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Sources of Hematopoietic Stem Cells

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1. Introduction

Hematopoietic stem cell transplantation (HSCT) has the potential to cure a variety of malignant and non-malignant diseases. Sources of hematopoietic stem cells for transplantation have expanded progressively since the beginning of the modern era of transplantation in the late 1960s. Although bone marrow was the main source of stem cells in the early years of transplantation, in the past 10 to 15 years peripheral blood has assumed increasing importance. The initial impetus for the use of PBSCs for transplantation was to be able to offer transplantation to patients who were not candidates for the use of bone marrow cells (tumor contamination of the marrow or those with hypocellular marrows). Subsequent studies demonstrated that PBSCs could be mobilized from the bone marrow with either hematopoietic growth factors (GM-CSF, G-CSF) or a combination of chemotherapy and growth factors, which increased the number of hematopoietic progenitors collected from the blood by 10- to 1000- fold compared with steady-state conditions. Umbilical cord blood represents the newest source of stem cells for transplantation. At now peripheral blood is the main source in the autologous setting. Within the allogeneic setting, multiple sources of stem cells are possible and include those derived from individuals related or unrelated to the patient.

Hematopoietic progenitor cell (HPC) products contain hematopoietic stem and lineage-committed progenitor cells capable of providing hematopoietic and immune reconstitution after myeloablative or reduced-intensity preparative regimens. HPCs administered intravenously migrate to the marrow, where they adhere, expand, self-renew (stem cells only), and differentiate. The differentiated cells are released into the blood, restoring blood counts and immunity. The time from administration of HPCs to recovery of adequate or normal blood counts is variable. Recipients of peripheral blood stem cells recover counts faster than recipients of bone marrow. Cord blood tends to be the slowest to engraft.

The minimum number of HPCs necessary for engraftment in a myeloablated recipient has not been established. Different products have widely different numbers of progenitors and stem cells. However, eligibility criteria for some protocols usually dictate a minimum number of cells to be collected and infused.

Several methods are used to measure the number of cells in an HPC collection. Simple cell count may be adequate for many marrow collections. Most centers use flow cytometric enumeration of CD34+ cells for the majority of cellular products. The discovery of the CD34 antigen in the early 1980s revolutionized our understanding of hematopoiesis. Cells expressing CD34 are capable of reconstituting hematopoiesis in lethally irradiated animals and humans, indicating that the putative hematopoietic stem cell expresses CD34.

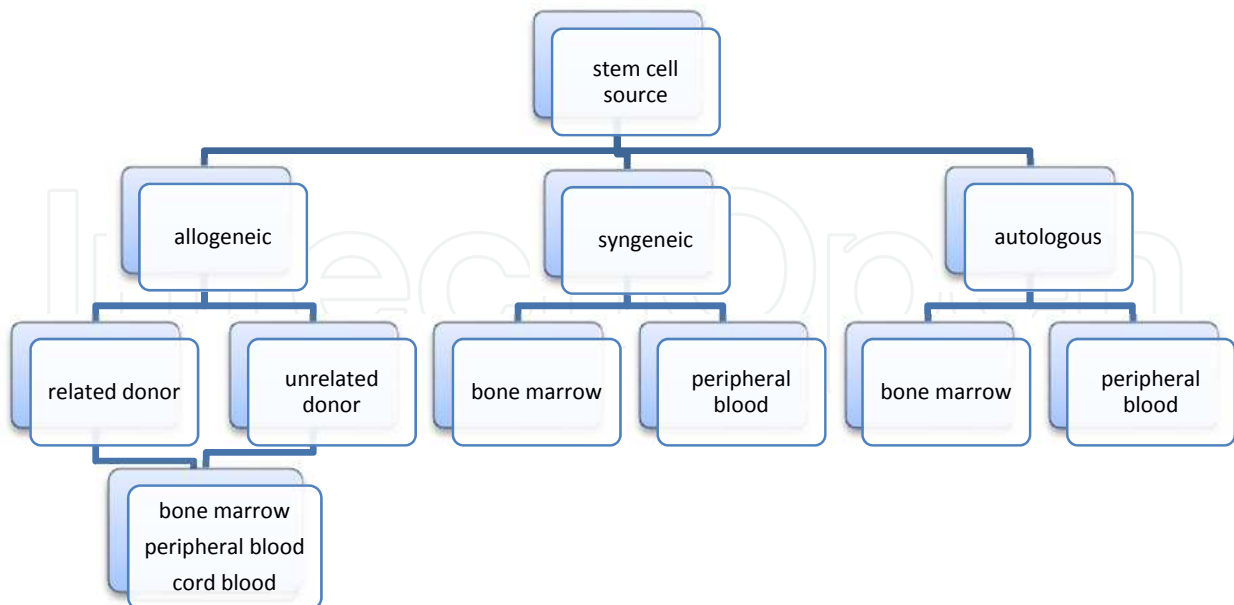


Fig. 1. Sources of haematopoietic stem cells for different types of transplantation

This type I transmembrane glycoprotein is expressed on:

1- 3% of bone marrow mononuclear cells

0,01- 0,1% of peripheral blood mononuclear cells

0,1- 0,4% of umbilical cord blood cells

2. Marrow as a source of stem cells

Marrow is collected in the day surgery suite using either general or regional anesthesia. With proper fluid and blood replacement, overnight hospitalization should not be required. For the healthy donor, the risks of serious complications from either general or regional anesthesia are about the same. Spinal or epidural anesthesia avoid the nausea that may occur with general anesthesia, especially for young women, but hypotension from loss of vascular tone in the lower extremities often occurs as the volume of marrow is collected. General anesthesia is preferable for the donor with comorbid disorders such as cardiovascular or cerebral vascular disease because of the better control of donor airway and lower risk of hypotension during the harvest procedures. Local anesthesia is acceptable only if a very limited harvest is being performed, as large quantities of lidocaine are cardiotoxic and local anesthesia does not achieve anesthesia of the marrow space. The technique of bone marrow harvest is straightforward and involves repeated aspirations of small volumes (10ml) of marrow. The marrow is removed sterilely from both posterior iliac crests by two operators simultaneously to minimize anesthesia time. Occasionally marrow is obtained from the anterior iliac crest or sternum. Typically we do two puncture through the skin and multiple bone punctures. Do not take more than 25 ml of bone marrow per kilogram of donor - this is the upper limit on the volume of collected bone marrow. The marrow is placed in a sterile container with an electrolyte solution and an appropriate anticoagulant.

The cell suspension is passed through sterile filters to remove fat, bone particles, and cellular debris [4,5]. For patients with a history of radiation or tumor involvement of one pelvic crest, adequate cells can be harvested from the anterior and posterior crests of the other side [6-8].

