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Cellular Therapy

Adoptive Immunotherapy with Cord Blood for the Treatment of Refractory Acute Myelogenous Leukemia: Feasibility, Safety, and Preliminary Outcomes

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A B S T R A C T

Adoptive immunotherapy has shown efficacy in patients with relapsed/refractory acute myelogenous leukemia (AML). We conducted a prospective evaluation of cord blood (CB)-based adoptive cell therapy following salvage chemotherapy in patients with AML or myelodysplastic syndrome (MDS) and describe the safety and early outcomes of this approach. To enhance the antileukemic effect, we selected CB units (CBUs) with a shared inherited paternal antigen (IPA) and/or noninherited maternal antigen (NIMA) match with the recipients. Furthermore, the CBUs had total nucleated cell (TNC) dose $<2.5 \times 10^7$/kg and were at least 4/6 HLA-matched with the patients; a higher allele-level match was preferred. Heavily pretreated adult patients with AML/MDS were enrolled. CBU searches were performed for 50 patients. CBUs with shared IPA targets were identified for all, and CBUs with NIMA matches were found for 80%. Twenty-one patients underwent treatment (AML, primary induction failure, n = 8; refractory relapse, n = 10, including 7 recipients of previous allogeneic HSCT; blast crisis chronic myelogenous leukemia, n = 1; MDS, n = 2). Most received combination chemotherapy; those not fit for intensive treatment received a hypomethylating agent. Response was defined as <10% residual blasts in hypocellular bone marrow at approximately 2 weeks after treatment. Ten of the 19 evaluable patients responded, including 5 of the 7 recipients of previous transplant. Response was seen in 4 of 4 patients with full CBU-derived chimerism, 2 of 2 of those with partial, low-level chimerism and 4 of 12 of the recipients with no detectable CBU chimerism. The most common adverse events were infections (bacterial, n = 5; viral, n = 2; fungal, n = 5). Grade IV acute graft-versus-host disease (GVHD) developed in 2 patients with full CBU chimerism; 2 other patients had grade 1 skin GVHD. A total of 11 patients died, 7 from disease recurrence and 4 from infections (1 early death; the other 3 in remission at the time of death). Overall, 12 patients proceeded to allogeneic HSCT; of those, 7 had responded to treatment, 3 had not (and had received additional therapy), and 2 had persistent minimal residual disease. In conclusion, the use of CB as adoptive immunotherapy in combination with salvage chemotherapy for patients with refractory AML/MDS is feasible, can induce disease control, can serve as a bridge to allogeneic HSCT, and has an acceptable incidence of adverse events. Alloreactivity was enhanced through the selection of CBUs targeting a shared IPA and/or NIMA match with the patients. CBUs with lower cell doses, already available in the CB bank and unlikely to be adequate grafts for adult transplants, can be used for cell therapy within a short time frame.

INTRODUCTION

Patients with relapsed or refractory acute myelogenous leukemia (AML) have limited treatment options and a generally poor prognosis. The outcomes after allogeneic hematopoietic stem cell transplantation (HSCT) are poor in such patients [1–4]. Additional medical interventions to induce remission have been exhausted in most cases. Adoptive cell therapy using donor cells from partially matched related donors has been used to induce an antileukemic effect without durable engraftment [5]. Guo et al [6,7] reported this approach for treatment of older patients with AML with encouraging response rates.

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Another source of cells that can induce strong antileukemic effect is umbilical cord blood (CB). Unrelated cord blood units (CBUs), fully tested and stored, can be available on demand to treat patients, avoiding the complicated logistics required for haploidentical donors. Moreover, recent studies have highlighted improved outcomes of unrelated CB transplantation compared with transplantation from adult donors [8,9]. It has been hypothesized that the superior graft-versus-leukemia (GVL) effects result from the fetal-maternal interactions during pregnancy; small numbers of maternal cells present in the CB can react against specific HLA targets in the recipients, and/or CB cells may be already primed against certain maternal HLA antigens that may be shared with the recipient [10,11].

In the present study, we prospectively evaluated the feasibility and tolerability of infusing CBUs selected to have specific alloreactive properties, after salvage chemotherapy, in patients with refractory AML or MDS. Here we report our preliminary results.

METHODS

Patients

The study was performed under US Food and Drug Administration IND 16423 and has been listed on ClinicalTrials.gov (identifier NCT02508324). All patients were treated at New York Presbyterian Hospital-Weil Cornell Medicine, and all CBUs were obtained from the National Cord Blood Program (NCBP). The study was approved by the Institutional Review Boards of both institutions.

