Haplo-identical allografting with post-transplant cyclophosphamide in high-risk patients

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Abstract

Haplo-identical transplants (Haplo-Tx) are an important alternative for patients with hematological malignancies who lack a HLA-identical donor. Seventy-one T-replete Haplo-Tx were performed in 70 high-risk patients at our center; 22/70 (31%) patients with refractory/relapsed leukemia received sequential salvage therapy (SeqTh) with high-dose chemotherapy followed by Haplo-Tx during the chemotherapy-induced neutropenia. Graft-versus-host disease (GVHD) prophylaxis consisted of post-transplant cyclophosphamide (days + 3 and + 4) with tacrolimus and mycophenolic acid. After a median follow-up of 29.2 months, 3-year overall survival (OS) and event-free survival (EFS) were 43.8 and 40.2%, while 3-year cumulative incidences (CIs) of non-relapse mortality (NRM) and relapse (RI) were 27 and 33%. Day 100 and day 400 CI of grade III–IV acute and moderate-severe chronic GVHD were 11 and 15%. Three-year RI was significantly lower in patients in complete remission (CR) versus those not in CR at the time of transplant (21.5 vs. 48%, $p = 0.009$) and in patients who received PBSC as compared to BM (22 vs. 45%, $p = 0.009$). In patients treated with SeqTh, 3-year OS was 19%, while 3-year RI and NRM were 52 and 28% at a median follow-up of 50 months. Overall, Haplo-Tx was feasible in heavily pretreated high-risk patients without a suitable HLA-identical donor.

Keywords Allogeneic transplant · Haplo-identical · High-risk · Acute leukemias · Sequential therapy

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Introduction

Allografting is potentially curative for several hematological malignancies. Donor availability remains a major limitation. A HLA-identical sibling is currently considered the optimal donor at most centers. However, approximately only one third of the patients of Caucasian origin eventually find one in the family and 70% find a suitable unrelated donor. These figures are lower for individuals of ethnic minorities [1, 2]. Family haplo-identical donors and umbilical cord blood (UCB) units have become important alternatives for those patients without a suitable HLA-matched donor.

Haplo-identical hematopoietic stem cell transplantation (Haplo-Tx) is associated with intense bi-directional allo-reactivity. Different programs with both T-cell depleted (TCD) and T-cell repleted modalities were proposed over the decades to prevent graft vs. host disease (GVHD) while

sparing the graft vs. leukemia effects. High rates of non-relapse mortality (NRM) were however observed [3, 4]. More recently, other Haplo-Tx strategies were developed [5] either by refinements of T-cell depletion [6–8] or by intensified immune suppression [9–11]. The currently most commonly used T-cell replete (TCR) Haplo-Tx modality was pioneered by the Johns Hopkins group. In this setting, the administration of post-transplant cyclophosphamide (PT-Cy) plays a pivotal role [12–20]. Preclinical studies showed that PT-Cy targets allo-reactive T-cells generated early after Haplo-Tx, sparing non-dividing lymphocytes and hematopoietic stem cells mainly due to increased expression of protective aldehyde dehydrogenase (ALDH) [21–23]. In particular, Tregs resistance to cyclophosphamide was recently correlated with lower GVHD rates [24, 25].

At our center, the preferred alternative for patients who lack a HLA-identical sibling has been a HLA-matched or partially matched (8/8 or 7/8 matched) unrelated donor. In 2010, we implemented a Haplo-Tx program with PT-Cy for high-risk patients without a suitable donor. Here, we report our experience that helped to define our center policy.

Patients and methods

Patients

Seventy-one Haplo-Tx were performed in 70 patients with high-risk hematological malignancies at the Transplant Center of the Department of Oncology, Presidio Molinette, AOU Città della Salute e della Scienza di Torino, Torino, Italy, between January 2010 and January 2017. Fourteen patients, with shorter follow-up, were also part of a retrospective registry study by the European Bone Marrow Transplantation Group (EBMT) [26]. Patient and disease characteristics are summarized in Table 1. The hematopoietic cell transplantation comorbidity index (HCT-CI) was assessed as previously described [27]. Disease risk index (DRI) was calculated for patients who underwent Haplo-Tx as first transplant with the exception of 2 with rare diseases (blastic plasmocytoid dendritic cell neoplasm and plasmablastic lymphoma) [28]. All patients gave written informed consent to the proposed treatment and to the use of medical records for research purposes. The study was approved by the Center Ethical Committee and conducted according to the Declaration of Helsinki.

Definition of haplo-identical donor

HLA-typing was performed at high resolution level at HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci. A haplo-identical donor was defined as a family donor with a shared haplotype and at least two HLA-mismatches at the allele level on the unshared haplotype [29].

