Matched unrelated donor transplantation *versus* haploidentical transplantation with post-transplant cyclophosphamide in children with acute myeloid leukemia: a PDWP-EBMT study

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Abstract

In children with acute myeloid leukemia (AML) who lack a human leukocyte antigen (HLA) identical sibling, the donor can be replaced with an HLA-matched unrelated donor (MUD) or a haploidentical donor (haplo). We compared outcomes of patients <18 years with AML in first and second complete remission (CR1 & CR2) undergoing a hematopoietic stem cell transplantation (HCT) either with a MUD with anti-thymocyte globulin (ATG) (N=420) or a haplo HCT with post-transplant cyclophosphamide (PT-CY) (N=96) after a myeloablative conditioning regimen (MAC) between 2011 and 2021, reported to the

European Society for Blood and Marrow Transplantation. A matched pair analysis was performed to adjust for differences among groups. The final analysis was performed on 253 MUD and 95 haplo-HCT. In the matched cohort, median age at HCT was 11.2 and 10 years and median year of HCT was 2017 and 2018, in MUD and haplo-HCT recipients, respectively. The risk of grade III-IV acute graft-*versus*-host disease (aGVHD) was significantly higher in the haplo group (hazard ratio [HR]=2.33, 95% confidence interval [CI]: 1.18-4.58; P=0.01). No significant differences were found in 2 years overall survival (OS; 78.4% *vs*. 71.5%; HR=1.39, 95% CI: 0.84-2.31; P=0.19), leukemia-free survival (LFS; 72.7% *vs*. 69.5%; HR=1.22, 95% CI: 0.76-1.95; P=0.41), CI of relapse (RI; 19.3% *vs*. 19.5%; HR=1.14, 95% CI: 0.62-2.08; P=0.68) non-relapse-mortality (NRM; 8% vs. 11%; HR=1.39, 95% CI: 0.66-2.93; P=0.39) and graft-*versus*-host free relapse-free survival (GRFS; 60.7% *vs*. 54.5%, HR=1.38, 95% CI: 0.95-2.02; P=0.09) after MUD and haplo-HCT respectively. Our study suggests that haplo-HCT with PT-CY is a suitable option to transplant children with AML lacking a matched related donor.

Introduction

Childhood acute myeloid leukemia (AML) is a rare and heterogeneous disease, with an incidence of seven cases per million children younger than 15 years.[1] Improvements in genomic characterization, disease response monitoring,[2] as well as the introduction of new drugs[3] and advances in allogeneic hematopoietic cell transplantation (HCT) techniques[4] have resulted in improved outcomes with survival rates reaching 70% in high-income countries. First line treatment approaches for pediatric AML include four or five cycles of myelosuppressive chemotherapy followed by HCT for high-risk patients,[5] while HCT is offered in second clinical remission for other cases.[5,6]

In recent years, the development of HCT from alternative donors, preferably from matched unrelated donors (MUD), has provided the means to offer HCT, when no human leukocyte antigen (HLA)-matched family donor is available. However, in up to 40% of patients a MUD cannot be identified in international donor registries.[7] Recently, HCT from relatives sharing only one HLA-haplotype with the recipient (haplo-HCT) has emerged as a suitable alternative, with the great advantage that haplo-donors may be available for almost all patients, in due time, with a flexible schedule and additional cellular therapies being readily available.

In haplo-HCT, different techniques to overcome the HLA barrier[8-12] have been developed for improving immune reconstitution and graft-*versus*-leukemia with no excess of graft-*versus*-host disease (GVHD). In the last years, the use of T-cell replete haplo-HCT with post-transplant cyclophosphamide (PT-CY),[8] has rapidly increased across the world, showing identical clinical outcomes to matched donors (MD) in several retrospective studies in adults.[8]

Many studies demonstrated that PT-CY given after graft infusion can eliminate alloreactive T cells while preserving hematopoietic cells as well as memory and regulatory T cells.[13,14] In pediatric patients affected by acute leukemia, promising clinical results have been reported,[13,15-19] but specific data on AML are lacking.

Here, we compared the results of a haplo-HCT with PT-CY to a MUD HCT using anti-thymocyte globulin (ATG) for the treatment of children affected by AML reported to the European Society for Blood and Marrow Transplantation (EBMT) registry.

