Bone Marrow Transplantation (2011) 46, 1581–1582
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LETTER TO THE EDITOR

Successful hematopoietic SCT from non-identical twins to two sisters with β-thalassemia major by using preimplantation genetic diagnosis and HLA typing

Bone Marrow Transplantation (2011) **46,** 1581–1582; doi:10.1038/bmt.2010.344; published online 17 January 2011

Current therapy protocols, including regular blood transfusion and chelation, have considerably increased the survival and quality of life for patients with thalassemia, although hematopoietic SCT (HSCT) still remains the only available curative option. However, only 25–30% of patients have an HLA-compatible family donor. Preimplantation genetic diagnosis (PGD) is a method for the diagnosis of genetic diseases in the early embryonic period so that implantation of affected embryos can be avoided, and the potential need for termination of pregnancy is eliminated in families who have genetic diseases. PGD also may offer an HLA-genoidentical sibling donor for an affected child. Recently, a few successful HSCTs have been reported in some genetic diseases using HLA-matched siblings born with PGD.[1–4] Here, we report, as the first case in the literature as far as we know, two HLA non-identical siblings with β-thalassemia major (TM) who underwent HSCT from dizygotic twin siblings born after PGD.

Case 1: A 15-year-old girl suffering from TM was admitted to our hospital for HSCT. She had been treated with regular RBC transfusions and chelation therapy. She was assigned to class II according to classification proposed by Pesaro group.[5] She was treated with i.v. BU (12.8 mg/kg), CY (200 mg/kg) and anti-thymocyte globulin (ATG) (10 mg/kg/day for 3 days) as the conditioning regimen. GVHD prophylaxis was attempted by administering CsA and MTX. She underwent HSCT from her HLA-identical healthy brother using cord blood (CB) as well as BM as stem cell sources. The cell counts of the CB unit assessed before cryopreservation were 0.29×10^7 nucleated cells and 0.05×10^5 CD34+ cells per kg of recipient weight. Because of low-cell counts with expected risk of graft failure, BM from the same donor was used in addition to the CB unit. The number of infused nucleated cells was 0.15×10^7/kg, and $CD34^+$ cell number was 0.024×10^5/kg. The viability of the cells in CB was 75%. The BM infused on the same day provided 2.1×10^8 nucleated cells/kg and 4.7×10^6 CD34+ cells/kg. She achieved successful neutrophil and platelet engraftment on day 15 and 25, respectively. The chimerism evaluations done by using interphase fluorescence in situ hybridization yielded 92% of donor cells at the end of first month after transplantation, increasing to 97% by the end of 6 months and to 100% by the end of first year. She has been continued as transfusion free without any complications with 18 months follow-up.

Case 2: An 18-year-old girl diagnosed with TM had been regularly transfused for 15 years. She was admitted to our hospital for HSCT from her HLA-matched sibling, born after PGD. She had hepatomegaly of 3 cm below the right costal margin and was splenectomized. Liver biopsy revealed hepatic fibrosis, and she was assigned as class III Pesaro classification. She received the Pesaro 26 protocol for conditioning. Preparative regimen consisted of i.v. BU 11.2 mg/kg, CY 90 mg/kg and ATG 10 mg/kg once daily on −1 to +1. CsA and MTX were used for GVHD prophylaxis. As the cell counts of the CB unit were markedly lower than the minimum required for engraftment, and the weight difference between recipient and the donor was significant, G-CSF-mobilized BM was collected from the donor with approval of the parents. The total nucleated cells (TNC) and $CD34^+$ cell counts of the CB unit after thawing were 0.01×10^7 and 0.007×10^5 per kg of recipient body weight, respectively. G-CSF was administered to donor at the dosage of 10 μg/kg/day for 5 consecutive days and BM with 1.96×10^6 CD34+ cells/kg, and 3.6×10^8 TNC/kg was collected. Neutrophil and platelet engraftment occurred on day 13 and day 22, respectively. She experienced CMV reactivation and intra-atrial thrombus that was successfully treated with anticoagulant treatment. The chimerism evaluation with STR showed presence of 100% of donor cells at the end of the first month after transplantation. Although the percentage of donor cells decreased to 79% by the end of the third month, she has been well and transfusion free, with 5 months follow-up.

Following confirmatory molecular genetics analyses and HLA pattern tests for the family, the parents underwent standard *in vitro* fertilization (IVF) procedures. Then, as a part of the PGD procedure, single blastomeres were removed from the third day embryos; briefly, HLA typing and the mutation screening of β-globin gene were performed on preimplantation embryos by single-cell multiplex PCR using informative STR markers in the HLA locus together with the gene region containing the mutations of β-globin gene. Owing to HLA-incompatible embryos, the parents underwent second IVF cycle. According to PGD combined with HLA-typing results, embryos that were possible donors of HSCT for their affected sisters were selected and transferred, and twin pregnancy resulted. Healthy twins were delivered, each of whom were donors for each of their affected sisters.

