25 years of peripheral blood stem cell transplantation

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Abstract

Peripheral blood stem cell transplantation (PBSCT) is the most common transplantation procedure performed in medicine. Its clinical introduction in 1986 replaced bone marrow (BM) as a stem cell source to almost 100% in the autologous and to approximately 75% in the allogeneic transplant setting. This historical overview provides a brief insight into the discovery of circulating hematopoietic stem cells in the early 1960s, the development of apheresis technology, the discovery of hematopoietic growth factors and small molecule CXCR4 antagonist for stem cell mobilization, and in vivo experimental transplant studies that eventually led to clinical PBSCT. Also mentioned are the controversies surrounding the engraftment potential of circulating stem cells before acceptance as a clinical modality. Clinical trials comparing the outcome of PBSCT with bone marrow transplantation (BMT), registry data analyses and the role of the National Marrow Donor Program (NMDP) in promoting unrelated blood stem cell donation are referred to in the article.

Introduction

It has been 25 years since peripheral PBSCT was introduced as a transplant modality. This anniversary, therefore, marks the first clinical evidence that circulating hematopoietic stem cells are capable of completely and permanently regenerating a lymphohematopoietic system after myeloablative treatment. Even more fundamental and beyond transplant aspects, the capability of hematopoietic blood stem cells to regenerate and maintain equal cellular concentration throughout all marrow spaces in the human
body attributes to them a homeostatic function that had until then never been shown before in a clinical setting (Fig.1a).

**Experimental evidence of circulating stem cells**

The evolution of knowledge leading to PBSCT is quite remarkable. In the 1950s, the discovery of dividing, non-leukemic DNA-synthesizing cells in peripheral blood (PB) suggested the existence of a small number of circulating cells of “multipotential character”. Indirect evidence of the migration of stem cells via PB was first demonstrated in so-called shielding experiments, in which, after lethal total body irradiation, stem cells from shielded hematopoietic tissue areas entered the circulating blood and subsequently repopulated the irradiated BM (Fig.1b). Postradiation parabiosis experiments, followed later by cross-circulation experiments in large animals, provided clear evidence that circulating stem cells were capable of reconstituting an entire lymphohematopoietic system after myeloablative treatment (Fig.1c). The basic concept of blood stem cell transplantation was formulated, a concept that can be seen as a repeat of embryonic hematopoietic development in which the vascularized cellular matrix becomes colonized through extravasation of circulating multipotent hematopoietic progenitors cells.

The logical next step was to harvest white blood cells and, among them, putative stem cells to reconstitute lymphohematopoietic function in myeloablated BM (Fig.1d). In the early 1960s, the term “blood stem cell” as a transplantable cell population was introduced. BM rescue studies after autologous and allogeneic blood stem cell transplantation into lethally irradiated large animals followed at Columbia University in
Cooperstown, New York\textsuperscript{7} and at the University of Washington School of Medicine in Seattle, Washington,\textsuperscript{8} but were not pursued at a clinical level because of the rarity of circulating stem cells at steady state compared with BM as a stem cell source.

**Development of clinical apheresis technology**

The development of the continuous-flow apheresis technology at the National Institutes of Health (NIH) in the early 1960s and its first clinical application at The University of Texas MD Anderson Cancer Center in Houston, Texas, was a critical next step. The design of the NCI-IBM blood cell separator allowed two to three times the patient’s blood volume to be processed resulting in large numbers of white blood cells to be collected in a matter of only 2 to 3 hours.\textsuperscript{9} It was, therefore, conceivable that large amounts of blood stem cells, a small subpopulation of white blood cells, could be harvested as well using the apheresis technology (Fig.1d). In the early 1970s, a first attempt at MD Anderson Cancer Center collecting colony-forming stem cells by continuous-flow apheresis was successful,\textsuperscript{10} thus laying the foundation for clinical PBSCT as an equivalent to BMT, albeit apheresis-derived stem cell yield was still significantly inferior to that of multiple BM aspirations.

