Unrelated cord blood transplantation and post-transplant cyclophosphamide

The use of unrelated cord blood transplantation (UCBT) has been declining over the years, for patients who lack an hyman leukocyte antigen (HLA)-matched donor, due to the competition of haploidentical grafts.[1] However UCBT may have advantages, such as a strong graft-*versus*-leukemia (GVL) effect.[2,3] There are disadvantages, such as delayed engraftment and extensive chronic graft-*versus*-host disease (GVHD), may be as high as 30%, after double UCBT.[4] We have reported a first patient with acute myeloid leukemia (AML) undergoing a successful UCBT using reduced dose post-transplant cyclophosphamide (PTCY), cyclosporin (CSA) and mycophenolate (MMF) for GVHD prophylaxis.[5] We are now reporting the interim analysis of 14 patients undergoing UCBT in our Center, on the same clinical trial (*clinicaltrials gov. Identifier: NCT03802773*), approved by our local Ethical Committee (ID 2382).

Eligible for the study were patients who lacked a suitable haploidentical related or a 7/8 unrelated donor. The study enrolled 14 patients, including 12 females and two males, with a median age at transplant of 56 years (range, 36-68); the median weight was 58 kg (range, 50-85 kg) (Table 1). Patients were grafted between 2017 and 2023. Diagnosis, phase of the disase, are detailed in Table 1, together with HLA allele matching and number of CB infused cells x10^7/kg; CB units were prioritized on the following criteria: cell dose, DRB1 match, non ABO major mismatch. The median Hematopoietic Cell Transplant-Co-Morbidity Index (HCT-CI) was 4 (range, 0-7).

The conditioning regimen in 13 of 14 patients, was a combination of thiotepa 10 mg/kg, fludarabine 150 mg/m^2 and busulfan 3,2 mg/kgx3 (TBF3); with high HCT-CI and or advanced age the days of busulfan infusion were reduced to 2 (TBF2) or 1 day (TBF1), thiotepa and fludarabine remaining at the dame dose; one patient in very poor clinical conditions (patient number 12) received a combination of fludarabine cyclophosphamide and total body irradiation 2 Gy.

All patients received PTCY (30 mg/kg on days +3 and +5), combined with a calcineurin inhibitor (all patients received CSA apart from one patient who received tacrolimus, starting on day 0); mycophenolate mofetil (MMF) from day +1 to +36 was given to the first ten patients and was omitted in the last four patients, following an amendment to the study (16/07/2020). Subcutaneous granulocyte colony-stimulating factor, 250 ug/day, was given daily from day +5 until neutrophil engraftment.

All patients received an optimal $CD34^+$ dose - according to European Society for Blood and Marrow Transplantation guidelines - with a median of CD34 cells 1.57x10^5/kg and total nucleated cells 3.05x10^7/kg (range, 1.8-5.0). Two patients were not evaluable for engraftment, because of early sepsis (patients number 3 and 9) (*Stenotrophomonas maltophilia* and multidrug-resistant *Klebsiella pneumoniae*). One patient was not evaluable because of autologous leukemic recovery.

The cumulative incidence of engraftment in the 11 evaluable patients, was 82%: two patients (18%) had primary graft failure (PrGF) - one was rescued with a mismatched unrelated donor hematopioetic stem cell transplant (HSCT), one died on day +54. PrGF occurred in two of eight (25%) patients grafted with UCB matched at four of eight alleles, and in none of four patients grafted with UCB matched at five of eight alleles; PrGF occurred in one of seven remission and in one of five relpased/refractory (R/R) patients, and both occurred in patients receiving the highest intensity conditioning (TBF3), and a high cell dose (3.1 and 5.0x10^7/kg) (Table 1). The time to first neutrophil engraftment was 23 days (range, 15-33) and the median time to platelet engraftment was 38 days (range, 34-42). Two patients had acute GVHD grade 1 and one patient had grade 2 GVHD. Minimal chronic GVHD was diagnosed in one patient and moderate chronic GVHD in two patients. The cumulative incidence of acute GVHD grade 2 was 7%. The cumulative incidence of moderate chronic GVHD at 3 years is 11%. CSA was discontinued at a median interval of 141 days (range, 97-390). The median absolute CD4 counts on day +50 was 75 cell/cmm (range, 50-116).

The overall actuarial survival at 3 years is 50% (95% confidence interval [CI]: 23-76%), with a median overall survival of over 1,000 days (range, 7-3,002): when stratified for remission status this was 75% for remission patients and 17% for R/R patients.

The 3-year CI of transplant-related mortality (TRM) is 21%; and for remission or R/R patients it is 25% and 17%, respectively; the 3-year CI of relapse is 12% and 66%, respectively.

