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

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27 **Abstract**

28 Graft-versus-host disease (GvHD) is an important challenge and one of the major causes of morbidity  
29 and mortality in children after hematopoietic stem cell transplantation (HSCT). Herein, we report our  
30 institution's experience of a goal-oriented Bayesian monitoring for cyclosporine (CsA) used alone as  
31 GvHD prophylaxis during the post-transplant period in pediatric patients with Thalassemia Major  
32 (TM) or Sickle Cell Anemia (SCA) undergoing HLA-matched HSCT. We also studied evolution of  
33 chimerism. Twenty-six consecutive patients (14 SCA; 12 TM) underwent matched sibling donor  
34 (MSD) HSCT from 2004 to 2014. All patients received a myeloablative conditioning regimen. GvHD  
35 prophylaxis consisted of 20 mg/kg of antithymocyte globuline (ATG) in conditioning regimens then  
36 CsA alone in the post-transplant period. Target CsA trough blood concentration (TBC) was 150 +/- 20  
37 ng/ml. At last follow-up, all patients are alive and free of disease, even in case of mixed chimerism.  
38 Engraftment occurred in all patients. No patient developed grade II-IV acute GvHD, 4 patients  
39 developed acute grade I skin GvHD and only one presented with chronic pulmonary GvHD. A better  
40 control of GvHD and immunosuppression by a strict monitoring of CsA TBC as described herein is  
41 promising and could play a crucial role. Further investigations are required but this study opens new  
42 perspectives to improve survival and safety of HSCT from alternative donors in TM and SCA to levels  
43 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.

## 75 **Materials and methods**

### 76 *Subject eligibility*

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

### 85 *Transplantation procedure*

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

### 98 *GvHD prophylaxis*

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

#### 109 *CsA monitoring*

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

#### 117 *Individualization of CsA regimen*

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

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

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

#### 141 *Relationship between CsA exposure and aGVHD*

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

#### 145 *Grading of GvHD*

146 aGvHD was graded according to the revised Glucksberg scale (20). Four grades were defined  
147 according to the number and severity of localizations of the disease: grade I (minor aGvHD), grade II  
148 (moderate aGvHD), grade III et IV (severe aGvHD). The diagnosis of chronic GvHD (cGvHD) was  
149 based on clinical and histological criteria and was defined by the presence of GvHD signs after day  
150 100.



151 *Evaluation of chimerism*

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

161           Full donor engraftment and a total replacement of RHC by donor hematopoiesis after HSCT  
162 was defined as complete chimerism (CC), and the simultaneous presence of both donor and RHC was  
163 defined as mixed chimerism (MC). As previously reported (23–25), the status of MC could be  
164 transient (TMC) that could evolve early after HSCT towards graft failure or CC, while MC was  
165 considered as persistent (PMC) when donor and recipient cells permanently coexisted during the post-  
166 transplantation follow-up period. Moreover, to better follow the evolution of TMC, 3 categories were  
167 defined, TMC level 1 when the number of RHC was lower than 10%, level 2 when this was between  
168 10 and 25%, and level 3 when this was greater than 25%. PMC could evolve into improving PMC  
169 (continuous increase in the proportion of donor cells), stable PMC (fluctuations in the percentage of  
170 RHC), or worsening PMC (progressive decrease of the proportion of donor cells).

171 *Statistical Analysis*

172           Outcomes were compared using the Mann-Whitney U test and Chi<sup>2</sup> test, as appropriate. All  
173 statistical analyses were performed using SPSS version 12.0.

## 174 **Results**

### 175 *Patients*

176 A total of 28 consecutive patients with TM or SCA underwent HSCT in our institution from  
177 July 2004 to December 2014. Two patients were excluded: one patient for whom cyclosporine was  
178 stopped at day 4 post-transplant because of localized seizures, and another who was transplanted from  
179 a mismatched UD. Twenty-six patients were therefore included in the study. The sex ratio was 1, the  
180 median age at the time of transplantation was 6 years (range: 2-14 years). One patient had a second  
181 HSCT after failure of the first one that was performed in 2002 (outside the study period). Twenty-five  
182 patients had an HLA-identical MSD and 1 patient received a transplant from a phenotypic HLA-  
183 identical relative. The stem cell source was bone marrow in 21 patients (81%), cord blood in 2 patients  
184 (8%), and combined bone marrow and cord blood from the same sibling in 3 patients (11%). No  
185 peripheral blood stem cells were used (Table 1).

