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Stem Cell Review Series

Transplantation of hematopoietic stem cells from the peripheral blood

Jan Jansen *, Susan Hanks, James M. Thompson, Michael J. Dugan, Luke P. Akard

Indiana Blood and Marrow Transplantation, Beech Grove, IN, USA

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Abstract

Hematopoietic stem cells can be collected from the peripheral blood. These hematopoietic stem cells (HSC), or better progenitor cells, are mostly expressed as the percentage of cells than react with CD34 antibodies or that form colonies in semi-solid medium (CFU-GM). Under steady-state conditions the number of HSC is much lower in peripheral blood than in bone marrow. Mobilization with chemotherapy and/or growth factors may lead to a concentration of HSC in the peripheral blood that equals or exceeds the concentration in bone marrow. Transplantation of HSC from the peripheral blood results in faster hematologic recovery than HSC from bone marrow. This decreases the risk of infection and the need for blood-product support. For autologous stem-cell transplantation (SCT), the use of peripheral blood cells has completely replaced the use of bone marrow. For allogeneic SCT, on the other hand, the situation is more complex. Since peripheral blood contains more T-lymphocytes than bone marrow, the use of HSC from the peripheral blood increases the risk of graft-versus-host disease after allogeneic SCT. For patients with good-risk leukemia, bone marrow is still preferred, but for patients with high-risk disease, peripheral blood SCT has become the therapy of choice.

Keywords: peripheral blood stem cells • hematopoietic stem cells • bone marrow • stem-cell transplantation • CD34 • colony-forming unit granulocyte macrophage • CFU-GM • mobilization

* Correspondence to: Jan JANSEN,
Indiana Blood and Marrow Transplantation, 1500 Albany, #911, Beech Grove, IN 46107, United States of America.
Tel.: 317-865.5500
Fax: 317-782.6316
E-mail: jjansen@ibmtindy.com

Introduction

Stem cells have received a large amount of attention recently, both in the scientific and the lay press. The attention in the scientific press focuses on the specific value of embryonal stem cells, which probably have true pluripotentiality, and somatic adult stem cells, which may either be equivalent to embryonal stem cells or far more limited in their potential. Discussions in the lay press focuses on the religious, moral, legal, and practical issues involved with the harvesting of embryonal stem cells and the uncertainty about the use of stem cells in the treatment of patients [1,2].

Long before the worldwide obsession with all types of stem cell started, the concept of stem cells was fully accepted in the field of hematology. Although initially the hematopoietic stem cells (HSC) were thought capable of only producing blood cells (unipotentiality), more recently several studies have suggested that HSC may also show plasticity and mature into new muscle cells (*e.g.*, to replace damaged myocardium), liver cells, or even bone cells [3,4]. This plasticity of HSC is still debated with controversy focusing on contamination of HSC with other types of stem cell.

Historical aspects

In 1909 in a scientific presentation in Berlin, the Russian biologist Alexander Maximow claimed that among the small lymphocytes in the peripheral blood a small number of cells circulated that had, or might be capable of reacquiring, pluripotentiality, He called these cells "gemeinsame Stamzellen" [5]. For several decades few attempts were made to confirm or clinically exploit this concept of stem cells [6]. The field of clinical transplantation of HSC did not start until the late 1940's, when experiments in mice showed that shielding of the spleen allowed animals to survive otherwise lethal total body irradiation (TBI) [7]. It took several years before it was established that transplantation of HSC and not the infusion of humoral factors was responsible for the survival of the mice [8]. Starting in the late 1950's several groups tried to exploit these concepts in the treatment of patients with leukemia. The group in Seattle (USA) standardized the collection and infusion of hematopoietic progenitor cells from the bone marrow [9], and Mathé's group in Paris was the first to report long-term survival of an adult patient with acute leukemia who received a bone-marrow transplant from several relatives [10]. After a hiatus of several years because of universally disappointing results of allogeneic bone-marrow transplantation, the field took off in the late 1970's when patients were selected who did not have end-stage disease and who were in better clinical condition, and when HLA-typing became commonplace [11]. Virtually all of these clinical transplants used allogeneic or autologous bone marrow (BM) as the source of stem cells. At that point in time, peripheral blood as a source of stem cells was still considered inadequate to permanently reconstitute hematopoiesis [12].

Several developments were responsible for a complete shift in the practice of stem-cell transplantation (SCT). First, *in-vitro* techniques were developed to quantify HSC (or more precisely defined as the more limited hematopoietic progenitor cells (HPC)) in the laboratory. Cultures assays were able to document the number of HPC. At different levels of maturation they were called Colony Forming Units for granulocytes/macrophage (CFU-GM) or for erythroid cells (CFU-e; BFU-e), or multipotential colonies (CFU-GEMM), and even more immature progenitors (LTC-IC, cobblestone area forming cells). These assays revealed that the peripheral blood of healthy donors in steady state contained a small number of stem cells, although much fewer than found in the BM [13,14]. Actually, Van Bekkum's group reported on a small lymphocyte which they held responsible for colony formation, the "CMOMC" ("Cell Meeting Our Morphological Criteria") [15]. A decade later, the presence of glycoprotein CD34 was detected on the surface of immature hematopoietic cells [16]. This glycoprotein is present of colony-forming cells and myeloblasts but is lost at the level of the promyelocyte. The peripheral blood of healthy individuals was found to have a small number of CD34+ cells, although again at a much lower level than human BM. A close correlation exists between the number of CD34+ cells and CFU-GM in peripheral blood stem-cell collections (Fig. 1).

