

Peripheral blood stem cell transplantation ; an update

Tsutomu Watanabe, Yoichi Takaue and Yoshifumi Kawano

Department of Pediatrics, The University of Tokushima School of Medicine, Tokushima, Japan

Abstract: Patients with a number of different malignancies have been treated with high-dose chemotherapy and peripheral blood stem cell transplantation (PBSCT). PBSC already replaced bone marrow as the source of autologous hematopoietic progenitor support. This is due to ease of collection, rapid engraftment and less possibility of tumor cell contamination in the graft. Furthermore, allogeneic transplantation of granulocyte colony-stimulating factor (G-CSF) mobilized PBSC is now being increasingly performed. Recent advance of clinical PBSCT and new strategies are stressed in this review. New strategies include CD 34⁺ cell purification, *ex vivo* expansion of PBSC and PBSC as a target cell for gene therapy. Major future advance may occur better understanding of the mechanism of mobilization and the biology of PBSC. *J. Med. Invest.* 44 : 25-31, 1997

Key Words: *peripheral blood stem cell transplantation, mobilization, autologous, allogeneic, CD34⁺ cell*

INTRODUCTION

The field of stem cell transplantation continues to advance very rapidly (1). The term "bone marrow transplantation" now has become less used. Instead of bone marrow cells, peripheral blood stem cells (PBSC), or cord blood cells are increasingly used for hematopoietic rescue operations. In the 1980s, we recognized that hematopoietic progenitor cells circulated in the blood during the marrow recovery phase of myelosuppressive chemotherapy and could be concentrated for transplantation by repeated aphereses (2, 3). Thereafter hematopoietic growth factors came into clinical use, which markedly improved the efficiency of PBSC collection and accelerated hematopoietic recovery following transplantation (4). Over the last decade, the use of PBSC and better supportive care techniques have made high-dose chemotherapy comparatively safe with less than a 2-3% mortality rate in experienced centers (5). Thus, it has now been widely applied even in community hospitals (6). Recently, PBSC also began to be applied as an allogeneic stem cell source for the treatment of hematologic malignancies (7). Nonetheless, the exact mechanism of stem cell mobilization is poorly understood, and the biology of PBSC remains incompletely defined. In this review, we update recent progress in this field.

PERIPHERAL BLOOD AS A STEM CELL SOURCE

Mobilization

There is little question against the need to mobilize progenitor/stem cells for practical collection. Mobilization of progenitor cells from the bone marrow (BM) to

the PB can be achieved with myelosuppressive chemotherapy alone, or in combination with hematopoietic growth factors, or with hematopoietic growth factor alone administered in a hematologic steady state. Myelosuppressive chemotherapy was the first measure used for mobilization (8). In this way the timing of apheresis is rather unpredictable and toxicities such as neutropenic fever are inevitable (9). With the advent of growth factors, myelosuppressive chemotherapy alone is less used for mobilization. Mobilization methods by growth factors alone are certainly more convenient since collection days can be more precisely timed. Granulocyte colony-stimulating factor (G-CSF) or granulocyte/macrophage colony-stimulating factor (GM-CSF) alone enhance mobilization in both cancer patients and normal healthy donors (10, 11). In the allogeneic setting, G-CSF is exclusively used because its administration is safer and less toxic compared with other available cytokines including GM-CSF (12). Other cytokines or combinations of current or new growth factors, i.e., stem cell factor, interleukin-3 and thrombopoietin, are being studied as more efficient mobilizers (13). However, additional advantages of these factors to G-CSF are still unknown.

The exact mechanism by which progenitor cells are mobilized is unknown. Evidence is now emerging that cell adhesion molecules (CAMs) may be involved in the process of mobilization (14, 15). We studied several CAM expressions on PB and BM CD 34⁺ cells and found a significantly lower expression of integrins such as very late antigen-4 (VLA-4), leukocyte function antigen-1 (LFA-1) and LFA-3, and a significantly higher expression of L-selectin and CD44 on PB CD34⁺ cells compared with BM CD34⁺ cells. This suggests that decreased expression of CAMs play a role in progenitor cell mobilization. Progenitor cell mobilization might occur as a stochastic process and involve the selection of CD34⁺ cell with low

Received for publication August 1, 1997 ; accepted August 15, 1997.

¹ Address correspondence and reprint requests to Tsutomu Watanabe, M.D., Ph.D., Department of Pediatrics, The University of Tokushima School of Medicine, 3-18-15, Kuramoto-cho, Tokushima, Japan.

CAMs. Some role might be attributed to a loss of function of integrins, and the disruption of VLA-4 ligand receptor binding results in the release of progenitor cells in primates (16).

