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RESEARCH ARTICLE

Modified G-CSF/ATG-Based Haploidentical Transplantation Protocol in Pediatric Primary Hemophagocytic Lymphohistiocytosis: A Long-Term Follow-Up Single-Center Experience

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ABSTRACT

Background: Primary hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome caused by immune dysregulation. Hematopoietic stem cell transplantation (HSCT) represents the only option for long-term cure for primary HLH. However, only around 25% of patients have a fully HLA-matched donor.

Methods: In this retrospective study, we analyzed 42 pediatric patients with primary HLH who underwent haplo-SCT using the modified granulocyte colony-stimulating factor (G-CSF)/antithymocyte globulin (ATG)-based protocol. The conditioning regimen included 300–600 mg/m^2 etoposide (VP16), along with low doses of busulfan (Bu) (0.8–1.2 mg/kg every 6 hours on Days −8 to −6), cyclophosphamide (Cy) (10 mg/kg/day on Days −4 to −3), fludarabine (Flu) (30 mg/m^2/day on Days −5 to −3), and ATG (8–9 mg/kg total dose on Days −5 to −2) to reduce complications.

Results: All 42 patients achieved successful engraftment. Following a median follow-up period of 48.7 months, 32 of the 42 patients remained alive and disease free. The 2-year overall survival (OS) rate was 78.4%, and the 5-year OS rate was 73.7%. The 2-year failure-free survival (FFS) rate was 71.3%, and the 5-year FFS rate was 66.5%. Patients who achieved complete remission at the time of HSCT showed better OS ($p < 0.05$). The incidence of Grade III–IV acute graft-versus-host disease (GVHD) was 26.2%, and severe chronic GVHD was observed in 11.9% of patients. Thrombotic microangiopathy occurred in 13 patients, and veno-occlusive disease in two patients.

Conclusions: This modified G-CSF/ATG-based haploidentical protocol demonstrates significant potential for pediatric patients with primary HLH, exhibiting commendable effectiveness and safety.

Abbreviations: aGVHD, acute graft-versus-host disease; ATG, antithymocyte globulin; BMSC, bone marrow stem cells; Bu, busulfan; cGVHD, chronic graft-versus-host disease; CI, confidence interval; CR, complete response; Cy, cyclophosphamide; FFS, failure-free survival; G-CSF, granulocyte colony-stimulating factor; haplo-SCT, haploidentical donor hematopoietic stem cell transplantation; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; OS, overall survival; PBSC, peripheral blood stem cells; PR, partial response; TMA, thrombotic microangiopathy; VOD, veno-occlusive disease; VP16, etoposide.

Juan Xiao and Xingcheng Yang contributed equally to this study.

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1 | Introduction

Primary hemophagocytic lymphohistiocytosis (HLH) is a critical, life-threatening disorder characterized by unrestrained activation of cytotoxic T lymphocytes, natural killer cells, and macrophages, leading to hypercytokinemia and immune-mediated damage across multiple organ systems [1]. Pathologically, HLH is generally categorized into primary (driven by an inherent genetic defect that impairs immune function) and secondary (without known potential inherited defects, typically triggered by infection, malignancy, or autoimmune disease). The prognosis of pediatric HLH is very poor if untreated; the median survival of pediatric patients is no more than 2 months [2]. Once HLH is diagnosed, treatment should commence immediately.

Hematopoietic stem cell transplantation (HSCT) represents the only option for long-term cure for primary HLH [3], with more than 50% of children who undergo HSCT surviving [1]. The time between disease onset and transplantation affects outcomes. Even if remission is hard to achieve, urgent HSCT should be considered for primary HLH patients to prevent disease progression and subsequent neurological sequelae [4]. Upon an HLH diagnosis, the search for a donor should begin promptly. However, only around 25% of patients have a fully HLA-matched donor [5]. Seeking a fully compatible donor can delay HSCT, adversely affecting the prognosis. Recent studies suggested that even with a haploidentical donor, HSCT should still proceed actively [6, 7].

Over recent years, the granulocyte colony-stimulating factor (G-CSF)/antithymocyte globulin (ATG)-based protocol (the so-called Beijing Protocol) has increasingly become the standard practice for patients requiring haploidentical donor stem cell transplantation (haplo-SCT) [8–10]. The standard conditioning regimen of the Beijing Protocol includes busulfan (Bu), cyclophosphamide (Cy), and ATG. The infused graft consists of G-CSF-stimulated bone marrow (BM) and peripheral blood stem cells (PBSCs).

However, current studies indicate that haploidentical donors have been identified as a risk factor for the occurrence of veno-occlusive disease (VOD), which is one of the primary causes of transplant-related mortality [11–14]. Meanwhile, the intensive chemotherapeutic agents, pathophysiological mechanisms of HLH, and conventional intensity conditioning regimens can lead to further damage in vascular endothelial cells, linked to an increased incidence of vascular complications, including VOD, which can further worsen the prognosis after HSCT [15–19]. As such, there is considerable interest in developing conditioning regimens with reduced toxicity or intensity, and thereby decreasing the high incidence of VOD post HSCT in pediatric HLH patients [20–22].