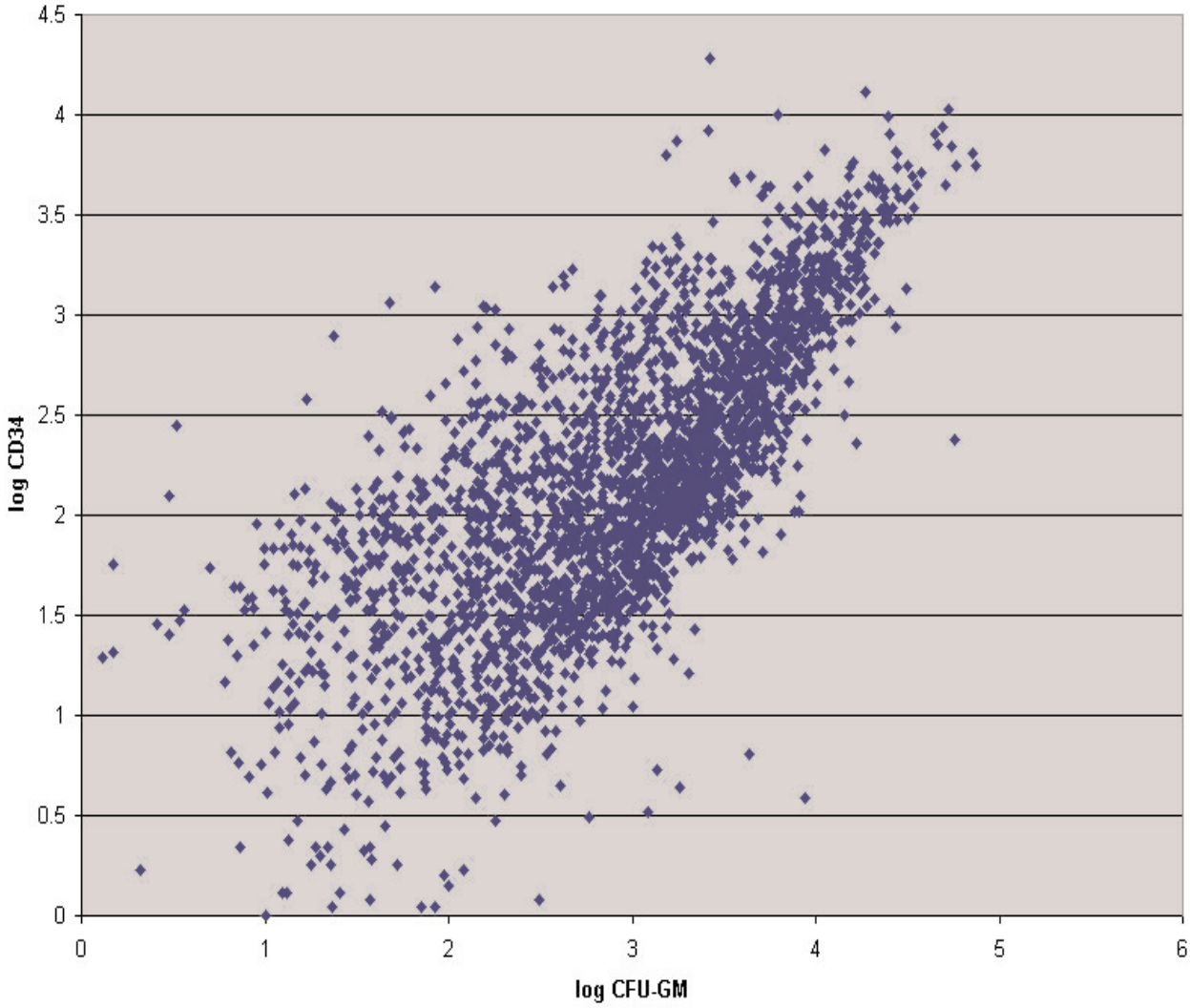

Fig. 1 Correlation between colony-forming unit granulocyte-macrophage (CFU-GM) and CD34+ cells in 2700 consecutive peripheral blood stem cell collections

Each collection derived from a single leukapheresis procedure of 2.5 X blood volume with the Cobe Spectra; data expressed as 10-log of both observations. (IBMT; unpublished data).

Second, Fliedner's group documented in a dog model that HSC obtained from the peripheral blood could permanently reconstitute irradiated animals, just like BM could [17]. Stem cells from the peripheral blood did not peter out, as previously had been assumed. An occasional clinical allogeneic transplant, in which the donor refused to give BM but was willing to undergo several leukapheresis procedures, proved the principle in humans [18].

