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Nonmyeloablative Peripheral Blood Haploidentical Stem Cell Transplantation for Refractory Severe Aplastic Anemia

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A B S T R A C T

New transplant approaches are urgently needed for patients with refractory severe aplastic anemia (SAA) who lack a matched sibling or unrelated donor (UD) or who have failed UD or cord blood transplant. Patients with refractory SAA are at risk of later clonal evolution to myelodysplastic syndrome and acute leukemia. We report our pilot findings with haploidentical hematopoietic stem cell transplantation (haploHSCT) using uniform reduced-intensity conditioning with postgraft high-dose cyclophosphamide in 8 patients with refractory SAA or patients who rejected a prior UD or cord blood transplant. Six of 8 patients engrafted. Graft failure was associated with donor-directed HLA antibodies, despite intensive pre-HSCT desensitization with plasma exchange and rituximab. There was only 1 case of grade II skin graft-versus-host disease. We show that haploHSCT can successfully rescue refractory SAA patients who lack donor-directed HLA antibodies but not in the presence of donor-directed HLA antibodies. This novel protocol for haploHSCT for SAA has been adopted by the European Group for Blood and Marrow Transplantation Severe Aplastic Anaemia Working Party for a future noninterventional, observational study to further evaluate its efficacy.

INTRODUCTION

Patients with severe aplastic anemia (SAA) who lack a matched sibling or unrelated donor (UD) and who fail to respond to immunosuppressive therapy are at increased risk of death from infection and hemorrhage and later clonal evolution to myelodysplastic syndrome and acute myeloid leukemia. Risk factors for myelodysplastic syndrome/acute myeloid leukemia include older age, short telomeres, presence of monosomy 7, prolonged use of granulocyte colony-stimulating factor (G-CSF), and multiple courses of immunosuppressive therapy [1-5]. Hence, there is a need to explore alternative donor hematopoietic stem cell transplantation (HSCT) using either a haploidentical family donor [6-12] or cord blood [13,14], because this is a potentially curative option. However, most studies report poor outcomes, due to poor engraftment, and high risk of graft-versus-host disease (GVHD) with haploidentical HSCT (haploHSCT). Attractions of haploHSCT are graft availability for most patients, less expensive procedural cost than cord HSCT, and shorter time to procurement of the graft. However, published data are limited and mostly restricted to children and young adults [7-11].

Post-transplant cyclophosphamide (CY) has been used in haploHSCT for hematological malignancies to selectively deplete donor alloreactive T cells and thereby reduce acute GVHD [15,16]. In the setting of aplastic anemia, high-dose CY without stem cell support can salvage a significant proportion of refractory patients, but this approach has not been met with widespread enthusiasm because of prolonged pancytopenia after therapy [17]. A nonmyeloablative regimen with postgraft CY was reported in 2 patients with hemolytic paroxysmal nocturnal hemoglobinuria and 1 with both paroxysmal nocturnal hemoglobinuria and sickle cell disease, resulting in sustained engraftment and absence of GVHD in 2 patients [11]. In 2011, Dezern et al. [18] reported the first use of post-transplant CY for GVHD prophylaxis in 2 high-risk SAA patients. They used matched sibling donors and myeloablative conditioning, with both patients engrafting and no GVHD [18]. The Hopkins group also reported 17 cases using the mini-haploidentical approach with post-transplant CY in sickle cell disease. There were no cases of mortality or GVHD, but primary graft failure was a problem [19].

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In this study we used a uniform conditioning regimen using reduced-intensity conditioning with post-transplant CY for haploHSCT in 8 SAA patients, 4 with refractory SAA and 4 after failed UD or cord blood HSCT. We also used G-CSF mobilized peripheral blood stem cells (PBSCs) instead of bone marrow (BM) to achieve a high dose of infused $CD34^+$ cells for engraftment.

