TRANSPLANTATION

Bone marrow transplantation for thalassemia from alternative related donors: improved outcomes with a new approach

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Key Points

- A novel approach to BMT for thalassemia using related phenotypically matched or 1-antigen–mismatched donors improved transplant outcomes.
- BMT from phenotypically matched or 1-antigen–mismatched donors is associated with a high thalassemia-free survival rate (94%).

Bone marrow transplantation (BMT) performance can be limited by a lack of ideal donors, and the role of alternative donor hematopoietic cell transplantation in thalassemia is not well established. Here we used a new treatment protocol (Pc 26.1) in 16 thalassemia patients to perform BMT using phenotypically HLA-identical or 1-antigen–mismatched relatives (related donors [RDs]). We compared these results with HLA-matched sibling (matched sibling donors [MSDs]) BMT in 66 patients. The entire RD group and 88% of MSD group had sustained engraftment. Rejection incidence was 0% in the RD and 12% (95% confidence interval [95% CI], 6%-21%) in MSD groups ($P = .15$), with respective thalassemia-free survival probabilities of 94% (95% CI, 63%-99%) and 82% (95% CI, 70%-89%) ($P = .24$). Transplant-related mortality was 6% (95% CI, 1%-26%) in the RD group and 8% (95% CI, 3%-16%) in the MSD group ($P = .83$). The intensified new protocol was not associated with increased nonhematologic toxicity. The present data show that the Pc 26.1 preparative regimen allows thalassemia patients to safely undergo BMT from RDs who are not HLA-matched siblings, with transplant outcomes similar to patients with MSD grafts. (*Blood*. 2013;122(15):2751-2756)

Introduction

Advances in patient care have led to significant improvements in the life expectancy of patients with thalassemia.[1,2] However, the aging of this population leads to increasing development of new complications, such as lung disease, heart disease, and endocrine disorders that lead to morbidity and premature mortality.[3] Hematopoietic stem cell transplantation is the only well-established curative treatment of thalassemia major and shows excellent long-term results,[4-6] but allogeneic bone marrow transplantation (BMT) is often limited by a lack of suitable donors.

Transplant outcomes are better when using an HLA-matched sibling donor (MSD), but the probability of finding such a donor varies considerably among different ethnic populations. In North America and Europe, <30% of patients have an HLA-MSD,[7] while the likelihood of finding a MSD could be as high as 60% to 70% in most Middle East and Asian countries where large families are common.[8,9] For the remaining patients, alternative related or unrelated donors can be found, but many countries lack appropriate registries and the high cost of a search can make it practically impossible to find an unrelated donor. Thus, patients without a MSD could benefit from a related HLA phenotypically matched or 1-antigen–mismatched donor. In most communities of the Middle East, North Africa, and West Asia, consanguineous marriage is traditional, accounting for 20% to >50% of marriages.[10] Importantly, an extended family search in such populations results in the identification of an HLA-matched nonsibling donor for up to 13% to 18% of patients.[11,12] The probability of finding an HLA-matched related donor (RD) in a Middle East population (65.5%-80%) is similar to the probability of finding a matched unrelated donor (10%-75%, depending on the race and ethnicity of the patient) (http://www.marrow.org). Moreover, approximately 13% of patients lacking an HLA-identical sibling donor could have a 1-antigen–mismatched RD.[13]

Historically, the use of alternative RDs (either phenotypically identical or 1-antigen–mismatched donors) has been associated with high engraftment failure, severe graft-vs-host disease (GVHD), and low disease-free survival in cases of hematologic malignancies or aplastic anemia.[14,15] In 2000, our group published a study examining alternative related unmanipulated BMT for thalassemia, and we reported high rates of graft failure and GVHD and low disease-free survival rates.[16] However, since then, there have been major improvements in donor identification, HLA typing, conditioning therapies, transfusion medicine, antimicrobials/antifungals, immunosuppression, and supportive care, which have improved transplant outcomes. Additionally, in 2005, we adopted a new transplant approach for alternative RD transplantation in thalassemia. We hypothesized that low disease burden due to preconditioning cytoreduction/immunosuppression followed by an intensified conditioning regimen would decrease the incidence of rejection while minimizing associated

Submitted July 5, 2013; accepted August 13, 2013. Prepublished online as *Blood* First Edition paper, August 20, 2013; DOI 10.1182/blood-2013-07-513473.

