**Title : Nonmyeloablative matched stem cell transplantation with** **optional reinforced stem cell infusion for hemoglobinopathies**

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**Running title:** Nonmyeloablative SCT for hemoglobinopathies

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**Abstract**

Nonmyeloablative (NMA)-conditioning stem cell transplantation (SCT) with alemtuzumab and low-dose total body irradiation (TBI) corrects the abnormal sickle cell disease (SCD) phenotype in the absence of graft-versus-host disease (GVHD). However, NMA regimens are rarely applied to patients with thalassemia major (TM). In this study, β-TM (N = 9) and SCD (N = 4) patients were equally effectively treated for eradicating the abnormal hemoglobin phenotype. However, to avoid prolonged immunosuppression after 1 year post-SCT, a two-stage strategy was developed, wherein a mixed donor chimerism was initially achieved using the protocol developed by the National Institute of Health (NIH), before facilitating donor chimerism using reinforced hematopoietic stem cell (SC) infusion in specific cases requiring protracted immunosuppression. A majority of the patients (N = 10), which include seven patients with β-TM, presented stable mixed chimerism (MC). Four out of the 5 reinforced infusion recipients achieved complete chimerism. An overall survival rate and event-free survival at 4 years of 91.7% (95% CI, 53.9–98.8) was achieved, with a thalassemia-free survival rate in β-TM patients of 87.5% (95% CI, 38.7–98.1). This study is the first to report successful NMA SCT to achieve stable MC and correct abnormal hemoglobin phenotype in adult patients with TM.

**Keywords:** Nonmyeloablative conditioning, sickle cell disease, thalassemia major

**Introduction**

Allogeneic stem cell transplantation (alloSCT) remains ~~only~~ a curative treatment option for various disorders, including malignant and non-malignant hematologic disorders. The rates of transplantation-related morbidity and mortality are often unacceptable, especially for patients with benign hematologic disorders, such as β-thalassemia major (β-TM) and sickle cell disease (SCD). Adult patients with these diseases, associated with both disease- and treatment-related organ complications, may be unable to tolerate conventional myeloablative (MAC) or reduced-intensity conditioning (RIC) transplantation, resulting in significant transplantation-related morbidity and mortality. Therefore, non-myeloablative (NMA) conditioning can be considered as a feasible treatment option for these patients. However, earlier studies reported minimal toxicity after alloSCT with NMA regimen using low-dose total body irradiation (TBI) plus fludarabine in patients with β-TM and SCD patients, but resulted in only transient donor engraftment with consequent graft failure (GF) [1]. Recently, the National Institute of Health (NIH) developed the NMA regimen using alemtuzumab with low-dose TBI, whose application resulted in stable mixed-donor chimerism enough to the production of donor-type red blood cells and the reversion of the sickle cell phenotype. It also resulted in very low incidence of graft-versus-host disease (GVHD), which was associated with a low rate of treatment-related mortality (TRM) [2]. As for β-TM, because it has different biological and clinical features with SCD, including robust proliferation of bone marrow (BM) and allo-sensitization due to multiple transfusions, it is unclear whether this low-intensity conditioning is enough to overcome these features. Although conditioning with low-dose TBI plus alemtuzumab resulted in successful outcomes, the main population in previous studies were patients with SCD. In this setting, thalassemia-specific outcomes could not have been detailed so far.

In addition, a significant portion of the patients who received NMA regimen with the NIH protocol were unable to cease immune-suppressive medications (ISM), because their donor T-cell chimerism did not reach 50% after one year, with a graft failure rate of 13% at x years [2]. It is currently unclear how long patients who could not achieve a donor T-cell chimerism of over 50% should receive immunosuppression. Various complications due to protracted ISM administration should be a major concern. To address these issues, we have established a two-stage strategy; a mixed donor chimerism using NMA conditioning was initially achieved, followed by facilitating donor chimerism using optional reinforced hematopoietic stem cell (SC) infusion in cases requiring prolonged immunosuppression. Also, patients who experienced impending graft failure (GF) also received subsequent reinforced SC infusion. Herein, we will describe allogeneic SCT using NMA conditioning with alemtuzumab and low-dose TBI, with the optional reinforced SC infusion for patients with β-TM and SCD.

