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ARTICLE

Haploidentical CD3 or α/β T-cell depleted HSCT in advanced stage sickle cell disease

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Abstract

Despite significant improvements in the supportive care, sickle cell disease (SCD) leads to significant morbidity and mortality. Allogeneic hematopoietic stem cell transplantation (HSCT), the only curative option, is limited due to matched donor availability. This could be met with T-cell-depleted haploidentical HSCT. Twenty advanced-stage SCD patients, median age 15 years, and 9 patients, median age 14 years, were transplanted with CD3/CD19- or TCRαβ/CD19-depleted grafts and from matched sibling donors (MSDs). The conditioning consisted of ATG, thiotepa, fludarabine, and treosulfan. The median follow-up in the T-haplo-HSCT and the MSD patients was 21 (9–62) and 25 (7–60) months, respectively. The OS in the T-haplo-HSCT and MSD was 90% and 100%, respectively. In the T-haplo-HSCT group, two patient succumbed to a CMV pneumonitis and a macrophage activation syndrome (MAS). One patient in the T-haplo-HSCT group requires renal replacement therapy because of BK virus nephritis. None developed grade III–IV acute GvHD. In the T-haplo-HSCT and in the MSD, 20% and 22%, respectively, developed a mild or moderate chronic GvHD. These results demonstrate the feasibility, safety, and efficacy of T-haplo-HSCT also for adult advanced stage SCD patients.

Introduction

Sickle cell disease (SCD) is the most frequent inherited hemoglobinopathy worldwide [1]. SCD causes progressively debilitating and life-threatening complications. Despite significant improvements in preventive and therapeutic modalities, the morbidity and mortality of this chronic multi-organ disease has not improved significantly in the last decades [1, 2] with a significantly reduced life expectancy and a high disability and unemployment rate.

To date, allogeneic hematopoietic stem cell transplantation (HSCT) from a human leukocyte antigen (HLA)-identical sibling donor (matched sibling donor, MSD) is the only curative option offered currently only to patients with severe SCD. The reported overall survival (OS) and event-free survival (EFS) exceeds 90% and 80%, respectively, and EFS greatly improved to 95% for patients transplanted after 2006 [3, 4].

However, this standard approach is limited by a MSD or a matched unrelated donor (MUD) availability of <20% [5–7]. Although MSD and MUD donors are generally considered equivalent in OS and disesase free survival (DFS) in leukemia patients, the current results in SCD are

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not quite as satisfactory, in particular with regard to acute and chronic GvHD (aGvHD and cGvHD) [8].

Partially HLA-mismatched first-degree related donors increase the donor pool to an average of 2.7 donors per patient [9]. In addition to immediate donor availability, the incidence of aGvHD and cGvHD compares favorably with MSD or MUD donors [8, 10–13].

In haploidentical HSCT (haplo-HSCT), two different T-cell depletion procedures are available: in vitro T-cell depletion of peripheral blood stem cells (CD3/CD19 or TCRαβ/CD19; T-haplo-HSCT) and posttransplantation cyclophosphamide for in vivo T-cell depletion (post-CY-haplo-HSCT). Post-CY-haplo-SCT seems very intriguing due to its simplicity, ubiquitous availability, and fast immune recovery but has a high risk of aGvHD and cGvHD, and rejection observed until now [12, 14].

Here we report our results of 20 patients with advanced-stage SCD transplanted with CD3/CD19- and TCRαβ/CD19-depleted haploidentical grafts in comparison with 9 patients transplanted from a MSD using an almost identical regimen.

Material and methods

Patients

We treated 20 patients with high-risk SCD to determine the feasibility, safety, and EFS of a T-cell-depleted haploidenticalHSCT. For proof-of-concept, the program started in Regensburg with a CD3/CD19-depleted graft completing a series of 15 patients and was consecutively implemented in Berlin using a TCRαβ/CD19-depleted graft in 5 patients. The patient and donor characteristics and the inclusion criteria for HSCT are summarized in Tables 1 and 2. Pre-transplant management included hydroxyurea (over at least 1 year) in all patients and transfusion programs in patients with CNS risk factors.

High-resolution molecular typing was performed in donors and recipients to characterize HLA class I (A, B, C) and II (DRB1, DQB1) loci. A direct cross-match assay was performed with all eligible donors to detect donor-specific anti-HLA antibodies. In all T-haplo-HSCT patients, no HLA-identical family or HLA-matched MUD donor was available. Written informed consent was obtained from all patients or parents/legal guardians. Fertility preservation was offered to all patients before HSCT. Patients with an indication for HSCT and MSD were treated according to our institutional guidelines.

