Pretransplant Immunosuppression followed by Reduced-Toxicity Conditioning and Stem Cell Transplantation in High-Risk Thalassemia: A Safe Approach to Disease Control

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Abstract

Patients with class 3 thalassemia with high-risk features for adverse events after high-dose chemotherapy with hematopoietic stem cell transplantation (HSCT) are difficult to treat, tending to either suffer serious toxicity or fail to establish stable graft function. We performed HSCT in 18 such patients age ≥7 years and hepatomegaly using a novel approach with pretransplant immunosuppression followed by a myeloablative reduced-toxicity conditioning regimen (fludarabine and i.v. busulfan [Flu-IV Bu]) and then HSCT. The median patient age was 14 years (range, 10 to 18 years). Before the Flu-IV Bu + antithymocyte globulin conditioning regimen, all patients received 1 to 2 cycles of pretransplant immunosuppression with fludarabine and dexamethasone. Thirteen patients received a related donor graft, and 5 received an unrelated donor graft. An initial prompt engraftment of donor cells with full donor chimerism was observed in all 18 patients, but 2 patients developed secondary mixed chimerism that necessitated withdrawal of immunosuppression to achieve full donor chimerism. Two patients (11%) had acute grade III-IV graft-versus-host disease, and 5 patients had limited chronic graft-versus-host disease. The only treatment-related mortality was from infection, and with a median follow-up of 42 months (range, 4 to 75), the 5-year overall survival and thalassemia-free survival were 85%. We conclude that this novel sequential immunosuppressive pretransplantation conditioning program is safe and effective for patients with high-risk class 3 thalassemia exhibiting additional comorbidities.

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is the sole available curative therapy for patients with severe β-thalassemia, providing potential cure in approximately 80% of recipients [1]. However, several reports have suggested the existence of a subset of patients with worse outcomes. This subgroup includes older patients with normal organ damage due to iron overload and/or evidence of immunization to donor histocompatibility antigens.
through multiple blood transfusions. These patients, classified by Lucarelli et al. [1] as “class 3,” demonstrate high transplantation-related mortality (TRM; ~40%) and rejection rates (~16%).

The current risk classification of patients with severe thalassemia who are undergoing HSCT fails to recognize another high-risk subgroup, however: patients age ≥7 years with a liver ≥5 cm in size. These patients constitute what Mathews et al. [2] defined as a very-high-risk subset of a conventional high-risk class 3 group. The adverse impact of age and liver size was further validated by a Center for International Blood and Marrow Transplantation Research study that summarized HSCT results at centers outside Italy and confirmed a higher TRM in patients age >7 years and those with hepatomegaly [3]. The patients in these high-risk class 3 subsets are at elevated risk for graft rejection and regimen-related toxicity, especially veno-occlusive disease, leading to multiorgan failure and death. This finding stimulated the investigation of several novel conditioning regimens [4-7].

We previously reported the use of reduced-intensity conditioning in 8 patients with class 3 thalassemia, 6 of whom survived thalassemia-free but 2 of whom rejected their grafts [5]. In the present study, we wanted to test the hypothesis that sequential pretransplant immunosuppression (PTIS) with fludarabine (Flu) and dexamethasone (Dex) before reduced-toxicity conditioning for HSCT would safely suppress recipient T cell function and permit sustainable engraftment while modulating host cell-mediated antigen presentation to alter the development of graft-versus-host disease (GVHD), ultimately resulting in improved disease control.

**PATIENTS AND METHODS**

This study was approved by the Ethical Committee of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University. All patients provided written informed consent.

Eighteen patients with severe thalassemia (14 with β-thalassemia/hemoglobin E and 4 with homozygous β-thalassemia) treated between December 2007 and December 2012 were included in this study. Two patients (3 and 4) who had undergone previous HSCT and experienced graft rejection were enrolled for a second HSCT. The cohort included 7 males and 11 females, with a median age of 14 years (range, 10 to 18 years). All 18 patients had hepatomegaly (liver ≥2 cm below the costal margin [3]), and 7 had undergone splenectomy. The median ferritin level was 3100 ng/mL (range, 869 to 8350 ng/mL). All patients were Lucarelli class 3 [1].

HLA typing was performed using a DNA-based, high-resolution technique with sequence-specific oligonucleotide primers for class I and class II loci. Thirteen patients received a related donor graft and 5 received an unrelated donor graft. Two of 13 patients in the related-donor group and 2 of 5 patients in the unrelated-donor group had 1-antigen-mismatched donor (Table 1).

