Unrelated donor bone marrow transplantation for thalassemia: the effect of extended haplotypes

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Allogeneic bone marrow transplantation (BMT) from a genotypically identical family donor is an accepted therapeutic option for homozygous beta-thalassemia. However, only a minority of patients have access to this curative procedure. The aim of this study is to explore the feasibility of matched unrelated transplants in thalassemia and the possibility of reducing the risk of immunologic complications through careful selection of donor/recipient pairs. Since November 1992, 32 patients (age range, 2-28 years) have been enrolled. There were 4 patients assigned to risk-class I, 11 to risk-class II, and 17 to risk-class III of the Pesaro classification. Extended haplotype analysis and family segregation studies were employed for identification of suitable donors. Of the 32 donor/recipient pairs, 24 were identical for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1, and DQB1 loci; 7 pairs were identical for 2 extended haplotypes, and 15 pairs shared one extended haplotype. Grade II-IV acute graft-versus-host disease (GVHD) developed in 11 cases (41%) and chronic GVHD in 6 (25%) out of 24 patients at risk. There are 22 patients (69%) who are alive and transfusion-independent after a median follow-up of 30 months (range, 7-109 months). There were 6 patients (19%) who engrafted and subsequently died from transplant-related complications. In 4 cases (12.5%) graft rejection was observed within 30 days and it was followed by autologous reconstitution. Out of 22 patients with a donor identical for at least one extended haplotype, there are 19 who survived, 17 of them being transfusion-independent. Among the 10 recipients who did not share any extended haplotype with the donor, only 5 are alive without thalassemia and 3 patients died. Of the 6 patients who died, 5 belonged to risk-class III and only 1 to risk-class II. BMT from well-selected unrelated donors may offer results comparable to those obtained in transplantations using HLA-identical family donors, especially for patients with lesser iron overload. (Blood. 2002;99:4350-4356)

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Introduction

Allogeneic bone marrow transplantation (BMT) from a genotypically identical family donor has radically changed the prognosis of patients with homozygous beta-thalassemia. For young patients at an early stage of disease, the reported percentage of transfusion-free survival and the mortality risk were 91% and 8%, respectively.1,2 Transplantation performed on older, heavily iron-loaded patients, in particular those with liver abnormalities, has a less satisfactory outcome, the probability of transfusion-free survival and mortality being 51% and 32%, respectively.3

The probability of finding an HLA-identical donor within the family is less than 30% in western countries.4 For the remaining 70% of patients with thalassemia, there was until recently no option other than treatment based on chronic transfusion and iron-chelating therapy. Although optimization of protocols for transfusion and chelation has dramatically improved the life expectancy of these patients, complications of iron overload cannot be completely avoided.5,6 Full compliance with a chronic transfusion regimen and a daily, lifelong injective chelation therapy has been shown to be difficult to maintain with advancing age.7 Multiple endocrine dysfunctions, myocardiopathy, progressive liver fibrosis, and consequences of posttransfusion viral infections affect the quality of life and increase the mortality risk with age.7,9

During the past 10 years, BMT from unrelated donors has been increasingly employed for hematologic malignancies and life-threatening inborn errors.10-12 At first, results were characterized by elevated mortality due to the high frequency of transplantation-related complications, mainly acute and chronic graft-versus-host disease (GVHD) and graft failure. These complications were most likely related to HLA differences either not tested or not revealed by the techniques for HLA typing available at that time.13-15 Therefore, BMT with volunteer unrelated donors for beta-thalassemia did not meet consensus, due to the high risk of transplantation-related death.

Recent years have witnessed a progressive increment in the number of unrelated-donor BMTs, mainly due to the increase in the number of volunteer donors worldwide; some studies have reported results comparable to those obtained in transplantations using HLA-matched family donors, and this improvement may be attributed to the introduction of high-resolution molecular techniques for histocompatibility testing.16-20

There is increasing evidence that the entire structure of an HLA extended or ancestral haplotype is generally identical, except for rare variations at the centromeric and telomeric extremities, in HLA-matched unrelated individuals belonging to the same or a
different ethnic group. Therefore, 2 extended haplotypes shared by donor and recipient guarantee a very high probability of identity of the 2 HLA regions both for the typed A, B, C, DR, and DQ genes and for the nucleotide sequences between these genes where there may be polymorphism, potentially relevant to BMT. Moreover, several studies support the hypothesis that reduction of postransplantation immune-mediated complications is attained when donor/recipient pairs share one or 2 extended haplotypes.

