**Title: Nonmyeloablative matched sibling stem cell transplantation with** **the 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 benign and malignant hematologic disease. The incidences 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, frequently associated with both disease- and treatment-related organ complications, may be unable to tolerate alloSCT using conventional myeloablative (MAC) or reduced-intensity conditioning (RIC). 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, of which application resulted in stable mixed donor chimerism sufficient 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 incidence 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, β-TM -specific outcomes could not have been detailed so far.

In addition, a significant portion of the patients who received alloSCT using NMA conditioning of the NIH protocol were unable to discontinue 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 over 50% should been received immune-suppression. Various complications due to protracted immunosuppressive drug administration must 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 the optional reinforced hematopoietic stem cell (SC) infusion in cases requiring prolonged immunosuppression. Patients who experienced impending graft failure also received the optional reinforced SC infusion. Herein, we describe the outcomes of 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 β-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 of 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 post-transplant one year, we carefully tapered the sirolimus, if donor T-cell chimerism was maintained 50% or more in the absence of GVHD. If PB donor T-cell chimerism declined to 50% or less after post-transplant one year or impeding graft failure was suspected, patients received the optional reinforced unmanipulated SC infusion from the same donor 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 BM (aplasia) at day 28 or counts recovery with transfusion dependency (autologous recovery). Secondary graft failure was defined as when initial engraftment followed by irreversible cytopenia [3]. Because we thought that patients who showed rapid decline of PB donor WB 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**

We have tried to adhere to the US and Thalassemia International Federation guidelines, Standards of care guidelines for thalassemia and Guidelines for the clinical management of thalassemia [4-6]. Briefly, we have attempted to maintain target pre-transfusion hemoglobin level was between 9 and 10.5 g/dL through transfusing packed red cell every 2-5 weeks. Furthermore, if patients have cardiac dysfunction, they received packed red cell transfusion with a higher hemoglobin level of 10-12 g/dL. We have attempted to maintain post-transfusion threshold for hemoglobin level of 14 g/dL or less with a mean level of 12 g/dL. The prophylaxis with acyclovir and sulfamethoxazole-trimethoprim was administered to prevent herpes virus reactivation and pneumocystis jirovecii, respectively. We performed regular cytomegalovirus (CMV) DNA RQ-PCR monitoring, followed by pre-emptive ganciclovir treatment.

**2.5 Statistical analysis**

The primary outcomes of this study were event free survival (EFS) and overall survival (OS), which were estimated with Kaplan-Meier estimates. The EFS was calculated from the date of transplantation to any occurrence of an event or last follow-up. Event was defined as primary and secondary graft failure or any cause of death, with being censored if patients were alive without event. The OS was calculated from the date of transplantation to any cause of death or last follow-up, with being censored if patients 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). Thalassemia-free and SCD-free survival were calculated from the date of transplantation to β-TM-specific and SCD-specific event, respectively, or last follow-up.

**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). There were major and minor ABO mismatches transplantations between the donor and recipient (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**

The detailed overall transplant-related outcomes are described 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 4 (30.8%) patients did not experience neutrophil or platelet nadir during peri-transplantation period, respectively. Others achieved neutrophil and platelet engraftments at a median of 14.5 (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 (UPN #03) with β-TM and one (UPN #05) with SCD patients (7.7%) who showed rapid decline of PB WB donor chimerism to approximately 20% (34% and 22%, respectively) during with occasional transfusion requirement during early post-transplantation period (less than post-transplant 3 months). In addition, 5 (38.5%) patients failed to maintain PB donor T-cell chimerism over 50% after post-transplant one year. Because two (one with β-TM and one with SCD; UPN #10 and #06, respectively) refused to receive the optional reinforced SC infusion, 5 patients proceeded to the optional reinforced SC infusion after TNI conditioning at a median of post-transplant 15.1 months (range, 3.9–37.6), when they had a median PB donor T-cell chimerism of 30.0% (range, 17.0–40.0). 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. When we calculated the estimated the optional reinforced SC infusion-free survival rates at 12 and 24 months, they were 83.9% (95% CI, 49.4–95.7) and 66.1% (95% CI, 32.5–85.8), respectively (Fig. 2).

