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Favorable outcomes with durable chimerism after hematopoietic cell transplantation using busulfan and fludarabine-based reduced-intensity conditioning for pediatric patients with hemophagocytic lymphohistiocytosis

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Background
The incorporation of a reduced-intensity conditioning (RIC) regimen in hematopoietic cell transplantation (HCT) for patients with hemophagocytic lymphohistiocytosis (HLH) has decreased early mortality but is associated with a high rate of mixed chimerism and graft failure. Here, we present a successful single-center experience using busulfan and a fludarabine-based RIC regimen for the treatment of HLH.

Methods
The medical records of pediatric patients with HLH who underwent HCT using a busulfan/fludarabine-based RIC regimen between January 2008 and December 2017 were reviewed retrospectively.

Results
Nine patients received HCT with a busulfan/fludarabine-based RIC regimen. Three patients had primary HLH, and the other six patients had secondary HLH with multiple reactivations. All three patients with primary HLH had *UNC13D* mutations. All patients achieved neutrophil and platelet engraftment at a median of 11 days (range, 10–21) and 19 days (range, 13–32), and all eight evaluable patients had sustained complete donor chimerism at the last follow-up. Two patients (22%) experienced grade 2 acute graft-versus-host disease (GVHD). Two patients (22%) developed chronic GVHD, and one died from chronic GVHD. One patient (11%) experienced reactivation 4 months after HCT from a syngeneic donor and died of the disease. The 8-year overall survival and event-free survival rates were 78%. No early treatment-related mortality within 100 days after HCT was observed.

Conclusion
Our experience suggests that a busulfan/fludarabine-based RIC regimen is a viable option for pediatric patients with HLH who require HCT.

Key Words Hematopoietic stem cell transplantation, Pediatric, Hemophagocytic lymphohistiocytosis

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a severe, often overwhelming, systemic hyperinflammatory syndrome. HLH can be grouped into primary forms, with underlying genetic susceptibility to developing HLH, and secondary forms, with no identified genetic causes. Chemoimmunotherapy has markedly improved outcomes in HLH. However, primary HLH cannot be cured with chemoimmunotherapy,

and further curative measures are required to correct genetic susceptibility.

Allogeneic hematopoietic cell transplantation (HCT) is the only curative treatment for primary forms of HLH, including its multiple reactivated secondary forms [1-3]. Earlier experiences of HCT in HLH with myeloablative conditioning (MAC) approaches reported significant treatment-related mortality (TRM), with overall-survival (OS) rates ranging from 43% to 65% [4-10]. Most mortalities occurred in the first 6 months after HCT with MAC regimens.

High TRM following MAC has prompted the use of reduced-intensity conditioning (RIC) regimens. In particular, RIC regimens containing alemtuzumab, fludarabine, and melphalan improved OS by over 80% with a lower incidence of TRM than traditional busulfan and cyclophosphamide-based MAC regimens [1, 9, 11-13]. However, substantial graft failure rate and mixed chimerism remain to be issues [2, 13, 14].

Therefore, we designed a fludarabine-based RIC regimen containing a submyeloablative dose of busulfan and cyclophosphamide with or without anti-thymocyte globulin (ATG). Therefore, we evaluated the safety and effectiveness of fludarabine- and busulfan-based RIC regimens before allogeneic HCT in patients with primary or refractory HLH.

MATERIALS AND METHODS

Patients

We retrospectively reviewed the medical records of pediatric patients with HLH who underwent allogeneic HCT with a busulfan/fludarabine-based RIC regimen between January 2008 and December 2018. The Institutional Review Board of Asan Medical Center approved the procedure for reviewing medical records (2013–0781). Patients diagnosed with primary HLH or those who experienced multiple reactivations with no pathognomonic genetic defects received allogeneic HCT as a definitive treatment. The diagnosis and reactivation of HLH were defined using the HLH 2004 diagnostic criteria [15]. PCR-based direct sequencing analysis involving all coding exonic sequences and their flanking intronic sequences of *PRF1*, *UNC13D*, and *STX11* genes were performed at diagnosis to screen primary HLH, and patients whose genetic testing revealed no genetic abnormality were designated as having presumed secondary HLH.

Treatment

All patients diagnosed with HLH were treated with chemoimmunotherapy according to the HLH 2004 protocol containing dexamethasone, etoposide, and cyclosporine [15]. The conditioning regimen included rabbit ATG (Thymoglobulin) on days 9 to 7 (7.5 mg/kg), fludarabine on days 8 to 4 (150 mg/m^2), busulfan on day 5 to 4 (6.4 mg/kg), and cyclophosphamide on days 3 to 2 (60 mg/kg).