2.1 Toxicity and adverse events associated with bone marrow collection

Anesthesia complications present the major health risk to the donor. Marrow aspiration is generally well tolerated. Major complications occur in approximately 0.27% of healthy allogeneic donors and up to 0.97% of autologous transplant patients [9-10]. Complications include hemorrhage and infections at skin puncture sites. Severe hematomas and neuralgias rarely occur, but attention to pelvic anatomy is required to decrease the risk of damage to vessels and nerves lying under or adjacent to the iliac crest harvest sites. Irritation of the sacral nerves may result from needle penetration through the pelvic bone or from blood tracking into the nerve roots and requires several months of convalescence. Localized pain is common, may last for several days, and may require a brief period of medication with opioid/acetaminophen combinations [4]. In a survey of almost 500 donors for unrelated marrow transplantation, the average time for recovery was 15.8 days, although 10% of donors required more than 30 days for self-reported complete recovery [11]. Most donors are able to return to routine activities 1 to 2 days after harvesting. In a study of related donors, an equivalent level of pain was reported by donors undergoing bone marrow harvesting and those receiving filgrastim for mobilization of PBSC [12]. Minor complications occur in 6-20% of marrow donations [11]. These include such events as hypotension, syncope, severe post-spinal headache, excess pain, unexpected hospitalization and minor

Symptom	Women %	Men %
Tired	85	76
Collection site pain	78	75
Back pain	67	68
Nausea	63	40
Sore throat	62	57
Pain sitting	62	57
Lightheadedness	53	42
Headache	40	32
Vomiting	39	17
Intravenous site pain	37	23
Fever	22	22
Bandage pain	19	26
Bleeding at site	10	8
Fainting	7	5

Table 1. Symptoms reported by National Marrow Donor Program (NMDP) bone marrow donors, 1987-2000 (n=9601) [8]

infections. In National Marrow Donor Program (NMDP) observation the frequency of serious adverse events following marrow donation is estimated at 0,1-0,3%. Reactions to the anesthetic agents administered may be adverse reactions, hypersensitivity or idiosyncratic reactions. One NMDP donor experienced laryngospasm following extubation. Additionally, several NMDP donors have experienced profound bradycardia during anesthesia, including regional anesthesia (spinal and epidural), that required emergency treatment. Death has occurred among normal marrow donors. A recent review of 7857 marrow collections reported two deaths [13].

2.2 Quantity of bone marrow cells for transplantation

Generally, 10-20ml marrow/kg of donor weight is harvested to achieve a minimum mononuclear cell (MNC) count of 2×10^8 to 8×10^8 MNC/kg of recipient body weight, although ideally up to $4,0 \times 10^8$ to 8×10^8 MNC/kg is preferred to compensate for cell loss during processing and to ensure adequate engraftment. The only setting in which higher numbers of MNC/kg definitely have been shown to be of benefit is aplastic anemia, in which low cell counts have been associated with an increased risk of rejection. Marrow contains mature red cells, white cells, platelets, mast cells, fat cells, plasma cells, committed progenitors of all lineages and hematopoietic stem cells. The most common modifications of allogeneic marrow are to decrease the volume of ABO-incompatible red cells, remove ABO- incompatible plasma, isolate CD34+ cells and remove donor T lymphocytes. The most common modification of autologous marrow is to reduce the volume by removing plasma and red cells before cryopreservation [4].

2.3 "Rich" bone marrow

Pretreatment of the marrow donor with filgrastim (granulocyte- colony-stimulating factor G-CSF) may increase the number of myeloid progenitor cells harvested and decrease the period of posttransplant aplasia to that achievable by PBSC transplantation [14]. Hematologic recovery in patients who are treated with autologous stem cells taken from bone marrow after G-CSF stimulation (rich bone marrow - RBM) is faster than in patients without G-CSF. In the Polish study [15] were estimated engraftment outcomes of patients who received bone marrow unstimulated or stimulated with G-CSF. The median and range for neutrophil engraftment times in this study when they used stimulated bone marrow were comparable with those published by Lemoli et al. [16]. It seems that a better method of obtaining stem cells from bone marrow is the RBM. Using of stimulated bone marrow can faster engraftment comparing to non-stimulated bone marrow and can help patients, who fail to collect adequate number of stem cells from their peripheral blood. It is generally accepted that RBM engraft more rapidly than unstimulated bone marrow and that RBM appear to have similar engraftment times to peripheral blood stem cell transplantations suggesting that it is prior G-CSF exposure, not the anatomic site, which influences engraftment [14,17,18]. CD34+ cell dose now is being correlated with transplant outcomes, with more rapid engraftment kinetics, possibly lower transplant-related mortality, and better overall survival, for example, in recipients of allogeneic products containing higher quantities of CD34+ cells. Otherwise patients receiving filgrastim- primed bone marrow had significantly less steroid-refractory acute graft-vs-host disease (GvHD), less chronic GvHD and fewer days of immunosuppressive therapy. Download stimulated bone marrow may

favor the acquisition of more hematopoietic cells and facilitate reconstitution after myeloablative therapy with no significant increase in risk of GvHD [19].

	PLT> 20	ANC> 0.5 G/L	Length of hospitalization
Median time to recovery (RBM)	12,6 days	13,0 days	17,3
Median time to recovery (bone marrow without stimulation of G-CSF)	18,8 days	17,8 days	23,1

Table 2. Time to recovery of platelets (PLT) and neutrophils (ANC) and length of hospitalization when we used bone marrow after stimulation G-CSF and without stimulation of G-CSF [15]

3. Peripheral blood stem cells (PBSC)

Stem cells were detected in the peripheral blood of mice in 1962 and in human in 1971 [20]. However, interest in peripheral blood stem cell transplantation did not develop until mid-1980s. During 1985-1986 several centers from different parts of the world reported encouraging results of the autologous transplants using hematopoietic progenitor cells collected from peripheral blood [21,22]. The first allogeneic transplant with peripheral blood progenitor cells was reported in 1989 [23]. Use of PBSC became more common after 1995 with the publication reports of successful allogeneic transplants with PBSC [24-26]. By stimulating the donor with either hematopoietic growth factors, or chemotherapy and growth factors, a sufficient number of circulating stem cells for marrow rescue can be collected in one to three apheresis procedures. Mobilized PBSC products are routinely used as an alternative source of HSCs for transplantation [27,28]. A PBSC collection is performed with a cell separation device originally developed for therapeutic leukapheresis, plasmapheresis and platelet donation procedures. This apheresis device uses a centrifuge to separate and collect mononuclear cells, including HSCs, from the blood. In order to achieve an adequate HSCs for transplantation it is necessary to process 12 to 25 liters of blood or 2.5 to 6.0 times the patient's/ healthy donor's calculated blood volume. Investigators have reported that the yield of CD34+ cells increases continuously as more blood volumes are processed. Although up to six times the donor's blood volume can be safely processed, some donors are not able to tolerate 4 or 6 hours being connected to an apheresis machine. Therefore, these donors require repeated collections on sequential days once the peripheral CD34+ count has increased to acceptable levels (>10 CD34+ cells/ μ l) for collection. There are currently three different commercially available apheresis instruments. In each case, instrument settings such as inlet flow rate, centrifuge speed, collect pump flow rate and anticoagulant: whole blood ratio vary, depending on the target cell type to be collected. The three instruments operate differently. The Amicus (Baxter) and the COM.TEC (Fresenius) are more automated, computer-controlled instruments; however, the Spectra is more widely used [1,29]. Because an apheresis procedure is used to collect PBSC products, they contain very few erythrocytes or granulocytes, compared to marrow, and are primarily composed of mononuclear cells (MNCs). PBSC products also contain larger numbers of HPCs than either marrow or cord blood, and therefore facilitate faster engraftment and shorter hospital stays.

Patients who have been heavily pretreated with multiple rounds of chemotherapy or radiation therapy often mobilize poorly and require multiple collection episodes [30].

