# Targeted busulfan-based conditioning unrelated donor bone marrow transplantation for Diamond-Blackfan anemia

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**Abstract**

Hematopoietic cell transplantation corrects a hematological phenotype of Diamond-Blackfan anemia (DBA). Reduced conditioning has been recommended for patients with the cancer predisposition, but appropriate intensity is required to prevent graft failure. We conducted unrelated-donor bone marrow transplantations in six consecutive transfusion-dependent patients (median age, 5.4 years; range, 1.8–23) after targeted-busulfan (60–75 mg/L×h of cumulative AUC) and fludarabine regimen without irradiation. All obtained chronic graft-versus-host-disease-free complete donor chimerism, including one unrelated cord blood transplantation rescue. The rescued 7-year-old girl had the longest transfusion-dependency (>40 months) and highest ferritin level (>2,000 ng/mL) pretransplant. Intermediate-intensity targeted busulfan-based conditioning may cure DBA.

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**Abbreviations:**

| AUC | Area-under-the-curve |
|---|---|
| BMT | Bone marrow transplantation |
| BU | Busulfan |
| CB | Cord blood |
| DBA | Diamond-Blackfan anemia |
| EBMT | European Blood and Marrow Transplantation Group |
| ESID | European Society for Immunodeficiencies |
| FLU | Fludarabine |
| GVHD | Graft-versus-host disease |
| HCT | Hematopoietic cell transplantation |
| IBMFS | Inherited bone marrow failure syndrome |
| MAC | Myeloablative conditioning |
| MEL | Melphalan |
| PRCA | Pure red cell aplasia |
| rATG | Rabbit anti-thymocyte globulin |
| RIC | Reduced intensity conditioning |
| RP | Ribosomal protein |
| RRT | Regimen related toxicities |
| TBI | Total body irradiation |
| Treo | Treosulfan |

## ABSTRACT

Hematopoietic cell transplantation corrects a hematological phenotype of Diamond-Blackfan anemia (DBA). Reduced conditioning has been recommended for patients with the cancer predisposition, but appropriate intensity is required to prevent graft failure. We conducted unrelated-donor bone marrow transplantations in six consecutive transfusion-dependent patients (median age, 5.4 years; range, 1.8–23) after targeted-busulfan (60–75 mg/L×h of cumulative AUC) and fludarabine regimen without irradiation. All obtained chronic graft-versus-host-disease-free complete donor chimerism, including one unrelated cord blood transplantation rescue. The rescued 7-year-old girl had the longest transfusion-dependency (>40 months) and highest ferritin level (>2,000 ng/mL) pretransplant. Intermediate-intensity targeted busulfan-based conditioning may cure DBA.

## INTRODUCTION

Diamond-Blackfan anemia (DBA) is a rare inherited bone marrow failure syndrome (IBMFS) characterized by hypoproliferative and proapoptotic erythropoiesis along with malformations and cancer predisposition.[1] The pure red cell aplasia (PRCA) arises from haploinsufficiency due to a loss of function mutation in ribosomal protein (RP)-encoding genes. Corticosteroids are the first-line treatment for PRCA in infancy, but >20% of patients result in transfusion-dependency. Patients are predisposed to myeloid malignancy and solid tumors. In pediatric patients, the 5-year chronic graft-versus-host-disease (GVHD)-free survival rate following allogeneic hematopoietic cell transplantation (HCT) has improved to 87.0%, including mostly histocompatible sibling or unrelated donors.[2] HCT can achieve a hematological cure transfusion-dependent young patients if a suitable donor is available. Nevertheless, conditioning of alternate donor HCT is carefully personalized according to organ dysfunctions associated with the ribosomopathy by itself and iron-overload. Regarding cancer predisposition and the high risk of complications, reduced intensity conditioning (RIC) is preferable; however, there is limited information on unrelated donor HCT.[3-7] To optimize the HCT regimen for DBA, we assessed the outcomes of recent cases with alternate donor bone marrow transplantation (BMT)

following targeted busulfan (BU)-based intermediate intensity conditioning.

## METHODS

Among 7 patients with RP-mutated DBA who received HCT in Kyushu University Hospital (n=6) and Kobe Children's Hospital (n=1) from 2000 to 2020, the latest six patients received targeted BU and fludarabine (FLU) conditioning in the first HCT. BU was administered intravenously four times daily for four days. The cumulative area-under-the-curve (AUC) of BU was calculated by JMP Pro 11 (version 11.2.0 for Windows; JMP Inc., SAS Institute Japan) based on the results of a test-dose using the Dried Blood Spot method. Based on the transfusion-dependent periods and serum ferritin levels, the target of cumulative AUC was adjusted to 60–75 mg/L×h (65˜80% of the conventional myeloablative cumulative AUC [85–95 mg/L×h]). FLU was administered at 180 mg/m$^2$ in four to six divided doses. Rabbit anti-thymocyte globulin (rATG) (2.5–5.0 mg/kg Thymoglobulin, Genzyme, Boston, MA) was added in five patients. Tacrolimus and short-term methotrexate were used for acute GVHD prophylaxis in all patients. The date of engraftment was defined as the first of three consecutive days when the neutrophil count was >0.5x10$^9$/L. Donor chimerism was assessed by sex chromosome fluorescence *in situ* hybridization or short tandem repeats.