Methods

Study design and definitions

This multicenter retrospective study was conducted on behalf of the Pediatric Diseases Working Party (PDWP) of EBMT after approval by the institutional review board of the PDWP. Data were collected according to EBMT rules. Patients or legal guardians provided written informed consent for data collection and analysis in accordance with the Declaration of Helsinki. Included were pediatric patients (age <18 years) diagnosed with AML, transplanted in first or second complete remission from 2011 to 2021, who underwent a first allo-HCT using either a 10/10 MUD with ATG or a haploidentical family donor (≥2 recipient-donor HLA mismatch number) in a haplo-HCT with PT-CY. Transplants were performed in 117 EBMT centers. All patients received a myeloablative conditioning (MAC) regimen, and the graft source was bone marrow (BM) or peripheral blood, according to the transplant center policy. In patients receiving *ex vivo* T-cell depletion, alemtuzumab or a combination of ATG and PT-CY was excluded.

Outcomes

The primary objective was to compare the leukemia-free survival (LFS) of AML patients receiving either a haplo-PT-CY or a MUD-ATG HCT. The secondary objectives were the comparison of overall survival (OS), non-relapse mortality (NRM), relapse incidence (RI), incidence of both acute GVHD (aGVHD) and chronic GVHD (cGVHD), GVHD-free/relapse-free survival (GRFS) in both groups. LFS was defined as time from HCT to first event of relapse or death. OS was defined as the time from HCT to death from any cause. RI was defined as the time from HCT to the first event of leukemia recurrence. Non-relapse mortality (NRM) was defined as death without evidence of relapse. RI and NRM were mutually competing events. GRFS was defined as the time from HCT to the first event among grade 3-4 aGVHD, extensive cGVHD, relapse, and death.[20] Grade 2-4

aGVHD and cGVHD were assigned and graded using standard criteria.[21, 22] Competing events of aGVHD and cGVHD were relapse and death. Cytogenetics abnormalities were classified according to the genetically defined prognostic stratification of the 2017 European Leukemia Net cytogenetic classification system.[1] Neutrophil recovery was defined as the time from HCT to the first of 3 consecutive days with neutrophil counts above 0.5x10^9/L; platelet recovery was defined as independence from platelet transfusion for at least 7 days with a platelet count of more than >20×10^9/L. Death and consecutive HCT were competing events.

Statistical analysis

The overall population included 420 MUD and 96 haplo PT-CY. A matched pair analysis was performed to reduce or eliminate confounding factors: the matching included exact match on disease status, cell source and age group at HCT, and nearest match on year of HCT, age at diagnosis, female donor to male recipient, Lansky score and cytomegalovirus match. A maximum of three MUD matched patients was allowed for one haplo PT-CY. There were three controls for 73, two for 12 and one for ten haplo PT-CY. For one haplo PT-CY it was impossible to find a control. Finally, a match could be identified for 253 MUD and 95 haplo PT-CY. Median values with respective interquartile ranges (IQR), were used to express quantitative variables while frequencies and percentages were used for categorical variables. On the unmatched population, differences between MUD and haplo PT-CY on quantitative and qualitative variables have been tested using χ^2 and Wilcoxon tests respectively. LFS, OS and GRFS were estimated using the Kaplan-Meier estimator. All outcomes with competing events were estimated using the cumulative incidence function. Median follow-up was estimated using the reverse of the Kaplan-Meier method. All outcomes were censored at last follow-up or at 2 years due to a different follow-up between the groups. Differences in outcomes were tested using a Cox model including a cluster term for each pair. Results are expressed as hazard ratio (HR). Outcomes and HR are presented with their 95% confidence interval (CI). All tests are two-sided with an error rate fixed at 5%. Analyses were done using R statistical software version 4.0.2 (http://www.R-project.org), and matching was performed using the MatchIt package.

