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ORIGINAL ARTICLE

# Donor-derived marrow mesenchymal stromal cell co-transplantation following a haploidentical hematopoietic stem cell transplantation trail to treat severe aplastic anemia in children

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## Abstract

Haploidentical hematopoietic stem cell transplantation (haplo-HSCT) is associated with an increased risk of graft failure and severe graft-versus-host disease (GVHD). Recent studies have shown that mesenchymal stromal cells (MSCs) display potent immunosuppressive effects and can support normal hematopoiesis. In a multi-center trial, we co-transplanted culture-expanded donor-derived bone marrow MSCs (BM-MSCs) into 35 children with severe aplastic anemia (SAA) undergoing haplo-HSCT. All 35 patients (100%) achieved hematopoietic reconstitution and showed sustained full donor chimerism. The median time for myeloid engraftment was 14 days (range 10–22 days), while that for platelet engraftment was 18 days (range 9–36 days). The incidence of grade II–IV acute GVHD and chronic GVHD was 25.71 and 22.86%, respectively. The overall survival rate was 85.71% with a median of 22 months (range 3.5–37 months). The combined transplantation of haploidentical HSCs and BM-MSCs into children with SAA without an HLA-identical sibling donor is relatively safe and may represent an effective new therapy to improve survival rates and reduce the risk of graft failure.

**Keywords** Haploidentical hematopoietic stem cell transplantation · Children · Severe aplastic anemia · Bone marrow mesenchymal stromal cells

## Introduction

Human leukocyte antigen (HLA) fully matched allogeneic hematopoietic stem cell transplantation (allo-HSCT) has proven to be one of the most efficacious therapies for pediatric cases of severe aplastic anemia (SAA), with an overall survival rate of 92% for 17 years [1]. However, only less than 30% of adolescent SAA patients can successfully find a HLA fully matched sibling or non-relative donor especially in China [1, 2]. As an alternative strategy, haploidentical hematopoietic stem cell transplantation (haplo-HSCT) has recently been used to treat patients who are either non-responsive to immune inhibitors (refractory SAA) or who relapsed after successful initial treatments (relapse SAA) [3, 4]. The main challenges associated with haplo-HSCT for the treatment of SAA is the high graft-failure rate and the development of graft-versus-host disease (GVHD) [5, 6]. Mesenchymal stromal cells (MSCs) are characterized by a high proliferation rate, their multi-directional differentiation, and low immunogenicity. According to recent reports, administered MSCs can secret cytokines to improve hematopoietic stem cell transplantation (HSCT) engraftment and decrease the incidence and severity of GVHD [7–10].

While there were several reports on the use of allo-HSCT combined with donor bone marrow MSC transplantation to treat both children and adults with SAA to achieve promising clinical results [11–13], no multi-center clinical studies with larger

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numbers of pediatric patients in this area are available thus far. The aim of this study was to conduct a summarized analysis focusing on the rates and kinetics of hematopoietic engraftment, and the incidence and severity of GVHD in 35 children with SAA using allo-HSCT combined with donor bone marrow MSC (BM-MSC) transplantation in a multi-center clinical study.

## Subjects and methods

### Patient selection for trial

A total of 35 SAA patients including 18 males and 17 females with a median age of 11.5 years (range 3–18 years) were enrolled in the study after informed parental consent was obtained from January 2014 to December 2016 (Table 1). The haplo-HSCT with BM-MSC protocol for the treatment of SAA was approved by the ethics committee. Patients were treated with haplo-HSCT and MSCs in the General Hospital of Guangzhou Military Command, the Guangzhou First People's Hospital, the Second Affiliated Hospital of Sun Yat-Sen University, the Affiliated Nanfang Hospital of Southern Medical University, and the First Affiliated Hospital of Harbin Medical University from January 2014 to December 2016 (Table 1). The trial was registered at ClinicalTrials.gov under the identifier NCT02247973.