Except one (UPN #13) not eligible for discontinuing due to follow-up duration of one year or less, 5 of 7 patients who did not receive the optional reinforced SC infusion were able to successfully discontinue sirolimus at a median post-transplant 14.1 months (range, 3.9–18.4). They achieved stable PB donor WB and T-cell mixed chimerism of a median of 89% (range, 85–95) and 73% (range, 46-75) at the time of last follow-up, respectively. All patients who received the optional reinforced SC infusion achieved PB donor WB and T-cell mixed or complete chimerism of a median of 99% (range, 86–100) and 99% (range, 93–100) at the time of last follow-up, respectively. Three (UPN #03, #04 and #05) of these patients discontinued sirolimus at the optional reinforced SC infusion 16.8, 7.8, and 15.5 months, respectively. As a whole, 8 (57.1%) patients discontinued sirolimus at the time of last follow-up (Fig. 1). Consequently, patient who experienced primary and secondary graft failure was not observed at all.

On the other hand, of the three patients (UPN #04, #12, and #13) with major ABO mismatches, one (UPN #04) proceeded to the optional reinforced SC infusion at post-transplant 20.7 months, who achieved complete donor chimerism and discontinued sirolimus at the optional reinforced SC infusion 7.8 months. Another (UPN #12) discontinued sirolimus after post-transplant 14.7 months with a stable PB donor WB and T-cell mixed chimerism (89% and 46% at the last follow-up), not requiring the optional reinforced SC infusion. The other has a follow-up duration of less than one-year as described above. Of the three patients (UPN #03, #07, and #08) with minor ABO mismatch, only one (UPN #03) required the optional reinforced SC infusion, whereas other two successfully discontinued sirolimus without requiring the optional reinforced SC infusion.

The overall 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**

Of the 8 β-TM patients, except the above-mentioned with follow-up duration of less than one year, one (UPN #03) patient experienced impending graft failure with occasional red cell transfusion requirement. Others achieved stable mixed chimerism until post-transplant one year. However, three patients of them (UPN #02, #10 and #11) failed to maintain donor T-cell chimerism over 50% after post-transplant one year, but one (UPN #10) refused further procedure and is currently receiving sirolimus. Including one patient with impending graft failure, a total of three (UPN #02, #03 and #11) received the optional reinforced SC infusion each at post-transplant 37.6, 3.9, and 15.1 months, respectively. Other four patients 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 time of follow-up, total seven patients maintained stable mixed or complete chimerism. Except one patient with follow-up duration of less than one year, the median hemoglobin levels for male and female patients significantly improved from 7.7 g/dL (range, 7.4–9.4) and 8.0 g/dL (range, 7.6–9.1) before receiving our strategy, respectively, to 12.8g/dL (range,12.5– 13.7) and 14.3 g/dL (range, 11.0–15.4), respectively, at the time of last follow-up (*P* < 0.01 and *P* = 0.01, respectively). After patients received our strategy, there was no one who required red cell transfusion, regardless of the need for the optional reinforced SC infusion, and hospitalization due to any cause. 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 (Table 2).

**3.2.2. Sickle cell disease**

Of the 4 patients with SCD, one (UPN #05) with impending graft failure received the optional reinforced infusion at post-transplant 7.2 months. Other three patients achieved stable mixed chimerism until post-transplant one year: One patient (UPN #09) showed sustained donor T-cell chimerism (more than 50% after post-transplant one year) and discontinued sirolimus at post-transplant 18.4 months. Others showed persistently low donor T-cell chimerism after post-transplant one year. One patient (UPN #06) refused the optional reinforced infusion and is currently receiving sirolimus. Another (UPN #04) received the optional reinforced infusion at post-transplant 20.7 months, who achieved complete donor WB and T-cell chimerism 99.0% in both at last time of follow-up. The hemoglobin level of only one male patient improved from 8.8 g/dL before receiving our strategy to 13.8 at the time of last follow-up. The median hemoglobin levels of female patients also, but not significantly, improved from 8.4 g/dL (range, 8.1–8.7) to 13.5 g/dL (range, 10.1–15.3) (*P* = 0.11). The patients achieved the same percentage of HbS as their donors’ after receiving our strategy; from median 72.9% to 37.4% (most recent) and all patients achieved donor type HbS. At the last time of follow-up, there was no SCD patient who suffered disease-related complications. All genetic abnormalities of hemoglobin were also replaced by donor-type gene, regardless of whether they received the optional reinforced SC infusion or not (Table 2).