Table 1 Patients and transplants characteristics

	N (%)
Patients; Haplo-Tx	70; 71
Median age, years (range)	49 (21–70)
Male	36 (51%)
Hematological disease	
AML	47 (67)
ALL-B, ALL-T	9 (13)
Aggressive NHL	6 (9)
MDS	3 (4)
CLL	1 (1)
CML-BC	2 (3)
BPDCN	2 (3)
Disease risk index (DRI)[a]	
Intermediate	34 (48.5)
High/very high	20 (28.5)
Not applicable	16 (23)
2nd allogeneic transplant[b]	12 (17)
3rd allogeneic transplant[b]	2 (3)
Sequential therapy	22 (31)
HCT-CI ≥ 3[b]	34(48)
Conditioning regimen[b]	
Thiotepa + busulfan + fludarabine	46(65)
Thiotepa + melphalan + fludarabine	2 (3)
Fludarabine + TBI 12 Gy	2 (3)
Fludarabine + cyclophosphamide + TBI 2 Gy	19 (27)
Fludarabine + melphalan + TBI 2 Gy	1 (1)
Fludarabine	1 (1)
GVHD prophylaxis[b]	
Tacrolimus + MMF + PT Cy 50 mg/kg +3/+4	66 (93)
CSA + MMF + PT Cy 50 mg/kg +3/+5	4 (7)
Stem cell source BM/PBSC[b]	42 (59) / 29 (41)
Median donor age, years (range)	38 (16–66)

Haplo-Tx haplo-identical transplant, *AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *NHL* non-Hodgkin lymphoma, *MDS* myelodysplastic syndromes, *CLL* chronic lymphocytic leukemia, *CML-BC* chronic myeloid leukemia–blastic crisis, *BPDCN* blastic plasmocytoid dendritic cell neoplasia, *HCT-CI* hematopoietic cell transplantation comorbidity index, *TBI* total body irradiation, *GVHD* graft versus host disease, *MMF* mycophenolate mofetil, *PT Cy* post-transplantation cyclophosphamide, *CSA* cyclosporine A, *ATG* anti-thymocyte globulin, *BM* bone marrow, *PBSC* peripheral blood stem cells

[a] Calculated for patients at 1st Haplo-Tx

[b] Calculated on 71 transplants

Transplantation and graft vs. host disease prophylaxis

Bone marrow (BM) was used as stem cell source in 42/71 (59%) transplants and granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood stem cells (PBSC) in 29/71 (41%). Target cell doses were ≥ 3×10^8 total nucleated

cells (TNCs) for BM and ≥ 5×10^6 CD34+ cells/kg for PBSC. Conditionings employed are illustrated in Table 1. GVHD prophylaxis consisted of PT-Cy 50 mg/kg on days + 3 and + 4, tacrolimus and mycophenolic acid (MMF) from day + 5 in 66/71 transplants (93%). Four out of 71 (6%) enrolled in a multicenter clinical trial received PT-Cy 50 mg/kg on days + 3 and + 5 plus, cyclosporine from day − 1 and MMF from day + 1 as per protocol. MMF was stopped at days + 35 and + 28, respectively. Tacrolimus and cyclosporine were tapered off by day +180 in the absence of GVHD. Acute and chronic GVHD were diagnosed according to standard criteria [30, 31].

Supportive care and infection control

All patients received daily G-CSF from the day after the second PT-Cy infusion until absolute neutrophil count (ANC) engraftment. During neutropenia, patients received prophylactic quinolones or cephalosporins. Antifungal prophylaxis included fluconazole in 11/71 (15%) patients and echinocandins in 48/71 (66%) (micafungin in 46/48, caspofungin in 2/48). Moreover, 12/71 (17%) patients received secondary antifungal prophylaxis with mold-active triazoles (voriconazole in 6/12, posaconazole in 3/12) or liposomal amphotericin-B (in 3/12). Long-term prophylaxis against herpes virus and *Pneumocystis jirovecii* was performed in all patients. Preemptive antiviral therapy was initiated when a cytomegalovirus (CMV) DNA viral load ≥ 10.000 copies/ml by polymerase chain reaction assay was detected in peripheral blood. Diagnosis of invasive fungal infections (IFI) was defined according to the revised European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) definitions [32].

