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Alexandra Gauthier, Nathalie Bleyzac, Nathalie Garnier, Kamila Kebaili, Philippe Joly, et al.. Goal-Oriented Monitoring of Cyclosporine Is Effective for Graft-versus-Host Disease Prevention after Hematopoietic Stem Cell Transplantation in Sickle Cell Disease and Thalassemia Major. *Biology of Blood and Marrow Transplantation*, 2020, 26, pp.2285 - 2291. 10.1016/j.bbmt.2020.01.016 . hal-03492822

HAL Id: hal-03492822

<https://hal.science/hal-03492822>

Submitted on 21 Nov 2022

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Goal-oriented monitoring of cyclosporine is effective for graft-versus-host disease prevention after Hematopoietic Stem Cell Transplantation in Sickle Cell Disease and Thalassemia major

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Conflict of interest: none

Abstract

Graft-versus-host disease (GvHD) is an important challenge and one of the major causes of morbidity and mortality in children after hematopoietic stem cell transplantation (HSCT). Herein, we report our institution's experience of a goal-oriented Bayesian monitoring for cyclosporine (CsA) used alone as GvHD prophylaxis during the post-transplant period in pediatric patients with Thalassemia Major (TM) or Sickle Cell Anemia (SCA) undergoing HLA-matched HSCT. We also studied evolution of chimerism. Twenty-six consecutive patients (14 SCA; 12 TM) underwent matched sibling donor (MSD) HSCT from 2004 to 2014. All patients received a myeloablative conditioning regimen. GvHD prophylaxis consisted of 20 mg/kg of antithymocyte globuline (ATG) in conditioning regimens then CsA alone in the post-transplant period. Target CsA trough blood concentration (TBC) was 150 +/- 20 ng/ml. At last follow-up, all patients are alive and free of disease, even in case of mixed chimerism. Engraftment occurred in all patients. No patient developed grade II-IV acute GvHD, 4 patients developed acute grade I skin GvHD and only one presented with chronic pulmonary GvHD. A better control of GvHD and immunosuppression by a strict monitoring of CsA TBC as described herein is promising and could play a crucial role. Further investigations are required but this study opens new perspectives to improve survival and safety of HSCT from alternative donors in TM and SCA to levels compatible to that obtained with matched-sibling donors.

44 Introduction

45 Thalassemia major (TM) and sickle cell anemia (SCA) are the two most widespread inborn
46 hemoglobinopathies worldwide. TM was the first congenital hemoglobinopathy to be cured by
47 allogenic hematopoietic stem cell transplantation (HSCT) (1), and SCA rapidly followed (2,3). Other
48 than gene therapy (4), HSCT remains the only available curative treatment for these two diseases.
49 Provided that an HLA-identical sibling donor is available, it should be performed as soon as possible
50 for TM and in case of complications for SCA (5). Outcomes have substantially improved over the past
51 3 decades; currently more than 90% of children's survive HSCT with myeloablative conditioning and
52 geno-identical donor in hemoglobinopathy (6,7).

53 Despite improvement in immunosuppressive prophylaxis over the past decade, graft-versus-
54 host disease (GvHD) remains an important challenge and one of the major causes of morbidity and
55 mortality in children transplanted from matched-sibling donors (MSD). It is reported that grade II-IV
56 acute GvHD (aGvHD) occurred in 10 to 31% of patients transplanted for TM or SCA (including 2 to
57 17% grade III-IV aGvHD) (8–15), probably because of differences in the GvHD prophylaxis protocol
58 employed. Indeed, post-transplant GvHD prophylaxis has been based on either cyclosporine A (CsA)
59 alone, or CsA combined with methotrexate or mycophenolate mofetil. Furthermore, an international
60 expert panel now recommends systematic use of anti-thymocytes globulin (ATG) in conditioning
61 regimens for SCA and TM to obtain partial *in vivo* T-cell depletion before transplant and prevent
62 rejection and GvHD but administration and dosage of ATG varied widely between centers (3,5,8–
63 10,12–15).

