Review Article

Hematopoietic Stem Cell Transplantation

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Hematopoietic stem cell disorders such as severe aplastic anemia, myelodysplastic syndrome, paroxysmal nocturnal hemoglobinuria and clonal disorders, *e.g.,* acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, lymphoma and multiple myeloma are fatal conditions. Secondaries from solid tumors, *e.g.,* neuroblastoma, Wilm's tumor and rhabdomyosarcoma in children involving the hematopoietic system are also fatal. High dose chemotherapy or radiotherapy either alone or in combination, not only eradicates the tumor cells but also destroys the hematopoietic stem cells. Reconstitution of hematopoietic stem cells offers a definitive therapy for a variety of disorders and it has been a focus of intense research over the last 3-4 decades(l-5).

Hematopoietic stem cells were initially obtained from the bone marrow of a donor and were infused intravenously into recipient. This procedure is commonly known as bone marrow transplantation (BMT). The hematopoietic stem cells can be obtained either from genetically identical twin (syngenic) or from an HLA identical matched sibling (allogenic). Another source of hematopoietic stem cells is from the peripheral blood used for stem cell transplantation (PBSCT). The probability of getting a HLA matched donor is about 30-35%(4). It is becoming increasingly difficult to find suitable HLA matched sibling donors as the family sizes are getting smaller. To overcome this problem, bone marrow registeries have been established in the developed countries from volunteer donors to find suitable HLA matched unrelated donor (MUD) for those patients who do not have matched sibling donor.

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HLA Matching

Success of hematopoietic stem cell transplantation is dependent upon accurate HLA matching. Standard serological method using alloantisera for class I (HLA-A,B) and class II (HLA-DR-DQ) antigens along with family haplotype segregation studies are essential to determine the genotypic identity. Recently a number of molecular techniques have been developed using dimensional isoelectric focusing for identification of HLA-A, B and C antigen variants. DNA molecular studies have been employed to identify a better match for the HLA-DR and DQ alleles(6). Some centers use polymerase chain reaction for fingerprinting the HLA-DR matching. These advances have helped to identify the perfect HLA matching. Mixed lymphocyte culture assays have been used to determine the functional significance of HLA disparity. However, the results of mixed lymphocyte culture studies are unable to predict the acute graft versus host disease (GVHD)(3). To overcome the disparity of mixed lymphocyte culture studies, functional assays

using dilution assay of cytotoxic T-lymphocyte and T-helper lymphocyte precursor cells have been developed(7,8). These studies have been successful in predicting the development of acute GVHD.

HLA studies have revealed that matching of minor histocompatability anitgens is equally important besides matching for the major histocompatability complex.' Acute GVHD in HLA identical sibling transplants develops due to the mismatching of minor histocompatability antigens. Minor histocompatible molecules which are recognized by the T cells of the donor. The reactivity of donor T cells against recipients minor histocompatability antigens can be evaluated by using limiting dilution studies(9). All these studies have helped in identifying an accurate HLA match and in predicting the possibility of acute GVHD.

Hematopoietic Progenitor Cell Assays

Definition of stem cells has been a subject of controversy and presently it is based upon the long term hematopoietic reconstitution. Various methods have been developed to identify the hematopoietic progenitor cells.

Colony Cultures Assays

Colony forming assays were developed initially to identify hematopoietic progenitor cells. The number, growth rate and morphological features on the semisolid agar plates were the basis of the culture assays in the presence of growth factors. Every colony, belonged to particular cell lineage. However, these studies were not well standardized, difficult to interpret and were time consuming. It is now believed that long term culture-initiating cells should form the basis of progenitor stem cells(10).

Phenotypic Characterization

A monoclonal body against KG-Ia Cells has been developed to identify the CD-34 antigen as a marker for hematopoietic cells(11). One to three per cent of normal bone marrow mononuclear cells were identified as immature progenitor cells(12,13). CD-34 cells, when correlated with the colony forming assays generated multipotent granulocyte-erythroidmacrophage-megakaryocyte colony forming units (CFU-GEMM), burst forming erythroid units (BFU-E), erythroid colony forming units (CFU-E), granulocyte-macrophage colony forming units (CFU-GM) and megakaryocyte colony forming units (CFU-MEG)(14,15). Further studies revealed that more immature subpopulation of CD34+ . progenitor cells which constitute 5-20% of bone marrow cells express multiple lineage specific antigens (CD 5, CD 10, CD 33, CD 71)(16,17). These observations indicate that CD-34 antigen identifies hematopoietic progenitor cells in various stages of maturation and serves as a better indicator for identification of hematopoietic progenitor cells.

Cell Compartments

Cells from the bone marrow, peripheral blood and umbilical cord generate almost equivalent number of CFU-GEMM, CFU-E, CFU-GM and CFU-MEG colony forming units(12). It has been observed that CD-34+ cells in the peripheral blood are always in equilibrium with the bone marrow and usually do not exceed 10% of fraction in the bone marrow(13). Umbilical cord blood is more comparable to adult bone marrow in the hematopoietic progenitor cell number(16,18).