### 186 *HSCT outcomes*

187 At the time of writing, all patients are alive and free of TM or SCA (overall survival (OS)  
188 100% and disease free-survival (DFS) 100%). All transplants resulted in sustained engraftment of  
189 donor cells with no late graft rejection. Median follow-up was 107.5 months.

190 The median time to neutrophil recovery ( $> 500 / \text{mm}^3$ ) after HSCT was 22 days (range: 10-  
191 41), and the median time to platelet recovery ( $> 20 \text{ G/L}$ ) after HSCT was 23 days (range: 12-66). The  
192 median time to neutrophil and platelet recovery was, respectively, 21 and 22 days for transplants of  
193 cells originating from bone marrow, 28 and 48 days for cells originating from cord blood, and 26 and  
194 24 days for cells from both bone marrow and cord blood. The median duration of intravenous CsA  
195 administration before switch to the oral route was 22.5 days.

### 196 *Acute and chronic GvHD*

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

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

#### 206 *Other HSCT complications*

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

#### 216 *Relationships between aGvHD and CsA TBC*

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

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

#### 226 *Evolution of chimerism*

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

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

#### 241 *Relationship between busulfan AUC and HSCT outcomes*

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

#### 246 *Relationship between chimerism and HSCT characteristics*

247 No significant difference was found for chimerism according to indication after HSCT ( $p =$   
248 0.39). Among the 14 patients who experienced CMV reactivation, 4 had CC, 4 had stable PMC, 4 had

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

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

## 258 **Discussion**

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

263           Statistical analysis of potential differences in CsA TBC according to GvHD was not possible  
264 because of the very low number of GvHD events. It is noteworthy that patients only experienced grade  
265 1 aGvHD. However, a slight difference in mean CsA TBC was found, especially in the first week post  
266 HSCT. A CsA TBC greater than 150 ng/mL seems therefore to be a relevant target to prevent GvHD  
267 in these transplantations and should be obtained as soon as possible, as suggested previously in other  
268 contexts (17). Furthermore, although viral reactivation and/or infections affected most patients herein,  
269 there was no lethal infection and only one patient had severe EBV reactivation. It is also of note that  
270 the preparative regimen and GvHD prevention strategy used in our patients was very well tolerated,  
271 resulting in no cases of transplant-related mortality, compared to previously published reports that  
272 have variable TRM between 4-37% (3,8,10,12,14). In the international study of result of HLA-  
273 identical sibling HSCT, 7% died, with the most common cause of death being infection (7). The  
274 strategy of CsA monitoring in our patients may have contributed to the absence of TRM through a  
275 more controlled immunosuppression, and this effective prevention of GvHD strategy abrogated the  
276 need for methotrexate or mycophenolate mofetil, further decreasing toxicity. The use of a single  
277 immunosuppressant drug is likely to reduce the incidence of adverse reactions and so may be a safer  
278 post-transplant approach. However, immunosuppression-related toxicity was not completely avoided  
279 which could be in relation to the high dose of ATG used (20 mg/kg dose total) that seemed to be  
280 effective for engraftment and prevention of GvHD. It could therefore be interesting to monitor ATG  
281 in order to improve the results in terms of toxicity (26–29).