METHODS

Eight patients were transplanted, 4 had refractory acquired SAA and 4 failed to engraft after UD (n = 3) or cord blood (n = 1) HSCT. Of the 3 patients who had failed previous UD HSCT, 2 had failed 2 previous UD transplants. Six of 8 patients had acquired idiopathic SAA. Two patients had secondary severe marrow aplasia, 1 after chemotherapy for Hodgkin disease and who subsequently failed UD HSCT and 1 who failed 2 UD HSCTs for myelodysplastic syndrome. Median age was 32 years (range, 19 to 57) and median disease duration 46 months (range, 6 to 91). Family donors were siblings (n = 5), parents (n = 2), and children (n = 1), and HLA matching was 5/10 (n = 6) and 6/10 (n = 2). Five patients were included in the previously reported multicenter series [20].

Conditioning regimen was fludarabine 30 mg/m^2 (days −6 to −2), CY 14.5 mg/kg (days −6 and −5), and total body irradiation 2 Gy (day −1). Unmanipulated G-CSF–mobilized PBSCs were infused at a median $CD34^+$ cell dose of 6. 2×10^6/kg (range, 1.8 to 8.3). GVHD prophylaxis was CY 50 mg/kg/d (days +3 and +4), tacrolimus for 9 months maintaining trough drug at 10 to 15 μg/L with tapering between 9 and 12 months, and mycophenolate 15 mg/kg until day +35.

Median follow-up of survivors was 14.8 months (range, 7.2 to 44.4). Median Karnofsky and HCT-comorbidity index scores were 80% (range, 50 to 90) and 3 (range, 0 to 5), respectively. Neutrophil and platelet engraftment and acute and chronic GVHD were defined as previously reported [21]. Chimerism was assessed in unfractionated BM and peripheral blood $CD3^+$ T cells and $CD15^+$ granulocyte populations. Full donor chimerism was defined as >95% donor hematopoietic cells and mixed chimerism as 5% to 95% cells [21,22]. All patients were screened for cytomegalovirus, Epstein-Barr virus, and adenovirus at twice weekly intervals for the first 3 months. HLA antibodies were measured routinely pretransplant in all patients using microbead flow cytometry assay with mean fluorescence intensity read-out for serum antibody level.

RESULTS

Six patients had sustained neutrophil engraftment; median time to neutrophil engraftment was 18.5 days (range, 16 to 23), and 5 sustained platelet engraftment with a median time of 26 days (range, 21 to 27) (Table 1). Full donor chimerism in unfractionated cells and CD3 and CD15 lineages was achieved and maintained at last follow-up (Table 2). These results are different from the pattern of chimerism as previously reported from our group using fludarabine, CY, alemtuzumab (FCC) conditioning regimen for SAA patients transplanted from sibling or matched UDs, where a high incidence of mixed T cell chimerism in the presence of full donor myeloid chimerism was observed, associated with a very low incidence of chronic GVHD [22]. We therefore compared BM trephine cellularity at days +28 and +100 and at 1 year after haploHSCT with results using FCC conditioning for sibling and UD HSCT from our center. As seen in Supplementary Figure 1, the median BM cellularity at each time point was not significantly different between the 2 groups.

Two patients (patients 6 and 8) who failed to engraft died on days +60 and +137 from sepsis. Both had multiple HLA antibodies directed against the donor which persisted at high level despite treatment with rituximab (375 mg/m^2 weekly for 4 weeks) and plasma exchanges pretransplant (Figure 1A,B). In contrast, sustained engraftment occurred in patients with no HLA antibodies. For patient 6, there was a reduction in the HLA-B51, donor-directed, antibody level, but despite this the patient failed to engraft. The second patient with multiple donor-directed HLA antibodies achieved a significant reduction in antibody levels for 3 antibodies but not for HLA-DR53 specific antibody.

Cytomegalovirus and Epstein-Barr virus viremia occurred in 2 and 5 patients, respectively, but no cases of cytomegalovirus disease or Epstein-Barr virus post-transplant lymphoproliferative disorder occurred. There was no adenovirus viremia. One patient developed Guillain-Barré syndrome (patient 2) with no viral pathogens identified. No response to intravenous immunoglobulin occurred, but some improvement was found with plasmapheresis. During this episode the patient required a prolonged period of intubation during a 92-day intensive care unit admission, which was complicated by recurrent respiratory infections with multiple organisms. At the time of death he was independent of platelet transfusions with the use of eltrombopag and required RBC transfusion approximately 6 weekly.