Quantitative determination of CD 34+ cells subpopulation is often difficult. Peripheral CD 34+ cells may express myeloid CD 33 and panleucocyte CD 45 antigens more often than bone marrow. Similarly CD-71 transfusion receptor antigen on the

peripheral blood are present in smaller number(13). Umbilical cord blood CD 34+ also express CD 33 antigen more often than bone marrow cells. Umbilical cord blood CD 34+ were richer in the subpopulation of immature CD 38 subpopulation(19). The differences in various compartments of progenitor subpopulation is likely to have an impact on the hematopoietic reconstitution. However, the source of the progenitor have greater impact on the hematopoietic recovery.

Bone Marrow Transplantation (BMT) versus Peripheral Blood Stem Cell Transplantation (PBSCT)

BMT was initiated earlier but now PBSCT has become more popular because of the better understanding of various cell compartments and ease of collection of hematopoietic cells. Multiple advantages of PBSCT over BMT include: *(a)* easy and inexpensive method of cell collection even as an outpatient procedure; *(b)* reduced period of cytopenia; (c) lower incidence of infectious complications(20,21) and *(d)* its use in patients with bone marrow involvement, following irradiation of pelvis and (e) presence of few tumor cells.

Over the last two decades, several technical advances have taken place which have made the peripheral blood stem cell (PBSC) collection safe and efficient by automated blood cell separators. Cryopreservation and multiple collections can be made in the plastic bags. PBSC collection can either be increased manifold by use of growth factors or by pairing chemotherapy and cell collection during the rebound recovery period. Efforts are in progress to improve the PBSC collection. Theoretically there are many advantages of PBSCT; however, in clinical practice the results of PBSCT are not superior over BMT.

The disadvantages of PBSCT include: (a) central vascular access; *(b)* need of large volume transfusions; (c) need for a long period for adequate harvest; and (d) adverse effects of chemotherapy and cytokines.

Cell Mobilization

It is a process of transient shift of hematopoietic stem cells from the bone marrow to the peripheral blood which enables better PBSC collection. Normally the progenitor cells (CD 34+) constitute nearly 0.1% of the peripheral blood which is nearly 10% of the bone marrow concentration. Many drugs such as cyclophosphamide, daunorubicin, cytosine arabinoside and etoposide have no cytotoxic effect on the stem cells and result in increase in number of stem cells during recovery from the myelosuppresion. Cyclophosphamide (4-7 g/M^2) is administered intravenously and stem cell collection is done when total leucocyte count reaches 1000/(µl(6). Three to six aphareses are essential for desired number of stem cells(22).

Use of cytokines like granuiocyte macrophage-colony stimulating factors (GM-CSF) or granulocyte-colony stimulating factors (G-CSF), in the doses of 5-10 µg/ kg/day increases the CFU-GM number by 50-100 folds between 5-7th day of therapy (23-25). It allows harvesting of sufficient number of CFU-GM ($1x10^5$/kg) for most patients. Recently, the relative efficacy of various growth factors has been evaluated(26). It was observed that combination of interleukin-3 (IL-3) and GM-CSF has maximum effect on the cell mobilization. Mega-karyocyte progenitors cells were not mobilized by G-CSF. Combination of cytokines have resulted in the increase of trilineage committed progenitor cells. The procedure of collection and preservation of hematopoietic stem cells have been reviewed recently(27-29).

Tumor Cell Contamination of PBSC

It was believed that PBSC collection will have lesser number of tumor cells as compared with the autologous BMT. However, the studies by sensitive techniques of detection have revealed that contamination of PBSC with malignant cells is commoner than envisaged(30). On the contrary, studies by other workers have failed to demonstrate the significant stimulation of tumor cells by growth factors(31,32). Recently, the gene on the stem cells has been marked to identify whether stem cells used in the autologous BMT contain tumor cells which may be responsible for relapse(33). They have suggested that effective purging will be essential to improve the event free survivors for diseases requiring autologous BMT. Multiple purging protocols using drugs such as VP-16 and 4-hydroperoxycyclopho-sphamide (4 HC)(34) or use of monoclonal antibodies specific to various malignancies have been successful for separating the malignant cells(35).

Cord Blood Stem Cell Transplant (CBSCT)

HLA matched suitable donor for allogenic BMT or PBSCT is usually available in nearly 30% of cases. In the absence of suitable donor, BMT centers in the developed countries make an effort to search a list of 2 million volunteer registry for National marrow donor programme. Inspite of these efforts, one is able to find a unrelated suitable donor in less th-an 40% of cases(36). Placental blood which is universally discarded is rich in hematopoietic progenitor cells. Attempts to capitalize this source have been fruitful, as evident by the success of over 200 CBSCT in the World(36). CBSCT have not only been successful with complete HLA matched sibling but even with partial match (difference of single antigen or allele). Success of CBSCT from unrelated donors has' been documented (37). In addition, it was observed that CBSCTs that differed from recipients even by three HLA antigen or allele were successfully engrafted and produced all three cell lines without any significant graft versus host disease (GVHD). It is believed that cord blood stem cells from a fetus will be sufficient for a child weighing upto 30 kilograms. However, success has been reported in engrafting of cord blood stem cells from an unrelated donor in a 26 year old woman weighing 55 kg with chronic myeloid leukemia(38). She only received a relatively low dose of 10 million nucleated cells per kilogram of her body weight. It is possible that cord blood stem cells have greater capacity for proliferation and self renewal than bone marrow(39). Further studies are needed to assess the GVHD and use of CBSCT in adults. It is important to study whether the absence of GVHD will influence, in any way, the survival of patients with leukemia.