64 In the teaching hospitals of Lyon (France), GvHD prophylaxis for pediatric patients consists of
65 high dose of ATG prior to transplant and CsA alone in the post-transplant period. CsA is characterized
66 by large inter- and intra-individual pharmacokinetic variability, and the administration of standard
67 doses of CsA leads to variable responses in patients exposed, from inefficacy to toxicity. In previous
68 studies, we showed that controlling early CsA exposure in pediatric patients was a determining factor
69 for aGvHD occurrence in MSD as well as in unrelated donor (UD) HSCT; the lower the CsA systemic

70 exposure during the first 2 weeks post-HSCT was, the more severe was aGvHD (16,17). We use close
71 monitoring of early post-transplant CsA trough blood concentration (TBC) and maximum *a posteriori*
72 probability (MAP) Bayesian adaptive control of CsA dosage (18). Herein, we report our institution's
73 experience of this dosing method for CsA used alone as GvHD prophylaxis during the post-transplant
74 period in pediatric patients with TM or SCA undergoing HLA-matched HSCT.

Materials and methods

Subject eligibility

A retrospective review of pharmacokinetic monitoring of CsA and outcomes of patients with TM or SCA who received an HSCT in our institution from July 2004 to December 2014 was conducted. During this period all patients received 2-hour IV infusions of CsA twice daily. In order to obtain a homogeneous cohort of patients and interpretable results, patients were included in the present study if they were transplanted from a MSD and if CsA was tolerated at least until engraftment to correctly assess the impact of CsA monitoring on GvHD incidence. Data were collected until October 2018. The study was declared to the national data protection commission (*Commission nationale de l'informatique et des libertés*, CNIL).

Transplantation procedure

All patients received a myeloablative conditioning regimen with intravenous busulfan (D-10; D-9; D-8 and D-7) combined with cyclophosphamide (D-5; D-4; D-3 and D-2) (200 mg/kg total dose) (Figure 1). Therapeutic drug monitoring of busulfan plasma concentrations and Bayesian dose adjustment was performed to adjust doses in order to obtain a target area under the curve (AUC) between 900 and 1100 $\mu\text{M}\cdot\text{min}$ (18). All patients received 20 mg/kg total dose rabbit ATG (Genzyme, Cambridge, MA, USA) at D-7; D-5; D-3 and D-1, to prevent both graft rejection and GvHD (Figure 1). In order to prevent neurological complications related to administration of busulfan and CsA, in particular for SCA patients, patients received prophylactic clonazepam throughout hospitalization. Systematic screening by blood PCR was made every week in case of seropositive donor and/or recipient status for *cytomegalovirus* (CMV) and *Epstein Barr Virus* (EBV). *Human herpesvirus 6* (HHV-6), *adenovirus* (ADV) blood PCR and BK virus urinary PCR was performed in case of clinical or biological symptoms.

GvHD prophylaxis

In addition to ATG given prior to transplant to obtain partial *in vivo* T-cell depletion, GvHD prophylaxis during the post-transplant period consisted of CsA alone; methotrexate and mycophenolate mofetil were not used anytime. CsA was first administered twice daily by 2-hour intravenous infusion. CsA was started on 5 mg/kg/day the day before transplantation and dosing was actualized after each CsA TBC measurement. According to the possible intra-individual pharmacokinetic variability, each new CsA blood level was implemented in a Bayesian model, following a procedure described below, in order to estimate a new set of individual pharmacokinetic parameter values, which were then used to stimulate doses to reach the target TBC, if the previous measured one was off-target (18). When oral medication could be tolerated, CsA was given orally every 12 hours.

CsA monitoring

Measurements of CsA whole blood concentrations were all performed on peripheral venous blood samples using an immuno-enzymatic assay (technique EMIT or antibody conjugated magnetic immunoassay method ACMIA). The limit of detection was 30 ng/mL. During intravenous treatment, CsA TBC was measured 2 or 3 times weekly starting from the day +1 after transplantation to maintain CsA TBC in the target interval. After the switch from intravenous to oral medication, CsA TBC was measured twice weekly during the first month post-transplantation, always on peripheral sites to avoid analytical issues with TBC measurements as described elsewhere (19).