Although the peripheral blood of healthy individuals contains fewer than 0,1% HSCs, this number increases dramatically during recovery from cytotoxic therapy and even more so more so then recombinant CSFs such as G-CSF are administered. Various mobilizing techniques are used by centers and generally consist of growth factor administration alone or in combination with various types of chemotherapy. G-CSF and high-dose cyclophosphamide (CY) are the most commonly used agents [28, 31-33]. Currently, almost all of autologous and a majority of allogeneic transplants are performed with PBSC. Advantages of PBSC over bone marrow include [21,34]:

- Elimination of the need of general anesthesia, pain and other side effects of bone marrow aspiration.
- Patients with bone marrow metastases could be transplanted with autologous PBSC as there is a potential for tumor cell free collection.
- The hematological recovery with PBSC was faster than bone marrow significantly reducing the time to transfusion independence.

PBSC products contain larger numbers of T cells than marrow collections, and thus present a greater risk of causing graft-versus-host disease (GvHD) in the allogeneic setting, although the rate of acute GvHD is less than originally feared [35-39]. In a prospective randomized study comparing PBSC donation with bone marrow, PBSC products contained double the number of CD34+ cells, eight fold more of T and NK cells than bone marrow collection. The advantages to the recipient were faster recovery of both neutrophil and platelets with PBSC compared to marrow without increasing risk of graft-vs-host disease. Another multi-center study also reported faster neutrophil and platelet recovery but significantly more frequent acute and chronic GvHD in PBSC recipients than recipients of bone marrow cells. There were no significant differences in transplant related mortality, relapse rate and overall survival were found [40-42].

3.1 Mobilization regimens

Recent advances in stem cell mobilization techniques have exploited the interactions between stem cells and the bone marrow microenvironment. Composed of stromal cells, endothelial cells, osteoblasts and other matrix components (collagens, fibronectins, proteoglycans), the bone marrow microenvironment anchors hematopoietic stem cells through a wide range of adhesive interactions [43]. Hematopoietic stem cells express a broad array of cell surface receptors, namely the adhesion molecules lymphocyte function-associated Ag-1, very late Ag-4, and Mac-1; the chemokine receptors CXCR4 and CXCR2; the cell surface glycoproteins CD44 and CD62L; and the tyrosine kinase receptor c-kit [43-45]. The bone marrow stroma contains stromal cell-derived factor-1 (SDF-1), CXC chemokine GRO- β , vascular cell adhesion molecule-1, kit-ligand, P-selectin glycoprotein ligand-1 and hyaluronic acid, all of which are cognate ligands for the stem cell adhesion molecules [43-45]. Data from a number of preclinical models showed that inhibition of these receptor-ligand interactions resulted in enhanced progenitor cell mobilization [46-48].

Several exogenous hematopoietic cytokines that can mobilize hematopoietic progenitor cells into circulation are now available. Granulocyte colony stimulating factor (G-CSF) (filgrastim, lenograstim, pegfilgrastim) and granulocyte macrophage colony stimulating factor (GM-CSF) (sargramostim) are most commonly used for mobilization. They are used

	G-CSF	GM-CSF	Erythropoietin	Plerixafor
Adhesion factors	↓	Additive effects with G-CSF. May inhibit osteoclast activation.	Exact mechanism unknown, but additive when used in combination with G-CSF or GM-CSF	
CXCR4	↓ or ↑			No effect or ↓
SDF-1	↓			
MMPs	↑			
Neutrophil elastase	↑			

MMP- matrix metalloproteinase; SDF-1: stromal-derived factor 1 α

Table 3. Factors using for mobilization- mechanism of action [31]

alone to mobilize hematopoietic stem cells into circulation in healthy donors for allogeneic transplantation and in patients who are in complete remission or in heavily pretreated ones for autologous transplantation. Approximately 4 days after the daily administration of G-CSF at a dose of 8-20 mcg/kg body weight, there is an increase in the number of CD34+ cells in the blood of patients and healthy stem cell donors. With this method, the progenitor cell peak is reached between days 5 and 6 [49]. The extent of mobilization is determined by the age of the patient, the diagnosis, the earlier cytotoxic therapy, the dose of G-CSF and the sequence of G-CSF administration [50]. Kroger et al [51] showed that mobilization was more effective if the total dose of G-CSF was divided into two equal fractions and given in the morning and in the evening rather than as a single dose once daily. G-CSF administered every 12 h at doses of 5 μ g/kg provides better CD34+ cell yield than 10 μ g/kg once a day in normal donors which may translate into a decrease in the number of apheresis required to obtain enough numbers of CD34+ cells for allogeneic PBSC transplant.

3.2 Which growth factor should we choose?

Filgrastim is commonly used to mobilize peripheral blood stem cells. Pegylation of filgrastim (pegfilgrastim) leads to prolongation of its half- life without loss of activity. Attachment of the polyethylene glycol (PEG) moiety reduces renal excretion and masks proteolytic cleavage sites resulting in elevated G-CSF serum levels for up to 14 days after a single injection. While filgrastim is also cleared by the kidneys, pegfilgrastim is mainly eliminated via a neutrophil-mediated clearance mechanism [52-54]. The initial results of using pegfilgrastim obtained in limited numbers of patients with solid tumours showed that pegylated G-CSF was principally capable of mobilizing haematopoietic progenitor cells [54]. In 2001 and 2003, two studies were designed for the treatment of myeloma patients. The treatment regimens were comparable and employed DT-PACE [55,56] as mobilization chemotherapy. In the first study patients received twice a day filgrastim, until completion of stem cell collection; in the second study two doses of pegfilgrastim were administered after DT-PACE. After a cycle of DT-PACE, pegfilgrastim 6 mg was given subcutaneously on days

+6 and +13. If the WBC count $> 100 \text{ G/l}$ by day +13 the second dose of pegfilgrastim was not administered. Investigators found some advantages when pegfilgrastim was used:

- A higher percentage of patients collected $15 \times 10^6/\text{kg}$ in the first three days ($p < 0.001$)
- The median number of CD34 cells/kg collected on day 1 was higher ($p = 0.004$)
- The median number of growth factor injections was 2 versus 26 ($p < 0.0001$)
- Post-transplantation neutrophil recovery was faster after first and second transplant ($p < 0.001$)
- Platelet recovery was faster after first transplant (when less stem cells were infused) ($p = 0.01$)
- Authors concluded, that pegfilgrastim may be considered the standard of care for stem cell mobilization.

Kobbe et al. [54] found that in patients with multiple myeloma, a single dose of 6 mg pegfilgrastim after cyclophosphamide ($4\text{g}/\text{m}^2$) is equally effective in terms of the mobilization of haematopoietic progenitor cells as daily administration of conventional G-CSF. There is no increase in this effect if the dose is doubled to 12 mg. This finding is consistent with the results of a recently published, blinded, placebo-controlled multicenter study conducted in patients with malignant lymphomas [57]. These results were also confirmed by other groups of investigators [58,59]. There are no studies on the use of pegfilgrastim to mobilize haematopoietic stem cells in patients with acute leukemia. Pegylated G-CSF has not also been studied in the so-called "poor mobilizers".