Third, the introduction of hematopoietic growth factors, such as filgrastim (G-CSF) and sargramostim (GM-CSF), not only shortened the duration of neutropenia after cancer chemotherapy, but also resulted in an increased number of circulating CD34+ cells and CFU-GM. In the steady state the concentration of CD34+ cells in BM is up to 100 times higher than in peripheral blood, but stimulation with G-CSF resulted in concentrations of CD34+ cells or CFU-GM in the peripheral blood that were similar to BM ("mobilization") (Fig. 2) [19]. Similar increases in CD34+cells and CFU-GM were observed on the rebound after aggressive chemotherapy, even without the use of hematopoietic growth factors [20].

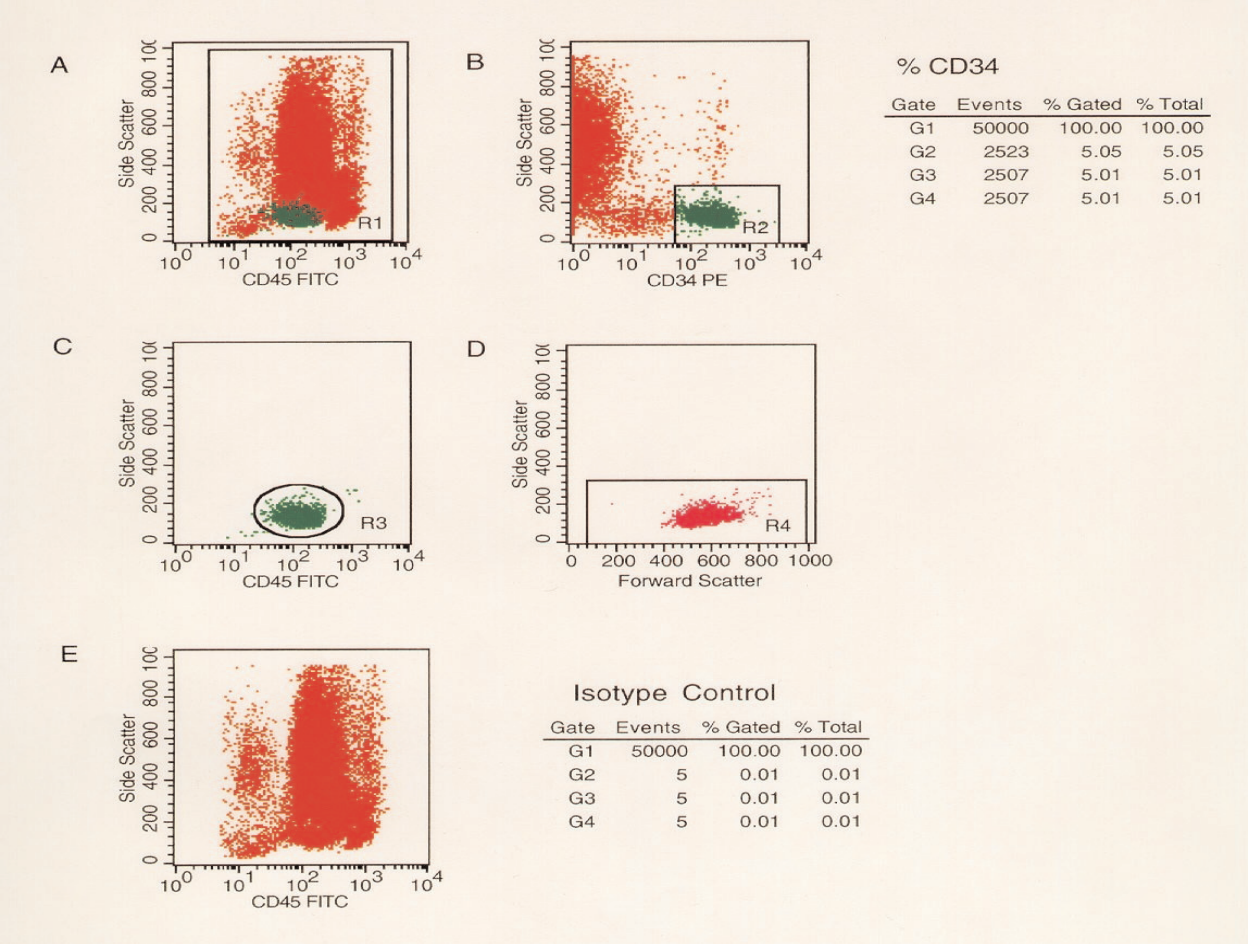

Fig. 2 Determination of CD34+ cells in a peripheral-blood stem cell collection (after mobilization).

