

# ALTERNATIVE SOURCE OF STEM CELLS FOR ALLOGENEIC TRANSPLANTATION

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## Introduction

Bone marrow cells have been, in the past, the only source of stem cells for autologous or allogeneic transplantation. Recently, mobilized peripheral blood and cord blood hematopoietic stem cells have been increasingly used. In vitro and in vivo studies comparing the advantages and disadvantages of each method are necessary to assess the indications for alternative sources of hematopoietic stem cell transplantation.

## I. Allogeneic Bone Marrow

Until recently, the method of collection of bone marrow hematopoietic stem cells has been exclusively marrow aspiration from the posterior iliac crests under general anesthesia. This procedure has a very low incidence of complications, but the risks of general anesthesia - including death - cannot be minimized. Persistent back pain is observed in 30% of the cases. The risk of transmission of viral infections has been very limited with the use of autologous blood red cell transfusions.

Bone marrow aspiration can be performed without age limit; infants a few weeks old have been successfully used as donors for familial allogeneic bone marrow transplantation. Young donors have a clear advantage, with a better yield of stem cells and a decreased risk of graft-versus-host disease (GVHD).

Bone marrow aspirates are highly contaminated with peripheral blood, which explains why a volume of approximately 1 liter is necessary, in adults, to obtain a number of mononuclear cells of  $3 \times 10^8/\text{kg}$ . In 111 recent bone marrow grafts, the counts showed:  $2.3 \times 10^8/\text{kg}$  MNC (0.5-7),  $6.3 \times 10^4/\text{kg}$  (0.97-24.5) CFU-GM, and  $3.27 \times 10^6/\text{kg}$  (0.07-15.4) CD34+ cells.

Standardisation of methods of counting stem cells has been impaired by the absence of reproducibility of CD34+ cells counts and clonogenic tests. It is known that engraftment correlates with the number of mononuclear cells infused, but this number might vary in function of donor and recipient variables. The minimum number of stem cells necessary for long-term engraftment is not known.

In order to prevent GVHD, several methods of T-cell depletion have been used, including negative selection with monoclonal antibodies, positive selection of CD34+ cells with immunomagnetic beads or biotin avidin columns. This manipulation gives an enrichment of CD34+ cells with a 2 to 3 logs T-cell depletion. In allogeneic bone marrow transplantation, these methods of T-cell depletion are mostly used in family HLA-mismatched or in unrelated transplantation. It has been shown that T-cell depletion increases the risk of rejection and leukemic relapse. This complication can be overcome by increasing the dose of conditioning and the number of cells infused. Other protocols investigated a selective T-cell depletion or lymphocyte add-back after transplant. Clearly all these methods are experimental and need evaluation before routine use.

## **II. Mobilized Peripheral Blood Stem Cells**

Various cytokines have the property to mobilize CD34+ bone marrow cells. The most widely used cytokine is G-CSF,<sup>(1)</sup> but addition of other synergistic cytokines such as IL-3, KL or FLT3L might increase the efficiency of stem cell collection. The advantages of peripheral blood stem cell (PBSC) collection are the absence of general anesthesia and the ability to collect 10 times more stem cells. The increase of stem cells facilitates engraftment; this is crucial in mismatched transplants or in manipulated transplants. Short-term side-effects of G-CSF are limited to bone pain and malaise, but in some cases a central catheter must be used with possible complications. Thrombocytopenia can be observed. The main concern is the possibility of increasing the risk of leukemia or cancer after the use of growth factors in healthy donors. For this reason, several teams are reluctant to use mobilized PBSC in children and in unrelated donors except in cases of second donation after bone marrow rejection. In our center, a typical G-CSF mobilized allogeneic PBSC infusion (11 cases) gives  $8.12 \times 10^8/\text{kg}$  (3.25-49.8) MNC,  $7.14 \times 10^6/\text{kg}$  (3.15-39.7) CD34+ cells,  $47.5 \times 10^4/\text{kg}$  (14.2-390) CFU-GM, and  $2.73 \times 10^8/\text{kg}$  (0.74-17.8) CD3 T. The second concern is the risk of increasing acute and chronic GVHD because of the high number of peripheral blood lymphocytes infused. Surprisingly, preliminary results do not show increased incidence or severity of acute GVHD in HLA-identical bone marrow transplantation. This might be due to a modification of lymphocyte subsets toward an increased number of type 2 helper cells, which have a suppressive activity.<sup>(2)</sup> In contrast, chronic GVH seems to increase at more than 50% in HLA-identical PBSC transplants. These results show that PBSC allogeneic transplants are still experimental and must be assessed in large multicenter randomized studies comparing bone marrow to peripheral blood as it is currently performed in the European Blood and Marrow Transplant Group (EBMT).