Results

Patients and transplant characteristics

Patient and transplant characteristics are summarized in Table 1. Overall, 96 and 420 patients receiving a haplo PT-CY or MUD HCT, respectively, fulfilled the inclusion criteria. Median age at transplant was 9.8 (IQR, 3.0-15.2) years for haplo PT-CY and 7.7 (IQR, 2.8-13.6) years for MUD (*P*=0.03). Patients in the haplo group were transplanted more recently (median 2018 for haplo *vs.* 2016 for MUD, *P*<0.01). In both

groups the majority of patients were in CR1 at transplant (72.9% haplo *vs.* 70.5% MUD; *P*=0.63).
Cytogenetic risk at diagnosis was not significantly different in the two groups (good: 21.3% *vs.* 13.6%, intermediate: 48% *vs.* 44.3%, poor: 30.7% *vs.* 42.1% in haplo PT-CY and MUD respectively).
Donors were older in the haplo PT-CY than in the MUD cohort: 35.7 years (IQR, 29.9-42.7) *versus* 27.7 years (IQR, 23.3-34.2; *P*<0.01). BM was the most frequently used stem cell source in both groups (65.6% for haplo *vs.* 63.6% for MUD; *P*=0.71).
Conditioning regimen was MAC, mainly based on busulfan (Bu), with Bu/fludarabine (66.7%) based in the haplo PT-CY and Bu/CY (28.8%) or Bu/CY/melphalan (38.3%) being the most common in MUD.
Post-HCT immunosuppression consisted mainly of cyclosporine (CSA) plus mycophenolate mofetil (MMF) in the haplo PT-CY (52.1%) and CSA plus methotrexate (MTX) in the MUD cohort (68.6%).
The baseline characteristics of the matched cohort are described in Table 2.

Engraftment, acute and chronic graft-*versus*-host disease

The CI of day 60 neutrophils and day 180 platelet recovery for haplo PT-CY was 90.4% (95% CI: 82-95) and 92.2% (95% CI: 82.6-96.6) while for MUD it was 97.1% (95% CI: 93.9-98.6) and 93.8% (95% CI: 89.1-96.4) respectively.
The 100 day grade 2-4 aGVHD was 36.7% (95% CI: 26.8-46.6) for haplo PT-CY and 28.7% (95% CI: 23.2-34.4) for MUD and CI of grade 3-4 aGvHD was 14.4% (95% CI: 8.1-22.5) *versus* 6.4% (95% CI: 3.8-9.9), respectively.
2-year CI of cGVHD was 22.4% (95% CI: 13.4-32.8) and 18.5% (95% CI: 13.5-24.1) for haplo PT-CY and MUD respectively; 2-year CI of extensive cGVHD was 6.6% (95% CI: 2.4-13.8) and 8.6% (95% CI: 5.3-13) for haplo PT-CY and MUD (Table 3).
According to donor type, there were no statistically significant differences between groups on the incidence of grade 2-4 aGVHD (HR=1.26, 95% CI: 0.84-1.89; *P*=0.27), cGVHD (HR=1.81, 95% CI: 0.75-4.37; *P*=0.19) and extensive cGVHD (HR=1.01, 95% CI: 0.25-4.03; *P*=0.99). The risk of grade 3-4 aGVHD was significantly higher in haplo PT-CY (HR=2.33, 95% CI: 1.18-4.58; *P*=0.03) (Table 4).

Main outcomes

Results of the punctual estimation of outcomes after matched-pair analysis are summarized in Table 3.
The 2-year CI of RI was 19.5% (95% 11.4-29.2) for haplo PT-CY *versus* 19.3% (95% CI: 14.4-29.4) for MUD. The 2-year NRM was 11% (95% CI: 5.2-19.1) *versus* 8% (95% CI: 5-11.9) after haplo PT-CY and MUD respectively.
The 2-year OS was 71.5% (95% CI: 59.1-80.7) and 78.4% (95% CI: 72.2-83.4), the 2-year LFS was 69.5% (95% CI: 57.7-78.6) and 72.7% (95% CI: 66.3-78.1) and the 2-year GRFS was 54.5% (95% CI: 42.5-65) and 60.7% (95% CI: 53.8-66.9) for

Table 1. Patients and transplant characteristics.