Collection and storage

Cell separators widely used for PBSC collections are Spectra (Cobe Laboratories), CS 3000 (Baxter Health Care Corp.) and AS 104 (Fresenius AG). Harvesting PBSC is safe and does not involve the risk of general anesthesia (17) or multiple invasive marrow aspirations. Adverse effects related to apheresis procedures occurred in less than 3% of adults (18). These included moderate hypovolemia and symptomatic hypocalcemia with perioral paresthesia which usually promptly resolved with infusion of calcium gluconate.

For cryopreservation of PBSC, a simple freezing method using 6% hydroxyethyl starch (HES), 5% dimethyl sulfoxide (DMSO) and 4% albumin has been used (19). This procedure is simple and requires less processing time compared with the conventional method which employs controlled-rate freezing by a programmed freezer and use of 10% DMSO (20). For storage of PBSC, a -135°C mechanical freezer replaced the storage in liquid nitrogen at some transplant centers. PBSC isolated from apheresis products can be stored in a -80°C mechanical freezer, but the duration of frozen storage should not exceed 1.5 years (21).

Monitoring PBSC collection efficiency

Colony forming units-granulocyte/macrophage (CFU-GM) and CD34⁺ cells are the two widely accepted indicators of the hematopoietic reconstitutive capacity of transplanted cells. The dose-effect relationship between the CFU-GM content of the graft and the speed of recovery following PBSCT has been shown in several studies (22). Standardization of CFU-GM measurements remains elusive due to the variations in methodology used in different laboratories. The measurement of CD34⁺ cells using a flow cytometer is much quicker than CFU-GM assays, which require at least 2 weeks to obtain the final results (23). However, CD34⁺ cell enumeration needs the level of stringency to measure rare event at the level of 0.1% to 3% (9). Issues in achieving standardization in CD34⁺ cell enumeration include gating strategy, CD34 antibody and degree of cell debris (24).

Characterization of PBSC

The phenotypic analyses of mobilized CD34⁺ cells are also extensively studied. The most striking differences observed regarding phenotypic profiles of mobilized CD34⁺ cells were the very high percentage of CD34⁺ cells coexpressing CD33 and CD13 molecules and the low percentage coexpressing CD10 and CD19 compared with BM CD34⁺ cells (25). Furthermore, PB CD34⁺ cells express less c-kit and CD71, suggesting that the mobilization of progenitor cells involves the down-regulation of c-kit (8). Mobilized PBSC showed a low percentage of circulating cells in the S phase despite

growth factor administration. This may be due to a selective release from BM cells in the G₀-G₁ phase and/or to the expression of critical cell cycle-related adhesion receptors.

AUTOLOGOUS TRANSPLANT

Initially, PBSCT was used for patients who were not eligible for BM harvest because of tumor involvement, extensive marrow fibrosis following local irradiation or contraindication to general anesthesia (1). Although there was a concern regarding long-term hematopoiesis following PBSCT, recent clinical data supported the durability of normal hematopoiesis after autografting with PBSC. Furthermore, proof of long-term engraftment following infusion of PBSC was obtained by gene-marking studies (26, 27). Since 1990, the number of PBSCT has far exceeded that of BMT in the autologous setting, and nowadays, PBSC have been used in 90% of autologous transplants (28). The major advantage of using PBSC over BM cells is the rapid hematopoietic reconstitution, which results in a shorter duration of antibiotic use, fewer blood product transfusions and early hospital discharge. Although autologous PBSCT should not by themselves lead to a different tumor control outcome than autologous BMT. Various analyses show no advantage of PBSCT over BMT in terms of overall survival rate [Table 1] (29-34). However, economic assessments of PBSCT have proven a direct cost saving effect associated with a reduction in the duration of hospitalization compared with BMT (35, 36). In some cases, PBSCT are performed largely on an outpatient basis to save admission costs (37). In addition, the collection process for PBSC is less invasive than BM harvest, and the donor can avoid general anesthesia. PBSCs may be harvested in an outpatient setting. These properties may increase the pool of unrelated donors in allogeneic transplants.

Indications for autologous PBSCT have changed with time and the types of illnesses treated with this approach continue to increase, within a range of chemosensitive tumors. High-dose therapy with PBSCT has become an established therapeutic option for patients with relapsed, but still chemotherapy-sensitive aggressive non-Hodgkin's lymphomas (NHL) (5). PBSCT has also been shown to produce long-term disease-free survival in selected patients with refractory and advanced Hodgkin's disease (38). Intensified treatment with high-dose melphalan supported by PBSC as a means of overcoming drug resistance yields complete remission even in patients with high-risk and advanced multiple myeloma (39). Yet, earlier transplantation is recommended before the development of drug resistance and end organ damage. Breast cancer has now become the disease most frequently treated with autologous PBSCT worldwide (40). Leading indications moved from refractory or metastatic breast cancer to high-risk or locally advanced breast cancer as an adjuvant consolidative therapy. Germ cell tumors are also a good indication for autologous PBSCT. Although autologous PBSCT also has been performed for childhood cancers such as neuroblastoma,

rhabdomyosarcoma and Wilms' tumor, the number of cases is still small and its role for these indications remains to be determined. For acute leukemias, many trials involving autologous BMT or PBSCT have been performed in patients with AML.

