Bone Marrow, Peripheral Blood, or Umbilical Cord Blood: Does the Source of Allogeneic Hematopoietic Progenitor Cells Matter?

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Introduction

Rescue of the blood and immune cell production by transplantation of donor Hematopoietic Progenitor Cell (HPC) preparations has become an indispensable intervention for malignant and non-malignant diseases. When a suitable donor is identified, currently two types of procedures may be employed to obtain progenitor cells. (i) Aspiration of 500 to maximal 1500ml Bone Marrow (BM) blood under general anesthesia from the iliac crest or (ii) harvest of progenitor cells by apheresis of mononuclear cells from peripheral blood (PB) after pretreatment with substances like Granulocyte-Colony Stimulating Factor (G-CSF) or a CXCR4 antagonist, which mobilize progenitor cells from bone marrow. The decision for one of the two procedures mainly depends on donor characteristics (e.g. condition of veins needed for access to apheresis or contraindications against anesthesia) and preferences of the donor and/or the transplant center. In recent years, as a third source of progenitor cells, blood from placenta and umbilical cord (cord blood, CB) has become available.

The legislation regulating the procurement and use of medicinal products of human origin has changed in the past decades. While for a long time blood products had been regarded as replacement of physiological substances, they became subject to the pharmaceutical legislation in the year 1989 under the impression of massive transmission of viruses such as HIV. Meanwhile, in Europe also a legal frame for blood components for transfusion has been established [1]. In Germany, providers of HPC preparations have to submit an application in order to obtain approval by the Paul-Ehrlich-Institute (PEI), which is a German higher competent authority responsible for blood products, clinical trial, marketing authorization and vigilance. Such applications comprise description of harvesting and manufacturing procedure, testing for safety, quality and functionality, supported by quality and non-clinical data (e.g. investigating excipients like cryo-preservatives) and clinical data supporting its use as bone marrow replacement.

According to the European legislation [2], clinical trials have to be conducted following Good Clinical Practices. The sponsors of clinical studies have to submit applications to an independent ethics committee for review and to a competent regulatory authority, which for HPC is in Germany the PELA quality and non-clinical documentation needs to be provided with each clinical trial application.

One of the major problems in stem cell transplantation is to find a suitable cell preparation for each patient, since the donor cells need to be immunologically matched to the recipient, particularly to his HLA antigens [3]. Usually, closely related donors would be preferred, but in certain circumstances also haploidentical related or unrelated donors may be chosen [4].

In this paper, the three sources of HPC, i.e. BM, PB and CB, are compared, with a particular focus on comparison of clinical outcomes. In addition, an anonymized overview will be presented of clinical trials with HPC which have been approved by the PEI in the years 2005 to 2010, covering not only the transplantation for rescue of bone marrow function, but also applications in tissue regeneration.

Comparison of BM, PB and CB Preparations

The choice of the preparation for a given patient usually depends on the availability of a suitable donor. In many indications, HPC from either BM or PB can be used; if no such preparation is available, CB may alternatively be used. Since the therapy is complex and often under pressing time constraints due to the patient’s deteriorating condition, all three types of HPC may be used more or less interchangeably according to their availability. An overview of some general aspects is provided in Table 1.

In contrast to e.g. stable plasma derivatives, where tight specifications can be set and batch release is performed by manufacturer and official control laboratories, each HPC preparation is a unique medicine. Nevertheless, the collection of HPC should follow accepted standards and the quality of HPC has to be assured by laboratory testing of their composition, including differential cell counts with particular focus on the determination of surface antigens such as CD34 [5]. Functional assays, such as determination of Colony Forming Units (CFU) are difficult to standardize. Different processing procedures may be necessary depending on e.g. minor (plasma reduction) or major (red blood cell reduction) ABO incompatibility, on HLA mismatch (T cell depletion or CD34+ cell selection) or on storage duration requirements (cryo-preservation). Variations of measured quality parameters may be enhanced by different preparation steps. Moreover, there is a great individual variability of donors’ bone marrow and blood and thereby the HPC collected from them. Therefore, specifications for HPC usually cover a broad range for key parameters such as volume, total nucleated cell and CD34+/CD45+ cell content.