Variables	Modalities	N=516 N (%)	MUD N=420 N (%)	Haplo PT-CY N=96 N (%)	P
Age in years at HCT	median (IQR)	8.5 (2.8-13.7)	7.7 (2.8-13.6)	9.8 (3-15.2)	0.03
Age in years at HCT	0-7	231 (44.8)	200 (47.6)	31 (32.3)	Not done
	7-12	107 (20.7)	82 (19.5)	25 (26)	-
	12-18	178 (34.5)	138 (32.9)	40 (41.7)	-
Disease status at HCT	CR1	366 (70.9)	296 (70.5)	70 (72.9)	0.63
	CR2	150 (29.1)	124 (29.5)	26 (27.1)	-
Molecular remission	No	49 (15.6)	41 (15.9)	8 (14)	0.73
	Yes	266 (84.4)	217 (84.1)	49 (86)	-
	Missing	201	162	39	-
Year of HCT	Median (IQR)	2017 (2014-2019)	2016 (2014-2018)	2018 (2017-2020)	<0.001
	Range	2011-2021	2011-2021	2011-2021	-
Relation to donor	Parent	-	-	70 (83.3)	Not done
	Sibling	-	-	14 (16.7)	-
	Missing	-	-	12	-
Donor age in years	Median (IQR)	29.1 (23.8-36.3)	27.7 (23.3-34.2)	35.7 (29.5-42.7)	<0.001
	Missing	104	91	13	-
Cell source	BM	330 (64)	267 (63.6)	63 (65.6)	0.71
	PB	186 (36)	153 (36.4)	33 (34.4)	-
Patient sex	Female	242 (46.9)	197 (46.9)	45 (46.9)	0.99
	Male	274 (53.1)	223 (53.1)	51 (53.1)	-
Female donor to male recipient	No	434 (84.1)	365 (86.9)	69 (71.9)	<0.001
	Yes	82 (15.9)	55 (13.1)	27 (28.1)	-
Lansky or KPS	<90	119 (23.1)	104 (24.8)	15 (15.6)	0.06
	≥90	397 (76.9)	316 (75.2)	81 (84.4)	-
CMV-IgG match (donor-recipient)	Neg to Neg	120 (23.3)	112 (26.7)	8 (8.3)	< 0.001
	Neg to Pos	130 (25.2)	123 (29.3)	7 (7.3)	-
	Pos to Neg	60 (11.6)	50 (11.9)	10 (10.4)	-
	Pos to Pos	206 (39.9)	135 (32.1)	71 (74)	-
Cytogenetic risk	Good	66 (14.9)	50 (13.6)	16 (21.3)	0.09
	Intermediate	199 (44.9)	163 (44.3)	36 (48)	-
	Poor	178 (40.2)	155 (42.1)	23 (30.7)	-
	Missing	73	52	21	-
GVHD prophylaxis	CSA+MTX based	289 (56)	288 (68.6)	1 (1)	Not done
	CSA based	71 (13.8)	66 (15.7)	5 (5.2)	-
	CSA+MMF based	67 (13)	17 (4)	50 (52.1)	-
	CSA+MMF+MTX based	12 (2.3)	9 (2.1)	3 (3.1)	-
	Other	77 (14.9)	40 (9.5)	37 (38.5)	-
Conditioning regimen	BuCyMel based	162 (31.4)	161 (38.3)	1 (1)	Not done
	BuCy based	131 (25.4)	121 (28.8)	10 (10.4)	-
	BuFlu based	123 (23.8)	59 (14)	64 (66.7)	-
	Treo based	88 (17.1)	70 (16.7)	18 (18.8)	-
	Other combinations	12 (2.3)	9 (2.1)	3 (3.1)	-

MUD: matched unrelated donors; haplo: haploidentical; PT-CY: post-transplant cyclophosphamide, HCT: hematopoietic stem cell transplantation; IQR: interquartile range; CR: complete remission; BM: bone marrow; PB: peripheral blood; KPS: Karnofsky Performance status, CMV: cytomegalovirus; GVHD: graft-*versus*-host disease; CSA: cyclosporin; MTX: methotrexate, MMF: mycophenolate mofetile; Bu: busulfan, Flu: fludarabine; Cy: cyclophosphamide; Mel: melphalan; Treo: treosulfan; Pos: positive; Neg: negative.

Table 2. Patients and transplant characteristics in the matched pair cohort.