Statistical analysis

Primary end-points were overall survival (OS) and event-free survival (EFS). OS was defined as the time from transplant to death from any cause, while EFS as the time from transplant to progression/relapse/death from any cause, whichever occurred first. Alive patients were censored at the date of last contact (04/30/2017). Patients who received more than one transplant were censored as alive at the date of the second/third transplant. Survival curves were estimated by the Kaplan-Meier method and compared using the log-rank test. The composite end point of GVHD-free/relapse-free survival (GRFS) after transplant was calculated as described by Holtan et al. [33]; OS and EFS were also analyzed by the Cox proportional hazards model, comparing the two arms by the Wald test and calculating 95% confidence intervals. The following covariates were tested as risk factors: donor age (≥ 40 vs. < 40 years), recipient age (≥ 40 vs. < 40 years), donor/recipient sex-mismatch (female into male vs. other), disease status at transplant (active disease vs. complete remission (CR), patients who received SeqTh were considered with active disease), time from diagnosis to transplant (> 1 vs. ≤ 1 year), HCT-CI (≥ 3 vs. 0–2), stem cell source (bone marrow vs. peripheral blood), median cell doses infused/recipient weight (≥ 7.3×10^6 CD34+/kg vs. < 7.3×10^6 CD34+/kg, or ≥ 4.6×10^8 TNC/kg vs. < 4.6×10^8/kg TNC/kg), intensity of the conditioning (non-myeloablative/reduced intensity vs. myeloablative) [34], DRI. Cumulative incidence (CI) analyses were calculated for the following events: acute GVHD [competing events (CE): relapse/death without acute GVHD); chronic GVHD (CE: relapse/death without chronic GVHD); NRM (CE: relapse), RI (CE: death without relapse), neutrophil (ANC) and platelet (PLT) engraftment by stem cell source (CE: death without engraftment), CMV reactivation (CE: death without CMV reactivation), and IFI occurrence (CE: relapse/death without IFI). CI was estimated by the Gray test and by the competing risks regression model comparing the risk factors by the Fine-Gray test [35, 36]. Patient characteristics were tested using the Fisher's exact test for categorical variables and the Mann-Whitney test for continuous ones. All results for continuous variables were expressed as median (range). All reported *p* values were obtained by the two-sided exact method, at the conventional 5% significance level. Data were analyzed as of May 2017 by R 3.2.3 (R Foundation for Statistical Computing, Vienna-A, http://www.R-project.org).

Results

Donor selection and study population

An unrelated donor search was initially started for 53/70 patients (76%). Twenty-seven did not find a suitable donor while 18 (34%) relapsed during the donor search and underwent Haplo-Tx. For 17/70 (24%) patients, a donor search was not started because of age older than 65 years (n = 4), disease progression (n = 4), or graft failure (n = 1) after a first allograft from a sibling donor, very aggressive or refractory disease (n = 8) requiring an urgent transplant. Overall, 13 patients for a total of 14 transplants underwent Haplo-Tx as second or third allograft due to prior graft failure after Haplo-Tx (n = 1) or disease relapse after a previous allograft from UCB units, sibling, or unrelated donors (n = 12).

Median donor age was 38 years (range 16–66). Haploidentical donors were offspring in 34/71 (48%) transplants, siblings in 21/71 (30%), parents in 15/71 (21%), and a first-degree cousin in 1/71 (1%). Female donor into male recipient was used in 19/71 (27%) transplants. Forty-seven/71 (66%) recipient/donor pairs were fully allele-mismatched on the unshared haplotype, 15/71 (21%) had 4 mismatches, and 9/71 (13%) ≤ 3.

Thirty-one/70 (44%) patients, including those who underwent SeqTh, had active disease at the time of transplant. HCT-CI was ≥ 3 in 48%. Median time from diagnosis to

Haplo-Tx as first transplant (n = 56) was 7.5 months (range 2.9–114 months).

Cell doses and engraftment

Median cell doses infused were 5.9×10^8/kg TNC (range 1.8–20.0 × 10^8/kg) and 7.3 × 10^6 CD34+/kg (range 4.1–15.5 × 10^6/kg) for BM and PBSC, respectively. No statistically significant differences were observed in CI of acute GVHD, chronic GVHD, ANC, and PLTS engraftment comparing patients who received graft with cell doses above or below the median. Median ANC recovery occurred on day + 18 (range 14–24 days) for BM and on day + 15 (range 9–20 days) for PBSC (p = 0.001). Median PLTS recovery occurred on day + 27 (range 12–347) for BM and on day + 21 (range 10–192) for PBSC (p = 0.004). CI of ANC recovery at day + 30 were 97.6% for BM, and 86.2% for PBSC (p = 0.535); CI of PLTS at days + 30, + 60, and + 90 were 58.5, 73.2, and 78.9% for BM, and 69, 72.4, and 76% for PBSC (p = 0.434).

Acute and chronic GVHD

Overall CI of acute grade II-IV GVHD was 45% at day + 100 (Fig. 1a), whereas grade III–IV acute GVHD was 11% at day + 100. Median day of onset of acute GVHD was day 25 (range 7–100). CI of acute GVHD was significantly higher in patients who received PBSC versus BM (69 vs. 29%, p < 0.001). Day-400 CI of overall chronic GVHD and moderate-severe chronic GVHD were 38.5 and 15%, respectively (Fig. 1b); median day of onset was day + 196 (range 104–340). Overall, chronic GVHD was mild, moderate, and severe in 12/20 (60%), 4/20 (20%), and 4/20 (20%) of the evaluable patients. CI of overall chronic GVHD was 33.8% in patients who received PBSC and 38.2% in BM (p = 0.753). One-year GRFS of 56 patients who received Haplo-Tx as first allograft was 30.5% (Fig. 1c).