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morbidity and mortality in thalassemia patients who receive transplant using RDs who are not HLA-matched siblings. In the present study, we prospectively and comparatively evaluated the transplantation outcomes in this context.

Methods

This study was a prospective, single-center investigation of the safety and efficacy of a novel preparative regimen for transplantation in patients with thalassemia from RDs who were not HLA-matched siblings. Between September 2005 and January 2012 at the Mediterranean Institute of Hematology, Policlinic of Tor Vergata of Roma, 16 consecutive patients with thalassemia received their first BMT from RDs who were phenotypically matched or 1-antigen–mismatched. These patients were prepared for transplantation following treatment protocol 26.1 (Pc 26.1). To elucidate the efficacy and safety of RD transplants, we compared the transplant outcomes of these patients with 66 thalassemia patients treated by HLA-matched sibling (MSDs) BMT between July 2004 and January 2012 and who had the same risk-class characteristics as patients treated according to Pc 26.1. The treatment protocol was approved by the Mediterranean Institute of Hematology institutional review board, and the parents of all patients provided written informed consent, in accordance with the Declaration of Helsinki.

HLA typing

All patient–donor pairs were analyzed by 4-digit sequence-based typing or sequence-specific polymerase chain reaction (PCR-SSP) at the HLA-A, HLA-B, HLA-C, HLA-DRB, and HLA-DQB1 loci. Four-digit HLA-DPB1 typing was prospectively performed by PCR-SSP and by PCR sequence-specific oligonucleotide probing (PCR-SSOP).

Treatment protocols

Treatment protocol 26.1 consisted of a preconditioning phase and a conditioning regimen. It was initially designed for second transplants,[17] but the very encouraging results led us to also apply it in patients receiving transplants from donors who are not HLA-matched siblings. During the preconditioning phase, patients received hydroxyurea (HU) at 30 mg/kg per day and azathioprine (AZ) at 3 mg/kg per day from day −45 pretransplant and fludarabine (FLU) at 30 mg/m^2 per day from day −16 through day −12. The conditioning regimen consisted of oral busulfan (Bu) at 14 mg/kg total dose (for 2 patients) or weight-based targeted intravenous Bu (i.v.Bu), thiotepa (TT) (10 mg/kg per day), cyclophosphamide (Cy) (200 mg/kg total dose), and rabbit antithymocyte globulin (ATG) (Thymoglobulin; Genzyme, Lyon, France) 12.5 or 10 mg/kg total dose.

In the MSD group, the conditioning regimen for class 2 patients consisted of oral Bu at 14 mg/kg total dose or weight-based targeted i.v.Bu and Cy at 200 mg/kg total dose ± TT 10 mg/kg per day (31 patients). Class 3 younger (26 patients) and adult patients (9 patients) before conditioning were given cytoreduction/immunosuppression with HU at 30 mg/kg per day, AZ at 3 mg/kg per day from day −45 pretransplant, and FLU at 20 mg/m^2 from day −16 through day −12. Their conditioning regimens consisted of oral Bu at 14 mg/kg total dose or weight-based targeted i.v.Bu, and Cy 160 mg/kg (younger patients) or the same dose of Bu, and CY 90 mg/kg ± TT10 (adult patients). As GVHD prophylaxis, all patients received cyclosporine, low-dose methylprednisolone, and a modified short course of methotrexate.[18]

All patients received a bone marrow infusion at 36 hours after the last dose of Cy. Acute and chronic GVHD were diagnosed and the severity was scored according to published criteria.[19,20]

Regimen-related toxicity

Regimen-related toxicity was scored using the NCI Common Toxicity Criteria, version 2.0. During the first 30 days after transplantation, we scored the presence of oral mucositis and/or central nervous system, gastrointestinal, hepatic, pulmonary, cardiac, and renal toxicity. Any blood culture that was positive for bacteria or fungal species was documented as bacteremia or fungemia, respectively.