We conducted a pilot study, approved by the Italian Bone Marrow Transplant Group (GITMO) and by the Italian Bone Marrow Donor Registry (IBMDR), aimed at exploring both the feasibility of BMT from a marrow unrelated donor (MUD) in thalassemia and the possibility of reducing the risk of immunologic complications by selecting donor/recipient pairs sharing extended haplotypes or parts of them.

### Patients, materials, and methods

#### Clinical characteristics of patients

From November 1992 to May 2001, 32 patients with thalassemia major were enrolled into this study by 4 BMT centers in Italy. The study received approval by the local institutional review board. After detailed explanation of the procedure and its risks, informed consent was obtained from all patients or from parents in the case of minors. Particular emphasis was placed on the option of continuing conventional disease management with transfusions and chelation therapy. Pretransplantation patient characteristics are shown in Table 1. There were 11 female patients and 21 male patients, age range being 2-28 years (median, 14 years).

Prior to transplantation, all patients underwent a complete check-up and were assigned to one of 3 classes of risk according to the criteria proposed by Lucarelli et al. Risk factors were (1) hepatomegaly, (2) liver biopsy revealing the presence of portal fibrosis, and (3) the quality of pretransplantation iron chelation.

The classification of liver iron overload was based on the scheme of Sciot and portal fibrosis was defined in each patient as mild, moderate, or severe.

One patient, aged 4 years, did not undergo liver biopsy because his parents refused the procedure and he was assigned to risk-class I for the absence of the other 2 risk factors.

Of 32 patients, 8 were positive for hepatitis C virus (HCV)–RNA and 2 of them had antibodies to hepatitis B virus due to previous clinical infection.

In 3 HCV-RNA–positive patients, liver histology revealed chronic active hepatitis. In the remaining 5 patients, liver biopsy indicated a diagnosis of chronic persistent hepatitis. There were 24 patients who had positive cytomegalovirus (CMV) serology.

Out of 32 patients examined, 4 were assigned to risk-class I, 11 to risk-class II, and 17 to risk-class III.

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<th>Conditioning regimen</th>
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*Patient shared 2 extended haplotypes.
†Patient shared one extended haplotype.
‡Take followed by rejection.

aGVHD indicates acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; R, regular; Ir, irregular; NO, none; MI, mild; MO, moderate; SE, severe; BU, busulphan; CY, cyclophosphamide; TT, thiota; NC, nucleated cells; NE, not evaluable; L, limited; E, extensive.
HLA typing and donor/recipient matching

Patients, donors and, when feasible, family members were typed for the currently recognized HLA-A, -B, and -Cw antigens using monolocus-specific antisera according to a standard 2-stage National Institutes of Health (NIH) complement-dependent microcytotoxicity test. Typing for HLA-DR and -DQ was performed using Dynabead-purified B cells in a microcytotoxicity assay.

DNA for molecular typing was extracted from whole blood collected in neuroaminidase-treated ethylenediaminetetraacetic acid (EDTA). Alleles at the HLA-A, -B, -Cw, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, and -DPB1 loci were identified by polymerase chain reaction–single strand polymorphism (PCR-SSP) (Dynal, Oslo, Norway) and sequence-based typing. Amplification and sequencing of HLA class I and class II genes were performed as previously described, using standard big dye terminator cycle-sequencing chemistries supplied with the ABI sequencing kit. Reactions were analyzed on the Applied Biosystems 310 Automated DNA sequencer (Applied Biosystems, Foster City, CA). Alleles were assigned according to DNA sequences published by the Nomenclature Committee. According to DNA sequences published by the Nomenclature Committee.