**Table 1** Characteristics of SAA patients for haplo-HSCT ($n = 35$)

| Variable | Data |
|---|---|
| Patient demographics | |
| Median age, year (range) | 11.5(3–18) |
| Sex | |
| Male | 17(48.57%) |
| Female | 18(51.43%) |
| Type of AA | |
| SAA, *n* (%) | 19(54.29%) |
| VSAA, *n* (%) | 16(45.71%) |
| Donor–recipient sex match | |
| Female–male | 7(20%) |
| Female–female | 4(11.43%) |
| Male–male | 10(28.57%) |
| Male–female | 14(40%) |
| Donor–recipient relationship for haplo-HSCT | |
| Mother–child | 9(25.71%) |
| Father–child | 20(57.14%) |
| Siblings | 6(17.14%) |
| HLA mismatched | |
| Haplo 5 locus mismatched | 23(65.71%) |
| Haplo 4 locus mismatched | 5(14.29%) |
| Haplo 3 locus mismatched | 3(8.57%) |
| Haplo 2 locus mismatched | 3(8.57%) |
| Haplo 1 locus mismatched | 1(2.86%) |
| ABO pairs | |
| Pairs | 25(71.43%) |
| Un-pairs | 10(28.57%) |
| Pretreatment blood cell count | |
| Median WBC, $10^8$/kg(range) | 15.4(5.6–26.9) |
| Median $CD34^+$ ($\times 10^6$/kg) | 3.7(0.49–16.9) |
| BM-MSCs ($\times 10^6$/kg) | 34(14–65) |

*haplo-HSCT* haploidentical hematopoietic stem cell transplantation, *MNC* mononuclear cell, *SAA* severe aplastic anemia, *VSAA* very severe aplastic anemia

This is a subgroup analysis, all patients were refractory to immunotherapy, and they were required to meet specific inclusion and exclusion criteria [13]. Patients met the following inclusion criteria: (1) in line with the 2009 Edition (UK), aplastic anemia diagnostic criteria for SAA or very severe SAA; (2) age < 18 years old; (3) no HLA-identical sibling donor; (4) HLA-mismatched related donors with ≥ 5/10 HLA-matched loci; (5) no serious infection or acute hemorrhage; (6) cardiac ultrasound examination showed left ventricular ejection fraction >50%; (7) both transaminase and serum creatinine level no more than twice the upper limit of normal value; (8) no acute contagious disease; (9) ability to understand and willingness to sign a written informed consent document by guardians; and (10) Eastern Cooperative Oncology Group score of 0–2 points. Any patient with SAA receiving conventional immunosuppressive therapy alone was excluded from the study cohort.

### Conditioning regimen

The modified intravenous (IV) conditioning regimen was based on our previous protocol for haploidentical HSCT [9], which included the administration of busulfan (Bu) at a dose of 0.8 mg/kg/6 h (days − 7 to − 6; total dose: 6.4 mg/kg), cyclophosphamide (Cy) at a dose of 50 mg/kg/day (days − 5 to − 2; total dose 200 mg/kg), and antithymocyte globulin (ATG) (rabbit, Genzyme Polyclonals SAS, Lyon, France) 2.5 mg/kg/day (days − 5 to − 2; total dose 10 mg/kg).

### Stem cell collection

Stem cell donors received recombinant human granulocyte-colony-stimulating factor (rhG-CSF) at a dose of 5 μg/kg/day for 5 continuous days before transfusion. BM cells were collected on day 01. The target volume was 15–20 ml/kg of recipient weight. PBSCs were harvested on day 02. The target mononuclear cell count from BM and peripheral blood and was $\geq 6 \times 10^8$/kg and $CD34^+$ cells $\geq 2 \times 10^6$/kg of recipient weight respectively. This corresponded to day 0 in the recipient cycle. The first day of stem cell infusion was designated as day "01"; the second day of infusion was day "02." Stem cell transfusion was performed on days 01 and day 02 (Fig. 1).

### BM-MSC preparation and transfusion

Mononuclear cells were isolated from 30 mL of donor bone marrow and then cultured in serum-free medium (αMEM; UltraGRO) for 7–10 days. These cells expressed the surface markers of MSCs (CD29, CD30, CD90, and CD105) and

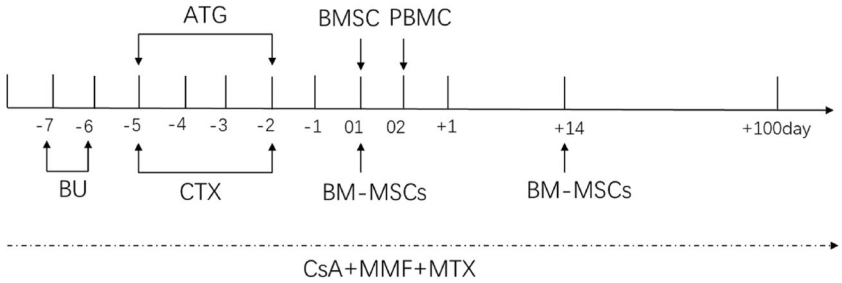


**Fig 1** Conditioning regimen for patients rec.eiving haplo-HSCT. Busulfan (BU): 3.2 mg/kg was administered on days − 7 and − 6. Cyclophosphamide (CTX): 40 mg/kg was administered on 4 consecutive days (days − 5 to − 2). Human antithymocyte globulin (ATG): 2.5 mg/kg was administered on 4 consecutive days (days − 5 to − 2)

