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ORIGINAL ARTICLE

Selective T-cell depletion targeting CD45RA as a novel approach for HLA-mismatched hematopoietic stem cell transplantation in pediatric nonmalignant hematological diseases

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Abstract

Severe aplastic anemia and congenital amegakaryocytic thrombocytopenia are rare bone marrow failure syndromes. Treatment for aplastic anemia consists of hematopoietic stem cell transplantation (HSCT) from a matched sibling donor or immunosuppressant drugs if there is no donor available. Congenital amegakaryocytic thrombocytopenia is a rare autosomal recessive disease that causes bone marrow failure and has limited treatment options, except for transfusion support and HSCT. In the absence of a suitable matched sibling donor, matched-unrelated, haploidentical, or mismatched donors may be considered. A 2-step partial T-cell-depletion strategy can remove CD45RA+ naïve T cells responsible for graft-versus-host disease (GvHD) while preserving memory T cells. Five patients underwent transplantation using this strategy with rapid neutrophil and platelet recovery. Acute and chronic GvHD ≥ grade 2 appeared in two and one patient, respectively. No severe infections were observed before day + 100. A high (60%) incidence of transplant-associated microangiopathy was observed. Three patients (60%) remain alive, with a median follow-up of 881 (range 323–1248) days. CD45RA-depleted HSCT is a novel approach for patients lacking a suitable matched donor; however, further improvements are needed.

Keywords Haploidentical · Bone marrow failure · CD45RA depletion · T depletion · Aplastic anemia · Immune reconstitution

Introduction

Severe aplastic anemia (SAA) is a rare, life-threatening condition that causes bone marrow failure. Therapeutic options for SAA are primarily immunosuppressive treatment (IST) or hematopoietic stem cell transplantation (HSCT). HSCT from a matched sibling donor (MSD) has been the standard of care, although only 30–40% of patients have a fully matched donor in the family. If an MSD is not available, other options include a second IST cycle or a matched-unrelated donor (MUD) HSCT [1]. Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare autosomal recessive disease causing bone marrow failure that presents with severe thrombocytopenia at birth and related hemorrhagic complications. With time, it can evolve into SAA and even leukemia. Treatment options are limited to transfusion support and HSCT [2, 3].

In absence of a fully human leukocyte antigen (HLA)-matched donor, mismatched unrelated and mismatched related (haploidentical) transplants have occasionally been performed using various approaches for patients with SAA and CAMT. This approach provides the possibility of a donor for virtually all patients, with immediate availability. However, the high rate of graft failure (GF) and graft-versus-host-disease (GvHD), delayed immune recovery (IR), and severe infections can be major drawbacks [4–7].

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One approach using haploidentical donors has been to ex vivo T-cell-depleted grafts using $CD34^+$ cell enrichment, with infusion of high doses of $CD34^+$ cells from mobilized peripheral blood to overcome the HLA barrier. Early studies using mega-doses of $CD34^+$ cells reported rapid engraftment, but with a higher number of associated infectious complications related to the delayed IR [8]. To overcome these problems, selective elimination of $\alpha\beta^+$ T cells (which leaves NK cells and $\gamma\delta^+$ T cells in the graft) has been evaluated in nonmalignant conditions, including IST-refractory SAA and CAMT, allowing for a faster IR [9].

A novel 2-step partial T-cell-depletion strategy can remove naïve T cells responsible for GvHD and preserve $CD34^+$ progenitor cells and $CD45RA^-$ memory T cells with specificity for opportunistic pathogens. When utilized in leukemia patients, rapid engraftment with low rates of corticoid-responsive GvHD and a low incidence of viral complications was observed [10]. Previously, we reported our experience using this strategy with 25 consecutive high-risk patients with leukemia, and $CD45RA^-$ depleted haplo-HSCT was well tolerated, with rapid engraftment and a low risk of severe acute and chronic GvHD. Although viral reactivations of cytomegalovirus (CMV) and adenovirus were well controlled, we observed a high rate of human herpesvirus-6 encephalitis [11].

However, this approach has not yet been reported in the context of bone marrow failure syndromes. Therefore, here, we describe our experience with $CD45RA^+$ T-cell-depleted HSCT in patients with SAA and CAMT.

Methods

Patients

From February 2017 to July 2018, we enrolled five pediatric patients diagnosed with nonmalignant diseases who needed allogeneic transplantation but lacked a suitable HLA-matched donor (Table 1). The median age at transplantation was 9 (range 2–13) years. One patient (patient 4) received transplantation from 9/10 mismatch unrelated donors with a mismatch at HLA*A; cell processing was similar to the other four patients who received transplantation from a haploidentical donor. The only exclusion criterion was a poor clinical condition (Lansky score < 60%). The study protocol was approved by local ethics committees; informed consent was obtained from the patients themselves or from their legal guardians.

