

ORIGINAL ARTICLE

The changing role of stem cell transplantation in childhood

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Over the past decade, relevant improvements and refinements have significantly changed the indications, technique and results obtained with allogeneic hematopoietic SCT (HSCT) in childhood. A fundamental turning point in the history of allogeneic HSCT is represented by the use of placental blood, which was first employed in 1988 in a patient with Fanconi anemia, successfully transplanted with cord blood cells from an HLA-identical sibling. Since then, thousands of children were given an allograft of cord blood-derived hematopoietic progenitors, mainly from an unrelated donor. This large clinical experience has documented that, as compared with BMT, cord blood transplantation (CBT) is associated with reduced incidence and severity of GvHD. The outcome of recipients given unrelated CBT has been reported to be at least as good as that of patients transplanted with either BM or peripheral blood mobilized cells of an unrelated volunteer. Another emerging strategy of HSCT is that of using HLA-partially matched relatives as donors of hematopoietic progenitors. The infusion of a huge number of positively *in vitro*-selected CD34+ cells, with the concomitant removal of T cells, has been demonstrated to permit sustained engraftment of donor hematopoiesis, without the occurrence of GvHD in the majority of patients transplanted from an HLA-disparate relative. In adults given this type of transplantation, the most favorable results have been reported for AMLs and when the donor displays alloreactivity of natural killer cells. It remains to be definitively proved whether these findings documented in adults maintain their value in pediatric patients transplanted from an HLA-disparate family donor. Finally, the last few years have witnessed the emergence of approaches of adoptive cell therapy aimed at optimizing the results of allograft through strategies able to reinforce immune competence against pathogens, as well as against tumor cells, or at modulating donor T-cell alloreactivity.

Bone Marrow Transplantation (2008) **41**, S3–S7;
doi:10.1038/bmt.2008.45

Keywords: HSCT; children; CBT; HLA-partially matched relatives; HSCs

Introduction

Allogeneic hematopoietic SCT (HSCT) is a treatment largely employed for children affected by a number of hereditary and/or hematological conditions, of both malignant and non-malignant origin, which are reported in Table 1.¹ Through this procedure, thousands of subjects have been cured from their original disease.

Nearly 40 years have elapsed since the first successful BMT;^{2,3} since then, significant changes and improvements have been reported, with particular regard to the source of stem cells employed, to the optimization of techniques of HLA-typing, the use of HLA-disparate family donors and the development of strategies of adoptive cell therapy.

The revolution of cord blood transplantation

As mentioned before, BMT was first performed in 1968 on two children affected by inherited diseases, namely severe combined immune deficiency and Wiskott–Aldrich syndrome,^{2,3} and, for the past two decades, it has been practically the only possible source of hematopoietic stem cells (HSC) employed for transplanting in patients in need of an allograft.

In 1988, the first transplant in a child affected by Fanconi anemia using cord blood cells of a sibling, collected at the time of delivery was performed, which was successfully introduced by Gluckman *et al.*⁴ The safety of the newborn during the collection of placental blood,⁵ as well as the demonstration of efficacy of cord blood transplantation in several disorders,^{6–9} have provided the clinical basis for starting large programs of collection, characterization, cryopreservation and storage of HSC from the umbilical cord blood (UCB) to be employed for transplantation even of a non-consanguineous patient. UCB banks are now well established in most developed countries, with advantageous integration with registries of volunteer BM donors. The current situation can be summarized as follows: although accurate and complete records are not available, it can be estimated that, to date, at least 300 000 U of cord blood from a non-consanguineous donor have been successfully collected, characterized, and cryopreserved, and they have facilitated the realization of at least 3000 transplantation procedures.

Umbilical cord blood offers the advantages of easy procurement, the absence of risks to donors, the reduced

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Table 1 Main indications to allogeneic hematopoietic SCT in childhood

<i>ALL</i>	SCID
In I CR ^a	
In II CR	Immunodeficiency with hyper IgM
In III or further CR	
AML in I or further CR	Leukocyte adhesion deficiency
CML	Omenn syndrome
Myelodysplastic syndromes	Wiskott–Aldrich syndrome
Hodgkin and non-Hodgkin lymphoma	Chediak–Higashi syndrome
Selected types of solid tumors ^b	X-linked lymphoproliferative disease
Severe aplastic anemia	Kostmann syndrome
Fanconi anemia	Chronic granulomatosis disease and other severe neutrophil function disorders (that is, Schwachmann syndrome)
Dyskeratosis congenital	Life-threatening, hereditary platelet disorders (that is, Glanzmann thromboasthenia, Bernard–Soulier syndrome)
Diamond–Blackfan anemia	Hemophagocytic lymphohistiocytosis
Thalassemia major	Selected types of mucopolysaccharidoses
Sickle cell disease	Selected types of peroxisomal and lysosomal disorders
Infantile malignant osteopetrosis	Selected types of life-threatening autoimmune disorders resistant to conventional treatments

^aPatients at high risk of recurrence (that is, t(9;22) or t(4;11); T-ALL with poor prednisone response, high levels of minimal residual disease).