were negative for CD34, CD45, and CD14 [14, 15]. The second-passage cultured cells were cryopreserved at 2–3 infusion doses waiting to be resuscitated and cultured to become a final product. The third passage [16] of freshly cultured BM-MSCs was infused according to the patient's body weight at $1 \times 10^6$ cells/kg. The first BM-MSC infusion was performed 6 h before bone marrow stem cell infusion and completed within 30 min on day 01. The second BM-MSC infusion was performed on day 14 following stem cell transplantation. Body temperature, blood pressure, pulse, and electrocardiogram readings were closely monitored during and within 24 h after the infusion window.

### GVHD prophylaxis

GVHD prophylaxis consisted of the IV administration of CsA at 2.5 mg/kg/day in divided doses beginning the day before transplantation (day − 7), and it was continued thereafter. Mycophenolate mofetil (MMF) was administered orally at a dose of 0.25 g every 12 h from days − 7 to + 30. Methotrexate (MTX) was administered via IV at a dose of 15 mg/m$^2$ on day + 1 and at a dose of 10 mg/m$^2$ on days + 3, + 6, and + 11 in the absence of severe mucositis. In the absence of GVHD, the oral cyclosporine dose was reduced weekly by approximately 5% beginning on or near 6 months, and therapy was usually discontinued by 1 year after transplantation. Acute and chronic (c)GVHDs were treated according to institutional practices.

### Supporting treatment

All children were admitted in class 100 laminar flow clean-room wards after a skin-care bath, with oral administration of fluconazole to prevent fungal infection, oral administration of sulfamethoxazole to prevent pneumocystis carinii infection, and heparin sodium administration of 80 U/kg/day (− 10~+ 30 days) to prevent hepatic vein occlusion. G-CSF at a dose of 5 μg/kg from day + 1 was given by subcutaneous injection until granule implantation. In addition, irradiated erythrocyte and platelet suspensions were also given to maintain the patients' hemoglobin and platelet levels, which exceeded 60 g/L and $20 \times 10^9$/L, respectively.

### Hematopoietic reconstitution and implantation assessment

Granulocyte implantation was defined as the first day of 3 continuous days, whereby the absolute neutrophil count was $\geq 0.5 \times 10^9$/L; conversely, platelet implantation was defined as the first day of 7 continuous days whereby the platelet levels were $\geq 20 \times 10^9$/L in the absence of further platelet infusion. Short-tandem repeat-polymerase chain reaction (STR-PCRs) DNA fingerprinting was used to measure chimerism in bone marrow on + 30 days to determine the success of implantation. If the donor and recipient were of a different sex, the bone marrow sex chromosome transformation was used to measure implantation efficiency, whereas if there was blood-type incompatibility between the donor and recipient, blood-type change was used to determine implantation efficiency.

### Statistical analysis

The primary endpoint of the study was the overall survival rate from transplantation. Secondary endpoints included implantation rate and the incidence and severity of aGVHD and cGVHD. Patients who died before implantation were excluded from the analyses of aGVHD, cGVHD, and implantation. Only those patients who survived > 100 days were analyzed for cGVHD. The overall survival rate was evaluated using the Kaplan–Meier estimate. The incidence of aGVHD and cGVHD was also evaluated by the Kaplan–Meier estimate. The statistical software package (SPSS 16.0; IBM Corporation, Armonk, NY, USA, 1989–2007) was used for comparative analyses.

## Results

### Characteristics of patients and their donors

Clinical assessments of the patients and donors are shown in Table 1. A total of 35 patients who were diagnosed with SAA were enrolled in the study. Of these, 16 patients (45.71%) were considered to have very severe SAA. Patients and their

donors were haploidentical. The patients' median age was 11.5 years (range 3–18 years). The median mononuclear cell and $CD34^+$ cell counts were $15.4 \times 10^8$ and $3.7 \times 10^6$/kg, respectively.