Eligibility included a confirmed diagnosis of AML (primary induction failure [PIF] or relapsed refractory) [12–14] or MDS (refractory anemia with excess blasts [RAEB] I or RAEB II) after failure of 2 or more cycles of chemotherapy, including relapse after allogeneic HSCT. Patients age <60 years with AML had to have failed conventional-dose cytarabine therapy. Patients age >60 years with AML and all patients with RAEB I or RAEB II were included after failure of at least 1 chemotherapy regimen including either cytarabine or a hypomethylating agent. Patients with minimal residual disease (MRD) at the time of evaluation and who met the other study criteria were eligible for treatment. Patients with acute promyelocytic leukemia were not eligible. A Karnofsky Performance Status score of >70 was required, and patients with persistent severe toxicities from previous treatment, uncontrolled concomitant illness, or unacceptable impairment of hepatic, renal, or cardiac function were not eligible.

CBU Selection

CBU searches were performed using the NCBP standard search algorithm (NCBP online access; Websearch). Additional selection criteria for the study included a pre-cryopreservation total nucleated cell (TNC) dose of .5 to 2.5×10^7/kg, a CBU HLA match of 4 to 6/6 (HLA-A and -B, intermediate resolution; -DRB1, high resolution) with the patient, and CBU with shared inherited paternal antigen (IPA) targets and/or noninherited maternal antigen (NIMA) match with patient (Table 1). CBUs with a greater HLA allele match were preferred in the final selection. Donors targeted by donor-specific anti-HLA antibodies were avoided.

Chemotherapy Treatment and CBU Infusion

Chemotherapy regimens for reinduction were selected based on the patient's previous treatments and ability to tolerate intensive therapy. Most patients received combination chemotherapy; in those considered unfit for intensive therapy, single-agent decitabine was used.

Unrelated CBUs were infused within 72 hours after completion of the chemotherapy regimen and no sooner than 24 hours after administration of the last dose of chemotherapy. CBUs were thawed and washed according to the center's validated procedures. Patients did not receive any graft-versus-host disease (GVHD) prophylaxis or additional immunosuppression. Granulocyte colony-stimulating factor was not used routinely.

HLA Typing

Patient HLA typing and CBU confirmatory HLA typing were performed at high resolution for -A, -B, -C, and -DRB1 loci (G-level typing) by HistoGenetics (Ossining, New York). Maternal samples of the CBU were also HLA-typed to assign maternal and paternal antigens.

Patients were tested for donor-specific anti-HLA antibodies at the Rogosin Institute (New York, New York), using flow cytometry; a specificity was considered positive at a mean fluorescence index value of ≥2000 [15].

Table 1
Example of NIMA/IPA Assignments

	HLA-A		HLA-B		HLA-C		HLA-DRB1	
Patient	**02:01**	26:01	38:01	39:01	12:03	17:01	**11:04**	13:02
CBU	02:01	26:01	38:01	51:01	04:01	12:03	04:01	13:02
Mother	24:02	26:01	38:01	35:02	04:01	12:03	11:04	13:02

Shown is an example of patient (patient 4) and selected CBU HLA typing. IPAs are shown in blue in the CBU HLA typing. The shared IPA target is shown in blue bold in the patient's HLA typing (-A locus).
NIMAs are shown in red in the maternal HLA typing. The NIMA match is shown in red bold in the patient's HLA typing (-DRB1 locus).
CBU and mother are identical for the HLA-C locus, so maternal/paternal antigens could not be defined.
Interpretation: CBU is 4/6-matched, 5/8 allele-matched with the patient, with NIMA match at HLA-DRB1 and a shared IPA target at HLA-A.

Definition of Objectives

The primary objective of the study was to examine the safety of treatment with salvage chemotherapy followed by CBU infusion in patients with refractory AML. Adverse events were graded according to the Common Terminology Criteria for Adverse Events version 4.3 from initiation of the study treatment until the occurrence of an event—death, HSCT, or relapse, whichever occurred first. Acute and chronic GVHD were graded according to consensus criteria [16,17].

Serious adverse events were grade III-IV acute GVHD, with an incidence of >10% considered unacceptable, and unexplained prolonged myelosuppression, defined as no blood count recovery for >30 days after completion of chemotherapy without evidence of residual or recurrent leukemia/MDS, with an incidence of >10% also considered unacceptable.

Stopping rules were in place in the event of excessive incidence of complications attributed to the intervention. Because these stopping rules were not reached, the protocol continued to accrue patients. Cytokine release syndrome (CRS) was anticipated, and CRS management approaches were included in the study protocol.

The secondary objective was assessment of response. Response to treatment was defined as effective cytoreduction, that is, <10% residual blasts in a hypocellular bone marrow (BM) or no blasts in an acellular BM (BM aplasia) obtained at approximately 14 days after CB cell infusion [10]. Several patients with effective cytoreduction proceeded to allogeneic HSCT, so formal assessment of treatment response (ie, BM evaluation after recovery of peripheral blood counts) was not possible.

We also evaluated the feasibility of identifying CBUs that met the study criteria within the time frame needed to treat the patients. The number of CBUs identified per patient, their characteristics, and the time for completion of CBU evaluation were recorded throughout the study period.

