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Outcomes of Children with Hemophagocytic Lymphohistiocytosis Given Allogeneic Hematopoietic Stem Cell Transplantation in Italy

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A B S T R A C T

We report on 109 patients with hemophagocytic lymphohistiocytosis (HLH) undergoing 126 procedures of allogeneic hematopoietic stem cell transplantation (HSCT) between 2000 and 2014 in centers associated with the Italian Pediatric Hematology Oncology Association. Genetic diagnosis was *FHL2* (32%), *FHL3* (33%), or other defined disorders known to cause HLH (15%); in the remaining patients no genetic abnormality was found. Donor for first transplant was an HLA-matched sibling for 25 patients (23%), an unrelated donor for 73 (67%), and an HLA-partially matched family donor for 11 children (10%). Conditioning regimen was busulfan-based for 61 patients (56%), treosulfan-based for 21 (20%), and fludarabine-based for 26 children (24%). The 5-year probabilities of overall survival (OS) and event-free survival (EFS) were 71% and 60%, respectively. Twenty-six patients (24%) died due to transplant-related causes, whereas 14 (13%) and 10 (9%) patients experienced graft rejection and/or relapse, respectively. Twelve of 14 children given a second HSCT after graft failure/relapse are alive and disease-free. Use of HLA-partially matched family donors was associated with higher risk of graft failure and thus with lower EFS (but not with lower OS) in multivariable analysis. Active disease at transplantation did not significantly affect prognosis. These data confirm that HSCT can cure most HLH patients, active disease not precluding successful transplantation. Because in HLH patients HLA-haploidentical HSCT performed through $CD34^+$ cell positive selection was found to be associated with poor sustained engraftment of donor cells, innovative approaches able to guarantee a more robust engraftment are warranted in patients given this type of allograft.

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INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening, hyperinflammatory syndrome characterized by cytopenia, fever, hepatosplenomegaly, coagulation disorders, and multiorgan dysfunction. It affects children and adolescents with a higher incidence in the first years of life. HLH can be triggered by infection, autoimmune disease, or cancer. In one-third of cases primary immune deficiency resulting in impaired killing of infected cells by T cells or natural killer cells is present (familial HLH [fHLH]) [1]. The genetic defect underlying fHLH results in impaired formation and release of cytotoxic granules and is caused by genes directly implicated in the secretory lysosome-dependent exocytosis pathway (*PRF1* in FHL2, *UNC13D* in FHL3, *STX11* in FHL4, *STXBP2* in FHL5) [2]. HLH can also be part of clinical syndromes with other associated manifestations, such as Chédiak-Higashi syndrome, Griscelli syndrome type 2, Hermansky-Pudlak syndrome type 2, and X-linked lymphoproliferative disease types 1 and 2 [3]. Approximately 70% of fHLH in Southern Europe is caused by *PRF1* and *UNC13D* mutations [4].

Chemoimmunotherapy with dexamethasone, etoposide, and cyclosporine A can control the inflammatory manifestation in around 60% to 80% of the cases [5-7]. However, in patients with familial/genetic, relapsing, or severe and persistent disease, allogeneic hematopoietic stem cell transplantation (HSCT) is the only established curative treatment [8].

HSCT in a patient with HLH was first reported in 1986, and many case series have since then been described [9]. Significant transplant-related mortality (TRM) was reported in earlier experiences, with an overall survival (OS) ranging between 45% and 65% [10-12]. This observation has prompted the use of conditioning regimens less toxic than the traditional busulfan-based myeloablative regimen. The use of fludarabine or treosulfan permitted to gradually reduce TRM with better outcomes [13,14]. The major drawbacks related to the use of less toxic regimens are a relevant incidence of mixed chimerism and overt rejection [15,16]. In this study we present the outcomes of a cohort of 109 patients affected by HLH who underwent HSCT in centers affiliated with the Italian Paediatric Haematology Oncology Association (AIEOP) network between 2000 and 2014.

METHODS

In this study we collected data reported to the AIEOP Stem Cell Transplantation Registry and selected patients according to all the following criteria: (1) diagnosis of fHLH, a genetic disorder predisposing to HLH, or clinical HLH without genetic markers not responding to chemoimmunotherapy treatment or relapsing after treatment [17]; (2) HSCT performed in 1 of the centers participating in the AIEOP network; and (3) transplantation date between January 1, 2000 and December 31, 2014. Whenever indicated, the centers were contacted for further information about patient status before HSCT, details of the procedure, and outcomes. We excluded patients without adequate data available. Forty-two patients included in this cohort (38.5% of the overall population) have been previously reported [18].

Patients or their legal guardians signed written informed consent for collection, analysis, and publication of relevant data. Genetic diagnosis was centrally performed at Meyer Children Hospital, Firenze, Italy, as previously described [1].