## Implantation

All 35 patients (100%) achieved hematopoietic reconstitution and sustained full donor chimerism within 30 days after HSCT (Table 2). The median time for myeloid engraftment was 14 days (range 10–22 days), while for platelets, implantation was 18 days (range 9–36 days). Further, the 35 patients sustained complete stable donor chimerism without late graft failure.

## GVHD

Of the 35 patients, 17 (48.57%) experienced aGVHD after transplantation, including 8 (22.86%) with grade I, 5 (14.29%) with grade II, 2 (5.71%) with grade III aGVHD, and 2 (5.71%) with grade IV aGVHD (Table 2). At 100 days after transplantation, the cumulative incidence of grades II–IV aGVHD was 25.71% and that of grades III–IV was 11.42% (Fig. 2). The four patients with grade III aGVHD or IV aGVHD received additional treatment with methylprednisolone CD25 monoclonal antibody, and CsA was substituted for FK-506. Three of these patients were cured and one patient died.

Of the 35 patients who survived longer than 100 days after transplantation, the majority ($n = 25$; 71.42%) remained disease-free whereas a minority ($n = 8$; 22.86%) developed cGVHD. Among these 8 patients, 5 (62.5%) showed limited cGVHD and 3 (37.5%) showed extensive cGVHD (Table 2; Fig. 3).

**Table 2** Clinical outcomes of haplo-HSCT for 35 patients with SAA

| Outcome | Data |
|---|---|
| Engraftment ($n = 35$) | |
| Neutrophil engraftment (days) | 14(10–22) |
| Platelet engraftment (days) | 18(9–36) |
| Engraftment | 35(100%) |
| Acute GVHD ($n = 35$) | |
| None | 18(51.43%) |
| | 8(22.86%) |
| II | 5(14.29%) |
| III | 2(5.71%) |
| IV | 2(5.71%) |
| Chronic GVHD ($n = 35$) | |
| None | 25(71.42%) |
| Limited | 5(14.29%) |
| Extensive | 3(8.57%) |
| Viremia ($n = 22$) | |
| CMV | 11(31.43%) |
| EBV | 17(48.57%) |
| EBV-associated PTLD | 1(2.86%) |
| Mortality ($n = 5$) | |
| Cause | |
| Graft loss | |
| GVHD | 1(2.86%) |
| Infections | 4(11.43%) |

*haplo-HSCT* haploidentical hematopoietic stem cell transplantation, *PTLD* post-transplant lymphoproliferative disorder

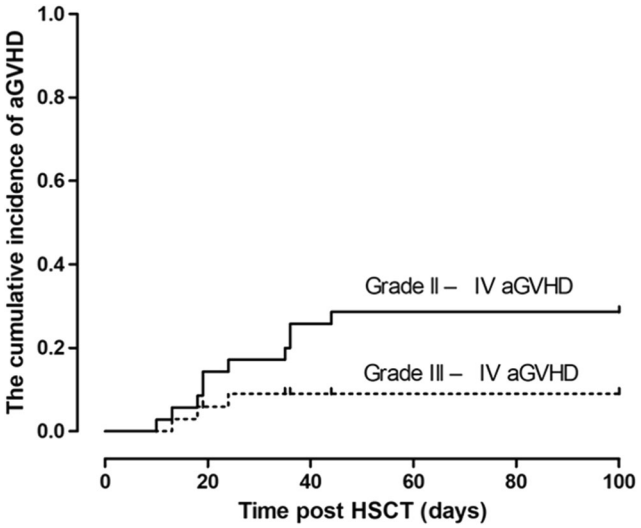


**Fig. 2** The Kaplan–Meier curve for the cumulative incidences of Grade II–IV aGVHD and Grade III–IV aGVHD ($n = 35$ patients)

## Complications

All patients received the conditioning regimen on schedule without encountering any significant transplantation-related bowel, renal, or liver toxicity. No patients experienced infusional toxicity during the infusion of BM-MSCs; however,

