Pre-emptive treatment of early unstable mixed chimerism in a Chinese thalassemia major patient by graded peripheral blood stem cell infusions

The thalassaemias are common in Hong Kong Chinese. 8% of the population are heterozygous carriers of a- and b-thalassaemia mutations.[1] Bone marrow transplantation (BMT) from an HLA-identical sibling donor is an established treatment for b-thalassaemia major but failure of donor marrow engraftment with subsequent autologous marrow recovery results in the recurrence of b-thalassaemia. Mixed chimerism (MC) occurs when a mixture of donor and recipient cells repopulates the recipient's marrow. Early unstable MC with a large proportion of residual host cells (RHC) predicts recurrence of the underlying disorder.[2] We report on a 17-month-old Chinese b-thalassaemia major patient who developed unstable mixed chimerism (MC) with 56% residual host cells (RHC) at 2 months and imminent graft rejection after allogeneic bone marrow transplantation (BMT). Peripheral blood stem cells (PBSC) from the original donor were infused to the patient in a graded incremental fashion to displace the RHC and augment the donor graft on days 129, 148, and 166 post-BMT. A conditioning regimen consisting of low dose total body irradiation of 2 Gray and anti-thymocyte (rabbit) globulin of 5mg/Kg/day for 3 days was administered prior to the first PBSC infusion. The patient was monitored longitudinally by DNA chimerism studies, haemoglobin pattern and ABO groupings. A cumulative dose of 5.4×10^8/Kg T cells and 2.2×10^6/kg $CD34^+$ cells was given. Full donor chimerism was achieved with development of treatment responsive hepatic graft versus host disease. Serial DNA chimerism studies can identify early unstable MC in thalassaemia and may guide the timing of therapeutic intervention.

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Case history

A 17-month-old Chinese girl was diagnosed with b-thalassaemia major at the age of 6 months when she presented with severe anaemia (haemoglobin 5.9 g/dL). Molecular analysis showed homozygous codon 41/42(dTCTT) b0 mutation. She had been transfused monthly for 10 months before BMT without chelation therapy. Hepatomegaly was not present but liver biopsy showed grade 3 haematochromatosis with minimal fibrosis indicating Pesaro Class II status. She underwent allogeneic matched sibling BMT from her elder sister who has b-thalassaemia trait. The patient and transplant characteristics are summarized in Table 1. Neutrophil engraftment (>0.5×10^9/L) occurred on day 15 and platelet engraftment occurred on day 19 (>20×10^9/L) and day 21 (>50×10^9/L), respectively. Engraftment status was closely monitored by serial DNA chimerism studies which showed 44%, 42% and 31% donor cells at 2, 3 and 4 months post-BMT, respectively (Table 2), and the patient remained red cell transfusion-dependent, indicating imminent graft rejection and autologous regeneration. In order to salvage the donor marrow graft, a mini-PBSC transplantation using the same sibling donor was performed on day 129 after low-dose total body irradiation (TBI) of 2 Gray and rabbit ATG of 5 mg/kg daily for 3 days. She became pancytopenic. Graded increments of donor PBSCs and T lymphocytes, collected by leukopheresis after granulocyte-colony stimulating factor mobilization, were further infused on day 148 and 166. A total cumulative dose of 5.4×10^8/kg T cells and 2.2×10^6/kg $CD34^+$ cells were given (Table 1). Cytopenia recovered in parallel with a rise in the proportion of donor cells. Full donor chimerism was documented from day 255 onward. She became red cell transfusion-independent since day 215 (Table 2). The procedure was complicated by grade III liver GVHD on day 204, which responded to a combination of prednisolone of 2 mg/Kg/day, cyclosporin A 3 mg/kg/dose twice daily, mycophenolate mofetil 500 mg/day and ursodeoxycholic acid 5 mg/kg/day. At 18 months post-BMT, she remained red cell transfusion independent and had 100% donor chimerism with mild grade chronic liver GVHD.

Methods of monitoring engraftment kinetics

Donor/recipient chimerism was analysed prospectively by using a semi-quantitative polymerase chain reaction (PCR) analysis of microsatellite markers with variable tetranucleotide repeats as previously described.[3] Briefly, DNA was extracted from peripheral blood leucocytes and amplified by PCR with flurochrome labeled primers for the markers D5S818 and TH01. Chimerism was enumerated based on the relative intensities of alleles polymorphic for the donor and recipient. ABO cell grouping after BMT was performed by incubating commercial antisera (Immuncor, Norcross, GA) with 5% recipient red cells at ratio of 2:1 for one hour at room temperature, followed by centrifugation for 15 seconds at 3000rpm. The end point was read macroscopically and microscopically to detect mixed field or weak reactions. Serum grouping was performed by incubating serum of recipient with ABO red cells using the same protocol as described above. For negative reactions, the procedure was repeated at an increased serum to red cell ratio of 4:1. Haemoglobin pattern analysis was performed by high performance liquid chromatography using Variant Hb testing system (Bio-Rad Laboratories, Hercules, CA). The different haemoglobins (including HbF) were identified by retention time and quantified.