**Sequential Pretransplant Immunossuppression**

Ten patients received 1 cycle if PTIS and 8 received 2 cycles of PTIS, which consisted of Flu 40 mg/m²/day i.v. for 5 days and Dex 25 mg/m²/day i.v. for 5 days. This was administered every 28 days for 1 or 2 cycles before the start of the conditioning regimen. PTIS is given to suppress recipient T cell function and facilitate sustainable engraftment, while decreasing the risk of GVHD and improving disease control. Two patients in the first 10-patient group, who received only 1 cycle of Flu-Dex, had unstable donor chimerism in the first 100 days post-HSCT. Because there was no clinical toxicity from the Flu-Dex, we administered 2 cycles, 28 days apart, in the next 8 patients. In addition, all patients had received hydroxyurea 20 mg/kg/day daily for at least 3 months before entering our program [5].

**Conditioning Regimen**

The reduced-toxicity conditioning regimen consisted of fludarabine 35 mg/m² i.v. once daily for 6 days (day -9 to day -4), busulfan 130 mg/m² i.v. once daily for 4 days (day -9 to day -6), and rabbit antithymocyte globulin (ATG; Thymoglobulin; Sandoz-Genzyme Canada, Ontario, Canada) 1.5 mg/kg/day from day -3 to day -1. This regimen was modified from regimens described by Russell et al. [8] and de Lima et al. [9].

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RTR indicates reduced-toxicity regimen; MMRD, mismatched related donor; MUD, matched unrelated donor; FC, full donor chimerism; MC, mixed donor chimerism; NA, not available.
GVHD Prophylaxis
GVHD prophylaxis was started 3 days before HSCT. Patients who received a related donor transplant were given cyclosporine, and those who received an unrelated donor transplant were given tacrolimus. All patients who received a related donor transplant were given cyclosporine, and those who received an unrelated donor transplant were given tacrolimus.

Monitoring of Chimerism
Chimerism was monitored from engraftment and every 2 weeks during the first 100 days after HSCT, using variable number of tandem repeats analyses (for donor–recipient sex match) and fluorescein in situ hybridization for X and Y chromosomes (for donor–recipient sex mismatch).

T Cell Function Testing
T cell function was tested by phytohemagglutination (PHA) stimulation before and after sequential PTIS. Proliferation of T cells was detected by a carboxyfluorescein diacetate succinimidyl ester (CFSE) assay as described previously. T cell proliferation was evaluated based on a stimulation index, which was calculated as the percentage of CFSElowCD4 T cells (with PHA) divided by the percentage CFSElowCD4 T cells without PHA [10].

Statistics
Survival probability was estimated by the Kaplan-Meier method.

RESULTS
Regimen-Related Toxicity, Infections, and GVHD
Four patients had grade 1-2 mucositis (22%), and 3 patients had mild, reversible veno-occlusive disease (16%). One patient had shingles, 1 had generalized herpes zoster, and 1 had BK virus cystitis. Another 3 patients had cytomegalovirus reactivation. Four patients (22%) had acute grade II GVHD, and none had acute grade III-IV GVHD, although both patients who experienced secondary graft weakness and had immunosuppression held subsequently developed acute grade III GVHD that resolved with systemic steroid administration. Thus, the overall incidence of grade III-IV GVHD was 11%. Five patients (28%) developed limited chronic GVHD, and none had extensive chronic GVHD.

Engraftment and Graft Rejection
T cell–replete PBSCs were given to all but 1 patient, who received bone marrow. The median infused cell dose was $9.4 \times 10^6$ CD34+ cells/kg (range, 4.67 to $19.26 \times 10^6$ CD34+ cells/kg). All patients developed full donor chimerism with engraftment. Immunosuppressive treatment was successfully weaned and discontinued by 6 months post-HSCT in the patients who received a matched related donor graft and by 12 months post-HSCT in those who received a matched unrelated donor graft. However, 2 patients (patients 3 and 8) subsequently suffered secondary mixed chimerism within the first 100 days (65% and 86% donor chimerism, respectively), and immune suppression was withdrawn for 1 to 2 weeks until full donor chimerism was re-established. The median time to neutrophil engraftment was 12 days (range, 11 to 18 days), and that to platelet engraftment was 18 days (range, 12 to 42 days).