Central nervous system (CNS) involvement was considered present if a patient had any of the following findings: elevated cerebrospinal fluid (CSF) WBC count, clinical symptoms consistent with CNS involvement (ie, seizures or focal or global neurologic deficit), or magnetic resonance imaging abnormalities consistent with CNS involvement. Patient status before HSCT was defined according to the following criteria: complete response, normalization of all diagnostic clinical and laboratory abnormalities associated with HLH; partial response, sustained normalization of 3 or more of the diagnostic parameters previously validated [17] and no apparent progression of other parameters; and nonresponse, normalization of 2 or less diagnostic parameters or clear progression of other aspects of HLH disease.

After HSCT, disease relapse was defined as recurrence of symptoms typical of HLH with re-establishment of recipient hematopoiesis. Rejection was defined as immunologically mediated graft failure.

Definitions and Statistical Analysis

Primary endpoint was event-free survival (EFS), defined as the probability of being alive and in continuous complete remission (CR) at last follow-up. To estimate EFS probability death from any cause, relapse, and graft failure (whichever occurred first) were considered events. Occurrence of stable mixed chimerism without signs and symptoms of HLH was not considered an event. Full donor chimerism was defined as presence ≥ 95% leukocytes of donor origin in peripheral blood or bone marrow. Secondary endpoints were OS, time to neutrophil and platelet recovery, incidence of relapse, TRM, and acute and chronic graft-versus-host disease (aGVHD and cGVHD). Probabilities were calculated from date of transplantation until the event or last follow-up.

Neutrophil engraftment was defined as achieving an absolute neutrophil count $\geq .5 \times 10^9$/L for 3 consecutive days with no evidence of autologous recovery (ie, <5% leukocytes of donor origin in peripheral blood or marrow). Platelet engraftment was defined as achieving a platelet count $\geq 20 \times 10^9$/L unsupported through platelet transfusions for 7 days. aGVHD occurrence was evaluated in all patients, whereas cGVHD was evaluated only in patients surviving beyond day +100 after HSCT. aGVHD and cGVHD were graded according to previously published criteria [19,20].

Quantitative variables were reported as median value and range, whereas categorical variables were expressed as absolute value and percentage. Probabilities of EFS and OS were calculated using the Kaplan-Meier estimates. Cumulative incidence functions were used to estimate incidence of relapse and TRM in a competing risks setting, because death and relapse compete with each other. To estimate aGVHD and cGVHD incidences, relapse and death were considered as competing events.

A comparison with 2-sided $P < .05$ was considered to be statistically significant. Variables reaching $P < .10$ in univariate analysis for EFS estimations were reported in detail and included in Cox proportional hazard regression models using a backward stepwise selection. Statistical analysis was performed using NCSS 10 statistical software (2015; NCSS, LLC., Kaysville, UT [www.ncss.com/software/ncss]) and R 2.5.0 software package (http://www.R-project.org) [21,22]. Analysis used January 31, 2016 as the reference date.

RESULTS

Patient Population

One hundred twelve patients with HLH who underwent 129 transplant procedures were reported the AIEOP HSCT registry. Three patients were not assessable for this study due to lack of data; thus, the final analysis included 109 patients and 126 transplant procedures performed in 16 AIEOP centers. Sixty-five patients (60%) were males and 44 (40%) were females. Median age at diagnosis was 1 year (range, 27 days to 18 years), whereas median age at first transplantation was 2 years (range, 4 months to 20 years). Mean time interval between diagnosis and first HSCT was 289 days (range, 26 to 1844). The median time interval between diagnosis and transplantation was 160 days (range, 35 to 1844) for patients with a genetic abnormality known to cause HLH and 237 days in the remaining patients (range, 26 to 553; not significant). Patient and HSCT characteristics are summarized in Table 1.

Genetic testing was performed for 94 of 109 patients (86%). Mutation of *PRF1* was found in 31 patients (32%), of *UNC13D* in 32 patients (33%), of *STXBP2* in 2 patients (2%), of *RAB27A* in 6 patients (6%), of *SH2D1A* in 5 patients (5%), of *BIRC4* in 2 patients (2%), and of *LYST* in 1 patient (1%). No known gene abnormality was found in 15 patients. CNS involvement at diagnosis was recorded for 79 patients (72% of the overall population) and was present in 30 patients (38%): 17 (22%) had elevated cerebrospinal fluid WBC count, 20 (25%) had clinical symptoms consistent with HLH, and 7 (9%) had magnetic resonance imaging abnormalities consistent with HLH.

