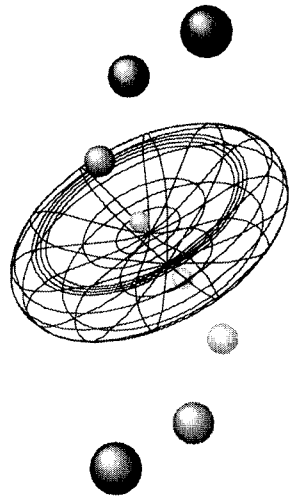
REVIEW ARTICLE



SERIES
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The Importance of Hemopoietic Stem Cells in the Treatment of Hemo-oncological Diseases

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Key Words

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INTRODUCTION

By transplanting hemopoietic progenitor and stem cells, it is possible to improve significantly the treatment results in a number of severe diseases of the hemopoietic and immune systems, as well as in malignant diseases of the blood and in tumors. The effectiveness of this method is based on the fact that it is now possible to escalate cytostatic and radiotherapeutic strategies, whose application had *previously* been limited by their lethality resulting from bone marrow toxicity. The results in recent years have shown that these procedures, in conjunction with biological substances (growth factors, interleukins), are breaking new ground in oncology therapy[1]. Sources of these hemopoietic cells may be bone marrow or circulating blood from patients themselves, their relatives, unrelated donors or from umbilical cord blood. High-dose chemotherapy/radiotherapy followed by transplantation of autologous or allogeneic peripheral blood stem cells has attracted widespread interest during the past 5 years due to the surprisingly low incidence of adverse effects. Impressive results have been reported in the scientific literature[2], particularly when stem cell mobilizing therapy is given prior to harvesting[3].

Totipotential cells, capable of either self-renewal or differentiation, head the hierarchical structure of hemopoiesis and are responsible for the production of blood cells throughout life. From these cells, lineage-specific progenitors develop which, in turn, continuously replenish the cell pool of the circulating blood. Hemopoietic stem cells also develop, by way of intermediary monocytic stages, into dendritic cells, Langerhans and *Kupffer cells*, osteoclasts, alveolar and tissue macrophages, synovial lining cells (Type A) and microglia[4].

So far it has not been possible to identify human pluripotential stem cells. Clinical observations indicate that they are present among the more highly differentiated progenitor cells[5]. Hemopoietic progenitor cells can be identified by CD34 antigen expression on the surface of mono-nuclear cells. Under steady state conditions, this antigen can be detected in approximately 1-3% of the mononuclear

cell (MNC) population in the bone marrow, in approximately 0.1-0.2% of the MNC in the circulating blood, and in 0.8-1.2% of the MNC in cord blood[6]. Using flow cytometry and multiple markers, it is also possible to detect very early progenitor/stem cells of phenotypes $CD34^+$, $CD38^-$, DR^+ and $Thy1^+$ [5-7].
Mobilization by chemotherapy and/or hemopoietic growth factors, now common adjuncts, results in increased release of lineage-specific progenitor cells and a variable percentage of pluripotential stem cells from the bone marrow into the circulating blood. These are harvested from the circulating blood by cytopheresis, cryo-preserved and stored in liquid nitrogen until used[8]. Cell harvesting is initiated when daily flow cytometric monitoring shows a minimum concentration of 0.4 X $10^6/\mu L$ CD34-positive cells in the blood. The losses during freezing using programmable freezers are virtually negligible, however, cell damage during thawing continues to be a problem.

INDICATIONS FOR TRANSPLANTING PERIPHERAL BLOOD STEM CELLS

The use of peripheral blood stem cells (PBSCs) for transplantation following chemotherapy/radiotherapy in well-defined malignant diseases of the blood and malignant tumors allows an increase in cytostatic therapy dosage up to 10 times *or more* that previously given. This improves the chance of eliminating residual tumor cells and can overcome multiple drug resistance.