It is justified to establish cord blood banks to expedite CBSCT. These banks would need to maintain good control on the quantity of collection, processing and cryopreservation (40). Further, they will need to maintain the universal standards to enable the exchange of supply of cord blood units to other centers. With the development of cord blood banking, proper testing of HLA and screening for various infections and genetic disorders will become essential. It is anticipated that the number of CBSCT will outnumber the BMT or PBSCT in the near future.

Clinical Application

Hematopoietic stem cells transplantation using bone marrow or PBSC has been in practice for over a decade for multiple indications in children *(Table I)*. Besides

these indications, the transplantation in adults have been undertaken for several diseases such as chronic lymphocytic leukemia, multiple myeloma, breast cancer, ovarian cancer and germ cells tumors which have been reveiwed elsewhere along with the complications of BMT(3,41-44). Studies on hematopoietic stem cells transplantation generally include both adults and children and therefore in the present review the overall outcome of the BMT has been considered.

Acute Myeloid Leukemia

With the current chemotherapy protocols, cure is possible only in 20-25% of patients. Thus allogenic and even autologous BMT have been undertaken to improve the event free survival (EFS). Allogenic BMT have resulted an overall EFS in 50% of patients in first remissin and 20-30% of cases in patients with second or third remission.

TABLE I- *Indications for Hematopoietic Stem Cell Transplantation in Children.*

Malignant Conditions	Nonmalignant Conditions
Acute myeloid leukemia	Aplastic anemia
Acute lymphoblastic leukemia	Pure red cell aplasia
Chronic myeloid leukemia	Thalassemias
Myelodysplastic syndromes	Sickle Cell anemia
Hodgkin's disease	Immunodeficiency states
Nonhodgkin's lymphomas	Myelofibrosis
Wilm's tumor	
Neuroblastoma	
Ewing's sarcoma	
Embryonal rhabdomyosarcoma	

In three randomized clinical studies, the results of BMT have been compared with chemotherapy(45-47). In one of them, the results were similar(45) while in other two patients undergoing allogenic BMT had significant better EFS(46,47). In a prospective study(48) the projected EFS at 4 years was 55% and 48% in patients with allogenic and autologous BMT while it was 30% for patients on intensive chemotherapy. However, the authors concluded that overall survivals after successful complete remission were similar in the above three groups. Another study has also reported that the results of allogenic BMT were better when compared with the chemotherapy group(49). On comparing the results of PBSCT in 28 patients with the data of autologous Bone Marrow Transplant Registry of 683 matched patients, it was observed that EFS were identical in these two groups of patients(50).

There is a theoretical risk that the autologous BMT may be contaminated with residual leukemia cells. Purging of bone marrow by mafosfamide has improved the survivals in the European study(51). Recently EFS of 57% has been observed in patients who received purged BM (4 HC and/or etoposide) as compared with 32% in patients who received unpurged marrow. It is expected that with further improvement in purging techniques, the results of autologous BMT or PBSCT are likely to improve further.

Acute Lymphoblastic Leukemia (ALL)

Currently the EFS in children with ALL varies between 70 to 80% for the newly diagnosed cases. Presently patients at high risk are considered for BMT or PBSCT and both these procedures have resulted in frequent relapses(52). Recently polymerase chain reaction (PCR) has been utilized to identify the minimal residual disease(53).

Presently allogenic BMT is recommended for: *(a)* children in second or third remission; and (c) young adults with high leucocyte count at presentation. In a recent study form International Bone Marrow Transplant Registry (IBMTR) of 38 patients with resistant ALL, BMT resulted in successful remission in 89% of patients with EFS in 23%(54). Therefore allogenic BMT may successfully cure some patients who are resistant to chemotherapy(54,55).

In a French multicentric study, of 572 evaluable adult patients with ALL, 436 (76%) subjects achieved complete remission(56). Of 116 patients who underwent allogenic BMT, 43% of them had 3 years of EFS while EFS in 96 patients, each, with chemotherapy and autologous BMT was 32% and 39%, respectively. Thus all these studies have shown that allogenic BMT offers better EFS in patients with high risk ALL.