At diagnosis, 9 patients were enrolled in the HLH-94 protocol [5], 41 patients were enrolled in the HLH-04 trial [7,18],

Table 1
Patient and Transplant Characteristics

	No. of Patients or Median	Percentage or Range
Gender		
Male	65	60
Female	44	40
Genetic diagnosis		
FHL2	31	28
FHL3	32	29
Griscelli syndrome	6	5
XLP1	5	5
Other	7	7
No known genetic defect	15	14
Study not performed	13	12
Median age at diagnosis (range)	1 yr	(27 days to 18 yr)
Median age at transplant (range)	2 yr	(4 mo to 20 yr)
CNS involvement		
Present	30	28
Absent	49	45
Data not available	30	27
Treatment before transplant		
HLH-1994 protocol	9	8
HLH-2004 protocol	41	38
Euro-HIT-HLH protocol	3	3
Other	8	7
Unknown	48	44
Disease status at first transplant		
First CR	24	22
More advanced CR	5	5
Partial response	17	16
No response	54	50
Preemptive	2	2
Unknown	7	6
Conditioning regimen		
Busulfan-based conditioning	61	56
Busulfan-cyclophosphamide	10	9
Busulfan-etoposide	18	17
Busulfan-fludarabine	6	6
Busulfan-thiotepa	22	20
Other busulfan-based conditioning	5	4
Fludarabine-based conditioning	26	24
Fludarabine-melphalan	12	11
Fludarabine-melphalan-thiotepa	9	8
Other fludarabine-based conditioning	5	5
Treosulfan-based conditioning	21	20
Treosulfan-fludarabine-thiotepa	15	14
Treosulfan-fludarabine	5	4
Treosulfan-fludarabine-cyclophosphamide	1	1
Other conditioning regimen	1	1
Donor type		
Matched sibling donor	25	23
Matched unrelated donor	73	67
HLA partially matched family donor	11	10
Stem cell source		
Bone marrow	70	64
PBSCs	18	17
Umbilical blood graft	21	19
Serotherapy		
ATG	76	70
Alemtuzumab	7	6
No serotherapy	26	24
T cell depletion		
Ex vivo T cell depletion	8	7

ATG indicates antithymocyte globulin.

3 in the Euro-HIT-HLH trial (EudraCT#2011-002052-14), 4 received personalized treatment, 2 patients were transplanted without any other treatment because of a diagnosis of *BIRC4* mutation before developing clinical HLH, and for 48 patients data on frontline treatment received were not available. Two patients were treated with an anti-IFN-γ monoclonal antibody in the context of a clinical trial (EudraCT#2012-003632-23, NCT01818492) [23]. Multiple intrathecal injections of methotrexate were used for preventing/treating HLH-related neurologic involvement.

Transplant Procedure

Ninety-five patients received 1 transplant, whereas 14 received more than 1 HSCT because of rejection in 8 patients or disease relapse in 6 patients (preceded by rejection in 1 case): 2 transplants were performed in 12 cases, whereas 3 and 4 transplants were performed in 1 case each. Twenty-seven HSCT were performed between 2000 and 2004, 36 between 2005 and 2009, and 46 between 2010 and 2014.

Disease status at first HSCT was known for 102 of 109 patients (94%); 71 patients had active disease (no response, 54; partial response, 17), 24 were in first CR, and 5 were in later CR. Two patients received HSCT due to diagnosis of *BIRC4* mutation before developing clinical HLH and were analyzed together with patients in CR. Conditioning regimen was busulfan-based for 61 patients, treosulfan-based for 21 patients, fludarabine-based for 26 patients (often in combination with melphalan), and melphalan-etoposide for 1 patient (for further details see Tables 1 and 2). Patients given a fludarabine-based preparation were considered to have received a reduced-intensity conditioning, whereas those prepared with either busulfan or treosulfan were allocated to the myeloablative conditioning regimen group.

The donor for the first transplant was an HLA-matched sibling donor for 25 patients, an unrelated volunteer selected using high-resolution HLA typing for 73 patients, and an HLA-partially-matched family donor for 11 patients. Seventy patients were transplanted with bone marrow–derived stem cells, 18 with peripheral blood stem cells (PBSCs; mainly used in patients transplanted from an HLA-disparate relative), and 21 with umbilical cord blood. The mean dose of mononuclear cells was 6.4 cells $\times 10^8$/kg for bone marrow grafts (range, 2.5 to 27.3) and 11.2 cells $\times 10^7$/kg for cord blood grafts (range, 2 to 29.2). The mean dose of $CD34^+$ cells for PBSC grafts was 15.9 cells $\times 10^6$/kg (range, 2 to 24.8).