Two formulations of recombinant human (rh) G-CSF, one glycosylated form and one non-glycosylated, are available. The glycosylated form, lenograstim, possesses at least 25% greater bioactivity *in vitro*. Some comparative studies into the preparation's potential to mobilize haematopoietic stem cells were performed to assess the potential greater efficacy of lenograstim *in vivo*. Ataergin S et al. [60] investigated whether a 25% reduced dose of lenograstim at $7.5 \mu\text{g}/\text{kg}/\text{day}$ is equivalent to $10 \mu\text{g}/\text{kg}/\text{day}$ filgrastim for autologous peripheral blood stem cell mobilization and transplantation. The two evaluated patients' cohorts were similar in regard to disease, sex, body, weight, body surface area, conditioning regimens, previous chemotherapy cycles and radiotherapy. Each growth factor was administered for 4 consecutive days. The first PBSC apheresis was done on the 5th day. In the posttransplant period, the same G-CSF was given at $5 \mu\text{g}/\text{kg}/\text{day}$ until leukocyte engraftment. No significant difference was seen in the median number of CD34+ cells mobilized, as well as the median number of apheresis, median volume of apheresis, percentage of CD34+ cells, and CD34+ cell number. Leukocyte and platelet engraftments, the number of days requiring G-CSF and parenteral antibiotics, the number of transfusions were similar in both groups in the posttransplant period. In conclusion, lenograstim $7.5 \mu\text{g}/\text{kg}/\text{day}$ is as efficacious as filgrastim $10 \mu\text{g}/\text{kg}/\text{day}$ for autologous PBSC mobilization and transplantation.

Another study [61] explored the efficacy of the IGEV regimen (ifosfamide, gemcytabine, vinoreblin and prednisone) combined with a fixed dose of lenograstim ($263 \mu\text{g}/\text{day}$) to mobilize PBSCs in 90 Hodgkin's lymphoma patients. An adequate number of CD34+ cells ($> 3 \times 10^6/\text{kg}$) were collected in 98,7% mobilized patients. Hematological and non-hematological side effects were acceptable, and no toxic deaths occurred. These results confirm that the IGEV regimen with lenograstim support can be used successfully and safely to mobilize PBSCs.

Kopf B et al.[62], in 2006 conducted a prospective randomized clinical trial to assess the mobilizing efficacy of filgrastim, lenograstim and molgramostim (GM-CSF) following a disease-specific chemotherapy regimen. In conclusion, all three growth factors were efficacious in mobilizing peripheral blood progenitor cells with no statistically significant difference between CD34+ cell yield and the different regimens, and the time to apheresis is likely confounded by the different mobilization regimens. An advantage on platelet recovery with molgramostim, suggested by other authors [63,64] was not confirmed by results of this study.

3.3 G-CSF in conjunction with chemotherapy

Growth factor combined with chemotherapy are given for patients, who are candidates to autologous transplantation and need additional treatment as debulking therapy or to achieve complete/ partial remission before transplantation [65]. A variety of chemotherapeutic agents are used with G-CSF or GM-CSF to mobilize hematopoietic stem cells for autologous transplantation. Administration of CY with G-CSF is widespread, as this regimen mobilizes hematopoietic stem cells effectively and is highly active against tumor cells [65]. Successful chemomobilization regimens used in combination with G-CSF are often disease oriented. Some of the most frequently used chemotherapeutic regimens in lymphoma patients include IEV (ifosfamide, epirubicin and etoposide), DHAP and ESHAP (etoposide, Ara-C, methylprednisolone and cisplatin) [66,67]. Patients with other hematologic malignancies are frequently treated with ICE (ifosfamide, carboplatin and etoposide) plus G-CSF for mobilization [68].

Chemomobilization is widely used in clinical practice because the addition of a myelosuppressive chemotherapy agent to a cytokine mobilization regimen results in higher CD34+ cell yields, which may promise better outcomes for patients. In particular, mobilization with CY and G-CSF rather than with G-CSF alone improves CD34+ cell collection significantly in patients with either MM [69,70] or NHL [71,72]. However, it has been noted that the use of CY plus G-CSF severely depletes T cells and spares regulatory T cells, which could negatively affect immune reconstitution [73]. The benefits of adding chemotherapeutic agents to a G-CSF mobilization regimen may be offset by the increased risk of complications to the patients. Compared with mobilization regimens using G-CSF alone, chemomobilization is associated with increased morbidity, greater risk of infection, more hospital admissions, transfusions, antibiotic therapy and considerably greater cost overall. [28]. Although treatment-related mortality is rare, significant morbidity related to neutropenia that can often require hospitalization has been described, and many reports point to greater resource utilization with chemomobilization than with cytokine-alone mobilization [28].

Koumakis et al [74] compared various time schedules of granulocyte colony-stimulating factor (G-CSF) treatment in a clinical model of patients who received high-dose cyclophosphamide (4.5 g/m²). They found, that:

- G-CSF administration after high-dose cyclophosphamide has a similar effect upon the incidence and duration of severe leukopenia and thrombocytopenia
- Severe leukopenia is shorter when G-CSF starts up to 72 hours after high-dose of cyclophosphamide
- The length of G-CSF administration and its cost is also in favor of early initiation of treatment as well as the number of febrile days and antibiotic use

- Delayed (> 72 hours) or supportive treatment indicate more febrile days, antibiotic use and higher cost when compared to the early groups

For these reasons, preemptive rather than therapeutic administration of G-CSF is indicated in patients who receive high-dose cyclophosphamide as treatment for mobilization CD34+ cells into peripheral blood.

3.4 Toxicity of growth factors

Cytokine-mobilized PBSC collected from healthy sibling or unrelated donors are increasingly used as stem-cell source in the allogeneic setting. PBSCs have been shown to be superior to BM-derived stem cells during the early posttransplant course. The high CD34+ cell yield in PBSC grafts significantly shortens the posttransplant aplasia and the need for blood component support, especially platelets [40,41]. Both cytokine administration and harvest procedure cause unphysiological conditions in healthy individuals. The effects of rhG-CSF on peripheral blood count and leukocyte function, cytokine release and response, coagulation parameters and metabolic changes have been reviewed by Anderlini et al [75,76]. Limited data exist on the late effects of cytokine mobilization and PBSC collection. Until now, there is no convincing evidence that rhG-CSF administration leads to an increased risk to develop hematological malignancies in healthy individuals [77]. Leitner GC et al [78] investigated the actual quality of life (QoL) and health status of the donors as well as the need for medical treatment since PBSC donation by a questionnaire (151 donors were evaluated). The questionnaire was sent to donors at a median of 4 (range: 0.2-11) years after donation. rhG-CSF mobilization as well as subsequent PBSC collection is shown to be well tolerated in the short- and long-term profiles in these sibling donors. It had no negative influence on health status and QoL in the majority of them. Investigators observed no increased risk for hematological or oncological disorders. However, to acquire profound knowledge about rhG-CSF- and donation-related long term risks, consecutive monitoring of more donors for at least 10 years has to be performed. Hasenclever and Sextro [79] stated that to exclude or to show a 10-fold increase in the 10-year cancer incidence, a long-term prospective follow-up of several thousand donors for at least 10 years would be necessary. In 2009 the report from European Group for Blood and Marrow Transplantation Group was published [80]. Three hundred and thirty-eight allogeneic transplant teams from 35 European countries were asked to report numbers of fatalities, severe adverse events and hematologic malignancies occurring among their hematopoietic stem cell donors. 51024 first allogeneic hematopoietic stem cell transplantations were evaluated, of which 27770 were bone marrow and 23254 peripheral blood. They observed five donor fatalities, one after a bone marrow donation and four after peripheral blood donation (incidence 0.98 per 10000 donations; 95% CI 0.32-2.29), 37 severe adverse events (7.25/10000; 95% CI 5.11-9.99), of which 12 in bone marrow donors (4.32/10000; 95% CI 2.24-7.75) and 25 in peripheral blood donors (10.76/10000; 95% CI 6.97-15.85; $p < 0.05$) and 20 hematologic malignancies (3.92/10000; 95% CI 2.39-6.05), of which 8 after donating bone marrow and 12 after donating peripheral blood stem cells. The observed incidence rate of hematologic malignancies did not exceed the expected incidence in an age- and sex-adjusted general population. Authors concluded, that hematopoietic stem cell donation is associated with a small but define risk of fatalities and serious adverse events. True incidences might be higher, due to potential underreporting by study design. A continuous, standardized donor follow-up is needed to define donor risk groups and to monitor intermediate and long-term sequelae.