Infections

Median day of CMV reactivation was day + 40 (range 23–152) in 38/70 (54%) patients. Six/38 (15%) had multiple CMV reactivations (range 2–4). No CMV disease occurred. CI of CMV reactivation was 53% at 3 months. Four/70 (6%) showed Epstein-Barr virus (EBV) reactivation. Hemorragic cystitis developed in 12/70 (17%) at a median of day +43 (range 31–102); BK viruria was detected in 10/12 (83%). Overall, 15/70 (21%) experienced IFI, probable in 14/15 and proven in 1/15. Proven IFI was caused by *Aspergillus fumigatus* and *Aspergillus terreus* detected by bronchoalveolar lavage culture. Median time of onset was day + 73 (range 3–575). Breakthrough IFI was observed in 4% of the patients who received mold-active prophylaxis and 27% in those who received fluconazole. CI of probable-proven IFI was 20 and 22% at 12 and 24 months. By Gray test, the development of IFI was not influenced by age (p = 0.051), acute grade II–IV GVHD (yes vs. no, p = 0.566), acute grade III–IV GVHD (yes vs. no, p = 0.229), and chronic GVHD (yes vs. no, p = 0.694), conditioning regimen (non-myeloablative/reduced intensity vs. myeloablative, p = 0.839), and stem cell source (BM vs. PBSC, p = 0.966).

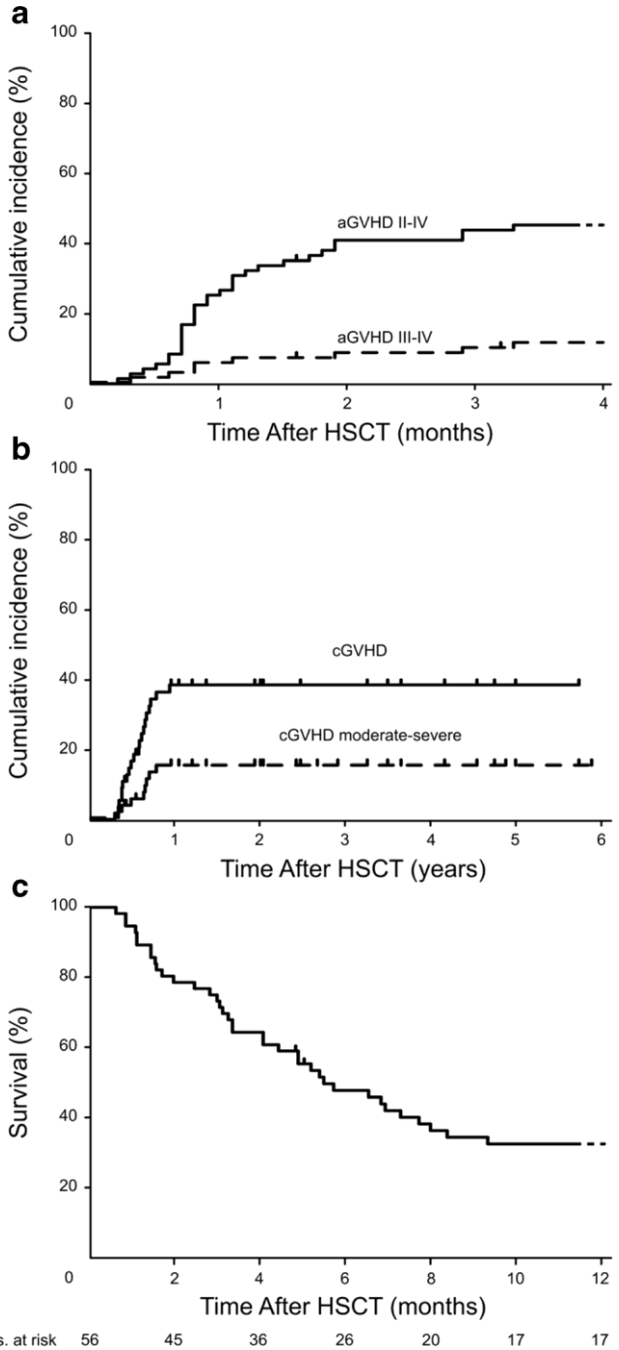

Fig. 1 a Cumulative Incidence of acute grade II-IV GVHD and grade III–IV GVHD at day100 (71 transplants). b Cumulative Incidence of overall chronic GVHD and moderate-severe chronic GVHD at day400 (71 transplants). c BGVHD relapse-free survival^ (GRFS) (56 patients who received Haplo-Tx as first allograft)

Clinical outcomes

Median OS and EFS were 24 and 14.5 months (Fig. 2a). Thirty-four out of 70 (48.5%) patients died. Median follow-up of survivors was 29.2 months (range 1.4–71.2 months). Median follow-up was 42.2 months (range 1.4–71.2) and 23.7 months (range 3.2–59) for patients receiving BM and PBSC, respectively. Three-year OS and EFS were 43.8 and 40.2%. For patients in CR (n = 40) at the time of Haplo-Tx, median OS and EFS were not reached at a follow-up of 29.2 months. Three-year OS was 54.6%, 3-year EFS was 52.3% (Fig. 2c). No statistically significant differences in OS and EFS were observed in patients who received myeloablative vs. non-myeloablative/reduced intensity conditionings (53.5 vs. 48.0%, p = 0.515, and 42.4 vs. 35.9%, p = 0.283, at 2 years respectively), or PBSC vs. BM (60.3 vs. 47.5%, p = 0.586, and 55.3 vs. 32.7%, p = 0.257, at 2 years, respectively). By univariate analysis, only DRI had a significant impact on OS (Table 2). A formal multivariate analysis was not performed given the sample size of our cohort.