For graft-versus-host disease (GVHD) prophylaxis, patients received cyclosporine one day before infusion and mycophenolate mofetil from the day of infusion. Granulocyte-colony stimulating factor was started on day 5 until the absolute neutrophil count (ANC) reached 3.0×10^9/L. For infection prophylaxis, patients received micafungin for fungi, acyclovir or ganciclovir for viruses, and trimethoprim-sulfamethoxazole or pentamidine for Pneumocystis jiroveci. Chimerism was routinely checked on D28, D90, D180, D360, and then annually.

Definitions and post-HCT monitoring

Neutrophil and platelet engraftments were defined as achieving an ANC $\geq0.5\times10^9$/L for 3 consecutive days with no evidence of autologous recovery and achieving a platelet count $\geq20\times10^9$/L without transfusion support for 7 days, respectively. Disease status was defined according to the following criteria: complete response (CR), normalization of all diagnostic clinical and laboratory abnormalities associated with HLH; partial response (PR), sustained normalization of three or more of the diagnostic criteria previously validated and no apparent progression of other criteria; and nonresponse (NR), normalization of two or fewer diagnostic criteria or clear progression of other aspects of HLH disease. Complete donor chimerism was defined as the presence of $\geq$95% leukocytes of donor origin in the peripheral blood or bone marrow, mixed chimerism as the presence of $\geq$5% autologous cells, and graft failure as the absence of hematopoietic cell recovery at day 42 or autologous reconstitution. Diagnosis and grading of acute and chronic GVHD were performed using established criteria [16, 17]. EBV and CMV infection or reactivation was periodically monitored using quantitative PCR.

Statistical analysis

OS was defined as the time between HCT and death or last follow-up. To estimate event-free survival (EFS), death from any cause and relapse (whichever occurred first) were considered events. The Kaplan–Meier method was used to estimate survival rates, and all results were expressed as the estimated probability of survival with a 95% confidence interval. Statistical analyses were performed using IBM SPSS Statistics for Windows version 24 (IBM).

RESULTS

Patient and transplant characteristics

Nine patients with HLH underwent busulfan/fludarabine-based RIC HCT between January 2008 and December 2017. Three patients had primary HLH, and the other six had multiple reactivated secondary HLH. All three patients with primary HLH had mutations in *UNC13D*. Two out of six patients with secondary HLH had underlying conditions, such as chronic active EBV infection (CAEBV), autoimmune disease, and systemic lupus erythematosus (SLE). Five patients received HCT from matched sibling donors (MSDs) and four received HCT from unrelated donors (URDs). Of the five patients who received HCT from MSDs, one received an urgent syngeneic sibling at his third

reactivation. Eight patients (89%) experienced HLH reactivation prior to HCT, and five patients (56%) experienced two or more reactivation events. Patients received HCT at a median of 6.1 months (range, 3.1–28.7) after a diagnosis of HLH. Eight patients, except one who received HCT from a syngeneic sibling donor, achieved more PR at the time of HCT: five patients achieved CR and three patients achieved PR (Table 1).

Engraftment and chimerism

A median of 8.35×10^6/kg of CD34 cells (range, 2.84–11.91) was infused into patients. All patients achieved neutrophil and platelet engraftment at a median of 11 days (range, 10–21) and 19 days (range, 13–32) after HCT. Of the nine patients, all eight patients, except one who received HCT from a syngeneic donor and could not be assessed for chimerism, continued to have sustained complete donor chimerism at the time of the last evaluation. Seven patients rapidly achieved complete donor chimerism, while one had mixed chimerism (74%) until 3 months after HCT. The patient finally achieved complete chimerism without additional intervention, such as donor lymphocyte infusion (DLI) and a second HCT (Table 2).

GVHD and acute complications

Table 3 shows transplant outcomes. Two (22%) patients, who received HCT due to multiple reactivated secondary HLH, developed acute GVHD; one had grade 2 skin GVHD, and the other one had grade 2 skin and gut GVHD, which were resolved after using conventional steroid therapy with calcineurin inhibitors. Two (22%) other patients developed chronic GVHD: one patient with secondary HLH and CAEBV had extensive chronic GVHD involving the lung, liver, skin, mouth, and eyes and died from multi-organ failure during immunosuppressive therapy 2 years after HCT, and the other patient had limited disease involving the skin and mouth, which resolved with local treatment. Five patients experienced CMV reactivation. All five patients received preemptive antiviral treatment, and no patient experienced CMV disease. Two patients developed EBV reactivation, and no patient experienced post-transplant lymphoproliferative disease. Two patients developed BK virus-associated hemorrhagic cystitis and recovered after supportive care. No other fatal acute complication including veno-occlusive disease

Table 1. Patient characteristics before HSCT.