Donors and grafts

T-haplo-grafts were obtained from a relative (age: median 41 years) who shared at least one HLA haplotype (Tables 1 and 2). Patients with a MSD (age: median 13 years) received a bone marrow (BM) allograft. In T-haplo-HSCT, $CD3^+$ and TCRαβ-positive T cells, respectively, as well as $CD19^+$ B cells were depleted with the CliniMACS device as described previously [15]. Targeted cell counts post manipulation were $>1 \times 10^7$/kg body weight (BW) $CD34^+$ cells, $<1 \times 10^5$/kg BW $CD3^+$ (or $TCR\alpha\beta^+$) cells, and $<5 \times 10^5$/kg BW $CD19^+$ cells (Table 3). In five MSD patients with a major blood group incompatibility, erythrocyte depletion of the graft was performed.

Conditioning regimen and immune therapy

Prior to conditioning, a partial exchange transfusion was used to reduce peripheral hemoglobin S levels to below 30%. The conditioning regimen for T-haplo-HSCT patients was almost identical to our institutional standard containing ATG-Neovii® 15 mg/kg but given on days −10 to −8 instead days −3 to −1 for MSD patients, thiotepa 5 mg/kg on days −8 and −7, fludarabine 40 mg/m^2 on days −7 to −4, and treosulfan 14 g/m^2 on days −5 to −3. In 17 patients (5 MSD and 12 T-haplo-HSCT), GvHD prophylaxis consisted of cyclosporine (CsA) (target level: 120 to 150 ng/ml) and mycophenolate mofetil (MMF) 600 mg/m^2 starting day −3 and day −1, respectively (Fig. 1). Because of neurological complications such as posterior reversible encephalopathy syndrome (PRES) in five patients, CsA was subsequently substituted by FK506 (0.01 mg/kg/day over 20 h infusion; target level: 7 ng/ml). Duration of immunosuppression was scheduled >6 months for T-haplo-SCT and ≤6 months for MSD, depending on chimerism. In case of a mixed or falling chimerism ≤90%, split-chimerism analyses were performed and immunosuppression was continued until donor CD3 chimerisms reached 50%.

Supportive care

Supportive care was performed according to institutional guidelines in addition to levetiracetam for anticonvulsive prophylaxis. In case of differences in the pre-transplantation cytomegalovirus (CMV) immunity between donor and recipient, ganciclovir three times per week was used as antiviral prophylaxis post transplant. All patients were monitored weekly for Epstein–Barr virus (EBV), CMV, human herpes virus 6 (HHV6), adenovirus (ADV) and BK-polyomavirus (BKV) until day +100 post transplantation.

Chimerism analysis

Peripheral blood chimerism was analyzed weekly up to day +100 and monthly thereafter. In patients with a mixed chimerism (chimerism <90%; MC) split-chimerism analyses were performed via PCR in mononuclear cell fractions, sorted