Class III HLA antigens C4A and C4B were determined by immunofixation electrophoresis of neuroaminidase-treated EDTA plasma. BF alleles were determined by high-voltage electrophoresis in agarose gel, followed by immunofixation with anti-human factor B antibodies.

Extended or ancestral haplotypes

The combination of alleles encoded by 2 or more HLA loci on a single chromosome is defined as a haplotype. Because certain alleles are strongly associated with one another due to linkage disequilibrium (LD), only a relatively small number of haplotypes can be observed. The HLA haplotypes that show a high LD among their class I, class II, and class III alleles are considered extended or ancestral haplotypes. These haplotypes are conserved and therefore can be shared by apparently unrelated subjects. Therefore, 2 unrelated individuals who share 2 extended haplotypes are highly likely to be identical, not only for the routinely tested HLA class I and class II genes, but for the entire major histocompatibility (MHC) region where there are many other genes that have an important role in antigen presentation and immune response. Several mechanisms, including selection pressure, recombination suppression, and preferential transmission, may explain the conservation and frequency of extended or ancestral haplotypes in the different populations. In our study, HLA extended haplotypes were identified and defined by referring to the data provided by Rendine et al and Contu et al for the Italian population and to data from the 10th and 11th International Histocompatibility Workshop and the National Marrow Donor Program (NMDP) donor registry for other populations (Tables 2, 3).

Donor characteristics

There were 29 bone marrow donors identified within the IBMDR. 2 were found in the German National Bone Marrow Donor Registry, and one was found in the French Bone Marrow Donor Registry. Median interval between donor search and transplantation was 5 months (interval from 1 to 20 months). There were 16 female donors and 16 male donors, age range being 25 to 52 years (median, 36 years). For 24 donors, at least one informative family member was typed for HLA haplotype deduction. In the remaining 8 cases, the haplotypes were assigned on the basis of the presence of at least one extended haplotype well-defined in the population. Of these 8 cases, 4 could be considered to share a single extended haplotype with the patients: HLA-A2, -A30, -Cw5, -B18, and -DR3 for pair no. 7; HLA-A30, -Cw5, -B18, and -DR3 for pair no. 21; and HLA-A2, -Cw7, -B18, and -DR3 for pair nos. 26 and 30. The first 2 haplotypes are in strong LD in the Sardinian population, the third haplotype is in LD in the Greek and Sardinian populations (Tables 2, 3).

Transplantation regimen, GVHD prophylaxis, and supportive therapy

Data regarding conditioning regimen and number of cells infused are given in Table 1. The first 4 patients received transplants after a preparative regimen including busulfan (BU) and cyclophosphamide (CY). Oral BU (3.5 mg/kg per day), was administered 3 times per day for 4 days (total dose 14 mg/kg) followed by 50 mg/kg per day intravenous CY for 4 days (total dose 200 mg/kg) in 2 patients and 40 mg/kg per day CY for 4 days (total dose 160 mg/kg) in the other 2 patients. As 2 of these 4 patients did not have sustained engraftment, in the remaining 28 patients the conditioning regimen was modified as follows: 14 mg/kg BU, 10 mg/kg thiotepa (TT), and 200 mg/kg CY for 14 patients (risk classes I and II); 14 mg/kg BU, 10 mg/kg TT, and 160 mg/kg CY for 8 patients aged less than 16 (risk class III); 14 mg/kg BU, 10 mg/kg TT, and 120 mg/kg CY for 6 patients aged more than 16 (risk class III: adults). TT was divided into 2 doses and administered on day minus 6.

Marrow was infused 36 hours after the last dose of CY. The median bone marrow nucleated cell dose was 3.8 × 10^6/kg of recipient weight (range, 1.8-11.6 × 10^9/kg). All patients received 3 mg/kg per day cyclosporine (CSP) intravenously from day −2 to day +30 and short-term methotrexate (MTX) for GVHD prophylaxis. The CSP schedule was switched to 9 mg/kg per day orally as soon as oral administration could be tolerated; from day +60 the dose was tapered until discontinuation at one year.