Variables	Modalities	MUD N=253 N (%)	Haplo PT-CY N=95 N (%)
Age in years at HCT	Median (IQR)	11.2 (4.9-14.6)	10 (3-15.4)
Age in years at HCT	0-7	87 (34.4)	31 (32.6)
	7-12	51 (20.2)	24 (25.3)
	12-18	115 (45.5)	40 (42.1)
Disease status at HCT	CR1	178 (70.4)	69 (72.6)
	CR2	75 (29.6)	26 (27.4)
Molecular remission	No	26 (16)	7 (12.5)
	Yes	136 (84)	49 (87.5)
	Missing	91	39
Year of HCT	Median (IQR)	2017 (2014-2019)	2018 (2017-2020)
Relation to donor	Parent	-	69 (83.1)
	Sibling	-	14 (16.9)
	Missing	-	12
Donor age in years	Median (IQR)	27 (23.2-34.2)	35.7 (29.5-42.89
	Range	18.4-57.4	4.4-54.3
	Missing	48	13
Source of cells	BM	163 (64.4)	62 (65.3)
	PB	90 (35.6)	33 (34.7)
Patient sex	Female	123 (48.6)	44 (46.3)
	Male	130 (51.4)	51 (53.7)
Female donor to male recipient	No	217 (85.8)	68 (71.6)
	Yes	36 (14.2)	27 (28.4)
Lansky or KPS	<90	62 (24.5)	15 (15.8)
	≥90	191 (75.5)	80 (84.2)
CMV IgG match donor-recipient	Neg to Neg	50 (19.8)	7 (7.4)
	Neg to Pos	64 (25.3)	7 (7.4)
	Pos to Neg	35 (13.8)	10 (10.5)
	Pos to Pos	104 (41.1)	71 (74.7)
Cytogenetic risk	Good	30 (13.7)	15 (20.3)
	Intermediate	123 (56.2)	36 (48.6)
	Poor	66 (30.1)	23 (31.1)
	Missing	34	21
GVHD prophylaxis	CSA+MTX based	169 (66.8)	1 (1.1)
	CSA based	46 (18.2)	5 (5.3)
	CSA+MMF based	11 (4.3)	49 (51.6)
	CSA+MMF+MTX based	3 (1.2)	3 (3.2)
	Other	24 (9.5)	37 (38.9)
Conditionning regimen	BuCyMel based	89 (35.2)	1 (1.1)
	BuCy based	71 (28.1)	10 (10.5)
	BuFlu based	36 (14.2)	63 (66.3)
	Treo based	51 (20.2)	18 (18.9)
	Other combinations	6 (2.4)	3 (3.2)

MUD: matched unrelated donors; haplo: haploidentical; PT–CY: post-transplant cyclophosphamide, HCT: hematopoietic stem cell transplantation; IQR: interquartile range; CR: complete remission; BM: bone marrow; PB: peripheral blood; KPS: Karnofsky Performance status, CMV: cytomegalovirus; CSA: cyclosporin; MTX: methotrexate, MMF: mycophenolate mofetile; Bu: busulfan; Flu: fludarabine; Cy: cyclophosphamide; Mel: melphalan; Treo: treosulfan; Pos: positive; Neg: negative.

Table 3. Survival outcomes after matched-pair analysis.

Outcomes	N=348	MUD N=253	Haplo N=95
	Estimation, % (95% CI)	Estimation, % (95% CI)	Estimation, % (95% CI)
OS 2-year	76.6 (71.2-81.2)	78.4 (72.2-83.4)	71.5 (59.1-80.7)
LFS 2-year	71.8 (66.3-76.6)	72.7 (66.3-78.1)	69.5 (57.7-78.6)
RI 2-year	19.4 (15.1-24.2)	19.3 (14.4-24.9)	19.5 (11.4-29.2)
NRM 2-year	8.8 (6-12.2)	8 (5-11.9)	11 (5.2-19.1)
aGVHD 2-4 100 days	30.8 (26-35.7)	28.7 (23.2-34.4)	36.7 (26.8-46.6)
aGVHD 3-4 100 days	8.5 (5.8-11.8)	6.4 (3.8-9.9)	14.4 (8.1-22.5)
cGVHD 2-year	19.5 (15-24.4)	18.5 (13.5-24.1)	22.4 (13.4-32.8)
cGVHD Ext 2-year	8.2 (5.3-11.8)	8.6 (5.3-13)	6.6 (2.4-13.8)
GRFS 2-year	59 (53-64.4)	60.7 (53.8-66.9)	54.5 (42.5-65)
Neutrophil engraftment 60 days	95.3 (92.3-97.1)	97.1 (93.9-98.6)	90.4 (82-95)
Platelet engraftment 180 days	93.3 (89.5-95.8)	93.8 (89.1-96.4)	92.2 (82.6-96.6)