Healthy donors who were mobilized using lenograstim and who were undergoing peripheral hematopoietic cell collection with apheresis were enrolled in a surveillance protocol. The study was conducted by Martino et al [81]. The median dose of lenograstim was 10 µg/kg (range 5-15). 184 healthy donors have been assessed with a median follow-up of 62 months (range 2-155). The short-term adverse events:

- Bone pain 71,2%
- Headache 27,7%
- Insomnia 22,3%
- Fatigue 19,0%
- Nausea 12,0%
- Fever 5,4%
- Increased spleen size 4,3%

No vascular disorders, no cardiac disease

Long-term follow-up included monitoring of adverse events, neoplastic disease or other pathologies:

Type of adverse event	Number of donors suffered from	Time of occurrence after donation [months]
Transit ischaemic attack	1	39
Ankylosing spondylitis	1	28
Secondary polyglobulia	1	50
Lung cancer	1	19
No haematological disease was observed		

During hematopoietic stem cell mobilization in healthy donors, slight thrombocytopenia is common and is attributed to the leukapheresis procedure or to splenomegaly. The platelet depletion is a recognized effect of continuous flow leukapheresis, particularly large-volume leukapheresis [75].

About one-third of neutropenic patients chronically treated with rh-G-CSF develop palpable splenomegaly and there have been reports of spontaneous spleen rupture in rhG-CSF or cyclophosphamide plus rh G-CSF- mobilized individuals or even in patients treated with rhG-CSF or rhGM-CSF after chemotherapy for acute leukemia or lymphoma. Few data are available on changes in spleen size as a result of a brief course of rhG-CSF [82]. Picardi et al. evaluated spleen size, comparing palpation with ultrasound (US)-evaluated longitudinal diameter and volume, in 13 healthy donors and 22 patients with a hematological malignancy who were undergoing PBSC mobilization with rhG-CSF-including regimens. When evaluated by sensitive methods, rhG-CSF caused spleen enlargement in almost all individuals treated. US-calculated volume proved to be an excellent method, much better than longitudinal diameter, for detecting non-palpable splenomegaly induced by rhG-CSF [83].

G-CSF has been also discussed as the causative agent for the occurrence of [84-94]:

- Sweet syndrome
- Leukocytoclastic vasculitis
- Interstitial pneumonitis
- Adult respiratory distress syndrome (ARDS)
- Pyoderma gangraenosum

- Capillary leakage
- Stroke
- Acute gouty arthritis
- Iritis
- Severe anaphylactoid reactions
- Non-traumatic rupture of the spleen
- Severe pyogenic infection (painful perianal abscess and apical abscess)

3.5 How can we predict the optimal time for adequate collection of peripheral blood progenitor cells after chemotherapy ?

The absolute number of circulating CD34+ cells was found to correlate closely with the CD34+ cell yield of the corresponding leucapheresis product. The number of CD34+ cells circulating in the peripheral blood reliably predicts both:

- The number of CD34+ cells
- The number of CFU-GM harvested

By monitoring the level of circulating CD34+ cells during the mobilization period, generally starting at about day 9 or 10, the day to perform the leucapheresis can be planned. The optimal time for collecting PBSC is when a peripheral blood sample contains at least 20×10^3 circulating CD34 cells/ml. This value provided at least 2×10^6 CD34+ cells/kg in a single leucapheresis, harvested the following day, in 94% of the collections regardless of the patient's diagnosis or mobilization regimen [95]. In Hill's study [96] a total of 168 adult patients with haematological malignancy were primed using low-moderate dose cyclophosphamide ($1.5-3 \text{ g/m}^2$) with G-CSF $5-10 \text{ } \mu\text{g/kg}$ per day. Harvesting was booked and peripheral blood counts first checked between 6 and 10 days post-priming. The peripheral blood CD34+ cell count correlated significantly with harvest yield ($r= 0.8448$, $p < 0.0001$). A peripheral blood CD34+ count $\geq 10/ \mu\text{l}$ predicted a collection of $\geq 2 \times 10^6/\text{kg}$ (positive-predictive value of 61%, negative-predictive value 100%). There was no benefit to checking the peripheral blood CD34+ count or booking apheresis before day 9 post-cyclophosphamide.

3.6 What is the minimal CD34+ cells threshold collected from peripheral blood for sufficient engraftment?

What is the optimal CD34+ cells number for transplantation?

Gandhi et al.[97] advocated a minimum CD34+ threshold of $> 1.0 \times 10^6/\text{kg}$ in patients without extensive prior chemoradiotherapy, and $\geq 2.0 \times 10^6/\text{kg}$ in all other patients. In this study all patients infused with grafts containing CD34+ cell doses between 1.0 and $2.0 \times 10^6/\text{kg}$ engrafted by day 51. The only variable associated with slow platelet recovery was exposure to stem cell toxins (BCNU, melphalan, CCNU and mustine). The majority of patients with CD34+ $> 1.0 \times 10^6/\text{kg}$ achieved rapid and sustained engraftment and the only predictive factor of delayed recovery is prior exposure to stem cell toxins.

Villalon et al. [98] analyzed the factors affecting mobilization and engraftment in autologous peripheral blood progenitor cell transplantation according to the number of CD34+ re-infused ($< 2.0 \times 10^6/\text{kg}$ CD34+ vs $> 2.0 \times 10^6/\text{kg}$ CD34+). They found that,:

- Neutrophil and platelet engraftment was significantly longer with $< 2.0 \times 10^6/\text{kg}$ (12 vs 10 days, $p = 0.014$ and 16 vs 13 days, $p = 0.0001$ respectively)

- Platelet recovery was affected by exposure to alkylating agents ($p = 0.04$), refractory disease ($p = 0.02$), and AML ($p = 0.0001$), but only the last two variables remained significant in Cox regression ($p < 0.01$)
- Granulocyte engraftment was longer in CML (univariate, $p = 0.04$) and in refractory disease (multivariate, $p = 0.02$)
- In patients re-infused with $> 2.0 \times 10^6/\text{CD34+}/\text{kg}$, the Cox model did not identify prognostic factors for haematopoietic recovery

They concluded, that although mobilization schedules and disease status influenced not only the yield of progenitor cells, but also the engraftment kinetics, the number of CD34+ re-infused was the main predictor of hematopoietic recovery. While engraftment succeeded in most of the cases, the re-infusion of $> 2.0 \times 10^6/\text{kg}$ resulted in significantly shorter recovery times. Thus, for autologous stem cell transplantation, it is common practice to infuse at least $2.0 \times 10^6/\text{CD34+}/\text{kg}$ to ensure rapid engraftment. But investigators [99-102] found, that when comparing patients receiving at least $5 \times 10^6/\text{kg}$ and $2-5 \times 10^6/\text{kg}$ CD34+ cells there are:

- A significant reduction in the median number of days with fever
- Incidence of fever
- Duration of antibiotic treatment
- Faster neutrophil recovery

There was no significant difference in the number of platelet or red cell transfusions

Thus, transplantations with stem cell dose of at least $5.0 \times 10^6/\text{kg}$ reduce infectious complications and should thereby increase the safety of this type of therapy while reducing duration (and cost) of antibiotic therapy.