Chronic Myeloid Leukemia

Presently for patients (<45 years of age), allogenic BMT, if undertaken within one year of diagnosis offers cure in 50-70% of patients. However, results of allogenic BMT in patients with accelerated phase and in blast crisis vary between 15-25% and 15% of cases, respectively(57,58). The outcome of autologous BMT in 200 patients over 8 centers has been reviewed. All twenty eight cases with blast crisis died within 3 years of transplant while the median survival in 30 patients with accelerated phase was 39.9 months. In the 142 patients transplanted in chronic phase, the median survival had not been reached within seven years of follow up(59). PBSC harvested at diagnosis has been used to transplant 14 Philadelphia positive patients during chronic phase. Thirteen patients engrafted and survived for more than one year, three for several years not requiring further therapy and two became Philadelphia negative(60). Progress on cell identification and separation techniques will permit for vitro selection, better purging and expansion of normal hematopoietic stem cells for BMT/PBSCT(61,62). With these studies it is expected that one will be able to use purified PBSC for the treatment of CML.

Hodgkin's Disease

Bone marrow is frequently involved in refractory Hodgkin's disease. In a study, 72 patients were treated with high dose chemotherapy and unmobilized PBSC for transplantation(63). Complete and partial remission was observed in 50% and 35% respectively of the 68 evaluable patients. In 33% of survivals there was no progression of the disease at 46 months. Similarly high dose chemotherapy followed by autologous BMT has resulted in complete remission in 50% of patients with EFS of 20-30% at 2-3 years(63). Autologous BMT if undertaken when the disease is minimal *(i.e.,* soon after relapse) has resulted in improved survivals as against those in whom multiple chemotherapeutic regimens have failed(65).

Non-Hodgkin's Lymphoma (NHL)

Results of autologous and allogenic BMT in patients with NHL resistant to chemotherapy are identical(66,67). However, the results are better in those patients who are chemosensitive with minimal disease and with good performance status(67). Paired hematopoietic stem cells have been cultured from the bone marrow and PBSC and tumor cells were observed in 36% and 5% of cases, respectively. Patients treated with PBSCT fared better than those who underwent BMT(3). In a study of 158 patients with intermediate or high grade NHL, results indicate an EFS at 3 years in 70% of patients (n=53) undergoing PBSCT as compared with 32% of cases (n=105)

who underwent BMT(68). Complete remission in 51% of patients with actuaril survival of 57 months in 41 (39%) of patients with bone marrow involvement has also been documented(69). Thus it is expected that PBSCT will outnumber BMT in most centers for NHL as the results with PBSCT are better because of lesser contamination by tumor cells.

Solid Tumors

BMT is being undertaken more frequently for solid tumors with bone marrow infiltration. The results of 25 children who underwent high dose chemotherapy alongwith autologous BMT have been recently reported(70). Seventeen (68%) children achieved complete EFS between 14-90 (media 34) months. Non-randomized studies of high dose chemotherapy with autologous BMT have revealed EFS in 20-40% of children with Stage IV neuroblastome(71,72). There is a need of controlled studies to evaluate the results from multicentric centers on myeloablative therapy supported by autologous BMT or PBSCT versus conventional chemotherapy in children with various solid tumors.

Aplastic Anemia

The International Bone Marrow Transplant Registry (IBMTR) has reported results of 595 patients with aplastic anemia following allogenic BMT from HLA identical siblings. Five year survival was observed in 69% of patients receiving cyclosporin-A and 56% of those patients receiving methotrexate for GVHD. Presence of infections and multiple transfusions before BMT, use of multiparous or multitransfused women donors, and older patients were associated with a poor prognosis(73). Experience with family donor transplants (other than HLA sibling donor) is limited. IBMTR, in a study of 60 patients, reported that the probability of EFS for 2 years was 27% for related family donor transplants as compared to 67% for HLA identical sibling donor transplants. Risk of BMT failure increased significantly with HLA disparity(74). These studies have revealed that BMT is the treatment of choice for young patients (<40 years of age) with severe aplastic anemia and should preferably be undertaken soon after the diagnosis. Patients who are old or who do not have HLA matched sibling donor should be treated with alternative methods of treatment.

Thalassemia

BMT is being done at multiple centers for Thalassemia. However, the maximum number of children have undergone BMT in Italy. Three distinct prognostic factors have been identified (hepatomegaly of more than 2 cm, portal fibrosis and poor chelation therapy). Class I children v/ere those who did not have any of the above factor, Class II children had one or two of the above factors while Class III children had all three risk factors. In 484 children, the authors observed EFS in 94%, 84% and 67% of children for Classes, I, II and III, respectively(75). Results from other studies are not as good. However, the results between various centers cannot be compared as selection criteria and classification of various subgroups are not identical. BMT at present offers complete cure for those children who have HLA matched donor, are young and well chelated.

Sickle Cell Anemia

Sickle cell anemia is another life threatening inherited disorder associated with sickle cell crisis. Allogenic BMT offers complete cure. Thirty six of 42 symptomatic children without chronic organ damage had successful allogenic BMT. All these children became asymptomatic and had normalization of the hemoglobin electro-

phoresis(76). Bone marrow rejection was observed in 5 cases and two of them engrafted again following second BMT. Multicentric controlled studies are essential to evaluate the results of BMT in a larger number of patients which need to be compared with prophylactic hydroxyurea therapy.