MUD: matched unrelated donors; haplo: haploidentical; CI: confidence interval; OS: overall survival; LFS: leukemia-free survival; RI: relapse incidence; NRM: non-relapse mortality; a: acute, c: chronic; GVHD: graft-*versus*-host disease; ext: extensive; GRFS: graft-*versus*-host free, relapse-free survival.

haplo PT-CY and MUD respectively (Figure 1).
The most common causes of death were disease recurrence (61.9% in the haplo and 59.6% in the MUD group, respectively), infections (28.6% *vs.* 19.1%), and GVHD (9.5% *vs.* 8.5%).
There were no statistically significant differences between groups on RI (HR=1.14, 95% CI: 0.62-2.08; *P*=0.68), NRM (HR=1.39, 95% CI: 0.66-2.93; *P*=0.39), OS (HR=1.39, 95% CI: 0.84-2.31; *P*=0.19), LFS (HR= 1.22, 95% CI=0.76-1.95; *P*=0.41) and GRFS (HR=1.38, 95% CI: 0.95-2.02; *P*=0.09) (Table 4).

Discussion

Allogeneic HCT from MUD remains the standard of care in patients who lack a MSD, especially for pediatric patients in which transplant-related toxicities and late effects are particularly relevant with regard to a long life expectancy.[23-28] The recent emergence of HLA haplo-HCT extended the availability of donors to most pediatric patients without a MD.[29] Significant improvements of supportive care and the technical development of highly effective T-cell depleted and T-cell replete HLA-haploidentical platforms, have resulted in improved outcomes and reduced transplant-related mortality for patients undergoing haplo-HCT,[30] independently of the haplo platform used.[31,36,37]
T-cell-depleted haploidentical platforms have been successfully used despite high costs for graft processing and specialized expertise.[11,32,33] Of particular interest, Locatelli *et al.*[10] reported the outcome of a cohort of 80 children with acute leukemia transplanted from a haplo donor after αβ T-cell and CD19+ B-cell depletion. All children received a myeloablative conditioning and ATG for GVHD prophylaxis

Table 4. Impact of donor type for outcomes censored at 2 years in the matched cohort.

Outcome	HR (95% CI)	*P*
OS	1.39 (0.84-2.31)	0.19
LFS	1.22 (0.76-1.95)	0.41
RI	1.14 (0.62-2.08)	0.68
NRM	1.39 (0.66-2.93)	0.39
aGVHD 2-4	1.26 (0.84-1.89)	0.27
aGVHD 3-4	2.33 (1.18-4.58)	0.01
cGVHD	1.81 (0.75-4.37)	0.19
Ext cGVHD	1.01 (0.25-4.03)	0.99
GRFS	1.38 (0.95-2.02)	0.09

HR: hazard ratio; CI: confidence interval; OS: overall survival; LFS: leukemia-free survival; RI: relapse incidence; NRM: non-relapse mortality; a: acute, c: chronic; GVHD: graft-*versus*-host disease; ext: extensive; GRFS: graft-*versus*-host free, relapse-free survival.

and graft rejection; no post-transplant GVHD prophylaxis was given. The 5-year OS was 72% and LFS 71% with no differences in AML and ALL. TRM was 5% and RI was 24%. No patients developed grade 2-4 aGVHD with visceral involvement, grade 3-4 aGVHD or cGVHD. Bertaina *et al.*[12] compared the outcomes of 98 children treated with αβ T-cell depletion compared with 127 MUD and 118 mismatched UD (MMUD). Five-year LFS was not significantly different in the three groups (67%, 55%, and 62%, respectively), while a lower incidence of aGVHD was reported in patients treated with T-cell depleted haplo-HCT, compared with MUD and MMUD (2-4 aGVHD was 35% *vs.* 44% *vs.* 16%, respectively). On the other hand, T-cell-replete haplo-HCT approaches are mainly based on PT-CY, pioneered by the John Hopkins

group in the adult setting.[8,34] This platform is now the most widely adopted strategy because it is cost-effective and easily replicated. In recent years, emerging evidence also supports the use of this platform in the pediatric population with hematological malignancies.[19,35]

