

T cell-depleted hla-haploidentical stem cell transplantation in thalassemia young patients

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Introduction

The cure for thalassemia involves correcting the genetic defect in a hematopoietic stem cell that results in reduced or absent β -globin synthesis and an excess of α -globin dimers. Intracellular precipitation and accumulation of α -dimers results in ineffective erythropoiesis and hemolytic anemia. Replacing the abnormal thalassaemic marrow with allogeneic normal or heterozygous stem cells carrying the functional gene restores appropriate β -globin chain synthesis. Eighty to ninety per cent of patients transplanted from an HLA-identical sibling or parent become ex-thalassaemic after transplant.^{1,2} Haploidentical hemopoietic stem cell transplantation has been explored as an option for treating patients with leukemia who lack an HLA-identical sibling or parent donor. However, severe graft versus host disease and high graft failure/rejection rates have limited the application of this transplant modality for patients with thalassemia. Advances using high doses of T cell-depleted peripheral blood stem cells (PBSCs) and intensive pre-transplant conditioning regimens have helped to overcome these limitations. Grafts containing mega doses of enriched CD34⁺ progenitor cells can be achieved by combining bone marrow with G-CSF-mobilized PBSCs. Thereafter, T-cells can be removed

by positive selection for CD34. Limiting the numbers of CD3⁺ cells in the graft might allow retention of rapid engraftment kinetics provided by the mobilized PBSCs while reducing the risk of extensive GVHD. In this pilot study, we used a similar approach involving mega dose haploidentical positively selected CD34⁺ marrow and peripheral hematopoietic stem cell transplantation to treat patients with thalassemia who lack an HLA-identical familial or unrelated marrow donor. Positive selection of CD34⁺ stem cells results in an approximately 3-4 log reduction of CD3⁺ cells, which reduces the risk of GVHD but increases the risk of graft failure.³⁻⁵ Adding a defined dose of CD3⁺ marrow cells to the cellular suspension at the time of transplant can help to reduce the graft rejection rate. In contrast to positive selection of stem cells, marrow graft depleted of CD3⁺ and CD19⁺ cells contains significant amounts of monocytes, NK cells, dendritic cells, precursor T-cells, and other cell types that may play important roles in engraftment while accelerating the post-transplant immune reconstitution. Therefore, in a second prospective phase of this pilot study, we evaluated the use of haploidentical CD3⁺/CD19⁺-depleted marrow graft combined with CD34-selected mobilized PBSCs and CD3⁺ marrow cells that were added back at the time of infusion.^{6,7}

Here, we report the outcomes of 31 children with thalassemia who were transplanted from haploidentical donors, 27 was mothers, two brothers and two fathers.^{8,9}

Graft processing and transplant procedures

All donors received recombinant human G-CSF 15 μ g/kg/day in two daily subcutaneous boluses to mobilize PBSCs. CD34⁺ cells from leukapheresis and bone marrow harvests were selected using the CliniMACS one-step procedure (Milteny Biotech, Germany) for 14 donors. Two-step selection (CD34 positive selection leukapheresis followed by negative selection using anti-CD3 and anti-CD19 monoclonal antibodies) of bone marrow cells was employed for eight donors. We attempted to suppress erythropoiesis by intensive hypertransfusion and chelation. Between day -59 and day -11 before the transplantation, 40 mg/kg deferoxamine was continuously infused through a central venous catheter each 24 hours. Red cells were transfused every 3 days to maintain the hemoglobin level between 140 and 150 g/L (14 and 15 g/dL). During this time interval hydroxyurea 60 mg/kg daily and azathioprine 3 mg/kg daily were administered to eradicate marrow, and growth factors, granulocyte colony-stimulating factor and erythropoietin, were given twice weekly to maintain stem cell proliferation in the face of hypertransfusion, thereby facilitating the effect of the hydroxyurea. Fludarabine was administered at a dosage of 30 mg/m²/d from day -17 through day -13. Starting on day -10, 14 doses of

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Key words: thalassemia, stem cell transplantation.

Received for publication: 4 May 2011.
 Accepted for publication: 4 June 2011.