Table 1 Characteristics of patients and donors undergoing T-depleted haplo-HSCT

Pat.	Age (years)	Sex	Disease	Indication for transplantation	Donor age (years)	Donor sex	Donor relation	HLA match	T-cell depletion	Recipient ABO	Donor ABO
1	13	F	HbSS	VOC, ACS, CD, Acc-TCD*, Allo-Im, H, SI, SN	43	M	Father	5/10	CD3/CD19	AB+	O+
2	18	F	HbSS	VOC, ACS, CD, ON, SI, Acc-TCD*	45	M	Father	5/10	CD3/CD19	O+	A+
3	4	M	HbSS	VOC, CD, ON, Acc-TCD*	30	F	Mother	5/10	CD3/CD19	B+	B+
4	31	F	HbS/β-Thal	VOC, CP, SI, SN, Allo-Im	33	M	Cousin	7/10	CD3/CD19	O+	A+
5	27	F	HbS/β-Thal	VOC, CP, ON, ACS	26	F	Sister	7/10	CD3/CD19	A+	A+
6	16	M	HbSS	VOC, CD, ACS	46	M	Father	5/10	CD3/CD19	O+	O+
7	3	M	HbSS	VOC, ACS, ON, SI	35	M	Father	8/10	CD3/CD19	A+	O+
8	21	F	HbSS	VOC, ACS, CD, PH, Allo-I	48	M	Father	6/10	CD3/CD19	O+	O+
9	10	M	HbSS	VOC, ACS, CD	56	M	Father	5/10	CD3/CD19	O+	O+
10	28	M	HbSS	VOC, CD, Pp, SI, SN, PH	16	F	Sister	6/10	CD3/CD19	A+	A+
11	21	M	HbS/β-Thal	VOC, ACS, ON, H	47	M	Father	6/10	CD3/CD19	A+	A+
12	21	M	HbSS	VOC, ON, SI	15	F	Sister	6/10	CD3/CD19	B+	O+
13	5	M	HbSS	VOC, ACS, CD, ON	40	F	Mother	7/10	CD3/CD19	O+	O-
14	21	M	HbSS	VOC, ACS, CD, Acc-TCD*	17	F	Sister	5/10	CD3/CD19	O+	O+
15	21	M	HbSS	VOC, ACS, ON	26	M	Brother	5/10	CD3/CD19	B+	O+
16	9	M	HbSS	VOC, ON, OM, H	31	M	Father	5/10	TCR αβ $^{+}$/CD19	A+	A+
17	12	F	HbSS	VOC, ACS	54	M	Father	5/10	TCR αβ $^{+}$/CD19	A+	O+
18	11	F	HbSS	VOC, ACS	44	M	Father	5/10	TCR αβ $^{+}$/CD19	O-	O+
19	11	F	HbSS	ACS, CD	42	M	Father	5/10	TCR αβ $^{+}$/CD19	O+	B+
20	13	M	HbSS	VOC, CD	49	F	Mother	5/10	TCR αβ $^{+}$/CD19	O+	B+

ACS acute chest syndrome, *Acc. TCD* accelerated transcranial doppler*, *Allo-I* allo-immunization, *CD* cerebrovascular disease (include infarcts and/or strokes), *CP* chronic pain, *F* female, *H* hepatopathy (included: hepatomegaly and/or siderosis and/or infarcts), *HbSS* homozygous sickle cell disease, *HbS/β-Thal* compound hetereozygous HbS and β° thalassemia disease, *HLA* human leukocyte antigens, *M* male, *OM* osteomyelitis, *ON* osteonecrosis, *PH* pulmonal hypertension, *Pp* priapism, *SI* splenic infarct (including splenectomy), *SN* sickle cell nephropathy, *VOC* vaso-occlusive painful crises

*In case of transcranial doppler analyses (TCD), the time-averaged mean of the maximum velocity (TAMMX) was measured at the Aa. carotis interna, cerebri anterior, media, posterior, and basilaris

Table 2 Characteristics of patients and donors undergoing matched sibling-SCT (MSD)

Pat.	Age (years)	Sex	Disease	Indication for transplantation	Donor age (years)	Donor sex	Donor relation	Recipient ABO	Donor ABO
1	22	F	HbSS	VOC, ACS, CD, H	18	M	Brother	O+	A+
2	9	F	HbS/β-Thal	VOC, ACS, CD, ON, H, Allo-I	4	F	Sister	O+	A+
3	10	F	HbSS	VOC, ACS, Acc. TCD, H, Allo-I	14	M	Brother	A+	A+
4	23	F	HbSS	VOC, ACS, CD, ON	15	F	Sister	A+	B+
5	25	M	HbS/β-Thal	VOC, ACS, CD, ON, SN	20	M	Brother	O+	A+
6	23	M	HbSS	VOC, ACS, ON	13	F	Sister	O+	O+
7	14	M	HbS/β-Thal	VOC, ACS	8	F	Sister	A+	A+
8	11	M	HbSS	VOC, PH	5	F	Sister	A+	A+
9	11	M	HbS/β-Thal	VOC, ACS	3	F	Sister	B+	A+

ACS acute chest syndrome, *Acc. TCD* accelerated transcranial doppler*, *Allo-I* allo-immunization, *CD* cerebrovascular disease (include infarcts and/or strokes), *CP* chronic pain, *F* female, *H* hepatopathy (include hepatomegaly and/or siderosis and/or infarcts), *HLA* human leukocyte antigens, *HbSS* homozygous sickle cell disease, *HbS/β-Thal* compound heterozygous HbS and β° thalassemia disease, *M* male, *OM* osteomyelitis, *ON* osteonecrosis, *PH* pulmonal hypertension, *Pp* priapism, *SI* splenic infarct (including splenectomy), *SN* sickle cell nephropathy, *VOC* vaso-occlusive painful crises

*In case of transcranial Doppler analyses (TCD), the time-averaged mean of the maximum velocity (TAMMX) was measured at the Aa. carotis interna, cerebri anterior, media, posterior, and basilaris