There was only 1 case of acute GVHD (grade II skin) and no chronic GVHD; however, follow-up was short, with a median follow-up of patients of 12.2 months (range, 3.2 to 40.4). The conditioning regimen was well tolerated, with no hemorrhagic cystitis. One patient (patient 3) delivered a healthy baby at term on day +560 post-transplant. The observation that this regimen does not appear to cause gonadal failure is supported by a previous report of 2 successful pregnancies using this regimen [23].

DISCUSSION

We show in a small cohort of patients with aplastic anemia that haploHSCT using a uniform, nonmyeloablative conditioning regimen with post-transplant CY and with PBSCs as stem cell source rather than BM is a feasible option. Remarkably, the procedure was able to rescue all 4 patients who had primary graft failure after a matched UD transplant or cord blood HSCT, including 2 patients with nonengraftment after 2 unrelated grafts. Furthermore, our patients had a high HCT-comorbidity index and poor performance status. This series includes 2 patients with secondary aplasia, 1 due to chemotherapy for Hodgkin lymphoma. Although the mechanism underlying the marrow failure in these 2 cases is different from those with idiopathic SAA, these cases also illustrate the efficacy of this approach after failed matched UD or cord blood donor HSCT in the presence of severe pancytopenia and a hypocellular BM.

In contrast to our previous finding of mixed T cell chimerism with sustained myeloid engraftment using alemtuzumab-based conditioning for matched sibling and UD HSCT for SAA, in this study we observed sustained donor T cell and myeloid engraftment in all assessable patients. We used G-CSF–mobilized stem cells in preference to BM cells to optimize the infused stem cell dose for halpoHSCT. Stem cell dose is especially important in SAA, where there is an inverse correlation between stem cell dose and graft rejection [24]. The concern with PBSCs of an increased risk of GVHD was not borne out in our small series. We previously reported a high incidence of mixed T cell chimerism with sustained myeloid engraftment and a low incidence of chronic GVHD in SAA patients transplanted from matched sibling donors or UDs using the FCC conditioning regimen [22]. In contrast, in this study of haploHSCT, we showed full donor T cell and myeloid chimerism in engrafted patients. We therefore were interested to know if hematopoietic recovery was associated with a higher degree of BM cellularity after haploHSCT compared with FCC HSCT. However, we showed no significant difference between the 2 groups, but the patient numbers in the

Table 1
Patient Characteristics and Outcomes

Patient No., Age (yr), Gender/Donor	Disease and Severity	Previous Therapy for Aplasia Pre-HaploHSCT	Previous HSCT and Outcome	HCT-CI/ Karnofsky Score	CD34 Stem Cell Dose ($\times 10^6$/kg)	Engraftment (Neutrophil and Platelet)	GVHD (Acute and Chronic)	Median Follow-Up (mo)/Survival Status
1, 19, F/mother	SAA-MDS	ATG + CSA × 2, NR ATG + MMF, PR, relapse MMF-NR	Double cord-GF	0/80	6.7	Day +18 Day +21	None	40.4/alive
2, 51, M/sister	Therapy-related severe aplasia for Hodgkin disease	ATG + CSA, NR	MUD-GF	3/70	4.5	Day +18 No platelet engraftment	None	22/dead Sepsis
3, 23, F/sister	SAA/hPNH	ATG + CSA, PR, relapse	None	3/80	5.8	Day +16 Day +26	Grade II acute GVHD Skin	12.2/alive
4, 57, F/brother	SAA/hPNH	ATG + CSA, CR, relapse, oxymetholone, eculizumab eltrombopag	MUD-GF MUD-GF and Epstein-Barr virus PTLD	5/50	4.9	Day +20 Day +27	None	9.3/alive
5, 22, M/brother	VSAA	ATG + CSA, NR	None	1/90	6.9	Day +23 Day +27	None	3.2/alive
6, 20, M/father	VSAA	CSA, NR ATG + MMF, NR oxymetholone, NR danazol, NR	None	1/90	6.7	Non-engraftment HLA antibody positive	None	2.1/dead Graft failure/sepsis
7, 50, M/son	Therapy-related severe aplasia for MDS-RCMD	None	MUD-GF MUD-GF	4/50	8.3	Day +19 Day +25 100%	None	33.4/alive
8, 41, M/mother	VSAA/PNH	ATG + CSA × 2, CR, relapse × 2, oxymetholone, PR, relapse, eculizumab	None	5/80	1.8	Nonengraftment HLA antibody positive	None	3.1/dead

