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 contraindication 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 the 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 10^7/kg body weight and patients’ platelet count was higher with PB grafts containing > 8 x 10^4 CD3+ CD8+ T-cells /

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Received January 06, 2012; Accepted February 07, 2012; Published February 13, 2012


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J Blood Disord Transfus Hematologic Oncology: Diagnosis & Therapeutics ISSN: 2155-9864 JBDT, an open access journal
Interestingly, the size (and thus experience) of the center.

dose, use of total body irradiation, immunosuppressive medication and
e.g.; disease stage and pretreatment, type of T-cell depletion, CD34+ cell

Many variables influence the outcome of this very complex treatment,

transplantation of children with acute lymphatic leukemia [11] showed,

better in BM recipients. However, such results need to be interpreted

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

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.
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 with PB, in relation to CD3+ cell dose. CD34+ cell dose was
inversely correlated with treatment-related mortality, but the latter was
higher in PB recipients. The 4 year survival was decreased in patients
receiving > 2 x 10^6 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,
eg; 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
genome 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 hematopoesis 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 hematopoesis 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.
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 drawbacks such as delayed neutrophil and platelet engraftment [18] and increased risk of serious infections [19] which has been attributed to the naïve state of CB T cells and more potent suppressor function of CB compared to adult regulatory T cells. A further important hurdle against the use of CB in adults is the limited volume and thus CD34+ cell dose of average CB preparations. Strategies to overcome this limitation are the concept of double-unit CB transplantation [20], or ex vivo expansion [21] which still needs further refinement. A practical issue is that CB is so far less cost effective compared to BM and PB, mainly because a large number of > 500,000 CB has been banked worldwide, but only about 3,800 CB have been transplanted up to 2009 [22]; still public funding for CB banks is needed.

Anyway, one would expect that the different origin of BM, PB and CB and variations in cell composition and functionality should somehow translate into differential clinical effects and also safety profiles. It is understood that it is very difficult to elucidate such differences due to the highly individualized nature of the HPC preparations, diversity of disorders to be treated, influence of HPC processing steps, diversity of treatment modalities including co-medications and further confounding factors which even encompass the experience of the centers procuring and using the grafts.

It can even be expected that with further progress of experimental cognition and technology, more refined processing and thus further variations in the cellular composition of HPC will be introduced, which in turn will warrant advanced quality control methods. An example is the increasing use of new “mobilizing” agents such as plerixafor in addition to G-CSF, which leads to a higher content of more primitive progenitor cells in PB grafts [23]. Recent results suggest that genomic and proteomic analysis may become important, since variations in cell composition and functionality should somehow translate into differential clinical effects and also safety profiles.

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

<table>
<thead>
<tr>
<th>EudraCT Number</th>
<th>Preparation</th>
<th>Short title, Indication</th>
</tr>
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<tbody>
<tr>
<td>2005-000774-46</td>
<td>BM, autologous</td>
<td>BOOST-2, myocardial regeneration</td>
</tr>
<tr>
<td>2005-009686-33</td>
<td>BM, autologous</td>
<td>PROVASA, chronic limb ischemia [27]</td>
</tr>
<tr>
<td>2005-009699-19</td>
<td>BM, autologous</td>
<td>RENERVATE, diabetic neuropathy</td>
</tr>
<tr>
<td>2005-003629-19</td>
<td>BM, autologous</td>
<td>Myocardial regeneration</td>
</tr>
<tr>
<td>2006-005709-50</td>
<td>BM, autologous</td>
<td>CELLWAVE, Combined Extra corporal Shock Wave Therapy and Intracoronary Cell Therapy in Chronic Ischemic Myocardium</td>
</tr>
<tr>
<td>2005-004051-35</td>
<td>BM, autologous, CD133+</td>
<td>INSTEM, myocardial regeneration, combined with Trans Myocardial Laser Revascularisation (TMLR)</td>
</tr>
<tr>
<td>2006-00393-76</td>
<td>PB, allogeneic, CD3/CD19 depleted</td>
<td>Hematological disorders in pediatric patients</td>
</tr>
<tr>
<td>2005-004028-38</td>
<td>BM, autologous, CD133+</td>
<td>Chronic cardiac ischemia</td>
</tr>
<tr>
<td>2007-006404-11</td>
<td>PB, autologous, CD133+</td>
<td>PERFECT, Intramyocardial transplantation for Improvement of Post-Infarct Myocardial Regeneration in Addition to Surgery</td>
</tr>
<tr>
<td>2007-004874-14</td>
<td>BM, autologous</td>
<td>REPAIR, Reinfusion of Enriched Progenitor cells And Infarct Remodeling in non-ST elevation AMI</td>
</tr>
<tr>
<td>2008-007684-23</td>
<td>CB, autologous</td>
<td>Umbilical cord blood to reverse DM type I in children</td>
</tr>
<tr>
<td>2006-001269-40</td>
<td>PB, autologous, CD34+</td>
<td>Severe lupus erythematoses</td>
</tr>
<tr>
<td>2007-006016-33</td>
<td>PB, autologous, CD3/CD19 deletion</td>
<td>Reduced conditioning in adult leukemia patients</td>
</tr>
<tr>
<td>2008-004625-42</td>
<td>BM, autologous</td>
<td>AMI; intramyocardial application</td>
</tr>
<tr>
<td>2007-003514-34</td>
<td>PB, autologous</td>
<td>Comparing chemotherapy with stem cell transplantation in elderly with AML</td>
</tr>
<tr>
<td>2008-003658-13</td>
<td>PB, autologous</td>
<td>EWING 2008; Ewing sarcoma, chemotherapy versus chemotherapy + HPC</td>
</tr>
<tr>
<td>2009-008368-28</td>
<td>BM, autologous</td>
<td>Ischemic cardiomyopathy, intramyocardial application</td>
</tr>
<tr>
<td>2007-004517-34</td>
<td>PB, autologous, CD3/CD19 depletion</td>
<td>Relapsed or refractory AML (including children)</td>
</tr>
<tr>
<td>2010-013856-61</td>
<td>PB, autologous</td>
<td>multiple myeloma; combined with lenalidomide/dexamethason</td>
</tr>
<tr>
<td>2010-019377-15</td>
<td>BM or PB, allogeneic</td>
<td>AML; allogeneic HPC versus chemotherapy</td>
</tr>
<tr>
<td>2008-001699-27</td>
<td>PB, autologous</td>
<td>Poor-risk CLL</td>
</tr>
<tr>
<td>2010-018487-42</td>
<td>PB, allogeneic</td>
<td>MDS; 5-Azacytidine versus 5-Azacytidine + allogeneic HPC</td>
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</tbody>
</table>

AMI: Acute Myocardial Infarction; AML: Acute Myeloid Leukemia; CLL: Chronic Lymphatic Leukemia; DM: Diabetes mellitus; MDS: Myelodysplastic Syndrome.
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.

References

21. Yang H, Robinson SN, Lu J, Decker WK, Xing D, et al. (2010) CD3(+) and/or CD14(+) depletion from cord blood mononuclear cells prior to ex vivo expansion culture improves total nucleated cell and CD34(+) cell yields. Bone Marrow Transplant 45: 1000-1007.