A. R1 gated on CD45+ cells in population; the green population shows where theCD34+ population is in relationship to all CD45+ cells.
B. R2 gated on CD45+ cells that are also CD34+ from R1
C. R3 gate shows tightening of population in R2 to exclude cells staining with CD34 that do not fit stem-cell granularity criteria.
D. R4 gate shows CD34+ cells that also fit stem-cell population size and granularity criteria: 5.01% CD34+ cells
E. shows cell staining with isotype control in the CD45+ (IBMT; unpublished data)
G1-G4: cells inside the R1-R4 gates

The use of peripheral blood stem cells (PBSC) in clinical autologous transplantation started in the mid 1980's simultaneously in several countries [21,22]. The use of autologous PBSC rapidly spread and actually has nearly completely replaced the use of BM as the source of HSC for transplantation [21-24]. The use of PBSC for allogeneic transplantation did not take off until the early 1990's, and also has become widespread over the last number of years [22,25].

How are peripheral blood stem cells obtained?

Even when hematopoietic growth factors and/or chemotherapy are used to "mobilize" PBSC, the enormous number of blood cells needed requires the use of leukapheresis procedures that can process large volumes of donor blood (Fig. 3). In healthy donors, one or two collection procedures are performed on consecutive days, and result in a CD34+

Table 1 Engraftment data after stem-cell infusion.

	Median Days to Absolute Granulocyte Count >0.5 x 10^9/l	Platelet Count >20 x 109/l
Autologous bone marrow (no GF*)	24	20
Autologous bone marrow (with GF)	20	19
Autologous PBSC (no GF)	11	11
Autologous PBSC (with GF)	10	11
Allogeneic bone marrow (no GF)	17	22
Allogeneic PBSC (no GF)	14	13

(IBMT data base)
*= hematopoietic growth factor given daily after stem-cell infusion

cell dose that is adequate in the vast majority of transplants [26]. Typically, each leukapheresis procedure lasts 2-3 hours, and a total of 10-18 liters of blood are processed (2-3 x total blood volume). The blood vessels of many donors can be accessed through catheters in one or two antecubital veins, but occasionally a temporary apheresis catheter needs to be inserted. In some patients, in particular after prolonged exposure to (alkylating) chemotherapy, even multiple leukapheresis procedures fail to obtain sufficient numbers of CD34+ cells to guarantee prompt and adequate hematologic recovery [27].

The dose of CD34+ cells needed for autologous stem-cell transplantation has been documented by many groups to be at least 2 x 10^6 per kg body weight of the recipient [26]. Infusion of smaller doses of CD34+ cells may well lead to delayed recovery of the platelet count to acceptable levels. Doses of <1 x 10^6/kg CD34+ often lead to delayed recovery of neutrophils, and in a proportion of patients hematologic recovery will never occur [28]. Increasing the CD34+ cell dose to >5 x 10^6/kg does not appear to decrease the duration of neutropenia, but results in even faster platelet recovery [29]. Since our group believes that a higher dose of CD34+ cells/kg will result in faster hematologic recovery, fewer transfusions, and consequently lower costs, we aim for a high dose (5-10 x 10^6/kg CD34+ cells) even in cases where this requires an additional collection episode. Such a large CD34+ cell dose also results in engraftment that is less susceptible to suppression by drug toxicity or viral infection.

Since under steady-state conditions, the number of CD34+ cells in the peripheral blood is only 1-5/mm^3, up to 100 liters of blood may need to be processed to reach the minimal transplant dose. Thus, collection of sufficient CD34+ cells from the steady-state peripheral blood of a donor/patient is not practical and prohibitively expensive [18]. Fortunately, "mobilization" procedures have

Table 2 Current Indications for Stem-cell Transplantation

	Allogeneic	Autologous
Leukemias		
Acute Myelogenous	+	+
Acute Lymphoblastic	+	+
Chronic Myelogenous	+	±
Chronic Lymphocytic	+	±
Lymphomas		
Non-Hodgkin's	+	+
Hodgkin's	±	+
Plasma Cell disorders		
Myeloma	+	+
Amyloidosis	-	+
Solid tumors		
Breast cancer	±	+
Ovarian cancer	-	+
Testicular cancer	-	+
Renal cell cancer	+	-
Brain tumors	-	±
Neuroblastoma	±	+
Ewing's sarcoma	-	+
Acquired bone marrow disorders		
Severe aplastic anemia	+	-
Myelodysplastic syndrome	+	±
Myeloproliferative disorders	+	-
Congenital disorders		
Immunodeficiencies	+	-
Wiskott Aldrich's	+	-
Fanconi's anemia	+	-
Thalassemia	+	-
Sickle cell anemia	±	-
Osteopetrosis	+	-
Storage diseases	±	-
Auto-immune diseases		
Scleroderma	+	+
Rheumatoid arthritis	-	+
Systemic lupus	±	+
Multiple sclerosis	-	+

