Treosulfan-Based Conditioning Regimen in Sibling and Alternative Donor Hematopoietic Stem Cell Transplantation for Children with Sickle Cell Disease

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Abstract. Background and objectives: Lack of suitable donors and regimen related toxicity are major barriers for hematopoietic stem cell transplantation (HSCT) in patients with sickle cell disease (SCD). The aim of the study is the assessment of efficacy and toxicity of Treosulfan-based conditioning regimen for SCD also when alternative donors such as mismatched unrelated donor and haploidentical donor are employed.

Methods: We report our single-center experience: 11 patients with SCD received HSCT with a Treosulfan/Thiotepa/Fludarabine/Anti-thymoglobulin conditioning regimen between 2010 and 2015. The donor was a matched sibling donor (n= 7), a haploidentical parent (n= 2), a matched unrelated donor (n= 1) or a mismatched unrelated donor (n=1). The haploidentical and mismatched unrelated donor grafts were manipulated by removing TCRαβ and CD19 positive cells.

Results: All patients survived the procedure and achieved stable engraftment. Stable mixed chimerism was observed in 5/11 patients. Grade III-IV regimen related toxicity was limited to mucositis and no grade III-IV graft-versus-host disease (GvHD) occurred. No SCD manifestation was observed post transplant and cerebral vasculopathy improved in 3/5 evaluable patients. Organ function evaluation showed no pulmonary, cardiac or renal toxicity but gonadal failure occurred in 1/4 evaluable patients.

Conclusion: Our data suggest that Treosulfan is associated with low toxicity and may be employed also for unrelated and haploidentical donor HSCT.

Keywords: Hematopoietic stem cell transplantation, Treosulfan, Sickle cell disease, Haploidentical, T-cell depletion.

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Introduction. Sickle cell disease (SCD) is the most frequent haemoglobinopathy worldwide. A point mutation in the beta-globulin gene alters the hemoglobin structure and results in chronic hemolytic anemia and increased blood viscosity. This leads to an heterogeneous phenotypic...
spectrum: increased morbidity and mortality is due to a higher susceptibility to infections, intermittent vaso-occlusive events and ischemic tissue injury with progressive organ dysfunction.¹

Hematopoietic stem cell transplantation (HSCT) is the most consolidated curative treatment.² When considering HSCT for SCD patients, expected SCD morbidity should be balanced against the risk of transplant related mortality and morbidity, keeping in mind that SCD is a disease with a life expectancy of over 50 years with contemporary treatment.³

More than 200 matched sibling donor (MSD) transplants after a myeloablative conditioning regimen based on Busulfan and Cyclophosphamide were reported. The limitations associated with this strategy are the significant regimen related toxicity and the risk of graft failure. The transplant associated mortality is around 2-8% and graft failure is observed in around 10-15% of patients.⁴,⁵,⁶,⁷,⁸,⁹,¹⁰,¹¹ Although better outcomes have been recently reported with the use of targeted Busulfan therapy, the use of Busulfan-based conditioning regimen is established only in the setting of MSD transplant and only few SCD patients in need of a HSCT have a matched sibling available.¹²,¹³,¹⁴,¹⁵ The use of alternative donors such as matched unrelated donor (MUD), mismatched unrelated donor (MMUD), unrelated umbilical cord blood (UCB) and haploidentical family members is associated with higher mortality and morbidity, due to pre-existing organ dysfunction, alloimmunisation and risk of GvHD.²,¹⁶ Moreover, suitable matched unrelated donors are difficult to find and experience is limited for umbilical cord blood or haploidentical donor HSCT.¹⁷,¹⁸,¹⁹,²⁰,²¹ In order to overcome the limitations of Busulfan-based conditioning regimen and allow the use of alternative donors, different conditioning strategies have been proposed.²²,²³ Recently, Treosulfan became attractive to substitute Busulfan due to its lower toxicity and good immune suppressive and myeloablative potential.²⁴,²⁵,²⁶,²⁷,²⁸ This led to the use of Treosulfan in patients with pre-existent morbidity (mainly primary immune deficiency or β-thalassemia) or receiving a second transplant for malignant disorders.²⁵,²⁹,²⁶,³⁰,³¹,³²,³³,³⁴,³⁵ For these reasons, since 2010, at our institution Busulfan was substituted with Treosulfan as standard conditioning regimen for SCD patients.