Table 3 Graft contents and engraftment characteristics

Graft content (median)	T-haplo-HSCT	CD3/CD19	TCR αβ/CD19	MSD
$CD34^+$ cells/kg BW	$13\ (8–78) \times 10^6$	$12\ (8–78) \times 10^6$	$19\ (9–38) \times 10^6$	$3\ (2–7) \times 10^6$
BM MNC/kg BW				$2.4\ (1.1–5.1) \times 10^8$
$CD3^+$ T cells/kg BW*	$14\ (4–706) \times 10^3$	$20\ (7–706) \times 10^3$	$13\ (4–17) \times 10^3$	
$CD19^+$ B cells/kg BW	$47\ (1–305) \times 10^3$	$45\ (1–305) \times 10^3$	$88\ (1–179) \times 10^3$	
Time to engraftment (median)				
Leukocyte $> 1 \times 10^9\,L^{-1}$	16 (10–29)	16 (10–29)	18 (16–20)	26 (16–41)
Granulocyte $> 0.5 \times 10^9\,L^{-1}$	19 (11–30)	19 (11–30)	18 (16–20)	27 (17–41)
Thrombocyte $> 20 \times 10^9\,L^{-1}$	10 (5–16)	10 (5–16)	9 (6–11)	21 (14–41)

Absolute cell doses per kilogram (kg) body weight (BW) and days of engraftment values are shown as median (minimum–maximum values). T-cell depletion indicates patients who underwent T-haplo-HSCT (both T-cell depletion procedures); CD3/CD19, patients only with CD3/CD19 T-cell depletion; TCR αβ/CD19, patients only with TCR αβ/CD19 T-cell depletion; MSD, patients with a HLA identical donor (matched sibling donor)

*Still remaining amount of TCR αβ / $CD3^+$ T cells in the graft

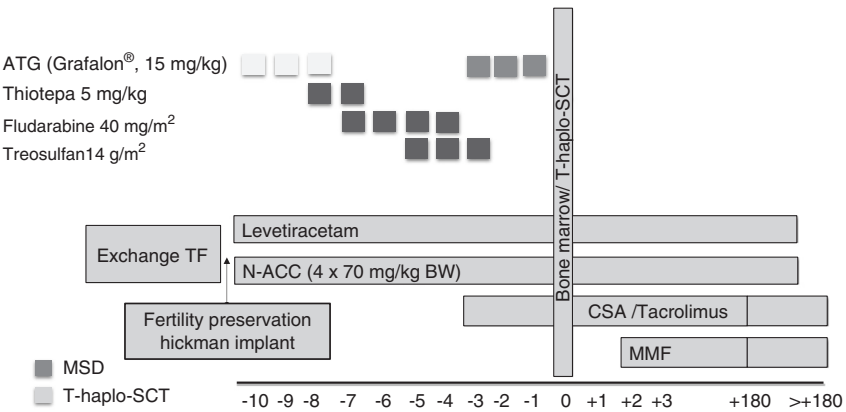

Fig. 1 Conditioning regimen for T-haplo-HSCT and MSD

Table 4 Immune reconstitution of patients after SCT for SCD

T- and Bcell recovery	T-haplo-HSCT	CD3/CD19	TCR αβ/CD19	MSD
$CD3^+$ T cells $> 0.2 \times 10^9\,L^{-1}$	128 (29–400)	130 (34–400)	97 (29–145)	65 (24–178)
$CD3^+$ T cells $> 0.5 \times 10^9\,L^{-1}$	173 (34–500)	183 (34–500)	145 (59–182)	82 (30–178)
$CD4^+$ T cells $> 0.2 \times 10^9\,L^{-1}$	181 (120–703)	181 (180–703)	183 (183–184)	180 (62–274)
$CD4^+$ T cells $> 0.5 \times 10^9\,L^{-1}$	277 (120–703)	277 (180–703)	253*	198 (120–356)
$CD8^+$ T cells $> 0.2 \times 10^9\,L^{-1}$	120 (33–213)	120 (33–213)	66 (60–72)	75 (40–147)
$CD8^+$ T cells $> 0.5 \times 10^9\,L^{-1}$	213 (33–292)	213 (33–292)	60[a]	81 (54–198)
$CD19^+$ B cells $> 0.1 \times 10^9\,L^{-1}$	80 (34–359)	86 (39–359)	46 (34–182)	83 (40–353)