**Patients and methods**

Patients

Patients (18 years or older) with an HLA-matched sibling donor (MSD) and hemoglobinopathies of β-TM and SCD were included in this study.

Transplant-related procedures

Similar to the NIH protocol, patients received a NMA conditioning regimen of alemtuzumab (Campath 1-H; 0.03 mg/kg for 1 day, 0.1 mg/kg for 1 day, then 0.3 mg/kg for 3 days; total dose 1.03 mg/kg on day –7 to –3) plus fractionated TBI (300–400 cGy for 1 day on day –2) [2]. Subsequently, peripheral blood (PB) stem cells (target CD34+ cell dose at least 10 × 106/kg) were mobilized with granulocyte-colony stimulating factor (G-CSF; 10 µg/kg filgrastim for 4 days) from MSD, and then administered to the patients. For the GVHD prophylaxis and maintaining sustained engraftment, all patients began sirolimus loading at a dose of 15 mg/day starting day –1, then 5 mg/day after day 0, followed by dose adjustment of target trough level of 10–15 ng/ml for the first 3 to 4 months. Thereafter, we attempted to maintain a trough level of sirolimus to 5–10 ng/L. We carefully had tapered the sirolimus after 1 year if donor T-cell chimerism was maintained over 50% in the absence of GVHD. If patient’s whole blood (WB) PB donor T-cell chimerism declined to < 50% after 1 year, they were administered reinforced infusion of SC from the same donors after total-nodal irradiation (TNI) at a single dose of 500 cGy. We thought that patients who showed rapid decline in donor chimerism approaching 20% during the early post-transplant period have greater risk of overt GF, regarding it an impending GF. Hence, these patients also received the SC infusion. Informed and written consent was obtained from all patients undergoing allo-SCT and reinforced SC infusion. This study was approved by the Institutional Review Board.

Definition of graft failure

Primary GF was defined as lack of neutrophil engraftment (absolute neutrophil count < 0.5×109/L) observed in 3 consecutive days within 28 days of post-transplant with cytopenia and a hypocellular marrow (aplasia) or count recovery with transfusion dependence (autologous recovery). Secondary GR was defined when initial engraftment was followed by subsequent development of cytopenia [3]. We defined impending GF as a rapid decline of donor chimerism to 20% or below without evidence of overt GF.

Supportive care

Antimicrobial prophylaxis with acyclovir and sulfamethoxazole-trimethoprim were administered to prevent herpes virus reactivation and pneumocystis, respectively, combined with regular monitoring for cytomegalovirus (CMV) DNA in the PB.

Statistical analysis

The primary outcomes of this study were overall survival (OS) and event free survival (EFS). OS was calculated from the date of transplantation to death from any cause. EFS was calculated from the date of transplantation to the any occurrence of an event. An event was defined as rejection or death; an SCD-specific event was defined as a clinical manifestation of SCD or a failure to achieve donor-type hemoglobin S (HbS). Any requirement for a red cell transfusion or failure to achieve thalassemia-free disease status was defined as a TM-specific event.

**Results**

Patient demographics and disease-related characteristics

Between April 2014 and March 2019, 13 consecutive adult patients of Arab ethnicity were included (Table 1). At transplantation, the median age of the patients was 31 (range, 24–34) years old. The proportion of female donor to male recipient transplantation were 15.4% (95% CI, 1.9–45.4) and 30.8% (95% CI, 9.1–61.4), respectively. Major and minor ABO mismatches between the donor and the recipient were included (N = 3, respectively). More detailed patient demographics and disease-related characteristics are described in Table 1. Patients received NMA conditioning allo-SCT from MRDs, with an optional reinforced SC infusion according to patients’ PB donor chimerism, at our institution. All patients were afflicted with hemoglobin disorders, including β-TM in 9 (69.2%) and SCD in 4 (30.8%) patients. A patient with β-TM (UPN #01), who was referred from another center, was included for a second SCT after complete graft loss with MAC SCT.