Prenatal diagnosis has been used extensively worldwide to prevent the birth of a child with a genetic disease. It is well known that successful results have been obtained for

some genetic diseases such as β-TM. However, potential termination of pregnancy can be a major limiting factor for some parents with an affected fetus, because of religious or cultural objections. PGD presents a good alternative option to prenatal diagnosis in this field. Furthermore, it may also provide an HLA-compatible sibling donor for treating the affected child with HSCT. As the first successful allogeneic HSCT was reported in 1982, a great many thalassemic patients have been cured after an allograft in most cases from an HLA-matched family donor.[6,7] Using an HLA-identical sibling donor, survival is excellent for young children who are in a low-risk group. Unfortunately, only 25–30% of thalassemic patients have an HLA-compatible sibling, meaning that the vast majority of patients lack a family donor. Although alternative stem cell sources, such as unrelated donor or CB, have been used in some reports of β-thalassemia patients, results are less favorable with a higher graft rejection and GVHD ratio. Additionally, the main limiting factor for unrelated CB transplantation is the limited number of stem cell counts. Also, ~25% cell loss was reported after thawing of CB units.[8] Therefore, it seems beneficial to add some BM from the same donor to avoid graft failure, which it is not possible with unrelated CB.

Parents who already have a child affected with β-TM would like to ensure that their next children will be healthy, while at the same time serving as an HLA-identical donor for their affected child. PGD combined with HLA typing seems an attractive option to achieve this aim. PGD applications resulting in successful HSCT in the affected sibling have, although limited, been reported in literature.[3,9,10] However, we believe that our cases are the first in the world to use dizygotic twin sibling donors, born after PGD, for their two thalassemic sisters. Unfortunately, the stem cell numbers in the CBs were poor; therefore stem cell collections from BM were added to supplement this. Our patients were 15 and 18 years old when HSCTs were performed. Thus, to obtain adequate cell numbers, BM harvesting and HSCT were done when the donors were aged 3 and 4 years, respectively. Now, both sisters are alive and transfusion free after 18 and 5 months of transplant.

In vitro fertilization and preimplantation selection of HLA-compatible healthy embryos are encouraging for couples with an affected child to initiate a pregnancy that will be able to provide stem cells for HSCT. However, there is no consensus worldwide as to whether it should be authorized, although significant ethical, legal and policy issues have been raised by this procedure. In many countries, PGD is either regulated liberally or not at all, and the practice is generally policed on a case-by-case basis, even permitting non-medical applications like gender selection. At the other extreme, in some countries, all the interventions with embryos including PGD are forbidden or restricted. In our country, PGD with HLA typing is allowed and reimbursed by the social security system, if a family has a genetically affected child without an HLA-compatible family donor. Another important issue is the ethical aspect of PGD for HLA compatibility with selection of an embryo as a donor. Discarding other embryos only because they are not HLA compatible with the candidate and also the risk for the future child being valued only as a donor and being neglected after birth may obviously raise ethical concerns. However, the desire for saving a life with less burden for parents compared with other alternatives such as natural conception, followed by PGD and termination, if the fetus is sick, seems reasonable. Aside from these debates, this form of prenatal diagnosis is a viable alternative for couples with religious, ethical and moral objections to induced abortions and who do not wish to face the associated emotional disappointments and risk of repeated cycles of conception, followed by termination of pregnancy. We believe that PGD with HLA matching offers a life-saving chance for patients with genetic disease such as β-TM who need HSCT but lack an HLA-genoidentical donor. However, it should be performed under strict supervision of national and ethics committees.

Conflict of interest

The authors declare no conflict of interest.

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References

1 Reichenbach J, Van de Velde H, De Rycke M, Staessen C, Platteau P, Baetens P *et al.* First successful bone marrow transplantation for X-linked chronic granulomatous disease by using preimplantation female gender typing and HLA matching. *Pediatrics* 2008; **122**: 778–782.
2 Bielorai B, Hughes MR, Auerbach AD, Nagler A, Loewenthal R, Rechavi G *et al.* Successful umbilical cord blood transplantation for Fanconi anemia using preimplantation genetic diagnosis for HLA-matched donor. *Am J Hematol* 2004; **77**: 397–399.
3 Qureshi N, Foote D, Walters MC, Singer ST, Quirolo K, Vichinsky EP. Outcomes of preimplantation genetic diagnosis therapy in treatment of beta-thalassemia: a retrospective analysis. *Ann N Y Acad Sci* 2005; **1054**: 500–503.
4 Goussetis E, Konialis CP, Peristeri I, Kitra V, Dimopoulou M, Petropoulou T *et al.* Successful hematopoietic stem cell transplantation in 2 children with X-linked chronic granulomatous disease from their unaffected HLA-identical siblings selected using preimplantation genetic diagnosis combined with HLA typing. *Biol Blood Marrow Transplant* 2010; **16**: 344–349.
5 Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baronciani D, Giardini C *et al.* Bone marrow transplantation in patients with thalassemia. *N Eng J Med* 1990; **322**: 417.
6 Gaziev J, Lucarelli G. Stem cell transplantation for hemoglobinopathies. *Curr Opin Pediatr* 2003; **15**: 24–31.
7 Yesilipek MA. Stem cell transplantation in hemoglobinopathies. *Hemoglobin* 2007; **31**: 251–256.
8 Karasu G, Uygun V, Kurt P, Ozturk Z, Kupesiz A, Hazar V *et al.* A. Viability and cellular recovery in cord blood transplantation: 5th Congress of the International Pediatric Transplant Association. *Pediatr Transplant* 2009; **13**(Supp 1): 151.
9 Kuliev A, Rechitsky S, Tur-Kaspa I, Verlinsky Y. Preimplantation genetics: improving access to stem cell therapy. *Ann N Y Acad Sci.* 2005; **1054**: 223–227.
10 Handyside A. Let parents decide. *Nature* 2010; **464**: 978–979.