Innovative approaches to hematopoietic stem cell transplantation for patients with thalassemia

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Since the first successful allogeneic transplant of hematopoietic stem cells, performed more than 20 years ago, hundreds of patients with thalassemia have been cured of their original disorder after receiving an allograft, in most cases from an HLA-identical family donor.[1-3] This extensive experience has allowed the identification of clinical parameters influencing post-transplant outcome, thus permitting more refined prognostic counseling for patients considering the option of hematopoietic stem cell transplantation (HSCT) or for their parents.[2-5] The risk of dying of transplant-related complications has been shown to be mainly dependent on patient age, iron overload and viral liver infections. In fact, adults, especially when affected by chronic active hepatitis, have a worse outcome than children;[5] among children, three classes of risk have been identified on the basis of regularity of previous iron chelation, liver enlargement and presence of portal fibrosis.[2,3] In pediatric patients who do not have liver disease and have received regular iron chelation (i.e. class 1 patients), the probability of survival with transfusion independence is over 90%, whereas for patients whose compliance to iron chelation is low and who show signs of severe liver damage (i.e. class 3 patients) the same probability is in the order of 60%. However, the most recent survey, reporting on a limited number of class 3 patients younger than 17 years, has suggested that the adoption of a hypertransfusion regimen during 2 months preceding HSCT, with the intent to reduce the expansion pressure on the erythron, together with the use of azathioprine and hydroxyurea to suppress hematopoiesis, and fludarabine to reduce the risk of rejection, may decrease the probability of treatment failure and improve post-transplant outcome also in this subgroup of patients.[6] Despite the fact that HSCT still remains the only curative treatment for patients with hemoglobinopathies, transplantation is associated with a non-negligible risk of both transplant-related mortality and morbidity. This risk must be taken into account also considering the relevant improvements achieved with conventional treatment, namely safe blood transfusions and effective iron chelation therapy. Conventional treatment has, in fact, dramatically improved both survival and quality of life of patients with thalassemia over the last two decades, and has changed a previously fatal disease with early death, to a chronic, slowly progressive disease compatible with prolonged survival[7-9] Patients born in the last 2 decades have a life expectancy, which, at least until 30 years of age, is comparable to that of age-matched healthy individuals, provided that good compliance with medical treatment is guaranteed. By contrast, whenever compliance with chelation therapy is poor, fewer than 10% of patients with thalassemia are expected to reach their 40th birthday.[9]

These latter considerations, indicating that HSCT is no longer a life-saving procedure but has become a strategy for rendering patients free of the need of transfusions and chelation, immediately raise the question of how can we make transplantation safer and more effective. Another important question, given the fact that, especially in Western countries, less than 25% of patients have a suitable compatible family donor, is how can we increase the number of patients treatable with HSCT. In the last few years, two innovative approaches have been tried to provide solutions to these problems, namely, cord blood transplantation (CBT) to make the procedure safer, and the use of unrelated volunteers, selected using high-resolution molecular typing of HLA-loci and donating bone marrow cells, to increase the number of patients who could potentially benefit from HSCT. As compared to adult hematopoietic stem cells, umbilical cord blood hematopoietic stem cells have many theoretical advantages for HSCT: they are enriched with *in vivo* long-term repopulating stem cells,[10] they can better restore host hematopoietic progenitor cell reservoir after transplantation,[11] they are able to expand in *in vitro* long-term culture and they can engraft SCID-human mice in the absence of additional human growth factors.[12]

In comparison with bone marrow transplantation (BMT), the main clinical advantage associated with CBT is the lower risk of both grade II-IV acute and chronic graft-versus-host disease (GvHD).[9,13] The low incidence of GvHD in CBT recipients renders this type of transplant particularly appealing for patients with thalassemia, as well as for all patients with non-malignant disorders. A recent study from the Eurocord cooperative group analyzed the outcome of 33 patients, mainly children with thalassemia in class 1 and class 2 who were given CBT from a sibling, HLA-identical in 30 cases and with a single HLA-disparity in the 3 remaining donor/recipient pairs.[14] No patient died of transplant-related complications, suggesting that related CBT for hemoglobinopathies is a safe procedure.[14] Seven out of these 33 patients did not have sustained engraftment of donor cells, although 2 of them obtained sustained engraftment after subsequent allo-

geneic BMT from the same donor. These graft failures can be at least partially explained with the observation that CBT recipients receive one log less stem cells than do BMT recipients and this disadvantage could be not entirely compensated for by the more favorable biological profile of placental progenitors. Patients who did not receive methotrexate as part of GvHD prophylaxis and who were treated with thiotepa during the preparative regimen had a remarkably better probability of thalassemia-free survival (above 90%); this indicates that, in optimal conditions, CBT offers a probability of success at least as good as that of BMT. As the incidence and severity of both grade II-IV acute and chronic GvHD were negligible, this study also provided support to the concept that the reduction of GvHD limits the occurrence of fatal complications, this rendering HSCT more widely applicable for thalassemia. While the use of cord blood hematopoietic stem cells from an HLA-identical family donor has opened new scenarios able to make the procedure safer, no consistent data are available for evaluating the role of CBT from an unrelated volunteer, mainly because there is a low chance of locating an HLA-identical unit from an unrelated donor.