Chimerism Assessment

Chimerism studies were performed in peripheral blood (PB) or BM samples as described previously [18]. PB samples were obtained weekly from the day of CBU infusion for 8 consecutive weeks or until allogeneic HSCT. PB chimerism assessment was usually performed on isolated $CD3^+$ and $CD33^+$ cell fractions. When the white blood cell count was $<4 \times 10^9$/L, unfractionated samples were analyzed. BM samples were evaluated for CBU chimerism when available.

Statistical Analysis

CBU characteristics and patient outcomes are described using descriptive statistics.

RESULTS

CBU Searches

Between June 2015 and April 2018, 50 patients underwent a CBU search, and 21 received treatment.

The mean CBU number evaluated per search was 130 (range, 7 to 250) with 57 potentially eligible CBU per patient (range, 5 to 159). A total of 174 CBU underwent testing, including HLA confirmatory typing. At least 1 eligible CBU was identified for each patient. All patients had at least 1 CBU with shared IPA targets, and 80% of patients also had NIMA-matched CBU.

As the study progressed, CBU selection was expedited, and during the last year (July 2017 to June 2018), the median time

for CBU identification was 12 days for 22 recipients, with 6 patients having at least 1 CBU identified and ready for shipment in <7 days. The timely CBU selection was facilitated by prioritizing CBU that had already undergone HLA confirmatory and maternal HLA typing among the large Inventory of NCBP.

Patient Characteristics

Of the 50 patients, 29 did not receive treatment because of progressive disease and death (n = 17), other clinical trials/treatments (n = 5), or allogeneic HSCT (n = 7).Twenty-one patients were treated (Table 2), including 13 women and 8 men, with median age of 51 years (range, 22 to 67 years) and a median weight of 62 kg (range, 47 to 122 kg). They had received a median of 3 previous treatment regimens (range, 1 to 6). The median HSCT Comorbidity Index (HSCT-CI) was 3 (range, 0 to 7) [19]. Eighteen patients had AML (PIF, n = 8; relapsed refractory, n = 10), 1 patient had myeloid blast crisis chronic myelogenous leukemia, and 2 patients had high-risk MDS refractory to hypomethylating agents. Nineteen patients had poor-risk karyotype and/or FLT3 mutation, and 7 had relapsed after previous HSCT. Two patients had MRD (patients 16 and 19), but no excess blasts at the time of enrollment. Thus, these 2 patients were not evaluable for response assessment as defined above. The median time from diagnosis to the start of treatment was 12 months (range, 2 to 80 months).

Combination salvage chemotherapy with cladribine, cytarabine, and mitoxantrone was used in 12 patients [20]. Other salvage regimens were used in 5 patients: cytarabine and cladribine in 2 and high-dose cytarabine and ruxolitinib (for a patient with JAK-2 mutation), intermediate-dose cytarabine, and mitoxantrone, etoposide, and cytarabine in 1 each [21]. Four additional patients were deemed unfit to receive intensive chemotherapy and were treated with decitabine [22].

Eleven patients had anti-HLA antibodies. The median PRA was 27% (range, 2% to 89%).

Characteristics of Infused CBUs

Data on cell doses, characteristics, and HLA matching of the infused CBUs are presented in Table 3.

The median pre-cryopreservation CBU TNC was 86×10^7 (range, 63 to 179×10^7), and the median TNC dose was 1.5×10^7/kg (range, 0.7 to 2.4×10^7/kg). The median post-thaw $CD34^+$ cell dose was 0.4×10^5/kg (range, 0.2 to 1.0×10^5/kg). CBUs delivered a median post-thaw $CD3^+$ cell dose of 3.2×10^6/kg (range, 1.8 to 6.2×10^6/kg). Overall, CBUs with lower cell doses than those currently selected for transplantation were used. Further, CBUs were "older," with a median cryopreservation time of 10 years (range, 5.7 to 17.1 years). CBU segments (n = 19) were evaluated before release of the unit for clinical use, in accordance with current practice, and found to have a median $CD34^+$ cell viability of 96% (range, 91% to 98%), indicating good quality of the frozen products.

HLA match was 6/6 for 1 patient, 5/6 for 13 patients, and 4/6 for 7 patients. The allele- level compatibility and presence of shared IPA targets and/or NIMA matches are shown in Table 3. Overall, 15 patients received CBU with shared IPA targets, 5 patients received CBU with both shared IPA and NIMA matches, and 1 patient had a CBU with NIMA match only.

Adverse Events

No immediate CBU infusion reactions were observed. Table 4 summarizes all adverse events and the time in which they occurred. Two patients (3 and 6) experienced grade 1 CRS, manifested by fever of unknown origin [23]. Eleven patients had grade 3-4 infection (5 bacterial, 2 viral, and 5 fungal infections). Four patients developed acute GVHD, 2 with grade I (skin) and 2 with grade IV (skin, liver, and gastrointestinal tract).