Disease monitoring after transplantation

Twenty days after transplantation, the first chimerism analysis was performed on bone marrow samples. The percentage of donor/recipient DNA was determined by PCR-based analysis of short tandem repeats (Profiler Plus Applera). At 60, 90, 180, and 365 days after transplantation, we determined myeloid and lymphoid chimerism.

Study end points and definitions of outcomes

The study end points were graft rejection (primary or secondary), thalassemia-free survival (TFS), overall survival (OS), GVHD, and transplant-related mortality (TRM). Primary graft rejection was defined as the presence of <15% donor cells (minimum level of persistent mixed donor chimerism observed in our patients) as determined by bone marrow and peripheral blood chimerism assays, with persistent pancytopenia by 28 days after transplantation. Secondary graft rejection was defined as a loss of donor-derived hematopoietic cells in bone marrow and peripheral blood (<15% donor cells) after initial graft function and return to erythrocyte transfusion dependence. TFS was defined as survival without graft rejection or death. OS was defined as time from transplant to death, irrespective of the cause. The day of neutrophil engraftment was defined as the first of 3 consecutive days during which the absolute neutrophil count was 0.5×10^9/L or higher. Platelet engraftment was defined as the first of 7 consecutive days during which platelet counts exceeded 20×10^9/L in the absence of transfusion.

Supportive care

For infectious disease prophylaxis, the patients were given systemic antibacterial and antifungal drugs until the neutrophil level exceeded 1.0×10^9/L. They also received acyclovir for herpes virus prophylaxis and trimethoprim/sulfamethoxazole for *Pneumocystis jiroveci* prophylaxis. Starting prior to conditioning and continuing until at least 100 days posttransplantation, patients were monitored weekly for Epstein-Barr virus, cytomegalovirus, adenovirus, and BK polyomavirus in the blood and/or urine using sensitive reverse-transcription PCR. During hematologic recovery, patients were tested twice weekly for cytomegalovirus pp65 antigen.

Statistical analysis

Data regarding pretransplant patient characteristics, transplant complications, and outcomes were collected prospectively. Patient-, disease-, and transplant-related variables were compared between 2 groups using χ^2 statistics for categorical variables, and the Mann-Whitney *U* test for continuous variables. Probabilities of survival and disease-free survival were estimated using the Kaplan-Meier estimator, and the 2 groups were compared using a log-rank test.[21] The cumulative incidence of graft failure/rejection, GVHD, and TRM was estimated, accounting for competing risks for each outcome.[22] Death with sustained engraftment was considered a competing risk for rejection, and rejection and death before GVHD as competing risk for GVHD. Between-group differences were assessed using Gray's test. All *P* values were 2-tailed and considered to be significant at <.05. All statistical analyses were performed using IBM SPSS Statistics 21.0 statistical software and the EZR statistical program.[23]

Results

Patient characteristics

Table 1 summarizes the characteristics of the patients, disease, and transplantation. The median ages of the RD and MSD groups were 9.6 years (range, 1.4-24 years) and 10 years (range, 1.8-27 years), respectively. Two patients in the RD and 9 in MSD groups were