**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 more was observed in one patient (UPN #11) at post-transplant 0.4 months. Other transplant-related complications, including CMV reactivation re-quiring pre-emptive therapy, CMV disease, herpes zoster, hemorrhagic cystitis and sinusoidal obstruction syndrome were also not observed in any patients. However, of 5 patients received the optional reinforced SC infusion, one (UPN #11) developed steroid-refractory acute grade III GVHD and subsequently died of pneumonia complicating adult respiratory distress syndrome at the optional reinforced SC infusion 1.4 months (post-transplant 16.5 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 SC infusion. The other (UPN #02) developed severe chronic oral GVHD, which was partially responsive to corticosteroid and received ruxolitinib for 5 months. At the last time of follow-up, we have attempted for this patient to discontinue sirolimus after confirming disappearance of chronic GVHD. This patient’s infused doses of CD34+ and CD3+ cells were 13.0 × 106/kg and 27.9 × 107/kg, respectively, which were the second highest in those who received the optional reinforced SC infusion. The other three did not develop any form of acute and chronic GVHD and discontinued sirolimus at the optional reinforced SC infusion 16.8, 15.5, and 7.8 months, respectively. As a whole, the cumulative incidence of acute grade III-IV GVHD at the optional reinforced SC infusion day 100 was 20.0% (95% CI, 0–48.4). The cumulative incidences of severe chronic GVHD at the optional reinforced SC 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 (UPN #02) in both at the optional reinforced infusion 2.9 and 2.8 months respectively. One patient (UPN #04) was hospitalized due to non-specific colitis at the optional reinforced infusion 0.6 months. Except for the above-described acute GVHD-related mortality, an additional TRM was not observed.

**3.4 Survival outcomes**

With a median follow-up duration of 31.5 months (range, 4.2 – 64.4), the EFS and OS 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 SCD-free survival rate at 4 years in SCD patients was 100% (Fig. 3d). 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. 3e).

**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 [7]. Therefore, the ideal conditioning for β-TM patients should be able to eradicate hyperplastic marrow and be sufficiently immunosuppressive to overcome the established allo-sensitization. Under this circumstance, MAC with busulfan and cyclophosphamide has been considered a standard regimen for these patients receiving alloSCT [8]. However, MAC or even RIC is associated with high TRM incidence in adult β-TM patients, who already have advanced disease with marked erythroid expansion and multiple comorbidities. Early experiences of alloSCT using MAC patients showed poor outcomes, with an overall and rejection-free survival rates of only 65% and 62%, respectively. The TRM incidence was significantly high, ranging from 28% to 37% depending on the cyclophosphamide dose [9]. 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 [2,10,11], it is 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 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 [12]. 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, achieving a stable graft function without the need for additional red blood cell transfusion support [13]. Along with these data, a reliable attainment 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 post-transplant one year and 4 (30.8%) did not require the 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 patients with the optional reinforced SC infusion 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%), although the paradigm of which peripheral blood donor T-cell chimerism > 50% should be maintained before tapering immuno-suppressive agent to avoid graft rejection should be confirmed by larger studies. 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 [14,15], which become to be a major concern. It was especially problematic in patients who should take life-long sirolimus such as solid organ transplantation recipients [16,17]. 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, hypertension and respiratory or urinary tract infections [18]. Therefore, protracted immuno-suppression with sirolimus can be associated with increased morbidities from these complications. 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, the prognosis was extremely poor; nearly half of patients 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 [19].

We attempted to overcome these limitations using the optional reinforced SC infusion after conditioning with 500 cGy TNI in the cases of being unable to discontinue sirolimus after post-transplant one year. At the time of this procedure, their 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 the 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 [13,20,21]. In addition, several adult SCD patients of the NIH study discontinued sirolimus earlier than required and did not experience graft rejection with sustained peripheral blood donor T-cell chimerism after post-transplant one year [2]. Therefore, 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.

Previous reports showed that incorporating alemtuzumab to the conditioning for depleting donor T cells contributed to reduce the incidence of acute and chronic GVHD in the setting of alloSCT using NMA conditioning [22,23]. Three patients, in our cohort, who received the optional reinforced infusion due to impeding graft failure in early post-transplant period did not experience acute and chronic GVHD at all, whereas two of those who received the procedure due to declined PB donor T-cell chimerism after post-transplant one year experienced severe acute or chronic GVHD. It suggests that T-cell depletion by alemtuzumab could contribute to prevent GVHD in patients who received the optional reinforced infusion not after post-transplant one year, but in early post-transplant period, which might be resulted from gradually attenuated effect over time of alemtuzumab. The above-mentioned results make us to consider that the optional reinforced SC infusion should be preferentially performed to patients with rapid decline of PB donor WB chimerism during the early post-transplant period.