Additional adverse events were multiorgan failure due to disseminated adenovirus viremia (n = 1), liver failure due to leukemia progression (n = 1), post-transplantation lymphoproliferative disease (n = 1), and respiratory failure due to sepsis (n = 1).

Chimerism

CBU-derived chimerism was detected in 6 patients as early as 1 week after CBU infusion. One patient had CBU chimerism detected at 6 months after CBU infusion. Two patients (12 and 20) had full CBU chimerism in $CD3^+$ and $CD33^+$ cells in peripheral blood for 2 to 3 months after treatment and CBU-derived peripheral blood count recovery. Two additional recipients had CBU chimerism without peripheral count recovery. Patient 2 had 96% CBU-derived chimerism in unfractionated peripheral blood but died on day +32, without a count recovery, from adenovirus infection that had preceded his enrollment on the protocol. Patient 6 had 100% CBU-derived chimerism in unfractionated peripheral blood at the initiation of conditioning for HSCT on day +30. Neither patient had any evidence of leukemia at the last evaluation. All 4 recipients with full CBU chimerism had relapsed after previous allogeneic HSCT.

Transient low-level CBU-derived chimerism at 1% was detected in 2 additional recipients: patient 1, in peripheral blood $CD3^+$ and $CD33^+$ cells, and patient 10, in unfractionated bone marrow.

Finally, patient 7 had CBU chimerism at 1% in blood $CD3^+$ cells at approximately 6 months after CBU infusion and 5 months after allogeneic HSCT.

GVHD

The 2 patients with full CBU chimerism and peripheral blood count recovery experienced grade IV aGVHD involving the skin and upper and lower gastrointestinal tracts. Patient 12 required treatment with glucocorticosteroids, tacrolimus, and ruxolitinib, and patient 20 responded to glucocorticosteroids. Two patients (6 and 9) experienced grade 1 skin aGVHD, biopsy-proven, that responded to topical glucocorticosteroids; of these, patient 6 had CBU chimerism, but patient 9 did not. No chronic GVHD was observed in 15 patients who survived beyond 100 days.

Leukemia Response and Further Treatment

Ten of the 19 evaluable patients had effective cytoreduction, including 4 with residual BM blasts <10% and 6 without residual blasts [24]. The 2 patients with MRD but without blasts at the time of treatment were not evaluable for disease response.

We did not find an association between CBU TNC/$CD34^+$ cell dose or $CD3^+$ cell dose and response in this small cohort of patients. A response was seen in all 4 patients who had full CBU-derived chimerism, both patients who had partial CBU-derived chimerism and in 4 of the 12 patients without evidence of CBU chimerism. Notably, 5 of the 7 patients who had relapsed after a previous allogeneic HSCT responded. Responses to the adoptive cell therapy and outcomes after HSCT are summarized in Table 2 and Figure 1.

Of the 10 patients who responded to treatment, 7 subsequently underwent HSCT, 2 did not (patients 12 and 20, who had CBU-derived hematopoiesis), and 1 died early. In addition, 3 patients without response received a clofarabine bridge and then proceeded to HSCT [25]. Finally, the 2 patients who were