+: established indication; ±: used in small numbers of patients; -: not used

allowed the collection of sufficient number of CD34+ cells from a much smaller volume of donor blood. The most frequently used early attempts at "mobilization" involved the recovery phase after high-dose chemotherapy [20, 21]. As an example, after a single-dose treatment with intravenous

cyclophosphamide 4 grams/m^2, patients become severely leukopenic (white blood cell count <<1x10^9/l). The white cells start to recover rapidly about 9-11 days after the administration of the chemotherapy drug. Once the WBC count exceeds 1x10^9/l, the concentration of CD34+ cells (as a percentage of total cells) is maximal and may well amont to 5-10% of all white cells in the blood! The number of CD34+ cells per liter, however, continues to increase for 2-4 more days and is probably maximal when the WBC count is about 10x10^9/l. This mobilization technique increases the concentration of CD34+ cells per liter up to 15-fold [21].This approach is effective, but can obviously only be used in patients, not in healthy donors. In addition, during the mobilization episode the patient is exposed to risk of bleeding and infection, and high-dose cyclophosphamie may also lead to organ toxicity. For healthy donors, mobilization with hematopoietic growth factor(s) only is to be preferred [19,30]. G-CSF (filgrastim) in a daily dose of 10 µg/kg subcutaneously for 4-5 days can increase the number of circulating CD34+ cells up to 50-fold. In the short term, this mobilization regimen appears benign although 80% of healthy donors will develop bone pains and 50% will develop headaches; long-term safety data are still incomplete [30]. Higher single daily doses filgrastim or split doses filgrastim twice a day result in slightly higher CD34+ cell collections than a single daily dose of 10 µg/kg, but also are more cumbersome [31]. GM-CSF (sargrasmostim) as a single mobilization agent appears less effective than G-CSF [32], but the combination of filgrastim and sargrasmostim may well be more effective than either drug alone [33].

A combination of chemotherapy and hematologic growth factors has been used for mobilization in the vast majority of candidates for autologous stem-cell transplant. Bonnadonna's group found a marked increase in CFU-GM in the peripheral blood of patients on the rebound after cyclophosphamide when they were also treated with daily GM-CSF [34]. A variety of chemotherapy drugs have been used for mobilization, including cyclophosphamide, ifosfamide, etoposide, taxol, cis-platinum and epirubicin [23]. Overall, the more myelosuppressive the mobilizing chemotherapy is, the better the collection of CD34+ cells. On the other hand, these more aggressive chemotherapy regimens are associated with more toxicity and with higher risk of admission [35]. Both GM-CSF (250 µg/m2 per day s.c.) and G-CSF (10 µg/kg/day s.c.) are effective in combination with chemotherapy, which is different from mobilization with hematopoietic growth factors alone where G-CSF is clearly superior to GM-CSF.

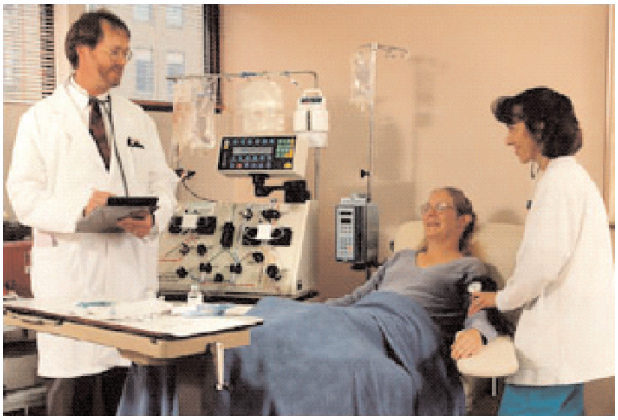

Fig. 3 Peripheral-blood stem-cell collection with the Cobe Spectra continuous-flow apheresis machine (GambroBCT, Lakewood, CO, USA)

Our group has been involved with randomized studies to determine whether mobilization with a combination of chemotherapy and G-CSF is more efficient than with G-CSF alone. The efficiency studied addressed both total collection of CD34+ cells and costs to cover the treatment and its side-effects [36,37]. In a matched control study, patients were first mobilized with G-CSF and PBSC were collected after 4-5 days. Then, after an interval of 1 week, a second mobilization was done with cyclophosphamide, etoposide, and G-CSF; again, PBSDC were collected. The mean daily CD34+ cell collections were nearly six-fold higher after the combination mobilization therapy; 57% of patients reached the goal of 5x10^6/kg CD34+ cells in one collection after combination therapy as compared to 13% after G-CSF alone. Resource utilization analyses, however, showed that it was less expensive to collect 5x10^6/kg CD34+ cells with G-CSF alone than with combination mobilizing therapy [36]. In a different study two consecutive mobilization attempts were made with the combination of cyclophosphamide, etoposide, and G-CSF. Whereas the first mobilization episode resulted in excellent CD34+ cell col-

lections, the second mobilization episode gave far smaller (75% less as determined both by CD34 and CFU-GM assays) PBSC collections [37]. These data suggest that stem-cell exhaustion is caused by the combination of chemotherapy and hematopoietic growth factors, but not by growth factors alone. Recently our group studied patients who received tandem autologous stem-cell transplants for multiple myeloma [38]. Attempts to collect CD34+ cells in a second mobilization episode 6 months after the first transplants were often unsuccessful. In fact, the number of collected CD34+ cells and CFU-GM was 90% less in the second collection episode, indicating that the mobilization chemotherapy and the preparative regimen (melphalan 200mg/m2) led to a high degree of stem-cell exhaustion that persisted for at least 6-9 months.

