**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**

**Background:** The NIH protocol for nonmyeloablative (NMA) conditioning allogeneic stem cell transplantation (alloSCT) with alemtuzumab and low-dose total body irradiation corrected the abnormal sickle cell disease (SCD) phenotype without the risk of graft-versus-host disease. AlloSCT using NMA conditioning had been rarely applied to β-thalassemia major (β-TM) patients.

**Methods:** To avoid prolonged immunosuppression, we developed a two-stage strategy. Mixed donor chimerism was initially achieved using the protocol developed by the NIH. Thereafter, we facilitated donor chimerism using the optional reinforced stem cell (SC) infusion in cases requiring protracted immuno-suppression or experiencing impeding graft failure.

**Results:** In this study, β-TM (n=9) and SCD (n=4) patients were equally effectively treated with eradicating the abnormal hemoglobin phenotype. Five patients, including four β-TM, achieved stable mixed chimerism without receiving optional reinforced SC infusion. All patients that received optional reinforced infusion recipients achieved complete (n=4) or mixed chimerism (n=1). The overall survival rate and event-free survival at 4 years of 91.7% (95% CI; 53.9–98.8) in both groups, with a thalassemia-free survival rate in β-TM patients of 87.5% (95% CI; 38.7–98.1).

**Conclusion:** This study is the first to report successful NMA conditioning alloSCT to achieve stable mixed chimerism correcting the abnormal hemoglobin phenotype in adult β-TM patients.

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

**1. INTRODUCTION**

Allogeneic stem cell transplantation (alloSCT) remains curative treatment option for various disorders, including benign and malignant hematologic disease. The incidences of transplantation-related morbidity and mortality is 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) conditioning or reduced-intensity conditioning (RIC). Therefore, alloSCT using non-myeloablative (NMA) conditioning can be considered an option for β-TM and SCD patients. However, earlier studies reported the minimal toxicity of alloSCT using an NMA regimen of low-dose total body irradiation (TBI) plus fludarabine in patients with β-TM and SCD, but it resulted in only transient donor engraftment and consequent graft failure [1]. Recently, the National Institutes of Health (NIH) developed an NMA regimen using alemtuzumab with low-dose TBI for SCD patients, the application of which of which application resulted in stable mixed donor chimerism sufficient to product donor-type red blood cells and reverse the sickle cell phenotype. It also resulted in a very low incidence of graft-versus-host disease (GVHD), which was associated with a low incidence of treatment-related mortality (TRM) [2]. However, in β-TM patients, because of biological and clinical features different from SCD, including robust proliferation of the bone marrow (BM) and allo-sensitization due to multiple transfusions, it is not clear whether this low-intensity conditioning is sufficient to overcome.

Furthermore, a significant proportion of patients who received alloSCT using NMA conditioning according to the NIH protocol were unable to discontinue immunosuppressive drugs 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 cannot achieve a donor T-cell chimerism over 50% should receive immunosuppressive therapy. The variety of complications following protracted immunosuppressive drug exposure is a major concern. To address these issues, we established a two-stage strategy. 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 immunosuppressive drug administration. Also, patients who experienced impending graft failure received the optional reinforced SC infusion. Herein, we describe the outcomes of β-TM and SCD patients who received our unique strategy.

**2. PATIENTS AND METHODS**

**2.1 Patients**

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

**2.2 Transplant-related procedures**

Similar to the NIH protocol, the 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 total body irradiation (TBI) (300 – 400 cGy on day –2) [2]. Subsequently, peripheral blood (PB) stem cells (target CD34+ cell dose of 10 × 106/kg) from the MSD were mobilized with granulocyte-colony stimulating factor (G-CSF; 10 µg/kg filgrastim for 4 days) and administered to patients without additional manipulation. For GVHD prophylaxis and sustained engraftment, all patients received sirolimus loading at a dose of 15 mg/day on day –1, then 5 mg/day after day 0, followed by dose adjustment of the target trough level of 10–15 ng/ml for the first three to four months. Thereafter, we attempted to maintain a trough level of sirolimus of 5 – 10 ng/ml. After post-transplant one year, we carefully tapered the sirolimus, if donor T-cell chimerism was maintained at 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, the 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 consent was 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 hypocellular BM (aplasia) on day 28 or counts recovery with transfusion dependency (autologous recovery). Secondary graft failure was defined as initial engraftment followed by irreversible cytopenia [3]. Because we thought that patients who showed a rapid decline in PB donor whole blood (WB) chimerism to approximately 20% during the early post-transplant period had a greater risk of overt graft failure, they were considered to have impending graft failure.