## **III. Cord Blood**

With the increasing number of cord blood transplants, large repositories of cord blood for allogeneic, unrelated or family transplants have arisen, and there is a need for standardization of cord blood collection, processing and storage procedures.

### ***Clinical Results of Cord Blood Transplantation***

The first cord blood transplantation was performed in 1988 in a patient who was affected by Fanconi anemia.<sup>(3)</sup> According to previously observed findings, showing that a single cord blood collected and cryopreserved at birth contained enough hematopoietic stem cells for long-term engraftment, the patient was conditioned for transplant for Fanconi anemia according to ongoing protocols and received thawed cord blood cells from his HLA-identical sibling. Eight years later he is doing well, apparently cured from his original disease. Since this first case, the number of cord blood collections and transplants has increased very quickly.<sup>(4-8)</sup> The donors were mostly HLA-identical siblings, but some successes have been reported with siblings different for 1, 2 or 3 HLA antigens. In addition, cord blood banks have been developing worldwide, and more than

150 unrelated cord blood transplants have been performed with HLA-matched or partially matched donors.

It can be concluded from a preliminary analysis of the results that cord blood transplant is a good alternative to bone marrow transplant. Results in children have been very good in various malignant and nonmalignant diseases. The overall results are very difficult to analyze because of the heterogeneity of this group of patients and because of the short follow-up time. Nevertheless, some conclusions can be given: engraftment was observed in 80% of the cases. In 59 cord blood transplants collected by Eurocord, the mean number of nucleated cells was  $0.5 \times 10^8/\text{kg}$  (range 0.1-9.8) and of CD34+  $0.5 \times 10^6/\text{kg}$  (0.1-5.4), and the median number of CFU-GM was  $2 \times 10^4/\text{kg}$ .<sup>(1-21)</sup> Despite the relatively low number of cells, engraftment has been observed in a limited number of adult patients. Median time to recovery was 22 days for neutrophils and 48 days for platelets. Absence of engraftment can be explained either by the poor status of the patient at time of transplant or by an insufficient number of stem cells infused. The addition of in vivo growth factors did not modify the speed of engraftment. GVHD was limited in patients receiving an HLA-identical sibling cord blood transplant. This decreased rate and severity of GVHD could be due to the young age of donor and recipient, to the absence of previous immunization or activation of donor cells by infectious agents, to the low number of lymphocytes present in one cord blood, to the immunosuppressive effect of contaminating mother's cells, or to the immaturity of immunological functions at birth. It is known that cord blood is enriched with immature naive lymphoid cells, some of which might have a suppressive phenotype. The diminution of incidence and severity of acute GVHD in unrelated or in family mismatched transplant has also been observed, but more comparative studies, more follow-up and more patients are needed for a definite statement.

