

From CD34 positive selection, to negative depletion approaches of CD3 and T-cell receptor (TcR) $\alpha\beta$ T lymphocytes in haploidentical transplant in Thalassemia and SCA patients

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Purpose and Conditioning

Feto-maternal microchimerism suggests immunological tolerance between mother and fetus. Thus, we performed primary hematopoietic stem-cell transplantation (HSCT) from mismatched mother to thalassemic patient without an HLA-identical donor. 42 patients with thalassemia major were conditioned with 60 mg/kg hydroxyurea and 3 g/kg azathioprine from day -59 to -11, 30 mg/m² fludarabine from day -17 to -13, 14 mg/kg busulfan starting on day -10 were administered orally 3 times daily over 4 days in the first 17 patients, and corresponding dose of busulfan given intravenously in the following 25 patients, and 200 mg/kg cyclophosphamide, 10 mg/kg Thiotepa, and 10 mg/kg ATG (Fresenius) daily from day -5 to -2. 34 patients received CD34+ mobilized peripheral and bone marrow progenitor cells;

Days of treatments	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0
Fludarabine 30 mg/m ² /d	█	█	█	█	█													
iv Busulfan dose weight based								█	█	█	█							
iv Cy 50 mg/kg daily												█	█	█	█			
Thiotepa 10 mg/kg													█					
ATG-F 10 mg/kg													█	█	█	█		

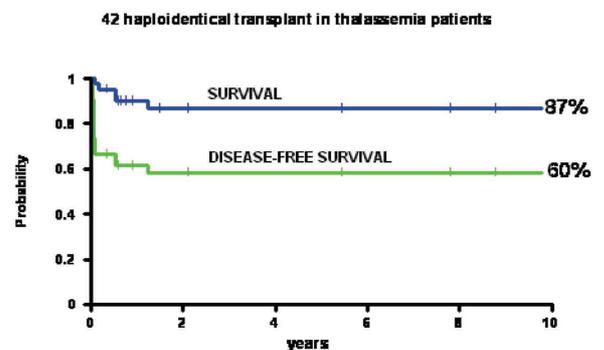
Graft Content

T-cell dose was adjusted to 2x10⁵/kg by fresh marrow cell add back at the time of transplant. Both groups received cyclosporine for graft versus host disease prophylaxis for two months post transplant. 3 patients died, 12 patients reject their grafts, and 27 showed full chimerism with functioning grafts at a median follow-up of 56 months. The overall survival, thalassemia free survival and mortality is 87%; 60% and 11%. To analyze immunohematologic reconstitution, particularly NK cells, we evaluated 13 thalassemia patients after 20 and 60 days and 1 year post transplantation with T cell-depleted HLA-haploidentical stem cells. NKs were the first lymphocytes to repopulate the peripheral blood.

Depletion of $\alpha\beta$ T Lymphocytes

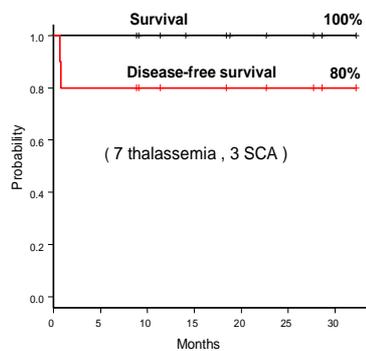
A significant increase in CD4+ and CD8+ markers paralleled an increase in CD3-CD16+ NKs, especially with full engraftment. In order to increase the T-cell depletion of PBSC while maintaining the anti-infectious and the engraftment facilitating effects of the depleted grafts, we have introduced **the depletion of $\alpha\beta$ T Lymphocytes using the CliniMacs System**. In addition to the Cd3 depletion, $\alpha\beta$ -depleted grafts contain large number of $\gamma\delta$ T lymphocytes.

Using this new strategies in 10 haploidentical transplant (7 thalassemia patients, 3 sickle cell patients), 8 patients showed full chimerism, 1 patient reject, with autologous recovery. In 1 cases we observed, after transplant failure, sustained full expression of HbF.



10 Haploidentical transplants in thalassemia e sickle cell anemia

Negative depletion approaches of CD3 $\alpha\beta$ T lymphocytes



8 patients we observed more rapid immunological reconstitution after 60 days post transplant, with an increase of T cells (CD4+ and CD8+), B cells (CD19+) and NK cells (especially CD3-CD16+, with cytotoxic potential) in the peripheral blood of these patients.

Conflict of interest: no relevant conflict of interest to disclose