In Bolwell study [103] investigators checked whether patients collecting high numbers of CD34+ cells ("super mobilizers") have a better outcome than other patients. Super mobilizers were defined as collecting a minimum of $8.0 \times 10^6/\text{CD34+}/\text{kg}$. In this study super mobilizers were younger and more likely to have received two or fewer prior chemotherapy regimens. Median CD34+ cell dose for the super mobilizing group was $13.7 \times 10^6 \text{ CD34+}/\text{kg}$ versus $4.4 \times 10^6 \text{ CD34+}/\text{kg}$ in the standard collecting group. The super mobilizer group had a superior overall survival ($p = 0.006$). In multivariable analysis, favorable disease status and younger age at transplant, and super mobilization were associated with improved survival.

It is reasonable to believe that the CD34+ cell dose has a positive influence on engraftment and survival. But, when compared with BM, PBSC grafts contain significantly more nucleated cells, more CD34+ hematopoietic stem cells, and more CD3+ lymphocytes [104,105]. It has been shown that granulocyte colony-stimulating factor (G-CSF)-mobilized CD34+ hematopoietic stem cells not only participate in engraftment, but also have an immunogenic role [106-108]. In the setting of T-cell-depleted allogeneic transplants using CD34+ positive selection as T-cell-depletion method, Urbano- Ispizua et al [109] could show that a high CD34+ cell dose not only does not improve the clinical results, but also actually may be associated with a poorer outcome. There is the suggestion, that transplantation with a higher CD34+ cell dose was detrimental in terms of chronic GvHD in allogeneic CD34+ cell-selected peripheral blood stem cell transplantation. Moreover, this association may also exist in the context of allogeneic unmanipulated PBSCT where a higher T-cell content or extremely high dose of CD34+ cells can be involved. It should be noted that CD34+ cells not only participate in engraftment, but also have an immunogenic role. However, although

cGvHD is a leading cause of late mortality in allogeneic settings, it also plays a positive role in preventing relapse, especially in advanced hematological malignancies with a high risk of relapse [110]. Sohn et al [111] investigated the impact of the CD34+ cell dose on chronic graft-versus-host disease and the clinical outcome in adult patients submitted to allogeneic peripheral blood stem cell transplantation from HLA-identical siblings. The patients were classified into "low" or "high" CD34+ cell dose groups based on whether they received less or more than a median CD34+ cell dose of $10.5 \times 10^6/\text{kg}$, respectively. There was a significant difference in the incidence of extensive cGvHD and relapse between the two groups. With a median follow up of 335 days, the 3-year survival estimate for whole population was 47.9%, while that for the low and high groups was 29.9 and 67.8% respectively ($p = 0.0434$). An inverse relation was noted between the relapse rate and the incidence of extensive cGvHD ($p = 0.043$). Authors concluded, that it would appear reasonable that the optimal dose of CD34+ cells should be determined based on the disease status or aggressiveness of the malignant cells in each patient. Thus, in the case of patients with a high risk of relapse, transplantation with a CD34+ cell dose $> 10.5 \times 10^6/\text{kg}$ would seem to be acceptable to minimize the risk of relapse. Mohty et al [110], Sohn et al [111] investigated whether there was a correlation between the composition of PBSC grafts (CD34+ and CD3+ cells) and hematological recovery, GvHD, relapse and relapse-free survival after myeloablative HLA-identical sibling PBST. Neither hematological recovery, acute or chronic GvHD, nor relapse, was significantly associated with CD3+ cell dose. Increasing CD34+ stem cells was associated with faster neutrophil and platelet recovery. The probability of extensive cGvHD at 4 years was 34% in patients receiving a "low" CD34+ cell dose ($< 8.3 \times 10^6/\text{kg}$) as compared to 62% in patients receiving a "high" CD34+ cell dose ($> 8.3 \times 10^6/\text{kg}$) ($p = 0.01$). At a median follow-up of 59 months, this has not translated into a difference in relapse. In patients evaluable for cGvHD relapse free survival was significantly higher in patients receiving "low" CD34+ cell dose as compared to those receiving a "high" CD34+ cell dose ($p = 0.04$). This difference was mainly because of a significantly higher cGvHD-associated mortality ($p = 0.01$).

3.7 Factors to predict the efficiency of blood progenitor cell mobilization

It is important to note that most patients mobilize adequate numbers of CD34+ cells using a regimen of G-CSF alone. Although the addition of chemotherapy improves CD34+ yield, this comes at the expense of increased short-term toxicity and, possibly, the increased risk of secondary myelodysplastic syndrome [112]. Even with chemotherapy-growth factor combination regimens, it may be difficult to achieve an adequate CD34+ cell yield in some patients. Several studies have identified predictors of poor PBSC yield.

- The most important factor is the amount of myelosuppressive therapy (chemotherapy +/- radiation therapy) received prior to mobilization
- Using stem cell toxic agents prior to mobilization: nitrogen mustard, procarbazine, melphalan, carmustine and > 7.5 g of cyclophosphamide
- The number of chemotherapeutic regimens > 6 and ≥ 11
- Duration of exposure to chemotherapy (> 12 months)
- Short time interval since last chemotherapy < 6 months and < 65 days
- Previous radiation therapy
- Hypocellular marrow
- Refractory disease

A recent scoring system based on previous therapy may be useful in predicting CD34-positive cell yield.

Treatment score was built by Drake et al [113], and then improved by others [114,115]. Treatment score was evaluated (as given in detail by Drake et al) [113] by Clark [114]. There was one exception in Clark study, in addition, arbitrarily allocated a score of 2 for ifosfamide per treatment cycle.

Briefly, chemotherapy drugs are assigned a toxicity factor as follows:

0. prednisolone, dexamethasone;
1. vincristine, vinblastine, bleomycin, alpha interferon;
2. cyclophosphamide, anthracyclines, cisplatin, etoposide, ifosfamide;
3. chlorambucil, procarbazine;
4. melphalan, carmustine, mechlorethamine, lomustine.

The number of courses of each drug received was multiplied by its toxicity factor, and the score for each drug administered was summed to yield an overall treatment score. An additional 2 points were added if mediastinal radiotherapy was administered. In this study was validated this scoring system on an independent group of 99 patients undergoing 103 harvesting episodes. In 61 patients mobilized with cyclophosphamide 1.5 g/m² and G-CSF, those with treatment scores less than 21 yielded significantly more CD34-positive cells than patients with scores greater than 63 ($P = 0.0012$). Previous treatment with melphalan or carmustine was associated with a significantly lower yield of CD34-positive cells ($P = 0.0001$). No relationship was seen between the time from previous chemoradiotherapy and harvest outcome. Patients with treatment scores less than 21 required a shorter duration of G-CSF therapy ($P = 0.05$). Similar findings were seen in 42 further mobilization cycles undertaken with alternative mobilization schedules. The data suggest that a score summarizing previous treatment can be used to predict CD34 yields, and could be of clinical use to identify poor PBPC mobilisers in advance. The next improvement in scoring system was done by Jantunen et al. [115]. Results are shown in the table.