In conclusion, a previous study tested the use of intrabone CB transplants with anti-thymocyte globulin (ATG) prophylaxis:[6] although engraftment was satisfactory, an update of that study on 127 patients (umpublished data), showed a TRM of 36%, of which 55% was due to infections and 22% to GVHD. For this reason we have tested a PTCY-based, as opposed to an ATG-based GVHD prophylaxis: the overall TRM in the first 14 patients, is 21%, with no case of severe acute or chronic GVHD, no case of GVHD-related death, and the actuarial 3-year survival for remission patients, is in excess of 70%. There have been two patients with primary graft failure, which is 18% of evaluable patients: one was rescued with a mismatched unrelated graft, the other deceased with aplasia. Intrabone injection of UCBT may be

Table 1. Clinical characteristics of patients.

	G/A	Diagn	Phase	HLA	Cells	Condit	Engr	Plt	GVHD		Status	FU	Cause
									Ac	Chr			
1	F/44	AML	CR2	5/8	4.2	TBF3	15	36	0	0	alive	3,002	-
2	F/54	ALL	CR1	5/8	4.5	TBF3	25	25	I	0	alive	2,740	-
3	F/59	sAML	REL	2/8	5.0	TBF1	-	-	-	-	dead	15	seps
4	M/56	AML	CR2	4/8	1.8	TBF3	22	40	0	mod	alive	2,568	-
5	F/49	sAML	REL	5/8	2.3	TBF3	18	34	0	min	alive	2,567	-
6	F/58	AML	CR1	4/8	3.1	TBF3	PGF	-	0	0	alive	2,446	-
7	F/66	sAML	REL	4/8	2.7	TBF1	25	40	0	0	dead	614	REL
8	F/63	AML	REL	5/8	2.7	TBF2	27	41	0	0	dead	561	REL
9	F/63	sAML	CR2	5/8	2.8	TBF2	-	-	-	-	dead	7	seps
10	M/43	AML	CR1	4/8	5.0	TBF3	PGF	-	-	-	dead	54	seps
11	F/46	AML	CR1	4/8	2.8	TBF3	23	42	II	mod	alive	2,127	-
12	F/53	FCL	REL	4/8	3.0	FC	18	38	0	0	dead	300	REL
13	F/68	AML	REL	2/8	4.1	TBF2	-	-	-	-	dead	55	REL
14	F/36	AML	CR2	5/8	3.6	TBF2	33	38	0	0	alive	1,364	-

Ac: acute; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; Cause: cause of death; Cells: number of cells infused x10^7/kg; Chr: chronic; Condit: conditioning regimen; CR: comple remission (1 or 2); Diagn: diagnosis; Engr: days of neutrophil engraftment; F: female; FC: fludarabine, cyclophosphamide; FCL: follicular cell lymphoma; FU: follow-up in days from transplant; G/A: gender/age in years; GVHD: graft-*versus*-host disease, (ac: grade 0-2) and Chr; HLA: number of human leukocyte antigen (HLA) matched alleles; Plt: days of platelet engraftment; M: male; min: minimal; mod: moderate; PGF: primary graft failure (see main text for details); REL: relapse; sAML: secondary AML; seps: sepsis; TBF: 13 patients received a combination of thiotepa, busulfan, fludarabine; TBF1, TBF2, TBF3 indicates days of busulfan.

one way to improve engraftment,[6] combined with low-dose PTCY. The cumulative incidence of acute and chronic GvHD was very low, which led to an amendment of the protocol, eliminating MMF. Because of a low incidence of GVHD, cyclosporin could be discontinued early at a median interval of 141 days from transplant. Sepsis remains a common complication of CB transplants, as demonstrated by our study population, in which all three transplant related-deaths occurred due to sepsis. Only one patient developed Epstein-Barr virus DNA-emia, requiring rituximab treatment, in contrast with prior trials including ATG.[7] Cytomegalovirus reactivation was seen in the first patients, but not later, due to the use of letermovir prophylaxis. The limitations of this study are currently related to the small number of patients enrolled; nevertheless we believe we have shown a proof of concept, suggesting PTCY as GvHD prophylaxis in UCBT. An amendment of the study has just been approved (ID 2382, amendment 26101; CET February 5, 2026): the study is now multicenter, it will include pediatric patients, the dose of PTCY will be further reduced to 25 mg/kg^2, and double CB units as well as intrabone infusion, will be included.

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No conflicts of interest to disclose.

Contributions

PC and AB designed the study, analyzed the data and wrote the manuscript. SG, EM, FS, MAL, FF, LDM, MR, CP, CGV, AR, LT, and SS provided clinical for the patients and data collection. SS and PC supervised the study.

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Data-sharing statement

Original data is available upon request.

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