HPC from BM, PB and CB are quite different in many aspects. Nevertheless, our knowledge about a possible impact of type and composition of transplanted HPC on parameters of clinical outcome is still incomplete. A retrospective study [6] of HPC transplantation for various (predominantly malignant) diseases compared cell composition of 181 PB and 94 BM grafts and clinical outcomes. While within the BM group, parameters of cellular composition did not correlate with hematopoietic recovery, Graft-Versus-Host Disease (GVHD) or survival, in the PB group survival rates at 1 year were higher with a CD34+ cell dose > 5 x 10^6 kg body weight and patients’ platelet count was higher with PB grafts containing > 8 x 10^9 CD3+ CD8+ T-cells /...
A study on HLA matched allogeneic transplantation for aplastic anemia [7] compared 225 patients receiving BM with 71 patients receiving PB grafts. Hematopoietic recovery was found to be similar, but grade 2 to 4 acute GVHD and mortality were higher in the PB group and the authors recommended BM as the preferred graft type. A comparison of 1,028 unrelated BM with 351 CB transplantations with myeloablative conditioning for acute leukemia or myelodysplastic syndromes [8] showed similar overall mortality. CB showed inferior neutrophil recovery, but lower risk of acute GVHD, lower risk of transplant-related mortality and similar risk of relapse; the authors therefore consider CB a reasonable alternative.

For only about one third of patients HLA-identical family donors are available, therefore HPC transplantation from mismatched donors, e.g. from haploidentical related donors was introduced [9]. However, this approach implicates transgression of the HLA barrier, thus increasing the risk of GVHD. One approach to improve the outcome is reduction of graft T-cells, which appear to mediate GVHD. On the other hand, there is concern that T-cell depletion might result in prolonged immunodeficiency predisposing to serious infections and reduce the much desired anti-leukemia effect of HPC transplantation. There are protocols and commercial devices for depletion of CD3+ and CD19+, or positive selection of CD34+ or CD 133+ cells. A randomized multicenter trial [10] compared CD34+ selected PB grafts with non-selected BM grafts, both from HLA identical sibling donors, for transplantation for hematological malignancies. PB grafts contained more CD3+ cells (despite selection) and more CD34+ cells; platelet and neutrophil recovery were faster, but grade 2 to 4 GVHD were higher in PB recipients. The 4 year survival was decreased in patients receiving > 2 x 10^5 CD3+ cells / kg body weight and was significantly better in BM recipients. However, such results need to be interpreted with much caution. As an evaluation of the European Group for Blood and Bone Marrow Transplantation (EBMT) of results of haploidentical transplantation of children with acute lymphatic leukemia [11] showed, many variables influence the outcome of this very complex treatment, e.g. disease stage and pretreatment, type of T-cell depletion, CD34+ cell dose, use of total body irradiation, immunosuppressive medication and interestingly the size (and thus experience) of the center.

In recent years, there are growing efforts to employ HPC in regenerative medicine applications, which are regulated under a special European Regulation for advanced therapy medicinal products [12]. Examples are the use of not substantially manipulated HPC preparations in cardiovascular indications (for review compare [13]) and in children with type 1 diabetes [14]. In these relatively new indications less than in "classical" HPC transplantation is known about the "active substance". This means that in many cases the cell type responsible for the desired effect is not entirely clear. Not only "hematopoietic" cells, but for instance also mesenchymal stem cells which can be found in BM and PB preparations appear to play a role in tissue repair [15]. In many new indications the mechanism of action, the significance of other cells contained in the preparation ("impurities") and the benefit risk ratio is not sufficiently elucidated. The situation is even more complex when HPC are used as starting material for novel medicines, e.g. in gene therapy or tissue engineering. Much more information is needed from non-clinical studies and clinical trials. There is only one aspect which reduces the obvious complexity: so far most of these approaches involve autologous cell preparations. Nevertheless, there are visions in the public domain to use e.g. CB as an indefinite source of tissue repair.

