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LETTER TO THE EDITOR

Haploidentical stem cell transplantation with $CD3^+$-/$CD19^+$-depleted peripheral stem cells for patients with advanced stage sickle cell disease and no alternative donor: results of a pilot study

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Sickle cell disease (SCD) is one of the most frequent inherited diseases and the most prevalent hemoglobinopathy worldwide.[1] The very divergent clinical manifestations seem to be based on interactions between sickle cells, endothelium and immune cells promoting the systemic vasculopathy with consecutive ubiquitous end organ damage.[2] Despite significant improvements in preventive and therapeutic modalities, SCD is a progressively debilitating and chronic multiorgan disease with a persistently high risk of morbidity and mortality.[1,3] Children with SCD can develop a progressive neurocognitive deficit without overt strokes. Their quality of life is significantly impaired with a high incidence of disability and unemployment.[4]

To date allogeneic hematopoietic stem cell transplantation (alloHSCT) is the only curative option and currently only offered to patients with serious SCD-related complications like strokes and acute chest syndromes and only if an HLA-matched related sibling donor (MSD) or matched unrelated donor (MUD)[5] is available. Myeloablative alloHSCT with bone marrow (BM) or cord blood from MSD and a busulfan (Bu)- based conditioning regimen combined with cyclophosphamide (Cy) or with thiotepa (TT) and fludarabine (Flu) are the standard of care.[5,6] One of the major improvements was the addition of anti-thymocyte globulin (ATG) to the conditioning regimen, which decreased the rejection rate from 22.6 to 3% and increased overall survival (OS) to 95%.[7]

Only a minority ($<14\%$) of SCD patients have a suitable HLA-identical sibling donor and the probability of finding a potential MUD is dependent on the ethnic and racial background.[8] Because of the significant progress in haploidentical transplantation we initiated a pilot study for advanced stage SCD patients with no potential MSD or MUD.

Over a period of 2 years we transplanted consecutively nine patients who failed hydroxyurea treatment (at least 1 year of treatment) using a treosulfan-based conditioning regimen and a $CD3^+$-/$CD19^+$-depleted stem cell graft from haploidentical first-degree relatives ($CD3^-$/$CD19^-$-haplo-SCT).

The patients' characteristics are summarized in Table 1. Disease status was confirmed molecularly in all patients and first-degree relative donors. While all patients were homozygous, all but one donor had a heterozygous SCD status. One was a healthy donor (patient 5). No suitable MSD or MUD could be identified and a written informed consent for the compassionate use therapy was obtained from all patients or parents/legal guardians. All nine patients received an allograft from a fully haplotype mismatched relative analyzed by high-resolution molecular typing. The patients received a $CD3^-$-/$CD19^-$-depleted haploidentical SCT and the total number of cell doses infused is summarized in Table 2.

Pre-transplant management included partial exchange transfusion to achieve hemoglobin S (HbS) levels below 30% immediately prior to conditioning and anticonvulsive prophylaxis with levetiracetam until discharge from the transplant unit. The myeloablative regimen for all patients consisted of ATG (Grafalon, Neovii, pharmaceutical AG, Rapperswil, Switzerland) 15 mg/kg/day (day −10 to −8), TT 5 mg/kg/day (day −8 and −7), Flu 40 mg/m^2/day (day −7 to −4) and treosulfan 14 g/m^2/day (day −5 to −3). Due to the high risk of graft rejection in these multiply transfused patients, immunosuppression consisted of cyclosporine and mycophenolate mofetil until day +120 post SCT. Chimerism analyses were performed molecularly from peripheral blood (PB) and BM[9] on a regular basis. In patients who presented with a mixed or falling chimerism below 90% or signs of GvHD, the immunosuppression was continued either until full chimerism was achieved or GvHD response to steroids was evident.

All nine patients included in this pilot study showed stable engraftment and at a median follow up of 26 months (range 6–42 months), eight of nine patients are alive. The average hospitalization was 45 days (median 38 days, range 30–97). Median leukocyte and granulocyte engraftment was achieved on days +15 and +18, respectively. Median platelet engraftment was achieved on day +11, summarized in Table 2. Limited analysis of immune reconstitution after $CD3^-$/$CD19^-$-haplo-SCT showed a delay in median recovery of T cells with $CD3^+ > 200$ at day +106 and >500 μL at day +128, respectively. Median B-cell engraftment ($CD19^+ > 100$ μL) was achieved on day +85 (Table 2). Especially, patient 8 showed at day +130 a significantly protracted regeneration of the T cells.