### ***Cord Blood Banking Collection and Freezing***

Following these preliminary encouraging data, several groups have been working to establish criteria for collection, evaluation of the number of stem cells, volume reduction, purification, selection of CD34+ cells, screening for infectious and genetic diseases, and freezing and thawing of cord blood for large scale banking.<sup>(10-11)</sup>

Cord blood banking for autologous or allogeneic transplants has several advantages: easy access, indefinite storage, speed of donor search, viral safety, and source of stem cells for expansion and gene therapy. For this reason several banks have been established in several countries; the most developed are in New York, Milan, Düsseldorf and Paris. Regulations and good medical and laboratory practice have been discussed as well as ethical aspects.<sup>(12)</sup> More than 15,000 samples have been frozen and are ready for use. The number of cord blood samples necessary for providing cells for a maximum number of patients will depend on the results of transplants performed with HLA-matched or partially mismatched unrelated donors. These results will be available only in several years. In order to have quicker answers, common prospective protocols must be applied to this type of transplant. For this reason, NIH and Eurocord transplant programs have established a network for connecting banks, standardizing procedures for the exchange of cord blood, collecting clinical results and performing prospective studies on cord blood transplants.

### ***Properties of Cord Blood Hematopoietic Stem Cells***

It is well known that the current methods of hematopoietic stem cell (HSC) quantification by measuring the number of CD34+ cells or of CFU-GM are not standardized and cannot be compared from one laboratory to another. Several authors showed that the number of LTC-CFC and CFU-GM per mononuclear cell from cord blood were 2.5-fold and twofold greater than those from peripheral blood cells, respectively.<sup>(13-15)</sup> Using a two-stage long-term culture system and limiting dilution techniques, scoring cobble stone areas of greater than 15 hematopoietic cells weekly for up to 15 weeks, Pettengell et al have shown that the incidence of putative stem cells in leukapheresis product and umbilical cord blood is at least comparable with that of bone marrow.<sup>(16)</sup>

Among several advantages, various authors have shown that cord blood was enriched with CD34+ cells and mostly with the more immature compartment rh123lo, CD38-.<sup>(17-18)</sup> Colonies obtained from these selected populations are larger, have a better replating capacity and grow longer in long-term culture.<sup>(19)</sup> Contrary to what has been observed in adult bone marrow, LTCBC-IC and presumably CB cells, capable of in vivo engraftment, reside in the CD34+, HLA-DR+ Rh 123dull fraction of CB. Thy-1 CD34+ CD45RAlo CD71lo expression on primitive cord blood progenitors have the highest in vitro proliferative potential, which suggests that Thy-1 is involved in early stem cell development.<sup>(20)</sup>

In contrast to adult bone marrow, purified progenitors obtained from umbilical cord blood undergo clonogenic maturation even in the absence of added growth factors.<sup>(21)</sup> Schibler et al conclude that production of growth factors occurs within culture dishes containing hematopoietic progenitors of umbilical cord origin; this autocrine or paracrine production of growth factors might explain some of their apparently unique features of in vitro growth.<sup>(22)</sup>

Unseparated or purified hematopoietic stem cells from cord blood were transplanted to severe combined immunodeficient mice.<sup>(23)</sup> High levels of multilineage engraftment, including myeloid and lymphoid lineages, were obtained with 80% of the donor samples as assessed by DNA analysis, fluorescence-activated cell sorting, and morphology. In contrast to previous and concurrent studies with adult bone marrow, treatment with human cytokines was not required to establish high-level human cell engraftment. There is growing evidence that the difference between adult and fetal hematopoiesis is not just a quantitative difference but that there are ontogeny-related differences related to differences of cell signaling and growth receptors requirements. Other evidence has been provided by the study of telomeric length, which decreases with age.<sup>(24)</sup>

Several studies show that the efficiency of gene transfer in cord blood is higher than in bone marrow cells, even in the absence of added hematopoietic growth factors. A first attempt has been performed by Kohn et al, who introduced the ADA gene into cord blood cells of three children with ADA deficiency whose cord blood was collected at birth, transfected during a short culture and reinfused to the infants. After two years, it has been shown that the gene was expressed in about 1% of the bone marrow population and

that the expression increases with age, suggesting that transfected cells might have a selective growth advantage.<sup>(25)</sup>

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