Therefore, it is reasonable to modify the G-CSF/ATG-based protocol to evaluate/exploit its strength and avoid unfavorable conditions in pediatric patients with primary HLH undergoing haplo-SCT. In our study, we used 300–600 mg/m^2 etoposide (VP16) in the conditioning regimen to further control the HLH and prevent relapse during the transplantation, along with a low dose of Bu (0.8–1.2 mg/kg every 6 hours), Cy (10 mg/kg/day), fludarabine (Flu) (30 mg/m^2/day), and ATG (8–9 mg/kg total dose on Days −5 to −2). We conducted a retrospective analysis of 42 pediatric patients diagnosed with primary HLH who underwent haplo-SCT with this modified protocol to assess its efficacy and safety.

2 | Materials and Methods

2.1 | Patients

This retrospective study includes 42 patients who were diagnosed with primary HLH and treated with haplo-SCT using the modified Beijing regimen at our institution between March 2016 and March 2023. The inclusion criteria were as follows: (i) meeting the HLH 2004/2009 diagnostic criteria; (ii) identification of pathogenic mutations after testing for internationally recognized HLH-related defect genes (such as PRF1, UNC13D, STX11, STXBP2, Rab27a, LYST, SH2D1A, BIRC4, ITK, AP3β1, MAGT1, CD27); and (iii) age under 18 years. All 42 patients underwent primary haplo-SCT, and four patients received secondary haplo-SCT after the graft failure of the primary HSCT. All stem cell sources utilized in the study comprised a combination of bone marrow stem cells (BMSC) and PBSCs. The cutoff date for follow-up was December 31, 2023. The study followed the Declaration of Helsinki and received approval from the Medical Ethics Committee of Beijing Jingdu Children's Hospital. The data are anonymous, and the requirement for informed consent was waived due to the retrospective nature of this study.

2.2 | Donors

High-resolution human leukocyte antigen typing was meticulously conducted using polymerase chain reaction amplification with sequence-specific primers. This process was aimed at identifying the specific types of HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQ loci. The donor for the initial haplo-SCT was not repeated as the stem cell donor for a second haplo-SCT in the same patient. All donors underwent EBV-DNA screening, NK cell function tests, and CD107a degranulation tests. Donors with normal NK cell activity and CD107a degranulation were considered for selection as a priority. Additionally, donors were thoroughly screened for the genes associated with HLH to exclude the possibility of them being carriers of this disease.

2.3 | Conditioning Regimen and HSCT Procedures

Upon diagnosis, all patients underwent induction chemotherapy following either the HLH-1994 or HLH-2004 protocol. For patients undergoing their first transplant, the conditioning regimen included VP-16/Bu/Flu/Cy/ATG: VP-16 (300–600 mg/m^2 total dose on Days −12 to −8), Bu (0.8–1.2 mg/kg every 6 hours on Days −8 to −6), Flu (30 mg/m^2/day on Days −5 to −3), ATG (8–9 mg/kg total dose on Days −5 to −2), and Cy (10 mg/kg/day on Days −4 to −3). Bu pharmacokinetics were monitored by measuring blood concentrations at four time points:

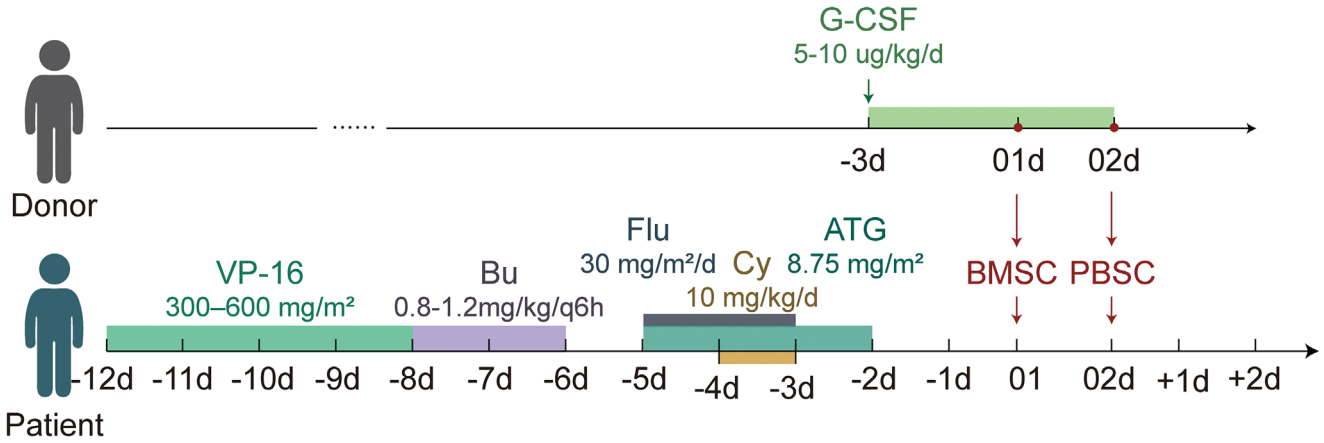

FIGURE 1 | The conditioning regimen of modified Beijing protocol and prophylactic therapy after transplantation. VP-16 (300–600 mg/m^2 total dose on Days −12 to −8), busulfan (Bu) (0.8–1.2 mg/kg every 6 hours on Days −8 to −6), Flu (30 mg/m^2/day on Days −5 to −3), ATG (8–9 mg/kg total dose on Days −5 to −2), and cyclophosphamide (Cy) (10 mg/kg/day on Days −4 to −3). All donors received injections of G-CSF (5–10 μg/kg/day on Days −3 to 02) from Day −3 until the last day of collection. Day 01 is the day when BMSC were infused, Day 02 is the next day when PBSC were infused, and the following day is referred to as Day +1.

0, 1, 2, and 4 hours after the initial dose to maintain the AUC value between 600 and 900. For those undergoing a second transplant, the regimen was adjusted to VP-16/Mel (120 mg/m^2 total dose on Days −6 to −5)/Flu/ATG. All donors received injections of G-CSF (5–10 μg/kg/day on Days −3 to 02) before hematopoietic stem cell collection to induce T-cell hypo-responsiveness in BM or PB grafts from healthy donors. Day 01 is the day when BMSC were infused, Day 02 is the next day when PBSC were infused, and the following day is referred to as Day +1 (Figure 1).

2.4 | GVHD Prophylaxis and Supportive Treatment

Graft-versus-host disease (GVHD) prophylaxis included a combination of cyclosporin A (2.5 mg/kg/day starting on Day −7), mycophenolate mofetil (300 mg/m^2 every 12 hours starting on Day −7), and anti-CD25 monoclonal antibody. If the HLH-2004 chemoimmunotherapy protocol was used prior to transplantation, the GVHD prophylaxis was adjusted to FK506 (0.03 mg/kg/day) combined with mycophenolate mofetil and anti-CD25 monoclonal antibody. Immunosuppressants were maintained for 12 months post transplant. Caspofungin was used for antifungal prophylaxis and sulfamethoxazole for pneumocystis pneumonia prophylaxis. Viral screening included quantitative PCR assays for EBV and CMV in plasma, initially conducted twice weekly during the first month post transplantation, then adjusted to weekly in the second month, biweekly in the third month, monthly from the third to the sixth month, and once every 3 months until 12 months. Antiviral prophylaxis included pre-transplantation ganciclovir and post-transplantation acyclovir. Intravenous immunoglobulin was administered post HSCT in the first month (at 400 mg/kg) on Days +1, +11, +21, and +31 (Figure 1). CMV-related complications were monitored in the central nervous system (CNS), retina, lungs, and gastrointestinal tract with symptomatic management, and CMV-specific T-cell immunotherapy was considered for refractory cases. VOD and thrombotic microangiopathy (TMA) management involved the administration of prostaglandin E1, defibrotide, plasmapheresis, and eculizumab to remove circulating factors that contribute to endothelial damage and to protect endothelial cells.

2.5 | Definitions

Neutrophil recovery is defined as achieving an absolute neutrophil count of at least 0.5 × 10^9/L, while platelet recovery requires a count exceeding 20 × 10^9/L without any platelet transfusions in the preceding 7 days. Chimerism is assessed in peripheral blood and bone marrow at the time of engraftment and at intervals of 3, 6, and 12 months post transplantation through short tandem repeat–polymerase chain reaction analysis of donor and recipient DNA. Full donor chimerism is defined as more than 95% donor-derived cells, mixed chimerism as 5%–95% donor-derived cells, and graft failure as the absence of hematologic recovery by Day +28 post HSCT, with neutrophil counts not exceeding 0.5 × 10^9/L and less than 5% donor chimerism. Complete response (CR) is defined as the total disappearance of all diagnostic clinical and laboratory abnormalities associated with HLH. Partial response (PR) is defined as the sustained normalization of three or more of the previously validated diagnostic parameters with no apparent progression of other parameters. Nonresponse is defined as the normalization of two or fewer diagnostic parameters or clear progression of other aspects of HLH disease. Overall survival (OS) is the time from HSCT to death from any cause or to the last follow-up, and failure-free survival (FFS) is the time from HSCT to the occurrence of death, graft failure, relapse, or last follow-up. Acute GVHD (aGVHD) was diagnosed according to the Glucksberg criteria [23], and chronic GVHD (cGVHD) was diagnosed according to the National Institutes of Health criteria for global cGVHD scoring (mild, moderate, or severe) [24]. The diagnosis of VOD is based on clinical and laboratory criteria according to the latest European Society for Blood and Marrow Transplantation guidelines [25], and the diagnosis of TMA follows the published criteria proposed by Jodele et al. [26]. Viral replication for EBV and CMV is confirmed when plasma viral loads exceed 5 × 10^2 copies/mL, with CMV disease defined by clinical symptoms indicative of CMV infection, classified either as

TABLE 1 | Genetic and clinic characteristics of patients.