Dose intensification and sequential use of agents to overcome drug resistance may benefit some patients, and interest in the concept of tandem transplants has been growing. The principal objective of this procedure is to reduce the size of a slowly growing tumor mass by repeated, closely timed courses of high-dose chemotherapy, each given with hematopoietic stem cell rescue. Tandem cycles of high-dose chemotherapy became feasible with autologous PBSCT, since abundant PBSC yields obtained by apheresis provides the opportunity to deliver more than one cycle of high-dose chemotherapy. These procedures are under intense investigation in the treatment of breast cancer, childhood cancers and multiple myeloma (41-43).

ALLOGENEIC TRANSPLANT

G-CSF mobilized allogeneic PBSC have been used without significantly increased severe GVHD while reproducing the rapid reconstitution seen in the autologous setting (44). A number of recent studies indicated that transplants using allogeneic PBSC had an obvious advantage for accelerated engraftment over conventional BMT (45). Although there was concern that a relatively high number of infused lymphocytes might result in unacceptably severe acute GVHD, this has not been observed in fully matched sibling pairs (46). The early survival rates were similar between allogeneic PBSCT and BMT [Table 1] (44,45). Nevertheless, there is a possibility that the risk of chronic GVHD may increase in allogeneic PBSCT (47).

Regarding stem-cell mobilization in normal donors, two clinical variables should be taken into consideration. The first is efficacy, defined as the ability to mobilize sufficient numbers of PBSC, and the second is toxicity, defined as the side effects that the donor may experience. Although

Table 1. Comparative studies of PBSCT vs BMT

Ref.	Type of transplant	Disease	Investigator /Type of study	Timing	Overall survival (PBSCT vs BMT)	Advantages of PBSCT over BMT
29	Auto	Follicular NHL (n=60)	Bastion, et al./ Comparison with published data	Variable Mostly PR	86% (2 yr.) vs 50% (5 yr.)	Low treatment-related death rate
30	Auto	HD & NHL (n=27 vs 31)	Schmitz N, et al. /Prospective randomized	Variable	87.1% vs 88.9% <i>p</i> =N. S. (median follow-up ; 311 days)	Rapid hematopoietic reconstitution Early discharge from hospital
31	Auto	HD (n=227 vs 227)	EGBMT/ Randomized	Variable	52.7% vs 65.3% <i>p</i> =.0198 (4 yrs)	Rapid hematopoietic reconstitution
31	Auto	NHL (n=128 vs 128)	EGBMT/ Randomized	Variable	52.7% vs 56.6% <i>p</i> =.4148 (4 yrs)	Rapid hematopoietic reconstitution
32	Auto	Adult ALL (n=12 vs 38)	Powles R, et al./ Non-randomized	First CR	11/12 vs 21/38 (median follow-up ; 40 months)	Decreased transplant-related toxicity
33	Auto	Multiple myeloma (n=43 vs 43)	Harousseau JL, et al./ Randomized	First remission induction	45% vs 45% <i>p</i> =0.37 (4 yrs)	Neutrophil recovery
34	Auto	Germ cell tumor (n=23 vs 24)	Beyer J, et al./ Randomized	Relapsed or refractory	41.7% vs 52.2% <i>p</i> =0.39	Rapid hematopoietic reconstitution
45	Allo	AML, ALL, CML, CLL, MDS, MPD, NHL, MM (n=22 vs 21)	Pavletic ZS, et al./ Prospective randomized	Variable	83% vs 75% (100 day) <i>p</i> =0.358	Faster engraftment, Shorter hospital stay
44	Allo	AML, NHL, ALL, HD, CML, MM (n=37 vs 37)	Bensing WI, et al./ Retrospective comparison	Relapse or >CR 2	50% vs 41% (estimated at 285 days) <i>p</i> =0.39	Faster engraftment, Fewer transfusions, No greater incidence of acute or chronic GVHD