Table 1. Patient, transplant, and graft characteristics

Variables	RD group	MSD group	*P*
Number of patients	16	66	—
Median patient age, y	9.6 (1.4-24)	10 (1.8-27)	.68
Patient sex			
Male, n	10	37	.78
Female, n	6	29	—
Risk class			
Class 1, n	1	0	.20
Class 2, n	5	31	.27
Class 3, n	10	35	.58
Median donor age, y	37.5 (2-53)	13 (1-34)	.11
Median AST, IU/L	38 (13-120)	34 (19-216)	.28
Median ALT, IU/L	48 (13-122)	38 (11-224)	.35
Median bilirubin, mg/dL	0.95 (0.4-1.5)	1.2 (0.3-5.0)	.12
Median serum ferritin, ng/mL	1867 (782-7944)	2219 (682-10 222)	.48
Median liver iron concentration, g/dry weight	14.7 (1.7-28.2)	16 (2-44)	.50
Median packed RBC units received pretransplantation	96 (13-350)	105 (11-400)	.86
Liver size >2 cm, n	11	41	.77
Splenectomy, n	4	20	.77
Median liver fibrosis score (Ishak et al[24])	2 (0-6)	2 (0-5)	.11
Hepatitis C (HCV-RNA positive), n	2	6	.65
Donor–recipient HLA matching			
Phenotypically matched, n	11		
One-antigen mismatched, n	5		
Matched siblings, n		66	
Donor–recipient CMV status			
Both positive, n	16	55	.11
Any positive, n		7	.33
Both negative		4	.58
Donor–recipient sex match			
Matched, n	9	30	
Mismatched, n	7	36	.57
ABO blood group compatibility			
Identical, n	7	40	.27
Major and/or minor incompatibility, n	9	26	
Preparation for transplant			
Preconditioning cytoreduction/ immunosuppression	16	BU14 /i.v.BU/CY200	
d −45 to d −12			
HU 30 mg/kg/d		±TT10 (n = 31)	
AZ 3 mg/kg/d		BU14 /i.v.BU/CY160	
d −16 to d −12			
Flu 30 mg/m^2/d		Preceded by HU, AZ, Flu	
Conditioning regimen		n = 26	
d −10 to d −7			
Oral BU14/weight-based i.v. Busilvex		BU14 /i.v.BU/CY90 ±TT10	
d −6			
TT 10 mg/kg/d		Preceded by HU, AZ, Flu	
d −6 to d −3			
Thymoglobulin 2.5 mg/kg/d		n = 9	
d −5 to d −2			
CY 50 mg/kg/d		—	
GVHD prophylaxis with CSA + methylprednisolone + CY or MTX (d 1) + MTX (d 3 and 6), n	16	66	
Median nucleated cells, × 10^8/kg	4.2 (1.8-10)	4.3 (1.3-8.7)	.75
Median CD34$^+$ cells, × 10^6/kg	4 (1-13)	6.3 (0.8-35)	.68

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CSA, cyclosporine; CY, cyclophosphamide; MTX, methotrexate; RBC, red blood cell; RD, HLA phenotypically identical or 1-antigen–mismatched RDs; TT, thiotepa.

>17 years old. The 2 groups were similar in terms of demographic characteristics. Before transplantation, most patients were heavily transfused and had moderately severe iron overload, as evidenced by high median serum ferritin and liver iron concentrations. The median liver fibrosis score was 2 in both patient groups. Most patients in both groups were in class 3 of risk. Of the 16 donors for the RD group, 11 (8 parents, 2 cousins, and 1 uncle) were phenotypically HLA identical, and 5 (3 siblings, 1 mother, and 1 aunt) were 1-antigen mismatched. Thirteen of these patients were from Middle Eastern countries, 2 patients were from South Asian countries, and 1 patient was from Eastern Europe. In 10 families, consanguinity was present.