Conditioning

The conditioning regimen included 800 cGy of total lymphoid irradiation over 4 days, 30 mg/m^2/day of fludarabine over 5 days, and cyclophosphamide 50 mg/kg/day for 4 days. On day 0, the patients received their first hematopoietic progenitor cell $CD34^+$-enriched graft. The following day, they received a second infusion of CD45RA-depleted cells.

Table 1 Patient and donor characteristics

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex	Male	Female	Male	Female	Male
Age at HSCT (years)	9	12	11	2	9
Disease	SAA	SAA	SAA	CAMT	SAA
Previous treatment	IST (2 courses)[a] eltrombopag	IST (2 courses) eltrombopag	IST (2 courses) eltrombopag	NA	IST (2 courses)
TL (Kb)/percentile	10/50	9.4/*p* 25–50	10/*p* > 50	NA	9.6/*p*25–50
Donor characteristics					
Type of donor	Haploidentical (mother)	Haploidentical (mother)	Haploidentical (brother)	MMUD (9/10)[b]	Haploidentical (father)
Sex	Female	Female	Male	Female	Male
CMV serology (D/R)	Pos/Pos	Pos/Pos	Pos/Pos	Pos/Pos	Neg/Pos
ABO	Compatibility	Compatibility	Incompatibility	Compatibility	Incompatibility
Anti-HLA antibodies (DSA)	No	No	No	NA	No
TL (Kb)/percentile	9.7/*p* > 50	9.7/*p* > 50	9/*p* > 50	NA	9.4/*p* > 50

HSCT hematopoietic stem cell transplantation, *SAA* severe aplastic anemia, *CAMT* congenital amegakaryocytic thrombocytopenia, *IST* immunosuppressive treatment, *MMUD* mismatch unrelated donor, *CMV* cytomegalovirus, *HLA* human leukocyte antigen, *DSA* donor-specific antibodies, *NA* not applicable, *TL* telomerase length

[a]Received 2 courses of immunosuppressive treatment. Second cycle with horse ATG and sirolimus

[b]Mismatched unrelated donor with mismatch in A (9/10 compatibility)

Supporting treatment

The administration of granulocyte-colony-stimulating factor (G-CSF) was started on day + 7. All patients received infectious prophylaxis with acyclovir from day − 1 to + 180 and if GvHD or immunosuppressive treatment were present. Antifungal prophylaxis with micafungin was prescribed from day-1 until IST was suspended. Trimethoprim-sulfamethoxazole was started with conditioning treatment until day-1 and was reinitiated after the neutrophil engraftment (> 1000/mm^3) until IST was stopped. Screening for CMV, Epstein–Barr virus (EBV), and adenovirus was performed weekly until day + 100 using a polymerase chain reaction test. Galactomannan antigen was performed twice a week until day + 100. BK virus study was performed when compatible signs and symptoms were observed.

GvHD prophylaxis and grading

GvHD pharmacological prophylaxis consisted of intravenous cyclosporine A 3 mg/kg/day or mycophenolate 15 mg/kg/bid starting at day − 1, maintained for 3 months and then slowly tapered. Acute GvHD (aGvHD) was graded according to standard criteria [12, 13], whereas chronic GvHD (cGvHD) was defined as mild, moderate, and severe [14] in patients surviving 100 days following transplantation.

Monitoring of immune reconstitution

Immune recovery was evaluated at the time of engraftment and then at 30, 60, 90, 180 and 360 days after transplantation. Immune reconstitution assessment included serum immunoglobulin measurement and total lymphocyte count, T cells, B cells, NK cells, and T-cell subsets (CD4$^+$, CD8$^+$, CD4$^+$ CD45RA$^+$, CD4$^+$ CD45RO$^+$, CD8$^+$ CD45RA$^+$, CD8$^+$ CD45RO$^+$).