Major transplant-related outcomes

The overall outcomes are summarized in Fig. 1 and Table 2. All patients received an initial PB SC infusion of median CD34+ cells of 14.6 × 106/kg (range, 8.8–33.3) and CD3+ cells of 55.1 × 107/kg (range, 15.8–77.3) after NMA conditioning. One (7.7%) and four (30.8%) patients did not experience neutrophil (<0.5 × 109/L) or platelet (<50 × 109/L) nadirs, respectively. The remaining patients achieved neutrophil and platelet engraftments at a median of 14.5 days (range, 12–21) and 14 days (range, 12–25), respectively. The cumulative incidences of neutrophil and platelet engraftments at day 28 were 100% for both. The median PB donor’s T-cell chimerism at 1 month was 84% (range, 3–97). There were two patients (one with TM and one with SCD) who showed rapid decline of donor chimerism in their WB to 20% (22% and 33%, respectively) during the early posttransplant period, with occasional transfusion requirement from 1.9 months and 6.2 months post-transplant, respectively.

β-thalassemia

There were 9 β-TM patients. One of them was ineligible for weaning from ISM, because the patient was under 1 year posttransplant at the time of data collection. Of the remaining 8 patients, 1 (UPN #10) had impending GF with occasional red cell transfusion requirement. The other 7 patients had stable mixed chimerism (MC). However, three of them failed to achieve sustained donor T-cell chimerism (more than 50%) after 1 year post-transplant and one refused the reinforced infusion and is currently taking ISM at 22.8 months post-transplant. Including the patient with impending GF, a total of three received the reinforced SC infusion each at 3.9, 15.1, and 37.6 months posttransplant, respectively. Of the 7 patients with stable MC, four showed sustained donor T-cell chimerism (more than 50% after 1 year post-transplant) and discontinued sirolimus each at 13.2, 14.1, 14.7 and 16.5 months post-transplant, respectively. Currently, all four maintain to be stable MC. The mean hemoglobin levels increased after SCT. The mean hemoglobin levels for women before HSCT were 8.0 g/dL (range, 7.4–9.5) vs 11.0 g/dL (range, 10.0–13.0) after SCT (at the most recent follow-up). For men, they were 8.0 g/dL (range, 7.4–9.2) before SCT vs 11.0 g/dL (range, 9.0–13.7) after HSCT. To date, no patients have required red cell transfusion regardless of the need for reinforced SC infusion or have required hospitalization. A genetic study revealed that all initial genetic abnormalities were corrected to donor-type genes, even before reinforced SC infusion.

Sickle cell disease

Of the 4 patients with SCD, the one with impending GF underwent reinforced infusion at 7.2 months post-transplant. The remaining 3 achieved stable MC; 2 of them showed persistently low donor T-cell chimerism after 1 year post-transplant. Of the 2, one of them refused reinforced infusion and continued ISM (WB and T-cell chimerism of 83% and 43%, respectively, at 27.3 months post-transplant). The other patient underwent reinforced infusion at 20.7 months post-transplant. The last patient with stable MC and sustained donor T-cell more than 50% after 1 year post-transplant (UPN #6) discontinued sirolimus at 18.4 months. The mean hemoglobin level improved in the patients; from median 6.0 g/dL to 13.5 g/dL in male patients, and from 7.5 g/dL to 11.5 g/dL in female patients. The recipients achieved the same percentage of HbS as their donors after SCT; from median 72.9% to 37.4% (most recent). No patients with SCD suffered disease-related complications and all patients achieved donor type HbS. Only 1 patient was hospitalized due to non-specific colitis. All genetic defects were also replaced by donor-type, regardless of the administration of reinforced SC infusion.

GVHD, complications, and survival

Before receiving reinforced SC infusions, none of the patients had developed acute and chronic GVHD. Infectious complications of grade 3 was observed in one (7.7%). CMV reactivation requiring pre-emptive therapy, CMV disease, sinusoidal obstruction syndrome, and herpes zoster were not observed in any patients.