Ref. : References, NHL : Non-Hodgkin's lymphoma, HD : Hodgkin's disease, AML : acute myelogenous leukemia, ALL : acute lymphocytic leukemia, CML : chronic myelogenous leukemia, CLL : chronic lymphocytic leukemia, MDS : myelodysplastic syndrome, MPD : myeloproliferative disorder, MM : multiple myeloma, EGBMT : the European Group for Blood and Marrow Transplantation, CR : complete remission, PR : partial remission, N.S. : not significant

mobilization with G-CSF thus far seems a safe procedure for normal donors, most donors still complain of general fatigue and myalgias/arthralgias which are relieved easily by analgesia. The platelet count decreased in most donors. Slight but significant decrease in platelet count is observed at the peak of the G-CSF induced leukocytosis. It is suggested that G-CSF administration reduced the capacity for platelet production (48). Furthermore, a decrease in platelet count was subsequently enhanced by an apheresis-related decrease. Although there has been no clinical report of life threatening bleeding, all donors should be carefully monitored. Another concern regarding donor safety is the potential long-term toxicities of G-CSF, which have to be determined with longer follow-up. The theoretical risk of developing leukemia and myelodysplastic syndrome (MDS) after G-CSF treatment may be of concern, but to date, there were insufficient cohorts to evaluate this possible risk (49). The issues still to be determined include more detailed knowledge of G-CSF dosage, optimal PBSC numbers, the dynamics and durability of engraftment and immunologic reconstitution of the recipient (50). The superiority of PBSCT over BMT, particularly from mismatched donors is unproven.

Nowadays, indications for allogeneic PBSCT include high-risk hematological malignancies such as acute leukemias, MDS and chronic myelogenous leukemia. Non-malignant hematologic disease such as severe aplastic anemias and congenital immunodeficiencies may also be good indications for allogeneic PBSCT.

NEW STRATEGIES

CD34 antigen is expressed on both hematopoietic progenitors and stem cells. The number of progenitor cells expressing CD34 in PB is known to increase following mobilization procedures. CD34⁺ cell purification technology continues to be improved to obtain cells in greater than 90% purity and greater than 3-log tumor-cell or T-cell depletion (51). Purposes of CD34⁺ cell purification are to purge tumor cells in the grafts for autologous PBSCT (52) and to deplete T-cells which contribute to the development of GVHD in allogeneic PBSCT (53). In addition, selected CD34⁺ cells have the advantage of reducing graft volume, facilitating storage and decreasing the amount of DMSO as well as cell lysis products. However, in the autologous setting clonogenic tumor cells are still present in CD34⁺ cell selected grafts, and their association with relapse is unknown. Recent advances in gene marking studies may provide much-needed information on malignant contamination in CD34⁺ selected grafts. Isolex 300 is now available for clinical use, although the cost of this equipment for processing exceeds one million yen. The cost-effectiveness should be evaluated carefully. Apheresis products undergoing selection of CD34⁺ cells have a greater yield and enrichment of progenitor cells compared with BM harvests collected from HLA-identical normal healthy donors (54).

Engraftment with CD34⁺ purified PBSC confirms that autologous CD34⁺ cells, alone, are sufficient to provide

hematopoietic rescue for myeloablated patients (55). Blood cell recovery following CD34⁺ cell transplantation is as rapid as after unmanipulated PBSCT, suggesting that there are no adverse effects to removing accessory cells, such as activated monocytes or lymphocytes, on blood cell recovery speed. There is some hope that most patients can have donors if transplants with allogeneic CD34⁺ cells obtained from haploidentical related donors are performed without deterioration of engraftment and GVHD. However, there is a concern that major risks of an allogeneic transplant with CD34⁺ cells include increased graft rejection, loss of GVL effect and development of serious viral infection and lymphoproliferative disorders due to delayed recovery of immune function (56). In this regard, add-back of T-cells into patients at an appropriate time following transplant might be a reasonable strategy.

There is considerable interest in the possibility of expanding stem cell *ex vivo*. *Ex vivo* expansion of progenitor cells is a new approach to abrogating cytopenia posttransplant or expanding a small aliquot of mobilized PBSC to provide sufficient progenitor cells for hematopoietic rescue. The specific combination of hematopoietic growth factors and the culture system and/or hematopoietic stroma were identified as important variables for *ex vivo* expansion of PBSC (57). However, the feasibility of this approach in a clinical setting still remains unclear in the lack of an *in vitro* assay for human stem cells. In addition, whether endogenous tumor cells from patients are concomitantly expanded in culture should be monitored carefully in the autologous transplant setting (58).

Clinical application of gene therapy using PBSC in patients with cancer or congenital metabolic diseases has been studied. An advantage of PBSC is that multiple collection procedures can be performed without invasive surgery; which may be an important consideration in gene therapy (59). The purification of CD34⁺ cells is again necessary to improve the transduction and/or long-term expression.