Considering the 109 first transplants, GVHD prophylaxis consisted of cyclosporine A in 25 cases, combination of cyclosporine A and short-term methotrexate in 55 cases, and combination of cyclosporine A and steroids in 20 cases. Post-transplant high-dose cyclophosphamide was used in 1 case, whereas in vitro T cell depletion performed through positive selection of $CD34^+$ cells was used in 8 patients transplanted from an HLA-mismatched relative.

Engraftment, Chimerism, and GVHD Occurrence

Neutrophil engraftment after first HSCT was obtained in 100 of 109 patients (92%) at a median time of 18 days (range, 9 to 57). Platelet engraftment after first HSCT was obtained in 87 of 109 patients (80%) at a median interval of 24 days (range, 9 to 105).

Stable mixed chimerism at time of last evaluation associated with good graft function and clinical remission of HLH was recorded in 6 patients. In these patients, donor contribution to hematopoiesis ranged from 5% to 97%. Fourteen patients received a second transplant. The reason for second HSCT was disease relapse (n = 6) or graft failure (n = 8). Subsequent transplants (n = 17, considering also third or fourth allografts) were performed with busulfan-based (n = 3), fludarabine-based (n = 5), treosulfan-based (n = 5), or other conditioning regimens (n = 4). The donor was a matched sibling donor for 2 procedures, an HLA partially matched

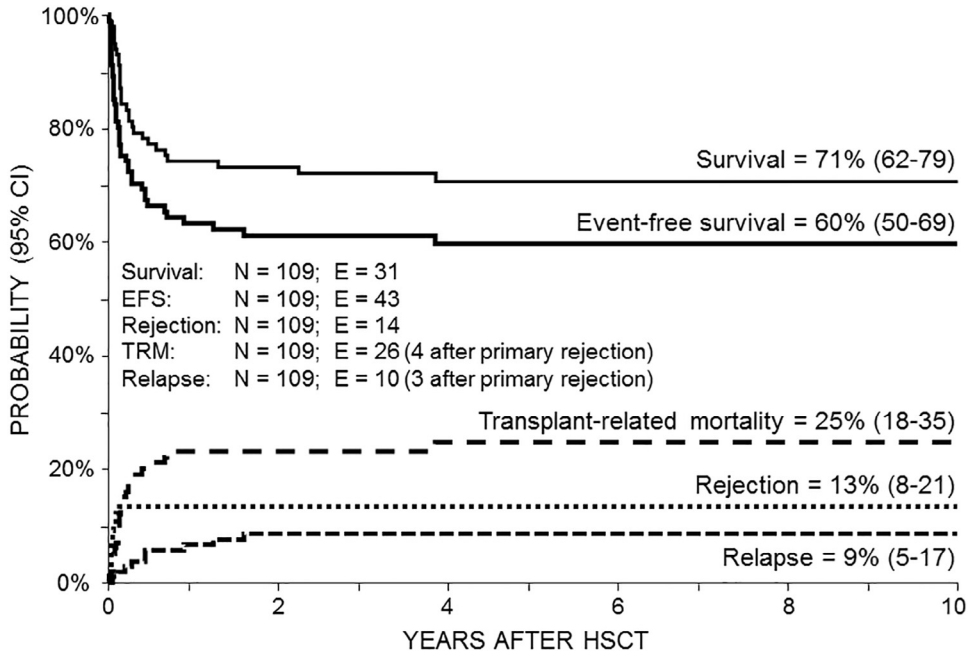

Figure 1. Five-year probability of OS and EFS and cumulative incidence of TRM, rejection, and relapse for 109 patients after the first HSCT.

family donor for 6 procedures, and an unrelated donor for 9 procedures. Stem cell source was bone marrow or PBSCs in 8 transplants each and cord blood for the remaining allograft.

Neutrophil engraftment after second transplant was obtained in 12 of 14 procedures (86%) at a median time of 17 days (range, 11 to 34). Platelet engraftment occurred at a median time of 24 days (range, 11 to 55).

Of the 14 patients who received a second transplant, 12 (86%) were alive and well at last follow-up; however, 1 of them required 2 further transplant procedures to achieve a good graft function. One patient died because of viral infection after the second transplant performed using a different unrelated donor, and another (transplanted from an HLA-matched sibling) died due to respiratory failure after the third transplant.

aGVHD was evaluated among the 115 transplants that resulted in donor engraftment. Grades II and III to IV aGVHD occurred in 29 (25%) and 11 (10%) transplants, respectively. Among 95 HSCT at risk, cGVHD was observed in 18 cases (19%) but was of limited severity in 9 cases (9%) and extensive in 9 cases (9%).

Clinical Outcome

The median observation time for surviving patients was 5.2 years (range, .9 to 14.9), whereas it was 54 days (range, 7 days to 3.8 years) for those who died. At time of the last follow-up 78 patients (72%) were alive, with a 5-year OS for the whole study population of 71% (95% confidence interval [CI], 62% to 79%) (Figure 1). There was no difference in terms of OS according to the type of donor used.