Variables	Number (%) or median (range)
No	42
Median age (range), years	2.46 (0.5–15.1)
Sex	20 Female/22 Male
Time to HSCT from diagnosis (months)	8.0 (3.5–72.0)
Response to initial therapy	
CR	22 (52.4%)
PR	19 (45.2%)
NR	1 (2.4%)
HLA-compatibility	
5/10	26 (61.9%)
6/10	7 (16.7%)
7/10	7 (16.7%)
9/10	2 (4.8%)
Genetic mutation	
UNC13D	17 (40.5%)
PRF1	9 (21.4%)
XIAP	6 (14.3%)
STXBP2	5 (12.0%)
SH2D1A	2 (4.8%)
LYST	1 (2.4%)

viral syndrome (with symptoms such as fever, fatigue, leukopenia, and/or thrombocytopenia) or tissue-invasive disease.

2.6 | Statistical Methods

Descriptive statistics were calculated, with categorical variables expressed as frequency (percentage) and quantitative variables presented as median (range). Patients lost to follow-up were censored at the point of their last recorded engagement. A Kaplan–Meier curve was drawn to determine the survival pattern. The log-rank test was utilized to compare statistical significance, represented by *p*-values. All tests were two-sided, and the level of significance was set at 0.05. The 95% confidence interval (CI) was calculated using log transformation. The statistical analyses were conducted using GraphPad 8.0.2.

3 | Results

3.1 | Patient and Disease Characteristics

The characteristics of the 42 primary HLH pediatric patients are summarized in Table 1. The median age of the patients was 2.46 years (ranging from 0.5 to 15.1 years). The median time from diagnosis to transplantation was 8.0 months (ranging from 3.5 to 72.0 months). Genetic diagnosis was confirmed in 39 patients, with the distribution of different mutant genes as follows: *UNC13D* in 17 cases (40.5%), *PRF1* in nine cases (21.4%), *XIAP* in six cases (14.3%), *STXBP2* in five cases (12.0%), *SH2D1A* in two cases (4.8%), and *LYST* in one case (2.4%). Before transplantation, 22 patients (52.4%) were in CR, 19 patients (45.2%) were in PR, and one patient (2.4%) had advanced disease. Nine patients (21.4%) had evidence of CNS disease before HSCT. A total of 27 patients (64.3%) had infections prior to transplantation, including bacterial infections in 21 cases (50.0%), fungal infections in eight cases (19.0%), and viral infections in one case (2.4%).

3.2 | Engraftment

All the donors were HLA-haploidentical, and most had HLA compatibility of 5/10 ($n = 26$, 61.9%). Out of 42 patients, all underwent haplo-SCT following the modified protocol. The median CD34 dose (BM+PBSC) used was 4.79×10^6/kg (range: 1.17–13.40). All patients achieved engraftment. Of them, 38 (90.5%) patients achieved complete chimerism after the first transplant, while four (9.5%) patients had mixed chimerism. Primary graft failure occurred in four patients, all of whom proceeded to a second transplant and achieved complete chimerism. For patients with successful transplants (38 primary transplants and four second transplants), the median times to neutrophil and platelet recovery were 12.0 days (range: 7.0–24.0) and 16.0 days (range: 8.0–130.0), respectively.

3.3 | Graft-versus-Host Disease

Twenty-three of 42 patients (54.8%) experienced aGVHD. Grade I–II aGVHD was observed in 12 of 42 patients (28.6%; aGVHD I, $n = 6$; aGVHD II, $n = 6$), and Grade III–IV aGVHD was observed in 11 patients (26.2%; aGVHD III, $n = 7$; aGVHD IV, $n = 4$). The median time to aGVHD occurrence was 30 days (range: 14.0–69.0 days) (Figure 2A). The cumulative incidence of total aGVHD was 54.8% (95% CI: 40.0%–67.3%) (Figure 2B). The cumulative incidence of Grade III–IV aGVHD was 26.2% (95% CI: 8.9%–47.7%) (Figure 2E). Among the Grade I–II aGVHD patients, the skin was most commonly affected (10 cases), followed by the liver (five cases) and intestines (four cases). Among the Grade III–IV aGVHD patients, the intestines were most commonly affected (10 cases), followed by the liver (seven cases) and skin (six cases) (Figure 2A). cGVHD was observed in 22 of 42 patients (mild, $n = 11$; moderate, $n = 6$; severe, $n = 5$) (Figure 2D). The median time to cGVHD occurrence was 7.2 months (range: 2.0–95.3 months). The cumulative incidence of cGVHD was 57.2% (95% CI: 42.0%–69.9%) (Figure 2F). Most patients affected by GVHD responded well to corticosteroid treatment, and there were no deaths related to aGVHD, but one patient died at 5.6 months due to bronchiolitis obliterans syndrome.