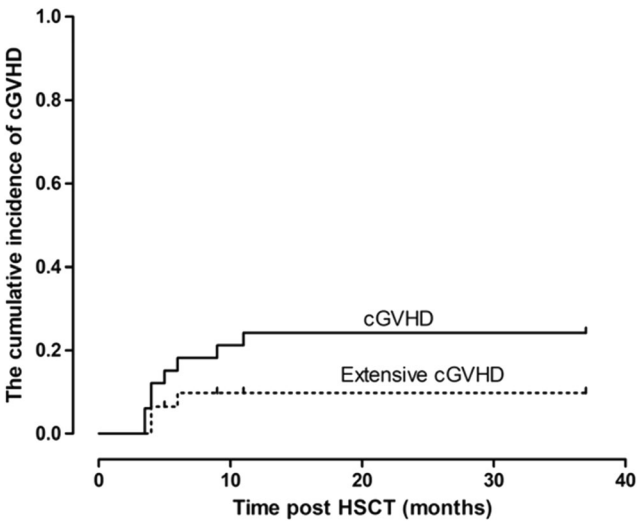


**Fig. 3** The Kaplan–Meier curve for the cumulative incidences of cGVHD and extensive cGVHD ($n = 35$ patients)

the majority of patients (22, 63%) developed varying degrees of fever associated with hematopoietic dysfunction, which was resolved after treatment with anti-inflammatory medication. In regard to infection, 11 patients developed CMV infection, 17 patients developed an EBV infection with 2 of the 28 patients developing a co-infection of CMV and EBV. One patient progressed to EBV-associated PTLD and died of viral infection at 130 days after transplantation and a second patient progressed to EBV accompanied with veno-occlusive disease (VOD) and died of pulmonary fungal infections at 115 days after transplantation. Two patients developed reversible posterior leukoencephalopathy syndrome (RPLS), and after the discontinuation of oral CsA and treated with sodium vedproate, the seizures were resolved. But they died of septic shock at 120 days and severe intestinal infection 105 days respectively (Table 3). All viral infections in the remaining 24 patients were treated with ganciclovir or foscarnet and gamma globulin, and all patients demonstrated a full recovery. Hemorrhagic cystitis was observed in 8 patients (22.86%) at 26–70 days (median 36) after transplantation. After hydration, alkalization of urine, and bladder flushing, all recovered within 2–3 weeks.

### Overall survival and treatment-related mortality

Patients were followed-up for a median of 22 months (range 3.5–37 months). None of the patients were lost to follow-up. Five patients died within the follow-up period. The causes of death included GVHD (1 patient) and infection (4 patients). The mean survival time was 21.4 months (Fig. 4). All 30 of the surviving patients (beyond 8 months) achieved a hematologic complete response.

## Discussion

Graft failure and rejection for patients with children SAA remains as one of the important and life-threatening complications in haplo-HSCT [17, 18]. Various transplant strategies have been introduced to decrease graft failure and GVHD over several decades [19–21]. MSCs are adult stem cells with self-replicating ability and multi-directional differentiation potential which exist in various tissues such as bone marrow, umbilical cord blood, umbilical cord tissue, placental tissue, and adipose tissue [22].

Some studies included our previous multi-center clinical study [13] which showed that MSCs can promote hematopoietic stem cell implantation, induce immune tolerance, prevent and treat GVHD, and repair tissue damage caused during pretransplant treatment [7, 23, 24]. But until now, there have been no multi-center trials to evaluate the efficacy of combined BM-MSCs and haplo-HSCT transplantation in children with SAA. On the basis of the encouraging results from our preceding work [13], we increased the sample size of children with SAA and further focused on the therapeutic effects of this

**Table 3** Causes of death and conditions of patients following haplo-HSCT for SAA

| Patient number | Survival time after HSCT (days) | Acute GVHD | Chronic GVHD | Engraftment | Complications | EBV-associated PTLD | Infection | Causes of death |
|---|---|---|---|---|---|---|---|---|
| 1 | 120 | Yes (IV) | Yes (extensive) | yes | Ion disonter | No | Yes (pneumonia) | GVHD |
| 2 | 130 | Yes (I) | No | Yes | No | Yes | Yes (EBV-associated PTLD) | Infection |
| 3 | 115 | Yes (III) | Yes (extensive) | Yes | Veno-occlusive disease | No | Yes (pulmonary fungal infections) | Infection |
| 4 | 120 | Yes (III) | Yes (extensive) | Yes | Reversible posterior Leukoencephalopathy syndrome | No | Yes (severe intestinal infection) | Infection |
| 5 | 105 | Yes (I) | Yes (I) | Yes | Reversible posterior Leukoencephalopathy syndrome | No | Yes (septicemia) | Infection |

*haplo-HSCT* haploidentical hematopoietic stem cell transplantation, *VOD* veno-occlusive disease, *SAA* severe aplastic anemia, *RPLS* reversible posterior leukoencephalopathy syndrome, *PTLD* post-transplant lymphoproliferative disorder