A total of 26 patients (24%) died due to transplant-related causes at a median of 53 days after HSCT; TRM was preceded by graft rejection in 4 cases. The cumulative incidence of TRM was 25% (95% CI, 18% to 35%). The number of fatal events according to the type of conditioning regimen used is shown in Table 2, whereas Table 3 summarizes the causes of death of the whole study population; veno-occlusive disease, lung aspergillosis, and multiorgan failure were the most frequent causes of death.

Graft failure was observed in 14 patients (13% of the whole population) at a median of 20 days after HSCT (range, 8 to 51). The cumulative incidence of graft failure was 13% (95% CI, 8% to 21%). Four of the 14 patients who rejected the

Table 2
Comparison of Outcome among Busulfan-Based, Treosulfan-Based, and Fludarabine-Based Conditioning Regimens

	Busulfan Based (n = 61)		Treosulfan Based (n = 21)		Fludarabine Based (n = 26)		Chi-Square *P*
Active disease at HSCT	38	(62)	13	(62)	20	(77)	N.S.
TRM	16	(26)	3	(14)	7	(26)	N.S.
Veno-occlusive disease	4	(7)	0	(0)	3	(11)	N.S.
Rejection	7	(11)	1	(5)	6	(22)	N.S.
Relapse	4	(7)	2	(10)	4	(15)	N.S.
Alive	43	(70)	18	(86)	17	(63)	N.S.
Alive and disease-free	37	(61)	15	(71)	14	(52)	N.S.

N.S. indicates not significant.

Table 3
Causes of Death

	Number of Transplants		Total
	First HSCT	Subsequent HSCT	
Disease progression	4	1	5
Veno-occlusive disease	7	0	7
Lung aspergillosis	5	0	5
Multiorgan failure	4	1	5
Viral infection (adenovirus/ cytomegalovirus)	3	0	3
cGVHD	2	0	2
aGVHD	1	0	1
Cerebral hemorrhage	0	1	1
Thrombotic microangiopathy	1	0	1
Unknown	1	0	1
Total	*28*	*3*	31

transplant died due to transplant-related causes (after a second HSCT in 1 case), whereas 3 subsequently developed an overt disease recurrence: 2 of them died due to disease progression and 1 was rescued by a second HSCT. The remaining 7 patients who rejected the first transplant are alive and disease-free after a second transplant.

A disease relapse was observed in 10 patients (9%) at a median of 163 days after HSCT (range, 41 to 585) and was preceded by a primary rejection in 3 cases. The cumulative incidence of relapse was 9% (95% CI, 5% to 17%). Seven of 10 patients who relapsed received a second HSCT; 5 of them are alive and disease-free.

Sixty-six patients were alive and disease-free after the first HSCT at time of last follow-up, with a 5-year probability of EFS of 60% (95% CI, 50% to 69%). Details on univariate analysis of variables potentially influencing EFS are shown in Table 4. The variables found to be statistically associated, in univariate analysis, with EFS were donor type and stem cell source. Patients transplanted from an HLA partially matched family donor had a significantly worse EFS (9%; 95% CI, 0% to 26%) than recipients of a matched family donor transplant (73%; 95% CI, 54% to 92%) or a matched unrelated donor allograft (63%; 95% CI, 52% to 74%; $P < .001$) (Figure 2). The main reason for the lower EFS of patients transplanted from an HLA partially matched family donor was graft rejection, which, however, as previously discussed, was largely rescued by a second allograft. Patients given PBSC transplantation had a significantly lower EFS probability (39%; 95% CI, 16% to 61%) as compared with bone marrow recipients (60%; 95% CI, 48% to 72%) or cord blood recipients (76%; 95% CI, 58% to 94%; $P = .0185$). Children who received the transplant within 6 months from diagnosis had a better EFS as compared with those transplanted later than 6 months from diagnosis (69% [95% CI, 56% to 81%] versus 50% [95% CI, 37% to 64%]), but this difference was not statistically significant ($P = .069$). In multivariate analysis (Table 5) only the use of a partially matched family donor confirmed its statistically significant association with a worse EFS probability, with a relative risk of 12.26 (95% CI, 2.82% to 53.35%; $P = .0008$).