REFERENCES

1. Kessinger A, Armitage JO: The evolving role of peripheral stem cell transplantation following high-dose therapy for malignancies. *Blood* 77: 211-213, 1991
2. Watanabe T, Takaue Y, Kawano Y, Koyama T, Huq MAHM, Shimokawa T, Ninomiya T, Aga Y, Inai T, Hino M, Takehara H, Komi N, Kuroda Y: Peripheral blood stem cell autotransplantation in treatment of childhood cancer. *Bone Marrow Transplant* 4: 261-265, 1989
3. Takaue Y, Watanabe T, Kawano Y, Koyama T, Abe T, Suzue T, Satho J, Shimokawa T, Ninomiya T, Kosaka M, Shimizu E, Ogura T, Kuroda Y: Isolation and storage of peripheral blood hematopoietic stem cells for autotransplantation into children with cancer. *Blood* 74: 1245-1251, 1989
4. Stadtmauer E, Schneider C, Silberstein L: Peripheral blood progenitor cell generation and harvesting.

- Semin Oncol 22 : 291-300, 1995
5. Armitage JO : The development of bone marrow transplantation as a treatment for patients with lymphoma-twentieth Richard and Hinda Rosenthal foundation award lecture. Clin Cancer Research 3 : 829-836, 1997
 6. Weaver CH, Schwartzberg LS, Hainsworth J, Greco FA, Li W, Buckner CD, West WH : Treatment-related mortality in 1000 consecutive patients receiving high-dose chemotherapy and peripheral blood stem progenitor cell transplantation in community cancer centers. Bone Marrow Transplant 19 : 671-678, 1997
 7. Bensinger WI, Weaver CH, Appelbaum FR, Rowley S, Demirer T, Sanders J, Storb R, Buckner CD : Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony-stimulating factor. Blood 85 : 1655-1658, 1995
 8. To LB, Haylock DN, Dowse T, Simmons PJ, Trimboli S, Ashman LK, Juttner CA : A comparative study of the phenotype and proliferative capacity of peripheral blood (PB) CD34⁺ cells mobilized by four different protocols and those of steady-phase PB and bone marrow CD34⁺ cells. Blood 84 : 2930-2939, 1994
 9. To LB, Haylock DN, Simmons PJ, Juttner CA : The biology and clinical uses of blood stem cells. Blood 89 : 2233-2258, 1997
 10. Duhrsen U, Villeval JL, Boyd J, Kannourakis G, Morstyn G, Metcalf D ; Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients. Blood 72 : 2074-2080, 1988
 11. Schmitz N, Dreger P, Suttorp M, Rohwedder EB, Haferlach T, Loffler H, Hunter A, Russell NH : Primary transplantation of allogeneic peripheral progenitor cells mobilized by filgrastim (granulocyte colony-stimulating factor). Blood 85 : 1666-1670, 1995
 12. Stroncek DF, Clay ME, Petzoldt ML, Smith W, Jaszcz W, Oldham FB, McCullough J : Treatment of normal individuals with granulocyte-colony-stimulating factor : donor experiences and the effects on peripheral blood CD34⁺ cell counts and on the collection of peripheral blood stem cells. Transfusion 36 : 601-610, 1996
 13. Moskowitz CH, Stiff P, Gordon MS, McNiece I, Ho AD, Costa JJ, Broun R, Bayer RA, Wyres M, Hill J, Jelace-Maxwell K, Nichols CR, Brown SL, Nimer SD, Gabrilove J : Recombinant methionyl human stem cell factor and filgrastim for peripheral blood progenitor cell mobilization and transplantation in non-Hodgkin's lymphoma patients-Results of phase I/II trial. Blood 89 : 3136-3147, 1997
