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

**1. INTROUDCTION**

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 alloSCT using conventional myeloablative (MAC) or reduced-intensity conditioning (RIC), resulting in significant transplantation-related morbidity and mortality. Therefore, alloSCT using non-myeloablative (NMA) conditioning can be considered as a feasible treatment option for these patients. However, earlier studies reported minimal toxicity after alloSCT using NMA regimen of 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 [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 alloSCT using NMA conditioning with the NIH protocol were unable to cease immunosuppressive drug, because their donor T-cell chimerism did not reach 50% after one year, with a graft failure rate of 13% [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 immunosuppressive drug administration should be a major concern. To address these issues, we have established a two-stage strategy; a mixed donor chimerism in alloSCT 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 also received optional reinforced SC infusion. Herein, we will describe alloSCT using NMA conditioning with alemtuzumab and low-dose TBI, with the optional reinforced SC infusion for patients with β-TM and SCD.

**2. PATIENTS AND METHODS**

**2.1 Patients**

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

**2.2 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 patients without manipulation. For the GVHD prophylaxis and maintaining sustained engraftment, all patients began to receive 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/ml. After one year, we carefully tapered the sirolimus, if donor T-cell chimerism was maintained 50% or more in the absence of GVHD. If whole blood (WB) PB donor T-cell chimerism declined to <50% after one year or impeding graft failure was suspected, patients were administered optional reinforced infusion of SC from the same donors after total-nodal irradiation (TNI) at a single dose of 500 cGy. Informed consents were obtained from all patients. This study was approved by the Institutional Review Board of Seoul St. Mary’s Hospital.

**2.3 Definition of graft failure**

Primary graft failure was defined as lack of neutrophil engraftment (absolute neutrophil count <0.5×109/l) with a hypocellular marrow (aplasia) at day 28 or counts recovery with transfusion dependency (autologous recovery). Secondary graft failure was defined as when initial engraftment was followed by subsequent cytopenia [3]. Because we thought that patients who showed rapid decline of PB WB donor T-cell chimerism to approximately 20% during the early post-transplant period have greater risk of overt graft failure, they were considered to have impending graft failure.

**2.4 Supportive care**

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

**2.5 Statistical analysis**

The primary outcomes of this study were event free survival (EFS) and overall survival (OS). The EFS was calculated from the date of transplantation to the any occurrence of an event or last follow-up. Event was defined as primary and secondary graft failure or any cause of death, patients being censored if they were alive without event. The OS was calculated from the date of transplantation to any cause of death or last follow-up, patients being censored if they were alive. In addition, any requirement for a red cell transfusion or failure to achieve thalassemia-free disease status was defined as a β-TM-specific event. An SCD-specific event was defined as a clinical manifestation of SCD or a failure to achieve donor-type hemoglobin S (HbS).

**3. RESULTS**

**3.1 Patient demographics and disease-related characteristics**

Between April 2014 and March 2019, 13 consecutive adult patients of Arabid ethnicity were included in this analysis (Table 1). They consisted of 6 (46.2%) male and 7 (53.8%) female with a median age of 31 (range, 24–34) years at transplantation. The proportion of female donor to male recipient pairs was 15.4% (95% CI, 1.9–45.4). Major and minor ABO mismatches transplantations between the donor and recipient were included (n = 3 in both). All patients were afflicted with hemoglobinopathies, including β-TM in 9 (69.2%) and SCD in 4 (30.8%). A patient with β-TM (UPN #01), who was referred after experiencing graft failure with previous MAC transplantation from another hospital, was included in our cohort. More detailed patient demographics and disease-related characteristics are described in Table 1.

**3.2 Major transplant-related outcomes**

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 receiving 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 others 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% in both. ~~The median PB donor’s T-cell chimerism at 1 month was 84% (range, 3–97).~~

There were one with β-TM and one with SCD patients who showed rapid decline of PB WB donor chimerism to approximately 20% (22% and 33%, respectively) during the early post-transplant period, with occasional transfusion requirement from 1.9 months and 6.2 months, respectively. (Impending graft failure in two patients; 15.4%) In addition, 5 (38.5%) patients experienced failure to maintain PB donor T-cell chimerism over 50% after post-transplant one year. Because two (one with β-TM and one with SCD) refused to receive the optional reinforced SC infusion, 5 patients proceeded to the optional reinforced SC infusion at a median of 15.1 months (range, 3.9–37.6), at a median PB donor T-cell chimerism of 30.0% (range, 17.0–40.0) after TNI conditioning. The median infusion doses of CD34+ and CD3+ cells were 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 in the optional reinforced SC infusion.

The optional 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). The detailed overall outcomes were summarized in Fig. 1 and Table 2.

At the last follow-up, no patient experienced primary and secondary graft failure. Changes in PB donor WB and T-cell chimerism of patients who received our strategies were shown in Fig. 4.