Preparation of the CD45RA-depleted graft

Donors were mobilized with G-CSF (10 µg/kg/day for 4 days) and harvested on day + 5 by 1–2 leukapheresis procedures. The first product was T-depleted using a CliniMACS device (Miltenyi Biotec, Bergisch Gladbach, Germany) to enrich the graft for CD34$^+$. The minimum cell dose required for CD34 was 5×10^6/kg. The unselected CD34$^-$ fraction was then processed for CD45RA$^+$ cell depletion using the CliniMACS device and Depletion 3.1 software. The maximum CD3$^+$ CD45RA$^+$ dose allowed was 1×10^4/kg and the minimum depletion of

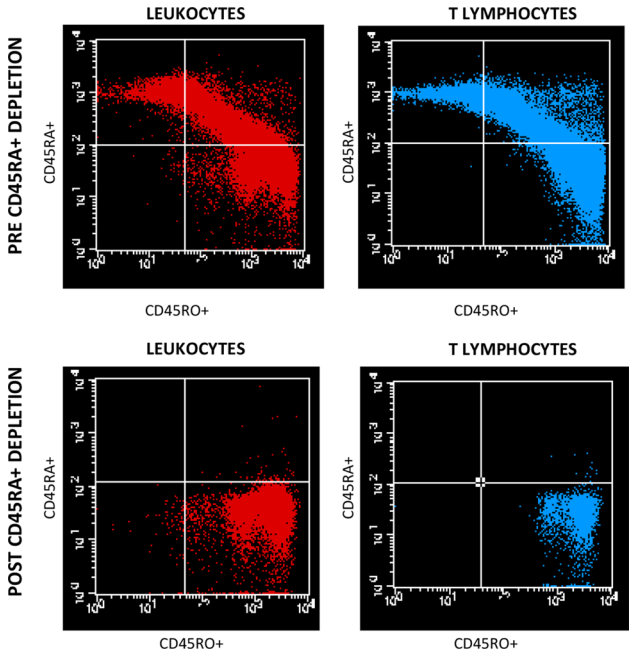

Fig. 1 Flow cytometry charts for leukocytes and T-lymphocytes in pre- and post-CD45RA-depletion cellular product

CD45RA$^+$ cells was ≥ 2.5 $\log_{10}$ (Fig. 1). The spillover of CD45RA$^-$ cells were frozen in separate aliquots. Outcomes were censored as of December 2020.

Engraftment definitions

Neutrophil engraftment was defined by the first of 3 consecutive days with an absolute neutrophil blood count > 0.5×10^9/L. Platelet engraftment was defined as the first day of platelet count > 20×10^9/L without transfusional support in the previous 7 days. Primary GF was defined as the absence of neutrophil recovery by day + 28 with platelet count < 20×10^9/L and Hb < 80 g/L. Secondary graft failure was defined as the presence of cytopenias in a patient who was already engrafted.

Telomere length determination

Telomere length was determined by Southern blots of enzymatically digested DNA, as previously reported from patients with inherited bone marrow failure [15, 16].

Results

Patients and cellular doses

Five patients underwent an HSCT from haploidentical (4) or mismatched unrelated donors (1) between February 2017

and July 2018. Donor characteristics are shown in Table 1. Patients received 2 cellular products: the $CD34^+$ fraction contained a mean of 6.58×10^6/kg cells (range 5–7.73) and the CD45RA-depleted fraction contained a mean of 3.84×10^7/kg cells (range 2.8–5). Graft composition is shown in Table 2. Median $\log_{10}$ depletion of $CD45RA^+$ cells was 4.99 (range 3.73–6.61).

Engraftment and GvHD

All patients engrafted neutrophils ($> 0.5 \times 10^9$/L) at a median of 10 days (range 9–11). Full donor chimerism was achieved by day +30 and maintained over time in all patients. Two of 5 (40%) patients (patients 1 and 3) developed aGvHD grade ≥2 with gut, liver, and skin involvement (histologically confirmed) rapidly responsive to steroids (details in Table 2). Chronic GvHD was observed in one patient (patient 1), with severe lung involvement confirmed by histopathology.

Infections

CMV reactivation was observed in the blood on days +21 and +23 in two patients (patients 1 and 2); and another patient (patient 3) presented with CMV-associated hemorrhagic cystitis on day +34. Additionally, there was one case of EBV reactivation on day +135 (patient 3) and two episodes of adenovirus reactivation on days +53 and +150 (patients 3 and 5). One patient presented BK reactivation on day +330 (patient 3), and another showed grade 4 hemorrhagic cystitis on day +165 (patient 2). Other infectious complications before day +100 included typhlitis in 1 patient (patient 2), which resolved with antibiotic treatment, and parainfluenza-1 virus pneumonitis and Clostridium difficile infection in another patient (patient 4). Adenovirus, BK virus, and parainfluenza reactivations did not need treatment with antiviral agents.

Follow-up and thrombotic microangiopathy

Three (60%) patients remain alive, with a median follow-up of 881 days post-transplant (range 323–1248 days). Two of the 5 patients (patients 1 and 2) died, on days 321 and 363, respectively. Patient 1 presented with severe lung cGvHD complicated by respiratory syncytial virus (RSV) pneumonia. Patient 2 presented with transplant-related thrombotic microangiopathy (TMA). Both patients needed invasive mechanical ventilation. TMA was observed in 3 (60%) of the five patients (patients 1, 2, and 3); these three patients were previously exposed to cyclosporine A during IST. All the patients with SAA had normal telomere-length values compared with their parents.