 14. Watanabe T, Dave B, Heimann DG, Lethaby E, Kessinger A, Talmadge JE : GM-CSF-mobilized peripheral blood CD34⁺ cells differ from steady-state bone marrow CD34⁺ cells in adhesion molecule expression. Bone Marrow Transplant 19 : 1175-1181, 1997
 15. Mohle R, Murea S, Kirsch M : Differential expression of L-selectin, VLA-4 and LFA-1 on CD34⁺ progenitor cells from bone marrow and peripheral blood during G-CSF-enhanced recovery. Exp Hematol 23 : 1535-1542, 1995
 16. Papayannopoulou T, Nakamoto B : Peripheralization of hematopoietic progenitors in primates treated with anti-VLA 4 integrin. Proc Natl Acad Sci USA 90 : 9374-9377, 1993
 17. Hosoya N, Miyagawa K, Mimura T, Hoshida S, Akazawa H, Kanda Y, Takahashi N, Hirai H, Maekawa K, Yazaki Y : Malignant hyperthermia induced by general anesthesia for bone marrow harvesting. Bone Marrow Transplant 19 : 509-511, 1997
 18. Torretta L, Perotti C, Dornini G, Danova M, Lovatelli F, Pedrazzoli P, Preti P, Da Prada GA, Pavesi L, della Cuna GR, Salvaneschi L : Circulating progenitor cell collection : Experience from 275 leukaphereses in various malignancies and in healthy donors. Hematologica 81 : 208-215, 1996
 19. Katayama Y, Yano T, Bessho A, Deguchi S, Sunami K, Mahmut N, Shinagawa K, Omoto E, Makino S, Miyamoto T, Mizuno S, Fukuda T, Eto T, Fujisaki T, Ohno Y, Inaba S, Niho Y, Harada M : The effects of a simplified method for cryopreservation and thawing procedures on peripheral blood stem cells. Bone Marrow Transplant 19 : 283-287, 1997
 20. Takaue Y, Abe T, Kawano Y, Suzue T, Saito S, Hirao A, Sato J, Makimoto A, Kawahito M, Watanabe T, Shimokawa T, Kuroda Y : Comparative analysis of engraftment after cryopreservation of peripheral blood stem cell autografts by controlled-versus uncontrolled-rate methods. Bone Marrow Transplant 13 : 801-804, 1994
 21. Valeri CR, Pivacek LE : Effects of the temperature, the duration of frozen storage, and the freezing container on in vitro measurement in human peripheral blood mononuclear cells. Transfusion 36 : 303-308, 1996
 22. Kawano Y, Takaue Y, Watanabe T, Saito S, Abe T, Hirao A, Sato J, Ninomiya T, Suzue T, Koyama T, Shimokawa T, Yokobayashi A, Asano S, Masaoka T, Takaku F, Kuroda Y : Effects of progenitor cell dose and preleukapheresis use of human recombinant granulocyte colony-stimulating factor on the recovery of hematopoiesis after blood stem cell autografting in children. Exp Hematol 21 : 103-108, 1993
 23. Diaz MA, Alegre A, Villa M, Granda A, de la Vega A, Ramirez M, Ruano D, Gonzalez A, Merino JM, Madero L : Pediatric experience with autologous peripheral blood progenitor cell transplantation : influence of CD34⁺ cell dose in engraftment kinetics. Bone Marrow Transplant 18 : 699-703, 1996
 24. Lumley MA, McDonald DF, Czarnecka HM, Billingham LJ, Milligan DW : Quality assurance of CD34⁺ cell estimation in leukapheresis products. Bone Marrow Transplant 18 : 791-796, 1996
 25. Tjonnfjord GE, Steen R, Eversen SA, Thorsby E, Egeland T : Characterization of CD34⁺ peripheral blood cells from healthy adults mobilized by