Allogeneic peripheral blood stem cell transplantation (PBSCT) following chemotherapy/radiotherapy is currently under extensive study. In addition to the advantage that PBSC offer with regard to rapid repopulation of the bone marrow and accelerated hemopoiesis, graft-versus-tumor effect may significantly improve the chance of disease-free survival. This is balanced by the probability of severe acute or chronic graft-versus-host (GvH) reactions if a high T cell count in the transplant remains uncorrected or of graft failure if the transplant is depleted of T cells.

At this time, allogeneic (both related and unrelated) PBSCT is used primarily in acute and chronic leukemias.

Extensive data is available pointing to acceptable results of high-dose regimes using autologous stem cell rescue in certain hematological diseases and a few solid tumors, and this method has been included among current therapeutic strategies. Extensive research is now being conducted into the suitability of this method especially in the context of a number of solid tumors.

CURRENT INDICATIONS

Hodgkin's disease

First line: if the progress of the disease gives rise to an unfavorable prognosis
- unsatisfactory response to first-line therapy (only partial remission or non-response),
- large mediastinal tumor mass.

Second line
- early recurrences sensitive to therapy (within the first year following the conclusion of the first-line therapy),
- accumulated late recurrences.

Non-Hodgkin lymphoma (low-grade, intermediate, and high-grade)

First line:if the progress of the disease gives rise to an unfavorable prognosis

- lymphoblastic NHL,
- failure of initial therapy (only partial remission or non-response),
- "bulky disease" (particularly with a large mediastinal tumor mass).

Second line
- all recurrences sensitive to chemotherapy.

Multiple myeloma

- Durie-Salmon tumor stages II and III responding to conventional chemotherapy.
- patients without related allogeneic donors and/or above 50 years of age.

Solid tumors

In principle, PBSCT is suitable for all high-risk tumors sensitive to chemotherapy. However, patients affected by the following tumor entities seem to benefit particularly from this method:

High-risk germ cell tumor
Patients with unsatisfactory response to primary therapy or with recurrences following cis-platinum chemotherapy (event-free survival (EFS) only approximately 30% after 2-4 years).

More recently PBSCT has been used as primary treatment in germ cell tumors with an unfavorable prognosis (large tumor cell mass, rapid progression; advanced disease).

High-risk carcinoma of the breast
Pre-menopausal carcinoma of the breast with a high risk of recurrence (more than 9 affected lymph nodes) in adjuvant situations. Final results are still being awaited. First results seem to indicate advantages over standard therapy in well-defined patient groups with stages II and III disease.

Pre-menopausal metastasizing carcinoma of the breast with complete or partial remission following standard chemotherapy. As early as 1992, progression-free 5-year survival rates of 15-30% could be shown in Phase II studies where autologous bone marrow had been tran-splanted[9].

StageIV neuroblastoma (with an EFS of 20-25%)

Recurrent or primary multifocal Ewing's sarcoma (with an EFS of more than 50% compared to conventionally treated control groups)

Small-cell bronchial carcinoma (limited disease) in patients up to 60 years of age
A significant chance of survival exists if a double transplantation is performed.

Carcinoma of the ovaries (as an adjuvant or neo-adjuvant method, promising initial study results)

Systemic hemoblastoses

Acute lymphoblastic and myeloid leukemia
Patients without related or unrelated allogeneic donor (escalating consolidation).
Chronic myeloid leukemia (CML)
Patients without related or unrelated allogeneic donor

Myelodysplastic syndrome (as in CML)

DESCRIPTION OF METHOD

Stem cell mobilization and separation

Stimulation of hemopoiesis, a side effect of conventional chemotherapy, is utilized for effective harvesting of stem cells. The number of circulating blood stem cells, which is normally very low, is significantly increased during cell growth following chemotherapy-related aplasia. The cell harvest can be increased up to 100-fold by administration of a growth factor (such as G-CSF, GM-CSF, Interleukin-3, stem cell factor, or a combination of factors)[10, 11]. Using a cell separator, the required amount of stem cells is harvested in one to three consecutive sessions, frozen and stored in liquid nitrogen at -196°C until used.