**Failed clinical PBSCT attempts**

In the mid 1970s, concerns were raised about the quality of circulating blood stem cells. They were identified as having a limited capacity for self-renewal and low proliferative potential. It was speculated that clonogenic blood-derived stem cells “…are victims of clonal senescence, and have been expelled as waste products from the bone marrow.”\textsuperscript{11}
Two clinical blood stem cell transplant attempts among identical twins, one in 1979 at the University of California at Los Angeles (UCLA)\textsuperscript{12} and the other in 1980 at the NIH,\textsuperscript{13} resulted in failed or incomplete lymphohematopoietic reconstitution. In the UCLA case the leukapheresis product was administered to the twin patient as six separate white blood cell transfusions over 14 days. The total amount of mononuclear cells transfused was $5.8 \times 10^{10}$/kg recipient body weight. Over 2 months after transfusion no platelet or granulocyte engraftment was documented. One may speculate that each individual white blood cell transfusion did not contain a sufficient amount of hematopoietic progenitor cells to enable engraftment. In the NIH case the leukapheresis-derived mononuclear cells were administered as daily infusions over 8 days. No recovery of granulocytes, monocytes or platelets was documented at 8 weeks after transfusion. The authors attributed engraftment failure to the low number of clonogenic hematopoietic progenitor cells transfused being only one-eighth the quantity used in BM transplantation. Subsequently, both patients were rescued with their twin’s marrow cells. It should be noted that, at that time, stem cell collection by apheresis was performed at steady state without any growth factor priming.

**First successful autologous PBSCTs**

The autologous transplant modality required a liquid nitrogen cryopreservation technique for large volume leukapheresis products. Such a method was developed allowing stem cells to accumulate over the course of several daily collections until an engraftment dose was reached.\textsuperscript{14}
Thus, with the apheresis and cryopreservation technologies in place, blood stem cell infusions were first attempted in 1981 at Hammersmith Hospital in London, England, in patients with accelerated phase chronic myeloid leukemia (CML); the patients’ stem cells had been harvested in earlier chronic phase with the intent of achieving a second chronic phase. In the same year, at Johns Hopkins Hospital in Baltimore, Maryland, a “normal” blood stem cell harvest and transplantation attempt was undertaken in a patient with CML in cytogenetic remission. After myeloablative treatment and blood stem cell infusion there was first evidence of fast and complete hematopoietic reconstitution, although long-term stem cell engraftment could not be documented.

It was another 5 years in 1986, before a patient with Burkitt’s lymphoma underwent myeloablative radio- and chemotherapy followed by PBSCT at Heidelberg University Hospital in Germany. Hematopoietic reconstitution was fast and complete. Twenty-five years later, the patient remains alive without evidence of disease and with normal lymphohematopoietic function.

In 1986 and 1987, similar reports of successful PBSCTs were documented by research groups at the University of Nebraska Medical Center, the Hospital Haut Leveque in Bordeaux, France, and the Royal Adelaide Hospital in Adelaide, Australia. In all of those initial PBSCT cases, stem cells were collected by multiple apheresis procedures at steady state without hematopoietic growth factor mobilization treatment.

**Strategies to increase peripheral blood stem cell concentration**

In contrast to the well established BM transplantation, the use of PB stem cells in the
mid 1980s seemed cumbersome mainly because in non-CML patients numerous steady-state apheresis procedures were required to achieve an engraftment stem cell dose. For PBSCT to be accepted as a mainstream treatment modality, strategies to temporarily increase PB stem cell concentration had to be developed. One strategy was to induce a rebound of PB stem cell concentration following non-myelotoxic chemotherapy and short-term myelosuppression.\textsuperscript{21} Another strategy was to temporarily expand the PB stem cell pool by administering hematopoietic growth factors.

**Hematopoietic growth factors and CXCR4 antagonist for optimizing stem cell yield**

**Hematopoietic growth factors**

Whereas the molecular cloning and clinical development of granulocyte colony-stimulating factor (G-CSF) initially focused on ameliorating the side effects of chemotherapy-induced cytopenia, it soon became obvious that G-CSF also expanded the circulating hematopoietic stem cell pool by mobilizing CD34\(^+\)38\(^-\) hematopoietic progenitor cells from extravascular BM sites to PB.\textsuperscript{22} In 1988, two stem cell mobilization studies in patients at the Dana Farber Cancer Institute in Boston, Massachusetts,\textsuperscript{23} and at Royal Melbourne Hospital in Australia\textsuperscript{24} were published. Researchers at the Dana Farber Cancer Institute used granulocyte-macrophage colony-stimulating factor (GM-CSF) while G-CSF was used at the Royal Melbourne Hospital; these studies provided clear evidence of an increased PB concentration of colony-forming cells by 60-fold and up to 100-fold over baseline, respectively. Follow-up studies showed a mobilization advantage
of G-CSF over GM-CSF, and today G-CSF is considered the gold standard of stem cell mobilization treatment. By the mid-1990s, with those two stem cell mobilization strategies (chemomobilization and/or G-CSF) in place, autologous PBSCT became increasingly accepted, eventually entirely replacing autologous BMT.