The limited number of hematopoietic progenitors contained in a unit has so far confined the use of CBT mainly to patients with a body weight not exceeding 40 kg. Potentially useful strategies for increasing the applicability of CBT also in patients with a body weight over this limit could be offered, in the near future, by *ex vivo* expansion of cord progenitors. The possibility of using cord blood cells for curing thalassemia may tempt a couple who has an affected child to conceive a new compatible, healthy sibling. In this regard, some bioethical considerations arise. Antenatal diagnosis of thalassemia is widely available and, besides determining whether a fetus is healthy, also makes it possible to evaluate HLA-compatibility with the affected child. Antenatal knowledge of lack of HLA-compatibility might lead parents to decide to terminate the pregnancy also in the case that the fetus is healthy, a decision that is highly questionable from an ethical standpoint. The recent demonstration that *in vitro* fertilization and pre-implantation selection of compatible, healthy embryos is feasible[15] is encouraging couples with an affected child to initiate a pregnancy with the certainty that a source of stem cells will be available for transplantation.[16] Reduced to its essential ethical-bioethical problematic issues, preimplantation genetic diagnosis for HLA-compatibility, with selection, on this basis, of an embryo programmed as an donor, entails weighing the desirable saving of a life (with optimum quality of success) against discarding a number of other embryos (not 'usable' for transplantation purposes). This choice of discarding other embryos only because they are not HLA-compatible with the candidate recipient may obviously raise ethical concerns.[17]

As already mentioned, only 25-30% of patients with diseases potentially curable with HSCT have a suitable, HLA-compatible sibling. Thus, the vast majority of patients who can benefit from an allograft lack a family donor. During the past 15 years, with the establishment of bone marrow donor registries, now including more than 9 million volunteers world-wide, the problem of increasing the number of patients who can be treated with a HSCT has been at least partially solved. In fact, a variable proportion of patients who need an allogeneic HSCT have been able to locate an unrelated donor, and transplantation from unrelated volunteer donors has been increasingly adopted for life-threatening inborn errors.[18,19] At the beginning of its use, mainly because of HLA polymorphism and the limits of conventional techniques for HLA-typing, unrelated donor HSCT was associated with a high incidence of transplant-related immune complications (namely graft failure and GvHD) which caused a relevant transplant-related mortality.[18-20] It is not surprising that recourse to this type of transplant for patients with thalassemia did not meet consensus until recently. In fact, in the last years, more precise characterization of HLA alleles using high-resolution molecular typing for both class I and class II loci has allowed to reduce the risk of both immune-mediated complications and fatal events.[21] These achievements have provided the rationale for considering the possibility of performing HSCT from unrelated volunteers in patients with hemoglobinopathies.

Some anecdotal reports have shown that unrelated donor HSCT is able to cure patients with thalassemia major.[22,23] The experience on unrelated donor HSCT for patients with thalassemia of the Italian cooperative group for bone marrow transplantation has recenty been described in detail.[24] In this study, 32 patients, aged between 2 and 28 years, were transplanted with bone marrow progenitors from an unrelated donor, prospectively selected using high-resolution molecular typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1 and DQB1 loci. Four and 11 patients were assigned to risk class 1 and to risk class 2, respectively, whereas the remaining 17 patients either belonged to risk class 3 or were adults. At time of reporting, 22 of these 32 patients (69%) were alive and transfusion-independent after a median follow-up of 30 months, whereas 4 and 6 had had graft failure or had died due to transplant-related complications, respectively. All deaths but one occurred in the risk class 3/adult group. A most recent, unpublished update on 51 class 1/class 2 patients, transplanted with bone marrow cells from an unrelated donor, has shown a probability of thalassemia-free survival in the order of 90%, with the risk of chronic GvHD being lower than 10% (Locatelli *et*

al., personal data). A recently published study has analyzed a total of 27 adult thalassemia patients transplants from an unrelated donor selected by high-resolution HLA molecular typing. Nineteen patients (70%) are alive and transfusion-independent after a median follow-up of 43 months (range 16-137), while the remaining 8 died from transplant-related causes.[25] As the main concern with the option of offering BMT to a thalassemia patient who is well chelated and in good health regards the possibility that either death or disability due to chronic GvHD ensue, this experience with transplantation using HSCT shows that both the risk of death and that of developing severe chronic GVHD are limited and do not exceed the level already largely accepted when using a family donor, provided that stringent criteria of compatibility are employed for selecting the donor. The main limitation of this experience is that, using such criteria, only about one-third of thalassemia patients who started the search found a suitable donor in a median time of 3-4 months.

In fact, the rigorous criteria established for considering a donor as suitable implies that a number of possible donors, who would have been acceptable for patients with other, more life-threatening diseases, are discarded. One theoretical possibility that would further widen the applicability of HSCT,would be to adopt less stringent criteria of HLA-matching for selecting donors with one or two allelic disparities and, with the aim of lowering the risk of GvHD associated with the increased immunogenetic disparity, to use some sort of *in vivo* serotherapy, which has recently been proven to reduce the incidence of both acute and chronic GvHD, in a dose-dependent manner, after the allograft from an unrelated donor.[26] However, the results achievable with HSCT from unrelated donors selected using less stringent criteria of compatibility with the recipients remain to be determined.

Along the same line, the realization of T-cell depleted HSCT from an HLA-partially matched relative is not routinely advisable, for the time being, for patients with hemoglobinopathies, due to the substantial risk of serious, often fatal, infectious complications. Haploidentical, T-cell depleted HSCT can be considered in extreme situations, such as that of a patient completely non-compliant with any type of chelation therapy and/or with immunization to allogeneic or autologous erythrocyte antigens rendering transfusion either impossible or life-threatening.

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