We present our single-centre experience of HSCT performed for SCD patients employing Treosulfan-based conditioning regimen also in haploidentical and MUD HSCT.

Methods. From April 2010 to December 2015, all SCD patients undergoing HSCT at the Pediatric Hematology-Oncology Unit of the University of Padova received a Treosulfan-based conditioning regimen and are described in this retrospective cohort study. Eligibility for HSCT was determined on the basis of published guidelines and criteria included cerebral vasculopathy, recurrent episodes of acute chest syndrome (ACS) or vaso-occlusive crises despite hydroxycarbamide treatment.³⁶ Donors were chosen on the basis of availability and considered in the following order: MSD, MUD, Haploidentical donor and MMUD. The preferred stem cell source was bone marrow or umbilical stem cells for MSD, bone marrow for MUD and T-depleted peripheral blood stem cells for haploidentical donors and MMUD. The use of a combination of umbilical cord blood and bone marrow was employed in MSD HSCT if the cellularity of the umbilical cord graft was low.²⁰ Before T-cell depleted or MUD HSCT, autologous bone marrow stem cells were harvested and cryopreserved in order to be re-infused in case of graft rejection, due to the higher risk of this event after T-cell depletion.³⁷ All patients received either red cell exchange transfusion or simple transfusion the day before the start of conditioning regimen in order to obtain a proportion of HbS < 30% and an Hb level ≥ 100g/L.

The haploidentical donors underwent PBSC collection after mobilization with subcutaneous Filgrastim 10 μg/Kg twice daily from day -5 to day -1 and once on the day of collection. PBSC were collected using a COBE® Spectra Apheresis System (BCT Terumo, Lakewood, CO). T-cell depletion was performed by removing TCRαβ positive and CD19 positive cells through immunomagnetic selection (CliniMACS; Miltenyi Biotec, Bergisch Gladbach, Germany).

All patients received Thiotepa (8 mg/kg or 10 mg/kg in 2 doses on day -7), Treosulfan (14 g/m²/day for 3 days from day -6 to day -4 ) and Fludarabine (40 mg/m²/day for 4 days from day -6 to day -3).²⁹ Fresenius® anti-thymocyte globulins (ATG) at the dose of 20 mg/kg/day were administered for 3 days in MSD and MUD transplants and 5 mg/kg/day for 4 days in T-
depleted transplants. Patient 8 and patient 11 received Rituximab (200 mg/m²) at day -1 to reduce the risk of EBV reactivation and GvHD after HSCT. Graft-vs-Host Disease (GvHD) prophylaxis for T-replete transplants consisted in short term Methotrexate (10mg/kg for 4 doses), Cyclosporine 1 mg/kg/day on days -7 to -2, and Cyclosporine aiming at a pre-dose level of 100-200 μg/L for 6 months post HSCT. No GvHD prophylaxis was given for T-deplete transplants. The supportive measures, diagnosis and treatment strategy for acute GvHD employed at our institution were recently described.

Engraftment and chimerism were serially tracked after HSCT. Samples were obtained every 2-3 weeks up to day +100 and monthly thereafter up to 18 months post-transplant in patient with complete donor chimerism. Follow up was longer for patients with mixed chimerism. Chimerism analysis was performed by PCR testing for informative short tandem repeats. Adverse events were graded according to common terminology criteria for adverse events (CTCAE) v4. Neutrophil and platelet recovery were defined as a neutrophil count ≥ 0.5×10⁹/L for 3 consecutive days and as a platelet count ≥ 50×10⁹/L independently of platelet transfusions for 7 consecutive days.