Clinical Outcome
With a median follow-up of 42 months (range, 4 to 75 months), the 5-year overall survival (OS) and thalassemia-free survival (TFS) rates were both 89% (95% CI, 56% to 96%), as shown in Figure 1A. Karnofsky/Lansky performance status was 100% in all surviving patients at the most recent follow-up.

Two patients (patients 3 and 8) developed secondary mixed donor chimerism and had immunosuppression held to allow re-establishment of full donor chimerism. The mixed donor chimerism resolved, but the ensuing grade III acute GVHD necessitated systemic corticosteroid administration. Unfortunately, 1 of these patients subsequently died of invasive pulmonary aspergillosis, and the other suffered a lethal cerebellar hemorrhage due to an unrelated trauma after discharge from the hospital.

T cell function tests were performed in 3 patients (patients 14, 16, and 18) who received 2 cycles of Flu-Dex. The data suggest that T cell function was indeed significantly suppressed after 2 cycles of sequential PTIS. The first assay was performed before the initiation of Flu-Dex, and the third assay was performed before the start of pretransplantation conditioning (Figure 1B). We tested T cell function only in these 3 patients because we performed this test only for the last 5 patients in our cohort, and the blood samples from patients 15 and 17 were missing. During ISPT, neither infection nor neutropenia occurred, and no patient developed kidney or brain toxicity.

DISCUSSION
Matched unrelated donor HSCT is a potentially curative option for patients with high-risk thalassemia, with clinical outcomes comparable to those from matched sibling donor...
HSCT [11]. Pretransplantation therapies include administration of hydroxyurea and azathioprine to eradicate marrow and hypertransfusion and aggressive chelation to suppress endogenous erythropoiesis [12,13]. Recently introduced reduced-intensity conditioning regimens carry some concerns regarding the risk of graft failure or rejection [11]. One of the major risk factors for graft rejection is mixed donor chimerism, which was found in approximately one third of the patients [14]. Several approaches have been taken to treat patients with mixed donor chimerism, including cessation of immunotherapy and use of donor lymphocyte infusion.

Previous studies have reported TFS rates of 50% to 80% after HSCT in patients with thalassemia with high-risk class 3 features, such as older age and hepatomegaly [2-4,6,7,15]. The present study demonstrates that a TFS rate of approximately 90% can be achieved. We included patients with not only high-risk class 3 features, but also comorbidities such as diabetes mellitus, extramedullary hematopoiesis, pulmonary hypertension, and previous splenectomy [16]; furthermore, some of the patients received grafts from mismatched donors. With this adverse risk information in mind, we suggest that our approach should be considered promising for use in this high-risk group. If our data are confirmed, then this novel strategy should be applied in standard-risk patients as well.

The conditioning regimen used in this study was based on our previously published nucleoside analog-alkylating agent platform concept [17], providing initial immune suppression from the nucleoside analog but with long-term stable engraftment facilitated mostly by the alkylating agent busulfan. We did not add irradiation, cyclophosphamide, or other alkylating agents to the regimen. This approach may reduce the risk for long-term toxicities, such as cardiopulmonary toxicity and secondary malignancies, but further study in a larger cohort is needed. The successful use of a myeloablative, reduced-toxicity regimen is hampered to only a minor extent by possible infertility; however, this will be addressed in the next-generation studies using a modified concept based on the platform described in this report.

Given that the persistence of recipient antigen-presenting cells in the post-HSCT period could potentially increase the risk of GVHD from the alloantigens present to donor T cells, our sequential pretransplant immunosuppression consisting of Flu and Dex not only suppresses recipient T cell function and facilitates sustainable engraftment, but also ensures the complete elimination of recipient antigen-presenting cells. Moreover, Flu-containing conditioning regimens, including the conditioning regimen used in this study (Flu, busulfan, and ATG) are reportedly associated with a decreased incidence of GVHD [18,19].

In conclusion, a key success factor in our safe and efficacious program is the sequential use of pretransplant immunosuppression followed by a reduced-toxicity conditioning program rather than striving for maximum intensity of the conditioning program itself, paired with a relatively high number of hematopoietic progenitor cells (on average >5 × 10<sup>5</sup>CD34<sup>+</sup> cells/kg of recipient weight [5,20]), which resulted in a low incidence of toxicity and durable engraftment in this group of patients with high-risk class 3 thalassemia.

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