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Haploidentical HSCT with post transplantation cyclophosphamide versus unrelated donor HSCT in pediatric patients affected by acute leukemia

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Abstract

T-cell replete hematopoietic stem cell transplantation (HSCT) from a haploidentical donor followed by high doses of cyclophosphamide has been demonstrated to provide the best chances of a cure for many children in need of an allograft but who lack both a sibling and an unrelated donor. In this study we retrospectively compared the outcome of pediatric patients undergoing T-replete haploidentical HSCT (Haplo) for acute leukemia with those undergoing transplantation from unrelated HLA-matched donor (MUD) and HLA mismatched unrelated donor (MMUD) from 2012 to 2017 at our Center. Both univariable and multivariable analyses showed similar 5-year overall survival rates for MUD, MMUD, and Haplo patients: 71% (95% CI 56–86), 72% (95% CI 55–90), and 75% (95% CI 54–94), respectively ($p = 0.97$). Haplo patients showed reduced event-free survival rates compared to MUD and MMUD patients: 30% (95% CI 12–49) versus 70% (95% CI 55–84) versus 53% (95% CI 35–73), respectively ($p = 0.007$), but these data were not confirmed by a multivariable analysis. Non-relapse mortality (NRM) and relapse incidence (RI) were similar for the three groups. Therefore, our data confirm that Haplo is a suitable clinical option for pediatric patients needing HSCT when lacking both an MUD and an MMUD donor.

Introduction

For many pediatric patients affected by acute leukemia, allogeneic hematopoietic stem cell transplantation (alloHSCT) can offer the best chances for long-lasting disease control [1], however only 25% of patients needing alloHSCT have an HLA-identical sibling and an unrelated donor can be identified in only 60% of the remaining cases [2]. Moreover, the likelihood of finding an unrelated donor strictly depends on the patient's ethnicity with higher probabilities for Caucasians compared to other ethnic groups [3]. For these patients, currently available options are transplantation from a cord blood unit or from a relative donor sharing only one HLA-haplotype with the recipient (Haplo HSCT) [2]. However, since 2009 the number of cord blood alloHSCTs has been in continuous decline and most of the transplant centers have adopted strategies based on Haplo HSCT [4,5,6]. Today, there are currently three strategies available when performing Haplo HSCT which are (1) based on the infusion of a high dose of Cyclophosphamide into the recipient, following stem cell infusion, to eliminate any alloreactive T-cells (CTX-Haplo HSCT) [7]. (2) the administration of a very intensive GvHD prophylaxis combined with the use of G-CSF primed bone marrow as stem cells source [8], and (3) the depletion of TCR alpha–beta and CD19+ cells from the graft [9]. In pediatric patients affected by acute leukemia promising clinical results have been reported, mainly with TCR alpha–beta CD19 depleted Haplo HSCT [10] and with CTX-Haplo HSCT [11].

Considering the increasing number of Haplo HSCT patients, it is crucial to understand if transplantation outcomes with these approaches are similar to those of more consolidated approaches such as matched unrelated donor (MUD) HSCT and mismatched unrelated donor (MMUD) HSCT.

CTX-HaploSCT has recently been compared to MUD and MMUD HSCT in some large retrospective trials with adult patients who were mainly affected by acute myelogenous leukemia with some authors concluding that CTX-Haplo HSCT results are similar to those of MMUD but inferior to those of MUD [12, 13], while others highlighting a comparable outcome with all three kinds of donor [14]. For acute lymphoblastic leukemia in adult patients, the results of CTX-Haplo HSCT have been reported to be very similar to those obtained by MUD and MMUD HSCT [15, 16]. Pediatric specific data at the time of this study are lacking so since it has been demonstrated that a patient's age plays a significant role in determining these CTX-Haplo HSCT results [17], we compared the results of CTX Haplo HSCT to MUD and MMUD HSCT in the treatment of children affected by acute leukemia in our single-center retrospective trial.