**2.4 Supportive care**

We tried to adhere to the Guidelines for the Management of Transfusion Dependent Thalassemia, Standards of Care Guidelines for Thalassemia and Guidelines for The Clinical Management of Thalassemia [4-6]. Therefore, we have attempted to maintain target pre-transfusion hemoglobin levels between 9.0 and 10.5 g/dL through transfusing packed red cell every two to five weeks. Furthermore, if the patients had cardiac dysfunction, they received packed red cell transfusion with a higher hemoglobin target level of 10 – 12 g/dL. We attempted to maintain post-transfusion hemoglobin threshold level of 14 g/dL or less with a mean of 12 g/dL. From the time the patients received conditioning, prophylaxis with acyclovir and sulfamethoxazole-trimethoprim was administered to prevent herpes virus reactivation and pneumocystis jirovecii, respectively. We performed regular cytomegalovirus (CMV) DNA qRT-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. EFS was calculated from the date of the initial SC infusion to any event occurrence or the last follow-up, whichever came first. An event was defined as primary and secondary graft failure or any cause of death, with censor of patients alive without events. The OS was calculated from the date of the initial SC infusion to any cause of death or the last follow-up, with censor of patients still alive. 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 the initial SC infusion to β-TM-specific and SCD-specific events, respectively, or the last follow-up, whichever came first.

**3. RESULTS**

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

Between April 2014 and March 2019, 13 consecutive adult patients of Arabic ethnicity were included in this study (Table 1). All patients had hemoglobinopathies, including β-TM in nine (69.2%) and SCD in four (30.8%). They consisted of six (46.2%) males and seven (53.8%) females 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 transplantations with major and minor ABO mismatches between the donor and recipient (n = 3 in both). A patient with β-TM (UPN #01), who was referred after experiencing graft failure with previous alloSCT using MAC 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 Figure 1 and Table 2. The initial PB SC infusion contained a median 14.6 × 106/kg (range, 8.8 – 33.3) CD34+ cells and 55.1 × 107/kg (range, 15.8 – 77.3) CD3+ cells after patients received NMA conditioning. One (7.7%) and four (30.8%) patients did not experience neutrophil or platelet nadirs, respectively, during the peri-transplantation period. The 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 groups.

There was one patient (UPN #03) with β-TM and one (UPN #05) with SCD (15.4%) that showed a rapid decline in PB WB donor chimerism to approximately 20% (34% and 22%, respectively), with occasional transfusion requirements during the early post-transplantation period (less than three months post-transplant). In addition, five (38.5%) patients failed to maintain PB donor T-cell chimerism over 50% after post-transplant one year. Of these patients, two (one with β-TM and one with SCD; UPN #10 and #06, respectively) refused to receive the optional reinforced SC infusion and five proceeded to receive the optional reinforced SC infusion after TNI conditioning at a median post-transplant time of 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 patients receiving optional reinforced SC infusions. The estimated 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 (Figure 2A). The cumulative incidence of achieving PB donor T-cell chimerism > 50% at four years in patients not proceeding to the optional reinforced SC infusion was 68.8% (95% CI; 4.5 – 89.8) (Figure 2B).

Except for one patient (UPN #13) not eligible for discontinuing immunosuppressive drug due to a follow-up duration of one year or less, five of seven patients who did not receive the optional reinforced SC infusion were able to successfully discontinue sirolimus at a median post-transplant time of 14.1 months (range, 3.9 – 18.4). They achieved median stable PB donor WB and T-cell mixed chimerisms of 89% (range, 85 – 95) and 73% (range, 46 - 75), respectively, at the time of the last follow-up. 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 of these patients (UPN #03, #04 and #05) discontinued sirolimus at the optional reinforced SC infusion times of 16.8, 7.8, and 15.5 months, respectively. In total, eight (57.1%) patients discontinued sirolimus at the time of the last follow-up (Figure 1). No patients were observed to experience primary or secondary graft failure.