Individualization of CsA regimen

A computer file was made for each patient starting CsA therapy, by using the file manager program included in the USC*PACK software (Laboratory of Applied Pharmacokinetics, USC* Pack P.C Collection Clinical Research Programs, Los Angeles, University of Southern California, School of Medicine, 1995, version 10.7). Each file contained anthropometric and treatment data such as age, weight, administered amounts of CsA, time of administration, infusion duration, blood sampling times and drug blood level measurements. The individual pharmacokinetic parameter values were then estimated for a two-compartment model, using a MAP (Maximum A Posteriori) Bayesian method also

implemented in USC*PACK. This method allows accurate estimation of the whole drug blood concentration vs time profile from only one to three drug blood level measurements, by combining available patient information and population information (defined as the distribution of pharmacokinetic parameter values obtained from similar patients treated previously according to a two-compartment-central c and peripheral p- model with the following values for the population PK parameters: volume of distribution $V = 2.00 \pm 1.5$ l/kg; elimination rate constant $K_{el} = 0.095 \pm 0.05$ h^{-1} ; absorption rate constant $K_{abs} = 0.533 \pm 0.866$ h^{-1} ; transfer rate constants K_{cp} and K_{pc} respectively 0.100 ± 0.268 and 0.300 ± 0.005 h^{-1}). Moreover, the analytical error is taken into account to determine pharmacokinetic parameter values, as each drug concentration measured is weighted by the reciprocal of the variance associated with the assay described above. This permits a credibility level to be attributed to each measured blood concentration, increasing the accuracy of the estimates. Individual pharmacokinetic parameter values are then used to simulate future CsA blood concentrations and consequently determine the doses necessary to reach CsA target blood concentrations at any time.

In TM or SCA where a graft-versus-leukaemia (GvL) effect is not required (in contrast to malignant diseases), target CsA TBC was 150 ± 20 ng/ml.

Relationship between CsA exposure and aGVHD

The mean CsA TBC for each patient was retrospectively calculated for different durations of post-transplantation exposure to CsA: first week, second week, third week, until hematopoietic recovery. The mean intravenous and oral daily doses of CsA administered were also studied.

Grading of GvHD

aGvHD was graded according to the revised Glucksberg scale (20). Four grades were defined according to the number and severity of localizations of the disease: grade I (minor aGvHD), grade II (moderate aGvHD), grade III et IV (severe aGvHD). The diagnosis of chronic GvHD (cGvHD) was based on clinical and histological criteria and was defined by the presence of GvHD signs after day 100.

Evaluation of chimerism

The presence of residual host cell (RHC) populations after HSCT was investigated using short tandem repeat polymerase chain reaction (STR-PCR) and real-time quantitative PCR (RQ-PCR) that have different sensitivities. The STR-PCR is an amplification method by multiplex PCR of repetitive DNA sequences (STR) (21,22), with a limit of detection of 5% of RHC; this technique was used in 24 patients. RQ-PCR was performed in 2 patients with a poor STR profile. The RQ-PCR method has a higher sensitivity (0.2% of RHC) and uses TaqMan technology (Applied Biosystems, Foster City, CA, US) and a set of specially designed primers and fluorogenic probes. In clinical routine, whole blood chimerism was performed. When clinical situation requires it, fractionated CD3 chimerism was performed, particularly in case of mixed chimerism.

Full donor engraftment and a total replacement of RHC by donor hematopoiesis after HSCT was defined as complete chimerism (CC), and the simultaneous presence of both donor and RHC was defined as mixed chimerism (MC). As previously reported (23–25), the status of MC could be transient (TMC) that could evolve early after HSCT towards graft failure or CC, while MC was considered as persistent (PMC) when donor and recipient cells permanently coexisted during the post-transplantation follow-up period. Moreover, to better follow the evolution of TMC, 3 categories were defined, TMC level 1 when the number of RHC was lower than 10%, level 2 when this was between 10 and 25%, and level 3 when this was greater than 25%. PMC could evolve into improving PMC (continuous increase in the proportion of donor cells), stable PMC (fluctuations in the percentage of RHC), or worsening PMC (progressive decrease of the proportion of donor cells).