Engraftment and graft failure/rejection

In total, 16 patients in the RD and 58 (88%) in the MSD group had sustained engraftment. The median times to an absolute neutrophil count of at least 0.5×10^9/L for the RD and the MSD group patients were 19 days (range, 14-29 days) and 20 days (range, 12-42 days) ($P = .41$), respectively, while the median times to a platelet count of at least 20×10^9/L were 24 days (range, 16-50 days) and 25 days (range, 15-172 days) ($P = .89$), respectively (Table 2). Graft rejection occurred in 8 MSD group patients (primary graft rejection in 4, secondary graft rejection in 4), but in none of the RD group patients. The cumulative incidence of rejection was 12% (95% confidence interval [95% CI], 6%-21%) in the MSD group and 0% in the RD group, which was not statistically significant ($P = .15$) (Figure 1). At 2 months after transplantation, all RD group and 52 MSD group patients exhibited complete myeloid and lymphoid chimerism.

GVHD

At 100 days, the cumulative incidence of acute GVHD (grades 2-4) in the RD group (19%; 95% CI, 4%-41%) was less than that in the MSD group (36%; 95% CI, 24%-48%), but the difference was not statistically significant ($P = .21$). The rates of grade 3 to 4 acute GVHD were 13% (95% CI, 2%-35%) and 7% (95% CI, 2%-17%), respectively ($P = .41$). Two patients in the RD group and 6 in the MSD group had chronic extensive GVHD, with cumulative incidences of 13% (95% CI, 2%-34%) and 11% (95% CI, 5%-20%), respectively ($P = .78$). At present, all patients are off immunosuppressive medication.

TRM and causes of death

One patient in the RD group and 5 patients in the MSD group died. The cumulative incidences of TRM at 100 days and 1 year, respectively, were 0% and 6% (95% CI, 1%-26%) in the RD group, and 8% and 8% (95% CI, 3%-16%) in the MSD group ($P = .77$). The 1 patient in the RD group that died was on combined immunosuppressive treatment of chronic GVHD with intestinal, liver, and skin involvement. He developed intestinal obstruction due to stenosis, required surgery, and died of postoperative intestinal bleeding. In the MSD group, the causes of death included severe acute GVHD in 1 patient, pneumonia in 2 patients, cardiac arrest during post-transplant splenectomy in 1 patient, and chronic lung GVHD in 1 patient.

Survival

At the time of survival analysis, 15 patients (94%) in the RD group and 61 patients in the MSD group (92%) were alive, with median follow-up durations of 72 months (range, 17-93 months) and 80 months (range, 25-107 months), respectively. The probabilities

Table 2. Clinical and hematologic parameters of patients treated according to Pc 26.1 before and after transplantation

UPN	Age (y)	Risk (class)	Donor	HLA	Treatment protocol	TNC, ($\times 10^8$/kg)	Days to ANC > 0.5 × 10^9/L	Days to PLT > 20 × 10^9/L	Acute GVHD grade	Chimerism (d +60, d +180)	Rejection	Outcome
1	9	2	Aunt	1 Ag# at locus DPB1	Pc26.1	4.4	19	22	2	100 D	No	Alive and cured
2	7	2	Father	Phenoidentical	Pc26.1	1.8	25	32	0	100 D	No	Alive and cured
3	9	3	Sister	1 Ag# at locus DPB1	Pc26.1	3.1	14	16	0	100 D	No	Alive and cured
4	7	2	Cousin	Phenoidentical	Pc26.1	5.2	19	22	0	100 D	No	Alive and cured
5	8	3	Brother	1 Ag# at locus DPB1	Pc26.1	2	20	24	0	100 D	No	Alive and cured
6	15	3	Mother	Phenoidentical	Pc26.1	5.3	18	18	0	100 D	No	Alive and cured
7	12	3	Cousin	Phenoidentical	Pc26.1	2.1	17	32	0	100 D	No	Alive and cured
8	15	3	Mother	1 Ag# at locus DPB1	Pc26.1	5	29	29	0	100 D	No	Alive and cured
9	12	3	Father	Phenoidentical	Pc26.1	6.7	26	50	0	100 D	No	Alive and cured
10	24	3	Father	Phenoidentical	Pc26.1	2.9	16	25	0	100 D	No	Alive and cured
11	6.4	3	Father	Phenoidentical	Pc26.1	4.8	14	29	2	100 D	No	Alive and cured
12	10.3	3	Brother	1 Ag# at locus A	Pc26.1	5.2	20	28	0	100 D	No	Alive and cured
13	1.4	1	Father	Phenoidentical	Pc26.1	10	23	25	0	100 D	No	Alive and cured
14	9	2	Father	Phenoidentical	Pc26.1	4	18	18	0	100 D	No	Alive and cured
15	14	2	Uncle	Phenoidentical	Pc26.1	2.8	21	24	0	100 D	No	Alive and cured
16*	21	3	Mother	Phenoidentical	Pc26.1	3.8	18	22	3	100 D	No	Dead (d +300)