Patient no.	Sex	Age at diagnosis, years	Diagnosis	Genetic mutation or underlying cause	No. of reactivation before HSCT	Duration of chemoimmunotherpy, months	Status at HSCT
1	M	0.3	Primary HLH	*UNC13D*	2	10.3	PR
2	M	7.6	Secondary HLH	Not identified	1	2.1	CR
3	F	0.6	Primary HLH	*UNC13D*	2	4.6	CR
4	M	9.9	Secondary HLH	Not identified	3	2.5	NR
5	M	8.5	Secondary HLH	EBV associated	1	2.3	CR
6	F	0.2	Primary HLH	*UNC13D*	0	5.3	CR
7	M	13.2	Secondary HLH	CAEBV	2	4.3	PR
8	F	1.9	Secondary HLH	EBV associated	2	4.6	CR
9	F	11.5	Secondary HLH	SLE	2	4.0	PR

Abbreviations: CAEBV, chronic active EBV infection; CR, complete response; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; NR, non-response; PR, partial response; SLE, systemic lupus erythematosus.

Table 2. Count of infused CD34+ cells and donor chimerism of each patient.

Patient no.	CD34+ cells, 10^6/kg	Donor chimerism, %					
		1 mo	2 mo	3 mo	6 mo	1 yr	2 yr
1	8.77	100	100	100	100	100	100
2	6.54	90	74	74	100	100	100
3	8.35	100	100	98	100	100	100
4	11.91	Syngeneic					
5	9.17	100	100	96	100	100	100
6	6.17	99	NA	95	100	100	100
7	2.84	95	100	100	100	100	Dead
8	10.05	98	100	100	100	100	100
9	7.46	99	NA	98	100	100	100

Abbreviation: NA, not available.

Table 3. Transplant characteristics and outcomes.

Patient no.	Time from diagnosis to HSCT, mo	Type of donor	Conditioning regimen	Acute GVHD	Chronic GVHD	[a]Performance score before HSCT, %	[a]Performance score after HSCT, %	Disease status	Survival
1	12.2	URD	FluBuCy	No	No	50	90	CR	Alive
2	28.7	MSD	FluBuCy	No	Yes, limited	70	100	CR	Alive
3	4.8	MSD	FluBuCy	No	No	90	100	CR	Alive
4	7.4	MSD (syngeneic)	FluBuCyATG	No	No	70	-	NR	Dead (DOD)
5	5.4	URD	FluBuCyATG	Yes (Gr 2, skin)	No	90	100	CR	Alive
6	6.1	URD	FluBuCyATG	No	No	90	100	CR	Alive
7	3.1	MSD	FluBuCy [b]VpATG	No	Yes, extensive	70	-	CR	Dead (TRM)
8	5.0	MSD	FluBuCy [b]VpATG	No	No	90	100	CR	Alive
9	28.2	URD	FluBuCyATG	Yes (Gr 2, skin and gut)	No	70	80	CR	Alive

[a]Performance score was evaluated using the Lansky score, which was not provided to patients who died. [b]Patients who developed HLH flare during conditioning received additional doses of VP-16 and dexamethasone.
Abbreviations: CR, complete response; DOD, death from disease; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; MSD, matched sibling donor; NA, not available; NR, nonresponse; PR, partial response; TRM, treatment-related mortality; URD, unrelated donor.

(VOD) was noted.

Reactivation and survival outcomes

Of the nine patients, eight had a sustained CR state without HLH reactivation. One patient with refractory secondary HLH, who received HCT from a syngeneic sibling donor, developed HLH reactivation 4 months after HCT and died of disease 7 months after HCT.

Although only one patient experienced disease relapse, three other patients developed some degree of immune reaction that was difficult to differentiate from HLH reactivation or disease relapse. Two patients (patients 7 and 8) developed fever and liver dysfunction 4 days prior to infusion and recovered after receiving additional etoposide and dexamethasone while receiving conditioning chemotherapy. The other patient (patient 9) developed fever, pleurisy, and dry eyes with increased ANA and anti-dsDNA antibody titers, which did not fulfill the HLH reactivation criteria at 9 months after HCT and improved after receiving prednisolone and hydroxyquinolone.

During the median 8.0-year follow-up (range, 0.5–12.7), the 8-year OS and EFS were both 78%. Two patients died: one due to treatment-related causes and the other due to disease reactivation. TRM due to acute toxicity within 100 days of HCT was not observed.