HCT-CI indicates HCT comorbidity index; MDS, myelodysplastic syndrome; ATG, antithymocyte globulin; CSA, cyclosporine A; NR, no response; MMF, mycophenolate; PR, partial response; GF, graft failure; MUD, matched unrelated donor; hPNH, hemolytic paroxysmal nocturnal hemoglobinuria; PTLD, post-transplant lymphoproliferative disease; VSAA, very severe aplastic anemia; RCMD, refractory cytopenia with multilineage dysplasia; CR, complete response.

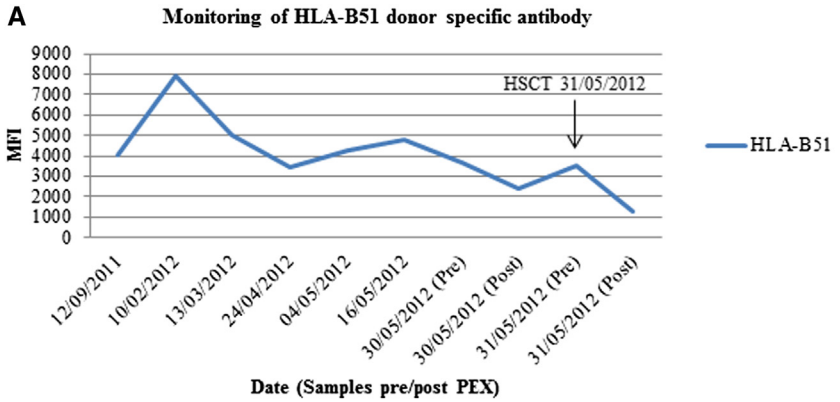
Table 2
Results of Chimerism

Days Post-Transplant	No. Patients Chimerism Tested (Total Assessable)	UF	(Range)	CD3	(Range)	CD15	(Range)
28	6 (8)	100	(99-100)	100	(100-100)	100	(98-100)
56	6 (7)	99	(98-100)	100	(99-100)	99	(98-100)
100	6 (6)	100	(99-100)	100	(99-100)	100	(99-100)
180	5 (6)	100	(96-100)	99	(96-99)	100	(96-100)
365	4 (4)	100	(99-100)	100	(99-100)	100	(100-100)

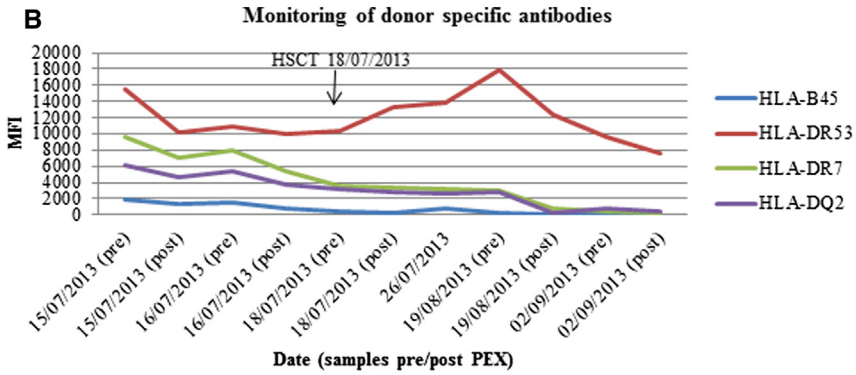
UF indicates unfractionated.
Values in the last 3 columns are median percentages.

haploHSCT group were small, and more patients need to be evaluated.