Hence, there are many open questions related to both therapeutic fields, in which HPC preparations are used, i.e. the "conventional" HPC transplantation for rescue of hematopoiesis and immune system and the development of advanced therapy medicinal products. In the years 2005 to 2010, a total of 22 clinical trials with HPC have been approved by the PEI (Table 2). For each study, the EudraCT number is listed; the sponsors of these studies and further details can be found in the EU Clinical Trials Register (https://www.clinicaltrialsregister.eu/). 15 of these trials explore the use of autologous HPC (in 10 studies BM, in 4 studies PB and in 1 study CB). Two of the 15 studies address the use of autologous HPC for rescue of hematopoiesis after intensive therapy of malignancies (one study each in Ewing sarcoma and multiple myeloma) and one an autoimmune disease. In the other 12 trials with autologous HPC treatment of cardiovascular or metabolic diseases is studied. None of the studies on tissue repair uses allogeneic HPC; all 7 studies with allogeneic HPC address hematological malignancies. 3 of these trials involve CD3/CD19-depleted PB preparations.

### Table 1: Characteristics of HPC preparations for allogeneic transplantation in accordance with guidelines of the German Medical Association after consultation with the PEI.

|                  | BM                                      | PB                                      | CB                                      |
|------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|
| **Mode of collection** | Usually in operation theatre, under general anesthesia, puncture of spinallacu posteriors, aspiration of up to 20 ml/kg body weight BM containing blood (corresponding to maximal 1500 ml or 25% of blood volume in children) anticoagulated with Citrate and / or heparin | Mobilization of progenitor cells from bone marrow with eg; G-CSF pretreatment, apheresis of mononuclear cells into citrate anticoagulant | After completed birth collection of blood from umbilical cord into citrate anticoagulant |
| **Typical processing steps** | Removal of larger particulate matter by filtration, depending on ABO compatibility plasma or red cell reduction; Selection and/or depletion of cells according to CD markers | If needed volume reduction; selection and/or depletion of cells according to CD markers | Depletion of red cells, volume reduction |
| **Storage** | Liquid storage after collection up to 72h at 2 to 6°C, or up to 48h at room temperature, or freezing within 72h after collection in a suitable cryoprotectant (e.g. 10% DMSO): ≤ 140°C (above or in liquid nitrogen) several years as validated | Liquid storage after collection up to 72h at 2 to 6°C, or up to 48h at room temperature, or freezing within 72h after collection in a suitable cryoprotectant (e.g. 10% DMSO): ≤ 140°C (above or in liquid nitrogen) several years as validated | Freezing within 48h in a suitable cryoprotective solution (e.g. 10% DMSO): ≤ 140°C (above or in liquid nitrogen) several years as validated |
| **Dose rate autologous** | ≥ 2 x 10^6 nucleated cells/kg body weight | ≥ 2 x 10^6 CD34+CD45+ cells/kg body weight | ≥ 1.5 x 10^6 nucleated or CD45+ cells/kg body weight |
| **Dose rate allogeneic** | ≥ 2 x 10^6 nucleated cells/kg body weight | ≥ 4 x 10^6 CD34+CD45+ cells/kg body weight | ≥ 3 x 10^6 nucleated or CD45+ cells/kg body weight |

kg body weight. A study on HLA matched allogeneic transplantation for aplastic anemia [7] compared 225 patients receiving BM with 71 patients receiving PB grafts. Hematopoietic recovery was found to be similar, but grade 2 to 4 acute GVHD and mortality were higher in the PB group and the authors recommended BM as the preferred graft type.
Concluding Remarks

The use of donor HPC for rescue of the blood and immune cell production is a well-established option for treatment of many malignant and non-malignant diseases. However, particularly studies on high intensity treatment of hematological malignancies focus predominantly on the definition of the most appropriate disease stage and response to pretreatment for inclusion of the patients, the modalities of chemo-/radiotherapy often called “conditioning” of the patient, or supportive treatment such as immune suppression to reduce the GVHD risk. The HPC itself is often regarded as “ancillary” and BM, PB and in recent years also CB grafts are used more or less interchangeably. The choice of HPC-source for a given patient often depends on the availability of a suitable preparation.

Particularly for pediatric patients, CB grafts are increasingly accepted as an alternative to BM and PB in hematological malignancies [16] and metabolic disorders [17]. There are several advantages of CB, e.g. the abundant availability and the collection without risk for mother and newborn. Moreover, CB grafts permit a higher degree of HLA disparity and lead to reduced incidence and severity of GVHD. However, there are apparently also some draw-backs such as delayed HLA disparity and lead to reduced incidence and severity of GVHD. Moreover, CB grafts permit a higher degree of HPC-source for a given patient often depends on the availability of a suitable preparation.