A number of other hematopoietic growth factors have been studied as mobilization agents (interleukin-3, stem-cell factor), but none was ultimately approved for routine clinical use. A newer approach is the use of drugs that inhibit the binding of SF-1α to its cognate receptor CXCR4. Treatment with AMD-3100 probably does not increase the total pool of CD34+ cells in the body, but enhances the release of these CD34+ cells from the BM to the peripheral blood [39,40]. In a recent preliminary study, patients were mobilized with chemotherapy and G-CSF. After the first stem-cell collection, a dose of AMD-3100 was given, leading to an enhanced collection of CD34+ cells the next day [39]. Similar results were obtained when only G-CSF was used as a mobilizing agent [40].

Whatever mobilizing and/or releasing regimen is used, in a small proportion of patients a sufficient dose of PBSC cannot be collected. Extensive chemotherapy may explain this failure to "mobilize" [27,28], but it is occasionally seen in patients who have received only moderate amounts of chemotherapy. In our experience a proportion of these "poor mobilizers" go on to develop myelodysplasia, even though the bone marrow does not show any evidence of an intrinsic bone-marrow disorder at the time of PBSC collection. In such patients our group strongly recommends against transplantation, since bone-marrow failure may persist for a long time, or even be permanent.

Why use peripheral blood in stead of bone marrow?

Stem-cell transplantation (SCT) is effective therapy for a wide variety of hematological or immunologic diseases, and for a number of neoplastic diseases (Table 1). For many of these diseases, SCT is currently the treatment of choice (first-line), whereas for other diseases SCT is only used as second-line treatment in patients who fail more conventional therapy. SCT can utilize either BM or PBSC as the source of stem cells. Occasionally a combination of BM and PBSC has been used.

In autologous SCT, few situations can be imagined in which BM is preferred over peripheral blood as the source of stem cells. When PBSC are collected in the steady state, the procedure is cumbersome and has no clear advantage over bone marrow. Indeed, such steady-state collections have only been considered when BM could not be obtained because of damage to the bone marrow (local radiation) or because of heavy tumor infiltration. PBSC obtained after mobilization, however, contain far more CD34+ cells than steady-state BM. The various mobilization regimens lead to a concentration of CD34+ in the peripheral blood that is similar to, or even higher than, the concentration of CD34+ cells in steady-state bone marrow. Whereas it is difficult to collect >20 ml bone marrow per kg body weight of the donor (typically 1,000-1,500 ml), even a single leukapheresis procedure may process 10-20 liters of blood. It is not uncommon that the number of CD34+ cells collected from the peripheral blood is 10-20 fold larger than from a BM harvest. The infusion of such a large number of CD34+ cells results in a more rapid hematologic recovery ("engraftment"), in particular of platelets (Table 2). In a recent series of 400 patients we found that AGC>>0.5 x 10^9/l were reached a median of 10 days after stem-cell infusion, while unsupported platelets >20 x 10^9/l were reached after a median of 11 days. The hematologic recovery at 30 days after transplant was also more complete after PBSC than after bone marrow transplantation. An additional advantage of PBSC is the lower risk that PBSC are contaminated with tumor cells that may reside in the bone marrow [41]. Few studies have systematically addressed this issue, but the few studies support the common sense of this approach. It is hard

to find any arguments for the continued use of BM in autologous stem-cell transplantation.

In allogeneic stem-cell transplantation the situation is far more complex. Hematologic recovery is probably faster with PBSC than with BM (Table 2). Yet in our experience overall engraftment is slightly slower after allogeneic than after autologous PBSC transplantation (Table 2). This finding suggests that the number of infused CD34+ cells is not the only important factor for hematologic recovery. The differences between BM and PBSC in the allogeneic setting concern not only CD34+ cells, but also other lymphocyte subpopulations, in particular T-lymphocytes. Donor T-lymphocytes are capable of inducing graft-versus-host disease (GvHD), and the infusion of native donor T-cells from the peripheral blood in order to improve engraftment [42] or enhance graft-versus-leukemia [43] has been shown to markedly increase the risk of acute and chronic graft-versus-host disease. When donor buffy-coat cells were infused after allogeneic SCT for severe aplastic anemia to decrease the risk of immunological graft rejection, a high proportion of the patients developed serious chronic graft-versus-host disease [42]. Similarly, the infusion of titrated doses of donor (T-) lymphocytes (DLI) to treat or prevent recurrence of leukemia, leads to GvHD in a considerable proportion of the patients [43]. Therefore, for many years fear of GvHD prevented the use of donor PBSC as the stem-cell graft in allogeneic transplantation [22]. In fact, the PBSC graft of the donor who was willing to donate non-mobilized peripheral blood but not BM, was extensively T-cell depleted prior to infusion [18]. The total number of infused T-cells, and their reactivity status, are both important for the risk of developing acute, and in particular chronic, GvHD. With T-cell replete allogeneic bone-marrow grafts, no clear correlation was found between the T-cell dose and the risk of acute GvHD [44]; on the other and, nearly complete removal of T-cells from the marrow graft prevents acute GvHD [45].