Discussion

According to Pesaro's experience on a group of 295 homozygous b-thalassaemia patients with a minimum follow-up of 2 years after matched sibling BMT, none of 200 patients with complete donor chimerism at 2 months post-BMT rejected the transplant while 33 of 95 patients (34.7%) with mixed chimerism (MC) observed within the first two months after BMT rejected the graft.[2] The amount of residual host cells (RHCs) can be graded into three different levels: MC level 1, when the RHCs are lower than 10%; MC level 2, when the RHCs are between 10-25% and MC level 3, when the RHCs are more than 25%. Eighteen of 19 patients with MC level 3 (RHCs > 25%) subsequently rejected their graft in contrast to lower rejection rates of 12.7% and 33%, respectively, in patients with MC levels 1 and 2.2 Similar data in other ethnic groups including the Chinese b-thalassaemia major patients are largely unknown. Li et al recently reported that mixed chimerism occured in 11 of 35 (31%) thalassaemia major patients after BMT in the Hong Kong Chinese.[4] Only one patient rejected the graft at 8 months while 6 patients had persistent MC up to 9 years after transplant. However, MC status at 2 months was not available. Results from our centre showed that 4 (22%) of 18 evaluable Chinese thalassaemia major patients had MC at 2 months. One patient had MC level

Table 1. Comparison of three approaches to treatment of graft rejection in thalassaemia

	Current study	**Li et al. [6]**	**Aker et al. [8]**
Therapeutic approach	Pre-emptive PBSC	Second transplant (PBSC)	Pre-emptive DLI
Pesaro class	II	I	II
Age	17 months	13 years	5.5 years
1st conditioning regimen	BU(20)CY(200)ATG(90)	BU(16)CY(200)ATG(90)	BU(16)CY(200)CAMPATH-IG(0.8)
Cell dosage	NC: 2.33 x 10^8/Kg CFU-GM: 8.80 x 10^4/Kg	NC: 3.5 x 10^8/Kg CD34: 4.8 x 10^6/Kg	NC: 6 x 10^8/kg
Marrow manipulation	No	No	T cell depletion
Neutrophil engraftment (0.5 x 10^9/L)	Day 15	Day 20	Day 14
Platelet engraftment (>50 x 10^9/L)	Day 21	Day 28	Day 36
% RHC at 2 m post-BMT	56%	3%	30%
Method of chimerism study	PCR analysis of microsatellite markers	XY-FISH	Cytogenetics & PCR of X- and Y-specific amelogenin gene (AMG-PCR)
Time of PBSC/DLI (days post-BMT)	129	280	84
2nd conditioning regimen	TBI(2 Gray)ATG(15)	ATG(90)	Nil
Total T cell and CD34 cell dosage (Number of infusions)	T cell: 5.4 x 10^8/kg CD34: 2.2 x 10^6/kg 3 infusions	T cell: 3.5 x 10^8/kg CD34: 3.5 x 10^6/kg 1 infusion	T Cell: 1 x 10^8/kg CD34: data not available 3 infusions
Complication	Grade III liver GVHD	No GVHD	Grade II oral GVHD
Outcome	100% donor chimerism at 18 months	100% donor chimerism at 26 months	100% donor chimerism at 4 years

PBSC, peripheral blood stem cell; DLI, donor lymphocyte infusion; BU, busulphan; CY, cyclophosphamide; ATG, anti-thymocyte globulin CAMPATH-1G, monoclonal rat anti-human CD52 antibody; NC, nucleated cell; CFU-GM, granulocyte macrophage colony forming unit PCR, polymerase chain reaction; XY-FISH, fluorescent in-situ hybridization of X and Y ch romosomes; GVHD, graft versus host disease

Table 2. Serial monitoring of engraftment kinetics by DNA chimerism, haemoglobin pattern and ABO groupings