Immunodeficiency Diseases

Currently BMT offers complete cure for immunodeficiency diseases such as Severe Combined Immunodeficiency Disease, Wiscott Aldrich syndrome, or Chediak Higashi syndrome. European Bone Marrow Transplant Group (EBMTG) have reported encouraging results for these groups of patients and have observed that presence of infection prior to BMT, absence of protected environment, and use of female donors for male patients were bad prognostic factors(77,78).

Hematopoietic Stem Cell Transplantation in India

In India at present, there are only five centers where BMT or PBSCT are being performed, such as All India Institute of Medical Sciences, New Delhi, Tata Memorial Cancer Hospital, Mumbai, Christian Medical College (CMC), Vellore; Apollo Hospital, Madras; and Adeyer Cancer Hospital, Madras. Till date over 225 transplants have been undertaken and half of these have been done at Vellore. Majority of transplants at Vellore have been undertaken for children with thalassemia, while at other centers, BMT is being done for a large variety of indications. Till date PBSCT have been done in nearly 25 cases. All these centers have a long waiting list of over two years for non malignant cases. Lack of space, trained staff, and the will of the institutions and the Government have been the main stumbling blocks in the development of centers for BMT. The cost of BMT in the developed countries varies between 20-60 lakhs. In our country, patients have to spend between 4-6 lakhs, besides the expenses borne by the hospital or institutions. Such a high cost is beyond the reach of the majority. Therefore, it is essential that the Government and voluntary organizations should come forward to help these patients. The Government should also provide financial assistance to institutions to develop BMT or PBSCT facilities which will benefit a large number of patients.

REFERENCES

1. Juttner CA, Fibbe WE, Nemunaitis J, Kanz L, Gianni AM. Blood Cell transplantation: Report from an International consensus meeting. Bone Marrow Transplantation 1994; 14: 689-693.
2. Kumar L, Gulati SC. Peripheral stem cell transplantation. Lancet 1995; 346: 9.
3. Lee JH, Klein HG. Collection and use of circulating hematopoietic progenitor cells. Heamt Oncol Clin N Amer 1995; 9:1-22.
4. Silberstein LE, Jefferies LC. Placental blood banking: A new frontier in transfusion medicine. New Eng J Med 1996; 335: 199-201.
5. Kurtzberg J, Laughlin M, Graham ML Smith C, Olson JF, Halperin EC, *et al.* Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. New Eng J Med 1996; 335: 157-166.
6. Kumar L, Goldman JM. Bone marrow transplantation for patients lacking an HLA identical sibling donor. Current Options in Hematol 1993; pp 234-239.
7. Schwarrer AP, Jiang YZ, Deacock S, Brookes PA, Barrett AJ, Goldman JM, *et al.* Comparison of helper and cytotoxic antirecipient T cell frequencies in an unrealted bone marrow transplantation. Transplantation 1994; 58:1198-1203
8. Schwarrer AP, Jiang YZ, Brookes PA, Barrett AJ, Batchelor JR, Goldman JM,

et al. Frequency of antirecipient alloreactive helper T cells precursors in donor blood and graft versus host disease after HLA identical sibling bone marrow transplantation. Lancet 1993; 341:203-206.

9. Martin PJ. Increased disparity for minor histocompatability antigens as a potential cause of increased GVHD risk in marrow transplantation form unrelated donors compared with related donors. Bone Marrow Transplant 1991; 8: 217-223.

10. Lee JA, Klein HG. Collection and use of circulating hematopoietic progenitor cells. TransfMedl995,9:l-22.

11. Civin CI, Strauss LC, Broval £, Fackler MJ, Schwartz JF, Shaper JH, *et al.* Antigenic analysis of hematopoiesis, III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-Ia cells. J Immunol 1984; 133:157-165.

12. Brandt J. Baird N, Lu L, Srour E, Hoffman R. Characterization of a human progenitor cell capable of forming blast cell con taining colonies *in vitro*.] Clin Invest 1988; 82:1017-1027.

13. Bender JG, Unverzagt KL, Walker DE, Lee WE, Vanepps DE, Smith DH, *et al.* Identification and comparison of CD 34 positive cells and their subpopulations from normal peripheral blood and bone marrow using multicolor flow cytometry. Blood 1991; *77*:2591-2596.

14. Andrews RG, Singer JW, Bernstein ID. Monoclonal antibody 12-8 recognizes a 115-kd molecule present on both unipotent and multipotent hematopoietic colony forming cells and their precursors. Blood 1986; 67: 842-845.

15. Civin CI, Loken MR. Cell surface antigens on human marrow cells. Dissection of hematopoietic development using monoclonal antibodies and multiparameter flow cytometry. Int J Cell Cloning 1987; 5: 267-288.

16. Sovalat H, Liang H, Wunder E, Henon P. Flow cytometry characterization of CD34+ cells in bone marrow, cytopheresis products and cord blood at birth. Int J Cell Cloning 1992; 10 (Suppl 1): 20-22.

17. Uchida N, Weissman IL. Searching for hematopoietic stem cells. Evidence that Thy 1-110 Lin-Sea 14 cells are the only stem cells in C 57 BI/Ka-Thy-L-110 bone marrow. J Exp Med 1992; 175:175-184.