Seven patients (53.8%) showed requirements for reinforced SC infusion (β-TM, N = 4; SCD, N = 3), including failure to achieve donor T-cell chimerism over 50% after 1 year and thereafter (N = 5) and impending GF (N = 2). Five patients proceeded to reinforced SC infusion at a median of 15.1 months (range, 3.9–37.6), with a median PB donor T-cell chimerism of 30.0% (range, 17.0–40.0). The median infusion of CD34+ and CD3+ cells was 10.7 × 106/kg (range, 8.5–14.4) and 24.7 × 107/kg (range, 16.3–52.9), respectively. No neutrophil or platelet nadirs were observed. Two patients did not receive reinforced SC infusion due to patient refusal. Of the 5 patients who underwent reinforced SC infusion, 1 patient (UPN #11) developed severe acute gut GVHD, which was refractory to corticosteroid, and subsequently died of pneumonia complicating adult respiratory distress syndrome 1 month after infusion. The number of CD34+ and CD3+ cells at the reinforced infusion was 8.5 × 106/kg and 52.9 × 107/kg, respectively, the highest amounts for all reinforced infusion recipients. One other patient (UPN #2) developed chronic oral GVHD, which was partially responsive to corticosteroid, and was subsequently administered ruxolitinib for 5 months. Currently, this patient discontinued ruxolitinib and is weaning from sirolimus. The remaining 3 patients did not develop any form of GVHD and discontinued ISM at 20.7, 22.7, and 28.5 months post-transplant, respectively (16.8, 15.5, and 7.8 months after reinforced SC infusion, respectively). The reinforced SC infusion-free survival rates at 12 and 24 months were 83.9% (95% CI, 49.4–95.7) and 66.1% (95% CI, 32.5–85.8), respectively (Fig. 2). Five (71.4%) of 7 patients (1 patient is not eligible for weaning) who did not receive reinforced SC infusion were able to successfully discontinue sirolimus at a median 14.1 months (range, 3.9–18.4). All patients who received reinforced SC infusion achieved complete donor chimerism in terms of PB WB and donor T-cell with a median of 99.0% (95% CI, 93–100) while patients without the infusion all presented stable WB MC with a median of 89% (range, 79–97). In total, 8 patients (57.1%) discontinued ISM at the time of analysis (Fig. 1).

As a whole, the cumulative incidence of acute GVHD grade III–IV at day 100 after reinforced SC infusion was 20.0% (95% CI, 0–48.4). Two patients who received CD3+ cells at a dose above the median (26.3 × 107/kg) developed chronic GVHD. The cumulative incidences of severe chronic GVHD at 12 months was 20.0% (95% CI, 0.4–63.2). To date, none of these patients have experienced graft failure. No patients without reinforced SC infusion developed acute or chronic GVHD. Both CMV reactivation requiring pre-emptive therapy and hemorrhagic cystitis were observed in one patient (16.7%). Infectious complications of grade 3 were observed in one in terms of PB WB and donor T-cell with a median of 99.0%. Except for the above-described GVHD-related death, additional TRM was not observed.

Of the 3 patients with major ABO mismatches, 1 patient proceeded to the administration of reinforced SC infusion at 20.7 months post-transplant, achieved complete donor chimerism, and was weaned from ISM. Another patient discontinued ISM with a stable MC, not requiring reinforced SC infusion. The remaining patient has yet to reach 1 year post-transplant. Of the 3 patients with minor ABO mismatch, only 1 patient required reinforced SC infusion, while the remaining 2 patients successfully discontinued ISM without the need for infusion.

With a median follow-up duration of 31.5 months (range, 4.2 – 64.4), the OS and EFS at 4 years were 91.7% (95% CI, 53.9–98.8), respectively (Fig. 3a and 3b). The thalassemia-free survival rate in β-TM patients was 87.5% (95% CI, 38.7–98.1) at 4 years (Fig. 3c). The cumulative incidence of achieving PB donor T-cell chimerism >50% in patients not proceeding to reinforced SC infusion was 68.8% (95% CI, 4.5–89.8) at 4 years (Fig. 3d). Changes in donor chimerism after allo-SCT are shown in Fig. 4.

**Discussion**

Unlike SCD patients, thalassemia patients do not require chemotherapy nor is their immunological system impaired. Moreover, they have a robustly hyperplastic and expanded marrow compartment and allosensitization as a result of multiple transfusions. Because of these TM-specific features, the ideal conditioning regimen should be able to eradicate hyperplastic marrow and be sufficiently immunosuppressive to overcome the established allosensitization. Under these circumstances, allogeneic SCT using MAC with busulfan and cyclophosphamide has been considered a standard for patients undergoing SCT [4]. However, MAC or even RIC is associated with higher TRM in adult patients who already have multiple comorbidities and advanced disease with marked erythroid expansion. Early experiences of MAC-SCT in adult patients suggested that the outcome of transplantation was poor, with an overall and rejection-free survival of only 65% and 62%, respectively [5]. The TRM was significant, ranging from 28% to 37%, depending on the dosage of cyclophosphamide. As such, a safer conditioning regimen that is able to induce stable donor chimerism is required for this population.