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 the 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 for patients with hemoglobinopathies, indicating that irradiation a plays a significant role in this disease population [24].

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. On the contrary, ABO mismatch can be responsible for graft failure, pure red cell aplasia and immune-mediated hemolysis in alloSCT using NMA conditioning [25-27]. Our study included three patients with major and minor ABO-incompatibility: one with major mismatch successfully withdrew sirolimus without the need for the optional reinforced SC infusion with achieving a stable mixed chimerism of a donor PB T-cell chimerism of 60%. Two of the three minor ABO-mismatch did not require the 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, consisted of 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]. It may be a major barrier for performing our strategy, as most patients with hemoglobinopathies do not have an acceptable MSD [28]. 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 the 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 the optimal reinforced SC infusion to minimize the incidence of GVHD without sacrificing donor engraftment, especially in patients who received the procedure after post-transplant one year. In addition, considering risk/benefit of our strategy, the optional reinforced SC infusion should be also restrictively performed to patients with rapid decline of PB donor WB chimerism during the early post-transplant period. Despite of these limitations, our results showed acceptable outcomes with a consistency of using uniform NMA conditioning and criteria for the 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, consisted 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 the 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 | Pre-transplant | | | ECOG PS | HCT-CI | Donor-recipient compatibility | |
| Disease status | Ferritin | Iron chelating agent/hyroxyurea | ABO | Sex |
| 01 | M/29 | β-thalassemia major | T/F dependency, Iron overloading | NA | NA | 1 | NA | NA | M → M |
| 02 | M/33 | β-thalassemia major | T/F dependency, Iron overloading | 8161 | Deferasirox → deferoxamine 2000 mg | 1 | 4 | O+ → O+ | M → M |
| 03 | M/34 | β-thalassemia major | T/F dependency, Iron overloading | 10559 | Defroxamine | 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 | 155.6 | Hyroxyurea 1000 mg | 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 | 77.60 | ND | 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 | 8005 | Deferasirox / Hydroxyurea 1000 mg | 1 | 2 | O+ → O+ | M → F |
| 07 | F/32 | β-thalassemia major, companying α-thalassemia trait | T/F dependency, Iron overloading | 1435 | Defroxamine | 1 | 2 | A+ → AB+ | M → F |
| 08 | F/31 | β- thalassemia major, companying α-thalassemia silent carrier | T/F dependency, Iron overloading | 4830 | Defroxamine | 1 | 3 | A+ → AB+ | F → F |
| 09 | F/24 | Sickle cell anemia | Previous APL (CR state), T/F dependency, Iron overloading | 1605 | Deferasirox 8750 mg | 1 | 3 | A+ → A+ | M → F |
| 10 | M/29 | β-thalassemia major | T/F dependency, Iron overloading | 8142 | Deferasirox 1500 mg | 2 | 2 | O+ → O+ | M → M |
| 11 | M/34 | β-thalassemia major, companying α-thalassemia silent carrier | T/F dependency, Iron overloading | 812.8 | Deferasirox 1500 | 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 | 1674 | ND | 1 | 2 | AB+ → B+ | M → F |
| 13 | F/34 | β-thalassemia major, companying α-thalassemia silent carrier | T/F dependency, Iron overloading | 929.9 | ND | 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; NA, not available; Hb, hemoglobin; APL, acute promyelocytic leukemia; CR, complete remission; MAC, myeloablative conditioning; alloSCT, allogeneic stem cell transplantation; Cy, Cyclophosphamide; ND, not done