Organ function was assessed pre-transplant and every year post-transplant by pulmonary function testing, echocardiography, growth and puberty evaluation and hormonal dosage (estradiol/testosterone, FSH, LH, T4 and TSH levels). Transcranial Doppler Ultrasonography (TCD) was performed for all patients prior to the initiation of chronic transfusion and pre-HSCT; data were evaluated according to the criteria defined by the STOP trial. Brain magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) were performed pre-transplant, and repeated one year post transplant and every two years thereafter if lesions were detected. MRI and MRA were evaluated according to a standardized scoring system. Cerebral vasculopathy was defined as abnormal Transcranial Doppler velocity associated with cerebral artery stenosis. Immune reconstitution was evaluated by total lymphocyte count, CD4+ cell count and immunoglobulin dosage. Data were recorded at day +30, +90, +180 and +365.

Results. Patients: Eleven consecutive children affected by SCD (7 females, 4 males) were transplanted at the Pediatric Hematology-Oncology Unit of the University of Padova. The origin of patients was African (n = 6), Caucasian (n = 4) or Caribbean (n = 1). Patient and transplant characteristics are summarized in table 1. Seven patients were diagnosed at birth due to family history, the remaining at their first disease manifestation, between 7 month and 5 years of age. The Hb genotype was HbSS (n = 10) or HbSβ0 (n = 1, P7). Before transplant patients were treated with chronic transfusion (n = 7), monthly red cell exchange transfusion (n = 2) and/or hydroxyurea (n = 5). Patients were eligible for HSCT due to cerebral vasculopathy (n=7), recurrent episodes of acute chest syndrome (ACS) or vaso-occlusive crises despite hydroxyurea treatment (n = 4). Patient 7 suffered also from recurrent splenic sequestration. The median age at HSCT was 6.5 years (range: 4 – 16.3 years). At the time of HSCT, only two patients had significant comorbidities: relapsing autoimmune hepatitis (P8) and an association of Chiari I malformation and syringomyelia (P11). Donor source was an HbS/A MSD (n = 5), an HbA/A MSD (n = 2), a haploidentical HbS/A parent (mother, n = 1 and father, n = 1), HbA/A MUD (n = 1) or HbA/A MMUD (n=1, 8/12 HLA loci donor/recipient matching). Stem cell source was bone marrow (n=6, median total nucleated cells, TNC = 4.9 x 10⁸/kg), combined bone marrow and umbilical cord blood (n=2, median TNC for bone marrow = 2.67 x 10⁸/kg; median TNC for cord blood = 2.44 x 10⁸/kg) or peripheral blood stem cells (PBSC) (n=3, two haploidentical grafts and one MUD graft, median CD34+ cells = 14.3 x 10⁹/kg). One apheresis was sufficient to reach the target dose of CD34+cell (10 x 10⁹/kg recipient) in both haploidentical donors. Both donors complained only grade I-II myalgia and fatigue.

Transplant-related outcomes: All patients achieved neutrophil and platelets engraftment at a median of 20 days (range: 15-34 days) and 22 days (range: 12-31 days) from HSC infusion, respectively. No patient experienced primary graft failure. All patients experienced Grade III anemia, grade IV thrombocytopenia and grade IV neutropenia. Grade III-IV non hematological toxicity occurred in 2 patients and consisted in grade IV stomatitis in one patient and acute
Table 1. Patient characteristics and outcomes. Abbreviations: ATG = antithymoglobulin; FLU = fludarabine in mg/m²; RITUX = rituximab; TREO = Treosulfan in g/m²; TT = Thiotepa in mg/kg.