Table 4
Univariate Analysis of Factors Influencing EFS

	No. of Patients	No. of Events	EFS (%)	95% CI	*P*
All patients	109	43	60	50-69	—
Genetic diagnosis					
PRF1 mutation	31	10	67	50-84	N.S.
UNC13D mutation	32	14	55	38-73	
Other diagnosis	18	5	72	52-93	
No genetic diagnosis	15	7	53	28-79	
Study not performed	13	7	45	17-73	
CNS involvement at diagnosis					
Present	37	16	57	41-73	N.S.
Absent	72	27	61	49-72	
Years of transplant					
2000-2004	27	13	52	33-71	N.S.
2005-2009	36	15	58	42-74	
2010-2014	46	15	67	54-81	
Time from diagnosis to HSCT					
<6 mo	56	17	69	56-81	.0699
≥6 mo	53	26	50	37-64	
Disease status					
Active disease (no or partial response)	71	31	56	44-68	N.S.
CR or preemptive	31	10	67	50-84	
Missing information	7	2	69	32-100	
Conditioning regimen					
Busulfan-based	61	24	60	47-72	N.S.
Fludarabine-based	26	13	51	32-70	
Treosulfan-based	21	6	70	50-90	
Donor					
MFD	25	6	73	54-92	**<.001**
MUD	73	27	63	52-74	
PMFD	11	10	9	0-26	
Stem cell source					
BM	70	27	60	48-72	**.0185**
PBSCs	18	11	39	16-61	
UCB	21	5	76	58-94	
No. of HSCTs*					
First HSCT	109	43	60	50-69	N.S.
Second HSCT	14	4	71	48-95	

Bold type was employed to highlight statistically significant data.
MFD indicates matched family donor; MUD, matched unrelated donor; PMFD, partially matched family donor; BM, bone marrow; UCB, umbilical cord blood.
* Data were considered for first HSCT only.

DISCUSSION

To the best of our knowledge the cohort of HLH patients receiving HSCT presented here is the largest ever specifically analyzed (for a comparative analysis on different outcomes with previously published cohorts see Table 6). Included were mainly patients with genetic diagnosis of fHLH. Thirty patients (28%) without a genetic diagnosis or not molecularly studied but fulfilling the internationally accepted HLH criteria were transplanted for refractory or relapsed HLH.

Our results confirm that allogeneic HSCT is capable of curing a large proportion of patients, irrespectively of the genetic defect responsible for the disease. The optimal timing for performing HSCT in HLH patients is a matter of debate, especially in cases with relapsed or refractory disease. In particular, it is unclear whether for relapsed or refractory disease aggressive second-line chemoimmunotherapy, aimed at reaching CR before transplant, is warranted. Some case series suggest that active disease at transplantation might be a risk factor, especially when an HLA-haploidentical donor is used [10,12]; however, other data indicate that initial response to treatment (CR after 2 months of treatment) could be more informative about the prognosis [11,17,24]. Moreover, in published experiences around 30% to 60% of patients have been transplanted with active disease, indicating that CR is difficult to obtain in many patients with HLH [11-13,15,17,24]. Our data could shed further light on this issue: Active disease at transplantation was not statistically associated with adverse outcomes, whereas, interestingly, patients had a trend for a worse outcome if the interval between diagnosis and transplantation was longer than 6 months. Thus, we speculate that

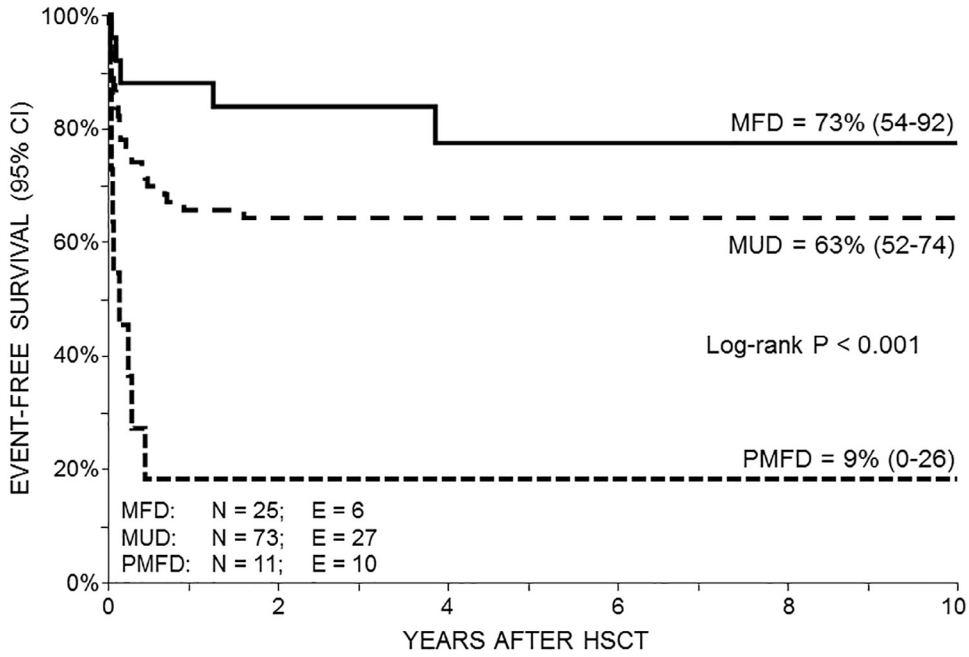

Figure 2. Five-year probability of EFS according to the type of donor used. MFD indicates matched family donor; MUD, matched unrelated donor; PMFD, HLA partially matched family donor.