Median (minimum–maximum) values are shown as days after SCT; T-cell depletion indicates patients who underwent T-haplo-HSCT (both T-cell depletion procedures); CD3/CD19, patients only with CD3/CD19 T-cell depletion; TCR αβ/CD19, patients only with TCR αβ/CD19 T-cell depletion; MSD, patients with a HLA identical donor (matched sibling donor)

[a]Only one patient in evaluation

from BM and peripheral blood (PB), respectively, on a BD FACSAria™ cell sorter (Becton Dickinson) [16]. For this, mononuclear cells (MNCs) were stained with anti-CD3-FITC, anti-CD14-PE (both BD Biosciences), anti-CD19-APC (eBioscience), and anti-CD45-PerCP (Miltenyi Biotec), and sorted into $CD45^+CD3^+$ (T cells), $CD45^+CD14^+$ (monocytes), and $CD45^+CD19^+$ (B cells). For isolation of erythroid precursors, BM cells were stained with anti-CD45-FITC and anti-CD235a-APC (both Miltenyi Biotec), and $CD45^-CD235a^+$ cells were sorted.

Results

Engraftment, graft failure/rejection and immunological reconstitution

All patients experience a primary engraftment with stable grafts. One patient developed a macrophage activation syndrome (MAS) with a late graft failure. The median time for leukocyte counts to reach $1 \times 10^9\,L^{-1}$ in T-haplo-HSCT and MSD was 16 and 26 days post-HSCT, respectively. Median recovery time for neutrophils ($>0.5 \times 10^9\,L^{-1}$) in T-haplo-HSCT and MSD was 19 and 27 days, and for thrombocytes ($>20 \times 10^9\,L^{-1}$) 10 and 21 days post-HSCT, respectively (Table 3).

T- and B-cell recovery is summarized in Table 4. The median time to reach $>0.5 \times 10^9\,L^{-1}$ $CD3^+$ T cells in the T-haplo-HSCT group compared with the MSD group was 173 and 82 days, respectively. $CD4^+$ T cells ($>0.5 \times 10^9\,L^{-1}$) in T-haplo-HSCT and MSD recovered by days 277 and 198, respectively. $CD8^+$ T cell ($>0.5 \times 10^9\,L^{-1}$) recovered by days 213 and 81 for T-haplo-HSCT and MSD, respectively. B-cell recovery in T-haplo-HSCT and MSD was on days 80 and 83 days, respectively (Table 4). These data indicate rapid recovery of neutrophils and thrombocytes following haplo-HSCT, whereas T-cell recovery is delayed due to stringent T-cell depletion.

Chimerism analysis

The median chimerism was 100% in both T-haplo-HSCT (59–100%) and MSD (84–100%).

We identified one patient with stable MC in MSD and four patients in T-haplo-HSCT, of whom one remains under immunosuppression with FK506. Interestingly, split-chimerism analyses in all patients with MC detected an almost complete chimerism in the glycophorin A-positive subset in the BM and the HbS fraction in the Hb electrophoresis was below 40% (after transplantation from heterozygous HbS donors; data not shown). In all remaining patients, the donor chimerism remained above 90% without immunosuppression.

Survival, regimen-related Toxicities, and infectious complications

In T-haplo-HSCT patients with a median follow-up of 21 months (range 9–62 months), the OS, EFS, and DFS were 90%. In MSD patients with a median follow-up of 25 months (range 7–60 months), the OS, EFS and DFS were 100% (Table 5). All surviving patients are transfusion independent and free of SCD-related complications. In the T-haplo-HSCT group, we observed a transplant-related mortality of 10% ($n = 2$ patients). No World Health Organization grade IV bacterial or fungal infection was observed. The most frequent early complications were headache, grade I–II mucositis, fewer of unknown origin, diarrhea, and episodes of pain in bone and muscles, mostly immediately before or during the early engraftment period (Table 5). Two patients developed transient grade III or IV toxicity: one patient in the T-haplo-HSCT group developed neuromuscular spasms with consecutive hemiplegia starting with conditioning. Magnetic resonance imaging showed ischemic areas distal of both anterior cerebral arteries. Symptoms resolved after intensified anticonvulsive and systemic anti-coagulation therapy. The second patient in the