^bStage IV neuroblastoma, renal cell carcinoma, very high risk Ewing sarcoma.

Table 2 Advantages associated with transplantation of umbilical cord blood cells in comparison with BMT

<i>For the recipient</i>
Prompt availability (with reduced waste of time between identification of an unrelated donor and SCT)
No risk of donor refusal
Reduced risk of both acute and chronic GvHD
Possibility of performing transplant using 1 or 2 antigen HLA-disparate donor
Low risk of viral contamination (that is, human cytomegalovirus, Epstein–Barr virus), with consequently low risk of transmission of infectious disease
<i>For the donor</i>
Ease and safety of collection, without the risks associated with general anesthesia (requested for marrow harvesting)
Lower incidence of psychological problems related to the figure of the child-donor and to possible transplant failure

risk of transmitting infections and, for transplants from unrelated donors, immediate availability of cryopreserved cells, the median time elapsing from the start of the search to transplantation being 3–4 weeks. Most importantly, mismatches up to two of the six antigens do not preclude the transplant feasibility, as T cells in cord blood are naive and less able, as compared with the counterpart in BM or peripheral blood, to cause GvHD.¹⁰ These, as well as other, advantages associated with the use of cord blood transplantation are detailed in Table 2.

Compared with children given matched unrelated BMT, UCB transplant recipients experience lower probability of sustained donor engraftment, delayed hematopoietic recovery, lower incidence of both acute and chronic GvHD and higher TRM in the early post-transplant period due to infectious complications (Table 3).^{6–10} The increased risk of fatal infectious complications in the early post-transplant period is mainly due to both the slow recovery of neutrophils and the lack of transfer of antigen-experienced (that is, memory) T cells, which significantly contribute to the early immunological reconstitution of children given either an unmanipulated allogeneic BM or PBSC

Table 3 Possible disadvantages associated with transplantation of umbilical cord blood cells in comparison with BMT

<i>For the recipient</i>
Increased risk of graft failure
Delayed platelet and neutrophil recovery
Absence of adoptive transfer of specific immunity toward infection agents due to fetal immune immaturity and lack of previous antigenic exposure
Theoretical increased risk of transmission of inherited disorders
<i>For the donor</i>
Ethical problems associated with donation (that is, increased propensity to conceive a child to save a child)

transplantation. An inverse correlation between the number of cells infused and the cumulative incidence of TRM has been repeatedly documented,^{9–11} with children given a lower number of cells per kilogram of recipient body weight being those at the highest risk of experiencing fatal complications.

Despite the low incidence of both acute and chronic GvHD, the risk of leukemia recurrence is not increased after transplantation of UCB. In the long term, the overall probability of survival of patients given cord blood transplantation from an unrelated donor has been reported to be at least as good as that of patients transplanted with either BM or peripheral blood mobilized cells of an unrelated volunteer. Particularly, promising results have been reported in children with AML transplanted with cord blood cells from an unrelated donor and in children with hemoglobinopathies given a related UCB transplantation.^{6,12}

Results of unrelated donor BMT have improved over time

Unfortunately, only 25% of patients who need an allograft of HSC have an HLA-identical sibling. Registries of volunteer BM donors are now well established in most

developed countries; they are advantageously integrated with UCB banks and it can now be estimated that a significant proportion of patients in need of HSCT and lacking an HLA-identical sibling have the possibility to locate a non-consanguineous donor suitable to be employed for transplantation.¹³ Mainly because of HLA polymorphism and the limits of conventional techniques for HLA-typing, increased difficulties for engraftment and augmented incidence of both acute and chronic GvHD have been initially reported in recipients of an unrelated donor HSCT, this leading to a final outcome of patients inferior to that reported using a compatible sibling as a donor.^{14,15} At that time, recourse to this type of transplant for patients in whom HSCT does not represent a life-saving procedure (that is, thalassemia) did not meet consensus. More recently, however, better selection of unrelated donors, with high-resolution molecular typing for both HLA class I and II antigens, as well as refinements in both prophylaxis and treatment of GvHD, has allowed to obtain a reduction of TRM and a relevant improvement over time in the outcome of patients transplanted from an unrelated volunteer;¹⁶ so when an HLA-fully matched or a single allelic-disparate donor is selected, results achievable with this type of transplant may be expected to be comparable with those of HSCT from a compatible relative.¹⁶ Support for this conclusion is provided by the recent results obtained using an unrelated donor in children with ALL in second CR, with juvenile myelomonocytic leukemia or with thalassemia.^{17–20}