In contrast, of the three patients with major ABO mismatches, one (UPN #04) proceeded to an optional reinforced SC infusion at post-transplant 20.7 months and achieved complete donor chimerism. Sirolimus was discontinued at the optional reinforced SC infusion time of 7.8 months. Another patient (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, respectively), not requiring an optional reinforced SC infusion. The other patient (UPN #13) had a follow-up duration of less than one-year. Of the three patients (UPN #03, #07, and #08) with minor ABO mismatches, only one (UPN #03) required an optional reinforced SC infusion, and the others successfully discontinued sirolimus without requiring optional reinforced SC infusion.

The overall changes in PB donor WB and T-cell chimerism of the patients who received our strategies are shown in Figure 3.

**3.2.1 β-thalassemia major**

Of the eight β-TM patients, except the above-mentioned patient with follow-up duration of less than one year, one (UPN #03) experienced impending graft failure and required occasional red cell transfusions. The others achieved stable mixed chimerism until one-year post-transplant. However, three of the patients (UPN #02, #10 and #11) failed to maintain donor T-cell chimerism over 50% after post-transplant one year. One patient (UPN #10) refused further procedure and is currently receiving sirolimus. Including one patient with an impending graft failure, a total of three patients (UPN #02, #03 and #11) received optional reinforced SC infusions at post-transplant times of 37.6, 3.9, and 15.1 months, respectively. The 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 follow-up, a total of seven patients maintained stable mixed or complete chimerism. Except for the above-mentioned patient with a follow-up duration of less than one year, the median hemoglobin levels for the 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) at the last follow-up (*P* < 0.01 and *P* = 0.01, respectively). After receiving the strategy, no patients required red cell transfusions, regardless of the need for optional reinforced SC infusions, and no hospitalization due to any cause. A genetic study revealed that all initial genetic hemoglobin abnormalities were corrected to the donor-type gene, regardless of whether or not the patient received the optional reinforced SC infusion or not (Table 2).

**3.2.2. Sickle cell disease**

Of the four patients with SCD, one (UPN #05) with impending graft failure received the optional reinforced infusion at post-transplant 7.2 months. The other three patients achieved stable mixed chimerism until one-year post-transplant. 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. The others showed persistently low donor T-cell chimerism after post-transplant one year. One patient (UPN #06) refused the optional reinforced SC infusion and is currently receiving sirolimus. Another (UPN #04) received an optional reinforced infusion at post-transplant 20.7 months and achieved complete PB donor WB and T-cell chimerisms of 99.0% at the last follow-up. The hemoglobin level of only one male patient improved from 8.8 g/dL before receiving our strategy to 13.8 g/dL at the time of last follow-up. The median hemoglobin levels of the female patients also improved from 8.4 g/dL (range, 8.1 – 8.7) to 13.5 g/dL (range, 10.1 – 15.3) (*P* = 0.11) but the difference was not significant. The patients achieved the same percentage of HbS as their donors’ after receiving our strategy; from a median of 73.0% (range, 64.5 – 89.3; pre-transplantation) to 37.4% (range, 30.6 - 40.3; at the time of the last follow-up). At the last follow-up, no SCD patients had disease-related complications. All genetic hemoglobin abnormalities were replaced by donor-type gene, regardless of whether or not they received the optional reinforced SC infusion (Table 2).

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

No patient had developed GVHD before receiving an optional reinforced SC infusion. Infectious complications of grade 3 or more were observed in one patient (UPN #11) at post-transplant 0.4 months. No oher 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 five patients that 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 an optional reinforced SC infusion time of 1.4 months (post-transplant 16.5 months). This patient’s infused CD34+ and CD3+ cell doses were 8.5 × 106/kg and 52.9 × 107/kg, respectively, which were the highest among those who received an optional reinforced SC infusion. The other patient (UPN #02) developed severe chronic oral GVHD, which was partially responsive to corticosteroid and received ruxolitinib for 5 months. At the last follow-up, discontinuation of sirolimus was being attempted in this patient after confirming the disappearance of chronic GVHD. This patient’s infused CD34+ and CD3+ cell doses were 13.0 × 106/kg and 27.9 × 107/kg, respectively, which were the second-highest among those who received the optional reinforced SC infusion. The other three did not develop any form of GVHD, and discontinued sirolimus at optional reinforced SC infusion times of 16.8, 15.5, and 7.8 months, respectively. In the total patients, the cumulative incidence of acute grade III-IV GVHD at optional reinforced SC infusion day 100 was 20.0% (95% CI, 0 – 48.4). The cumulative incidences of severe chronic GVHD at an optional reinforced SC infusion time of 12 months was 20.0% (95% CI, 0.4 – 63.2).