0	Prednisolone, dexamethasone, METHYLPREDNISOLONE
1	Vincristine, vinblastine, bleomycin, METHOTREXATE, alpha-interferon, CYTOSINE ARABINOSIDE
2	MITOGUAZON, cyclophosphamide, IFOSFAMIDE, cisplatin, anthracyclines, MITOXANTRONE, etoposide
3	Chlorambucil ^a , procarbazine, FLUDARABINE, DACARBAZINE
4	Melphalan, carmustine, mechlorethamine, lomustine

Changes to the original scoring system proposed by Drake et al [113] are shown in capital letters.

^aoral continuous treatment (4–6 mg/day) for a month=1 cycle.

Table 4. An improved chemotherapy scoring system [115]

3.8 Strategies of remobilization/ second-line stem cell harvest of patients who fail to achieve minimal progenitor thresholds at the first attempt

After an initial mobilization attempt, if too few CD34+ cells are collected to ensure prompt engraftment, patients often undergo additional mobilization attempts, which increase the risks associated with treatment [116]. Several salvage regimens have been developed to

improve mobilization in patients in whom a first mobilization attempt with G-CSF alone fails to result in collection of an adequate cell dose. In recent years, several investigational agents have been developed that may prove useful for amplifying yields of CD34+ cells without introducing additional toxicity. As the understanding of stem cell interactions with the BM microenvironment grows, new mobilizing agents will emerge.

STRATEGY
second PBSC mobilization using the same regimen
steady-state bone marrow
stimulated BM-“rich bone marrow”
Increase chemotherapy dose: 4-7 g/m ² CTX appears more effective than 2-4 g/m ²
Prolong duration between last chemotherapy and planned collection
Increase growth factor dose (up to 24 µg/kg G-CSF) and use twice daily schedule
Use investigational agents

Table 5. Strategies of remobilization/ second-line stem cell harvest of patients who fail to achieve minimal progenitor thresholds at the first attempt [27,28,117]

Mobilization agent	Mechanism
<i>Cytokines approved for mobilization</i>	
GM-CSF	Stimulates production of granulocytes and macrophages
G-CSF	Granulocyte expansion/activation, protease release and cleavage of adhesion molecules
<i>Chemotherapeutic agents commonly used for mobilization</i>	
CY	Expansion and activation of granulocytes after bone marrow suppression
Paclitaxel	
Etoposide	
<i>Investigational mobilization agents</i>	
Pegylated G-CSF	Granulocyte expansion/activation, protease release and cleavage of adhesion molecules
EPO	Stimulates erythropoiesis
Stem cell factor	G-CSF potentiation
Plerixafor	Disrupts CXCR4/SDF-1 α interactions
SB-251353	GRO-β analog involved in directing the movement of stem cells and other leukocytes
TPO	Regulates megakaryocyte development, G-CSF synergism
Parathyroid hormone	Activates osteoblasts, which produce HGFs in the stem cell niche

Abbreviations: CXCR4=chemokine receptor 4; GRO- β=a human CXC chemokine; SDF-1=stromal cell-derived-factor-1. TPO- thrombopoietin, HGFs- hematopoietic growth factors

Table 6. Mobilization agents currently used in auto-HSCT [27,28,117]

3.8.1 Erythropoietin- EPO

EPO, which is commonly used to preserve blood hemoglobin concentrations in patients undergoing chemotherapy, has also been shown to potentiate the mobilization effect of G-CSF or GM-CSF [118]. Although the mechanism of this cooperative effect is unknown, it is thought that expression of EPO receptors on CD34+ progenitor cells primed with G-CSF or GM-CSF may promote survival of these cells [119]. However, EPO use is generally regarded

as inefficient and, as such, has not become a standard of care. Studies evaluating the mobilization efficacy of EPO plus G-CSF or G-CSF alone in various doses have generated mixed results [120-122]. Further investigation is required to ascertain the clinical benefits of EPO combined with G-CSF [123].

3.8.2 High-dose G-CSF

High-dose G-CSF was investigated as a primary mobilization regimen throughout the 1990s. [49,124,125]. Although seldom used today for primary mobilization, high-dose G-CSF regimens are occasionally used for remobilization [126,127]. Although there is no standard protocol for high-dose G-CSF administration, doses ranging from 16 to 32 µg/kg s.c. daily to 12-16 µg/kg s.c. twice daily have been considered high-dose regimens even though some of the available data apply to patients with aplastic anemia, solid tumors or hematological malignancies other than NHL [124,126,128].

3.8.3 GM-CSF plus G-CSF

Significant synergism has been reported between GM-CSF and G-CSF in the formation of granulocytic colonies in vitro [129]. Mobilization regimens combining GM-CSF with G-CSF have consisted of sequential or concurrent administration of these agents at a range of doses (G-CSF, 5-10 µg/kg; GM-CSF, 5 µg/kg-250 µg/m²), with or without chemotherapeutic agents [130-133]. These combination regimens have not been shown to have substantial benefits over regimens that use G-CSF alone; therefore, GM-CSF and G-CSF are not commonly administered together for primary mobilization. However, the combination of G-CSF and GM-CSF is used as a salvage mobilization regimen when mobilization with G-CSF alone has been unsuccessful [126,134,135].

3.8.4 SCF

The c-kit ligand SCF is produced in BM stromal cells and acts as a potent co-mitogen for many hematopoietic growth factors [32]. Recombinant methionyl human SCF (ancestim, Stemgen, Amgen Inc.) administered sc. in combination with G-CSF has been shown to enhance mobilization and may fasten recovery in transplant recipients [32, 136]. The combination of SCF and G-CSF exerts a sustained mobilization effect that persists longer than does the effect of G-CSF alone, which persists for up to 7 days, as shown by an increase in the numbers of circulating CD34+ cells for up to 13 days in patients with breast cancer (BC) who received the combination treatment [32]. Despite the efficacy of SCF, its use is hindered by the infrequent occurrence of severe anaphylactoid reactions and the resultant need to closely monitor patients after SCF administration [137]. Although approved for use in Canada and New Zealand, ancestim is not currently available in the United States, and it is seldom used in Europe because of the relatively high risk of side effects.

3.8.5 Plerixafor

Plerixafor is a reversible bicyclam inhibitor of hematopoietic stem cells (HSC) binding to SDF-1α on marrow stromal cells via the chemokine receptor 4 (CXCR4) on HSC [138-140]. Plerixafor used in conjunction with G-CSF has been shown in a phase 2 studies to quickly and predictably enhance the numbers of CD34+ cells circulating in the peripheral blood [141]. In patients in whom mobilization with G-CSF either alone or in combination with chemotherapy has previously failed, CD34+ cell yields have been noted to increase by 5- to

100-fold in response to administration of plerixafor plus G-CSF [141,142]. Preliminary results of two phase 3 multicenter randomized placebo-controlled studies indicated that the addition of plerixafor to a G-CSF regimen resulted in greater efficacy than was seen with a regimen of G-CSF alone [140, 143]. In general, treatment with plerixafor and G-CSF was associated with side effects similar to those seen with treatment with G-CSF alone. Most treatment-related AEs appeared to be mild and transient. The most common AEs were gastrointestinal tract effects, such as diarrhea, nausea and vomiting, and injection-site reactions, such as erythema or edema [140, 143].