Table 2
Patient Characteristics and Outcomes

Patient	Age, yr	Sex	Disease	Disease Status	Cytogenetics/ Molecular Markers	Previous Regimens, n	ECOG	Chemotherapy	Donor Chimerism D etected	Response	HSCT after Treatment (Donor)	Time to HSCT, d	Outcome (Cause of Death)	PFS, d	OS, d
1	52	F	AML	RR	Mono 7	3	1	Decitabine x5	Yes, minimal	Yes, residual blasts	Yes (MaRD)	119	Died (relapse)	307	338
2	35	M	AML	RR after HSCT	Mono 7, del 13q/FLT3 ITD	5	2	CLAM	Yes, complete	Yes, aplasia	No	NA	Died (adenovirus)	32	32
3	55	M	AML	PIF	Complex/MLL, TP53	2	2	CLAM	No	No	No	NA	Died (relapse)	35	73
4	51	F	AML	RR	Tri 11/FLT3 ITD, MLL	5	1	CLAM	No	Yes, residual blasts	Yes (HC)	64	Died (relapse)	161	281
5	46	F	AML	RR	Tri 21/CEBPA, DNMT3A,NRAS, GATA2	2	2	MEC	No	Yes, residual blasts	Yes (MaRD)	46	Alive	643	643
6	67	F	AML	RR after HSCT	Complex/TP53, MLL	4	2	CLAM	Yes, complete	Yes, aplasia	Yes (HC)	30	Died (adenovirus)	278	278
7*	40	F	CML	Refrac-tory blast phase	Mono 7	2	1	CLAM	No	No	Yes (MaRD)	21	Died (influenza)	482	482
8	58	F	AML	PIF	FLT3 ITD, DNMT3A, RAD21, WT1, PTPN11, NPM	3	1	Decitabine × 10	No	No	No	NA	Died (relapse)	48	259
9	58	F	MDS	RR after HSCT	Del 11, t(9;X)/ MLL	3	2	Decitabine × 10	No	No	No	NA	Died (relapse)	90	117
10	53	F	AML	RR after HSCT	Del 5, inv 9/ Jak2, MLL, IDH1, CEBPA	2	1	CLAM	Yes minimal	Yes, aplasia	Yes (HC)	36	Died (infection)	255	255
11	66	M	MDS	PIF	MLL PTD, STAG2, SRSF2, TET2	1	1	CLAM	No	Yes, residual blasts	Yes (MUD)	119	Alive	268	268
12	49	F	AML	RR after HSCT	TP53, KRAS	4	2	Cytarabine/ cladribine	Yes complete	Yes, aplasia	No	NA	Died (relapse)	117	117
13	60	M	AML	PIF	MLL	3	3	IDAC	No	No	No	NA	Died (relapse)	37	65
14	38	F	AML	PIF	Complex/EZH2, KRAS, WT1	2	2	CLAM	No	No	Yes (HC)	64	Alive	194	194
15	59	M	AML	PIF	t(6;9)/CSF3R, STAG2, FLT3 ITD	2	0	CLAM	No	No	No	NA	Alive (relapse)	32	157
16	30	F	AML	PIF	Complex/TP53	3	0	Decitabine ×10	No	NE	Yes (dCBU)	66	Alive	139	139
17	22	M	AML	RR after HSCT	Tri 8/JAK 2, ZRSR2, RUNX1	5	3	Cytarabine/ ruxolitinib	No	No	Yes (HC)	24	Alive (relapse)	72	138
18	29	M	AML	PIF	Inv 3, mono 7	6	1	Cytarabine/ cladribine	No	No	No	NA	Alive (relapse)	12	138
19	64	F	AML	PIF	Complex/ IDH1, TP53, BCOR	2	1	CLAM	No	NE	Yes (MUD)	42	Alive	80	80
20	47	F	AML	RR after HSCT	FLT3 ITD, CEBPA (single), BCOR, DNMT3A, NPM1	5	2	CLAM	Yes, complete	Yes, aplasia	No	NA	Alive	73	73
21	35	M	AML	RR	Inv 16, mono 7, FLT3 TKD	3	2	CLAM	no	Yes, aplasia	Yes (MaRD)	25	Alive	62	62

CML indicates chronic myelogenous leukemia; RR, relapsed refractory; CLAM, cladribine, cytarabine, filgrastim, and mitoxantrone; MEC, mitoxantrone, etoposide, and cytarabine, IDAC, intermediate-dose cytarabine; NE, not evaluable; MaRD, matched related donor; HC, haploidentical CB transplant; MUD, matched unrelated donor; dCBU, double CBU; NA, not applicable.
Patient 7 had evidence of low-level CBU chimerism at 24 weeks after CBU infusion post-HSCT.

Table 3
CBU Characteristics

Patient	Precryopreservation TNCs, × 10^7	Precryopreservation TNC Dose, × 10^7/kg	Duration of Storage, yr	Post-Thaw CD34 Cell Viability, %	Post-Thaw Cell Dose		HLA Allele Match /6	HLA Allele Match/8	Shared IPA Targets	NIMA Match
					CD34, × 10^5/kg	CD3, × 10^6/kg				
1	100	1.6	13	95.20	.8	4.4	5	7	Yes	No
2	130	2.4	10	96.84	.5	4.5	5	5	Yes	No
3	127	1.5	9	97.05	.6	2.9	4	3	No	Yes
4	79	1.7	6	98.39	.7	4.0	4	5	Yes	Yes
5	69	1.2	14	95.40	.6	3.5	5	7	Yes	No
6	115	2.3	10	96.46	.9	5.2	5	7	Yes	Yes
7	79	1.4	9	93.64	.2	3.7	5	6	Yes	No
8	73	1.5	7	93.60	.3	2.4	4	5	Yes	No
9	151	1.9	12	95.33	.4	3.1	4	4	Yes	Yes
10	63	1.1	15	93.46	.2	3.2	5	6	Yes	Yes
11	179	1.9	10	96.52	.5	3.6	5	5	Yes	No
12	69	1.1	11	96.88	.5	2.8	5	6	Yes	No
13	94	1	16	ND	.2	1.8	6	8	Yes	No
14	134	1.5	10	95.83	.3	3.0	4	5	Yes	No
15	106	1.2	11	91.48	.2	2.0	4	5	Yes	No
16	88	1.4	17	ND	.2	4.4	4	5	Yes	No
17	80	1.6	11	90.71	.6	6.2	5	7	Yes	No
18	78	1.1	10	95.21	.2	2.3	5	5	Yes	Yes
19	65	1.3	7	96.31	1.0	2.2	5	6	Yes	No
20	86	1.5	12	98.24	.3	3.5	5	6	Yes	No
21	84	.7	7	97.37	.2	2.4	5	7	Yes	No

ND indicates not done.