When mobilized PBSC are used for allogeneic stem-cell grafting, acute GvHD does not appear to be more frequent than after the use of BM that is not depleted of T lymphocytes [25,31]. The fact that "mobilized" PBSC induce less GvHD than native peripheral blood cells [25,31,42] is still insufficiently explained, but decreased immunological reactivity of T-lymphocytes after exposure to G-CSF may be responsible for this observation [46]. When mobilized PBSC are used for allogeneic stem-cell grafting, acute GvHD appears to be delayed as compared to bone marrow [25,31]. Whereas the start of acute GvHD after matched sibling donor transplant using bone marrow occurs a median of 26 days after transplant, with PBSC the start often occurs more than 60 days after transplant. The severity of acute GvHD probably is not very different between bone marrow and PBSC. The risk of chronic GvHD, however, is clearly higher with the use of PBSC for allogeneic SCT [47].

This higher level of late-occurring reactivity between donor T cells and recipient tissues constitutes a double-edged sword. While the donor T cells can cause significant morbidity (and even mortality) in the recipient, they also can continuously attack the malignant cells in the patient. This "graft-versus-leukemia" effect is very obvious in chronic myelogenous leukemia, and probably also active in acute myelogenous leukemia (AML), myelodysplasia, low-grade lymphoma, and perhaps even in myeloma and chronic lymphocytic leukemia . In fact, this "graft-versus-leukemia" (or graft-versus-malignancy effect) forms the basis for non-myeloablative allogeneic stem-cell transplant. In this reduced toxicity approach, chemotherapy and/or radiation therapy are given in doses sufficient only to allow engraftment of donor hematopoietic cells in the patient. Destruction of as many tumor cells as possible, which forms the backbone of the more classical SCT approach, is not attempted under the non-myeloablative scenario [48, 49]. This reduced toxicity approach has rapidly gained in popularity. Older patients, and patients with less than optimal organ function, are not likely to tolerate a very aggressive chemotherapy/radiation therapy preparative regimen. They may still be able, though, to undergo a reduced toxicity preparative regimen. The vast majority of non-myeloablative SCT are performed with PBSC as the source of stem cells. Engraftment is likely to be fast, and the immunological reactivity of the PBSC against recipient tissues (and thus cancer cells) is probably more prominent than after BM grafts.

A topic of ongoing active ongoing study is whether to use BM or PBSC as the source of stem cells for allogeneic SCT. Some centers have

adopted the use of PBSC for virtually all allogeneic SCT. Other centers choose the source of stem cells on the basis of the stage of the disease and the clinical condition of the patient. For patients with a high risk of relapse and an HLA-identical related donor, PBSC are probably to be preferred [31]. The increased "graft-versus-leukemia" effect in those patients should outweigh the increased risk of chronic GvHD [31]. Several studies have suggested that for good-risk patients (HLA-identical related donor; low or average risk of relapse) BM and PBSC are equivalent [50,51]. More recent studies, however, have claimed that for good-risk younger patients, BM is to be preferred. Here, the balance between relapse and chronic GvHD has been tipped towards the less toxic BM option [52]. For matched unrelated donor (MUD) transplants the situation is not clear yet [53]. In fact, in the USA a study organized by the Clinical Trials Network (CTN) and the National Marrow Donor Program (NMDP) has just started. In this study the source of stem cells for transplants from matched unrelated donors is randomized between BM and mobilized PBSC. Informed consent needs to be obtained from both patient and volunteer donor; this requirement makes the study complicated. Currently, donor preference is often a factor is the choice of stem cells for MUD transplants.

For allogeneic SCT from partially matched related donors, the number of CD34+ cells infused appears to be of importance [54]. Megadoses of purified CD34+ cells (10 x 10^6/kg) can only be obtained from PBSC collections and not easily from bone marrow [54]. Overall, the pendulum appears to be swinging towards the use of PBSC.

Where do we go from here?

At the present time, there continue to be more questions than answers in the use of HSC for transplantation. The use of PBSC is only one of these questions. Donors may well prefer PBSC donation over BM donation. On the other hand, donors who have donated both PBSC and bone marrow, did not appear to have a clear preference for one modality over the other. Donors who donate bone marrow are completely asymptomatic up to the start of anesthesia. After surgery, they have the discomfort of the punctures from their iliac crests, which lasts from 2-7 days, and of the recovery from general anesthesia. Donors of PBSC have a 50-80% chance of bone pains and headaches prior to donation. The pain disappears within 48 hours of the last G-CSF injection. The collection procedure itself is painless except for the needle insertions. In donors who do not have good peripheral vein access, an apheresis catheter needs to be inserted, with its own risks.