CI of NRM was 25, 27, and 27% at 1, 2, and 3 years post-Haplo-Tx, respectively, whereas cumulative RI was 22, 33, and 33% at 1, 2, and 3 years (Fig. 2b). Three-year RI was significantly lower in patients in CR at the time of transplant (21.5 vs. 48%, p = 0.009) and in patients who received PBSC as stem cell source (22 vs. 45%, p = 0.009).

Sequential high-dose chemotherapy and haplo-identical transplant

Overall, 22/70 (31%) high-risk patients were transplanted during the chemo-induced neutropenia after high-dose salvage chemotherapy (Table 3). Conditioning, myeloablative in 14/22 (64%) and non-myeloablative in 8/22 (36%), was started at a median of 9 days (range 4–15) after the last day of chemotherapy. Six/22 (27%) had received a previous allograft. BM was used as stem cell source in 18/22 (82%) patients. All patients but 2, who presented with blast crisis of chronic myeloid leukemia, suffered from refractory (n = 12) or secondary AML (n = 8). One/22 patients (4%) experienced primary graft

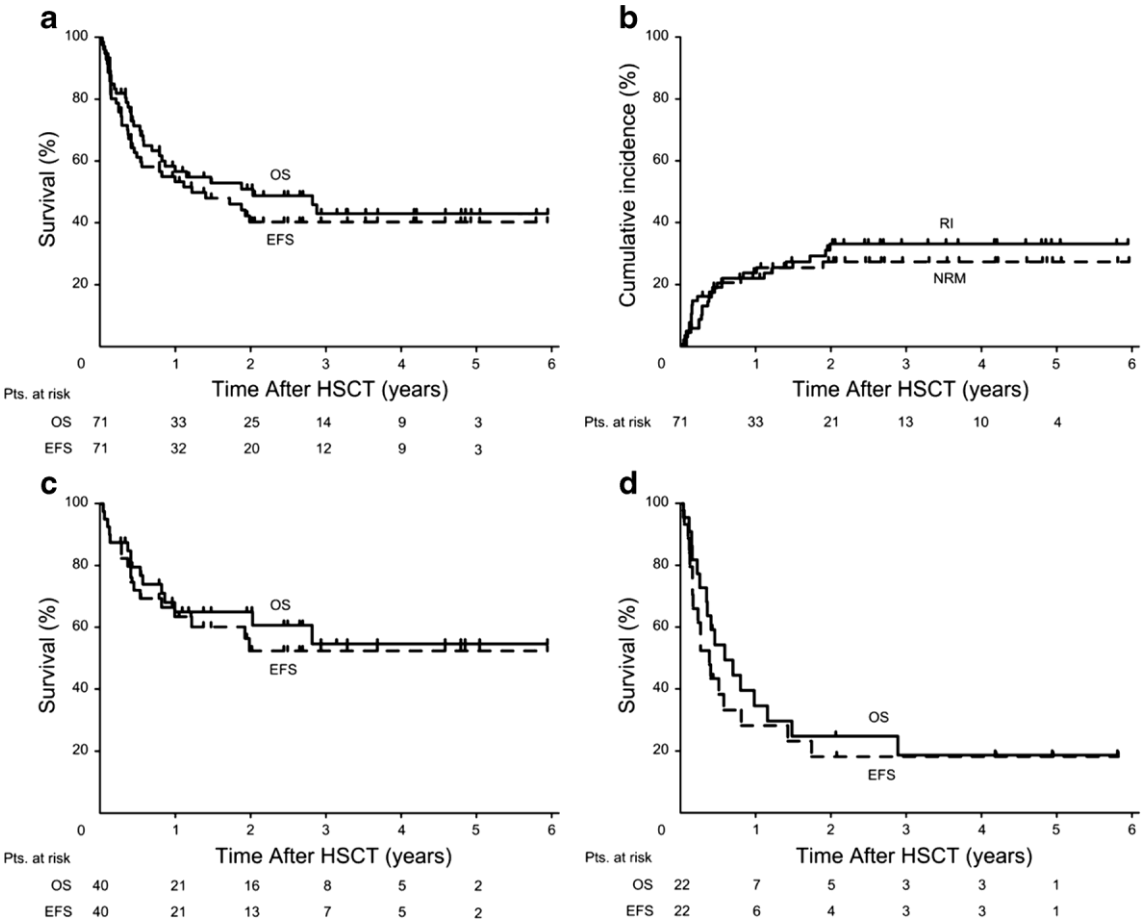

Fig. 2 a Overall survival (OS) and event-free survival (EFS) and b relapse incidence (RI) and non-relapse mortality (NRM) of the entire patient population (71 patients). c OS and EFS of 40 patients who were in complete remission at the time of Haplo-Tx. d OS and EFS of 22 patients who received sequential chemotherapy and allografting