MRD-positive before CBU infusion had undergone HSCT. Overall, 12 of the 21 patients proceeded to HSCT, at a median of 44 days after cell therapy (range, 21 to 119 days). All patients received a fludarabine/melphalan-based reduced-intensity conditioning regimen; the graft sources are listed in Table 2.

Overall Survival, Progression-Free Survival, and Causes of Death

At the time of this report, 10 patients were alive, with a median follow-up of 146 days, including 7 patients in CR and 3 with relapse (patients 15, 17, and 18). Eleven patients have died, at a median of 255 days after CBU infusion. Leukemia relapse was the cause of death in 7 patients. Four patients died without evidence of leukemia. Patient 2 died on day +32 without blood count recovery; he was found to have adenovirus viremia before CBU infusion. Patient 7 died from influenza B and fulminant cardiomyopathy at 461 days after undergoing matched related donor HSCT (at 482 days after CBU infusion). Patient 10 died from nontuberculosis mycobacterial infection at 219 days after haploidentical cord HSCT (255 days after CBU infusion).

Seven of the 12 HSCT recipients are alive at a median follow-up of 147 days (range, 70 to 651 days), and 6 remain in CR. Two died from relapse on days +281 and +338 after HSCT, and 3 died from infection while in CR on days +219, +278, and +482.

The median overall survival from the start of treatment was 147 days (range, 32 to 643 days). Patients who subsequently underwent HSCT had a median survival of 261 days (range, 62 to 643 days).

Table 4
Adverse Events and Time after CBU infusion

Adverse Event (no. of Events)	Time after CBU Infusion	
	≤30 d	>30 d
Grade 1 CRS (2)	2	
Grade I-IV aGVHD (4)	2	2
Grade III-IV aGVHD (2)		2
Infections (12)		
Bacteremia (5)	5	
Adenovirus viremia (2)	1	1
Candidemia (2)	2	
Mucor pneumonia (1)		1
Fungal pneumonia (2)	2	
Other (4)		
Multiorgan failure	1	
Respiratory failure	1	
Liver failure	1	
Post-transplantation lymphoproliferative disease		1

DISCUSSION

In this prospective study, we evaluated the combination of salvage chemotherapy and CB-based adoptive immunotherapy for the treatment of patients with refractory AML and MDS. Our results establish the safety, feasibility, and preliminary efficacy of this approach.

The patients selected for this study had extremely adverse characteristics. They were heavily pretreated patients with primary refractory or relapsed refractory AML who had received a median of 3 previous regimens, including 7 recipients of previous allogeneic HSCT. Most had high-risk cytogenetics and/or molecular abnormalities. Similar patient cohorts have been shown to have dismal outcomes, with a median survival of 1.5 months [1–3]. For example, the induction mortality rate for first salvage chemotherapy for 285 patients with PIF AML who failed 1 cycle of high-dose cytarabine was 16% at M.D. Anderson Cancer Center [1]. Giles et al [4] reported a 25% rate of induction death in 594 patients with relapsed AML undergoing second salvage therapy.

Given the poor results after chemotherapy alone, some groups have reported on adoptive cell therapy, where the donor cells are thought to mediate transient GVL effects and then be rejected. Colvin et al [5] reported 13 patients with refractory AML who received 100 cGy of total body irradiation and infusion of haploidentical T cells. There were 5 durable complete responses and 4 partial responses, all of which occurred without overt chimerism. Guo et al [6] reported on