| n | sex | age at transplant (years) | HSCT indication | Conditioning | Treatment pre HSCT | Donor | Donor genotype | Stem cell source | Grade III-IV non hematological complication | GvHD | Chimerism | HbS proportion | Follow up (years) |
|---|-----|--------------------------|------------------|--------------|------------------|-------|----------------|----------------|-------------------------------------------|------|-----------|----------------|------------------|
| 1 | M   | 3.9                      | Recurrent ACS/VOC| TREO42 TT8 FLU160 ATG | Hydroxycarbamide | MSD | HbAA | BM |                            | 100% donor | 0          | 6.5            |                  |
| 2 | F   | 9.1                      | Cerebrovascular disease | TREO42 TT8 FLU160 ATG | Chronic transfusion | MSD | HbSA | BM |                            | 41% donor | 40.4       | 5.6            |                  |
| 3 | F   | 6.5                      | Cerebrovascular disease | TREO42 TT8 FLU160 ATG | Chronic transfusion | MSD | HbSA | BM |                            | 53% donor | 40.3       | 5.6            |                  |
| 4 | F   | 6.5                      | Cerebrovascular disease | TREO42 TT8 FLU160 ATG | Chronic transfusion + hydroxycarbamide | MSD | HbSA | BM + UCB |                            | 100% donor | 38.8       | 4.3            |                  |
| 5 | F   | 13.8                     | Cerebrovascular disease | TREO42 TT8 FLU160 ATG | Monthly red cell exchange transfusion | Haploidentical father | HbSA | TCRαβ/CD19 depleted PBSC | 88% donor | 40.6       | 3.9            |                  |
| 6 | M   | 4.1                      | Recurrent ACS/VOC | TREO42 TT8 FLU160 ATG | Hydroxycarbamide | MSD | HbAA | BM + UCB |                            | 100% donor | 0          | 2.3            |                  |
| 7 | M   | 4.3                      | Recurrent ACS/VOC | TREO42 TT8 FLU160 ATG | Chronic transfusion | MSD | HbAjβ | BM | Acute disseminated encephalomyelitis | 100% donor | 0          | 2.2            |                  |
| 8 | F   | 16.3                     | Cerebrovascular disease | TREO42 TT10 FLU160 ATG RITUX | Monthly red cell exchange transfusion | Haploidentical mother | HbSA | TCRαβ/CD19 depleted PBSC | Grade IV mucositis | Grade II gastrointestinal aGvHD | 100% donor | 39.8       | 1.5            |                  |
| 9 | F   | 10.5                     | Recurrent ACS/VOC | TREO42 TT8 FLU160 ATG RITUX | Chronic transfusion + hydroxycarbamide | MUD | HbAA | BM | Grade II gastrointestinal aGvHD | 100% donor | 0          | 1.1            |                  |
| 10| F   | 4.1                      | Cerebrovascular disease | TREO42 TT8 FLU160 ATG | Chronic transfusion + hydroxycarbamide | MSD | HbSA | BM |                            | 37% donor | 41.4       | 1              |                  |
| 11| M   | 6.5                      | Cerebrovascular disease | TREO42 TT10 FLU160 ATG RITUX | Chronic transfusion | MMUD | HbAA | TCRαβ/CD19 depleted PBSC | EBV reactivation | Grade II gastrointestinal aGvHD | 61% donor | 0          | 0.8           |                  |
disseminated encephalomyelitis in one patient. All toxicity resolved completely. Grade I-II gastrointestinal acute GvHD was diagnosed in 3 patients (haploidentical transplant, n = 1; MUD transplant, n = 1, MMUD transplant, n=1). These patients were successfully treated with calcineurin inhibitors (n = 3), steroid (n = 1) and extracorporeal photochemotherapy (n = 3) as per institutional protocol. No grade III-IV acute GvHD or chronic GvHD were observed. No secondary graft failure was observed.

Full donor chimerism was demonstrated in 6/11 patients and stable mixed chimerism was observed in 5/11 patients (45%). Donor hematopoiesis ranged from 37% to 90%, but did not affect the HbS proportion: HbS was absent after a transplant from HbA/A MUD (n=1) or compatible with HbS carrier (median 40.5%, range 40.3–41.4%) in patients transplanted from a HbS/A MSD (n=4). The lymphocyte count reached normal values for age at day +180 in all patients except P11. Median time to reach a CD3+CD4+ cell count higher than 400/µL was 148 days for T-replete grafts (range 57–203 days) and 245 days (range 237–253 days) for T-deplete grafts. Immunoglobulin replacement was necessary only in one patient (P11) who experienced EBV reactivation and received one dose of rituximab. Despite serial monitoring no viral reactivation was documented in any other patient.