After the patients received the optional reinforced SC infusions, both CMV reactivation requiring pre-emptive therapy and hemorrhagic cystitis were observed in one patient (UPN #02), at the optional reinforced infusion 2.9 and 2.8 months, respectively. One patient (UPN #04) was hospitalized due to non-specific colitis at an optional reinforced infusion time of 0.6 months. Except for the above-described acute GVHD-related mortality, no additional TRM was 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 four years were 91.7% (95% CI, 53.9–98.8) in both patient groups (Figure 4A and 4B). The thalassemia-free survival rate at four years in β-TM patients was 87.5% (95% CI, 38.7–98.1) (Figure 4C). The SCD-free survival rate at four years in the SCD patients was 100%.

**4. DISCUSSION**

Unlike SCD, β-TM patients do not require chemotherapy nor is their immunological system impaired. However, they have a robustly hyperplastic and expanded BM compartment with allo-sensitization as a result of multiple transfusions [7]. Therefore, the ideal conditioning for β-TM patients should be able to eradicate the hyperplastic BM and be sufficiently immunosuppressive to overcome the established allo-sensitization. In this circumstance, MAC with busulfan and cyclophosphamide has been considered a standard regimen for β-TM 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. Their 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 several studies of alloSCT using NMA conditioning for adult SCD patients have been reported [2,10,11], they are currently very scarce in adult β-TM patients. However, by analogy to the behavior of malignant tissue, the 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 previously been reported using a mixed pediatric population with β-TM and SCD. The outcomes were disappointing with only transient engraftment, followed by overt graft failure [1]. In contrast, several studies have supported the observation that full donor chimerism is not mandatory for the clinical success of alloSCT 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 study, transient mixed chimerism did not necessarily lead to graft rejection and eventually evolved 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 three months after transplantation, once persistent mixed chimerism is established, the patients seemed no longer at risk, achieving a stable graft function without the need for additional red blood cell transfusion support [13]. 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 seven (77.8%) achieved stable mixed chimerism for more than post-transplant one year and four (30.8%) did not require the optional reinforced SC infusion due to the maintenance of PB donor T-cell chimerism over 50% after one year. These results suggest that stable mixed chimerism could be induced in a significant proportion of β-TM patients without the need for 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 could be successfully applied to adult β-TM patients who achieved a stable mixed chimerism and the correction of abnormal hemoglobin phenotypes.

One of themajor limitationsof the NIH protocol is the need for long-term immunosuppression in patients with persistently low donor T-cell chimerism (< 50%), although the paradigm of which PB donor T-cell chimerism > 50% should be maintained before tapering immunosuppressive agent to avoid graft rejection should be confirmed by larger studies. In addition, nearly half of the patients should have continued sirolimus with a median duration of 2.1 years [2]. Several studies reported that a cumulative duration of immunosuppression over two years was associated with an increased incidence of secondary malignancies [14,15], which is a major concern. It was especially problematic in patients who take life-long sirolimus such as solid organ recipients [16,17]. Although no one developed a secondary malignancy in the patients who received the NIH protocol, the follow-up duration of the study was not long enough to determine its’ true incidence. Sirolimus-related adverse drug reactions include anemia, thrombocytopenia, lipid metabolism disorder, new-onset diabetes, hypertension and respiratory and urinary tract infections [18]. Therefore, protracted immunosuppression 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 of approximately 13% [2]. Moreover, in a previous study analyzing the outcomes of secondary alloSCT for β-TM patients, 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 three 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 patients 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 an optional reinforced SC infusion. However, several reports suggested that a myeloid chimerism of around 20% was enough to achieve a functional graft characterized by normal hemoglobin level with no need for red cell transfusions and decreased serum iron level, and a limited degree of erythroid hyperplasia [13,20,21]. Several adult SCD patients in the NIH study discontinued sirolimus earlier than required and did not experience graft rejection with sustained PB donor T-cell chimerism after post-transplant one year [2]. Notably, in a fatal case reported herein, the number of CD3+ cells infused was the highest among the patients who received optional reinforced SC, which could account for the development of severe acute GVHD. Although no meaningful statistical conclusion can be drawn in our study due to the limited number of patients, the development of GVHD tended to be associated with the number of CD3+ cells infused, as expected. Therefore, careful attention should be paid in the selection of optimal candidates and the optimization of the infused cell dose, especially considering the significant risk of GVHD observed in our study.