Subjects and methods

Patients

Our study included all patients undergone first alloHSCT for hematological malignancies from (1) an HLA-MUD, (2) an HLA-MMUD, (3) a related HLA-haploidentical donor (Haplo) at our Center between January 1, 2012 and December 31, 2017. All data were retrieved retrospectively from clinical records according to the policy approved by our Institutional Committee on Medical Ethics and after obtaining informed consent from parents or legal guardians. Both the selection of the donor and HLA typing were performed according to the Italian Bone Marrow Donor Registry Standard of Practice that includes high-resolution molecular typing of loci HLA-A, -B, -C, DRB1, and DQB1. MUD was considered a ten out of ten antigens HLA-MUD and MMUD a nine out of ten antigens HLA-MUD. Haploidentical donors were family members with one identical HLA-haplotype and the other mismatched haplotype, as previously defined [18]. The decision to perform Haplo HSCT was based on the absence of both ten out of ten antigens and nine out of ten HLA-MUD. In the case of Haplo HSCT, following our previous observations [11], our first choice of donor was the patient's mother independently of the patient's sex. Where the mother presented clinical contraindications to stem cell collection or if the patient presented specific anti-HLA antibodies, we chose the patient's father or another close relative. In cases where patients had positive donor-specific anti-HLA antibodies and no other related donor was available, our patients were treated according to previously reported indications [19]. The Haplo HSCT was based on the non-myeloablative (NMA) conditioning regimen reported by Luznik et al. [7] including Cyclophosphamide (29 mg/kg), Fludarabine (150 mg/mq), and total body irradiation (TBI) (2 Gy in single fraction). The GvHD prophylaxis was based on the administration of a high dosage of Cyclophosphamide (total dose 100 mg/kg) on days +3 and +4 after HSCT, Tacrolimus and Mofetil Mycophenolate. The MUD and MMUD HSCT were performed using a full myeloablative conditioning (MAC) regimen including the association of TBI (1200 cGy in six fractions for a total of 3 days of treatment), Thiotepa (10 mg/kg) and Cyclophosphamide (120 mg/kg) or Busulfan (16 mg/kg), Cyclophosphamide (120 mg/kg), and Melphalan (140 mg/mq). The GvHD prophylaxis in these latter cases included rabbit anti-human thymocytes globulins (ATG-Grafalon, Neovii or Thymoglobuline, Sanofi), Cyclosporine and short course of Methotrexate.

We included patients receiving either bone marrow or peripheral blood hematopoietic stem cells as stem cell source, while cord blood HSCTs were excluded. Our patients underwent clinical and hematological assessments both before and after transplantation following our Center Standard Operating Policies.

Definitions and endpoints

The main aim of this study was to compare the overall survival rates (OS) of patients who had undergone Haplo HSCT to the OS rates of patients who had undergone MUD and MMUD HSCT at our Center for acute lymphoblastic leukemia and acute myelogenous leukemia. The secondary endpoints were differences in terms of event-free survival (EFS), non-relapse mortality (NRM), relapse incidence (RI), incidence of both acute (aGvHD) and chronic GvHD (cGvHD), achievement of full donor cell engraftment, and immune recovery in the three groups of patients. For OS, EFS, NRM, and RI an initial set of univariable analyses were performed considering the following variables: HSCT type, sex, age, disease type, disease risk index (DRI), and occurrence of both aGvHD and cGvHD. Following this, the same variables were combined in multivariable analysis models. DRI was evaluated as previously described [20] and aGvHD and cGvHD were diagnosed and graded according to the published criteria [21, 22].

OS is defined as the probability of survival irrespective of the disease state at any point in time. If the patient is still alive at the end of the study data are censored on the date of the last follow-up.

EFS is defined as the probability of survival with complete disease remission and with sustained donor cell engraftment. If the patient is still alive at the end of the study, in complete disease

remission and without any signs of both primary and secondary graft rejection, data are censored on the date of the last follow-up.

NRM is defined as the probability of dying without the occurrence of a previous relapse. If the patient experienced relapse or is still alive by the end of the study, data are censored on the relapse date or on the date of the last follow-up.

RI is defined as the probability of having had a relapse. If the patient died without experiencing relapse or is still alive by the end of the study, data are censored on the date of death or on the date of the last follow-up, respectively.

In the Haplo CTX HSCT group, we also retrospectively investigated the impact of NK alloreactivity on RI by analyzing for killer immunoglobulin receptors (KIR) in the donor and recipient, as previously described [23].