Organ damage and SCD-related outcomes: The median follow-up was 2.35 years (range: 0.8–6.5 years). All patients are alive and well. No episode compatible with acute chest syndrome, stroke or other sickle cell disease manifestation occurred.

No patient experienced renal or hepatic dysfunction following transplantation.

TCD was normal for patients without cerebral vasculopathy. Data for patients with cerebral vasculopathy are reported in Table 2. Brain MRI and MRA data are available for 10 patients. Three patients had normal pre-transplant brain imaging. Two patients had cerebral vasculopathy before transplantation, but no post-transplant imaging. Five patients had alteration on the pre transplant MRI and evaluable data on follow-up (Table 3). Resolution or improvement of the vascular stenosis was detected in 3/5 patients. Last post-transplant evaluation was performed after a median of 1315 days (range: 268–1417).

Pre-transplant organ function was within normal limits for all patients. Post-transplant lung function evaluation was performed in 7 patients (P1-3 and P5–8) and was normal for all of them after a median of 1104 days from transplant (range 369–2304 days). Hormonal function was evaluated in 7 patients (P1-3 and P5–8) after a median of 804 days from transplant (range 205–1518 days). Height, weight and thyroid function were normal for all explored patients. Three patients were pre-pubertal at last assessment. Puberty was evaluable in 4 patients (P1, P2, P5 and P8: 1 male and 3 females): 3 had normal pubertal development and 1 patient (P8) experienced secondary gonadal failure. Follow-up echocardiography (data available for 7 patients) and eye examination (data available for 5 patients) were normal.

Table 2. Brain imaging before and after HSCT for patients with cerebral vasculopathy. WHM: white matter hyperintensity; n/a: not available

| patient | Magnetic resonance angiography | White matter changes |
|---------|-------------------------------|----------------------|
|         | Before HSCT | After HSCT                      | Before HSCT                          | After HSCT |
| 1       | Moderate stenosis | Significant reduction of stenosis | Mild WHM in the hippocampus and temporal cortex | normal |
| 2       | Severe stenosis | Stable | WHM in the periventricular region | gliosis of affected region |
| 3       | Bilateral severe stenosis | Resolution of stenosis | WHM in the semioval centers | gliosis of affected region |
| 4       | Bilateral mild stenosis | n/a | Absent | n/a |
| 5       | Bilateral moderate stenosis | Significant reduction of stenosis | Minimal WHM in the left subcortical temporal region | Stable |
| 8       | Bilateral moderate stenosis | n/a | Absent | n/a |
| 10      | Bilateral severe stenosis | stable | Absent | Absent |
| 11      | Bilateral severe stenosis | n/a | Bilateral WHM in the semioval centers | n/a |
Discussion: We report a retrospective case series of 11 SCD patients who received HSCT after a Treosulfan-based conditioning regimen. Sustained engraftment was observed in all patients. Stable mixed chimerism was detected in a significant proportion of patients (45%), did not change after the discontinuation of immunosuppressive treatment and resulted in a cure of SCD for all patients. Previous experiences have demonstrated that full donor chimerism is not needed to cure SCD due to the survival advantage for donor red cell in peripheral blood: pulmonary, gonadal and central nervous system status can be significantly ameliorated also when stable mixed chimerism is obtained. Indeed, no clinical manifestation correlated with SCD occurred after HSCT in our cohort. Since cerebral vasculopathy was the cause for transplant in 7 patients, we focused our attention on the evaluation of TCD and brain MRI and MRA. Chronic transfusions resulted in normalization of pre-transplant TCD in all patients receiving this treatment. However, cerebral artery stenosis persisted on pre-HSCT MRA for all patients. Post-HSCT MRA data, evaluated with a standardized scoring system, were available for 5 patients and showed either a stabilization of the stenosis or amelioration. Although improvement in vascular stenosis has been previously described in patients treated with chronic transfusion, hydroxyurea or HSCT, the rate of improving patients in our cohort compares favorably with previous reports. These satisfactory outcomes could be possibly due to the screening program for cerebral vasculopathy performed at our center that led to the fact that all patients were transplanted before any clinically evident stroke.