Post-transplant adoptive $CD45RO^+$ lymphocyte infusion

Three patients received post-HSCT $CD45RA^-$ depleted donor lymphocyte infusions (DLIs) due to lymphopenia and viral reactivations (patients 1, 2, and 3) (Table 2). Two patients received 2 infusions (patients 1 and 2) and another patient received only one (patient 3). The mean $CD3^+$ $CD45RO^+$ dose per infusion was 2.38×10^7/kg (range 0.83–4). The median CD4/CD8 ratio infused in the DLI was 3.69. All DLIs were infused during GvHD prophylaxis, except for second DLI in patient 2 (day +194). After DLI, the viral load decreased in all patients.

A fourth patient (patient 5) received 1×10^6/kg $CD3^+$ $CD45RO^+$ prophylactic DLI on days +30, +60 and +90 (Table 2). After immunotherapy with DLI, only one patient of four who received a DLI presented with de novo grade I skin aGvHD (patient 2). In the other patients, GvHD was not related to DLI.

Immune reconstitution

On day +30, immune reconstitution showed a mean lymphocyte count of 0.54×10^3/μl (range 0.38–0.67), with a mean of 0.33×10^3/μl (range 0.21–0.47) T cells, a mean of 0.158×10^3/μl (range 0.03–0.35) NK cells, and very low B cells mimicking the graft. T-cell counts increased progressively, achieving a mean of 0.682×10^3/μl (range 0.18–1.49) T cells, a mean of 0.255×10^3/μl (range 0.006–0.59) NK cells, and a mean of 0.15×10^3/μl (range 0.0002–0.58) B cells at 6 months after HSCT (Fig. 2).

Discussion

Our results using CD45RA-depleted HSCT in haploidentical and mismatched unrelated donors for children with bone marrow failure syndromes show rapid neutrophil recovery within 10 days, similar to other T-cell-depletion strategies. GF was not observed. Grade > II acute and severe chronic GvHD appeared in 2/5 and 1/5 patients, respectively. Acute GvHD was universally responsive to steroids. Given the frequency of GvHD was higher than expected in the first three patients, we decreased the maximum allowed $CD45RA^-$ and $CD45RA^+$ T-cell doses in the graft to 3×10^7/kg and 1×10^4/kg, respectively. In addition, DLI with $CD3^+$ $CD45RA^-$ T cells was also decreased to 1×10^6 T cells/kg/dose. No severe infections were observed before day +100. CMV reactivation occurred in three patients, being the most common infectious complication.

Recently, new strategies for haploidentical HSCT with unmanipulated grafts have been developed for nonmalignant diseases. Post-HSCT cyclophosphamide has been

Table 2 Graft composition and postransplantation evolution

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
1° cellular product CD34-positive selection					
$CD34^+ \times 10^6$/kg	6.26	7.73	6.41	7.4	5
2° cellular product CD45RA-depletion					
$CD45RA^+ \times 10^4$/kg	0.011	0	0.2	0.36	0.046
$CD45RO^+ \times 10^7$/kg	5.03	5.01	2.85	2.8	3.57
$CD3^+ \times 10^7$/kg	1.65	1.57	0.82	0.24	0.97
$CD3^+$ $CD45RA^+ \times 10^4$/kg	0	0	0.18	0.36	0
$CD3^+$ $CD45RO^+ \times 10^7$/kg	1.49	1.55	0.81	0.24	1
$CD4^+$ CD45RA + $\times 10^4$/kg	0.002	0	0.2	0.08	0
$CD4^+$ $CD45RO^+ \times 10^7$/kg	1.46	0.72	0.26	0.032	0.52
$CD8^+$ $CD45RA^+ \times 10^4$/kg	0	0	0.16	0.36	0
$CD8^+$ $CD45RO^+ \times 10^7$/kg	–	0.2	0.09	0.006	0.01
Log $CD45RA^+$ depletion	6.56	4.49	4.58	6.61	3.73
B cells $\times 10^4$/kg		0.55	1.4	0	0.051
NK cells $\times 10^4$/kg		3.3	0.16		1.4
Conditioning treatment	TLI 200 cGy/d (4 days), fludarabine 30 mg/m^2/day (5 days), cyclophosphamide 50 mg/kg/d (4 days)[a]				
GvHD prophylaxis	MP	CsA	MP[b], steroids	CsA, steroids[c]	CsA, MP[d]
Neutrophil engraftment (days)[f]	11	10	9	9	11
Platelet engraftment (days)[g]	12	13	10	16	14
Graft failure[h]	No	No	No	No	No
Number of RBC transfusions before day + 30	3	4	3	4	4
Number of platelet transfusions before day + 30	4	5	3	6	5
Chimerism at day + 30	Full donor	Full donor	Full donor	Full donor	Full donor
Infectious complications	Parainfluenza 3 Rhinovirus Acinetobacter	Typhlitis HHV-6 reactivation	E. Coli Sepsis Enterobacter faecalis infection	Parainfluenza 1 pneumonitis Clostridium difficile	HHV-6 reactivation
CMV reactivation/disease	Reactivation (prophylaxis with acyclovir at time 2 DLI)	Reactivation (ganciclovir treatment at 2° DLI)	Hemorrhagic cystitis (foscarnet treatment at 1° DLI, valganciclovir treatment at 2° DLI)	No	No (prophylaxis with acyclovir at time 3 DLI)
EBV	No	No	Reactivation	No	No
ADV	No	No	Reactivation	No	Reactivation
BK	No	Hemorrhagic cystitis grade 4	Reactivation	No	No
Acute GvHD ≥ II	Gut grade 2 Skin grade 2 Liver grade 1 Global: III	No[e]	Gut grade 4 Skin grade 1 Global: IV	No	Gut grade 1 Skin 2–3 Global: II
Chronic GvHD	lung severe	No	No	No	No
TMA (day)	Yes (157)	Yes (148)	Yes (300)	No	No
Day of DLI	+ 16/+ 26	32/+ 194	+ 160	No	+ 30, + 60, + 90