Although there are reports of NMA-conditioning SCT in adult patients with SCD, there are currently no reports of successful engraftment with NMA conditioning in adult patients with TM [2, 6, 7]. By analogy with the behavior of malignant tissue, a large mass of rapidly proliferating hematopoietic tissue in TM is difficult to eradicate with a lower-intensity conditioning regiment and is more likely to recur after transplantation. NMA-conditioning SCT has been previously reported using a main population consisting of pediatric patients admixed with SCD and TM; the outcome was disappointing with only transient engraftment [1]. On the contrary, several studies support the observation that full donor chimerism is not mandatory in TM for the clinical success of SCT, as the persistence of even a small percentage of donor erythropoiesis may maintain the potential to correct the phenotypic expression of the disease, due to the competitive advantage of both donor peripheral erythrocytes and erythroid progenitors over their TM counterparts [8]. According to a long-term analysis, the occurrence of transient MC does not necessarily lead to graft rejection and evolves, in most cases, toward complete donor chimerism or a status of stable persistent MC [8]. In most reports, although the risk of rejection appears greatest in the first 2 months after transplantation, once persistent MC is established, patients seem to be no longer exposed to the risk of graft failure, showing a stable functional graft without the need for additional red blood cell transfusion support [9]. These data and the reliable achievement of stable MC by alemtuzumab with low-dose TBI provide a rational basis for the use of NMA-conditioning in SCT for patients with TM. Indeed, a major population in our study is adult patients with TM (9 of 13 patients, 69.2%), where 7 of them (77.8%) achieved stable MC for more than 1 year, and 4 patients did not require additional SC infusion due to their donor T-cell chimerism being maintained at over 50% after 1 year post-transplant, and thereafter. This result suggests that stable MC could be induced in a significant portion of β-TM patients without the need for additional manipulations. Two of the three patients with reinforced SC infusion maintain a complete chimerism. We believe that this is the first report to demonstrate that NMA SCT can be applied successfully for adult patients with TM, used to achieve a stable MC, and correct abnormal hemoglobin phenotypes.

One of themajor limitationsof the NIH protocol is the need for long-term immunosuppression in the SCT recipients who had a persistently low donor T-cell chimerism (<50%). Nearly half of the patient had to continue ISM with a median duration of 2.1 years. Under these circumstances, secondary malignancies and sirolimus-related complications can become major concerns. Several reports have found that a cumulative duration of immunosuppressive therapy of over 24 months is associated with an increased incidence of secondary malignancies [10, 11]. The risk of secondary malignancy is especially problematic in patients receiving solid organ transplantation taking life-long immunosuppression medication. Several studies have reported that the overall risk for malignancies was modestly increased among solid organ recipients [12, 13]. Although none of the patients developed a secondary malignancy with the NIH protocol, the follow-up duration of the study was not long enough to determine the true incidence. Sirolimus-related adverse drug reactions include hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, glucose intolerance, insulin resistance, new onset diabetes, anemia, and thrombocytopenia, as well as dermatological problems, gastrointestinal disorders, sinusitis, respiratory and urinary tract infections, and testicular dysfunctions [14]. Protracted immunosuppression with sirolimus can be associated with increased morbidities from these adverse reactions. Moreover, the study found a graft rejection rate of 13%. The prognosis of patients with TM who had experienced graft rejection was poor; nearly half of the patients (14/29) died of chronic GVHD, regimen-related toxicity, or secondary graft failure after the second SCT [15]. The three-year EFS and OS was 58% and 68%, respectively.