Donor chimerism was determined at day +45 ± 7 after alloHSCT, and then when clinically indicated, on whole bone marrow mononuclear cells by quantitative PCR of informative short tandem repeats in the donor and recipient, according to a method previously described [24].

Sustained donor cell engraftment was defined as the presence of more than 1000 neutrophils/mmc and more than 50,000 platelets/mmc for three consecutive days without transfusion support and with a chimerism showing more than 97% of the donor cells in the bone marrow after HSCT.

Graft rejection was defined as a lack of initial engraftment of donor cell graft (chimerism showing more than 50% of the recipient's cells) or loss of donor cell engraftment, independently from the peripheral cell blood count. In the case of graft rejection, for NRM and RI analyses, data were censored on the date of bone marrow assessment that showed a chimerism with more than 50% of the recipient's cells. Immune recovery was investigated by multi-color flow-cytometry on peripheral blood at different times after HSCT and included the absolute enumeration of total T-cells (CD3$^+$), Helper T-cells (CD3$^+$CD4$^+$), Cytotoxic T-cells (CD3$^+$CD8$^+$), NK-cells (CD16$^+$CD56$^+$), and B-cells (CD19$^+$CD20$^+$).

Statistical analysis

To identify baseline differences among the three groups of patients (MUD, MMUD, and Haplo), a two-tailed Fisher test was performed.

OS and EFS were calculated according to the Kaplan–Meier method with the significance between the observed differences being established by log-rank testing. Multivariable analyses on OS and EFS were performed using Cox's method. The NRM and RI were calculated as a cumulative incidence (CI) to adjust the analysis for competing risks: relapse and transplant-related death were considered competing risks, respectively. aGvHD and cGvHD were calculated as CI too. In these cases, disease recurrence and death by any cause were considered competing risks. The differences in terms of CI were compared using Grey's test. The NRM and RI multivariable analyses were performed using logistic regression. In the Haplo group, we also analyzed the impact on RI of NK alloreactivity in donor–recipient pairs according to KIR matching. To evaluate for differences in the achievement of the donor's cell engraftment, a two-tailed Fisher test was performed. Data concerning specific lymphocytes' sub-populations are reported as average ±standard deviation. A p value < 0.05 was considered statistically significant in all the analyses. All the statistical analyses were performed using NCSS (Hintze, 2001; NCSS PASS, Number Crunched Statistical System, Kaysville, UT, USA) and R 2.5.0 software packages.

Results

Patients

The study included 90 patients (49 males and 41 females) with a median age of 9 years (range 1–25) at the time of alloHSCT: 41 (45%) patients had received MUD HSCT, 26 (29%) MMUD, and 23 (26%) Haplo. The patients' characteristics are summarized in Table 1. No statistically significant baseline differences were observed in the three groups of patients (Table 1). Analyses used October 31, 2019

as reference date, the median follow-up time of patients enrolled in the study and who are still alive at the end of the study is 4 years (range: 1.4–7). In the Haplo group, two patients had positive donor-specific anti-HLA antibodies and they received specific treatment before starting the conditioning regimen.

Overall survival
The 5-year OS rate for the entire study population was 72% (95% CI 62–82). Patients who had undergone HSCT from MUD, MMUD, and Haplo showed a similar OS in univariable analysis (Table 2 and Fig. 1): 71% (95% CI 56–86), 72% (95% CI 55–90), and 75% (95% CI 54–94), respectively ($p = 0.97$). Among other variables investigated in univariable analysis (age, sex, disease type, DRI, aGvHD, and cGvHD), age and DRI showed a statistically significant correlation with OS rates (Table 2). In a multivariable analysis, OS was confirmed to be similar across the three study sub-groups and only age maintained a statistically significant correlation with OS. Patients older than 15 years showed a significant increased risk of mortality compared to younger children (HR 6.83 95% CI 1.6–30 $p = 0.007$) (Table 3), while for MUD and MMUD patients the main causes of death were infections and end-stage organ toxicities ($n = 13$, 72%), for Haplo patients the main cause of fatality was the recurrence of the original disease ($n = 3$, 60%).