Table 2 (continued)

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
DLI $CD3^+$ $CD45RO^+$/kg	4×10^7 4×10^7	1.55×10^7 1.55×10^7	8.3×10^6		1×10^6 1×10^6 1×10^6
Indication for DLI	CMV reactivation	CMV reactivation	EBV/ADV reactivation		Prophylaxis
TRM[i]	Yes	Yes	No	No	No
Follow-up (days)	323	363	1248	1247	881
Status/cause of death	Dead/SRV pneumonia	Dead/TMA	Alive	Alive	Alive

TLI total lymphoid irradiation, *MP* mycophenolate, *CsA* cyclosporine A, *RBC* red blood cells, *HHV-6* human herpes virus-6, *CMV* cytomegalovirus, *EBV* Epstein–Barr virus, *ADV* adenovirus, *GvHD* graft-versus-host disease, *NK* natural killer, *TMA* thrombotic microangiopathy, *DLI* donor lymphocyte infusion, *TRM* transplant-related mortality, *SRV* syncytial respiratory virus

[a]Patient 1 received TLI 200 cG/d (4 days)

[2]Patient 3 received 4 days of CsA, and due to toxicity was changed to MMF and steroids (dose 1 mg/kg/day) until day + 24 when he presented with signs and symptoms of GvHD and the steroid dose was increased to 2 mg/kg/day

[c]Patient 4 received cyclosporine until day + 29 when she presented with acute renal failure; it was decided not to reinitiate cyclosporine and maintain with prednisone 1 mg/kg/day, which was suspended 5 months after transplantation

[d]Patient 5 received cyclosporine for15 days and due to toxicity was changed to MMF

[e]Patient 2 presented grade 1 gut aGvHD (non-histologically confirmed) that responded to topical steroids (budesonide)

[f]Defined as the first of 3 consecutive days to achieve an absolute neutrophil count $>0.5\times10^9$/L

[g]Defined as the first day with platelet count $>20\times10^9$/L without receiving platelet transfusion in the following week

[h]Primary graft failure defined as the absence of neutrophil recovery by day + 28 with platelet count $<20\times10^9$/L and Hb < 80 g/L. Secondary graft failure defined as the presence of cytopenias in a patient who had already engrafted