All patients were treated in positive-pressure isolation rooms and received nonabsorbable oral antibiotics and a low-bacteria diet. Supportive therapy, as well as prophylaxis and treatment of infections, was homogeneous among participating centers. Prophylaxis for Pneumocystis carinii pneumonia was started after engraftment, with oral cotrimoxazole. CMV reactivation was monitored either by expression of the pp65 antigen or by direct PCR. Empirical broad-spectrum antibiotic therapy was started when patients became febrile, and antifungal therapy was added in the presence of either clinical evidence of fungal infection or fever persisting after 3 days of antibiotic therapy. All blood products administered after transplantation were irradiated with 30 Gy.

Neutrophil and platelet engraftment were defined as the first of 3 consecutive days with neutrophils more than 0.5 × 10^9/L and platelets more than 50 × 10^9/L, respectively. Acute and chronic GVHD were graded according to the Seattle criteria.

The first-choice drug for treatment of acute GVHD was steroids at a dosage of 2 to 10 mg/kg per day, depending on severity of GVHD.

Monitoring of chimerism

Chimerism was documented in situ Y chromosome hybridization of either bone marrow or blood samples in sex-mismatched donor/recipient pairs, by analysis of a variable number of tandem repeat (VNTR) polymorphisms and by microsatellite analysis of bone marrow and/or blood samples in the case of sex-matched pairs. Analysis of chimerism and patterns of hemoglobin synthesis were first performed on peripheral blood and bone marrow aspirates on days +2 and repeated at biweekly intervals until day +60 to document engraftment of donor hematopoiesis.

Statistical evaluation

For continuous variables with a symmetric distribution, the results are expressed as medians and ranges. Comparison between groups was performed by Fisher exact test. Survival probability was estimated by the product-limit method of Kaplan and Meier.

Results

From November 1992 to May 2001, 185 patients with thalassemia activated a search for locating an unrelated donor at one of the 4
BMT centers involved in this study. For 35 patients (19%) a suitable donor was identified. There were 3 patients who declined the transplantation procedure and 32 (17%) who received an unrelated donor transplant. Positive result of donor search ranged from 32% for BMT centers in Sardinia to 12% for the other BMT centers, mainly dealing with patients belonging to mixed ethnic groups. The patients who did not find a donor with the required characteristics continued conventional therapy.
None of the 6 recipients who shared 2 HLA-extended haplotypes and had sustained donor engraftment developed grade II to IV acute GVHD (Table 4), whereas in the remaining 21 evaluable patients who shared either a single or no extended haplotype, the overall occurrence of acute GVHD was 52% (P = .05; see also Table 4 for further details). A significant reduction of the incidence of acute GVHD was observed in the group of 14 patients who shared an identity for both DPB1 alleles with their donors, compared with the group of 18 patients who received bone marrow from a donor mismatched for either 1 or 2 DPB1 alleles (21% vs 61%, respectively, P = .05). Among the 14 patients (cases 1, 3, 10, 12, 17, 19, 23, 24, 26, 27, 28, 30, 31, 32) who achieved allogeneic engraftment without acute or chronic GVHD, 11 (78%) shared at least one extended haplotype with the donor and 8 (57%) were also identical at the DPB1 loci.

Of the 22 patients who had a donor identical for at least one extended haplotype, 19 survived, 17 of them being transfusion-independent. Among the 10 recipients who did not share any extended haplotype with the donor, only 5 are alive without thalassemia, and 3 patients died.

In 4 cases, donor marrow was rejected with complete autologous reconstitution and return to pretransplantation clinical status. In 2 of these patients, rejection occurred after transient engraftment of donor cells. As mentioned above, 2 of 4 cases of rejection were observed in the first series of 4 patients conditioned with BU and CY alone, whereas only 2 cases of rejection occurred in the second series of 28 patients whose conditioning regimen included TT. One of the 2 patients in whom rejection was observed after documented engraftment (case 4) was the only one lacking haplotype identity.