Ag#, antigen mismatch; ANC, absolute neutrophil count; D, % of donor chimerism; PLTs, platelets; TNC, total nucleated cells.
*Cause of death was chronic GVHD gut bleeding.

of OS were 94% (95% CI, 63%-99%) for the RD group and 92% (95% CI, 83%-97%) for the MSD group ($P = .83$). The respective probabilities of TFS were 94% (95% CI, 63%-99%) and 82% (95% CI, 70%-89%), with no statistically significant difference ($P = .24$) (Figure 2).

Regimen-related toxicities and infectious complications

In both groups, the most frequently observed toxicities were grade 2 elevations in aspartate aminotransferase and alanine aminotransferase, followed by grade 2 oral mucositis and diarrhea. No patients developed grade 3 or 4 toxicities. One patient in the MSD group developed moderate hepatic veno-occlusive disease that resolved with supportive care. Six patients in the RD and 10 patients in the MSD group had late-onset hemorrhagic cystitis ($P = .07$; Fisher's exact test). Three patients in the RD and 2 in the MSD group had cyclosporine-related neurotoxicity with seizures.

Gram-positive or gram-negative bacteremia was detected in 10 patients of the MSD group vs 6 patients in the RD group ($P = .07$). Three of the MSD and 1 of the RD group patients had pneumonia. In the RD group, all patients were positive for CMV serology before transplantation, and asymptomatic CMV reactivation occurred in 11 (69%) patients. In the MSD group, pretransplant CMV serology was positive in 58 of 66 patients, and CMV reactivation occurred in 38 (58%) of patients ($P = .57$). All patients with CMV reactivation received preemptive antiviral therapy and none developed CMV disease. Two patients in the RD group showed transient Epstein-Barr virus reactivation with very low viral load, and none developed posttransplant lymphoproliferative disease.

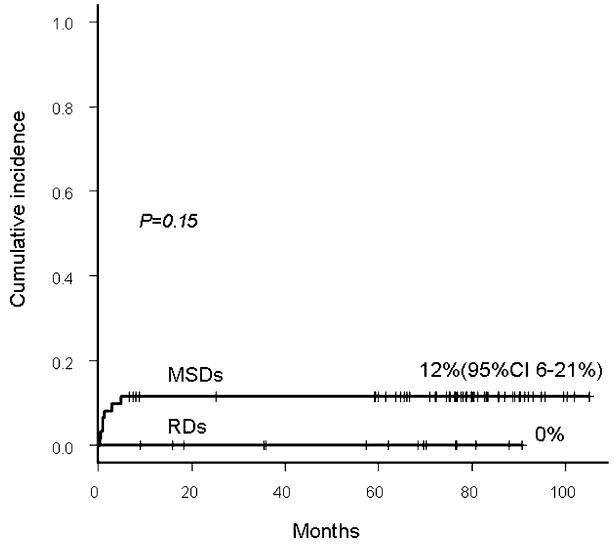

Figure 1. Cumulative incidence of graft rejection following transplantation in the RD and MSD groups.