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

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

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

312 and SCA with MSD. Such a donor may not always be available; for example, in the United States  
313 fewer than 1 out of 5 children with SCA are expected to have a MSD (33), and the availability of  
314 MSD in TM is estimated to 20-30%. However, suitable UD can be identified using stringent criteria  
315 for immunogenic compatibility. In TM, it is reported that using high resolution molecular typing for  
316 both HLA class I and class II loci and stringent criteria of compatibility with the recipient, unrelated  
317 HLA identical donors may offer similar results to those obtained with HLA-identical family donors  
318 (34,35). Recently, an international survey studied 1110 patients with TM who received transplants and  
319 confirmed similar OS and EFS between HLA-matched related and MUD transplantation (89% vs 87%  
320 and 86% vs 82%, respectively) (36). In SCA, MUD also appeared to be the next best option after  
321 MSD for HSCT but high rates of aGvHD and cGvHD was previously reported. In these studies  
322 conditioning regimens varied from myeloablative to reduced-intensity and umbilical cord blood was  
323 used (37). A better control of GvHD and immunosuppression by a strict monitoring of CsA TBC as  
324 described herein is promising and could play a crucial role in HSCT. Further investigations are  
325 required but this study opens new perspectives to improve survival and safety of HSCT from  
326 alternative donors both in TM and SCA to levels compatible to that obtained with MSD. Moreover,  
327 the recent description of a Bayesian network to predict therapeutic range achievement of initial CsA  
328 blood concentration after pediatric HSCT offers interesting perspectives to continue to improve  
329 monitoring of this drug characterized by a narrow therapeutic index (38).

## 330 **Conclusion**

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



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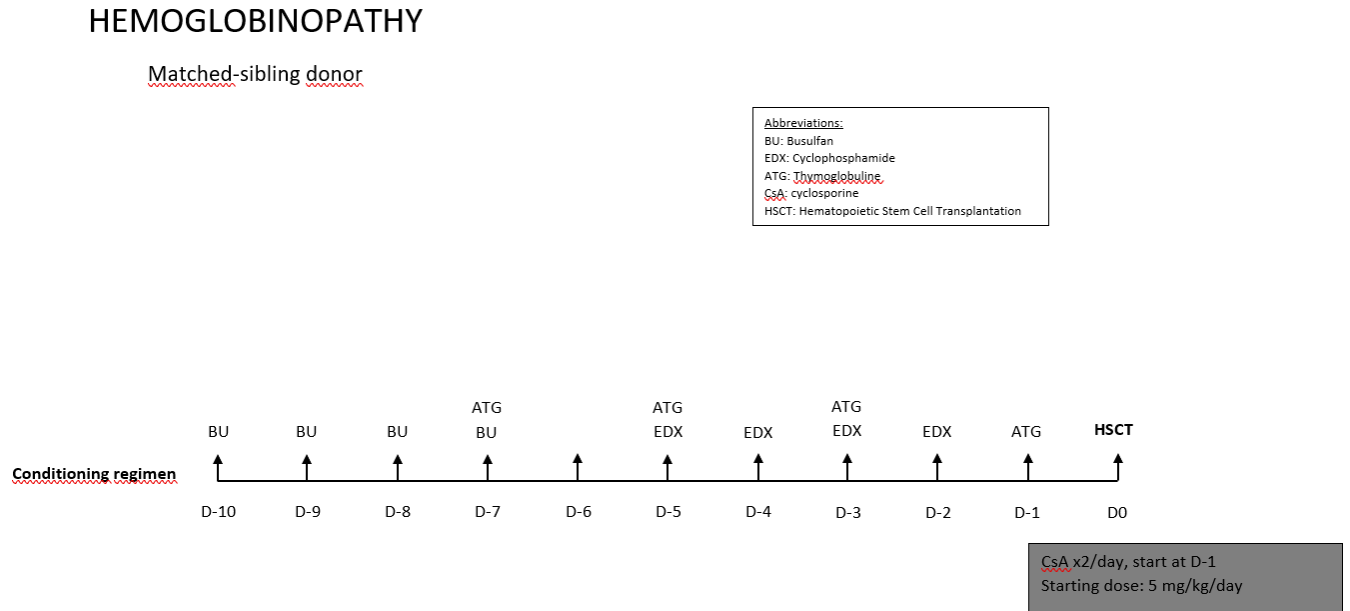
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# Figure

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 <sup>8</sup> /kg, median (range)			
Bone marrow	5.08 (1.38-8.84)		
Cord blood	0.59 (0.27-0.91)		
CD34+ x 10 <sup>6</sup> /kg, median (range)			
Bone marrow	6.6 (0.44-15.7)		
Cord blood	0.54 (0.15-0.93)		
T3 x 10 <sup>6</sup> /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 <sup>a</sup>		p
	CC n = 9	PMC n = 17	
Mean busulfan AUC per dose ( $\mu\text{M}\cdot\text{min}$ ) (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

<sup>a</sup> at last follow-up