3.4 | Infections After Transplant

At the time of HSCT, 17 out of 42 patients exhibited blood viral replication (EBV, $n = 13$; CMV, $n = 4$). After the transplant, viremia was detected in 35 out of 42 patients (83.3%). CMV was the most common, detected in 33 cases, followed by EBV in 11 cases, norovirus infection in one case, and varicella-zoster virus in one case. Among them, CMV disease occurred in seven cases, including CMV retinitis in three cases, CMV enteritis in two cases, CMV

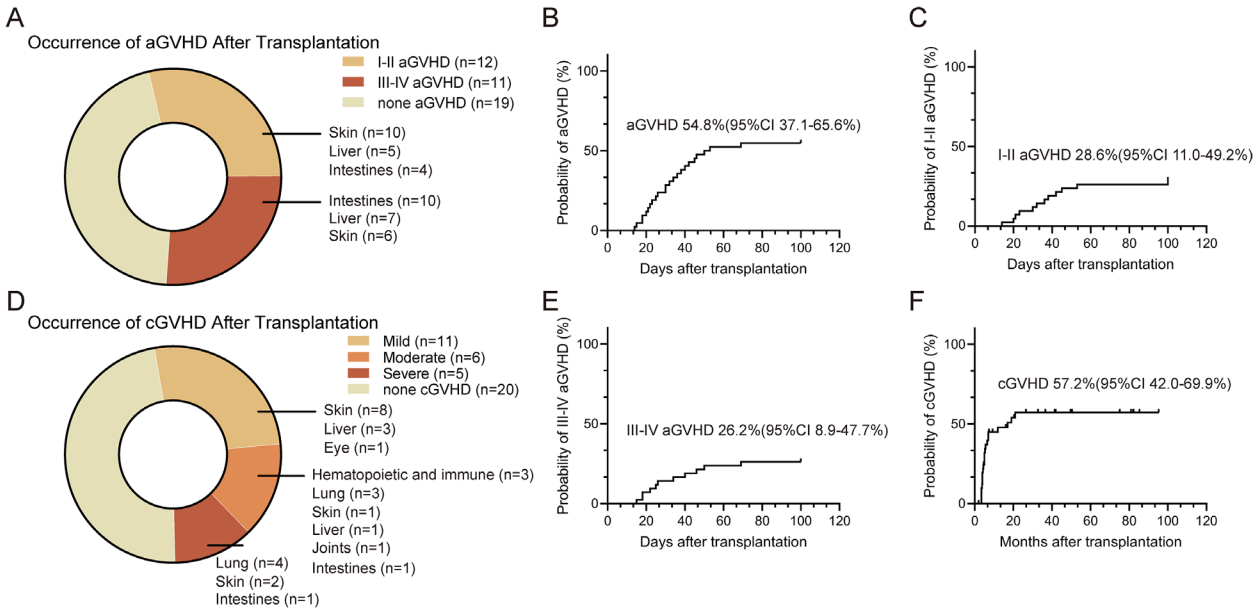

FIGURE 2 | GVHD features after transplantation. (A) Occurrence of aGVHD after transplantation. (B) Cumulative incidence of total aGVHD. (C) Cumulative incidence of Grade I–II aGVHD. (D) Occurrence of cGVHD after transplantation. (E) Cumulative incidence of Grade III–IV aGVHD. (F) Cumulative incidence of total cGVHD.

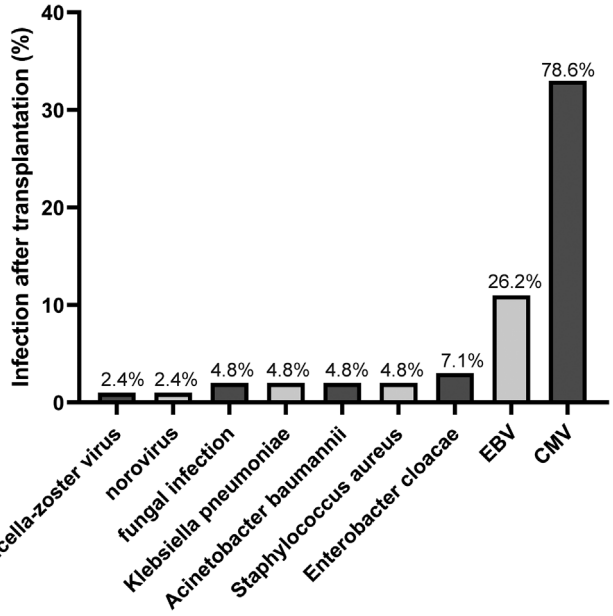

FIGURE 3 | Infection probability after transplantation.

pneumonia in one case, and CMV-related hemorrhagic cystitis in one case. In addition, there were eight cases of bacterial infection (*Klebsiella pneumoniae*, $n = 2$; *Acinetobacter baumannii*, $n = 2$; *Staphylococcus aureus*, $n = 2$; *Enterobacter cloacae*, $n = 2$) and two cases of fungal infection (Figure 3).