Discussion

Until recently, the results following transplantation were considered best when the donors are HLA-matched siblings. However, only approximately one-third of patients requiring transplantation will have such a donor available, while the remaining patients will require an alternative donor. Other possible donors can include phenotypically matched or mismatched RDs, unrelated donors, and unrelated cord blood donors. Because most donors in the Bone Marrow Donor Worldwide (BMDW) registry are of European descent, searches for patients of other ethnic backgrounds have a lower success rate, particularly for mixed-race patients. In developing countries, other major obstacles when searching for unrelated donors are the lack of national unrelated donor banks and the high cost of extensive donor screening. Therefore, patients without a

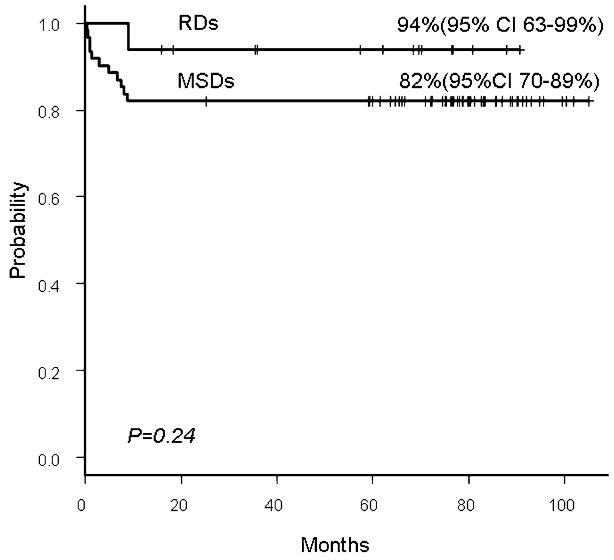

Figure 2. Kaplan-Meier probability of TFS following transplantation in the RD and MSD groups.

MSD could benefit from transplantation from an HLA-matched related nonsibling donor who is either phenotypically identical or 1-antigen mismatched. In certain areas of the world where large families are common, the probability of finding an HLA-matched RD is similar to that of finding a matched unrelated donor in the Western hemisphere.[11,12] Few studies have evaluated the role of related phenotypically HLA-matched or 1-antigen–mismatched donor transplantation in hematologic malignancies, and the results of these studies are conflicting. Some found comparable outcomes for related phenotypically HLA-matched or 1-antigen–mismatched donors and MSD,[25] while others found inferior results compared with HLA-matched siblings[15,26] but comparable outcomes with matched unrelated donors.[27-29] In 2000, we retrospectively evaluated outcomes of thalassemia treatment by transplantation from alternative donors using a standard conditioning regimen with BUCY ± antilymphocyte globulin.[16] Outcomes were poor with high rates of rejection and GVHD and low TFS rates. These findings clearly showed that the conditioning regimens used were not adequately myeloablative and immunosuppressive to ensure a high sustained engraftment rate, prevent severe GVHD, and promote a high TFS probability. Therefore, we adopted a new intensified treatment protocol for alternative donor transplantation in thalassemia.

To clarify the role of transplantation with the use of phenotypically matched or 1-antigen–mismatched RDs in the current era, we compared the outcomes of BMT with the use of these donors with HLA-matched sibling BMT. The 2 groups we analyzed were homogeneous in terms of disease and demographic characteristics. The main finding of our present study was that the new preparative regimen effectively prevented graft rejection in patients who received BMT from RDs who were not HLA-matched siblings, although most of these patients were in class 3 of risk who are considered to be at high risk of both rejection and TRM. The high sustained engraftment rate translated into a higher TFS rate of 94%. Significantly, the TFS observed in our patients was higher than previously reported results using related other than HLA-MSDs and was similar to that observed after HLA-MSD transplantation. A previous report from the International Bone Marrow Transplant Registry analyzed transplant outcomes in 60 patients with thalassemia who received bone marrow grafts from genotypically HLA-nonidentical RDs following conditioning with BUCY ± others. For 39 patients, information was available concerning the degree of HLA matching; 24 had phenotypically matched parental donors, and 11 had 1-antigen–mismatched and 4 had 2-antigen–mismatched RDs. TFS survival was 47%, with a 25% mortality rate.[30] A recent French multicenter retrospective study showed that of 6 thalassemia patients who received transplant from related phenotypically HLA-matched donors following BUCY ± ATG conditioning, only 1 patient was alive without thalassemia.[31] These data clearly demonstrate that, in terms of rejection and TFS, the present preparative regimen is superior to standard BUCY ± ATG/antilymphocyte globulin regimens for patients with thalassemia who receive alternative RD transplantation.