Chimerism analyses showed in all patients a complete chimerism (CC) off immunosuppression (defined as $>90\%$ donor cells) in the BM and in six patients in PB. Three patients developed a stable mixed chimerism (MC) in the PB with no SCD-related complications (Table 2). In those patients with a MC in the PB, line-specific chimerism analyses of red cell precursor (CD235a, glycophorine A) in the BM showed a complete engraftment. This is a frequent observation in patients with hemoglobinopathies after SCT, and unlike in hematological malignancies, it suffices to correct HbS-related disease.

The most frequently observed toxicities were limited pain crises in bone and muscles, grade 1–2 mucositis and diarrhea. No patient developed grade 3 or 4 toxicities. One patient developed neuromuscular spasms with consecutive hemiplegia starting with conditioning. Magnetic resonance imaging showed ischemic areas of both anterior cerebral arteries and no signs of posterior reversible encephalopathy syndrome in the magnetic resonance imaging. Symptoms resolved within days after increased anticonvulsive therapy. Patient 5 developed a transient impairment of the sensory and motoric cranial nerves V and VII, which resolved completely.

All patients received preemptive antiviral therapy, and three patients (33%) developed a CMV reactivation (>300 copies per μL) successfully treated with ganciclovir or foscarnet.

Table 1. Summary of patient characteristics who underwent T-haplo-SCT

Patients	*1 (17 y; f)*	*2 (13 y; f)*	*3 (4 y; m)*	*4 (31 y; f)*	*5 (27 y; f)*	*6 (16 y; m)*	*7 (3 y; m)*	*8 (22 y; f)*	*9 (10 y; m)*
Pain crises	13	6	6	Chronic pain (morphin dep.)	>25	>10	>5	>10	>10
Bone infarcts	Multiple	0	Multiple	Mult. degen. lesions	Multiple	0	Multiple	0	0
Osteomyelitis	0	0	0	0	0	0	0	0	0
Acute chest syndrome	2	2	1	0	3	1	2	3	4
Sepsis	0	1	0	0	0	1	0	1	1
TCD Flow	200 cm/s	>250 cm/s	240 cm/s	Normal	Normal	Normal +turb. flow	Normal + turb. flow	Normal + turb. flow	Normal
Abdominal organ status	Spleen infarct	Normal	Normal	Cholecystectomy	Normal	Splenectomy	Splenectomy Cholecystectomy	Cholecystectomy	Nephrocalcinosis Cholecystolithiasis
Cerebral infarcts bleedings	0	Small infarcts	0	Small infarcts	0	0	Small art. stenosis	Art. med. infarcts	Small infarcts

Abbreviations: SCD = sickle cell disease; TCD = transcranial Doppler analyses. For TCD the 'time-averaged mean of the maximum velocity (TAMMX)' is shown measured at the *Aa. carotis interna, cerebri anterior, media, posterior* and *basilaris*.

Table 2. Cell doses are given per kg of recipients body weight

Patients	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*Median*
Days post SCT	+1280	+1438	+941	+789	+782	+466	Ø (+423)	+339	+172	+786
Cell doses										
$CD34^+$ (10^6/kg BW)	12.7	10	20	10.9	10.9	17.9	77.6	11.9	12.2	12.2
$CD3^+$ (10^3/kg BW)	9.7	10.6	11.2	6.6	12.7	20	43.3	8.5	31.7	11.2
$CD19^+$ (10^3/kg BW)	45.2	10.6	23.3	1.5	131.3	19	56.7	8.8	114.5	23.3
Engraftment										
Leukocyte $>1\times10^9$/L	+16	+12	+18	+16	+16	+15	+14	+12	+14	+15
Granulocyte $>0.5\times10^9$/L	+18	+16	+19	+18	+22	+19	+18	+13	+14	+18
Thrombocyte $>20\times10^9$/L	+13	+11	+11	+16	+12	+11	+7	+9	+12	+11
T cell > 200/μL	+140	+103	+39	+90	+150	+30	+110	ny	+121	+106
T cell > 500/μL	+166	+144	+67	+112	+213	+34	Ø	ny	ny	+128
B cell > 100/μL	+87	+42	+67	+332	+105	+83	+39	+130	+272	+85
Chimerism										
PB	100%	100%	100%	88%[a]	74%	70%	100%	100%	99%	
BM	97%	100%	99%	99%[a]	98%	95%	98%	100%	97%	
HbS	40%	39%	38%	39%	0%	39%	12%	39%	38%	