**3.2.1 β-thalassemia major**

One of 9 β-TM patients was ineligible for discontinuing sirolimus, because the patient was less than post-transplant one year at the time of data collection. Of the remaining 8 patients, one (UPN #10) experienced impending graft failure with occasional red cell transfusion requirement. The others achieved stable mixed chimerism until post-transplant one year. However, three of them (UPN #00, #00 and #00) failed to achieve sustained donor T-cell chimerism (more than 50%) after post-transplant one year, but one (UPN #00) refused further procedure and is currently taking sirolimus at post-transplant 22.8 months. Including patients with impending graft failure, a total of three patients (UPN #10, #00 and #00) received the optional reinforced SC infusion each at post-transplant 3.9, 15.1 and 37.6 months, respectively. Other four patients (UPN #00, #00, #00 and #00) showed sustained donor PB T-cell chimerism (more than 50% after post-transplant one year) and discontinued sirolimus at post-transplant 13.2, 14.1, 14.7 and 16.5 months, respectively. At the last follow-up, total seven patients maintained stable mixed chimerism. The mean hemoglobin levels for male and female before receiving our strategy were 8.0 g/dL (range, 7.4–9.5) and 8.0 g/dL (range, 7.4–9.2), respectively. At the last follow-up, they have significantly improved to 11.0 g/dL (range, 9.0 – 13.7) and 11.0 g/dL (range, 10.0–13.0), respectively (*P* < 0.05 in both). After receiving our strategy, there was no patient who required red cell transfusion, regardless of the need for the optional reinforced SC infusion, and hospitalization. A genetic study revealed that all initial genetic abnormalities of hemoglobin were corrected to donor-type gene, regardless of whether they received the optional reinforced SC infusion or not.

**3.2.2. Sickle cell disease**

Of the 4 patients with SCD, one (UPN #00) with impending graft underwent the optional reinforced infusion at post-transplant 7.2 months. Remaining three patients (UPN #00, #00 and #00) achieved stable mixed chimerism until post-transplant one year: one patient ~ . The others showed persistently low donor T-cell chimerism after post-transplant one year. One patient refused the optional reinforced infusion and continued sirolimus ~~(donor WB and T-cell chimerism of 83% and 43% at post-transplant 27.3 months, respectively)~~. Another patient (UPN #06) underwent the optional reinforced infusion at post-transplant 20.7 months, who achieved stable mixed chimerism of donor PB T-cell chimerism more than 50% after post-transplant one year and discontinued sirolimus at 18.4 months. The mean hemoglobin levels for male and female before receiving our strategy were 6.0 g/dL (range, 0.0–0.0) and 7.5 g/dL (range, 0.0–0.0), respectively. At the last follow-up, they have significantly improved to 13.5 g/dL (range, 0.0 – 0.0) and 11.5 g/dL (range, 0.0–0.0), respectively (*P* < 0.05 in both). The patients achieved the same percentage of HbS as their donors after SCT; from median 72.9% to 37.4% (most recent). and all patients achieved donor type HbS. After receiving our strategy, there was no patient with SCD who suffered disease-related complications. Only one patient (UPN #00) was hospitalized due to non-specific colitis at post-transplant 0.0 months. All genetic abnormalities of hemoglobin were also replaced by donor-type gene, regardless of whether they received the optional reinforced SC infusion or not.

**3.3 Graft-versus-host disease and transplant-related complications**

Before patients received the optional reinforced SC infusions, no one developed acute and chronic GVHD. The infectious complications of grade 3 or worse was observed in one patient. Other transplant-related complications, including CMV reactivation requiring pre-emptive therapy, CMV disease, herpes zoster, hemorrhagic cystitis and sinusoidal obstruction syndrome were also not observed in any patients.

However, after 5 patients received the optional reinforced SC infusion, one (UPN #11) developed steroid-refractory acute GVHD grade III and subsequently died of pneumonia complicating adult respiratory distress syndrome after reinforced infusion 0.0 months. This patient’s infused doses of CD34+ and CD3+ cells were 8.5 × 106/kg and 52.9 × 107/kg, respectively, which were the highest in those who received the optional reinforced infusion. The other (UPN #02) developed chronic oral GVHD, which was partially responsive to corticosteroid, and had taken ruxolitinib for 5 months. At the last follow-up, we attempted for this patient to discontinue sirolimus after confirming disappearance of chronic oral GVHD. This patient’s infused doses of CD34+ and CD3+ cells were 8.5 × 106/kg and 52.9 × 107/kg, respectively, which were the second highest in those who received the optional reinforced infusion. The remaining three did not develop any form of acute and chronic GVHD and discontinued sirolimus at post-transplant 20.7, 22.7, and 28.5 months, respectively (reinforced infusion 16.8, 15.5, and 7.8 months, respectively). As a whole, the cumulative incidence of acute grade III-IV GVHD at the reinforced infusion day 100 was 20.0% (95% CI, 0–48.4). The cumulative incidences of severe chronic GVHD at reinforced infusion 12 months was 20.0% (95% CI, 0.4–63.2).