GVHD is one of the major complications that occur after 0- to 1-antigen–mismatched RD transplantation. The incidence of grade 2 to 4 acute GVHD was lower in the RD group than in the MSD group, but the difference was not statistically significant. The relatively low incidence of acute GVHD in our patients likely related to ATG use in the conditioning regimen, which is known to effectively reduce the rate of GVHD.[32]

Five donors in our study had a 1-antigen mismatch: 4 at locus DPB1 and 1 at locus A. Accumulated data indicate that hematopoietic cell transplantation from an HLA-DPB1–mismatched unrelated donor is associated with an increased risk of acute GVHD.[33] In thalassemia patients receiving matched unrelated donor transplantation, an HLA-DPB1 disparity in host-vs-graft direction was associated with increased incidence of rejection.[34] Moreover, a recent analysis of 627 HLA-matched sibling transplants (30 HLA-DPB1 mismatched), HLA-DPB1 mismatch was an independent risk factor for GVHD.[35] Among our 5 patients, only 1 with DPB1 mismatching developed grade 2 acute GVHD.

The present treatment protocol resulted in lower treatment-related mortality at day 100 and at 1 year, probably partly due to the PK-guided targeted i.v.Bu used in most patients.[36]

Despite the preexisting disease and treatment-related organ damage in the present patient group, the intensified preparative regimen was well tolerated and no significant toxicity was observed. Treatment-related toxicities were similar between the 2 patient groups. None of the patients experienced grade 4 toxicity.

The data from the present study clearly show that thalassemia patients treated with Pc 26.1 and receiving RD transplant have similar outcomes (rates of OS, TFS, GVHD, and TRM) to recipients of MSD transplantation. These findings are significant because they expand the availability of the treatment to more patients.

The present study is limited by the relatively small number of patients analyzed. However, this study was conducted on a homogenous group in terms of disease and treatment, which allows us to draw useful conclusions about the efficacy of Pc 26.1 in patients with thalassemia who undergo BMT from donors who are not HLA-matched siblings.

In conclusion, the novel treatment protocol Pc 26.1 effectively and safely prevented graft rejection and ensured a high TFS rate in patients who received BMT from RDs who were not HLA-matched siblings. Our data show that the use of phenotypically matched or 1-antigen–mismatched RDs results in outcomes similar to the use of MSD grafts. Importantly, the intensification of the treatment was not associated with increased nonhematologic toxicity, even though these patients suffer from preexisting organ damage due to iron overload and/or hepatitis. Our data support the use of related HLA-phenotypically matched or 1-antigen–mismatched donors as an acceptable alternative to matched

unrelated donors in patients with thalassemia. This finding is particularly important in certain areas where an unrelated donor search is impossible due to a lack of registries and/or prohibitive costs. Based on our results, we urge transplant physicians to conduct extended family studies in populations where consanguinity is common and the patient's haplotypes present at high frequency.

Acknowledgments

The authors thank the nursing staff for their dedication to the care of patients, and both the patients and the patients' families for participating in this study.

Authorship

Contribution: J.G. designed and performed the research, analyzed the data, performed the statistical analyses, and wrote the manuscript; G.L. designed the research and analyzed the data; and M.M., A.I., P.S., K.P., C.G., M.A., M.T., G.D.A., C.A., L.C., M.R., and D.A. performed the research.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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2013 122: 2751-2756
doi:10.1182/blood-2013-07-513473 originally published online August 20, 2013

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.