## RESULTS

### Pretransplant State of the Case-Series

From October 2015 to March 2021, 6 patients with DBA consecutively received unrelated-donor BMT due to steroid-resistant and transfusion-dependent anemia. The detailed clinical profiles are shown in **TABLE 1** . Mutations were identified in *RPS19* in 5 patients and *RPS26* in 1 patient. The median age at the time of BMT was 5.4 years (range, 1.8–23 years). All patients received unrelated-donor bone marrow (HLA-full allele-matched donor, n=3; HLA-one locus-mismatched donor, n=3). The median transfusion-dependent period was 31 months (range, 4–78 months) and the median pre-transplant serum ferritin level was 1164.4 ng/mL (range, 271.7–2174.0 ng/mL). The median cumulative AUC of BU was 61 mg/Lxh (range, 60–75 mg/Lxh), and the median dose per body weight was 12.0 mg/kg (range, 8.4–14.6 mg/kg). One patient had medication-free atrial septal defect at potential risk of transplantation. The other ribosomopathy-associated defects were not considered a risk of this conditioning. Three had over ferritinemia (>1000 ng/mL) but no evidence of organ dysfunction with hemosiderosis.

### Post-transplant Outcomes

Five patients achieved neutrophil engraftment at the median of 17 days (range, 13–21 days), and all had sustained >95% donor chimerism without donor lymphocyte infusion. One (Patient 4) developed sinusoidal obstruction syndrome and a graft failure despite well targeted BU. She had the longest transfusion-dependent period and the highest ferritin level of all patients (**Fig. 1** ). Two months later, umbilical cord blood (CB) transplantation was successfully performed after our conventional regimen FLU, melphalan (MEL) and low-dose total body irradiation (TBI). Histocompatible CB was obtained from the Japanese CB Bank Network. She is presently active in her school life with complete donor chimerism.

No grade >II acute GVHD or chronic GVHD occurred. Acute GVHD was well controlled by corticosteroids. Infectious complications occurred and were controlled in all 3 patients, including cytomegalovirus reactivation (n=2), human herpes virus-6 reactivation (n=1), non-tuberculosis mycobacteria infection (n=1), and herpes zoster (n=1). Autoimmune cytopenia occurred on treatment in one patient after complete engraftment. During the median follow-up period after HCT (42.5 months [range, 6–71 months]), no patients had neurological disability, neoplasms or pulmonary complications.

## DISCUSSION

Five transfusion-dependent DBA patients successfully underwent targeted BU-based conditioning 3 HLA-matched or 2 one-locus HLA-mismatched unrelated donor BMT; in the latter 2 cases, transplantation was at >10 and >20 years of age. The longest transfusion-dependent case, which showed the highest ferritinemia

had one graft failure but was rescued by CB transplantation after our established low-dose TBI regimen. The targeted BU-based intermediate intensity regimen was optimal for alternate donor BMT in DBA patients.

For successful HCT, the diverse clinical expression and high penetrance of DBA hamper the search for suitable family donors. Organ dysfunction associated with anomaly and/or iron overload increases the risk of regimen related toxicities (RRTs). Repeated transfusion augments the risk of rejection. RIC is preferable to reduce RRT and late complications in IBMFS. In a recent report from Brazil,[8] the 5-year overall survival rates after HLA-matched sibling donor, HLA-matched unrelated donor, and HLA-mismatched donor HCT were 80%, 73%, and 29%, respectively. In the EBMT database, the 3-year event-free survival rate of patients who received unrelated donor HCT was >80%.[9]In Japan,[3] 8 of 9 DBA patients underwent successful RIC-unrelated donor BMT, but all 9 patients received RIC regimen with both irradiation and alkylators. Appreciable intensity is required for complete donor chimerism to reduce the risk of graft failure and myeloid malignancy. TBI should be avoided in conditioning regimens for non-malignant disease, especially for cancer predisposition syndromes. BU or treosulfan (Treo) and FLU-based myeloablative conditioning (MAC) has been recommended for HCT for DBA patients.[10] Treo and alemtuzumab-RIC has been reported in a limited number of cases;[4-7] however, neither is licensed in Japan. Our targeted BU-based regimen was effectively used for children and adults. The dosage can be further personalized for wider ranges of circulating BU levels in infants.[11] Alemtuzumab is more effective than rATG in preventing GVHD in patients with non-malignant disorders.[12] Replacement of rATG with alemtuzumab may improve engraftment and decrease chronic GVHD without resulting in delayed immune reconstitution.[13] Because chronic GVHD increases the risk of post-transplant neoplasms in DBA patients,[14] rATG would be replaced by alemtuzumab. More recently, a critical precaution of Treo dose individualization has been reported in infants and children undergoing allo-HCT for non-malignant conditions.[15] Precise targeted conditioning is needed for pediatric patients even in the setting of BU or Treo.

Colorectal cancer, osteogenic sarcoma and cardiac Purkinje cell tumor have been reported in DBA patients, most frequently at a median of 9 years after MAC-HCT.[16-19] The combination of alkylators and TBI raises the risk of osteogenic sarcoma in pediatric patients after MAC-HCT;[20] however, there is no information on the effect of a single alkylator in RIC-HCT. The cancer risk and clonal hematopoiesis may be more accelerated with age in IBMFS. Cancer vigilance is needed to optimize the time and conditioning of DBA patients as well as the age at HCT.

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**Figure legend**

**Figure 1.** Relationship among the cumulative AUC of BU, the transfusion-dependent period, and ferritin levels.

Each number represents the patient number in Table 1. The radii of circles show the pre-transplant serum ferritin levels in individuals. White and black circles represent engraftment and rejection after the first HCT, respectively. Thin and thick shaded areas indicate the RIC and MAC range, respectively, in the EBMT/ESID guideline defined by the cumulative AUC of BU.

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Figure 1