3.5 | Toxicity

TMA occurred in 10 out of 42 patients, with a median time to occurrence of 40.27 months (range: 1.17–95.30 months). The cumulative incidence of TMA was 24.6% (95% CI: 7.3%–47.1%). Among the 10 patients with TMA after transplant, the intestines were affected in six cases, the kidneys in six cases, the liver in three cases, and the brain in one case. Most cases were mild, but four patients experienced fatal TMA, leading to death. VOD occurred in two patients, at 76 and 10 days after haplo-SCT, respectively, no deaths caused by it.

3.6 | Long-Term Follow-Up

As of December 31, 2023 (a median follow-up of 48.7 months [range: 2.0–95.3]), 32 of the 42 patients were alive and disease free, including three of four patients who underwent a second transplantation. The 2-year OS rate was 78.4% (95% CI: 62.5%–88.1%), and the 5-year OS rate was 73.7% (95% CI: 54.9%–85.4%). The 2-year FFS rate was 71.3% (95% CI: 55.0%–82.6%), and the 5-year FFS rate was 66.5% (95% CI: 48.4%–79.5%) (Figure 4). There was no significant difference in OS between patients undergoing their initial haplo-SCT and those who received a rescue HSCT, but patients who achieved CR at the time of haplo-SCT showed a better OS than patients who achieved PR at the time of HSCT. Through univariate Cox regression analysis, we found CR at transplantation ($p = 0.024$, HR = 0.166, 95% CI: 0.035–0.785) was associated with better OS, and Grade III–IV aGVHD ($p = 0.0003$, HR = 12.30, 95% CI: 3.145–48.140) was a risk factor for OS and FFS. Subsequently, variables with $p < 0.05$ from the univariate analysis underwent multivariate Cox analysis. Upon adjusting for pertinent indicators, we determined that CR at transplantation ($p = 0.023$, HR = 0.163, 95% CI: 0.034–0.780) and Grade III–IV aGVHD ($p = 0.0003$, HR = 12.311, 95% CI: 3.096–48.963) were independent risk factors for OS in pediatric HLH patients who received haplo-allo-SCT (Table 2).

Ten patients died after a median OS of 6.8 months (range: 2.0–53.2) after transplantation. Only one patient (2.4%) died within