Table 5 Outcome and SCT-related complications of patients after SCT for SCD

	T-haplo-HSCT	CD3/CD19	TCR αβ/CD19	MSD
Patients (female + male)	20 (8 F + 12 M)	15 (5 F + 10 M)	5 (3 F + 2 M)	9 (4 F + 5 M)
Median age (years)	15 (3–31)	21 (3–31)	11 (9–13)	14 (9–25)
Median follow-up (months)	21 (9–62)	26 (9–62)	18 (13–22)	25 (7–60)
Hospitalization (days)	40 (31–75)	39 (31–75)	44 (38–49)	44 (38–76)
OS/EFS	18 (90%)	13 (87%)	5 (100%)	9 (100%)
DFS	18 (90%)	13 (87%)	5 (100%)	9 (100%)
TRM	2 (10%)	2 (13%)	0 (0%)	0 (0%)
Early and late complications (number of patients)				
Headache	7	5	2	1
Bone pain	14	10	4	4
Neurological complications	7	5	0	2
PRES	3	1	1	1
Seizure	1	1	0	1
VOD	1	0	1	0
TAM/MAS	1	1	0	0
Viral reactivation[a]	13	9	0	6
Autoimmune disorders[b]	3	3	0	0

Median (minimum–maximum) number of patients are shown after SCT; T-cell depletion indicates patients who underwent T-haplo-HSCT (both T-cell depletion procedures); CD3/CD19, patients only with CD3/CD19 T-cell depletion; TCR αβ/CD19, patients only with TCR αβ/CD19 T-cell depletion; MSD, patients with a HLA identical donor (matched sibling donor). *BKV* polyomavirus, *CMV* cytomegalovirus, *DSF* disease-free survival, *EBV* Epstein–Barr virus, *EFS* event-free survival, *HSV* Herpes virus; *MAS* macrophage activation syndrome, *OS* overall survival, *PRES* posterior reversible encephalopathy/neurological complications (means peripheral neuropathy, neuralgia), *RV* rotavirus, *TAM* transplant-associated macroangiopathy,*TRM* transplant-related mortality, *VOD* veno-occlusive disease

[a]Patients with reactivation of CMV, HSV, EBV, RV, or BKV

[b]Patients with mild steroid-sensitive autoimmune disorders (hemolytic anemia ($n = 2$), immune neutropenia ($n = 1$), and immune thrombocytopenia ($n = 1$) as signs of cGvHD

MSD group developed a capillary leak syndrome following ATG, an opioid withdrawal syndrome, severe neuralgia, and disorientation combined with PRES under immunosuppression with CsA. After a switch of the immunosuppression to everolimus, all symptoms resolved completely. Relevant neurotoxicity (NT), in particular PRES, was exclusively observed with CsA (five patients) and occurred in the time before hematological recovery, which was not associated to steroid application or GvHD. NT resolved in all patients with switching immunosuppression to FK506 ($n = 3$) or everolimus ($n = 2$).

Seven patients (35%) in the T-haplo-HSCT group and three (33%) in the MSD group developed a CMV reactivation (Table 5). One patient in each group had a transient reactivation of BK virus (BKV) in the blood. One patient in the T-haplo-HSCT group had a prolonged BKV infection; despite intensive antiviral treatment, the patient developed a BKV-associated nephritis and requires dialysis treatment because of renal insufficiency.

One patient in the T-haplo-HSCT group developed a rotavirus diarrhea with high virus copy numbers in the stool ($>10^7$ ml^{-1}). The same patient developed subsequent severe CMV pneumonitis despite treatment with systemic antiviral therapy and infusion of CMV-specific T cells from the donor. He succumbed due to uncontrolled CMV pneumonitis. Transient reactivations of EBV, HHV6, or ADV with low viral load were observed without clinical relevance (Table 5).

One patient in the T-haplo-HSCT group, who suffered from a late graft failure (>day +180), developed a MAS and succumbed to a septicemia.

Graft vs. host disease

At 180 days post transplant, the cumulative incidence of aGvHD, grade I and II, was 35% in the T-haplo-HSCT group and 33% in the MSD group (Table 6). In all cases, aGvHD resolved within a few days of low-dose prednisolone (0.5–1 mg/kg BW). None of the patients developed aGvHD grade III–IV (Table 6). Four 4 patients (20%) in the T-haplo-HSCT group and two patients (22%) in the MSD group developed a steroid-sensitive, mild-to-moderate cGvHD with cutaneous, oral, ocular, and fascial involvement (Table 6) responding to primary treatment. None of the patients required salvage treatment for cGVHD.