Table 2 Univariate analysis

	OS			EFS		
	HR	95% CI	*p*	HR	95% CI	*p*
HCT-CI (≥ 3 vs. 0–2)	1.01	0.50–2.04	0.986	1.09	0.56–2.12	0.805
Conditioning regimen (myeloablative vs. non-myeloablative)	1.27	0.62–2.61	0.517	1.44	0.74–2.8	0.288
DRI (high/very high vs. intermediate)	2.36	1.07–5.22	0.034			
Donor/recipient sex match (F/M vs. other)	1.21	0.58–2.53	0.612	1.20	0.60–2.41	0.614
Stem cell source (BM vs. PBSC)	1.22	0.59–2.51	0.587	1.48	0.75–2.91	0.262

OS overall survival, *EFS* event-free survival, *HR* hazard ratio, *95% CI* 95 % confidence interval, *HCT-CI* hematopoietic cell transplantation comorbidity index, *DRI* disease risk index, *F* female, *M* male, *PBSC* peripheral blood stem cells, *BM* bone marrow

failure and was successfully rescued with a second haploidentical transplant from the same donor. Median follow-up of survivors was 50 months (range 5–69 months); 3-year OS was 19% (Fig. 2d), while 3-year RI and NRM were 52 and 28%, respectively. At day + 100/+ 400, CI of grade II–IV acute GVHD and chronic GVHD were 40.9 and 33.6%, respectively (Table 4). A statistically significant difference in clinical outcomes was observed in patients who received myeloablative vs. non-myeloablative/reduced intensity conditionings (OS, $p = 0.027$; EFS, $p = 0.007$).

Table 3 Characteristics of patients undergoing sequential chemotherapy and allografting

	Age	Sex	Status at Haplo HSCT	Previous Allo HSCT	Karyotype	BM Blasts at Seq. Th.	Reinduction regimen	Rest days	Conditioning regimen
#1	58	F	Refractory sAML from RAEB1	No	Monosomal	25%	MEC	9	TBF
#2	21	F	Refractory AML	Yes	inv(16), + 8	25%	AMSA+ARA-C	4	FLU+CY+TBI
#3	40	M	Refractory AML	Yes	Normal	72%	AMSA+ARA-C	10	FLU+CY+TBI
#4	37	F	Refractory AML	Yes	11q23 mut	17%	MEC	4	FLU+CY+TBI
#5	34	M	Refractory AML MDS related	No	t(3;3)	25%	MEC	4	FLU+CY+TBI
#6	50	F	Refractory sAML from CMML-2	No	Normal	43%	IDA+HD ARA-C*	15	TBF
#7	54	M	Refractory AML MDS related	No	Monosomal	9%	MEC	10	TBF
#8	42	F	Myeloid Blastic Crisis of CML	No	t(9;22)	34%	MEC	9	TBF
#9	41	F	Refractory AML	Yes	Normal	6%	CLOFA+ARA-C	6	FLU+CY+TBI
#10	55	M	Lymphoid Blastic Crisis of CML	No	t(9;22)	80%	HAM	6	TBF
#11	29	M	Refractory AML	No	+ 8	88%	CLOFA+ARA-C	7	TBF
#12	62	M	Refractory sAML	No	-7q	32%	CLOFA+ARA-C	8	TBF
#13	60	F	Refractory AML	No	Normal	10%	MEC	6	TBF
#14	46	F	Refractory AML	Yes	Normal	21%	CLOFA+ARA-C	9	FLU+MEL+TBI
#15	53	M	Refractory sAML from CNL	No	NA	20%	MEC	15	TBF
#16	51	F	Refractory AML Therapy-Related	No	Normal	55%	MEC	7	TBF
#17	54	F	Refractory AML MDS related	No	Monosomal	15%	IDA+HD ARA-C*	12	TBF
#18	48	M	Refractory AML	No	inv(3)	17%	CLOFA+ARA-C	10	TBF
#19	53	F	Refractory AML	No	Normal	50%	MEC	11	TBF
#20	41	M	Refractory AML	Yes	Complex	15%	MEC	11	FLU+CY+TBI
#21	58	M	Refractory AML	No	+ 21	75%	MEC	11	FLU+CY+TBI
#22	23	M	Refractory AML	No	Normal	52%	FAM	11	TBF