Statistical Analysis

Outcomes were compared using the Mann-Whitney U test and Chi² test, as appropriate. All statistical analyses were performed using SPSS version 12.0.

Results

Patients

A total of 28 consecutive patients with TM or SCA underwent HSCT in our institution from July 2004 to December 2014. Two patients were excluded: one patient for whom cyclosporine was stopped at day 4 post-transplant because of localized seizures, and another who was transplanted from a mismatched UD. Twenty-six patients were therefore included in the study. The sex ratio was 1, the median age at the time of transplantation was 6 years (range: 2-14 years). One patient had a second HSCT after failure of the first one that was performed in 2002 (outside the study period). Twenty-five patients had an HLA-identical MSD and 1 patient received a transplant from a phenotypic HLA-identical relative. The stem cell source was bone marrow in 21 patients (81%), cord blood in 2 patients (8%), and combined bone marrow and cord blood from the same sibling in 3 patients (11%). No peripheral blood stem cells were used (Table 1).

HSCT outcomes

At the time of writing, all patients are alive and free of TM or SCA (overall survival (OS) 100% and disease free-survival (DFS) 100%). All transplants resulted in sustained engraftment of donor cells with no late graft rejection. Median follow-up was 107.5 months.

The median time to neutrophil recovery ($> 500 / \text{mm}^3$) after HSCT was 22 days (range: 10-41), and the median time to platelet recovery ($> 20 \text{ G/L}$) after HSCT was 23 days (range: 12-66). The median time to neutrophil and platelet recovery was, respectively, 21 and 22 days for transplants of cells originating from bone marrow, 28 and 48 days for cells originating from cord blood, and 26 and 24 days for cells from both bone marrow and cord blood. The median duration of intravenous CsA administration before switch to the oral route was 22.5 days.

Acute and chronic GvHD

Grade I cutaneous aGvHD occurred in 4/26 patients and required only local treatment. One of these patients experienced grade I cutaneous aGvHD in relation with proven cyclosporine

underexposure at the 4th week. For this patient, aGvHD signs resolved one day after increasing the dose of CsA. Among these patients, 3 received bone marrow and 1 cord blood. No patient developed grade II-IV aGvHD. Limited chronic lung GvHD occurred in 1 patient, which was diagnosed at 3 months post-transplant following identification of a non-reversible obstructive respiratory disorder that was treated with systemic and inhaled corticosteroids. This patient did not have any other manifestation of cGvHD. This patient is not disabled in daily life because of his cGvHD. At last follow-up, the spirometry was satisfactory. Stem cell source was bone marrow in this patient.

Other HSCT complications

The conditioning regimen was well tolerated. Complications of HSCT are summarized in table 2. Liver veno-occlusive disease (VOD) accompanied by elevated bilirubin ($> 34 \mu\text{mol/L}$) was diagnosed in 2 patients (8%) and 1 of the latter required defibrotide treatment. Four patients had a documented bacterial infection and were successfully treated with antibiotics; none had invasive fungal infection. Thirteen patients had CMV reactivation (50%), but no CMV disease was observed. EBV reactivation occurred in 2 patients (8%), including 1 patient with severe lympho-proliferative syndrome (day 42). Cell source for this patient was bone marrow, and both pre-transplant donor and recipient EBV serologies were positive. HHV-6 reactivation occurred in 1 patient. There was no report of malignancy after HSCT.

Relationships between aGvHD and CsA TBC

No patient developed grade II-IV aGvHD, only 4 patients developed grade I aGvHD and one patient presented cGvHD. Statistical analysis was thus impossible because of the small number of patients in the GvHD group compared to the group without GvHD. During the first week post-transplantation, the mean (\pm SD) CSA TBC of patients who developed grade I aGvHD (n=4) was lower than mean CsA TBC of patient without aGvHD (n=22) ($136 (\pm 54)$ versus $146 (\pm 54)$ ng/ml). Likewise, the mean CSA TBC until recovery was lower, but to a lesser extent, in patients who developed aGvHD (158 ± 51 versus 163 ± 48 ng/ml). Due to lower CsA TBC during the first week,

CsA doses had to be increased and therefore mean CsA dose was higher in patients who developed grade I aGvHD (6.7 mg/kg/day) compared to those in patients without aGvHD (5.3 mg/kg/day).