Table 6 Incidence of GvHD of patients after SCT for SCD

		T-haplo-HSCT	MSD
Acute GvHD	Grade I–II	7/20 (35%)	3/9 (33%)
	Grade III–IV	none	none
Chronic GvHD	Mild/ moderate	4/20 (20%)	2/9 (22%)
	Severe	None	None

T-haplo-HSCT indicates patients who underwent haplo-HSCT with both T-cell depletion procedures; MSD, patients with a HLA identical donor (matched sibling donor)

Discussion

We analyzed the feasibility and efficacy of a T-cell-depleted haplo-HSCT in comparison with a MSD HSCT in patients with advanced-stage SCD using an almost identical transplant regimen. Due to donor availability, the groups are imbalanced but reflect the reality of HSCT in SCD. For one of the most relevant outcome parameters, a/c GvHD, the results are comparable between both groups. In particular, in the group of adult patients (>18 years), the outcome of T-Haplo-SCT was excellent with a OS, EFS, and DFS of 100% and compared favorably with MSD HSCT with a reported 5-year OS of 80% and an increasing incidence of cGvHD [4].

Although in United States and Europe 95% of SCD patients survive into adulthood due to improvement of conventional care, adults above the age of 20 years show a 25–30 years decrease in life expectancy compared with the general population with irremediable cardio-pulmonary and renal complications dominating mortality during adulthood [17–20]. In summary, SCD in developed countries is a chronic systemic vasculopathy, not remediable with conventional measures. However, due to a MD availability of <20%, the gold standard curative approach is limited to a minority of patients and even lower in mixed ethnicities [6, 21]. A retrospective analysis on behalf of the EBMT reported on 1000 MSD HSCT in SCD patients, most transplanted with a myeloablative conditioning regimen (87%) using BM as stem cell source (84%), the OS and EFS was 92.4% and 91.4%, respectively, with an increasing EFS over 95% for patients transplanted after 2006. The cumulative incidence of aGvHD grade II–IV and cGvHD was 14.8% and 14.3%, respectively. Twenty-three patients experienced GF and 70 patients died mostly due to GvHD or infection [4]. We observed in our series also an excellent outcome in our MSD group with an OS and EFS of 100%.

MUD HSCT is considered equivalent to MSD in childhood ALL [22]. Shenoy et al. [8] reported a poor outcome on 29 children aged 4 to 19 years, transplanted with a MUD. The 2-year OS and EFS was 79% and 69%, respectively. The incidence of aGvHD grade II–IV was 28% and cGvHD was 62%, with 38% classified as extensive, most likely caused by insufficient immunosuppressive therapy [8]. More recently, Cappelli et al. [23] reported an improved outcome from MUD-transplanted SCD patients. In 64 patients transplanted from related haploidentical ($n = 40$) or unrelated ($n = 24$) donors in the EBMT/Eurocord database, the OS was 89% in the haplo patients and 94% in the adult MUD patients. The rate of grade II–IV aGvHD or extensive cGvHD were in haplo-SCT with posttransplant cyclophosphamide vs. MUD 25 vs. 21%, and 28 vs. 18%, respectively [23].

So far haplo-HSCT in SCD patients is still considered experimental and should only be offered to patients with severe SCD-related complications. Post-CY-haplo-HSCT is almost ubiquitously available and intriguing due to the simplicity, but the high rate of aGvHD and cGvHD of up to 58% and 18% in malignant disease [24] is unacceptable in patients with a non-malignant disease where grade II–IV aGvHD and cGvHD reach 29% and 25%, respectively [25]. Bolanos et al. [14] reported from an OS from 100% and 0% GvHD, but a graft failure rate of 43% in 14 young adult SCD patients who received a post-cy-haplo-HSCT with a conditioning regimen containing ATG, cyclophosphamide, fludarabine, 2 Gy total body irradiation followed by MMF and tacrolimus or sirolimus for GvHD prevention. A transatlantic consortium therefore added a pre-conditioning with hydroxycarbamide and azathioprine over 2 months, as well as thiotepa to the original post-CY haplo-HSCT regimen. In 87 patients with hemoglobinopathies treated according to this protocol, 11 patients (12.6%, 6 SCD and 5 thalassemia patients) suffered from MAS resulting in death in 7 patients [26]. Due to these unexpected results, the strategy without pre-conditioning but with thiotepa is further pursued in the BMT CTN1507 trial with DFS and OS of 93% and 100% in 15 children, respectively. The median follow-up was 13.3 months and 2 patients had a aGvHD grade III–IV and one patient with a mild cGvHD. Viral reactivations were reported in nine patients (88%) with only one patient with virus-related disease (HHV6-related encephalopathy) [12]. Bolanos et al. [14] reported in 4 patients (28%) virus reactivations with CMV, EBV, and RSV with no virus-related disease.