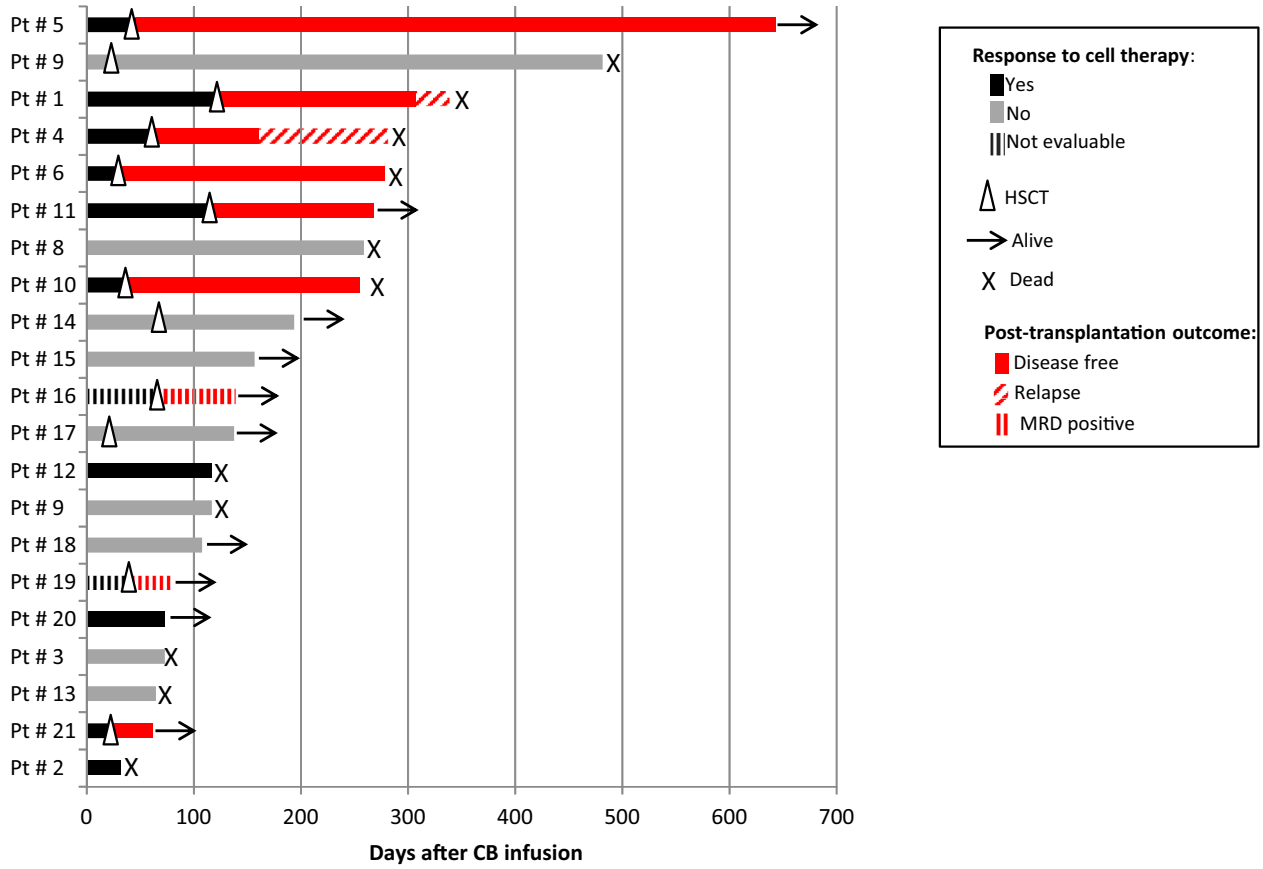

Figure 1. Response to CB adoptive cell therapy and outcomes. Of the 21 patients treated, 10 responded (black bars), 2 were not evaluable (MRD before and after cell therapy; blue bars), and 9 did not respond (gray bars). Twelve patients received HSCT at a median of 44 days after cell therapy (marked with a triangle). Seven patients were disease-free after HSCT (red bars), of whom 2 relapsed at a later point (diagonal red bars). The 2 patients with positive MRD before cell therapy underwent HSCT and remained positive after HSCT (orange bars). At the time of this report, 10 patients were alive, with a median follow-up of 146 days (arrows), and 11 had died, at a median of 255 days postinfusion (crosses).

58 patients aged 60 to 88 years with AML who were assigned at random to receive induction chemotherapy with cytarabine and mitoxantrone or the same chemotherapy plus granulocyte colony-stimulating factor-mobilized HLA-haploidentical stem cells. The CR rate and the 2-year probability of disease-free survival were significantly higher in this latter group. Persistent donor microchimerism was detected in 4 female patients who received male donor cells. Most recently, Guo et al [7] extended this approach and treated 185 elderly patients with newly diagnosed AML. They reported high CR rates, between 70% and 79%. Notably, the cumulative T cell doses that the patients received were as high as 8×10^8/kg, with very low rates of GVHD.

These studies suggest that cell therapy-mediated GVL effects can contribute to the induction of remission with minimal or transient chimerism. However, a limitation of this approach is the difficulty of organizing haploidentical donor cell infusions for newly diagnosed or relapsed patients requiring urgent treatment. This is illustrated by the fact that the 185 patients treated by the Guo group were accrued over an 8-year period in 12 centers in China, the United States, and Spain. These represent only a small fraction of the patients with AML diagnosed and treated at these centers during that period.

We took a different approach and used CB-based cell therapy to induce an antileukemic effect. CBUs already tested and stored are available promptly and represent the optimum cell source for patients requiring urgent treatment. Furthermore, the strong GVL effect of CB in allogeneic HSCT has been shown by several groups, with reports of a lower incidence of post-transplantation relapse compared with unrelated volunteer donor grafts [26–28]. The reduced rate of disease recurrence after CB transplantation may be explained by specific immunologic properties of CB resulting from the fetal exposure to the mother during pregnancy. Maternal lymphocytes cross the placenta and enter the fetal circulation, where they are exposed to the fetal cells expressing the inherited maternal antigens and inherited paternal antigens (IPAs). When transplanted (with the CB) into a recipient who shares the same antigens as the IPAs, the maternal cells are “primed” to recognize the targets. Retrospective analyses of transplantation outcomes have shown lower relapse rates for patients who received CBUs with shared IPA targets, particularly for 5/6 HLA-matched grafts, without an increase in the incidence of GVHD. Also during pregnancy, exposure of the fetus to maternal cells expressing NIMAs leads to the development of NIMA-specific responses. When transplanted into a recipient who shares the same antigen as the NIMAs, the CB cells may exert a potent effect, and clinical analyses have shown improved post-transplantation survival for recipients of NIMA-matched CB grafts [10,11,29].