Event-free survival
The 5-year EFS of the entire study population was 55% (95% CI 44–65). In univariable analysis patients who had undergone Haplo showed reduced EFS compared to MUD and MMUD patients: 30% (95% CI 12–49) versus 70% (95% CI 55–84) versus 53% (95% CI 35–73), respectively ($p = 0.007$). Among other variables investigated in univariable analysis (age, sex, disease type, DRI, aGvHD, and cGvHD), patient's age also showed a statistically significant correlation with EFS (Table 2). In the multivariable analysis we did not observe a correlation between HSCT type and EFS, while we highlighted that patients older than 15 years had a significant increased risk of disease recurrence, death in remission and autologous reconstitution compared to younger children (HR 6.12 95% CI 1.9–19.7 $p = 0.002$) (Table 3).

Non-relapse mortality
The 5-year NRM of the entire study population was 12% (95% CI 6–21). Patients who had undergone Haplo CTX HSCT showed no statistically significant different NRM compared to MUD and MMUD patients: 8% (95% CI 2–31) versus 15% (95% CI 7–31) versus 14% (95% CI 4–45), respectively ($p = 0.72$). Among other variables, only age showed a statistically significant correlation with NRM (Table 4) both in univariable and in multivariable analyses: patients aged between 5 and 10 years showed a reduced risk of NRM (HR $2.18 \times 10e-8$ 95% CI $6.25 \times 10e-8 - 7.59 \times 10e-7$ $p < 0.001$) compared to other age groups. Considering the incidence of complications that contributed to NRM, among the ten patients who died in disease remission in the MUD and MMUD group, we observed four cases of bacterial infection, two cases of viral infections, two cases of fungal infections, one case of secondary hemophagocytic lympho-histiocytosis, and one case of refractory heart failure. In the Haplo group, the only patient dying from NRM had central nervous system bleeding.
Relapse incidence
The RI of the entire study population was 16% (95% CI 10–26). Patients who underwent Haplo showed similar RI compared to MUD and MMUD patients: 33% (95% CI 19–58) versus 15% (95% CI 7–30) versus 26% (95% CI 14–50), respectively ($p = 0.25$). In univariable analysis, age showed a statistically significant correlation with RI (Table 5) and these data were also confirmed in multivariable analysis: patients over 15 years showed increased RI risk (HR 5.1 95% CI 1.2–22.3 $p = 0.027$) compared to younger patients. In the Haplo sub-group, KIR-mismatch patients did not have a different RI compared to KIR-matched patients [25% (95% CI 4–100) versus 36% (95% CI 20–66) ($p = 0.59$)].

GvHD incidence
Patients who had undergone Haplo HSCT showed a reduced incidence of aGvHD compared to patients who had undergone MUD and MMUD HSCT: 8% (95% CI 2–37) versus 14% (95% CI 6–30) versus 34% (95% CI 20–58), respectively ($p = 0.004$), while when considering cGvHD, the patients who had undergone Haplo HSCT showed a similar incidence of this complication compared to MUD and MMUD patients: 5% (95% CI 1–35) versus 10% (95% CI 4–27) versus 16% (95% CI 6–39), respectively ($p = 0.51$).
Donor cell engraftment
Full donor cell engraftment was achieved in 41 out of 41 (100%) of the evaluable patients had undergone MUD HSCT, in 25 out of 25 (100%) of the evaluable patients who had undergone MMUD HSCT, and in 17 out of the 23 (73%) evaluable patients who had undergone Haplo HSCT ($p < 0.0001$). Six patients from the Haplo group developed graft rejection. They were affected by acute myelogenous leukemia ($n = 2$) and acute lymphoblastic leukemia ($n = 4$). Five of them underwent a second hematopoietic stem cell transplantation using the same ($n = 2$) or an alternative ($n = 3$) haploidentical stem cell donor. All patients at their second HSCT had the same conditioning regimen and the same GvHD prophylaxis as that used for their first HSCT. As of October 31, 2019 they were all still alive: four of them are in complete disease remission with sustained donor cell engraftment, while only one experienced a second autologous reconstitution and he is currently alive and disease free. The only patient who did not undergo a second HSCT was lost to follow-up at +709 days after HSCT.

Immune recovery
All the patients in the three groups displayed very similar patterns in the recovery of the lymphocyte sub-populations (Fig. 2).