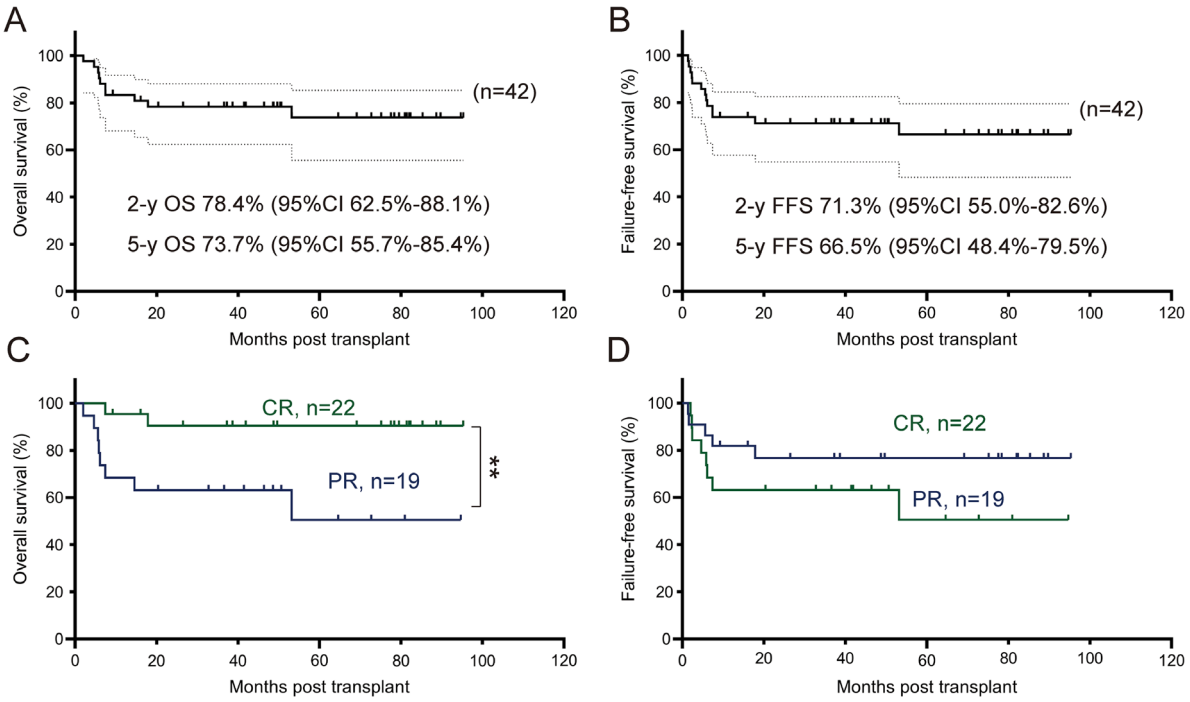

FIGURE 4 | Kaplan–Meier curve estimated overall survival and event-free survival rates after transplantation. (A) Overall survival. (B) Failure-free survival. (C) Overall survival of patients achieved complete response (CR) or partial response (PR) at the time of HSCT. (D) Failure-free survival of patients achieved CR or PR at the time of HSCT.

TABLE 2 | Multivariate Cox regression analysis.

	Univariate			Multivariate		
Characteristic	**95% CI**	**HR**	***p***	**95% CI**	**HR**	***p***
Sex	0.159–2.007	0.564	0.376			
Age	0.915–1.241	1.066	0.411			
CR at transplantation	**0.035–0.785**	**0.166**	**0.024**	**0.034–0.780**	**0.163**	**0.023**
Time between diagnosis and transplantation	0.944–1.043	0.992	0.760			
CD34 dose	0.566–1.136	0.802	0.214			
Time to neutrophil engraftment	0.905–1.188	1.037	0.603			
Time to platelet engraftment	0.993–1.026	1.010	0.253			
Grade I–II aGVHD	0.124–2.742	0.582	0.494			
Grade III–IV aGVHD	**3.145–48.140**	**12.30**	**0.0003**	**3.096–48.963**	**12.311**	**0.0003**
cGVHD	0.339–4.273	1.203	0.775			
Viral infection after transplantation	0.311–19.410	2.457	0.394			
TMA after transplantation	0.785–9.888	2.787	0.113			

100 days post transplantation due to a severe pulmonary infection. The main causes of deaths included severe infection ($n = 4$), TMA ($n = 4$), gastrointestinal bleeding ($n = 1$), and bronchiolitis obliterans syndrome ($n = 1$).

4 | Discussion

HSCT remains the only curative treatment for patients with primary HLH. However, in light of the risks associated with infections, the probability of early disease recurrence, and the risk of severe early-onset CNS complications, it is imperative to perform HSCT in HLH patients as early as possible. For these patients, an HLA-haploidentical donor is an attractive option, and the use of G-CSF/ATG-based regimens have shown favorable outcomes in haplo-SCT and hematological diseases [27]. Nonetheless, Data on its application in primary HLH pediatric patients are still scarce.

Here, we report a retrospective analysis of 42 pediatric patients with primary HLH who underwent haplo-SCT using the modified

G-CSF/ATG-based protocol. Our data showed that this protocol offers an effective treatment for long-term disease control and patient survival in pediatric primary HLH patients. In our study, a high rate of engraftment was noted, with all 42 patients achieving engraftment. During a median follow-up period of 48.7 months, the 2- and 5-year OS rates were 78.4% and 73.7%, respectively, and the 2- and 5-year FFS rates were 71.3% and 66.5%, respectively. These outcomes are comparable to those reported using MSD or URD donors in HLH [11, 14, 28–30]. Patients who achieved CR at the time of haplo-SCT showed a better OS than those who achieved PR at the time of haplo-SCT, suggesting that effectively controlling disease progression could improve allo-HCT outcomes. In addition, a high long-term OS rate was also seen in our HLH patients with CNS involvement. Nine patients (21.4%) had CNS involvement prior to haplo-SCT. Post transplant, their CNS symptoms improved to varying degrees. Patients with a history of seizures received prophylactic antiepileptic medications for 1 year after transplantation. Of the nine patients, four had no CNS symptoms after transplantation and achieved complete recovery, with learning abilities comparable to their peers. The remaining five patients continued to experience seizures, and one patient unfortunately died 183 days post transplant due to severe pulmonary infection. The other four required long-term antiepileptic treatment. Among these, one patient, who was comatose and in status epilepticus prior to transplant and did not respond to the transplant (NR), continues to experience recurrent seizures nearly 5 years post transplant, requiring three antiepileptic medications (levetiracetam, lamotrigine, and perampanel). The seizures of the other three patients are controlled with levetiracetam, and two of them have returned to school. All patients are regularly monitored in a joint neurology-transplantation clinic, where their neurological symptoms and neurodevelopment are assessed. Antiepileptic medications are adjusted as needed, and rehabilitation is provided accordingly.