The greatest challenge: transplantation of HSC from an HLA-haploidentical relative

Haploidentical transplantation represents an immediate alternative to almost all leukemia patients who fail to find a matched donor, whether related or unrelated, or a suitable cord blood unit. The infusion of a large number of CD34+ cells, with the concomitant removal of T cells, has been demonstrated to permit the sustained engraftment of donor hematopoiesis, without the occurrence of GvHD in the majority of patients transplanted from an HLA-disparate relative.^{21–23} Data from trials of HSCT from HLA-disparate family donors in adults demonstrate that an alloreactive natural killer (NK) cell response in the GvHD direction eradicates myeloid leukemia, improves engraftment and protects from T-cell-mediated GvHD.^{21–23} Donor-versus-recipient NK cell alloreactivity is a biological phenomenon unique to the mismatched transplant, which derives from a mismatch between donor NK clones (carrying specific inhibitory receptors for self-MHC class I molecules) and MHC class I ligands on recipient cells.²⁴ As patients given a T-cell-depleted HSCT from an HLA-disparate donor cannot benefit from the T-cell-mediated GVL effect, selection, whenever possible, of a donor with NK-alloreactivity is recommended to explore the GVL effect displayed by donor NK cells.^{21–24}

Life-threatening, either viral or fungal, infections occur with increased frequency in patients given a T-cell-depleted HSCT, as, in this case, the recipient cannot benefit from the contribution of adoptively transferred memory T-cells.^{21,22}

The use of cellular immunotherapy to prevent and/or treat infections is particularly attractive, as the primary defect contributing to the pathogenesis of infection-related complications appears to be the inability to mount an adequate pathogen-specific T-cell response.²⁵ Indeed, patients given a T-cell-depleted allograft have offered a unique opportunity to develop strategies of adoptive immune cell therapy, which are expected to play a major role in the future transplant approaches for optimizing the final outcome of subjects given an allograft of HSC.^{26–29}

A new actor in the scenario of transplantation: MSCs

MSCs are multipotent BM cells able to differentiate *in vitro* and *in vivo* into different tissues of mesenchymal origin.³⁰ Recent studies indicate that MSCs have immunosuppressive properties. MSCs may display their effect on all the cells involved in an immune response, including T and B lymphocytes, DC as well as NK cells.^{31–34} MSCs can suppress T-cell proliferation induced by allogeneic PBMCs and by mitogens, such as phytohemagglutinin, concanavalin A and anti-CD3/anti-CD28 antibodies, in a dose-dependent manner.^{31–34} At a high ratio with respect to the effector cells, MSCs also strongly inhibit *in vitro* activation of alloantigen-specific cytotoxic lymphocytes.^{31–34} Inhibition of T-cell proliferation and cytotoxicity did not require MHC compatibility between MSCs and responder lymphocytes, this supporting the concept that MSCs can be considered universal suppressors. The interaction between MSCs and human lymphocytes has been shown to favor the differentiation of CD4+CD25+ T lymphocyte subsets displaying a regulatory phenotype.³³ Interestingly, the calcineurin inhibitors, CYA and tacrolimus, are currently employed to prevent or treat GvHD and to enhance the immune suppressive effect of human MSCs.³⁵ Moreover, human MSCs display an inhibitory effect on alloantigen-induced DC differentiation and on APC maturation.^{31,33} Using a baboon skin graft model, it has been shown that infusion of *ex vivo* expanded donor or third-party MSCs prolonged the time to rejection of histoincompatible skin grafts.³⁶

The multiple immune suppressive properties of MSCs provide the biological explanation of the efficacy of MSCs in the treatment of patients with acute GvHD, even refractory to conventional treatment. After the seminal report by Le Blanc *et al.*³⁷ on a patient rescued by liver and gut acute GvHD resistant to multiple lines of immune suppressive therapy, other reports involving a fair number of patients have provided support to the concept that MSCs may represent a valuable option for allogeneic HSC transplantation recipients suffering from acute GvHD.^{38,39} Although the real efficacy of MSC infusion in the management of patients with GvHD remains to be proved in randomized trials comparing this treatment with more conventional approaches, there is no doubt that, in view of these exciting preliminary results, MSCs could represent one of the most innovative strategies for solving the problem of alloreactivity, which is still one of the most life-threatening complications of HSCT and which precludes a wider application of allograft.

Acknowledgements

This work was partially supported by grants from AIRC (Associazione Italiana Ricerca sul Cancro), CNR (Consiglio Nazionale delle Ricerche), MURST (Ministero dell'Università e della Ricerca Scientifica e Tecnologica), European Union (FP6 program ALLOSTEM) and Fondazione IRCCS Policlinico San Matteo to FL.

Conflict of interest

None of the authors declared any financial interests.

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