Discussion

Our study showed that AlloHSCT can offer one of the best chances of cure for the many children affected by acute leukemia. Unfortunately, however, there is a significant proportion of children for whom it is not possible to identify either an HLA-identical sibling or an acceptable HLA-MUD or MMUD. For these patients the use of an haploidentical donor followed by the administration of a high dose of cyclophosphamide is the most widely adopted strategy both for its simplicity and reduced cost [25]. While for adult patients undergoing Haplo CTX HSCT, several reports have highlighted outcomes comparable to those of more consolidated approaches such as MUD and MMUD HSCT unfortunately, pediatric specific data are still lacking. However, this is the first study which was entirely focused on a pediatric population which compared the outcomes of patients undergoing Haplo CTX HSCT with those of patients undergoing MUD and MMUD HSCT. Consistent with the results from studies involving adult populations [14,15,16], we have shown that patients who underwent HSCT from MUD, MMUD, and Haplo have similar OS rates [71% (95% CI 56–86), 72% (95% CI 55–90), and 75% (95% CI 54–94), respectively ($p = 0.97$)], and we can also confirm that this outcome measure is more likely related to factors independent from HSCT type (i.e., patient's age) as previously described [26]. Previous experiences based on NMA conditioning regimen in setting of pediatric Haplo HSCT with post transplantation Cyclophosphamide reported lower OS and EFS rates compared to our results [27], but we interpreted these differences as a consequence of an higher RI related to the inclusion in the previous study of high risk patients only, considering that our data confirmed NRM under 15%.
The reduction of the survival rate of patients over 15 years old is in accordance with previous observations in the literature that highlight that adolescents are usually affected by more aggressive hematological malignancies and that they also develop more serious transplant-related toxicities compared to younger children [28], but the low number of patients included in this group (total 14) prevents us from drawing any conclusion.

Considering that all the patients in the Haplo CTX HSCT received a NMA conditioning regimen and that no statistically significant baseline differences were identified according to the DRI of the three study sub-populations, the similar OS rate among the three groups is quite remarkable and our data may support the hypothesis that in the setting of hematological malignancies, the immunological aspects of HSCT are significant thus off-setting the intensity of the conditioning regimen.

When analyzing for EFS, in univariable analysis, we highlighted a statistically significant reduced probability of survival in disease remission and with full donor cell engraftment for Haplo HSCT patients compared to MUD and MMUD patients [30% (95% CI 12–49) versus 70% (95% CI 55–84) versus 53% (95% CI 35–73), respectively ($p = 0.007$)] that was not confirmed by multivariable analysis. Since in our study population we did not observe any significant correlation between HSCT type and RI and NRM, neither in univariable nor in multivariable analyses, we speculated that the reduced EFS of Haplo HSCT patients related to an increased risk of autologous reconstitution; in analyzing the donor cell engraftment we confirmed this hypothesis, showing that it was possible to achieve full donor cell engraftment in all the patients who had undergone MUD and MMUD HSCT, while six out of the 23 patients (73%) who had undergone Haplo CTX HSCT experienced autologous reconstitution. Unfortunately, since all the patients in the Haplo CTX group received a NMA conditioning regimen, whereas all patients in the MUD and MMUD group received a full MAC regimen, we were not able to investigate the role of the intensity of the conditioning regimen in determining the increased risk of autologous reconstitution in our specific population, compared also to previous reports about Haplo CTX HSCT, even if this aspect has been described when an RIC regimen was employed in this setting [29]. In order to reduce the risk of autologous reconstitution, one possible strategy may be the intensification of the conditioning regimen as it has been described both for non-malignant disorders [30] and for malignant disorders [31] by some single-institution trials.

Despite this, the majority of patients experiencing autologous reconstitution in our study underwent a second HSCT which was based on the same conditioning regimen and the same GvHD prophylaxis in every case. Following that, they showed similar OS rates compared to patients undergoing MAC MUD and MMUD HSCT.