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 Pediatric Reports 2011; 3(s2):e13
 doi:10.4081/pr.2011.s2.e13

busulfan (BU) 1 mg/kg were administered orally 3 times daily over 4 days (total dose 14 mg/kg over 4 days) in the first 17 patients, and corresponding dose of busulfan give intravenous in the following 14 patients, followed by intravenous cyclophosphamide (CY) 50 mg/kg daily on each of the next 4 days (total dose 200 mg/kg), and 10 mg/kg thiopeta, and 12.5 mg/kg anti-thymocyte globulin daily from days -5 to -2, (ATG-Fresenius S).

All patients received cyclosporine for GVHD prophylaxis for the first two months post transplantation.

Graft content

Eight patients received T cell-depleted peripheral blood progenitor cells (CD34⁺ immunoselection) and CD3⁻ and CD19⁻-depleted bone marrow stem cells. Median infused cell doses per kilogram of recipient body weight were CD34⁺: 15.2 \times 10⁶ (range, 8.2-26 \times 10⁶); CD3⁻ T cells: 1.8 \times 10⁵; and 0.27 \times 10⁶/kg CD19. Twenty-three patients received CD34⁺ mobilized peripheral and bone marrow progenitor cells. Positive selection was performed using the CliniMACS procedure. The CD34⁺ grafts contained a median of 14.2 \times 10⁶/kg CD34⁺ cells (range, 5.4-39 \times 10⁶/kg), 2 \times 10⁵/kg CD3⁺ cells, and 0.19 \times 10⁶/kg CD19⁺. No side effects were associated with graft infusion.

Results

All patients showed donor chimerism by day 14 after HSCT. Granulocytes count greater than 500/mL occurred after a median time of 13 days (range, 11-17). Seven patients rejected their grafts, surviving with thalassemia, 3 patients showed early mixed chimerism, which became persistent when observed respectively at 14, 38

and 42 months after the transplant. In 19 cases the transplantation was successful with complete allogeneic reconstitution. There were 2 patients who died from transplantation-related causes: one of these patients died on day +114 of Epstein-Barr virus cerebral lymphoma and one died on day +92 from CMV pneumonia. All the 22 cured children are not anymore transfusion-dependent with hemoglobin levels ranging from 10.3 g/dL to 13.8 g/dL and have an optimal quality of life (Figure 1).

Immunological reconstitution

Delayed immune reconstitution post transplant may be associated with a variety of functional and immunophenotypic abnormalities at BM level, due to augmented local production of inflammatory cytokines, increased T-cell activation, or intrinsic hematopoietic and stromal cell abnormalities. After 20 days post transplant, a significant decrease in total lymphocyte counts and depletion of CD4⁺ T cells were observed. *In vivo* and *in vitro*, hemato-lymphopoiesis occurs in association with the complex network of cell types found in the stroma, including non-hematopoietic (fibroblasts, adipocytes, and endothelial cells) and hematopoietic cells (macrophages and T cells). Progenitor cell growth and differentiation depend on their interaction with stromal cells. The prevalence of macrophage-like cells in long-term BM culture, rather than the typical fibroblast-like cells, suggests an altered composition of the BM stroma, possibly linked to an underlying inflammatory process within the BM microenvironment.¹⁰⁻¹² A central function of stromal cells is IL-7 production. Recent evidence shows that IL-7 acts as a master regulator of T-cell homeostasis, expanding both the naive and memory T-cell populations. Compared with controls, thalassemia patients exhibited altered stromal cytokine production at 20 days post transplant, characterized by decreased IL-7 levels. We can hypothesize that the delayed

immunoreconstitution of the T-cell compartment may be initially the result of altered generation of new T cells arising from hematopoietic progenitor cells with the interaction of impaired stromal cell function. NK CD56⁺bright cells develop more rapidly than other lymphocytes, but CD3-CD16⁺ NK cells (with cytotoxic potential) require more prolonged exposure to maturation factor (IL-2) in the BM. Interestingly, we observed higher percentages of NK CD56⁺bright cells 20 days post-transplant in patients with full engraftment, suggesting a role for donor NK cells in improved engraftment and in prevention of rejection by an attack of the host lympho-hematopoietic cells.