After patients received the optional reinforced SC infusion, CMV reactivation requiring pre-emptive therapy and hemorrhagic cystitis were observed in one in both. The Infectious complications of grade 3 or worse was observed in one patient. Except for the above-described acute GVHD-related mortality, an 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 one 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.

**3.4 Survival outcomes**

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) in both (Fig. 3a and 3b). The thalassemia-free survival rate at 4 years in β-TM patients was 87.5% (95% CI, 38.7–98.1) (Fig. 3c). The cumulative incidence of achieving PB donor T-cell chimerism > 50% at 4 years in patients not proceeding to the optional reinforced SC infusion was 68.8% (95% CI, 4.5–89.8) (Fig. 3d).

**4. DISCUSSION**

Unlike SCD patients, β-TM patients do not require chemotherapy nor is their immunological system impaired. However, they have a robustly hyperplastic and expanded marrow compartment with allo-sensitization as a result of multiple transfusions [4]. Therefore, the ideal conditioning for β-TM should be able to eradicate hyperplastic marrow and be sufficiently immunosuppressive to overcome the established allo-sensitization. Under these circumstances, MAC with busulfan and cyclophosphamide has been considered a standard regimen for β-TM patients receiving alloSCT [5]. However, MAC or even RIC is associated with high risk of TRM in adult β-TM patients, who already have advanced disease with marked erythroid expansion and multiple comorbidities. Early experiences of alloSCT using MAC in adult β-TM patients showed poor outcomes, with an overall and rejection-free survival rates of only 65% and 62%, respectively. The TRM incidence was significant, ranging from 28% to 37% depending on the dosage of cyclophosphamide [6]. Accordingly, safer conditioning regimens with inducing stable donor chimerism are required for this population.

Although there are several studies of alloSCT using NMA conditioning for adult SCD patients [2,7,8], they are currently very scarce for adult β-TM patients. By analogy with the behavior of malignant tissue, a large mass of rapidly proliferating hematopoietic tissue in β-TM is difficult to eradicate and is more likely to recur after transplantation, with a low-intensity conditioning. AlloSCT using NMA conditioning has been previously reported using a main population consisting of pediatric patients admixed with β-TM and SCD; the outcomes were disappointing with only transient engraftment [1]. On the contrary, several studies support the observation that full donor chimerism for the clinical success of alloSCT is not mandatory in β-TM patients, as the persistence of even a small percentage of donor-derived erythropoiesis may maintain the potential to correct the phenotypic expression of the disease, due to the competitive advantage of both donor-derived PB erythrocytes and erythroid progenitors over their β-TM counterparts . According to a long-term analysis, transient mixed chimerism does not necessarily lead to graft rejection and eventually evolves toward a status of stable persistent mixed chimerism or complete donor chimerism in most cases [9]. In most reports, although the risk of graft rejection appears greatest in the first two months after transplantation, once persistent mixed chimerism is established, patients seem to be no longer exposed to the risk of graft failure, showing a stable graft function without the need for additional red blood cell transfusion support [10]. Along with these data, a reliable achievement of stable mixed chimerism by alemtuzumab with low-dose TBI provides a rationale for alloSCT using NMA conditioning in β-TM patients. Indeed, a major population in our study was adult β-TM patients (9 of 13; 69.2%), of whom 7 (77.8%) achieved stable mixed chimerism for more than one year and 4 (30.8%) did not require additional optional reinforced SC infusion due to their PB donor T-cell chimerism being maintained at over 50% after one year. This result suggests that stable mixed chimerism could be induced in a significant portion of β-TM patients without requiring additional manipulations. In addition, two of the three (66.6%) patients with optional reinforced SC infusion sustainably maintain a complete chimerism. To the best of our knowledge, our report is the first to show that alloSCT using NMA conditioning can be successfully applied to adult β-TM patients who achieved a stable mixed chimerism with correcting abnormal hemoglobin phenotypes.