Evolution of chimerism

Chimerism status was followed over a median 78 months (range: 24-132). In the early post-transplant period, before the second month, 17 patients (65%) had a full donor engraftment, and 9 patients (35%) had MC (2 patients a TMC level 1, 4 patients a TMC level 2, and 3 patients a TMC level 3). The TMC level 3 status in the early post-transplant period of 1 patient was related to EBV reactivation with severe lympho-proliferative syndrome occurring at day 42 post-transplant (chimerism at 1-month post-HSCT: RHC 10% then RHC 38% at 2 months (50% on CD3 lymphocytes), and a maximum 55% RHC at 6 months). This patient was treated by intensifying immunosuppressive therapy and 4 injections of rituximab. The treatment was effective, marrow status improved to PMC level 2 at 8 years post-HSCT (79% donor).

At last follow-up, the proportion of donor engraftment ranged from 63 to 100%. In the 17 patients with an initial CC, 8 patients (47%) remained CC whereas 9 patients (53%) achieved a status of PMC, stable for 41% of them. In the 9 patients with an initial TMC, 1 achieved CC whereas 8 remained PMC, improving or stable PMC for 7 of them (table 3). All patients became free of thalassemia or sickle cell anemia and were transfusion independent.

Relationship between busulfan AUC and HSCT outcomes

The mean \pm SD busulfan AUC per dose was not significantly different between patients with full donor engraftment and those with PMC (1030 ± 115 versus 948 ± 146 μ M.min, $p=0.20$). One severe VOD was diagnosed and required defibrotide treatment; for this patient the mean busulfan AUC per dose was 861 μ M.min.

Relationship between chimerism and HSCT characteristics

No significant difference was found for chimerism according to indication after HSCT ($p = 0.39$). Among the 14 patients who experienced CMV reactivation, 4 had CC, 4 had stable PMC, 4 had

improving PMC, and 2 had worsening PMC. The 4 patients with EBV reactivation (2 in early post-transplant period) had PMC at last follow-up. Among them, donor chimerism decreased to a minimum of 45% in 1 patient who had severe lymphoproliferative syndrome; intensification of immunosuppressive therapy and rituximab prevented rejection and then donor chimerism gradually increased. The only patient with a HHV6 reactivation had improving PMC. PMC was not more frequent in patients who experienced viral reactivation than those who did not ($p = 0.50$; Table 4).

The mean \pm SD total nucleated cells in patients with full donor engraftment was not significantly higher than those in patients with PMC (4.63 ± 3.01 versus $4.13 \pm 1.02 \times 10^8/\text{kg}$, respectively, $p = 0.90$; Table 4).

Discussion

In this retrospective study analyzing outcomes after HLA-matched transplantation in children with TM and SCA, we found no graft rejection and a low rate of GvHD; moreover, all patients were alive and free of disease at last follow-up. These results are likely to be attributable, at least in part, to the strategy of CsA monitoring and the high doses of ATG used.