### Discussion

The majority of thalassemia patients do not have an HLA-identical donor within the family. For this reason, in recent years BMT centers engaged in the cure of hemoglobinopathies have been exposed to increasing questioning and pressure from both patients and families to explore the possibility of transplantation utilizing alternative donors.

Use of partially matched family donors carries a high risk of rejection, GVHD, and mortality, and has been confined to particular clinical situations.45

![Figure 1](figure1.png)

**Figure 1.** Kaplan-Meier probabilities of survival, thalassemia-free survival, nonrejection mortality, and rejections for 32 thalassemia patients who received transplants from HLA-matched unrelated donors (between parenthesis: 95% confidence limits at 2 years).

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### Table 3. List of the HLA extended haplotypes found in the 22 unrelated donor/recipient pairs

<table>
<thead>
<tr>
<th>Extended haplotypes</th>
<th>Numbers shared</th>
<th>Race</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-A30-Cw5-B18-DR3</td>
<td>12*</td>
<td>C, S</td>
</tr>
<tr>
<td>HLA-A2-Cw7-B58-DR2</td>
<td>6</td>
<td>C, S</td>
</tr>
<tr>
<td>HLA-A2-Cw5-B18-DR3</td>
<td>3</td>
<td>C, S</td>
</tr>
<tr>
<td>HLA-A1-Cw6-B57-DR10</td>
<td>2</td>
<td>C, S, A</td>
</tr>
<tr>
<td>HLA-A33-Cw6-B14-DR1</td>
<td>2</td>
<td>C, S</td>
</tr>
<tr>
<td>HLA-A2-Cw7-B18-DR5</td>
<td>2</td>
<td>C, S</td>
</tr>
<tr>
<td>HLA-A1-Cw7-B8-DR3</td>
<td>1</td>
<td>C</td>
</tr>
<tr>
<td>HLA-A2-Cw2-B27-DR2</td>
<td>1</td>
<td>C, S</td>
</tr>
</tbody>
</table>

C indicates Caucasian; S, Sardinian; A, Asian (ethnic groups in which these haplotypes are found in linkage disequilibrium).

*One donor/recipient pair was homozygous for this haplotype.
Recently published reports indicate that the outcome of unrelated donor transplantations in patients with leukemia today is comparable with that of transplantations from identical family donors.16-18 This is due to the better compatibility between donor and recipient afforded by DNA methods and to the compensatory effect of a stronger graft-versus-leukemia over the increased risk of GVHD. In thalassemia, where GVHD represents a complication without benefit for the patient, it is mandatory to minimize the immunologic risks of BMT before accepting unrelated donor transplants as an established therapeutic option.

So far, there are only a few anecdotal reports19-21 on BMT from unrelated donors in thalassemia. This is the first large series of consecutive thalassemia patients reported from unrelated donors. In our cohort, rejection and mortality rates were 12.5% and 19%, respectively. Sixty-nine percent of our patients are alive with sustained engraftment of donor hematopoiesis, this leading to a projected thalassemia-free survival of 66%.

In our series, only one death was observed among 15 class I and class II patients (7%), the other 5 occurring among 17 class III patients (29%). In class III risk patients, transplantation-related mortality is high (32%) even when the donor is an HLA-identical sibling.3,6 Overall survival and thalassemia-free survival (93% and 80%, respectively) in the 15 patients of class I and class II risk groups were comparable with those obtained in transplantations from an HLA-identical family donor.3,47

The relatively low incidence of acute and chronic GVHD (41% and 25%, respectively) confirms that a careful immunogenetic selection of donor/recipient pairs has an important role in reducing the incidence of this complication. In fact, the remarkable stability of the extended haplotypes21,36,48 and the data deriving from MLC studies49 support the hypothesis that 2 unrelated individuals sharing 2 HLA-extended haplotypes are nearly always practically HLA-genoidentical, just as if they had inherited the HLA haplotypes from the same parents. Therefore, it is reasonable to hypothesize that the histocompatibility differences between a pair of HLA-genoidentical siblings and a pair of unrelated individuals sharing 2 extended haplotypes exclusively reside in minor histocompatibility antigens (mHAg) located outside the HLA region. Moreover, haplotype matching, even when it is not for complete extended haplotypes, makes it possible to include parts of them (telomeric or centromeric portion of extended haplotypes) that are common in populations worldwide.50