active disease at transplantation could be indicative of more aggressive disease, potentially not adequately responding even to additional second-line rescue treatment. If so, the best frontline therapy should be selected to achieve adequate disease control, without postponing HSCT at more than 6 months from diagnosis. Treating patients for a longer time with the scope of obtaining CR before transplantation could expose to the risk of deterioration of the general status, thus making the outcome of transplant worse. Whether the use of novel approach to HLH by immunotherapy, such as that based on the use of an anti-IFN-γ monoclonal antibody (EudraCT#2012-003632-23, NCT01818492), may lead to better rates of CR at time of transplantation in refractory patients remains to be definitively confirmed [23].

Donor availability plays an important role in deciding when to perform a transplant. Our data indicate that although a matched sibling donor is the donor of choice, an unrelated donor selected using high-resolution molecular typing of HLA loci can be used with comparable patient's outcome. Our study confirms also that the use of umbilical cord blood is a feasible option [17,25]. In particular, 21 patients (19%) who received a cord blood allograft had outcomes comparable with those of patients given bone marrow cells. Probably, young age at HSCT with a consequent favorable ratio of number of cells infused per kilogram of recipient body weight makes this kind of procedure more appropriate for HLH patients than in other clinical settings.

Table 5
Multivariate Analysis of Factors Influencing EFS (Data Were Considered for First HSCT Only)

Variable	Relative Risk	(95% CI)	*P*
Interval diagnosis, HSCT			
>6 mo vs. <6 mo	1.15	.59-2.24	.68
Donor			
MUD vs. MFD	2.16	.85-5.49	.11
PMFD vs. MFD	12.26	2.82-53.35	.0008
Stem cell source			
Cord blood vs. bone marrow	.48	.18-1.28	.14
Peripheral blood vs. bone marrow	.63	.21-1.87	.41

Our data indicate that, so far, the use of HLA partially matched family donors is associated with an increased risk of graft failure, however, largely rescued by a second allograft in line with the data reported in 2 previously published studies [16,26]. Investigation of new approaches to HLA-haploidentical transplantation, such as that based on the infusion of the graft after the selective depletion of TCR αβ-T cell/CD19⁺ cells, is urgently needed to improve the rate of sustained donor engraftment and to more confidently offer a prompt transplant option also to patients lacking a matched donor [27].

The main causes of death in our cohort were complications related to HSCT, namely veno-occlusive disease, lung aspergillosis, and multiorgan failure; HLH relapse only accounted for 5 deaths. Indeed, busulfan-based myeloablative conditioning for HLH patients has been reported to be associated with a high rate of infections, veno-occlusive disease, and possibly a higher incidence of pulmonary complications [10-12,28-30]. To overcome these issues, in the mid-2000s use of fludarabine-melphalan reduced-intensity conditioning was introduced, leading to less TRM and better outcome, although at the expense of higher frequency of mixed chimerism, secondary graft failure, and relapse rates [13,16,31-33]. Excellent results were reported with the use of treosulfan-based conditioning regimens, which, despite being myeloablative, seem to be associated with less extramedullary toxicity [14,15]. Our cohort is the only one in which the 3 above conditioning regimens have been used in a significant number of patients and outcomes could be directly compared (Tables 2 and 6). Although no statistically significant differences were observed, a trend toward better OS and EFS after treosulfan-based conditioning was evident. In our