Abbreviations: BM = bone marrow; BW = body weight; cGvHD = chronic GvHD; HbS = hemoglobin S; ny = not reached yet; PB = peripheral blood; SCD = sickle cell disease; SCT = stem cell transplantation. Engraftment, immune reconstitution, percentage of HbS and chimerism from SCD patients after T-cell-depleted haploidentical stem cell transplantation by last evaluation. At the time of death patient 7 was off immunosuppression and had a complete chimerism with 12% HbS in the PB. [a]Patient 4 at time point of chimerism evaluation still under immunotherapy with ruxolitinib, because of moderate cGvHD.

Patient 7 developed, despite immediate systemic antiviral therapy, a severe CMV pneumonitis (day +100). He was treated with CMV-specific T cells from the donor (father) after he developed first clinical signs of CMV disease but succumbed to a pulmonary insufficiency. In all other patients, reactivation of EBV, adenovirus, HHV6 or BK-polyomavirus were transient with low systemic viral load and without any signs of disease. Four patients presented with abnormal arterial flow velocities (transcranial Doppler analyses) before HSCT (Table 1), which decreased to levels below 150 cm/s 6–12 months post transplant.

At day +100 post transplant, the cumulative incidence of acute GvHD grade 1–2 was 56% (5 patients), which resolved within a 5 days of low-dose prednisolone (1 mg/kg/day). None of the patients developed acute GvHD grade 3–4. One patient (patient 4) developed a steroid-sensitive moderate chronic GvHD on day +140 post transplant with mild fasciitis, mild oral and ocular as well as mild cutaneous chronic GvHD.

Today, HSCT remains the only curative therapy for patients with SCD. So far, it is only offered to patients with a matched donor (MD). In a series of 87 SCD patients who were transplanted from MSD after a myeloablative conditioning regimen with Bu and Cy, the 6-year OS was 93.1% and the event free survival (EFS) was 86.1%, respectively.[5]

With the continuous advancement in haploidentical transplantation techniques, two competing haploidentical concepts are currently available: T-cell depletion (depletion of $CD3^+/CD19^+$ or α/β T cells/$CD19^+$) and post-transplantation Cy (post-Cy-haplo-SCT). Post-Cy-haplo-SCT is intriguing due to the simplicity and therefore almost ubiquitous availability but is still associated with a high rate of acute and chronic GvHD of up to 58% and 18%, respectively.[10] In a study of 14 young adult SCD patients who received a post-Cy-haplo-SCT Bolanos-Meade *et al.*[11] reported no transplant-related mortality (TRM) or GvHD, but a graft failure rate of 43%. *In vitro* depletion of T cells ($CD3^+$ or α/β T cells) and B cells ($CD19^+$) from PBSC grafts using the CliniMACS system is a well-established modality with an extremely low incidence of acute GvHD of only 13% and chronic GvHD of almost 0%, respectively.[12] So far, only limited reports exist for T- and B-cell-depleted haploidentical SCT for patients with SCD.

At this time, T-cell-depleted haploidentical SCT in comparison to post-Cy-haplo-SCT appears to have a similar OS, disease free survival (DFS) and TRM.[10–12] While the incidence of acute or chronic GvHD might be even lower with the use of T-cell-depleted haplo-SCT compared to post-Cy-haplo-SCT, the delay of early immune reconstitution and extended immunodeficiency after T-cell-depleted haplo-SCT can possibly lead to a higher incidence of viral infections, which remains the major causes of morbidity and mortality.[13] Bolanos-Meade *et al.* reported in their series with post-Cy-haplo-SCT three patients with viral reactivation but no viral disease. Similar results in incidence of viral infections are reported from studies in pediatric patients with malignant or non-malignant diseases who underwent a haplo-SCT with T-cell depletion.[14,15]

It remains to be proven in a prospective study, if the limited results achieved in this pilot study using a $CD3^-/CD19^-$-haplo-SCT regimen could be a valid curative option for SCD patients with no available donor.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

JF, BP and SC reviewed the literature, analyzed the data and wrote the manuscript. JF, BP, KR, DW, EH and SC were involved in the clinical management of the patients. All authors contributed to the intellectual content of this paper and approved the final manuscript.

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