One of themajor limitationsof the NIH protocol is the need for long-term immunosuppression in patients with a persistently low donor T-cell chimerism (< 50%). Nearly half of the patients should have continued sirolimus with a median duration of 2.1 years [2]. Considering several reports found that a cumulative duration of immunosuppression over 2 years is associated with an increased incidence of secondary malignancies [11,12], which become to be a major concern. It was especially problematic in patients who should take life-long sirolimus such as solid organ transplantation recipient [13,14]. Although no one developed a secondary malignancy in patients who received NIH protocol, the follow-up duration of the study was not long enough to determine true incidence. In addition, sirolimus-related adverse drug reactions include anemia, thrombocytopenia, lipid metabolism disorder, new-onset diabetes, and hypertension and respiratory or urinary tract infections [15]. Therefore, protracted immuno-suppression with sirolimus can be associated with increased morbidities from these reactions. The NIH protocol study, despite of long-term immunosuppression, found a graft failure rate reached to approximately 13% [2]. Moreover, in a previous report analyzing the outcomes of secondary alloSCT for β-TM patients experiencing graft failure, prognosis was extremely poor; nearly half of patients (14/29; 48.3%) died of regimen-related toxicities, chronic GVHD and recurring graft failure. Consequently, the EFS and OS at 3 years were only 58% and 68%, respectively [16].

We attempted to overcome these limitations using optional reinforced SC infusion after conditioning with 500 cGy TNI in cases of being unable to discontinue sirolimus after one year. At the time of this procedure, the median donor WB and T-cell chimerism were 35% and 23%, respectively. To date, no patient (including two patients with impending graft failure) experienced graft loss after optional reinforced SC infusion. However, since several reports suggested that a myeloid chimerism of around 20% is enough to achieve functional graft characterized by normal hemoglobin level, no need for red cell transfusion, no serum iron level increment and a limited degree of erythroid hyperplasia [10,17,18], careful attention should be paid in the selection of optimal candidates and cell dose infused, especially considering the risk of GVHD. Notably, in a fatal case reported herein, the number of CD3+ cells infused was the highest among patients who received optional reinforced SC, which can account for developing severe acute GVHD. Although no meaningful statistical conclusion can be drawn in our study due to the limited number of patients, the development of acute and chronic GVHD tended to be associated with the number of CD3+ cells infused, as expected.

Our study included a β-TM patient with experiencing delayed graft failure after previous alloSCT using MAC without radiation. After receiving alloSCT using our NMA conditioning, this patient discontinued sirolimus with stable mixed chimerism without an optional reinforced SC infusion, suggesting that a conditioning with alemtuzumab plus low-dose TBI may overcome graft loss after the intensive conditioning. A previous study reported that an increased dose of TBI substantially reduced graft failure with maintaining the safety of haploidentical alloSCT using NMA conditioning, indicating that irradiation a plays a significant role in this disease population [19].

The NIH protocol excluded major ABO-incompatible donor-recipient pairs. In alloSCT using MAC, ABO-incompatibility is generally known to being not a hurdle in achieving complete donor chimerism. However, ABO mismatch can be responsible for graft failure, pure red cell aplasia and immune-mediated hemolysis in alloSCT using NMA conditioning [20-22]. Our study included three patients with major and minor ABO- incompatibility: one of them with major mismatch successfully withdrew sirolimus without the need for optional reinforced SC infusion with achieving a stable mixed chimerism of a donor PB T-cell chimerism of 60%. Two of the three (66.6%) minor ABO-mismatch did not require optional reinforced SC infusion and have currently discontinued sirolimus. These results suggest that ABO-incompatibility is not associated with an increased incidence of graft failure and should not be regarded as a contraindication for alloSCT using NMA conditioning 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 conclusive conclusions. Additional limitations include that alloSCT using NMA conditioning could only be applied only to patients with a suitable MSD as described previously [2]. This may be a major barrier for performing our strategy, as most patients with hemoglobinopathies do not have an acceptable MSD [23]. Lastly, although our strategy was effective in preventing patients from receiving long-term immunosuppression, there was on recorded fatality by developing of acute and chronic GVHD after an optional reinforcedSC infusion. Since infused number of CD3+ cells appear to be associated with these complications, further studies are needed to determine the optimal cell dose of optimal reinforced SC infusion to minimize the risk of GVHD without sacrificing donor engraftment. Despite of these limitations, our results showed acceptable outcomes with a consistency of using uniform NMA conditioning and criteria for optional reinforced SC infusion in both β-TM and SCD patients.

**5. CONCLISIONS**

Despite being retrospective and including a small number of patients, our study found that alloSCT using NMA conditioning of alemtuzumab plus low-dose TBI was effective in achieving stable mixed chimerism not only in SCD, but in β-TM patients. Our strategy of optional reinforced SC infusion was effective in preventing to continuing immunosuppression in these patients. However, future studies are needed in order to determine the optimal cell dose in optional reinforced infusions.

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**CONFLICT OF INTEREST**

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 after MAC (Treosulfan + Cy) alloSCT, 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 |

Abbreviations: UPN, unique patient number; ECOG PS, Eastern Cooperative Oncology Group performance status; HCT-CI, Hematopoietic Cell Transplantation-specific Comorbidity Index; T/F, transfusion; Hb, hemoglobin; APL, acute promyelocytic leukemia; CR, complete remission; MAC, myeloablative conditioning; alloSCT, allogeneic stem cell transplantation; 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.