HSCT with an in vitro T-cell- ($CD3^+$ or $TCR\alpha\beta^+$ cells) and B-cell (CD19)-depleted peripheral blood stem cells graft is a well-established haploidentical transplant modality with a low incidence of aGvHD (13%) and cGvHD (almost 0%) in patients with malignant diseases [11]. So far, experience is limited in T- and B-cell-depleted haplo-HSCT for patients with severe SCD. In 8 pediatric patients with SCD T-haplo-HSCT using a reduced intensity conditioning regimen yielded an OS of 75% with a DFS of 38% [27].

Although prospective comparative studies are missing, the incidence of aGvHD or cGvHD might be lower with the use of T-haplo-HSCT compared with post-CY-haplo-

HSCT. However, the delay of immune reconstitution after T-haplo-HSCT can lead to a higher incidence of viral reactivations with infectious complications being the major causes of morbidity and mortality after T-haplo-HSCT. Thirteen patients (65%) in the T-haplo-HSCT group and six patients (67%) in the MSD group presented viral reactivations (EBV, CMV, and BKV). In the MSD group, no related disease was observed. In the T-haplo-HSCT group, one patient developed a severe and fatal CMV pneumonitis and a prolonged rotavirus infection. One patient developed a renal insufficiency after a prolonged BKV infection. Similar results regarding incidence of viral reactivation or viral disease are reported in pediatric patients with malignant or non-malignant diseases who underwent either a T-haplo-HSCT with CD3/CD19- or TCRαβ/CD19-depleted grafts [28]. Retrospective analyses comparing median recovery times after CD3/CD19 or TCRαβ/CD19 depletion showed more rapid immune recovery after TCRα/β depletion. A reduced duration of immune deficiency after T-haplo-HSCT with TCRαβ/CD19-depleted grafts due to remaining γ/δ T cells may result in less infection episodes and improved clinical outcomes [28]. Gaziev et al. [29] reported on 14 children with hemoglobinopathies transplanted with TCRαβ/CD19-depleted grafts using a conditioning regimen with busulfan, thiotepa, cyclophosphamide, and ATG. The median follow-up was 3.9 years with an OS and DFS of 84% and 69%, respectively. CMV, EBV, ADV, and BKV reactivations were reported in 64%, 28%, 7%, and 23%, respectively [29]. The delayed immune reconstitution in our T-haplo-HSCT patients may correlate with an increased risk for viral reactivation (Tables 4 and 5).

Grade I–II aGvHD was observed in 35% T-haplo-HSCT and in 33% MSD, but no grade III–IV aGvHD in either group. In addition, 20% developed mild/moderate cGvHD in T-haplo-HSCT and 22% in MSD. No severe or steroid refractory cGvHD was observed in either group. Of note, five (two in the MSD and three in the T-haplo-HSCT group) of the six patients who developed cGvHD were beyond 20 years of age. Thus, our preliminary data confirm the incidence reported by Gluckman et al. in MSD, suggesting that the risk of GvHD increases with age at transplantation independent of the donor source [4]. Gaviez et al. [29] also reported about severe GvHD in his series of 14 SCD patients: 28% developed grade III–IV aGvHD and 21% presented with severe cGvHD. The same group reported a positive correlation between the incidence of PRES and aGvHD, both complications seem to share the systemic vascular pathology related to SCD, which progresses with age [29]. In our series, in patients beyond ($n = 16$) or below ($n = 13$) 18 years of age, the incidence of NT was 60% vs. 20% and the incidence of cGvHD was 31% vs. 9%. These results confirm the age-related incidence of cGvHD and transplant-related morbidity [4], and encourage the discussion to define the optimal time point for HSCT in SCD patients [30]. Nevertheless, the low incidences of aGvHD and mild/moderate cGvHD observed were similar to that reported in patients who received T-haplo-HSCT with TCR αβ/CD19-depleted grafts for malignant disease [31].

In conclusion, given the limited availability of MD, alternative approaches are needed in SCD. To our knowledge, this is the largest group of advanced-stage SCD patients transplanted almost identically using a T-depleted haplo-HSCT or a MSD. These preliminary results add to the increasing evidence for feasibility, safety, and efficacy of a T-haplo-HSCT using T-cell-depleted grafts, in order to offer a curative option to the majority of SCD patients even into adulthood. A prospective, stratified non-inferiority trial is in preparation (EudraCT number 2018–002652–33), in order to confirm these preliminary results.

Data sharing statement

For original data and protocols, please contact selim.corbacioglu@ukr.de. Deidentified individual participant data will not be shared.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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