- recombinant human granulocyte colony-stimulating factor. *Blood* 84 : 2795-2801, 1994
26. Drize N, Chertkov J, Sadovnikova E, Tiessen S, Zander A : Long-term maintenance of hematopoiesis in irradiated mice by retrovirally transduced peripheral blood stem cells. *Blood* 89 : 1811-1817, 1997
 27. Dunbar C, Cottler-Fox M, O'Shaughnessy M : Retrovirally marked CD34-enriched peripheral blood and bone marrow cells contribute to long-term engraftment after autologous transplantation. *Blood* 85 : 3048-3057, 1995
 28. Gratwohl A, Hermans J, Baldomero H for EBMT : Blood and marrow transplantation activity in Europe 1995. *Bone Marrow Transplant* 19 : 407-419, 1997.
 29. Bastion Y, Brice P, Haioun C, Sonet A, Salles G, Marolleau JP, Espinous D, Reyes F, Gisselbrecht C, Coiffier B : Intensive therapy with peripheral blood progenitor cell transplantation in 60 patients with poor-prognosis follicular lymphoma. *Blood* 86 : 3257-3262, 1995
 30. Schmitz N, Linch DC, Dreger P, Goldstone AH, Boogaerts MA, Ferrant A, Demuynck HMS, Link H, Zander A : Randomized trial of filgrastim-mobilized peripheral blood progenitor cell transplantation versus autologous bone-marrow transplantation in lymphoma patients. *Lancet* 347 : 353-357, 1996
 31. Majolino I, Pearce R, Taghipour G, Goldstone AH for the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation : Peripheral-blood stem-cell transplantation versus autologous bone marrow transplantation in Hodgkin's and Non-Hodgkin's Lymphoma : A new matched-pair analysis of the European Group for Blood and Marrow Transplantation registry data. *J Clin Oncol* 15 : 509-517, 1997
 32. Powles R, Mahta J, Singhal S, Horton C, Tait D, Milan S, Polland C, Lumley H, Matthey F, Shirley J, Williams H, Samaratunga I, Lakhani A, Millar J, Treleaven J : Autologous bone marrow or peripheral blood stem cell transplantation followed by maintenance chemotherapy for adult acute lymphoblastic leukemia in first remission : 50 cases from a single center. *Bone Marrow Transplant* 16 : 241-247, 1995
 33. Harousseau JL, Attal M, Divine M, Milpied N, Marit G, Leblond V, Stoppa AM, Bourhis JH, Caillot D, Boasson M, Abgrall JF, Facon T, Colombat P, Cahn JY, Lamy T, Troussard X, Gratecos N, Pignon B, Auzanneau G : Comparison of autologous bone marrow transplantation and peripheral blood stem cell transplantation after first remission induction treatment in multiple myeloma. *Bone Marrow Transplant* 15 : 963-969, 1995
 34. Beyer J, Schwella N, Zingsem J, Strohscheer I, Oettle H, Serke S, Huhn D, Siegert W : Hematopoietic rescue after high-dose chemotherapy using autologous peripheral-blood progenitor cells or bone marrow : A randomized comparison. *J Clin Oncol* 13 : 1328-1335, 1995
 35. Smith TJ, Hillner BE, Schmitz N, Linch DC, Dreger P, Goldstone AH, Boogaerts MA, Ferrant A, Link H, Zander A, Yanovich S, Kitchin R, Erder MH : Economic analysis of a randomized clinical trial to compare filgrastim-mobilized peripheral-blood progenitor-cell transplantation and autologous bone marrow transplantation in patients with Hodgkin's and non-Hodgkin's lymphoma. *J Clin Oncol* 15 : 5-10, 1997
 36. Hartmann O, Le Corroller AG, Blaise D, Michon J, Philip I, Norol F, Janvier M, Pico JL, Baranzelli MC, Rubie H, Coze C, Pinna A, Meresse V, Benhamou E : Peripheral blood stem cell and bone marrow transplantation for solid tumors and lymphoma : Hematologic recovery and costs. A randomized controlled trial. *Ann Intern Med* 126 : 600-607, 1997
 37. Meisenberg BR, Miller WE, McMillan R, Callaghan M, Sloan C, Brehm T, Kosty MP, Kroener J, Longmire R, Saven A, Piro LD : Outpatient high-dose chemotherapy with autologous stem-cell rescue for hematologic and nonhematologic malignancies. *J Clin Oncol* 15 : 11-17, 1997
 38. Bierman PJ, Bagin RG, Jagannath S, Vose JM, Spitzer G, Kessinger A, Dicke KA, Armitage JO : High dose chemotherapy followed by autologous hematopoietic rescue in Hodgkin's disease : Long term follow-up in 128 patients. *Ann Oncol* 4 : 767-773, 1993
 39. Bjorkstrand B, Ljungman P, Svensson H, Heramns J, Alegre A, Apperley J, Blade J, Carison K, Cavo M, Ferrant A, Goldstone AH, de Laurenti A, Majolino I, Marcus R, Prentice HG, Remes K, Samson D, Sureda A, Verdonck LF, Volin L, Gahrton G : Allogeneic bone marrow transplantation versus autologous stem cell transplantation in multiple myeloma : A retrospective case-matched study from the European group for blood and marrow transplantation. *Blood* 88 : 4711-4718, 1996
 40. Antman KH, Rowlings PA, Vaughan WP, Pelz CJ, Fay JW, Fields KK, Freytes CO, Gale RP, Hillner BE, Holland K, Kennedy MJ, Klein JP, Lazarus HM, McCarthy PL, Saez R, Spitzer G, Stadtmauer EA, Williams SF, Wolff S, Sobocinski A, Armitage JO, Horowitz MM : High-dose chemotherapy with autologous hematopoietic stem-cell support for breast cancer in North America. *J Clin Oncol* 15 : 1870-1879, 1997
 41. Haas R, Schmid H, Hahn U : Tandem high-dose therapy with ifosfamide, epirubicin, carboplatin and peripheral blood stem cell support is an effective adjuvant treatment for high-risk primary breast cancer. *Eur J Cancer* 33 : 372-378, 1997.
 42. Meisenberg BR, Miller WE, McMillan R : Mobilized peripheral blood progenitor cells (PBPC) in support of tandem cycles of high-dose chemotherapy (HDC). *Bone Marrow Transplant* 18 : 1087-1093, 1996
 43. Barlogie B, Jagannath S, Vesole DH, Naucke S, Cheson B, Mattox S, Bracy D, Salmon S, Jacobson J, Crowley J, Tricot G : Superiority of tandem autologous transplantation over standard therapy for