Previous reports showed that incorporating alemtuzumab into the conditioning regimen for depleting donor T cells contributed to reducing the GVHD incidence in the setting of alloSCT using NMA conditioning [22,23]. Three patients in our cohort who received an optional reinforced infusion due to impeding graft failure in the early post-transplant period did not experience GVHD at all, whereas two of those who received the procedure due to declining PB donor T-cell chimerism after post-transplant one year experienced severe acute or chronic GVHD. This suggests that T-cell depletion by alemtuzumab could contribute to preventing GVHD in patients who received the optional reinforced infusion not after post-transplant one year, but in early post-transplant period, which might have resulted from the gradually attenuating effect alemtuzumab over time. These results suggest that future optional reinforced SC infusion should be preferentially performed to patients with impending graft failure 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 alloSCT using intensive conditioning. A previous study reported that an increased dose of TBI substantially reduced graft failure while maintaining the safety of haploidentical alloSCT using NMA conditioning for patients with hemoglobinopathies, suggesting that irradiation 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 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 [25-27]. Our study included three patients with major and minor ABO-incompatibilities. One with a major mismatch successfully withdrew from sirolimus without the need for an optional reinforced SC infusion and achieved a stable mixed donor PB T-cell chimerism of 60%. Two of the three minor ABO-mismatches did not require optional reinforced SC infusions and discontinued sirolimus. These results suggest that ABO-incompatibility is not associated with an increased incidence of graft failure and must not be regarded as a contraindication for alloSCT using NMA conditioning consisting of alemtuzumab and low-dose TBI.

The retrospective nature and the small number of patients in this study make it difficult to draw definite conclusions. An additional limitation was that alloSCT using NMA conditioning could only be applied 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 from the development of acute GVHD after an optional reinforcedSC infusion. Since the number of CD3+ cells appears to be associated with these complications, further studies are needed to determine the optimal cell dose in the optional reinforced SC infusion to minimize the risk of GVHD without sacrificing donor engraftment, especially in patients who received the procedure after post-transplant one year. Considering the risk/benefit of our strategy, the optional reinforced SC infusion should also be preferentially performed in patients with rapidly declining PB donor WB chimerism during the early post-transplant period. Despite these limitations, our results showed acceptable outcomes in both β-TM and SCD patients with the consistent use of uniform NMA conditioning and criteria for optional reinforced SC infusions.

**5. CONCLUSIONS**

Despite its retrospective nature and the small number of patients, our study found that alloSCT using NMA conditioning, consisting of alemtuzumab plus low-dose TBI was effective in achieving stable mixed chimerism not only in SCD, but also in β-TM patients. Our strategy of an optional reinforced SC infusion was effective in preventing to protracted immunosuppression in these patients. However, future studies are needed to select the appropriate candidates and determine the optimal optional reinforced SC infusion cell dose.

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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 of packed red cell transfusions, the lowest hemoglobin levels of β-thalassemia major patients during pre-transplant 1 month are0 presented.

b Hemoglobin S levels in sickle cell anemia patients and hemoglobin level of β-thalassemia major patients just before transplantation are presented.

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

d Months after the optional reinforced stem cell infusion

**Figure Legends**

FIGURE 1 Overall outcomes of 13 patients who received our strategy. β-TM, β-thalassemia major; SCD, sickle cell disease; IS, immunosuppressive; TRM, treatment-related mortality; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease

FIGURE 2 Optional reinforced SC infusion-related outcomes. (A) the optional reinforced stem cells infusion-free survival and (B) the cumulative incidence of PB T-cell chimerism > 50% in patients not proceeding to an optional reinforced SC infusion

FIGURE 3 Changes in chimerism. (A) PB donor T-cell chimerism in the patients that received optional reinforced stem cell infusions, (B) PB donor whole-cell chimerism in the patients that received optional reinforced stem cells infusions, (C) PB donor T-cell chimerism of the patients that did not receive optional reinforced stem cells infusions, and (D) PB donor whole-cell chimerism of the patients that did not receive optional reinforced stem cells infusions. Black dot indicates optional reinforced stem cell infusion.

FIGURE 4 Survival outcomes. (A) Overall survival, (B) event-free survival, and (C) thalassemia-free survival