18. Broxmeyer HF, Gluckman F, Auerbach A. Human umbilical cord blood. A clinically useful source of transplantable hematopoietic stem/progenitor cells. Int J Cell Cloning 1990; 8 (Suppl 1): 76-91.

19. Bender JG, Unverzagt K, Walker DE, Myers S, Williams LB, Schwartzberg LS, *et al.* Phenotypic analysis and characterization of CD34+ cells from normal human bone marrow, cord blood, peripheral blood and mobilized peripheral blood from patterns undergoing autologous stem cell transplantation. Clin Immunol Immunopathol 1994; 70:10-18.

20. Bensinger W, Singer J, Appelbaum F, Lilleby K, Longin K, Rowley S, *et al.* Autologous transplantation with peripheral blood mononuclear cells collected after administration of recombinant granulocyte stimulating factor. Blood 1993; 81: 3158-3163.

21. Chao NJ, Schriber JR, Grimes K, Long GD, Negris RS, Raimondi CM, *et al.* Granulocyte colony-stimulating factor "mobilized" peripheral blood progenitor cells accelerate granulocyte and platelet recovery after high-dose chemotherapy. Blood 1993; 81:2031-2035.

22. McCarthy D, Goldman JM. Transfusion of circulating stem cells. CRC Crit Rev Clin Lab Sci 1984; 20:1-24.

23. Villeval JL, Duhrsen V, Marstyn G, Metcalf D. Effect of recombinant human granulocyte-macrophage colony stimulating factor on progenitor cells in patients with advanced malignancies. Br J Hematol 1990; 74: 36-44.

24. Demuynck H, Delforge M, Verhoef G, Zachee P, Vandenberghe P, Boogaerts M.

Comparative study of peripheral blood progenitor cell collection in patients with multiple myeloma after single dose cyclophosphamide combined with rhGM-CSF or rh G-CSF. Br J Hematol 1995; 90: 384-392.

25. Gluck S, Gagnon A. Neutropenic fever in patients after high dose chemotherapy followed by autologous hematopoietic progenitor cell transplantation and hu man recombinant granulocyte-macrophage colony stimulating factors. Bone Mar row Transplant 1994; 14: 989-990.

26. Kanz L, Brugger W, Mertelsmann R. Peripheral blood progenitor cells: Mobiliza tion, enrichment, *ex vivo* expansion and clinical use. *In:* Hematopoietic growth fac tors in Clinical Applications, 2nd edn. Eds. Mertelsmann R, Hermann F. New York, Marcel Dekker, 1995; pp 227-239.

27. Pretira RA, Razis E, Ciavarella D, Fan Y, Kuhns RE, Cook P, *et al.* Clinical and labo ratory comparison study of refrigerated and cryopreserved bone marrow for transplantation. Bone Marrow Transplant 1994; 13: 253-260.

28. Ossenkoppele GJ, Jonkoff AR, Huihgens PC, Nauta JJP, Vander HKG, Drager AM, *et al*. Peripheral blood progenitor cells mobilized by G-CSF (Filgrastim) and reinfused as unprocessed autologous whole blood shortern the pancytopenic period following high dose melphalan in multiple myeloma. Bone Marrow Trans plant 1994; 13:37-41.

29. Kamble R, Raju GMK, Kumar L, Kochupillai V. Blood stem cell transplan tation: Current concepts. Indian J Med Res 1997; 105-1-8.

30. Brugger W, Bross KJ, Glatt M, Weber F, Mertelsmann R, Kanz L. Mobilization of tumor cells and hematopoietic progenitor cells into peripheral blood of patients with solid tumors. Blood 1994; 83: 636-640.

31. Members of the EORTC Clonogeneic As say Screening Study Group. Human granulocyte macrophage colony stimulating factor modulates *in vitro* growth in only a minority of continuous human tumor cell lines. Eur J Cancer 1991; 27: 231-235.

32. Salmon SE, Liu R. Effects of granulocyte-macrophage colony stimulating factors on *in vitro* growth of human solid tumors. J Clin Oncol 1989; 7:1346-1350.

33. Brenner MK, Kill DR, Moen RC, Kance R, Mirro JRJ, Anderson WF, *et al*. Gene marking to trace origin of relapse after autologous bone marrow transplantation. Lancet 1993; 341: 85-86.

34. Gulati SC, Acaba L, Yahlom J, Reich L, Motzer R, Crown J, *et al.* Autologous bone marrow transplantation for acute myelogenous leukemia using 4-hydroperoxy-cyclophosphamide and VP-16 purged bone marrow. Bone Marrow Transplant 1992; 10:129-134.

35. Gulati SC, Lemoli RM, Acaba L, Igarashi T, Wasserheit C, Fraig M. Purging in au tologous and allogeneic bone marrow transplantation. Curr Opin Oncol 1992; 4:264-271.

36. Silberstein LE, Jefferies LC. Placental blood banking: A new frontier in transfu sion medicine. New Eng J Med 1996; 335: 199-201.