[i]Defined as death due to causes unrelated to the underlying disease

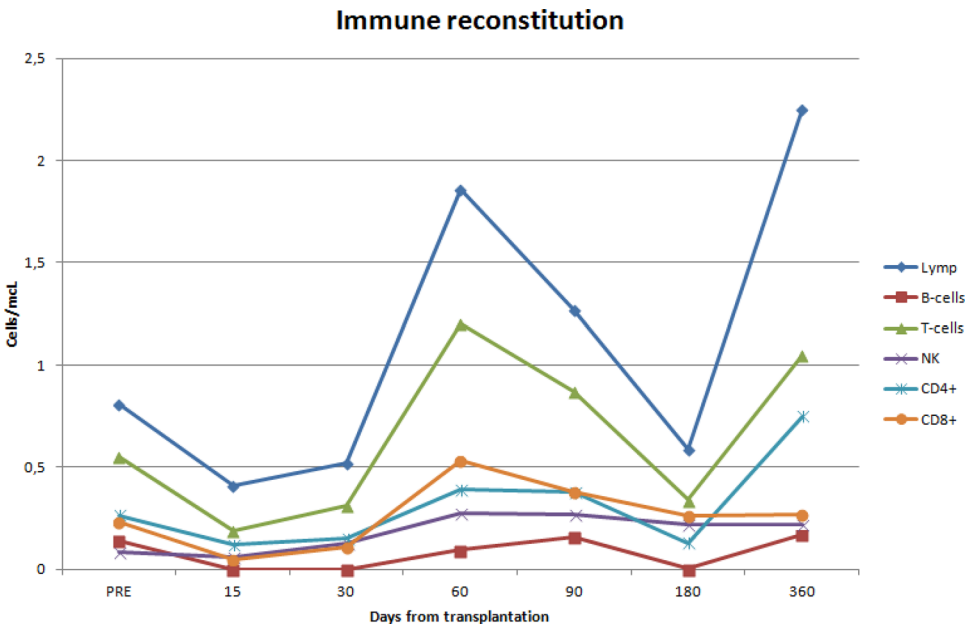

Fig. 2 Immune reconstitution pre and after transplantation on days + 15, + 30, + 60, + 90, + 180 and + 360 (median values)

used sporadically for patients with SAA and CAMT, especially in adults [7, 17, 18]. Another strategy includes G-CSF-primed bone marrow and G-CSF peripheral blood stem cells and significant immunosuppression for GvHD prophylaxis. Xu et al. reported 52 pediatric patients with SAA receiving haploidentical HSCT with median myeloid recovery of 12 days (range 10–22 days). No primary GF occurred; however, three patients experienced secondary GF. Acute GvHD, grades II–IV, was observed in 39.2% of patients, and cGvHD occurred in 38.1%. With a median follow-up of 744 days, overall survival (OS) was 84.5% [19].

Haploidentical HSCT strategy for pediatric patients using ex vivo T-cell depletion with purified high doses of $CD34^+$ cells from G-CSF mobilized donors presented high rates of GF and slow IR [9, 20]. Partial T-cell depletion can eliminate alloreactive cells from the graft while retaining cells that help faster IR and sustained engraftment. Several groups reported this approach for patients with nonmalignant disorders [9, 21]. A Korean group recently reported haploidentical HSCT using depletion of $\alpha\beta^+$ cells, retaining $\gamma\delta^+$ lymphocytes that enhance IR and are not implicated in GvHD. Five patients received αβ-depleted cells and 16 received CD3-depleted grafts [19]. Bertaina et al. reported 23 pediatric patients with nonmalignant disorders, including 4 with SAA and 1 with CAMT, using haploidentical donors and αβ/CD19 depletion. Conditioning differed according to primary disorder; however, all patients received anti-thymocyte globulin for preventing graft rejection and GvHD. There were four cases of GF. Hematological recovery was rapid, with a median of 13 (range 10–20) days to neutrophil engraftment. Three children developed grades I–II acute GvHD, and no cases of chronic GvHD were observed during a median follow-up of 18 months [9]. In this study, the only two patients who died were affected by SAA and died of RSV infection and TMA.

Partial T-cell-depleted transplantation from HLA-identical siblings was recently reported by Sanz et al. in 26 patients with SAA. There were seven pediatric patients and 19 adults. Engraftment was achieved at a median of 11 (range 10–14) days. Acute GvHD grade I was observed in two patients, but there was no grade II–IV GvHD. Only two patients presented mild skin cGvHD, and 1 had moderate skin and ocular involvement. The OS at 10 years was 81% [22].

The high incidence (60%) of TMA in our patients could be related to the prolonged exposure to calcineurin inhibitors prior to the transplant, the use of mismatch donors, and the use of cyclosporine as post-transplant immunosuppression, given the three patients had received two courses of IST with cyclosporine A. Two of the three patients received cyclosporine for GvHD prophylaxis, and the other patient received mycophenolate as prophylaxis and sirolimus for GvHD treatment. SAA has been linked to increased rates of TMA in previous studies, and has been mentioned as an underlying predisposition to complement activations or pre-existing endothelial injury [23, 24].