Statistical analysis of potential differences in CsA TBC according to GvHD was not possible because of the very low number of GvHD events. It is noteworthy that patients only experienced grade 1 aGvHD. However, a slight difference in mean CsA TBC was found, especially in the first week post HSCT. A CsA TBC greater than 150 ng/mL seems therefore to be a relevant target to prevent GvHD in these transplantations and should be obtained as soon as possible, as suggested previously in other contexts (17). Furthermore, although viral reactivation and/or infections affected most patients herein, there was no lethal infection and only one patient had severe EBV reactivation. It is also of note that the preparative regimen and GvHD prevention strategy used in our patients was very well tolerated, resulting in no cases of transplant-related mortality, compared to previously published reports that have variable TRM between 4-37% (3,8,10,12,14). In the international study of result of HLA-identical sibling HSCT, 7% died, with the most common cause of death being infection (7). The strategy of CsA monitoring in our patients may have contributed to the absence of TRM through a more controlled immunosuppression, and this effective prevention of GvHD strategy abrogated the need for methotrexate or mycophenolate mofetil, further decreasing toxicity. The use of a single immunosuppressant drug is likely to reduce the incidence of adverse reactions and so may be a safer post-transplant approach. However, immunosuppression-related toxicity was not completely avoided which could be in relation to the high dose of ATG used (20 mg/kg dose total) that seemed to be effective for engraftment and prevention of GvHD. It could therefore be interesting to monitor ATG in order to improve the results in terms of toxicity (26–29).

Mixed chimerism is frequently observed after HSCT for hemoglobinopathies (23–25,30). In the present study, two-thirds of patients had CC and one third MC in the early period post-transplant whereas the converse was found at last follow-up. No patient rejected his or her transplant, and all are

transfusion independent and free of disease at last follow-up. For TM patients, it has been reported that the probability of rejection was increased in the patients who had RHC above 25% early after HSCT, while most patients characterized by TMC with RHCs lower than 10% progressed towards CC or PMC rather than graft failure (25). Moreover, once PMC was established, patients no longer experienced graft failure. They did not require additional red blood cells (RBC) support, and were clinically cured by an incomplete, but functional graft, probably regulated by different immunological mechanisms and donor-host tolerance (23–25). Similar observations were made in SCA transplant patients with PMC (30). Thus, patients with hemoglobinopathies can have lifelong stable MC without rejection or symptoms of their initial disease following HSCT, unlike hematological malignancies in which RHC are often predictive of relapse. These findings are consistent with those reported herein; only 3 patients had TMC level 3 (RHC above 25%) in the early period post-transplant and progressed to improving or stable PMC. Another factor to consider is the mean busulfan AUC; the target busulfan AUC used in the present study was lower than currently recommended: between 900 and 1100 $\mu\text{M}\cdot\text{min}$ instead of 900-1500 $\mu\text{M}\cdot\text{min}$ (18). We compared busulfan mean AUC per dose between patients with CC versus patients with PMC, but no significant difference was found. In the literature, a significant association between partial donor engraftment and lower busulfan levels was observed (31). However, mean busulfan AUC of patients with partial donor engraftment was lower in this study as compared to the present one (862 ± 73 versus $948 \pm 146 \mu\text{M}\cdot\text{min}$, respectively) (31). Thus, in terms of toxicity, the frequency of VOD was low compared to previous reports (mean 13.7% in a report which analyzed 135 studies between 1979 and October 2007 (32), and 13% in the French cohort of TM (8)).

This study has several limitations. This was a single-center study, because other centers do not use this monitoring method for CsA. Thus, a low number of patients was included, and statistical analysis of potential differences between GvHD and CsA TBC was not possible. Furthermore, biases are inherent in retrospective studies, yet we can notice that we do not have essential missing data. At last, for some patients who have recently been transplanted, we could report only a short chimerism follow-up (24 months for 6 of them). Nevertheless, we confirmed good transplant prognosis in TM

and SCA with MSD. Such a donor may not always be available; for example, in the United States fewer than 1 out of 5 children with SCA are expected to have a MSD (33), and the availability of MSD in TM is estimated to 20-30%. However, suitable UD can be identified using stringent criteria for immunogenic compatibility. In TM, it is reported that using high resolution molecular typing for both HLA class I and class II loci and stringent criteria of compatibility with the recipient, unrelated HLA identical donors may offer similar results to those obtained with HLA-identical family donors (34,35). Recently, an international survey studied 1110 patients with TM who received transplants and confirmed similar OS and EFS between HLA-matched related and MUD transplantation (89% vs 87% and 86% vs 82%, respectively) (36). In SCA, MUD also appeared to be the next best option after MSD for HSCT but high rates of aGvHD and cGvHD was previously reported. In these studies conditioning regimens varied from myeloablative to reduced-intensity and umbilical cord blood was used (37). A better control of GvHD and immunosuppression by a strict monitoring of CsA TBC as described herein is promising and could play a crucial role in HSCT. Further investigations are required but this study opens new perspectives to improve survival and safety of HSCT from alternative donors both in TM and SCA to levels compatible to that obtained with MSD. Moreover, the recent description of a Bayesian network to predict therapeutic range achievement of initial CsA blood concentration after pediatric HSCT offers interesting perspectives to continue to improve monitoring of this drug characterized by a narrow therapeutic index (38).

