Harvesting and enrichment of hemopoietic progenitor cells mobilized into the peripheral blood of normal donors by granulocyte-macrophage colony-stimulating factor (GM-CSF) or G-CSF: potential role in allogeneic marrow transplantation. Blood 1995; 85:275-82.
55. Kernan NA, Collins NH, Juliano L, et al. Clonable T-lymphocytes in T-cell depleted bone marrow transplants correlate with developement of graft-versus-host disease. Blood 1986; 68:770-3.
56. Barnes R. Infections following bone marrow transplantation. In: Treleaven J, Barrett AJ, eds. Bone marrow transplantation in practice. London:Churchill Livingstone, 1992:281-2.
57. Weaver CH, Buckner CD, Longin K, et al. Syngenic transplantation with peripheral blood mononuclear cells collected after administration of recombinant granulocyte stimulating factor. Blood 1993; 82:1981-4.
58. Storb R, Prentice RL, Thomas ED, et al. Factors associated with graft rejection after HLA-identical marrow transplantation for aplastic anemia. Br J Haematol 1983; 55:573-85.
59. Bregni M, Magni M, Siena S, et al. Human peripheral blood hemopoietic progenitors are optimal targets of retroviral-mediated gene transfer. Blood 1992; 80:1418-22.
60. Kerr W, Fraser C, Chen B, et al. Gene transfer into higly purified CD34+ THY-1+ LIN- human peripheral blood hematopoietic stem cells. Blood 1994; 84(Suppl 1):402a.
61. Forni G, Parmiani G, Guarini A, Foà R. Gene transfer in tumor therapy. Ann Oncol 1994; 5:789-94.