In consideration of transplant toxicity, our data confirm that patients undergoing Haplo CTX HSCT have similar NRM rates compared to patients undergoing MUD and MMUD HSCT and, more specifically, when considering GvHD, they also underline that the GvHD prophylaxis based on the administration of high dose of Cyclophosphamide is able to significantly reduce the incidence of GvHD, especially in its acute form. As recently described by Wachsmuth et al. [32], the mechanisms responsible for the reduction in GvHD incidence observed with post transplantation cyclophosphamide may rely on the peripheral elimination of alloreactive T-cells, on the intra-thymic clonal deletion of alloreactive T-cell precursors, and on the expansion of regulatory T-cells, but unfortunately, in our study population we do not have any available data to support any of these hypotheses.

One of the main concerns in adopting a strategy based on a highly effective GvHD prophylaxis is the potentially increased risk of relapse. However, in our study the RI was not statistically different across the three groups of patients, as has been described when other kinds of Haplo HSCT have been compared to MUD and MMUD HSCT [33], and data for immune recovery at different times after transplantation suggest an equal graft versus leukemia effect. When analyzing the graft versus leukemia effect in CTX HSCT sub-group, we did not observe a protective KIR alloreactivity role against disease recurrence which is consistent with previous observations made in Haplo CTX HSCT [11, 34]. Needless to say, the low number of patients with KIR-mismatch included in our study and the reduced size of the entire study population, unfortunately prevents us from drawing conclusions in regard to this.

Our analysis of RI confirms that adolescents have an increased risk of disease recurrence that we interpreted as a consequence of the different disease biology in this age group that has been previously described both for ALL [35] and AML [36].

The main weakness of our study was the inclusion in the Haplo CTX HSCT group of only patients having received NMA conditioning regimen. This choice was mainly driven by our Center policy based on a wider experience in using this approach in comparison to MAC in the Haplo setting, in a time-frame when data about MAC in CTX Haplo HSCT [31] were not available yet. However, a more recent and larger multicenter retrospective trial showed no differences in terms of RI between patients undergoing Haplo CTX HSCT using either an RIC or an MAC [37], suggesting that this aspect may not be crucial in this setting. Some further significant limitations of our study are its retrospective design and the inherent heterogeneity of some of the patients' characteristics, that we tried, at least partially, to compensated by the use of a multivariable analysis.

In conclusion, our data confirm that CTX-Haplo HSCT is a suitable clinical option that can offer pediatric patients needing HSCT and lacking both an MUD and an MMUD donor similar opportunities for long-lasting disease control. The comparable risks of serious toxicity clearly have to be taken into account and furthermore, there are some aspects of this approach, such as the intensity of the conditioning regimen, that still need to be worked on. However, the results are promising, and it is hoped that in the future some prospective clinical trials will be run in the pediatric population to further improve the prognosis of children affected by acute leukemia and in need of an allograft.

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Table 1 Patients' characteristics.

MUD n = 41	MMUD n = 26	Haplo n = 23	p
Sex			
Male	23 (56%)	13 (50%)	13 (58%)	
				0.82
Female	18 (44%)	13 (50%)	10 (42%)	
Age			
0–5 years	15 (36%)	6 (23%)	4 (21%)	
6–10 years	12 (30%)	7 (27%)	11 (46%)	
				0.54
11–15 years	9 (22%)	7 (27%)	5 (21%)	
>15 years	5 (12%)	6 (23%)	3 (12%)	
Disease			
ALL	24 (59%)	20 (77%)	7 (29%)	
				0.75
AML	17 (41%)	6 (23%)	16 (71%)	
Disease risk index			
Low	10 (24%)	4 (15%)	3 (12%)	
Intermediate	25 (61%)	21 (81%)	18 (75%)	0.37
High	6 (15%)	1 (4%)	2 (13%)	

MUD matched unrelated donor, *MMUD* mismatched unrelated donor, *Haplo* haploidentical-related donor, *ALL* acute lymphoblastic leukemia, *AML* acute myelogenous leukemia.

Table 2 Overall survival (OS) and event-free survival (EFS): univariable analysis.