Healthy individuals were initially excluded from donating G-CSF-mobilized hematopoietic stem cells primarily because of concerns about triggering lympho-hematopoietic malignancies in genetically predisposed healthy sibling donors. Nevertheless, 5 years after the introduction of G-CSF as a stem cell mobilization agent in patients, data of a first mobilization study on healthy individuals were published by researchers at Sapporo Medical College in Japan. To ensure donor safety, initial guidelines for G-CSF-induced stem cell mobilization and collection in normal donors were formulated. A crucial next step was to open up PB stem cell donation to unrelated donors. A prospective clinical study was initiated by the NMDP for G-CSF-mobilized stem cell collections in second donation requests, followed in 1999 by an NMDP protocol for primary PB stem cell collection in unrelated donors. Stem cell collection data on 1488 G-CSF mobilized donors reported to the International Bone Marrow Transplant Registry (IBMTR) and EBMT revealed a comparable safety profile to BM harvesting.

A 2008 conference report on clinical and ethical issues of using hematopoietic growth factors in healthy stem cell donors gave a state of the art insight into the safety of G-CSF mobilization treatment, and came to the following conclusions:

1. severe but rare side effects of G-CSF mobilization treatment, including splenic rupture and sickle cell crisis, have been reported,
2. data from the NMDP show an overall rate of 0.6% serious adverse events attributed to stem cell mobilization treatment and collection,\textsuperscript{30}

3. no definite conclusions can be made regarding the long-term effects of G-CSF treatment in healthy donors, and

4. the balance between the risk to the donor and the benefit to the recipient raises ethical issues that are still being discussed.

According to the NMDP and its Be The Match Registry 76% of adult unrelated donors - more than 3100 - donated G-CSF-mobilized PB stem cells. However, in pediatric patients BM and umbilical cord blood (UCB) are the predominant unrelated stem cell sources.

**Plerixafor**

The interaction between the chemokine stromal cell-derived factor1 (SDF1 also known as CXCL12) and its receptor CXCR4 in BM plays a major role in the trafficking of progenitor cells between BM and PB.\textsuperscript{31} Whereas G-CSF induces stem cell mobilization by downregulating the expression of SDF1 on BM osteoblasts and by proteolytic cleavage of both SDF1 and CXCR4, plerixafor, a novel small molecule antagonist, reversibly inhibits the interaction of SDF1 with its cognate receptor CXCR4. With the safety of plerixafor in human volunteers documented,\textsuperscript{32} the first clinical study on the mobilization of hematopoietic progenitor cells in healthy individuals was published in 2003 by researchers at the University of Washington in Seattle, Washington.\textsuperscript{33} A follow-up study in 2005 clearly showed a synergistic effect of G-CSF and plerixafor on the mobilization of CD34\textsuperscript{+} cells.\textsuperscript{34} Thus, the combination of G-CSF and plerixafor has become the standard treatment regimen in difficult to mobilize patients. In 2008,
plerixafor was approved by the Food and Drug Administration (FDA) for PB stem cell mobilization in combination with G-CSF in patients with non-Hodgkin’s lymphoma and multiple myeloma. In 2009, two randomized phase III trials in patients with multiple myeloma and in patients with non Hodgkin’s lymphoma confirmed those earlier studies.

The central role of the SDF1-CXCR4 axis in mobilizing hematopoietic stem cells also applies to the release of leukemic blast cells and progenitor cells of various lymphohematopoietic malignancies and solid tumors. As with G-CSF, there is concern about mobilizing clonogenic tumor cells when using plerixafor. Long-term follow-up of patients transplanted with plerixafor-mobilized stem cell autografts is needed to evaluate its safety.

**Allogeneic PBSCT**

Until the mid-1990s, allogeneic PBSCT was not considered a transplant option because of its potential risk of inducing severe graft-versus-host disease (GVHD) owing to the 10-fold higher amount of donor T-cells contained in a PB allograft. Also, there was a hesitance to expose healthy stem cell donors to cytokine treatment for stem cell mobilization.