Reinduction regimens: MEC: mitoxantrone 6 mg/sqm day 1–4, etoposide 80 mg/sqm day 1–4, cytarabine 1 g/sqm day 1–4; AMSA+ARA-C: amsacrine 100 mg/sqm day 1–3, cytarabine 2 g/sqm day 1–3; IDA+HD ARA-C: idarubicin 17.5 mg/sqm day 3 and day 10, cytarabine 3 g/sqm BID day 1–2 and day 8–9; CLOFA+ARA-C: clofarabine 40 mg/sqm day1–4, cytarabine 1 g/sqm day 1–4; HAM: cytarabine 3 g/sqm BID day 1–3, mitoxantrone 10 mg/sqm day 1–3, FAM: fludarabine 25 mg/sqm day 1–5, cytarabine 2 g/sqm day 1–5, mitoxantrone 12 mg/sqm day 3–5

AML acute myeloid leukemia, *sAML* secondary AML, *RAEB1* refractory anemia with excess blasts-type 1, *MDS* myelodysplastic syndrome, *CMML-2* chronic myelomonocytic leukemia-type 2, *CML* chronic myeloid leukemia, *CNL* chronic neutrophilic leukemia, *NA* not available

*Cyclosporine A 12-h IV infusion days 3 and 10

Table 4 Outcome after sequential therapy approach

$N = 22$	1 year	2 years	3 years
Non-relapse mortality	28%	28%	28%
Relapse incidence	42%	52%	52%
Overall survival	34.5%	24.6%	19%
Event-free survival	30.3%	20.2%	20.2%
	Day + 100	Day + 400	
Grade II–IV acute GVHD	40.9%		
Grade III–IV acute GVHD	13.6%		
Chronic GVHD		33.6%	
Moderate/severe chronic GVHD		13.2%	

Discussion

The use of PT-Cy has allowed the rapid expansion of unmanipulated TCR Haplo-Tx [12–19]. In our series, most patients initially received BM and more recently PBSC, given the high risk of relapse. ANC engraftment occurred on day + 18 with BM and on day + 15 with PBSC, while PLTS recovery occurred on day + 27 with BM and on day + 21 with PBSC and secondary failure of engraftment was mainly due to florid relapse [37]. Overall, life-threatening grade III–IV acute GVHD and moderate-severe chronic GVHD were 15 and 11%. CI of acute GVHD was higher in patients who received PBSC versus BM while no difference in CI of chronic GVHD was observed. Differences in median follow-up between the two patient cohorts prevent however from drawing definitive conclusions. Of note, 1-year GRFS in patients who received Haplo-Tx as first allograft was 30.5% (Fig. 1c). This finding is particularly encouraging in heavily pretreated patients and is also a reliable surrogate of good quality of life. Overall, our findings are consistent with a larger EBMT registry study by Ruggeri et al. [26] where 451 patients with acute leukemias were analyzed. Use of PBSC rather than BM was significantly associated with increased risk of grade II–IV and III–IV acute GVHD while no differences were found in CI of chronic GVHD. A large US comparison on 681 patients with hematologic malignancy who underwent Haplo-Tx with PT-Cy and BM (n = 481) or PBSC (n = 190) grafts was also conducted [38]. Transplant outcomes were compared by graft type after adjusting for patient, disease, and transplant characteristics. ANC and PLTS engraftments were similar. Risk of grade II-IV and chronic GVHD was lower with BM as compared with PBSC. There were no differences in OS, with 2-year rates of 54 and 57% after BM and PBSC, respectively, and in NRM. Relapse risk was however higher after BM in patients with leukemia, but not with lymphoma. The authors conclude that both BM and PBSC grafts are suitable for Haplo-Tx though patterns of treatment failure differ.

Cell doses may play a role in clinical outcomes. In our study, CI of acute GVHD, chronic GVHD, neutrophil and PLTS engraftment did not statistically differ between patients who received grafts with TNC and CD34+ cell doses above or below the infused median cell doses. Several reports focused on the impact of CD34+ cells on transplant outcomes with discordant results. After myeloablative conditionings and allografts from sibling donors, Przepiorka et al. reported that doses higher than 8.2×10^6 CD34+/kg correlated with increased risk of grade II–IV acute GVHD while Zaucha et al. reported an increased risk of chronic GVHD with doses higher than 8×10^6 CD34+/kg [39, 40]. By contrast, two registry studies from the National Marrow Donor Program (NMDP) and the Center for International Blood and Marrow Transplant Research (CIBMTR) showed that CD34+ cell doses respectively higher than 4.5×10^6 and 6×10^6/kg correlated with reduced NRM and improved OS [41, 42]. Recently, Czerw et al. reported that doses higher than 8.25×10^6 CD34+/kg were associated with increased risk of grade III–IV GVHD, but had no significant impact on NRM, leukemia relapse, incidence of chronic GVHD, and OS in leukemia patients undergoing reduced-intensity allografting from unrelated donors [43]. Discrepancies are probably due to different disease categories, disease status at transplant, donor type, intensity of the conditioning, and GVHD prophylaxis. No prospective dose finding study on the impact of cell doses/graft composition on clinical outcomes has so far been reported.