Saglio *et al.*[15] retrospectively compared the outcome of 23 pediatric patients undergoing haplo-HCT with PT-CY for acute leukemia with patients undergoing HCT from MUD (N=41) and HLA-mismatched unrelated donor (MMUD) (N=26) from a single institution. Five-year OS, NRM and RI were not different for the three groups, confirming that haplo PT-CY is a suitable clinical option for pediatric patients. More recently, Srinivasan *et al.*[16] compared outcomes of haplo-HCT PT-CY with peripheral blood (N=26) to matched sibling donor (MSD) (N=31) and MUD HCT (N=47), both with

BM as stem cell source. Results showed that haplo-HCT PT-CY with peripheral blood had comparable outcomes to BM MSD and MUD HCT. Hong *et al.*[17] compared the outcomes of children and adolescents with high-risk acute leukemia who underwent haplo-HCT PT-CY (N=35) or MUD HCT (N=45) after a busulfan-based myeloablative conditioning. No differences were observed in the main outcomes. In a subgroup analysis of patients with AML (haplo, N=16; MUD, N=16), the 3-year GRFS, LFS, and OS rates in haplo-HCT and MUD groups were 80.8% *versus* 61.9%, 87.1% *versus* 73.9%, and 93.8% *versus* 85.6%, respectively.

Here, we report the largest series of children with AML treated with haplo PT-CY reported to the EBMT registry. Our results are in line with the previous single center reports and confirmed the overall feasibility of the PT-CY approach
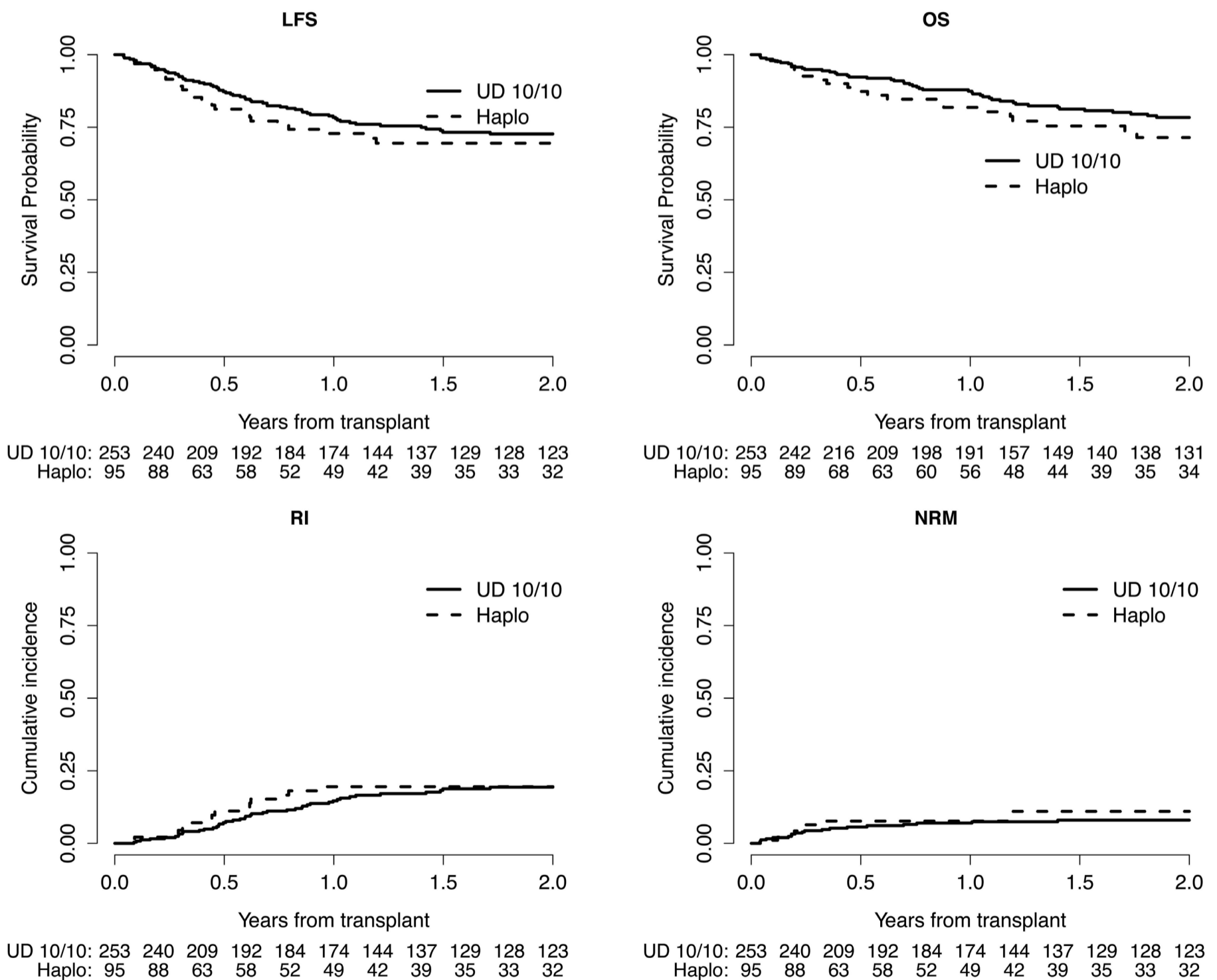
Figure 1. Two-years survival outcomes after matched unrelated donor and haploidential hematopoietic cell transplantation after matched pair analysis. Two-year leukemia-free survival (LFS), overall survival (OS), relapse incidence (RI) and non-relapse mortality (NRM) after matched unrelated donor (MUD) and haploidential hematopoietic cell transplantation in children with acute myeloid leukemia after matched pair analysis.