The CD45RA-depletion strategy allows for post-HSCT immunotherapy, and four patients received DLI of CD45RA-depleted cells to boost the IR. In three patients, DLI was prescribed to treat viral reactivations/infections and to increase lymphocyte counts. In these five infusions, the mean dose of $CD3^+$ $CD45RO^+$ cells was 2.38×10^7/kg/dose. In a separate patient, DLI was administered under a prophylactic regime on day + 30, + 60 and + 90 using lower doses (1×10^6/kg) of $CD3^+$ $CD45RO^+$. All DLIs were infused during GvHD prophylaxis, except for 2° DLI in patient 2 (day + 194). Even if immunosuppressive drugs impacted the IR in these patients, DLI showed a decrease in the CMV viral load in all patients. Maschan et al. used low-dose memory T-cell DLI after TCRαβ-depleted HSCT to improve the immune response to common pathogens in 53 patients and 134 infusions. This strategy used escalating doses of memory T cells at monthly intervals, with a dose range of $25–100 \times 10^3$/kg and $100–300 \times 10^3$/kg for MUD associated with a low risk of de novo GvHD but with a risk of reactivating preexisting GvHD [25]. The largest series of patients reported to date targeting $CD45RA^+$ cells as strategy to deplete naïve T cells but preserving memory T cells in hematological malignancies showed an increase in survival due to a reduction in transplant-related mortality without a significant increase in the rates of GvHD. In that series, the patients also received NK cells, so this strategy could help reduce GvHD [26].

In conclusion, we suggest that $CD45RA^+$ cell depletion in haploidentical and unrelated donors needs further improvement for children with bone marrow failure syndromes such as SAA and CAMT who lack a suitable matched related donor. Despite presenting rapid and sustained engraftment, the incidence of GvHD is still higher than expected. Therefore, reducing $CD3^+$ $CD45RA^+$ cells in the graft and DLI should be investigated. Our results showed lower rates of GvHD and infections using lower doses of $CD3^+$ $CD45RO^+$ cells; however, more experience is needed to confirm these data. In addition, we must mention the high incidence of TMA in this group of patients, which could be related to long-term immunosuppressive treatment. A change in the GvHD prophylaxis protocol avoiding cyclosporine could be beneficial in these patients to lower the risk of TMA, although a greater number of cases need to be evaluated to draw conclusions and to confirm this hypothesis regarding TMA and its association with immunosuppressive treatment in this group. This potential association, however, could underscore the importance of designing an effective GvHD prevention strategy that avoids prolonged use of calcineurin inhibitors in this heavily pretreated population. Although this T-cell-depletion approach also allows selectively depleted DLIs for post-HSCT immunotherapy to boost IR, the ideal dose regimen for minimizing GvHD toxicity and maximizing viral protection remains unclear.