Conclusion

Together, our results shown that CsA used alone during the post-transplant period and close monitoring of CsA TBC seems to be effective for GvHD prophylaxis in pediatric patients with TM or SCA undergoing HLA-matched HSCT. This study opens new perspectives to improve efficacy of HSCT from alternative donors in order to make transplantation accessible to a wider population of patients.

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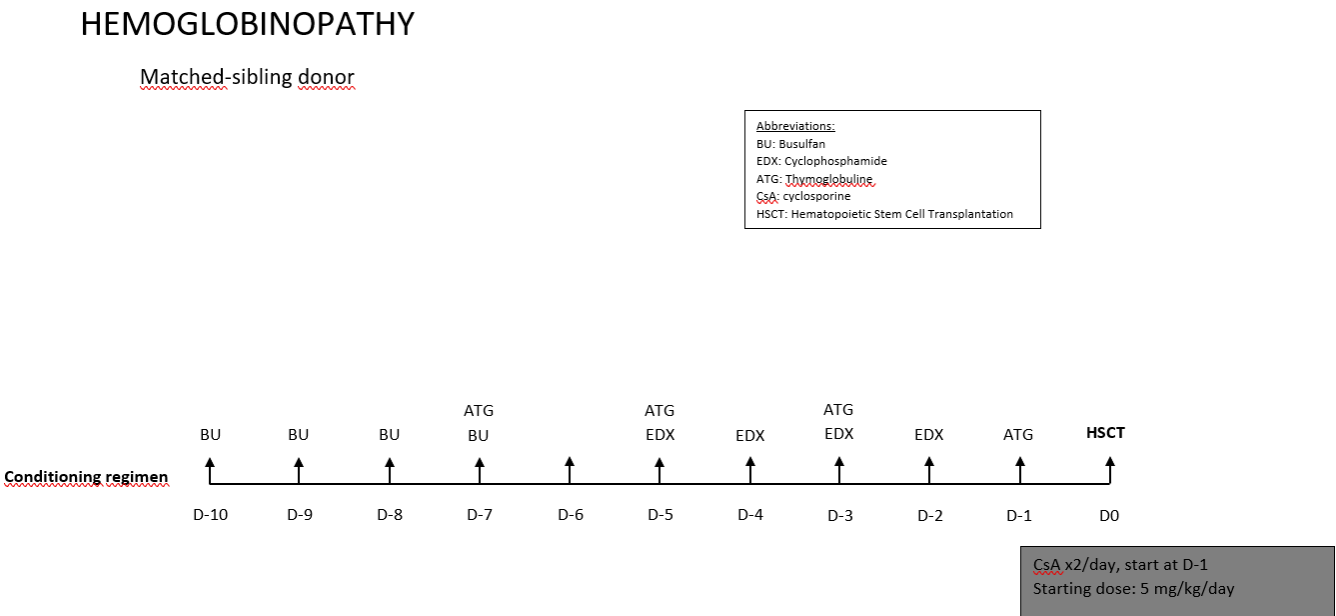
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Figure