PBSC are composed of a variety of subsets which may have their own influence on transplant biology. The large number of T-cells is responsible for the increased risk of chronic GvHD. *In-vitro* and *in-vivo* techniques are employed to decrease the number of T cells in the stem-cell graft. Martelli's group uses a combination of T-cell depletion and positive CD34 selection to obtain a stem-cell product that is very rich in CD34+ cells and virtually devoid of T lymphocytes [54]. *In-vivo* depletion of T cells involves the use of anti-T cell antibodies such as anti-thymocyte globulin or alemtuzemab (Campath-1H) which, given prior to stem-cell infusion, suppress both the host-versus-graft reaction and destroy most of the T-cells in the stem-cell graft [55]. Indeed, in our experience with either drug, we have only rarely observed GvHD in the first 60 days after allogeneic SCT. The draw-back of T-cell depletion is a decreased graft-versus-leukemia effect. Such T-cell depleted transplants are easier and better tolerated by the patient, but are they also less effective?[56] Longer follow-up is needed to answer that question, and randomized studies may well be necessary. Different centers have different selection criteria, and patient populations are not always comparable between centers. It may well turn out that history is repeating itself. In the 1980's *in-vitro* T-cell depletion of BM grafts was used extensively for allogeneic SCT in leukemia. The immediate post-transplant toxicity was markedly less than after T-cell replete transplantation [45]. Follow-up studies, however, documented that many of the patients relapsed and died from progressive disease. In particular, in chronic myelogenous leukemia the relapse rate was very high [45]. Thus, overall survival was affected negatively by this new approach. Most centers abandoned *in-vitro* T-cell depletion for BM transplants from matched sibling donors. Hopefully, we can prevent a repetition of this unfortunate episode.

The use of increasing doses of native T cells from the donor constitutes a possible approach. Under this scenario, mobilized PBSC are depleted of T-cells by *in-vitro* or *in-vivo* techniques. Then after the patient has engrafted and the effects of the post-transplant cytokine storm have abated, increasing doses of native donor T-cells are infused at 8-12 weeks' intervals to stimulate the graft-versus-leukemia effect [56]. A titration of the graft-versus-leukemia effect can perhaps be accomplished this way. Once clinically significant GvHD occurs, further T-cell infusions are not given.

For autologous SCT, the biology is more straight-forward. New mobilization techniques are needed for those patients who cannot be "mobilized" with current means. Infusing a PBSC product that is free of contaminating tumor cells would be very important. Purging procedures of autologous PBSC have so far never resulted in better overall survival. Therefore, both antibody and chemical purging methods have lost much of their popularity. New approaches to this field would be important.

Undoubtedly, the next decade will see a shift in clinical SCT towards non-hematological diseases. Although autologous SCT for locally advanced breast cancer appears to have completely lost its place, the recent randomized study from The Netherlands suggests a modest improvement in disease-free survival for patients after SCT [57]. Allogeneic SCT for cancers of breast and kidney have also received attention, and may signify a shift of treatment from chemotherapy to more immunological therapy [58]. The use of SCT for the therapy of patients with various auto-immune diseases (scleroderma, rheumatoid arthritis, systemic lupus) offers the hope of completely replacing the patient's immune system with an allogeneic stem-cell graft [59] or "resetting" the immunological clock with a T-cell depleted autologous stem-cell graft [60].

A completely new use of PBSC is the arena of tissue remodeling. Animal studies have suggested that stem cells may restore/replace myocardial cells after infusion into the coronary arteries after myocardial damage [3]. Currently, early studies in man are ongoing to see whether this approach is clinically feasible and effective in patients after myocardial infarction [61]. In these studies, BM or PBSC mononuclear cells are infused into the coronary arteries. This requires new technology for injecting cells that need to enter the myocardium. There is no consensus on whether true HSC from the peripheral blood can differentiate into mature cells outside the hematopoietic system [4,62,63]. The next decade will show whether these various differentiation pathways will translate into clinical benefits.

Conclusions

For the next couple of years, however, the main indication of PBSC will remain stem-cell transplantation for diseases such as listed in Table 1.

Currently, PBSC are preferred for all autologous SCT. In some cases where sufficient doses of PBSC cannot be obtained, BM is still an acceptable source of HSC, but these cases are few and mostly hematological recovery is slow in such patients.

For allogeneic SCT, the choice of the optimal stem-cell product is more difficult. Our group feels that PBSC are to be preferred in all cases in which the risk of recurrence of malignancy is more than average. In patients at low risk of recurrence, BM is still to be preferred as stem-cell source, because of the lower risk of chronic GvHD. Umbilical cord stem cells result in slower hematological recovery than either PBSC or BM. Since umbilical cord-blood cells are immunologically more naïve, a lesser degree of histocompatibility between donor and recipient is required for successful SCT. Thus, umbilical cord stem-cell transplants are reserved for patients who do not have a fully HLA-compatible related or unrelated stem-cell donor.

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