We observed viral reactivation but no life-threatening viral disease. CMV reactivated especially during the first 3 months post-transplant, but no CMV disease occurred [44]. Prospective monitoring of EBV DNA in blood is an institutional standard at our center while prophylaxis to prevent EBV reactivation is not given. Only 6% of our patients showed EBV reactivation. All patients remained asymptomatic and no post-transplant lymphoproliferative disorder was observed [45]. Hemorrhagic cystitis developed in 17% and most patients concomitantly showed BK viruria. Other viruses such as adenovirus and JC virus were not detected in the urine, and no major kidney complications were observed [46, 47]. Haplo-Tx patients are at high risk of developing IFI. Twenty-one percent of our patients developed fungal infections. Late infections were observed after mold-active primary prophylaxis had been stopped. A prospective evaluation of longer administration of mold active agents may be helpful.

Interestingly, 31% of our patients underwent a sequential approach—chemotherapy and allografting—to timely treat aggressive high-risk leukemia. Moreover, almost a third (6/22, 27%) had received a previous allograft. The sequential approach to treat myeloid malignancies was initially described by Schmid et al. [48–50]. In a prospective study, the combination of fludarabine, cytarabine, and amsacrine (FLAMSA) followed by reduced-intensity allografting from HLA-matched sibling or unrelated donors was associated with 2-year OS and 2-year EFS of 42 and 40% in 75 high-risk patients. More recently, Ringden et al. reported an outcome

analysis on 267 patients with relapse/refractory AML who received sequential chemotherapy including fludarabine, cytarabine, and amsacrine followed by a reduced-intensity allograft from 77 HLA-matched siblings and from 190 HLA-matched unrelated donors [51]. Incidence of acute grade II–IV and chronic GHVD was 32.1 and 30.2%, respectively. Three-year probability of NRM was 25.9%, of relapse 48.5%, of GRFS 17.8%, and of leukemia-free survival 25.6%. To our knowledge, we report on the largest experience of a sequential approach including Haplo-Tx. Outcomes of 30 refractory leukemia patients, where seven received intensive chemotherapy before Haplo-Tx, were described by Devillier et al. [52]. Overall and progression-free survivals were 37 and 32%. Of the seven patients who received a sequential approach, two were alive in CR at 11 and 14 months after Haplo-Tx. Though our cohort is relatively small, at a median follow-up of 50 months, 3-year OS was 19%. A survival advantage was seen with myeloablative conditionings. However, it is important to point out that some patients treated with non-myeloablative/reduced intensity regimens had also received a prior allograft increasing the risk of toxicity. A formal comparison between matched sibling versus unrelated donor or haplo-identical donor transplants has not been reported in this setting. However, Schmid et al. [49] observed a reduced risk of death from leukemia with transplant from unrelated donors. However, given a higher risk of non-relapse mortality, this finding did not translate into better OS as compared with transplants from HLA-matched sibling donor.

In retrospective analyses, outcomes of Haplo HSCT with PT-Cy appeared similar to those reported after HLA-matched sibling or unrelated donor transplants [53–57]. However, results should be validated in prospective randomized trials to avoid the inevitable limitations of retrospective comparisons. When compared with UCB transplants, Haplo HSCT with PT-Cy also showed equal or even better results either in retrospective and parallel prospective studies [58, 59]. A large multicenter phase III randomized trial comparing double unit UCB versus Haplo-Tx is currently ongoing (ClinicalTrials.gov: NCT01597778). Our donor selection policy has not changed over the study period. Our Bhierarchical^ selection includes first a HLA-identical sibling, second a fully or a 7/8 allele matched unrelated donor and third a haplo-identical family donor. Although Pidala et al. recently reported that only 5% of patients take longer than 59 days to find a suitable unrelated donor [60], an allograft may not be delayed especially in patients with refractory/relapsed malignancies. At our center, during the study period, 114 allografts from HLA-identical siblings and 212 from unrelated donors were performed. We estimate that with the implementation of the Haplo-Tx program, 15–20% of our patients who would not have found a suitable donor underwent a potentially curative allograft. However, our strategy by no means allows an unbiased comparison of clinical outcomes by donor type given the heterogeneity of patient cohorts and selection criteria. Thus, we strongly support the design of large multicenter prospective trials in the near future where clinical outcomes by donor type may reliably be compared and potential selection bias be reduced.

In summary, Haplo-Tx with PT-Cy is feasible in high-risk, heavily pretreated patients and can safely be part of a sequential Bchemotherapy and allografting^ approach. Prospective comparisons of long-term clinical outcomes by different donor types are eagerly awaited.

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Author contributions B.B, A.B, and L.G designed the study. L.B., L.G., A.B., and B.B. wrote the report. L.G., A.B., and B.B. supervised the clinical conduction of the study and data analysis. B.B, A.B., C.D., and L.G. supervised data collection, analyzed data, and reviewed and assisted in writing the manuscript. A.B., B.B., C.D., S.A., B.A., L.G., and M.F. recruited the patients. R.P. did the statistical analysis.

Compliance with ethical standards

The study was approved by the Center Ethical Committee and conducted according to the Declaration of Helsinki.

Conflict of interest statement The authors declare that they have no conflict of interest.

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