The method for donor/recipient selection reported here may require a prolonged search and reduce the probability of locating a suitable donor. However, it should be considered that the length of time from starting the search to performing the transplantation is not such a critical factor in thalassemia as it is, for example, in acute leukemia or congenital immune deficiencies. Patients with thalassemia receiving traditional medical treatment can maintain stable clinical conditions for a long period of time and therefore can wait until a donor with optimal immunogenetic characteristics is found. Moreover, it is possible that, due to the geographic distribution of thalassemia in selected areas, the probability of finding an unrelated identical donor may be higher than in the general population if in those areas large and representative marrow donor registries are established.36

Data on the role of HLA antigen DP molecules in transplantation outcome are contradictory.51,52 So far, it is quite difficult to differentiate the role of DP molecules from that of other HLA molecules as this would require the availability of genoidentical donor/recipient pairs different at the DP locus for a rare event of crossing over. Alternatively, an optimal model for this type of evaluation is represented by unrelated donor/recipient pairs different at the DP locus but sharing 2 extended haplotypes. In our study, although the number of cases is small to draw definitive conclusions, a significant increase in the incidence of immunologic complications was observed in DPB1-mismatched recipients.

In our study, 2 of the 4 cases of rejection occurred in the first 4 patients conditioned with the standard BU-CY regimen. It is possible that the dose of BU (14 mg/kg) was not sufficient to guarantee blood drug levels capable of determining engraftment in BMT from MUD.53 However, since in adult thalassemia patients increasing the dosage of BU carries the risk of increased mortality and toxicity,54 in the second series of patients a third drug, TT, was added. TT at a dose of 10 mg/kg has been demonstrated to intensify both the myeloablative and immunosuppressive effect of the conditioning regimen, without a significant increase of extramedullary toxicity.55 Our results show that the protocol BU-TT-CY, with dosage of CY adjusted for risk class, was well tolerated.

Although obtained in a limited cohort of patients, our results show that BMT from unrelated donors, especially when identical for at least one extended haplotype, may offer a probability of success comparable to that offered by transplantations using HLA-identical family donors. It is therefore quite reasonable to consider this type of transplantation as an acceptable therapeutic approach in thalassemia, at least for patients who are not fully compliant with conventional treatment and do not yet show irreversible severe complications of iron overload, provided that careful immunogenetic selection of marrow donors is maintained.

Acknowledgments

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References

4. Anasetti C, Etzioni R, Petersdorf EW, Martin PJ, Hansen JA. Marrow transplantation from

Table 4. Impact of matching for HLA extended haplotypes

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>No. of EH</th>
<th>Alive and well</th>
<th>Acute GVHD (II-IV)</th>
<th>Chronic GVHD</th>
<th>Rejection</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n %</td>
<td>n %</td>
<td>n %</td>
<td>n %</td>
<td>n %</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>5 71.4</td>
<td>0 0</td>
<td>0 0</td>
<td>1 14.2</td>
<td>1 14.2</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>12 80.0</td>
<td>6 46.1</td>
<td>2 16.6</td>
<td>1 6.6</td>
<td>2 13.3</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>5 50.0</td>
<td>5 62.5</td>
<td>4 57.1</td>
<td>2 20.0</td>
<td>3 30.0</td>
</tr>
</tbody>
</table>

EH indicates extended haplotype.


13. Santamaria P, Reinsmoen NL, Lindstrom AL, et al. Frequent HLA class I and DP sequence mismatches in serologically (HLA-A, HLA-B, HLA-DR) and molecularly (HLA- DRB1, HLA-DQA1, HLA-DQB1) HLA-identical unrelated bone marrow transplant pairs. Blood. 1994;83:280-287.


Unrelated donor bone marrow transplantation for thalassemia: the effect of extended haplotypes

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