Overall survival					Event-free survival				
Variable	*n*	Events	OS	95% CI	*p*	Events	EFS	95% CI	*p*
Sex									
Female	41	9	76%	63–90	0.42	16	60%	44–75	0.4
Male	49	14	69%	54–82		24	51%	37–65	
Age									
0–5 years	25	4	78%	61–95	**0.0014**	7	71%	53–89	**0.01**
6–10 years	30	5	81%	67–96		14	53%	35–71	
11–15 years	21	5	76%	57–94		8	62%	41–83	
>15 years	14	9	36%	10–60		11	21%	0–42	
Disease									
ALL	61	16	70%	58–82	0.94	28	53%	40–65	0.69
AML	29	7	73%	57–89		12	59%	41–76	
Disease risk index									
Low	17	1	94%	82–100	**0.01**	5	70%	48–92	0.17
Intermediate	64	17	70%	57–83		29	53%	41–66	
High	9	5	40%	12–77		6	33%	2–64	
Donor type									
MUD	41	11	71%	56–86	0.97	12	70%	55–84	**0.007**
MMUD	26	7	72%	55–90		12	54%	35–73	
Haplo	23	5	75%	54–94		16	30%	12–49	
Grade II–IV aGVHD[a]									
Present	17	4	76%	56–97	0.79	5	70%	48–92	0.16
Absent	72	18	72%	61–84		34	52%	40–64	
cGvHD[b]									
Present	10	4	76%	65–87	0.89	4	60%	30–90	0.68
Absent	74	30	59%	47–70		30	59%	47–70	

Table 3 Overall survival and event-free survival: multivariable analysis.

Variable		Overall survival			Event-free survival		
		Hazard ratio	95% CI	*p*	Hazard ratio	95% CI	*p*
Sex[a]	Male	1.96	0.4–1.75	0.21	1.37	0.6–2.8	0.39
	6–10 years	0.9	0.2–4.1	0.89	1.71	0.59–5	0.32
Age[b]	11–15 years	1.5	0.2–7.8	0.62	1.17	0.35–3.95	0.79
	>15 years	6.83	1.6–30	**0.007**	6.12	1.9–19.7	**0.002**
Disease[c]	AML	0.31	0.03–2.5	0.16	1.22	0.13–11.14	0.85
Disease risk index[d]	low	0.49	0.04–6.64	0.59	0.94	0.09–10.08	0.96
	high	1.7	0.16–18.3	0.2	0.91	0.09–9.38	0.94
Donor type[e]	MUD	0.9	0.3–2.8	0.86	0.45	0.18–1.13	0.08
	Haplo	0.9	0.23–3.5	0.89	1.95	0.82–4.7	0.13
Grade II–IV aGvHD[f]	present	0.8	0.25–2.6	0.71	0.55	0.19–1.65	0.29
cGvHD[g]	present	1.2	0.32–4.8	0.75	0.79	0.23–2.73	0.71

Table 4 5 years non-relapse mortality (NRM): univariable analysis.

Variable	n	Events	NRM	95% CI	p
Sex					
Female	41	2	5%	1–21	0.05
Male	49	8	19%	10–34	
Age					
0–5 years	25	2	12%	4–35	
6–10 years	30	0	–	–	**0.03**
11–15 years	21	5	23%	11–51	
>15 years	14	3	26%	9–70	
Disease					
ALL	61	7	12%	6–26	0.76
AML	29	3	13%	5–33	
Disease risk index					
Low	17	1	6%	0.8–39	
Intermediate	64	8	14%	7–27	0.76
High	9	1	11%	2–70	
Donor type					
MUD	41	6	15%	7–32	
MMUD	26	3	14%	4–45	0.72
Haplo	23	1	8%	2–31	
Grade II–IV aGVHD[a]					
Present	17	1	6%	1–39	0.51
Absent	72	8	12%	6–23	
cGvHD[b]					
Present	10	2	20%	6–69	0.16
Absent	74	5	8%	0–19	

ALL acute lymphoblastic leukemia, *AML* acute myelogenous leukemia, *MUD* matched unrelated donor, *MMUD* mismatched unrelated donor, *Haplo* haploidentical donor.
[a]89 evaluable patients.