After 60 days post transplant, a significant decrease in total lymphocyte counts, and depletion of CD4⁺ T cells expressing predominantly the CD45RA⁺CD62L⁺ phenotype were observed. Also the CD4⁺CD45RA⁺CD31⁺ T cell subset was significantly reduced in our cohort, suggesting a thymus involvement in these patients. Indeed, it is possible that the T-cell defect in thalassemia patients may occur at multiple levels, including egress from thymus.

NK CD56⁺bright cells develop more rapidly than other lymphocytes, but CD3-CD16⁺ NK cells (with cytotoxic potential) require more prolonged exposure to maturation factor (IL-2) in the bone marrow.^{16,17} Figure 2 reported the mixed chimerism condition in each of 3 patients. The recipients with full donor chimerism had 100% of CD3⁺ donor cells and 100% of donor NK cells, stable over time.

The individuals who had stable mixed chimerism, showed to have very low levels of CD3⁺ donor chimerism early after transplantation that increased over time, in parallel with high and stable levels of donor NK population, suggesting that donor NK cells might promote tolerance and donor engraftment.^{14,15} The levels of immunosuppression were uniform in all patients. No patient developed GVHD and all

patients came off immunosuppression at the same time.

Discussion

HSCT offers the only chance of cure for patients with thalassemia. Haploidentical transplantation may extend this possibility to the 50% to 60% of the patients who lack a suitably matched familial donor or an HLA-identical unrelated donor. The presence of fetal cells in maternal blood and of maternal cells in fetal blood (fetomaternal microchimerism) suggests that immunologic tolerance may exist between mother and offspring. The combination of a megadose of purified CD34 cells and a highly immunomyeloablative conditioning regimen is crucial for overcoming the barrier of residual antidonor cytotoxic T-lymphocyte precursors in T cell-depleted mismatched transplants and the addition of BMSCs (including NK cells, mesenchymal stem cells, T cells) to a T cell-depleted allograft may help promote engraftment and control GVHD.¹³ The reason of adding back BMSCs of 3x10⁵/kg in our patients was based on the assumption that a minimal threshold of lymphocytes for developing of GVHD was 10⁵/kg. The simultaneous addition of BMSCs (including stem cells, NK cells, monocytes, DCs, mesenchymal cells), may have a potential role for immunotolerance and engraftment. BM could be a site of T cell priming, because the antigen is accessible for presentation by BM DCs. In addition, an important role for CD4⁺ T cell on BM engraftment is described, not only promoting rejection of the few host cells after the conditioning regimen, but also allowing efficient HSC differentiation and reconstitution.

NKCD56⁺bright cells develop more rapidly than other lymphocytes, but CD3-CD16⁺ NK cells (with cytotoxic potential) require more prolonged exposure to maturation factor (IL-2) in the BM. No significant correlation has been observed between cell doses infused after T cell depletion or immunologic characteristics of the donors and engraftment in patients, but interestingly, we observed higher percentages of NK CD56⁺bright cells only 20 days post-transplant in patients with full engraftment, suggesting a role for newly generated NK cells in improved engraftment and in prevention of rejection by an attack of the host lymphohematopoietic cells. The higher percentages of CD3-CD16⁺ in mixed chimerism patients may have a possible role in control of host-cell escape and in maintaining the chimerism condition. The recipients with full donor chimerism had 100% of CD3⁺ donor cells and 100% of donor NK cells, stable over time. Pioneering studies by Velardi and colleagues revealed that patients with acute

31 HAPLOIDENTICAL TRANSPLANT IN THALASSEMIA



Figure 1. Results in 31 haploidentical transplant in thalassemia.