In 1989, the first attempt to transplant non-mobilized, T-cell-depleted PB stem cells into a human leukocyte antigen (HLA)-identical sibling at the University of Nebraska Medical Center in Omaha, Nebraska, was made. There was early and exclusively donor-derived hematopoietic engraftment. Grade 2 acute GVHD was limited to the skin. Since the patient died of infectious complications 32 days after transplantation, long-term
engraftment could not be demonstrated. Four years later, another single-case report on allogeneic stem cell transplantation was published by researchers at the Nottingham University Hospital in Nottingham, England.\textsuperscript{41} This time, G-CSF-mobilized stem cells were transplanted into an HLA-identical sibling. There was trilineage engraftment without GVHD. The follow-up, however, was only 58 days posttransplant, and again lacked evidence of sustained engraftment.

In 1995, the first clinical trials of successful allogeneic PBSCTs at MD Anderson Cancer Center,\textsuperscript{42} Fred Hutchinson Cancer Center,\textsuperscript{43} and Kiel University Hospital in Germany,\textsuperscript{44} proved concerns about inducing severe GVHD wrong. The incidence of acute GVHD was surprisingly similar to that in patients who underwent allogeneic BMT, and hematopoietic growth factor exposure in healthy individuals did not seem to impose an undue risk. Eventually, molecular evidence revealed that donor-derived multipotent stem cells transplanted into a conditioned recipient could permanently generate an entire lymphohematopoietic system. Indeed, at our institution the first patient with refractory acute myelogenous leukemia who received her brother’s blood stem cells has a 100% donor-derived lymphohematopoietic system and has been in complete and sustained remission for 16 years. The allogeneic transplant setting, therefore, provided definite clinical proof that hematopoietic progenitor cells mobilized into PB and harvested by apheresis can generate a complete and sustained lymphohematopoietic system.

UCB transplantation, an extension of PB stem cell harvesting into the newborn’s cord blood, was first introduced in 1989 at Hôpital Saint-Louis in Paris, France,\textsuperscript{45} and has become a widely used stem cell transplant option in children and, to a more limited extent, in adults. For a detailed review of the historical events leading to the clinical
introduction of UCB transplantation, and major follow-up studies, we refer to Eliane Gluckman’s paper published in 2009.46

**PB compared with BM as a source of hematopoietic stem cells**

**Clinical trials**

The unexpected initial finding of similar acute GVHD rates following allogeneic BMT and PBSCT prompted the design of randomized prospective clinical trials. In 1998, the results of the first multicenter trial of the European Group for Blood and Marrow Transplantation (EBMT) on 70 patients confirmed those early observations of similar incidence rates of moderate to severe acute GVHD and transplant-related mortality between patients who underwent PBSCT and patients who underwent BMT.47 Randomized trials, either multicenter or single center,48 followed. The clinical findings included faster platelet, neutrophil and immune recovery, higher occurrence of chronic GVHD and lower probability of relapse in patients following PBSCT. In 2001, a randomized multicenter trial on 172 patients that involved the Fred Hutchinson Cancer Research Center and the University of Washington Hospital in Seattle, Washington, the City of Hope Medical Center in Duarte, California, and Stanford University Hospital in Stanford, California, came to the following conclusions:

1. recovery of neutrophils and platelets was faster in patients who underwent PBSCT,

2. there was no significant difference between PBSCT and BMT in the cumulative incidence of grades II-IV acute GVHD at 100 days posttransplant,
3. there was no significant difference between PBSCT and BMT in the cumulative incidence of chronic GVHD at 2 years posttransplant, although a trend favoring less chronic GVHD after BMT was noticed, and

4. both, the estimated overall probability of survival and the rate of disease-free survival at 2 years posttransplant were better in patients who underwent PBSCT. The benefit of higher survival rates was seen primarily in patients with more advanced malignancy.

Clinical findings of an EBMT-initiated prospective randomized trial that involved 350 patients with standard-risk leukemia turned out to have a somewhat different outcome: the incidence of grades II-IV acute GVHD was found to be significantly higher in patients who underwent PBSCT. There was a higher incidence of chronic GVHD after PBSCT. On the other hand, there were no significant differences in overall survival or leukemia-free survival between the BMT and PBSCT groups at the 3-year follow-up.