	Haematological indices			**Hb Pattern**			**ABO groupings**	**DNA chimerism**	**Timing and cell dose of DLI/PBSC**
	Hb (g/dl)	MCV (fl)	Retics (%)	HbA (%)	HbF (%)	HbA2 (%)		% donor cells	
Donor	11	69	1	85.8	8.0	6.2	AB+		
Recipient									
At Diagnosis	5.9	67		19.1	79.6	1.3	O+		
Pre-BMT	8.3	83.4		89.2	8.0	2.8	O+		
Post-BMT									
Day 33	8.1	82.9	0.5	93.9	3.4	2.7	O+	39%	
Day 60	7.8	81.8	2.4	85.6	11.9	2.4	O+	44%	
Day 94	8.9	85.5	1.6	87.1	10.3	2.6	O+	42%	
Day 126	10.1	83.4		86.9	11.8	2.3	O+	31%	
Day 129									T cell: 6.3 x10^7/kg CD34: 0.2x10^6/kg
Day 136	9.4	82.8	0.1	87.5	10.1	2.4	O+	10%	
Day 148									T cell: 6.3x10^7/kg CD34: 0.2x10^6/kg
Day 150	8.7	82					AB+, O+	87%	
Day 163	8.1	82.1		92.4	5.8	2.6	AB+, O+	88%	
Day 166									T cell: 4.14x10^8/kg CD34: 1.8x10^6/kg
Day 177	7.9	82.0					AB+, O+	92%	
Day 198	7.1	82	3.4				AB+, O+	94%	
Day 215*	10.5	84.8	>5					94%	
Day 236	8.3	82.5						92%	
Day 255	12	87.6	2.3	85.9	9.9	5.2	AB+	100%	
Day 295	11.2	82		88.2	7.9	4.9	AB+	100%	
Day 385	11.3	78.2	2	85.9	10.2	4.9	AB+	100%	
Day 548	11.1	74		83.7	11.4	5.3	AB+	100%	

Hb, haemoglobin; MCV, mean corpuscular volume; Retics, reticulocytes; * patient became red cell transfusion-independent

1 who later achieved full donor chimerism. Three patients had MC level 3, with one patient rejecting the transplant and one patient achieving stable MC (SY Ha, unpublished data). The remaining patient is the subject in this report. Management of graft rejection or disease recurrence in thalassaemia patients is controversial. Early second transplant with additional course of conditioning regimen is associated with a high risk of treatment related mortality. Patients who reject the donor grafts but have autologous haematopoiesis do not urgently need a second transplant and intervention can be deferred until recovery from the toxic effects of the first conditioning regimen. Second transplants in Pesaro are generally preconditioned with 200 mg/kg cyclophosphamide and total lymphoid irradiation.[5] Interestingly, Li et al had successfully performed a second transplant in a Chinese thalassaemia major patient using PBSC at 10 months after the first BMT with only ATG pre-conditioning.6 Donor lymphocyte infusions (DLI) have been successfully applied to recurrent chronic myelogenous leukaemia after allogeneic BMT.7 Aker *et al.*[8] reasoned that the same therapeutic principles might hold true for non-malignant diseases. They reported the first successful use of graded increments of DLI at 84 days after T cell depleted BMT in a thalassaemia major patient with signs of imminent recurrence of the disease. Gradual elimination of the host haematopoietic cells was accomplished with conversion of unstable MC to 100% donor chimerism. Our patient had MC level 3 (56% RHCs) at 2 months and a continual increase in RHCs to 69% on day 126 (Table 2) indicating imminent graft rejection. To salvage the donor graft, either DLI or early second transplant with low toxicity conditioning regimen can be considered. However, DLI carries a significant risk of secondary marrow aplasia while patients rejecting the donor's graft are at risk of further graft rejection after the second transplant. We combined the two approaches by infusions of G-CSF mobilized PBSCs, in a graded incremental fashion, after reduced-intensity conditioning regimen. The graded increments of donor T cells displaced the RHCs and concomitant infusion of donor haematopoietic stem cells augmented the failing donor graft. The engraftment kinetics was closely monitored by longitudinal DNA chimerism studies which could guide the timing of PBSC infusions until a stable chimerism state was achieved. The DNA chimerism analysis was shown to be a more accurate assessment of the engraftment kinetics than red cell indices, haemoglobin electrophoresis and blood groupings which were all affected by frequent red cell transfusions. The therapeutic approaches of Li *et al.*[6] and Aker *et al.*[8] are compared with the current approach in Table 1. Li et al's was essentially an early second transplant with reduced intensity conditioning regimen whereas Aker et al.'s and ours were early pre-emptive interventions. We propose that the add-back donor stem cells in addition to DLI in our regimen may confer a greater safety margin than DLI alone by minimizing the period of cytopenia and susceptibility to infections. The treatment toxicity of the regimen is acceptable. Nevertheless, caution concerning the potential risk of severe GVHD related to DLI, particularly in the context of non-malignant diseases, is warranted. Infusing a high number of purified CD34+ T-cell depleted donor stem cells may be considered as an alternative treatment strategy in unstable mixed chimerism in thalassaemic patients. In conclusion, early detection of unstable MC by serial DNA chimerism studies may guide the clinical management of thalassaemia major patients after BMT. Graded DLI/PBSCs with mini-transplant type of conditioning regimen is a feasible method to eliminate residual host haematopoiesis and to convert unstable MC towards stable or full donor chimerism.

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