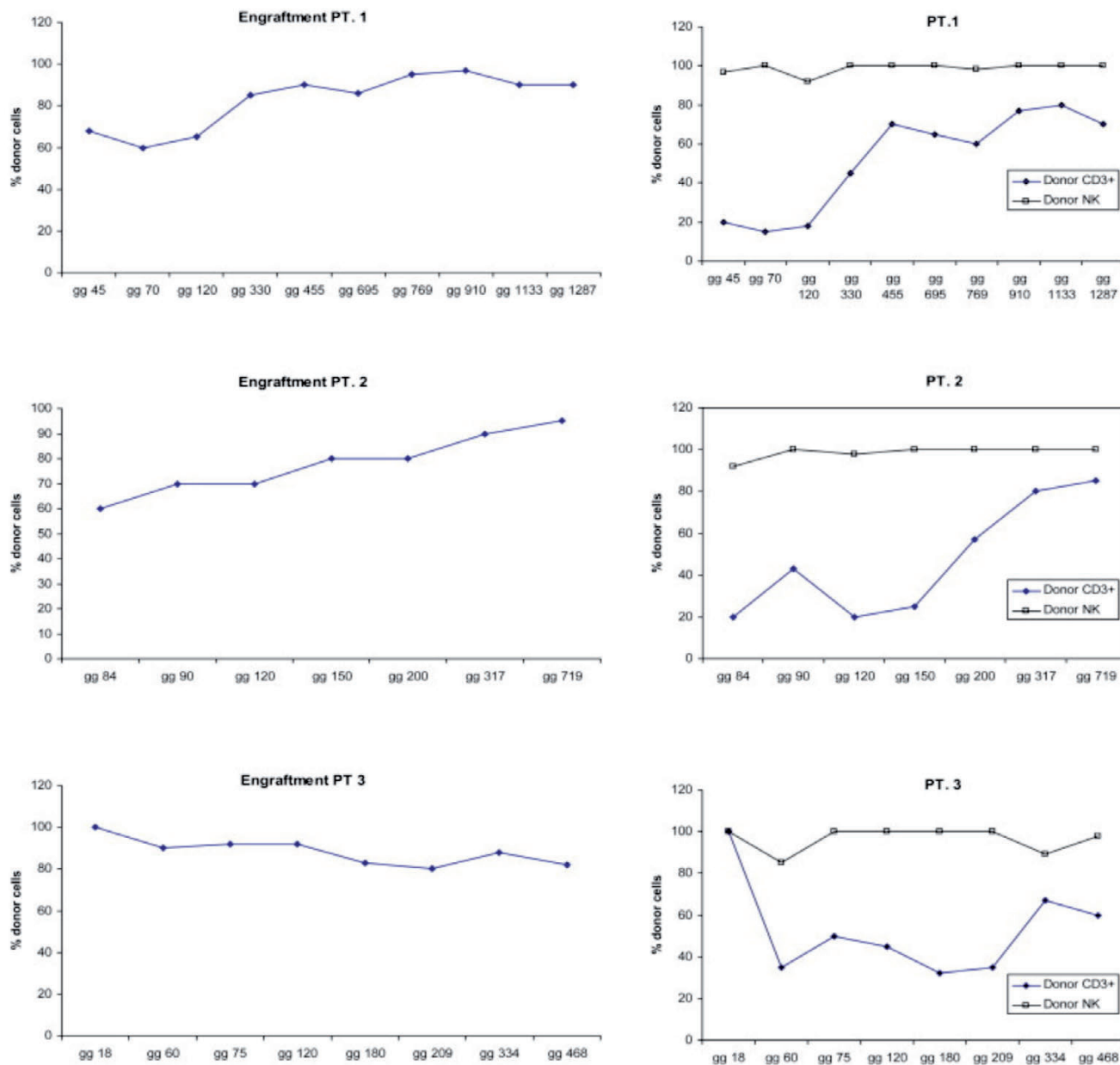


Figure 2. Mixed chimerism in 3 patients after haplo HSCT.

myelogenous leukemia transplanted from an NK alloreactive donor benefited from higher rates of engraftment and reduced rates of GVHD. The virtual abrogation of GVHD may be a consequence of NK cell-mediated killing of recipient antigen-presenting cells and. In our group, no patient showed signs of GVHD post-transplant. The beneficial effects could also be related to depletion of patient antigen-presenting cells and facilitation of engraftment as a result of the killing of T cells, removing patient lymphohematopoietic cells, and production of growth factors required for engraftment and for accelerating recovery of myelopoiesis. Our studies may also suggest NK subset analysis as a useful measure of

transplant outcome.

In conclusion, the transplant protocol described herein, appears to be well tolerated and effective for eradicating the hematopoietic system in patients with thalassemia. The incidences of transplant-related toxicities and severe infection were low.

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