In addition to surgery

In 2005-000774-46 BM, autologous BOOST-2, myocardial regeneration

In 2005-000968-33 BM, autologous PROVASA, chronic limb ischemia [27]

In 2005-000969-19 BM, autologous RENERVATE, diabetic neuropathy

In 2005-003629-19 BM, autologous Myocardial regeneration

In 2006-005709-50 BM, autologous CELLWAVE, Combined Extra corporal Shock Wave Therapy and Intracoronary Cell Therapy in Chronic Ischemic Myocardium

In 2005-004051-35 BM, autologous, CD133+ INSTEM, myocardial regeneration, combined with Trans Myocardial Laser Revascularisation (TMLR)

In 2006-000393-76 PB, allogeneic, CD3/CD19 depleted Hematological disorders in pediatric patients

In 2005-004028-38 BM, autologous, CD133+ Chronic cardiac ischemia

In 2007-006404-11 PB, autologous, CD133+ PERFECT, Intramyocardial transplantation for Improvement of Post-Infarct Myocardial Regeneration in Addition to Surgery

In 2007-004874-14 BM, autologous REPAIR, Reinfusion of Enriched Progenitor cells And Infarct Remodeling in non-ST elevation AMI

In 2008-007694-23 CB, autologous Umbilical cord blood to reverse DM type I in children

In 2006-001269-40 PB, autologous, CD34+ Severe lupus erythematoses

In 2007-006016-33 PB, allogeneic, CD3/CD19 depletion Reduced conditioning in adult leukemia patients

In 2008-004625-42 BM, autologous AMI, intramyocardial application

In 2007-003514-34 PB, allogeneic Comparing chemotherapy with stem cell transplantation in elderly with AML

In 2008-003658-13 PB, autologous EWING 2008, Ewing sarcoma, chemotherapy versus chemotherapy + HPC

In 2009-008368-28 BM, autologous Ischemic cardiomyopathy, intramyocardial application

In 2007-004517-34 PB, allogeneic, CD3/CD19 deletion Relapsed or refractory AML (including children)

In 2010-013556-61 PB, autologous multiple myeloma; combined with lenalidomide/dexamethasone

In 2010-019377-15 BM or PB, allogeneic AML; allogeneic HPC versus chemotherapy

In 2008-001699-27 PB, allogeneic Poor-risk CLL

In 2010-018467-42 PB, allogeneic MDS, 5-Azacytidine versus 5-Azacytidine + allogeneic HPC

Table 2: Clinical trials approved by the PEI in the years 2005 to 2010.

AMI: Acute Myocardial Infarction; AML: Acute Myeloid Leukemia; CLL: Chronic Lymphatic Leukemia; DM: Diabetes mellitus; MDS: Myelodysplastic Syndrome.
genotype of Ig-like receptor on donor natural killer cells has impact on both immunity status and infections [25] and relapse-free survival after unrelated HPC transplantation for acute myeloid leukemia [26]. If the clinical significance of such new insights can be further substantiated, methods to process the grafts accordingly and to verify the consistent efficacy of these methods will become very important. This is even more applicable to HPC used in regenerative medicine for advanced therapy medicinal products such as tissue engineering or gene therapy products.

Notwithstanding the acknowledgement of the very high degree of complexity, it will be necessary to increase our knowledge by clinical studies on the respective contributions of the different cell types contained in therapeutic HPC to both clinical efficacy and potential risks. Such enhanced insight will be crucial for further developing and optimizing HPC as important medicines, defining "active substances" and impurities, advancing processing technology and improving quality control. In this situation, it will be particularly important to prioritize questions and to conduct sufficiently sized clinical trials in a concerted way in order to have a sufficient number of patients studied to obtain meaningful answers. It is understood that this can only be done in close collaboration of scientific networks and well-designed multicenter studies. In this setting, it will be important to take all possible steps to approximate as far as possible all variables from the collection and processing of grafts over the treatment modalities and co-medications up to assessment and evaluation of clinical endpoints. In this respect, the application of Good Clinical Practices should not only be seen as a further burden, but also an important and helpful quality standard for these much awaited clinical trials.

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