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Declaration

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

References

1. Barone A, Lucarelli A, Onofrillo D, Verzegnassi F, Bonanomi S, Cesaro S, et al. Diagnosis and management of acquired aplastic anemia in childhood. Guidelines from the Marrow Failure Study Group of the Pediatric Haemato-Oncology Italian Association (AIEOP). Blood Cells Mol Dis. 2015;55(1):40–7.
2. Geddis A. Congenital amegakaryocytic thrombocytopenia and thrombocytopenia with absent radii. Hem Oncol Clin North Am. 2009;23(2):321–31.
3. Lackner A, Basu O, Bierings M, Lassay L, Schaefer U, Revesz T, et al. Haematopoietic stem cell transplantation for amegakaryocytic thrombocytopenia. Br J Haematol. 2000;109(4):773–5.
4. Dietz A, Lucchini G, Samarasinghe S, Pulsipher M. Evolving hematopoietic stem cell transplantation strategies in severe aplastic anemia. Curr Opin Pediatr. 2016;28(1):3–11.
5. Kumar R, Bonfim C, George B. Hematopoietic cell transplantation for aplastic anemia. Curr Opin Hematol. 2017;24(6):509–14.
6. de Latour RP. Transplantation for bone marrow failure: current issues. Hematol Am Soc Hematol Educ Program. 2016;2016(1):90–8.
7. Prata PH, Elkema D, Afansyev B, Bosman P, Smiers F, Diez-Martin JL, et al. Haploidentical transplantation and posttransplant cyclophosphamide for treating aplastic anemia patients: a report from the EBMT Severe Aplastic Anemia Working Party. Bone Marrow Transpl. 2019;55(6):1050–8.
8. Lacerda J, Martins C, Carmo J, Lourenço F, Juncal C, Ismail S, et al. Haploidentical stem cell transplantation with purified CD34+ cells after a chemotherapy-alone conditioning regimen in heavily transfused severe aplastic anemia. Biol Blood Marrow Transpl. 2005;11(5):399–400.
9. Bertaina A, Merli P, Rutella S, Pagliara D, Bernardo M, Masetti R, et al. HLA-haploidentical stem cell transplantation after removal of αβ+ T and B cells in children with nonmalignant disorders. Blood. 2014;124(5):822–6.
10. Bleakley M, Heimfeld S, Loeb K, Jones L, Chaney C, Seropian S, et al. Outcomes of acute leukemia patients transplanted with naive T cell–depleted stem cell grafts. J Clin Invest. 2015;125(7):2677–89.
11. Sisinni L, Gasior M, de Paz R, Querol S, Bueno D, Fernández L, et al. Unexpected high incidence of human herpesvirus-6 encephalitis after Naive T cell-depleted graft of haploidentical stem cell transplantation in pediatric patients. Biol Blood Marrow Transpl. 2018;24(11):2316–23.
12. Glucksberg H, Storb R, Fefer A, Buckner C, Neiman P, Clift R, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. Transplantation. 1974;18(4):295–304.
13. Harris A, Young R, Devine S, Hogan W, Ayuk F, Bunworasate U, et al. International, multicenter standardization of acute graft-versus-host disease clinical data collection: a report from the mount sinai acute GVHD International Consortium. Biol Blood Marrow Transpl. 2016;22(1):4–10.
14. Jagasia MH, Greinix H, Arora M, Hogan W, Ayuk F, Bunworasate U, et al. National Institutes of Health Consensus Development Project on Criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 diagnosis and staging working group report. Biol Blood Marrow Transpl. 2015;21(3):389-401.e1.
15. Gadalla S, Cawthon R, Giri N, Alter B, Savage S. Telomere length in blood, buccal cells, and fibroblasts from patients with inherited bone marrow failure syndromes. Aging (Albany NY). 2010;2(11):867–74.
16. Arias-Salgado EG, Galvez E, Planas-Cerezales L, Pintado-Berninchez L, Vallespin E, Martínez P, et al. Genetic analyses of aplastic anemia and idiopathic pulmonary fibrosis patients with short telomeres, possible implication of DNA-repair genes. Orphanet J Rare Dis. 2019;14(1):82.
17. Dezern AE, Zahurak M, Symons H, Cooke K, Jones RJ, Brodsky RA. Alternative donor transplantation with high-dose post-transplantation cyclophosphamide for refractory severe aplastic anemia. Biol Blood Marrow Transpl. 2017;23(3):498–504.
18. Esteves I, Bonfim C, Pasquini R, Funke V, Pereira N, Rocha V, et al. Haploidentical BMT and post-transplant Cy for severe aplastic anemia: a multicenter retrospective study. Bone Marrow Transpl. 2015;50(5):685–9.
19. Xu LP, Zhang XH, Wang FR, Mo X, Han T, Han W, et al. Haploidentical transplantation for pediatric patients with acquired severe aplastic anemia. Bone Marrow Transpl. 2016;52(3):381–7.
20. Arpacı F, Tezcan I, Kuzhan O, Yalman N, Uçkan D, Kürekçi A, et al. G-CSF-mobilized haploidentical peripheral blood stem cell transplantation in children with poor prognostic nonmalignant disorders. Am J Hematol. 2008;83(2):133–6.
21. Im HJ, Koh K-N, Seo J. Haploidentical hematopoietic stem cell transplantation in children and adolescents with acquired severe aplastic anemia. Korean J Pediatr. 2015;58(6):199.
22. Jodele S, Davies SM, Lane A, Khoury J, Dandoy C, Goebel J, et al. Diagnostic and risk criteria for HSCT-associated thrombotic microangiopathy: a study in children and young adults. Blood. 2014;124(4):645–53.
23. Sanz J, Moscardó F, Montoro J, Cano J, Guerreiro M, Dasi MA, et al. Partial T cell-depleted peripheral blood stem cell transplantation from HLA-identical sibling donors for patients with severe aplastic anemia. Biol Blood Marrow Transpl. 2020;26(1):83–7.
24. Dvorak CC, Higham C, Shimano KA. Transplant-associated thrombotic microangiopathy in pediatric hematopoietic cell transplant recipients: a practical approach to diagnosis and management. Front Pediatr. 2019;7:133.
25. Maschan M, Blagov S, Shelikhova L, Shekhovtsova Z, Balashov D, Starichkova J, et al. Low-dose donor memory T-cell infusion after TCR alpha/beta depleted unrelated and haploidentical transplantation: results of a pilot trial. Bone Marrow Transpl. 2017;53(3):264–73.
26. Mamcarz E, Madden R, Qudeimat A, Srinivasan A, Talleur A, Sharma A, et al. Improved survival rate in T-cell depleted haploidentical hematopoietic cell transplantation over the last 15 years at a single institution. Bone Marrow Transpl. 2019;55(5):929–38.