Table 2 Transplant-related outcomes

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| UPN | Cell dose of  1st stem cell infusion | | Cell dose of  reinforced SC infusion | | Pre-transplant  Hb or HbS | Post-transplant outcomes | | | | |
| CD34+  (× 106/kg) | CD3+  (× 107/kg) | CD34+  (× 106/kg) | CD3+  (× 107/kg) | Hb or HbS | Gene study | Chimerism  WB/T-cell (%) | GVHD | EFS/OS |
| 01 | 10.6 | NA | ND | | NA | Hb 13.0 g/dL at 61.4 mo | c.92+5G>C (homozygous → heterozygous, donor type) | 89/75 at 60.3 moc | No | 60.3/60.3 mo |
| 02 | 10.5 | 28.3 | 13.0 | 27.9 | Hb 7.4 g/dLa a | Hb 12.6 g/dL at 54.1 mo | c.92+5G>C (homozygous → heterozygous, donor type) | 99/100 at 46.6 moc | Severe cGVHD at 2.5 mod | 50.2/50.2 mo |
| 03 | 33.3 | 56.8 | 9.9 | 24.7 | Hb 7.8 g/dLa | Hb 13.7 g/dL at 21.6 mo | c.92+5G>C (homozygous → not detected, donor type) | 99/99 at 21.6 moc | No | 35.3/35.3 mo |
| 04 | 14.9 | 73.4 | 10.7 | 20.9 | HbS 70.8%b | HbS 30.6% at 29.4 mo | c.20A>T (homozygous → heterozygous, donor type) | 99/99 at 28.0 moc | No | 33.5/33.5 mo |
| 05 | 12.8 | 15.8 | 14.4 | 16.3 | HbS 64.5%b | HbS 34.5% at 22.7 mo | c.20A>T (homozygous → heterozygous, donor type) | 99/99 at 27.0 moc | No | 31.6/31.6 mo |
| 06 | 17.8 | 55.0 | ND | | HbS 89.3% b | HbS 40.3% at 21.9 mo | c.20A>T, C92+1G>A (homozygous → heterozygous, donor type) | 79/33 at 27.0 moc | No | 23.2/23.2 mo |
| 07 | 8.8 | 39.9 | ND | | Hb 7.6 g/dLa | Hg 15.4 g/dL at 18.7 mo | c.92+5G>C (homozygous → heterozygous, donor type) | 85/64 at 18.7 moc | No | 21.1/21.1 mo |
| 08 | 10.6 | 77.3 | ND | | Hb 9. g/dLa | Hb 14.4 g/dL at 18.0 mo | c.92+5G>C (homozygous → not detected, donor type) | 95.74 at 18.7 moc | No | 20.4/20.4 mo |
| 09 | 23.0 | 56.8 | ND | | HbS 75.1% b | HbS 40.3% at 23.3 mo | c.20A>T (homozygous → heterozygous, donor type) | 86/73 at 21.9 moc | No | 19.2/19.2 mo |
| 10 | 16.0 | 52.4 | ND | | Hb 7.7 g/dLa | Hb 12.5 g/dL at 7.3 mo | c.92+5G>C (homozygous → heterozygous, donor type) | 97/34 at 6.9 moc | No | 18.7/18.7 mo |
| 11 | 14.3 | 55.2 | 8.5 | 52.9 | Hb 9.4 g/dLa | Hb 6.2 g/dL at 16.5 mo | c.92+5G>C (homozygous → heterozygous, donor type) | 86/93 at 15.5 moc | aGVHD Gr III  at 0.4 mod | 16.5/16.5 mo |
| 13 | 17.1 | 56.8 | ND | | Hb 8.1 g/dLa | Hb 14.1 g/dL at 14.7 mo | C.93-22\_95del (homozygous → heterozygous, donor type) | 89/46 at 13.0 moc | No | 13.1/13.1 mo |
| 14 | 14.6 | 49.4 | ND | | Hb 7.8 g/dLa | Hg 11.0 g/dL at 3.7 mo | c.92+5G>C (homozygous → heterozygous, donor type) | 96/46 at 2.2 moc | No | 4.2/4.2 mo |

Abbreviations: UPN, unique patient number; Hb, Hemoglobin; WB, whole blood; GVHD, Graft-versus-host disease; EFS, event-free survival; OS, overall survival; NA, not available; ND, not done; cGVHD, chronic graft-versus-host disease; aGVHD, acute graft-versus-host disease; Gr, grade

a To avoid influences by packed red cell transfusions, the lowest Hg levels of β-thalassemia major patients during pre-transplant 1 month were presented.

b Hemoglobin S level of sickle cell anemia patients and Hemoglobin level of β-thalassemia major patients just before transplantation were presented.

c Peripheral blood donor whole blood/T-cell chimerism at the last time of follow-up were described.

d Months after the optinal reinforced stem cell infusion were described.

**Figure Legends**

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

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

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

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