37. Kurtzber J, Laughlin M, Graham ML, Smith C, Olson JF, Halperin EC, *et al.* Pla cental blood as a source of hematopoietic stem cell for transplantation into unrelat ed recipients. New Engl J Med 1996; 335: 157-166.

38. Laporte JP, Gorin NC, Rubinstein P, Lesage S, porthoi MF, Barbu V, *et al*. Cord-blood transplantation from anunrealed donor in an adult with chronic myeloid leukemia. New Eng J Med 1996; 333:167-170.

39. Lu L, Xiao M, Shan RN, Grigsby S, Bronmeyer HE. Enrichment characteriza tion and responsiveness of single primi tive CD34+ h human umbilical cord blood hematopoietic progenitors with high pro-

liferative and replacing potential. Blood 1993; 81: 41-48.

40. Me Cullough J, Clay ME, Fautsch S, Holzer D, Lie M. Proposed policies and procedures for the establishment of a cord blood bank. Blood Cells 1994; 20: 609-626.

41. Kumar L. Bone marrow transplantation. Current Status Medicine Update 1995; 6: 587-592.

42. Kumar L. Bone marrow transplantation current status part II. Medicine Update 1995; 6: 627-632.

43. Schiller G, Vesico R, Freytes C, Spitzer G, Sahebi F, Lee M, *et al.* Transplantation of CD34+ peripheral blood progenitor cells after high dose chemotherapy for patients with advanced multiple myeloma Blood 1995; 86: 390-397.

44. Motzer RJ, Bosl GJ. High dose chemotherapy for resistant germ cell tumours: Recent advances and future directions. J Nat Cancer Inst 1992; 94:1703-1709.

45. Champlin RE, Ho WG, Gale RP, Winson D, Selch M, Mitsuyasu R, *et al.* Treatment of acute myelogenous leukaemia: A prospective controlled trial of bone marrow transplantation versus consolidation chemotherapy. Ann Int Med 1985; 102: 285-291.

46. Zander AR, Keating M, Dicke K, Fabritus M. A comparison of marrow transplantation with chemotherapy for adults with acute leukemia of poor prognosis in the first complete remission. J Clin Oncol 1988; 6:1548-1577.

47. Appelbaum FR, Fisher LD, Thomas ED.The Seattle Marrow Transplant Team. Chemotherapy versus marrow transplan tation for adults with acute nonlymphoblastic leukemia: A five year follow up. Blood 1988; 72:179-184.

48. Zittoun RA, Mandelli F, Willemze R, dewitte T, Labar B, Resegoth L, *et al.* Autologous or allogenic bone marrow transplantation compared with intensive chemotherapy in acute myelogenous leukemia. New Eng J Med 1995; 332: 217-223.

49. Chopra R, Goldstone AH. Modern trends in the bone marrow transplantation for acute myeloid and acute lymphoblastic leukemia. Curr Opin Oncol 1992; 4: 247-258.

50. Reiffers J, Koerbling M, Labopin M, Gorin NC, Michallet M, Marit Cr, *et al.* Autologous blood stem cell transplantation versus autologous bone marrow transplantation for acute myeloid leukemia in first complete remission. Int J Cell Cloning 1992; 10 (Suppl 1): 111-113.

51. Labopin M, Gorin NC. Autologous bone marrow transplantation in 2502 patients with acute leukemia in Europe. Leukemia 1992; 6 (Suppl 4): 95-99.

52. Takaue Y, Hoshi Y, Watanabe A, Kawano Y, Watanabe T, Kanwano Y, *el al.* Collection and autografts of peripheral blood stem cells in children with acute leukemias and non-Hodgkin's lymphoma (NHL). Int J Cell Cloning 1992; 10 (Suppl 1): 152-154.

53. Yokota S, Hansen-Hagge TE, Ludwing WD, Reiter A, Raghavachar R, Kleihaver E, *et al.* Use of polymerase chain reaction to monitor minimal residual disease in acute lymphoblastic leukemia patients. Blood 1991; *77:* 331-339.

54. Hoelzer D. Treatment of acute lymphoblastic leukemia. Sem Hematol 1994; 31:1-15.

55. Biggs JC, Horowitz MM, Gale RP, Ash RC, Atkinson K, Helbig W, *et al.* Bone marrow transplantation may cure patients with acute leukemia never achieving remission with chemotherapy. Blood 1992; 80:1090-1093.

56. Fiere D for the French Group On Therapy for Adult Acute Lymphoblastic leukemia. Adult acute lymphoblastic leukemia: A multicentric randomized trial testing bone marrow transplantation as post remission therapy. J Clin Oncol 1993; 11:1990-2001.

57. Clift RA, Appelbaum FR, Thomas ED. Treatment of chronic myeloid leukemia

by marrow transplantation. Blood 1993; 82:1994-1996.

58. Goldman JM, Szydlo R, Horowitz MM, Gale RP, Ash RC, Atkinson K, *et al*. Choice of pretransplant treatment and timing of transplants for chronic myelogenous leukemia in chronic phase. Blood 1993; 82: 2235-2238.