We attempted to overcome this limitation using reinforced SC infusion after conditioning with TNI at a dose of 500 cGy in cases where patients were unable to be weaned from ISM after 1 year post-transplant. At the time of the reinforced SC infusion, the median WB and donor T-cell chimerism were 35% and 23%, respectively. To date, no patients, including the 2 patients with impending GF, have experienced engraftment failure in this study. However, since there are several reports that a myeloid chimerism of around 20% is enough to achieve functional grafts characterized by normal hemoglobin level, no need for red cell transfusion, no iron increment, and a limited degree of erythroid hyperplasia [9, 16, 17], careful attention should be paid in the selection of optimal candidates and in the cell dose infused when making decision on reinforced SC infusion due to the risk of developing GVHD. Notably, the fatal case reported herein resulted from severe acute GVHD, where the number of CD3+ cells infused was the highest among the reinforced SC recipients, which could account for the development of severe GVHD. Although no meaningful statistical conclusions can be drawn here due to the limited number of patients, the development of acute and chronic GVHD has a tendency to be associated with the number of CD3+ cells infused, as expected.

Our study includes a TM patient with complete graft loss after myeloablative conditioning regimen without radiation. This patient discontinued ISM with stable MC and did not require reinforced SC infusion, suggesting that NMA-conditioning with alemtuzumab and low-dose TBI is able to overcome engraftment failure after non-TBI MAC. A previous study reported that an increased dose of TBI substantially reduced graft failure while maintaining the safety of haploidentical SCT after NMA conditioning, indicating that irradiation a plays a significant role as a conditioning regimen in this disease population [18].

The NIH protocol excluded major ABO-incompatible donations. Blood group incompatibility is not a hurdle in MAC with complete donor chimerism. On the other hand, with NMA-conditioning, ABO mismatch can be responsible for graft rejection, pure red cell aplasia, and immune-mediated hemolysis [19-21]. Our study included three patients with major and minor ABO mismatches: one of the patients with major mismatch successfully withdrew from ISM without the need for additional SC infusion and has a stable MC status with a donor T-cell chimerism of 60%. Two of the three minor ABO mismatches did not require reinforced SC infusion and have currently withdrawn from immunosuppression. These results suggest that ABO incompatibility is not associated with an increased incidence of rejection and should not be regarded as a contraindication for NMA-conditioning SCT with alemtuzumab and low-dose TBI.

The fact that this study is retrospective and contained a small number of patients makes it difficult to draw concrete statistical conclusions from the results. Despite this limitation, our data shows some consistencies: inclusion of 13 consecutive patients, using uniform criteria for reinforced SC infusion, and uniform NMA conditioning. Additional limitations include the limited availability of suitable donors since the conditioning regimen could only be applied only to patients with HLA-matched related donors, as described previously [2]. This poses a major barrier for performing allo-SCT, as most patients with hemoglobin disorders do not have these donors [22]. Lastly, although our strategy was effective in preventing the patients from receiving long-term immunosuppression, there was on recorded fatality, as well as the possibility of developing of acute and chronic GVHD after reinforcedSC infusion. Since the number of CD3+ cells infused appears to be associated with these complications, further study will be needed to determine the optimal cell dose of the reinforced SC infusion to minimize the risk of GVHD while facilitating donor engraftment.

In conclusion, despite being retrospective and including a small number of patients, this study found that NMA-conditioning regimen using alemtuzumab with low-dose TBI was effective in achieving stable MC not only in SCD, but in β-TM patients as well. Our strategy of reinforced SC infusion is effective in preventing continuing immunosuppression in patients requiring protracted immunosuppression. However, future studies are needed in order to determine the optimal cell dose in reinforced infusions.

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**Competing interests**

The authors declare that they have no conflicts of interests.