In parallel studies focusing on pediatric patients with primary HLH, Al-Mofareh et al. described their cohort of 35 children with Griscelli syndrome Type 2 who underwent allogeneic HSCT. Their treatment protocol included a conditioning regimen comprising busulfan, cyclophosphamide, and etoposide. With a diversity of donor types, the study included 19 patients (54.3%) who received HLA-matched related marrow and 14 patients (40%) who were recipients of partially mismatched unrelated cord blood (UCB). The 5-year OS rate was 62.7%. Particularly, HLH patients in this cohort exhibited a more unfavorable prognosis, with a 5-year OS rate of only 53.3% ± 9.5% [14]. Another study by Greental et al. reported a retrospective analysis of 45 children with HLH who underwent allo-HSCT following either myeloablative or reduced-intensity conditioning. The majority of these children received HLA-matched (n = 26) or partially mismatched (n = 7) allo-HSCT, with a 5-year OS rate of 86% [30], exhibiting comparable outcomes to our study. Furthermore, Ouachée-Chardin et al. examined a larger cohort of 48 patients with HLH who underwent HSCT. In this group, 27 (56.2%) patients received haplo-SCT utilizing T-cell depleted strategies. They found a comparable overall event-free survival (EFS) rate between patients who received haplo-SCT and those with matched sibling donors (MSD) or unrelated donors (URD), with an OS rate of 58.5% [11].

Moreover, patients with HLH are known to be at high risk for VOD post transplant, attributed to the systemic hyperinflammatory syndrome, long-term infections, and pretransplant chemotherapy. In the study by Al-Mofareh et al., 20% (7/35) of HLH patients developed VOD following HSCT. Similarly, Ouachée-Chardin et al. reported a VOD incidence of 28% (17/60) in HLH patients post HSCT [11, 14]. Studies have found that the risk of VOD appears to be even greater in patients who undergo haplo-SCT [11]. In our study, we modified the conditioning regimen with 300–600 mg/m^2 VP16 to further control the HLH and prevent relapse during the transplantation, along with low doses of Bu, Cy, and Flu, aiming to mitigate the risk of transplantation complications. We observed few vascular complications in our cohort, with two cases (4.8%) of VOD.

Although the incidence of Grade III–IV aGVHD in our cohort was 26.2%, most cases responded well to corticosteroids, and there were no deaths caused by it. The occurrence of GVHD may be attributed to the T-cell repletion strategies employed in our cohort, and other proinflammatory conditions, such as active viral infections at the time of HSCT, donor type, and degree of match, which are also related to a higher risk of aGVHD. Our data demonstrate that although Grade III–IV aGVHD could be effectively managed post transplant, its occurrence still remains an independent prognostic factor for OS. These findings highlight the critical need to prevent aGVHD following transplantation.

Despite a relatively high incidence of EBV/CMV viremia post HSCT in our cohort, most cases were effectively managed, leading to no significant increase in mortality rates. This highlights the importance of rigorous monitoring and early interventions against viral infections during the post-transplant period.

In summary, our findings support the use of haplo-SCT with a modified G-CSF/ATG-based conditioning regimen as an effective and relatively safe treatment option for pediatric patients with primary HLH. However, to validate these preliminary results, future studies involving larger, multicenter cohorts and randomized controlled trials are essential. Further research should also include more comprehensive comparative analyses with other alternative haplo-SCT strategies, including high-dose post-transplantation cyclophosphamide protocols and other reduced-intensity/reduced-toxicity conditioning regimens. Such studies would provide a deeper and more holistic understanding of the role of haplo-SCT in treating pediatric HLH.

Author Contributions

Conceptualization: Y.S. and J.X. Data curation: J.X., N.W., S.F, Z.L., F.J., and J.C. Formal analysis: J.X. and X.Y. Investigation: J.X., N.W., S.F, Z.L., F.J., and J.C. Methodology: Y.S. and J.W. Project administration: Y.S. Resources: Y.S. and J.X. Supervision: Y.S., J.W., and J.X. Visualization, writing—original draft: J.X. and X.Y. Writing—review and editing: all authors.

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Ethics Statement

The study was carried out in accordance with the principles of the Declaration of Helsinki and its later amendments or comparable ethical standards, and was approved by the Medical Ethics Committee of Beijing Jingdu Children's Hospital through established procedures (approval number 2023-Y-002). Data of interest collected from the patients' medical records were secured as governed by the institutional policies on patient confidentiality and privacy. No informed consents were obtained since this was a retrospective review of data and all data items collected were already documented in medical charts as part of the patients' care and disease management documentation.

Conflicts of Interest

The authors declare no competing interests, and this work did not receive any financial support in any form from any funding agency.

Data Availability Statement

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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