in pediatric AML. Importantly, in our cohort haplo HCT was associated with higher incidence of grade 3-4 aGVHD and this could be related to the graft source itself, namely the content of CD3⁺ cells in the poeripheral blood stem cells as well to the differences in the overall GVHD prophylaxis in the two group. Importantly MUD recipients received ATG which is a key factor associated with reduced risk of GVHD. Whenever the combination of ATG and PTCY could be considered in the pediatric setting deserves further investigation. Overall, no differences in pivotal outcome parameters were found between haplo PT-CY and MUD. The results were consistent across the different disease status when we checked for interaction between the disease status and the donor type. We are aware of the limitations of this study, namely the retrospective nature, the short follow-up, due to the recent use of PT-CY in this setting and the limited data on infection and immune reconstitution due to the registry based analysis. However, we believe that our results are important and highlight the feasibility of this approach also in children. How the PTCY approach could be adopted also in the unrelated donor setting in the pediatric HCT deserves further investigation.

Eligibility criteria for HCT for each cooperative group are beyond the purpose of this multinational retrospective study. Nevertheless, eligibility for haplo-HCT may be either the same as for MUD or more restricted to higher risk patients only. Therefore, in case any unknown prognostic feature could not be adjusted within the matched pair analysis, the worst risk distribution would affect the outcome of the haplo PT-CY more than the MUD cohort. In the pair match analysis, the year of transplant was not considered as exact matching but using a propensity score. This resulted in a median year of transplant of 2017 and 2018 for MUD and haplo respectively. Consequently, the median follow-up

was different in the two groups. In order to solve this issue and make the groups more comparable, we censored the outcomes at 2 years preventing us to provide results at a longer period to avoid imbalance between the groups. Such a haplo platform may enlarge access to HCT to virtually all eligible pediatric patients with AML. Furthermore, the prompt availability and the flexibility of a family member may be crucial in the challenging HCT scheduling of rapidly evolving pediatric malignancies, such as high-risk AML. Albeit MUD HCT remains the standard of care, our study confirmed in a large international analysis the comparable results of haplo-HCT with PT-CY and MD HCT, so that pediatric patients with AML who either lack a MD or cannot afford a MUD HCT, can be safely transplanted without delay.

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