A large retrospective International Bone Marrow Transplant Registry (IBMTR) and EBMT registry analysis that involved 824 patients revealed the following data: the incidence of chronic GVHD at 1 year was significantly higher in patients after PBSCT, and treatment-related mortality rates and leukemia-free survival rates were in favor of patients undergoing PBSCT in advanced stages of leukemia. A later retrospective EBMT registry analysis of 3465 adult patients with AML and ALL revealed again a higher incidence of chronic GVHD after PBSCT. However, rates of acute GVHD, leukemia-free survival and overall survival were similar after BMT and PBSCT.

To test various clinical outcome parameters such as survival probability, engraftment characteristics, acute and chronic GVHD, relapse, and immunereconstitution in a more
GVHD-prone transplant setting, a phase III, randomized, multicenter, prospective comparative study of G-CSF-mobilized PBSCT versus BMT using HLA-compatible unrelated donors was designed. This trial was conducted under the auspices of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). The study met its accrual target of 550 patients and closed enrollment in September 2009. At the time of this writing patient outcome results have not been released.

**PB versus BM allograft composition**

The clinical outcome of stem cell transplantation is in part determined by the cellular composition of the stem cell graft.

The number of G-CSF-mobilized CD34+ hematopoietic progenitor cells contained in PB stem cell grafts is 3-fold higher than in BM grafts resulting in faster reconstitution of neutrophils and platelets after PBSCT as documented in most randomized trials and registry data analyses.

The approximately 10-fold higher number of CD3+ T-cells contained in an un-manipulated PB stem cell allograft did, against all predictions, not cause more severe or a higher incidence of acute GVHD. To explain these clinical findings, researchers at the Fred Hutchinson Cancer Research Center focused on the effects of G-CSF mobilization treatment on stem cell products. Data were presented showing that, compared to BM allografts, the 50-fold higher number of CD14+ monocytes and monocyte progenitors contained in G-CSF-mobilized PB stem cell allografts had a suppressive effect on donor T-cells. G-CSF mobilization treatment was also found to have an immunomodulatory effect on the graft. By mobilizing preferential T helper 2-inducing dendritic cells, the
cytokines interleukin-4 and interleukin-10 are produced, which are less inflammatory and, therefore, are associated with diminished GVHD-inducing ability.\textsuperscript{55} Cell subsets that are implicated in the graft-versus-leukemia (GvL) effect include CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells, and natural killer (NK) cells. The significantly higher number of T-cells contained in PB stem cell allografts together with a 3-fold higher percentage of NK-cells are believed to be associated with a more pronounced GvL effect, although clinical GvL data of various randomized trials, as mentioned above, are not conclusive. For a current and detailed understanding of the mechanisms underlying GVHD and GvL effect we refer readers to W. Shlomchik’s\textsuperscript{56} and Stanley Ridell’s reviews.\textsuperscript{57}

**Final remarks**

According to the Center for International Blood and Marrow Transplant Research (CIBMTR) database, 98\% of autologous stem cell transplants performed between 2002 and 2006 in patients older than 20 years were blood-derived. In the respective allogeneic transplant setting, 74\% were blood-derived, 24\% BM-derived and 2\% UCB-derived. The circulating blood has therefore emerged as the predominant source of hematopoietic stem cells for grafting.

Organ transplantation, including BM aspiration, has been traditionally a domain of surgery. In contrast, PB and UCB stem cell harvesting and processing has become the responsibility of transfusion medicine and blood banking. Hematopoietic “organ” donation has been replaced by “blood” donation lacking emotional, and, in some parts of the world, even religious limitations.
Translational research played a major role in the clinical development of PBSCT, and the evolution of PBSCT, in turn, has made a remarkable contribution to our understanding of stem cell biology.

Authorship

M.K. wrote the manuscript. E.F. provided archival material and documentation.

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**Legend**

Figure 1. Stem cell migration between BM and PB. A) Stem cells are in dynamic equilibrium between PB and extravascular BM sites. Stem cell migration occurs via circulating blood. B) Stem cell migration from non-irradiated BM via circulating blood to irradiated BM sites. C) Stem cell migration through cross-circulation from a normal BM donor to a total body irradiated recipient. D) Stem cell harvesting by apheresis. The apheresis-induced stem cell efflux from PB is compensated for by continuous stem cell influx from BM sites into circulating blood thus preventing the circulating stem cell pool from becoming exhausted during apheresis.
Figure 1

A

B

C

D

total body
irradiated

normal
hematopoiesis

shielded/
non-irradiated

irradiated

apheresis
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