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**Tables**

Table 1 Patients’ demographics and pre-transplant characteristics

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| UPN | Sex/Age | Diagnosis | Disease status | ECOG PS | HCT-CI | Donor → Recipient | |
| ABO compatibility | Sex compatibility |
| 01 | M/29 | β-thalassemia major | T/F dependency, Iron overloading | 1 | NA | NA | M → M |
| 02 | M/33 | β-thalassemia major | T/F dependency, Iron overloading | 1 | 4 | O+ → O+ | M → M |
| 03 | M/34 | β-thalassemia major | T/F dependency, Iron overloading | 1 | 5 | O+ → B+ | F → M |
| 04 | F/27 | Sickle cell β-thalassemia (homozygous HbSS), companying α-thalassemia silent carrier | T/F dependency, Recurrent sickle cell crisis | 1 | 0 | B+ → O+ | F → F |
| 05 | M/33 | Sickle cell anemia (homozygous HbSS type), companying α-thalassemia silent carrier | Recurrent sickle cell crisis, Regular Red cell exchange | 2 | 2 | B+ → B+ | M → M |
| 06 | F/26 | Sickle cell anemia (homozygous HbS/β type), companying α-thalassemia trait | Recurrent sickle cell crisis, Regular Red cell exchange | 1 | 2 | O+ → O+ | M → F |
| 07 | F/32 | β-thalassemia major, companying α-thalassemia trait | T/F dependency, Iron overloading | 1 | 2 | A+ → AB+ | M → F |
| 08 | F/31 | β- thalassemia major, companying α-thalassemia silent carrier | T/F dependency, Iron overloading | 1 | 3 | A+ → AB+ | F → F |
| 09 | F/24 | Sickle cell anemia | Previous APL (CR state), T/F dependency, Iron overloading | 1 | 3 | A+ → A+ | M → F |
| 10 | M/29 | β-thalassemia major | T/F dependency, Iron overloading | 2 | 2 | O+ → O+ | M → M |
| 11 | M/34 | β-thalassemia major, companying α-thalassemia silent carrier | T/F dependency, Iron overloading | 1 | 2 | B+ → B+ | F → M |
| 12 | F/25 | β-thalassemia major, companying α-thalassemia trait | Engraftment failure with MAC (Treosulfan + Cy), T/F dependency, Iron overloading | 1 | 2 | AB+ → B+ | M → F |
| 13 | F/34 | β-thalassemia major, companying α-thalassemia silent carrier | T/F dependency, Iron overloading | 1 | 2 | A+ → O+ | M → F |

*UPN* unique patient number *ECOG PS* Eastern Cooperative Oncology Group performance status *HCT-CI* Hematopoietic Cell Transplantation-specific Comorbidity Index *Flu* Fludarabine *Cy* Cyclophosphamide