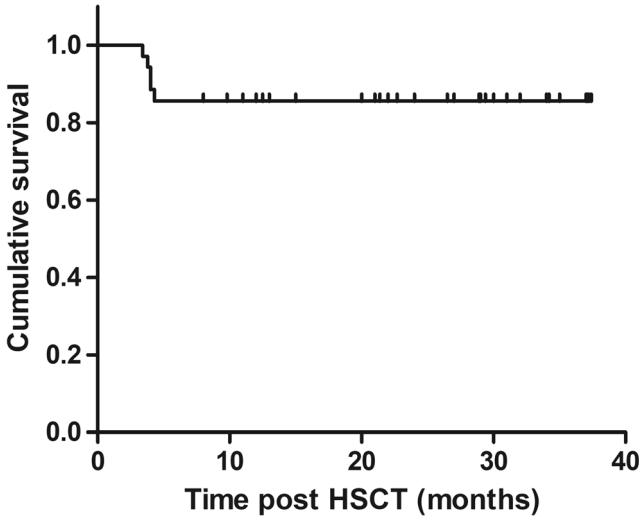


**Fig. 4** OS of SAA children after haplo-HSCT and MSC treatment ($n$ = 35)

treatment regimen on children with SAA to evaluate the safety and efficacy of BM-MSC and haplo-HSCT therapy.

In this study, all 35 children with SAA had between 1 and 5 mismatched loci. They exhibited rapid hematopoietic recovery after transplantation, in which the granulocyte and platelet implantation times were 14 days (range 10~22 days) and 18 days (range 9~36 days), respectively, with a full donor chimerism. The median time for myeloid and platelet implantation in our study were shorter than the median time reported in Wang Z et al. (granulocytes 16 days; platelets 22 days) [25]. GVHD is a common complication after transplantation, and it is a critical factor affecting transplantation success. Our study showed that the overall survival rate was 85.71%, and the incidence rates of II-IV aGVHD and cGVHD were 25.71 and 22.86%, respectively, which were lower than the risk rates reported in a similar recent study of 52 pediatric patients who received haplo-HSCT without MSC infusion (aGVHD 39.2 ± 0.5%; cGVHD 34.2 ± 0.5%) [4]. In another report of the co-transplantation of unrelated donor PBSCs and BM-MSCs, the incidence of grade II–IV aGVHD and cGVHD were 20.0% in SAA children [26]. The possible mechanisms by which MSCs could improve the outcomes of haplo-HSCT for children with SAA are the following. First, MSCs interact with the HSC niche secreting bioactive molecules to support proliferation and long-term growth of HSCs, thus influencing hematopoiesis [27]. Second, MSCs utilize multiple mechanisms to constrain both innate and adaptive immune responses that promote GVHD by soluble factors, such as transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), nitric oxide (NO), human leucocyte antigen G (HLA-G), chemokines, and also promote cell contact-dependent mechanisms [28, 29].Collectively, these limited but promising data suggest that the combined transplantation of haplo-HSCs and BM-MSCs in children with severe aplastic anemia without an HLA-identical sibling donor is safe and feasible.

Furthermore, CMV and EBV infections are opportunistic infections caused by low immune function. Although MSCs provide effective immune regulation through a variety of mechanisms, it has been found that the infusion of MSCs can lead to an increased CMV viral load and the incidence of CMV disease [30]. In this study, the infection rate of CMV patients was 31.43% and the EBV infection rate was 48.57%. One of the patients developed EBV-PTLD, and four patients died due to infection. A reduction in CMV infection after haplo-HSCT will only be achieved by hastening post-transplant immune reconstitution. However, immune reconstitution following haplo-HSCT is usually slower than that achieved after matched-sibling donor or matched-unrelated donor transplants [31].

In conclusion, in SAA children, BM-MSCs combined with haplo-HSCT infusion was a safe and efficacious method for the treatment of children with SAA. This approach might achieve faster hematopoietic implantation, and it may serve as an optional prevention route for cGVHD to effectively control against severe aGVHD. While this new therapeutic modality is promising, further verification of its safety and efficacy is required among a larger sample size of children with SAA. This study has some limitations. Primarily, there was no control group available to compare the efficacy and safety of the co-transplantation of BM-MSCs and haplo-HSCs. Thus, a case–control group comparison to evaluate treatment with HSC or BM-MSC alone is necessary to strengthen the integrity and statistical power of this study, as well as to validate our present results in future studies.

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## Compliance with ethical standards

The haplo-HSCT with BM-MSC protocol for the treatment of SAA was approved by the ethics committee.

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

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