A number of cytostatic drugs, particularly melphalan, have a destructive effect on the pluripotential stem cell. For this reason, some authors do not recommend their use for stem cell mobilization/tumor therapy despite their excellent tumoricidal effect[12]. This is contrary to our own experience, even after, sometimes very aggressive, pretreatment. To increase the yield of the stem cell harvest (mobilization), growth factors (G-CSF, GM-CSF) are to be used at dosages between 5 and 10μg/kg body weight. Growth stimulation using these factors is started 24 hours after completion of the stem-cell-mobilizing chemotherapy and ends on the last day of cell separation. At the same time, the proliferation of the mobilized cells to obtain more rapid hemopoietic reconstitution or to avoid adverse effects caused by cellular aplasia is promoted (priming). Shortly after stem cell infusion, primed precursors appear to proliferate and to differentiate into mature cells, so that the serious side effects observed when bone marrow stem cells are used for grafting do not materialize at all or only in a very mild form[10, 11].

Tumor cell contamination and graft purging

Studies with gene-marked tumor cells have shown that recurrences can be caused by re-implanted tumor cells[13]. Whenever a malignant growth is treated by high-dose therapy, contamination of the graft by tumor cells must be expected. But observations, including our own, indicate that recurrences probably occur only when a relatively high, although as yet undefined, number of tumor cells is re-implanted. Most of the time, recurrences can be traced back to ineffective treatment of the basic disease.

While the chemotherapeutic regime itself greatly reduces the number of circulating tumor cells *in vivo*, several additional methods may be used for cleaning (purging) the graft *in vitro*. The most popular of these is positive selection of CD34-positive stem cells, excluding the malignant cell population. On the other hand, with so-called negative selection the malignant cells can be removed. These two methods can be used in combination. Some centers perform *ex vivo* expansion of hemopoietic cells for eliminating malignant cells while at the same time increasing the percentage of transplantable progenitors[14].

Autologous PBSCT: advantages, prerequisites, performance

Some important advantages of using PBSC for transplantation following high-dose tumor therapy over untreated bone marrow stem cells are shown in ***Table 1***.

Potential candidates for high-dose therapy should not be older than 60 years nor suffer from any concomitant disorder adversely affecting the prognosis.

A further requirement for high-dose therapy with stem cell rescue is that the tumor responds to therapy and that the tumor cell mass is reduced to the minimum possible.

Table 1 Advantages of transplanting blood stem cells

- Rapid restoration of hemopoiesis following total bone marrow aplasia-inducing therapy.
- Reduced amount of supportive therapy required (antibiotics, antimycotics, blood products).
- Low transplant-related lethality (<5%).
- Short hospitalization phases, sometimes outpatient or combined inpatient/outpatient treatment possible.

The exact number of stem cells required for complete restoration of hemopoiesis in humans is not known. A minimum of 1 X 10^6 CD34-positive cells/kg body weight or 1 X 10^4 CFU-GM/kg body weight of the recipient should be present within the transplant.
The stem cells obtained previously are transfused via a central venous catheter one to two days after administration of the cytostatic chemotherapy/radiotherapy. During the early post-transplantation phase, bone marrow aplasia persists for 7 to 14 days. During this period the patient requires extensive clinical and laboratory monitoring. Normally the patient can be discharged and followed up as an outpatient at day 14 following the stem cell transfusion, provided the body temperature is normal and platelet support no longer required.

OUTLOOK

High-dose chemotherapy/radiotherapy with autologous PBSCT offers high-risk patients with certain systemic malignant diseases or solid tumors, a chance for longer survival, or, under certain conditions, even remission. The disease must not have reached the final stage, and the tumor must be sensitive to cytostatic drugs.

Intensive epidemiological, clinical, and molecular bio-logy research is underway to define prognostic factors which may extend the application of this treatment modality in malignant disease particularly in previously untreated patients. Further realization of the concept of *in vivo* expansion may permit the use of high-dose therapy in patients whose peripheral blood or bone marrow is severely contaminated with malignant cells or who have a low count of hemopoietic cells. Additionally hemopoietic progenitors have properties that make them ideally suited for gene manipulation yielding interesting new approaches to the treatment of a variety of diseases[15]. As far as PBSC therapy is concerned, fascinating new results may be anticipated particularly in tumor therapy.

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