Table 6
Results of Published Cooperative Studies on HSCT in HLH Patients

Author, period	No. of Patients	Conditioning Regimen	Type of Donor	Primary Engraftment	OS	EFS	GVHD	Notes
Horne et al. 1995-2000	86	BU/CY/VP-16: 41 Others: 45	MR n = 24 MUD n = 33 HAPLO n = 16 MMUD n = 13	90% HAPLO 81%	64% ± 10% at 3 years MR 71% ± 18% MUD 70% ± 16% HAPLO 50% ± 24% MMUD 54% ± 27%	—	aGVHD grades II-IV 32% MR 18% MUD 30% HAPLO 36% MMUD 58%	Graft failure: 3 Disease recurrence: 3
Sawada et al. 1990-2009	53	MAC: 37 (BU/CY/VP-16: 23) RIC: 16	MR n = 7 MMR n = 1 MUD n = 20 MMUD (1 loc) n = 18 MMUD (2 loc) n = 7	26/38 of assessable	65.4% ± 6.6% at 2 years	57.6% ± 6.9% at 2 years	Not reported	
Baker et al. 1989-2005	91	BU/CY/ VP-16 ± ATG: 73 Other 18	MUD n = 54 MMUD (1 loc) n = 32 MMUD (2 loc) n = 4	Neutrophil 85% Platelet 54%	52% at 1 year 45% at 3 years (Bu/CY/ VP-16 53%, Other regimens 19%)	—	aGVHD grades II-IV 41% aGVHD grades III-IV 24% cGVHD 25%	(OS Bu/CY/ VP-16 53%, Other regimens 19%)
Yoon et al. 1996-2008	19	BU/CY/ VP-16 ± ATG: 12 FLU-based: 5 Other: 2	MR n = 6 MUD n = 8 MMUD n = 5	16/19	73.3% at 5 years MR 85.7% MUD 87.5% MMUD 40%	—	aGVHD grades II-IV 5/16	TRM 21%
Ohga et al. 1995-2005	57 (14EBV-related)	MAC: 43 (Bu/CY/ VP-16: 31) RIC: 14	53 Allogeneic MR n = 8 MUD n = 24 HAPLO n = 4 MMUD n = 17 (UCB)	29/42 HLH 7/11 EBV-HLH	HLH 65% ± 7.9% EBV-HLH 85.7% ± 9.4% at 10 years	—	Not reported	TRM 11/42 in the HLH group
Messina et al. 2000-2014	109	BU-based: 61 TREO-based: 21 FLU-based: 26	MR n = 25 MUD n = 73 HAPLO n = 11	Neutrophil 92% Platelet 80%	71% at 5 years	EFS 60% at 5 years MR 73% MUD 63% HAPLO 9%	aGVHD grades II-IV 29% aGVHD grades III-IV 11% cGVHD 18%	TRM 25% CI graft failure 13% CI disease recurrence 9%

BU indicates busulfan; CY, cyclophosphamide; VP-16, etoposide; MR, matched related donor; MMUD, mismatched unrelated donor; HAPLO, haploidentical; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; FLU, fludarabine; UCB, unrelated cord blood; TREO, treosulfan; CI, cumulative incidence.

experience the high TRM (26%) observed with busulfan was not significantly different from that recorded with fludarabine-based regimens. Moreover, fludarabine-based conditioning exposed patients to a higher risk of graft failure/relapse and need of a second transplant (Table 2). On the other hand, treosulfan-based conditioning resulted in lower TRM (14%) with acceptable rates of graft failure (5%) or relapse (10%), this translating into a remarkably high rate of cured patients. The potential advantages deriving from the use of a treosulfan-based myeloablative conditioning regimens must be confirmed in properly designed, prospective randomized trials to be conducted in HLH patients.

Importantly, patients receiving a second transplant did not have worse outcomes than patients transplanted only once. This finding suggests that a second allograft may be safely offered to HLH patients in case of relapse or rejection, provided they did not develop significant end-organ damage due to either HLH itself or the first HSCT. In line with our data, a recently published study analyzed the outcome of 18 HLH patients given a second allograft because of HLH recurrence (10 patients) or low donor chimerism level only (8 patients) [26]. Ten of these patients were reported to be alive and disease-free, whereas fatalities were similarly distributed between patients with and without prior disease recurrence.

Our results on probabilities of 5-year OS and EFS obtained in a larger cohort of unselected children are slightly better as compared with some other similar cooperative studies (Table 6) [11,25,29,34,35]. This might be explained by the higher proportion of patients receiving fludarabine- or treosulfan-based conditioning in our cohort or by the fact that we included only patients transplanted after the year 2000.

The multicenter, retrospective design of our study has some intrinsic limitations, such as lacking information on pretransplant treatment in a relevant proportion of patients. Yet it describes well the current practice in HSCT for HLH.

In conclusion, our data indicate that in patients with HLH, allogeneic HSCT is able to cure two-thirds of patients, restoring normal immune response toward pathogens and abrogating the hyperinflammatory state typical of HLH. HSCT from an HLA-partially matched relative in patients with HLH is currently associated with unsatisfactory rate of engraftment, with new approaches needed to ameliorate this outcome. However, rejection and secondary graft failures are events that could be salvaged by a second allograft. Active disease does not preclude the chance of benefiting from transplantation, which should be ideally performed within 6 months from diagnosis. Finally, our data suggest that the use of treosulfan-based conditioning appears to be an attractive option to reduce TRM in this fragile population of patients.

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