- previously untreated multiple myeloma. *Blood* 89 : 789-793, 1997
44. Bensinger WI, Clift R, Martin P, Appelbaum FR, Demirer T, Gooley T, Lilleby K, Rowley S, Sanders J, Storb R, Buckner D : Allogeneic peripheral blood stem cell transplantation in patients with advanced hematologic malignancies : A retrospective comparison with marrow transplantation. *Blood* 88 : 2794-2800, 1996
 45. Pavletic ZS, Bishop MR, Tarantolo SR, Martin-Algarra S, Bierman PJ, Vose JM, Reed EC, Gross TG, Kollath J, Nasrati K, Jackson JD, Armitage JO, Kessinger A : Hematopoietic recovery after allogeneic blood stem-cell transplantation compared with bone marrow transplantation in patients with hematologic malignancies. *J Clin Oncol* 15 : 1608-1616, 1997
 46. Schmitz N, Bacigalipo A, Labopin M, Majolino I, Laporte JP, Brinch L, Cook G, Deliliers GL, Lange A, Rozman C, Garcia-Conde J, Finke J, Domingo-Albos A, Gratwohl A : Transplantation of peripheral blood progenitor cells from HLA-identical sibling donors. *Br J Hematol* 95 : 715-721, 1996
 47. Maajolino I, Saglio G, Scime R, Serra A, Cavallaro AM, Fiandaca T, Vasta S, Pampinella M, Catania P, Indovina A, Marceno R, Santoro A : High incidence of chronic GVHD after primary allogeneic peripheral blood stem cell transplantation in patients with hematologic malignancies. *Bone Marrow Transplant* 17 : 555-560, 1996
 48. Anderlini P, Przepiorcka D, Champlin R, Korbling M : Biologic and clinical effects of granulocyte colony-stimulating factor in normal individuals. *Blood* 88 : 2819-2825, 1996
 49. Welte K, Gahrilove J, Bronchud MH, Platzer E, Morstyn G : Filgrastim (r-metHuG-CSF) : the first 10 years. *Blood* 88 : 1907-1929, 1996.
 50. Bishop MR, Tarantolo SR, Jackson JD, Anderson JR, Schmit-Pokorny K, Zacharias D, Pavletic ZS, Pirruccello SJ, Vose JM, Bierman PJ, Warkentin PI, Armitage JO, Kessinger A : Allogeneic-blood stem-cell collection following mobilization with low-dose granulocyte colony-stimulating factor. *J Clin Oncol* 15 : 1601-1607, 1997
 51. Civin CI, Trischmann T, Kadan NS, Davis J, Naga S, Cohen K, Duffy B, Groenewegen I, Wiley J, Law P, Hardwick A, Oldham F, Gee A : Highly purified CD 34-positive cells reconstitute hematopoiesis. *J Clin Oncol* 14 : 2224-2233, 1996
 52. Handgretinger R, Greil J, Schurmann U, Lang P, Gonzalez-Ramella O, Schmidt I, Fuhrer R, Niethammer D, Klingebiel T : Positive selection and transplantation of peripheral CD34⁺ progenitor cells : Feasibility and purging efficacy in pediatric patients with neuroblastoma. *J Hematol* 6 : 235-242, 1997
 53. Urbano-Ispizua A, Rozman C, Martinez C, Martin P, Briones J, Rovira M, Feliz P, Viguria MC, Merino A, Sierra J, Mazzara R, Carreras E, Monsterrat E : Rapid engraftment without significant graft-versus-host disease after allogeneic transplantation of CD34⁺ selected cells from peripheral blood. *Blood* 89 : 3967-3973, 1997
 54. Hassan HT, Zeller W, Stocksvhlader M, Kruger W, Hoffknecht MM, Zander AR : Comparison between bone marrow and G-CSF mobilized peripheral blood allografts undergoing clinical scale CD34⁺ cell selection. *Stem Cells* 14 : 419-429, 1996.
 55. Shpall EJ, Jones RB, Bearman SI : Transplantation of autologous CD34⁺ hematopoietic progenitor cells into breast cancer patients following high-dose chemotherapy. *J Clin Oncol* 12 : 28-36, 1994
 56. Shlomchik WD, Emerson SG : The immunobiology of T cell therapies for leukemias. *Acta Haematol* 96 : 189-213, 1996.
 57. Brugger W, Heimfeld S, Berenson RJ, Mertelsmann R, Kanz L : Reconstitution of hematopoiesis after high-dose chemotherapy by autologous progenitor cells generated ex vivo. *N Eng J Med* 333 : 328-332, 1994
 58. Van Riet I, Juge-Morineau N, Schots R : Persistence of residual tumor cells after cytokine-mediated ex vivo expansion of mobilized CD34⁺ blood cells in multiple myeloma. *Br J Haematol* 96 : 403-411, 1997.
 59. Abe T, Ito M, Okamoto Y, Kim HJ, Takaue Y, Yasutomo K, Makimoto A, Yamaue T, Kawano Y, Watanabe T, Shimada T, Kuroda Y : Transduction of retrovirus-mediated NeoR gene into CD34⁺ cells purified from granulocyte-colony stimulating factor (G-CSF)-mobilized infant and cord blood. *Exp Hematol* 25 : 696-701, 1997