59. Me Glave PB, De Fabritis P, Deisseroth A, Goldman J, Barnett M, Reiffert, *et al.* Autologous transplants for chronic myelogenous leukemia: Results from eight transplant groups. Lancet 1994; 343:1486-1488.

60. Brito-Babupulle F, Bowcock SJ, Marcus RE, Apperley J, Thing KH, Dowding C, *et al.* Autografting for patients with chronic myeloid leukemia in chronic phase. Peripheral blood stem cells may have a finite capacity for maintaining hematopoiesis. Brit J Hematol 1989; 73: 76-81.

61. Lemoli RM. Characteriation and selection of benign stem cells in chronic myeloid leukemia. Hematologica' 1993; 78: 393-400.

62. Dubar CE, Stewart FM. Separating the wheat from the chaff. Selection of benign hematopoietic cells in chronic myeloid leukemia. Blood 1992; 79:1107-1110.

63. Kessinger A, Vose J, Bierman PJ, Armitage JO. High dose cyclosphosphamide, etoposide and carmustine and peripheral stem cell trans plantation for patients with relapsed Hodgkin's disease and bone marrow abnormalities. Int J Cell Cloning 1992; 10 (Suppl 1): 135-137.

64. De Vita Jr VT, Hubbard SM. Hodgkin's disease. New Eng J Med 1993; 328: 560-565.

65. Reece DE, Connors JM, Spinelli JJ, Barnett MJ, Fairey RN, Klingemash HC, *et al.* Intensive therapy with cyclophosphamide, carmustine, etoposide + cisplatin and autologous bone marrow transplantation for Hodgkin's disease in first relapse after combination chemotherapy. Blood 1994; 83:1193-1199.

66. Chopra R, Goldstone AH, Pearce R, Phillep T, Petersent F, Appelbaum F, *et al.* Autologous versus allogeneic bone marrow transplantation for non-Hodgkin's lymphoma: A case controlled analysis of the European Bone Marrow Transplant Group Registry data. J Clin Oncol 1992; 10:1690-1695.

67. Armitage JO. Treatment of non-Hodgkin's lymphoma. New Eng J Med 1993; 328:1023-1030.

68. Vose JM, Anderson JR, Kessinger A, Bierman PJ, Coccia P, Reed EC, *et al.* High dose chemotherapy and autologous hematopoietic stem cell transplantation for aggressive non Hodgkin's lymphoma. J Clin Oncol 1993; 11:1846-1851.

69. Kessinger A, Armitage JO. Peripheral stem cell transplantation for patients with non Hodgkin's lymphoma. Int J Cell Cloning 1992; 10 (Suppl 1): 127-128.

70. Garaventa A, Hartmann O, Bernard JL, Zucker JM, Pardon N, Castel V, *et al.* Autologous bone marrow transplantation for pediatric Wilm's tumor: The experience of the European Bone Marrow Transplantation Solid Tumor Registry. Med Pediatr Oncol 1993; 22:11-14.

71. Graham Pole JR. Myeloablative treatment supported by marrow infusions for children with neuroblastoma. *In:* High Dose Cancer Therapy. Eds. Armitage JO, Antman KH. Baltimore, Williams and Wilkins, 1992; pp 735-749.

72. Evans AE, August CS, Kamani N, Bunin N, Goldween J, Ross AJ, *et al.* Bone marrow transplantation for high risk neuroblastoma at the children's hospital of Philadelphia: An Update. Med Pediatr Oncol 1994; 23: 323-327.

73. Gluckman E, Horowitz MM, Champlin RE, Hows JM, Baciglupo A, Bigg JC *et al.* Bone marroe transplantation for severe aplastic anaemia: Influence of conditioning in graft versus host disease prophylaxis regimens on out come. Blood 1992; 79: 269-275.

74. Ash RC, Horowitz MM, Gale RP, Van-Bekkum DW, Casper JT, Gordon Smith EC, *et al.* Bone marrow transplantation from related donors other than HLA-identical siblings: effect of T-cell depletion. Bone Marrow Transplant 1991; 7: 443-452

75. Giardini C, Angelucci E, Lucarelli G, Galimberti M, Dolchi P, Baronciani D, *et al.* Bone marrow transplantation for thalassemia. Am J Pediatr Hematol Oncol 1994; 16: 6-10.

76. Vermylen C, Cornu G. Bone marrow transplantation for sickle disease: The European Experience. Am *J* Pediatr Hematol Oncol 1994; 16:18-21.

77 Fischer Landais S' F"edench W, Morgan G, Gerritsen B, Fasth A, *et al.* European experience of bone marrow transplantation for severe combined immunodeficiency. Lancet 1990; 336: 850-854.

78. Fischer A, Landais P, Friedrich W, Gerritsen B, Fasth A, Porta F, *et al.* Bone marrow transplantation (BMT) in Europe for primary immunodeficiencies other than severe combined immunodeficiency: A report from the European Group for BMT and the European Group for Immunodeficiency. Blood 1994; 83:1149-1154.