Table 5 5 years relapse incidence (RI): univariable analysis.

| Variable	*n*	Events	RI	95% CI	*p*
Sex					
Female	41	9	22%	12–39	0.94
Male	49	12	24%	15–39	
Age					
0–5 years	25	3	11%	3–33	
6–10 years	30	9	30%	17–51	**0.008**
11–15 years	21	2	9%	2–35	
>15 years	14	7	50%	29–84	
Disease					
ALL	61	15	24%	16–38	0.61
AML	29	6	20%	9–40	
Disease risk index					
Low	17	2	12%	12–38	
Intermediate	64	15	23%	14–40	0.15
High	9	4	44%	21–92	
Donor type					
MUD	41	6	15%	7–30	
MMUD	26	7	26%	14–50	0.25
Haplo	23	8	33%	19–58	
Grade II–IV aGvHD[a]					
Present	17	3	18%	6–49	0.52
Absent	72	18	25%	17–37	
cGvHD[b]					
Present	10	2	20%	6–69	0.74
Absent	74	17	23%	15–35	

ALL acute lymphoblastic leukemia, *AML* acute myelogenous leukemia, *MUD* matched unrelated donor, *MMUD* mismatched unrelated donor, *Haplo* haploidentical donor.
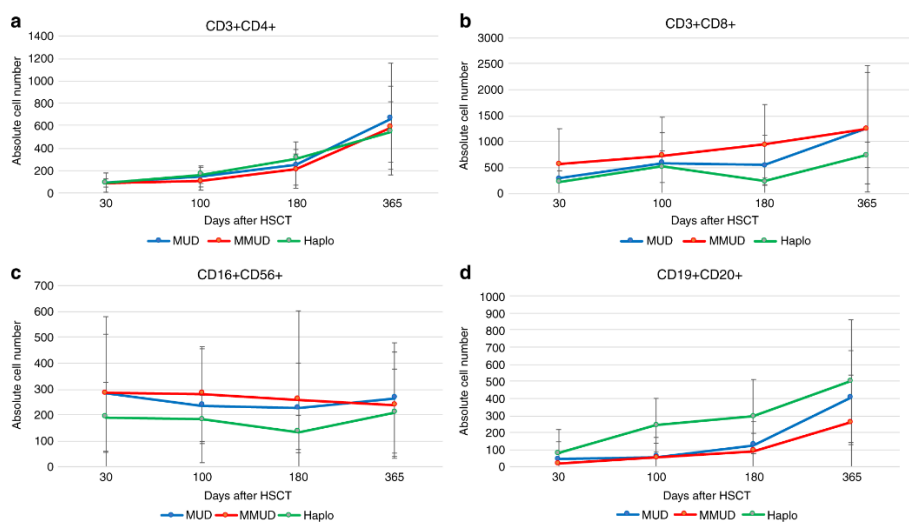[a]89 evaluable patients.
[b]84 evaluable patients.
Statistically significant values are in bold.

Fig. 1: Overall survival of patients included in the study.
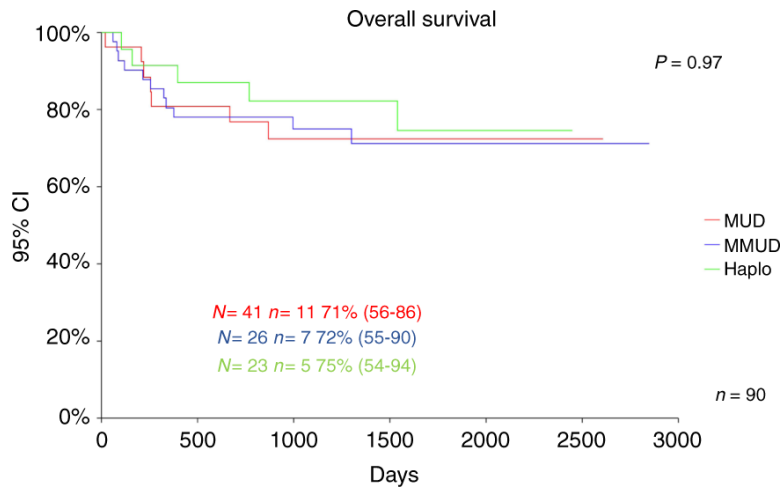
MUD matched unrelated donor, MMUD mis-matched unrelated donor, Haplo haploidentical donor.

Fig. 2

Mean ± standard deviation of (**a**) CD3$^+$CD4$^+$ cells, (**b**) CD3$^+$CD8$^+$ cells, (**c**) CD16$^+$CD56$^+$ cells, and (**d**) CD19$^+$CD20$^+$ cells at different time points after transplantation.