Table 2 Patients’ overall outcomes

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| UPN | Diagnosis | Cell dose of  1st stem cell infusion | | Cell dose of  2nd stem cell infusion | | Pre-transplant | Post-transplant outcomes | |
| CD34+  (× 106/kg) | CD3+  (× 107/kg) | CD34+  (× 106/kg) | CD3+  (× 107/kg) | Hb or HbS | Hb or HbS / Gene study / Chimerism, WB/T-cell (%) | GVHD / EFS/ OS |
| 01 | β-thalassemia major | 10.6 | NA | ND | | NA | Hb 13.0 g/dL @ 61.4 mo  c.92+5G>C (homozygous → heterozygous, donor type)  89/ 75@ 60.3 mo | No GVHD  EFS/OS@60.3 mo |
| 02 | β-thalassemia major | 10.5 | 28.3 | 13.0 | 27.9 | Hb 7.4 g/dLa | Hb 12.6 g/dL @ 54.3 mo  c.92+5G>C (homozygous → heterozygous, donor type)  99/100@ 46.6 mo | Severe cGVHD @ 2.5 mo after 2nd stem cell infusion  EFS/OS@50.2 mo |
| 03 | β-thalassemia major | 33.3 | 56.8 | 9.9 | 24.7 | Hb 7.8 g/dLa | Hb 13.7 g/dL @ 21.6 mo  c.92+5G>C (homozygous → not detected, donor type)  99/ 99@ 21.6 mo | No GVHD  EFS/OS@35.3 mo |
| 04 | Sickle cell β-thalassemia (homozygous HbSS), companying α-thalassemia silent carrier | 14.9 | 73.4 | 10.7 | 20.9 | HbS 70.8%b | HbS 30.6% @ 29.4 mo  c.20A>T (homozygous → heterozygous, donor type)  99/ 99@ 28.0 mo | Grade III aGVHD @ 0.2 mo after 2nd stem cell infusion  EFS/OS@33.5 mo |
| 05 | Sickle cell disease (homozygous HbSS type), companying α-thalassemia silent carrier | 12.8 | 15.8 | 14.4 | 16.3 | HbS 64.5%b | HbS 34.5% @ 22.7 mo  c.20A>T (homozygous → heterozygous, donor type)  99/ 99@ 27.0 mo | No GVHD  EFS/OS@31.6 mo |
| 06 | Sickle cell disease (homozygous HbS/β type), companying α-thalassemia trait | 17.8 | 55.0 | ND | | HbS 89.3% b | HbS 40.3% @ 21.9 mo  c.20A>T, C92+1G>A (homozygous → heterozygous, donor type), 79/33@ 27.0 mo | No GVHD  EFS/OS@23.2 mo |
| 07 | β-thalassemia major, companying α-thalassemia trait | 8.8 | 39.9 | ND | | Hb 7.6 g/dLa | Hg 15.4 g/dL @ 18.7 mo  c.92+5G>C (homozygous → heterozygous, donor type)  85/64@ 18.7 mo | No GVHD  EFS/OS@21.1 mo |
| 08 | β- thalassemia major, companying α-thalassemia silent carrier | 10.6 | 77.3 | ND | | Hb 7.6 g/dLa | Hb 14.4 g/dL @ 18.0 mo  c.92+5G>C (homozygous → not detected, donor type)  95/74@ 18.7 mo | No GVHD  EFS/OS@20.4 mo |
| 09 | Sickle cell disease | 23.0 | 56.8 | ND | | HbS 75.1% b | HbS 40.3% @ 23.3 mo  c.20A>T (homozygous → heterozygous, donor type)  86/73@ 21.9 mo | No GVHD  EFS/OS@19.2 mo |
| 10 | β-thalassemia major | 16.0 | 52.4 | ND | | Hb 7.7 g/dLa | Hb 12.5 g/dL @ 7.3 mo  c.92+5G>C (homozygous → heterozygous, donor type)  97/34@ 6.9 mo | No GVHD  EFS/OS@18.7 mo |
| 11 | β-thalassemia major, companying α-thalassemia silent carrier | 14.3 | 55.2 | 8.5 / 52.9 | | Hb 9.2 g/dLa | Hb 6.2 g/dL @ 16.5 mo  c.92+5G>C (homozygous → heterozygous, donor type)  86/ 93@15.5 mo | EFS/EFS@16.5 mo  Grade III aGVHD @ 0.4 mo after 2nd stem cell infusion |
| 13 | β-thalassemia major, companying α-thalassemia trait | 17.1 | 56.8 | ND | | Hb 6.6 g/dLa | Hb 14.1 g/dL @ 14.7 month  C.93-22\_95del (homozygous → heterozygous, donor type), 89/ 46@ 13.0 mo | No GVHD  EFS/OS@13.1 mo |
| 14 | β-thalassemia major | 14.6 | 49.4 | ND | | Hg 7.8 g/dLa | Hg 11.0 g/dL @ 4.2 month  c.92+5G>C (homozygous → heterozygous, donor type)  96/46 @ 2.2 mo | No GVHD  EFS/OS@4.2 mo |

*UPN* unique patient number *Hb* Hemoglobin *GVHD* Graft-versus-host disease *EFS* event-free survival *OS* overall survival *NA* not available *ND* not done *WB* whole blood *aGVHD* acute graft-versus-host disease *cGVHD* chronic graft-versus-host disease *Ig* immunoglobulin.

a To avoid influences by packed red cell transfusions, the lowest Hg levels of β-thalassemia major patients during pre-transplant 3 months were presented.

b Hemoglobin S level and serum immunoglobulin level of sickle cell anemia or hypogammaglobulinemia just before transplantation were showed.

**Figure Legends**

Fig. 1 Overall outcomes of 13 patients who received transplantation according to our strategy.

Fig. 2 Proportion of reinforced stem cells infusion-free survival.

Fig. 3 Survival outcomes. (a) Overall survival, (b) event-free survival, (c) thalassemia-free survival, (d) incidence of PB T-cell chimerism > 50%

Fig. 4 Changes in donor chimerism. (a) PB T-cell chimerism of the patients receiving reinforced stem cells infusion, (b) PB whole-cell chimerism of the patients receiving reinforced stem cells infusion, (c) PB T-cell chimerism of the patients not receiving reinforced stem cells infusion, (d) PB whole-cell chimerism of the patients not receiving reinforced stem cells infusion. Black dot indicates reinforced stem cell infusion.