For both haplo and cord blood HSCT, the presence of recipient HLA antibodies directed against the donor is associated with a high risk of graft rejection [15,16]. Most studies of haploHSCT for SAA do not report routine assessment of donor-directed HLA antibodies. High levels of donor-directed anti-HLA alloantibodies most likely explain the nonengraftment in 2 patients. Intensive desensitization with plasma exchanges and rituximab was insufficient to deplete the



HLA Type	HLA-A	HLA-B	HLA-Cw	HLA-DRB1	HLA-DQB1
Recipient	01:01/29:02	58:01/ 55:01	07:01/ 01:02	03:01/ 14:04	05:03/02:01
Donor	31:01/29:01	58:01/ 51:01	07:01/ 14:02	13:01/ 14:04	05:03/06:03



HLA Type	HLA-A	HLA-B	HLA-Cw	HLA-DRB1	HLA-DQB1
Recipient	23:01;30:01	41:01;42:02	17:01;08:02	11:01	06:02;03:19
Donor	02:01;23:01	45:01;41:01	08:02;41:01	11:01;07:01	06:02;02:02

Figure 1. (A) Patient 6. Changes in HLA antibody levels as measured by the mean fluorescence index (MFI). Recipient and donor HLA typing are shown in the table at the bottom. Before HSCT, this patient had multiple class I HLA antibodies, of which only HLA-B51 was donor directed. After intensive plasma exchanges and rituximab, the antibody level fell from a peak of 7946 MFI to 1304 immediately pre-HSCT. PEX indicates plasma exchange. (B) Patient 8. Changes in HLA antibody levels as measured by MFI. Recipient and donor HLA typing are shown in table at the bottom. Pretransplant, this patient had class I and II HLA antibodies, all of which were donor directed: HLA-B45, -DR7, -DQ2, and -DR53. (HLA-DR53 is directed against a protein encoded by the *DRB4* gene, which is in a haplotype with the DRB1*07:01.) After plasma exchanges and before HSCT, there was a further rise in the HLA-DR53 antibody, likely due to granulocyte transfusions given for invasive fungal infection.

antibodies sufficiently to prevent graft rejection. Despite universal leucodepletion of blood products, HLA alloimmunization remains a problem in up to 30% of patients with aplastic anemia [25,26]. In the setting of HLA matched sibling HSCT, this is due to minor histocompatibility antigens on leucocytes and red cell, and in mismatched HSCT, due to HLA antigens on leucocytes and platelets [27]. An HLA antibody level of 1500 mean fluorescence intensity is associated with graft rejection in the setting of T cell–depleted haploHSCT and >2500 in the setting of T cell–depleted matched UD HSCT due to anti-DPB1 HLA antibodies [28,29]. In our series, for HLA-B51 and HLA-DR53, graft rejection occurred at levels of 1304 and 7621, respectively. Other factors contributing to graft rejection in patient 8 were older donor age (mother's age of 68 years) [30], a low stem cell dose (1.8×10^6 CD34 cells/kg), and a female multiparous donor [28]. It may be that transplanting a much higher CD34 dose would aid engraftment in such cases, but this would potentially increase the risk of acute and chronic GVHD. Thus, for future cases, we agree that routine screening for HLA antibodies is essential pretransplant and, if present, as has been recommended for patients with hematological malignancies, an alternative donor lacking HLA antigen(s) against which recipient HLA antibody (ies) are directed should be used [16]. PBSCs have also recently been used for reduced-intensity haploHSCT with postgraft CY in the setting of hematological malignancy with no adverse impact on GVHD or survival compared with BM [20,31].

In our study of SAA we showed that haploHSCT using postgraft high-dose CY and PBSCs results in sustained engraftment and with minimal GVHD for those patients who lack donor-directed HLA antibodies. However, the presence of donor-directed HLA antibodies was associated with primary engraftment failure, despite attempts to remove the antibodies pretransplant with rituximab and plasma exchange. Because SAA patients have a particularly high risk of HLA alloimmunization, it is essential that all patients are screened pre-HSCT before considering such an approach, because the presence of donor-directed HLA antibodies should preclude the use of that donor.

We propose that this approach to haploHSCT now warrants further exploration in a larger cohort of SAA patients. The European Group for Blood and Marrow Transplantation Severe Aplastic Anaemia Working Party has adopted this protocol for a future noninterventional, observational prospective study of haploHSCT throughout Europe.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2014.06.028.

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