Quality of life

Six of the nine patients were alive and disease-free, with Lansky scores of 90% to 100% at a median of 8.0 years from HCT (Table 3). One patient (patient 9), who developed inflammatory reactions considered as a post-HCT autoimmune disease 9 months after HCT, was alive with a Lansky score of 80%. The patient underwent HCT for a secondary HLH and underlying SLE. The patient recovered from post-HCT autoimmune disease after receiving systemic steroids and hydroxyquinolone but underwent total hip replacement surgery due to steroid-induced osteonecrosis.

DISCUSSION

HLH is a life-threatening immunodeficiency characterized by severe systemic hyperinflammatory responses to infectious or other immune system triggers. Allogeneic HCT is the only curative treatment for HLH caused by genetic defects and multiple reactivated diseases. However, HCT in these patients is challenging because of pre-existing organ dysfunction, active infection, and persistent immune activation [13]. HCT with MAC regimens containing busulfan, cyclophosphamide, and etoposide resulted in high rates of TRM (OS, 43–65%), particularly associated with VOD [2, 4-9, 14, 18]. The advent of RIC regimens has led to substantial survival improvements, with a favorable toxicity profile and very low rates of early lethal toxicity, such as VOD. Most previous studies on HCT using RIC regimens in patients with HLH were based on melphalan as an alkylating agent combined with fludarabine. In these studies, patients had a significantly improved survival (OS, 51–92%) compared to those in MAC studies [11-14, 19] but had high rates of mixed chimerism (30–100%) and frequent need for secondary cellular therapy, including DLI and second HCT [13, 14, 19-21]. A recent study of HCT using a melphalan/fludarabine-based RIC regimen in 46 pediatric patients with HLH and primary immune deficiencies showed 1-year and 18-month OS rates of 80.4% and 66.7%, respectively. The incidence of acute GVHD was more than grade 2 and that of chronic GVHD was 17.4% and 26.7%, respectively. However, 43% of patients experienced graft failure or re-

quired a second intervention [13]. To overcome the increased risk of graft failure, investigators incorporated additional chemotherapeutic agents such as thiotepa and serotherapy agents such as alemtzutzumab and ATG in previous studies [1, 13, 22]. For serotherapy, it is well recognized that dose and timing in relation to the transplant have an impact not only on engraftment but also on the occurrence of GVHD, immune reconstitution, and viral reactivation [23]. We adopted a relatively high dose (7.5 mg/kg) of ATG in the distal part of conditioning to reduce GVHD occurrence, as well as to enrich engraftment.

However, risks and complications associated with unstable engraftment remain problematic. Some experts have advocated that further optimization using alternative chemotherapy such as treosulfan, sub-myeloablative conditioning including busulfan and thiotepa, and immunotherapy using anti-interferon-γ antibody and alemtuzumab could improve survival and lead to sustained engraftment [3, 13, 14, 22]. It is difficult to conclude which regimen is better for pediatric patients with HLH. Lack of experience with HCT in pediatric HLH patients impedes a direct comparison of each regimen.

In our study, RIC using fludarabine and sub-myeloablative busulfan resulted in excellent engraftment and chimerism outcomes, with all evaluable patients achieving neutrophil and platelet engraftment in complete donor chimerism without additional cellular therapy after HCT, except for one patient who received a syngeneic donor HCT and could not be assessed for chimerism. This result is promising compared to those of previous studies of HCT using both RIC and MAC regimens.

In addition to successful engraftment outcomes, favorable long-term survival is encouraging. For a relatively long median follow-up period of 8.0 years, the 8-year OS and EFS rates were both 78%. Most patients (78%) survived disease-free at the time of the last follow-up, with a favorable quality of life after HCT in terms of the Lansky score.

However, some patients (3/9) experienced some degree of inflammatory reaction during the conditioning period, which might be due to residual inflammation that could not be sufficiently suppressed by pre-HCT treatment. Since all three patients had more than twice the reactivation rate prior to HCT, additional strategies to mitigate residual inflammatory reactions, including alternative chemotherapy, immunotherapy, and targeted therapy, may be performed concurrently with HCT in high-risk patients.

Given the high rates of sustained donor chimerism and favorable long-term survival with improved quality of life, the busulfan/fludarabine-based RIC regimen is a viable option for pediatric patients with HLH who require HCT. However, further refinement is needed to control residual inflammation throughout HCT in patients at a high risk of reactivation. However, this study has limitations. It is a small, single-center retrospective study, and a larger prospective multicenter study and a study for direct comparison of conditioning regimens are needed to validate this observation.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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