Based on this work, CBUs for the present study were selected to have shared HLA targets between the IPAs of the CBU and the patient and also, if possible, a match between the NIMAs and the patient (Table 1).

Given the size of the NCBP inventory, a large number of already fully HLA-typed CBUs could be reviewed for each patient, making it possible to identify suitable CBUs for all patients. At least 1 CBU with shared IPA targets was identified for each patient, and CBUs with NIMA matches were identified for 80% of them. Following current CBU selection criteria for transplantation, we prioritized higher allele-matched units. Given that engraftment was not the aim of the study, selected CBUs had a lower cell dose (both TNC and CD34 cells/kg) compared with CBUs usually chosen for transplantation. They provided a median of 3.2×10^6 CD3 cells/kg per patient, far lower than the T cell doses infused in the haploidentical donor studies. CBUs were collected a median of 10 years before infusion; nonetheless, graft quality, as evaluated by $CD34^+$ cell viability in the segments, was excellent (median, 96%; all >90%). As a result, smaller, "older" CBUs, not often selected for transplantation, can mount an antileukemic effect when used as cell therapy.

Furthermore, as the study evolved, CBU selection was expedited, and CBU identification has become faster in the past year. In several cases, at least 1 CBU meeting study criteria could be identified at the start of the search. Such a timely approach is possible because maternal HLA typing has been completed for a large proportion of the CBU inventory in our bank, particularly for the older CBUs like those used in the present study.

Although treatment-related adverse events were frequent, they were in line with what would have been expected from salvage chemotherapy alone [4], and few were attributed to the CBU infusion. Adverse events were mostly opportunistic infections. Severe CRS or neurologic toxicities, such as those frequently encountered with chimeric antigen receptor T cell treatment, were not observed.

Four patients, all who had experienced relapse after previous HSCT, developed acute GVHD. Two of these patients had limited grade I skin aGVHD, and the other 2 patients, with durable CBU engraftment, had grade IV aGVHD. In both cases, aGVHD responded to immunosuppressive treatment. Our experience highlights the need for immediate intervention for treatment of GVHD, particularly in patients with CBU-derived engraftment.

CBU chimerism was detected in 6 patients. Persistence of the CBU cells, at least transiently, without full hematopoietic reconstitution seemed to correlate with response. All 6 patients who had detectable chimerism within 2 to 4 weeks after cell therapy responded, compared with only 4 of the 12 patients without detectable chimerism. In the latter group, it is also possible that transient chimerism might have been missed or that levels were too low to be detected.

We recognize several limitations of our study. The small sample size does not allow for statistical analysis and correlation of the various CBU- and patient-related characteristics with response. Furthermore, given come patients' comorbidities and high previous anthracycline exposure, we had to use multiple salvage chemotherapy regimens with variable intensity. We used this approach primarily as a bridge to HSCT, and the elimination of blasts allowed 7 of the responders to undergo allogeneic HSCT and 2 additional patients to remain disease-free for 2 to 3 months without HSCT. It is important to note that patients underwent HSCT within a short interval from cell therapy (median time to transplantation, 44 days; 3 patients proceeded to HSCT in <4 weeks); therefore, a definitive evaluation of the response to adoptive immunotherapy was not possible. Finally, our CBU selection criteria emphasized IPA sharing and NIMA matching, based on our previous experience. It is conceivable that different approaches (based on, eg, KIR type or intentional HLA mismatching), also might result in powerful GVL effects. More studies are needed to shed light on the mechanisms involved.

In conclusion, the use of CB as adoptive immunotherapy in combination with salvage chemotherapy in patients with refractory AML and MDS represents a feasible approach for disease control and a bridge to allogeneic HSCT with an acceptable incidence of adverse events. The potential for alloreactivity can be enhanced through the selection of CBUs targeting shared IPA and/or NIMA matches with the recipient. CBUs with lower TNC doses, already available in the public CB bank inventory and unlikely to be adequate grafts for adult transplants, can be used for cell therapy. We were able to treat 21 patients at a single center over a 2.5-year period and to identify appropriate CBUs for all 50 patients with searches within a short period, attesting to the feasibility of our approach. Additional prospective studies are needed to establish efficacy in AML, MDS, and possibly in other hematologic malignancies, and also to identify recipient and CBU-related predictors of response and of toxicity.

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