The safety profile of Treosulfan conditioning regimen was excellent and incidence of adverse events was comparable to previous reports: no transplant-related mortality was observed and grade III-IV non hematological toxicity was limited to mucositis which resolved completely without sequelae. The neurological event in our case series cannot be attributed with certainty to the Treosulfan conditioning. This toxicity profile is similar to results obtained in adult patients transplanted after a non-myeloablative conditioning. Grade I-II acute GvHD was observed in 3/11 patients in our cohort (27%) with no grade III-IV acute GvHD or chronic GvHD. The GvHD cases were all among patients receiving an alternative donor transplant, no GvHD was observed among the 7 patients receiving a MSD HSCT and all the patients experiencing GvHD responded rapidly to first line treatment. To the best of our knowledge, data regarding organ damage related to HSCT has not been previously reported for SCD patients undergoing HSCT after a Treosulfan-based conditioning regimen. In our cohort, the decline in pulmonary and renal function observed after Busulfan-based conditioning regimen was not present and growth, thyroid and cardiac function were preserved after HSCT. Although 3 patients had normal pubertal development, 1 patient that had reached puberty before HSCT and for whom pre-transplant ovarian cryopreservation was performed, experienced secondary gonadal failure. This event highlights the opportunity to attentively evaluate possible long term effects on reproductive health and propose mitigating strategies before HSCT.

Current knowledge about outcomes of Treosulfan based conditioning regimen in SCD is limited to a single-center experience reporting 15 patients who received a MSD or MUD HSCT. We have nearly doubled the number of reported patients and we have described the use of Treosulfan-based conditioning regimen for MMUD or haploidentical donor HSCT, which are considered investigational approaches in SCD. To mitigate the risk of rejection and GvHD, TCRαβ+ and CD19+ cell depletion was performed. In the MMUD setting, this approach was reported as safe and efficacious for patients with acute myeloid leukemia or Hurler syndrome but no SCD patient has been described yet. If further investigations will confirm its feasibility and efficacy, haploidentical or MMUD HSCT in SCD may open the possibility of cure for many patients.

Table 3. Transcranial Doppler Ultrasonography for patients with cerebral vasculopathy. Results were categorized as normal, conditional or abnormal according to the STOP trial criteria. For patients before initiation of chronic transfusion Before HSCT After HSCT 1 The patient did not receive chronic transfusion Abnormal Normal 2 Abnormal Normal n/a 3 Abnormal Normal n/a 4 n/a Normal n/a 5 Abnormal Normal n/a 8 Abnormal Normal n/a 10 Abnormal Conditional n/a 11 Abnormal Normal n/a

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patients without a MSD or MUD donor available. When employing alternative donors, a higher risk of GvHD and delayed immune reconstitution should be taken into account; however, in our experience, these drawbacks can be managed by supportive therapy and are outweighed by the satisfactory outcomes. Although PBSC mobilization with G-CSF in HbS heterozygous parents is often perceived as risky, no significant adverse events were reported and, in our experience, both the haploidentical donors underwent PBSC mobilization and collection safely.

The main limitation of our study is its retrospective nature and the report of a single center experience; sample size was also limited and warrants further confirmation. Moreover, in order to perform the TCRαβ and CD19 depletion, a facility with experience in stem cell manipulation is needed.

Conclusions. Our data show that HSCT after Treosulfan based conditioning regimen for SCD patients is effective and associated with low toxicity. End organ damage may be halted or even ameliorated as shown by the regression of cerebral vessel stenosis and white matter changes. This strategy is suitable also for alternative donor transplants and, if our data are confirmed in larger cohorts, could pave the way for expanding the access to HSCT also to SCD patients lacking a matched sibling or matched unrelated donor.

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