3.8.6 SB-251353

SB-251353 is another investigational mobilization agent currently in preclinical studies [144,145]. SB-251353 is an analog of GRO- β , a human CXC chemokine involved in directing the movement of stem cells and leukocytes. Although human data are lacking, this agent, when combined with G-CSF in rhesus monkeys, was shown to greatly increase mobilization of stem cells and progenitor cells in comparison with G-CSF alone. Further research is necessary to determine the efficacy and potential toxicities of this treatment in humans. [144,145].

3.8.7 Thrombopoietin- TPO

Endogenous TPO is the primary regulator of megakaryocyte development. Recombinant human TPO (rhTPO) has been shown to act synergistically with G-CSF to enhance stem cell mobilization [146]. This regimen has not been shown to be more efficacious or safer than existing mobilization regimens; however, a few studies document encouraging results [147]. AEs associated with the use of rhTPO plus G-CSF appear to be similar to those seen with the use of G-CSF alone [146,147], however, cytopenias owing to neutralizing antibodies to TPO have been reported in a small number of patients who were given rhTPO to treat chemotherapy-induced thrombocytopenia [148]. Currently, no TPOs have been approved by the FDA for mobilization [149].

3.8.8 Parathyroid hormone

Parathyroid hormone (PTH) activates osteoblasts, which produce hematopoietic growth factors in the stem cell niche, thereby increasing the numbers of circulating stem cells [150,151]. The efficacy and safety of PTH have yet to be established. In a recent phase 1 study, 20 patients with MM, NHL, HD or AML, in whom one or two previous mobilization attempts had failed, received escalating doses of 40, 60, 80 and 100 μ g of PTH (s.c.) for 14 days; PTH doses were combined with G-CSF 10 μ g/kg on the last 4 days of treatment [151]. Overall, 47% of patients in whom one previous mobilization attempt had failed reached the mobilization criterion of >5 CD34+ cells/ μ l in the peripheral blood, and 40% of patients who had previously experienced two failed mobilization attempts reached the mobilization criterion. No dose-limiting toxicity was evident, and PTH was well tolerated; AEs included headache, muscle pain, back pain, fatigue and hypothermia [151].

3.9 BM vs. PBSC for whom? [152]

BM for:

- a. Good risk younger patients
 - Children

- Aplastic anemia, CML in first chronic phase (CP1)
- b. PBSC for:
 - Advanced phase of diseases
 - If graft manipulation is needed
 - Reduced intensity transplants
 - Donor's preference

4. Umbilical cord blood

The use of allogeneic blood and bone marrow stem cell transplantations are limited by the availability of suitably HLA- matched donors. Only 30% of patients have HLA-identical sibling donors and through the National Marrow Donors Program and other registries worldwide nearly 75% of Caucasians, but far fewer racial minorities find suitably HLA-matched donors. In 1988 umbilical cord blood (UCB) hematopoietic stem cells from a related sibling were transplanted successfully into a 5-year-old child with Fanconi anemia. Fifteen years later, this patient is doing well with full donor hematopoietic and lymphoid reconstitution. UCB offers the advantages of easy procurement, no risk to donors, the reduced risk of transmitting infections, immediate availability of cryopreserved units and acceptable partial HLA mismatches. Nearly all patients can find at least one potential 4 of 6 HLA-matched UCB units through either Netcord or other banks. Limited cell dose has been the major limitation to the wide use of UCB for allogeneic transplantation.

The blood in the umbilical cord of newborn babies contains large numbers of stem cells, which have been shown to be capable of long-term engraftment in children and some adults after transplantation. Cord blood cells obtained from the umbilical cord at the time of delivery are used mainly for unrelated allogeneic stem cell transplantation. Patients without time to find an unrelated stem cell donor or who do not have a HLA 10/10 or 9/10 unrelated bone marrow graft donor should be considered for cord blood cells transplantation (CBCT). The cord blood cells may also be used for family- member transplantation, particularly in children for both malignant or nonmalignant diseases [153-155].

Advantages of CBCT compared with bone marrow or mobilized peripheral blood transplantations [156-161] :

- significantly faster graft availability (patients receiving CBCT in a median of 3-5 weeks earlier than those receiving an unrelated bone marrow graft),
- lack of risk to the donor,
- higher frequency of rare haplotypes compared to bone marrow donor registries,
- CD34+/CD38 cord blood cells proliferate more rapidly and generate large number of progenitor cells [162]
- extension of the donor pool due to tolerance of 1-2 HLA mismatches out of 6,
- lower incidence and severity of acute graft-versus-host disease,
- lower risk of infections by latent viruses - such as Epstein-Barr virus or cytomegalovirus

Disadvantages of CBCT compared with bone marrow or mobilized peripheral blood transplantations:

- cell dose is based per kilogram of recipient weight, which can be limiting. Target is 1,7-3,5 x 10⁷ total nucleated cells/kg,

- low number of hematopoietic progenitor cells- single cord blood unit transplantation (100ml) contains $0,3 \times 10^6$ CD34+ cells/kg (small recipient, below 40kg),
- in adults, single cord blood unit transplantation is associated with very delayed engraftment, combining two products seems to provide more rapid engraftment, albeit at the expense of higher rates of acute graft-versus-host disease,
- donor cannot be used for donor lymphocyte infusion,
- donor cannot be used for treatment of graft rejection or failure.

The cell dose infused is consistently an important marker for improved engraftment and survival. The lower dose of CD34+ cells translate into increased risk of graft failure, delayed hematopoietic engraftment [160] and delayed immune reconstitution [162,163]. Because of the delayed immune reconstitution infections are a serious problem in cord blood transplantation [164].

Many tests have been performed to enhance collection of hematopoietic stem cells in cord blood units. Examples include: in vivo or ex vivo expansion of cord blood cells [165,166], injecting cord blood cells directly into the bone marrow [167], use of double unit CBCT [168,169], use of reduced intensity conditioning regimen [170,171], coinfusion with a haploidentical T cell depleted graft [172,173] or mesenchymal stem cells [174].

5. Storage [1]

Hematopoietic progenitor cell products are stored using various methods depending on the required duration of storage. Products used fresh can be refrigerated for at least 24 hours before infusion. If there is a > 48-hour delay before infusion, most products are frozen to maintain viability. Most frozen products are stored in the vapor phase of liquid nitrogen (< -50 C). Products may be stored for up to 10 years although no longevity limit has yet been determined. Long-term storage is generally done in the liquid phase of liquid nitrogen. Hematopoietic progenitor cells have been frozen using cryoprotectant solutions. The most commonly used cryoprotectant is 10% DMSO and a protein additive such as human serum albumin.

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This book documents the increased number of stem cell-related research, clinical applications, and views for the future. The book covers a wide range of issues in cell-based therapy and regenerative medicine, and includes clinical and preclinical chapters from the respected authors involved with stem cell studies and research from around the world. It complements and extends the basics of stem cell physiology, hematopoietic stem cells, issues related to clinical problems, tissue typing, cryopreservation, dendritic cells, mesenchymal cells, neuroscience, endovascular cells and other tissues. In addition, tissue engineering that employs novel methods with stem cells is explored. Clearly, the continued use of biomedical engineering will depend heavily on stem cells, and this book is well positioned to provide comprehensive coverage of these developments.

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