Figure 1. Myeloablative conditioning regimen



Tables

Table 1. Patient, donor and HSCT characteristics

	Total population n = 26	Hemoglobinopathies	
		TM n = 12	SCA n = 14
Median age at transplant, years (range)	6 (2-14)		
Sex, female, n (%)	13 (50)		
Hemoglobinopathies, n (%)			
TM	12 (46)		
SCA	14 (54)		
Indications HSCT for SCA, n (%)			
Cerebral vasculopathy			5 (36)
Recurrent painful crises despite HU			4 (29)
Allo-immunization			1 (7)
Repeated acute splenic sequestration			1 (7)
Association			3 (21)
Pesaro risk for TM, n (%)			
I		2 (17)	
II		5 (41.5)	
III		0	
Missing data		5 (41.5)	
Stem cell source, n (%)			
Bone marrow	21 (81)		
Cord blood	2 (8)		
Bone marrow + cord blood	3 (11)		
Female donor/male recipient, n (%)	10 (38)		
Recipient CMV status, n (%)			
Positive	16 (62)		
Negative	10 (38)		
Recipient EBV status, n (%)			
Positive	20 (77)		
Negative	6 (23)		
Total nucleated cells x 10 ⁸ /kg, median (range)			
Bone marrow	5.08 (1.38-8.84)		
Cord blood	0.59 (0.27-0.91)		
CD34+ x 10 ⁶ /kg, median (range)			
Bone marrow	6.6 (0.44-15.7)		
Cord blood	0.54 (0.15-0.93)		
T3 x 10 ⁶ /kg, median (range)			
Bone marrow	63.0 (20.0-90.0)		
Cord blood	18.45 (15.5-21.4)		

HSCT: hematopoietic stem cell transplantation

TM: thalassemia major; SCA: sickle cell disease

HU: hydroxycarbamide

Table 2. Complications of HSCT

	Total population n = 26
aGvHD, n (%)	
Grade I, cutaneous	4 (15)
Grade II-IV	0
Hepatic/gastrointestinal	0
cGvHD, n (%)	
Lung	1 (0.04)
Viral reactivation, n (%)	
CMV	13 (50)
CMV disease	0
EBV	2 (8)
HHV6	1 (4)
BK virus	1 (4)
ADV	0
Infections, n (%)	
Septicemia	3 (12)
Pneumonitis	1 (4)
Invasive fungal infection	0
Veno-occlusive disease, n (%)	
Severe	1 (4)
Moderate	1 (4)
Toxicity, n (%)	
Mucositis	13 (50)
Anusitis	4 (15)
Gastrointestinal toxicity (diarrhea)	2 (8)
Macroscopic hematuria	2 (8)
HTA	1 (4)
Acute renal failure	1 (4)
Alloimmunization, n (%)	
Anti-HLA and anti-platelet	1 (4)

Table 3. Evolution of chimerism at last follow-up according to chimerism in the early post-transplant period (<2 months)

	Full donor engraftment n = 17	TMC level 1 n = 2	TMC level 2 n = 4	TMC level 3 n = 3
Complete chimerism, n (%)	8 (47)	/	1 (25)	/
Stable PMC, n (%)	7 (41)	/	1 (25)	1 (33)
Level 1	2			
Level 2	4		1	1
Level 3	1			
Improving PMC, n (%)	1 (6)	1 (50)	2 (50)	2 (67)
Level 1				
Level 2	1	1	1	2
Level 3			1	
Worsening PMC, n (%)	1 (6)	1 (50)	/	/
Level 1				
Level 2	1			
Level 3		1		

TMC: transient mixed chimerism (level 1: residual host cell (RHC)<10%; level 2: RHC 10 to 25%; level 3: RHC>25%)

PMC: permanent mixed chimerism

Table 4. Characteristics of patients with complete chimerism (CC) versus patients with persistent mixed chimerism (PMC)

	Chimerism ^a		p
	CC n = 9	PMC n = 17	
Mean busulfan AUC per dose (μ M.min) (SD)	1030 (115)	948 (146)	0.20
HSCT indication, n (%)			0.39
TM	5 (56)	7 (41)	
SCA	4 (44)	10 (59)	
Viral reactivation, n	4	10	0.50
Mean total nucleated cells, $\times 10^8/\text{kg}$ (SD)			
Bone marrow	4.63 (3.01)	4.13 (1.02)	0.90
Cord blood	0.27	0.91	

AUC: area under curve

^a at last follow-up