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ORIGINAL ARTICLE

Transplantation of *ex vivo* expanded cord blood cells using the copper chelator tetraethylenepentamine: a phase I/II clinical trial

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The copper chelator tetraethylenepentamine (TEPA; StemEx) was shown to attenuate the differentiation of *ex vivo* cultured hematopoietic cells resulting in preferential expansion of early progenitors. A phase I/II trial was performed to test the feasibility and safety of transplantation of CD133+ cord blood (CB) hematopoietic progenitors cultured in media containing stem cell factor, FLT-3 ligand, interleukin-6, thrombopoietin and TEPA. Ten patients with advanced hematological malignancies were transplanted with a CB unit originally frozen in two fractions. The smaller fraction was cultured *ex vivo* for 21 days and transplanted 24 h after infusion of the larger unmanipulated fraction. All but two units contained $<2\times10^7$ total nucleated cells (TNCs) per kilogram pre-expansion. All donor–recipient pairs were mismatched for one or two HLA loci. Nine patients were beyond first remission; median age and weight were 21 years and 68.5 kg. The average TNCs fold expansion was 219 (range, 2–620). Mean increase of CD34+ cell count was 6 (over the CD34+ cell content in the entire unit). Despite the low TNCs per kilogram infused (median $=1.8\times10^7$/kg), nine patients engrafted. Median time to neutrophil and platelet engraftment was 30 (range, 16–46) and 48 (range, 35–105) days. There were no cases of grades 3–4 acute graft-versus-host disease (GVHD) and 100-day survival was 90%. This strategy is feasible.

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Introduction

Allogeneic hematopoietic progenitor cell transplantation is the treatment of choice for a variety of malignant and nonmalignant diseases. Unfortunately, a suitable family donor is found for less than 30% of patients who might benefit from this therapy. Unrelated donor transplants offer an alternative source of hematopoietic cells for some patients without related donors, particularly those of Caucasian, Western European origin. Such unrelated donors require stringent human leukocyte antigen (HLA) matching to the patient due to the risk of graft-versus-host disease (GVHD) when mismatched grafts are used. Furthermore, unrelated donors are more difficult to find for minority patients, as minority donors are underrepresented in the donor registries. Finally, it can often take several months before the unrelated donor is identified and the hematopoietic cells procured, making this a suboptimal transplant for patients with rapidly progressive disease.

Umbilical cord blood (CB) grafts appear to cause less GVHD than bone marrow or mobilized peripheral blood grafts, allowing the use of mismatched donor–recipient pairs and thereby increasing the donor pool. Faster procurement is also a potential advantage, especially for patients with rapidly evolving hematologic malignancies.[1] Consequently, over the past several years there has been a dramatic increase in the number of patients grafted with CB, which has become a suitable alternative for children and young adults lacking HLA-matched related donors.[2,3]

Use of CB for transplantation is still limited by the relatively small number of available progenitor cells compared to bone marrow or mobilized peripheral blood. Accordingly, CB grafts are routinely and more successfully used in the pediatric transplantation setting. The NCBP program reports that only 20% of the CBUs in their inventory could suffice for a 75 kg patient according to the recommended threshold cell dose ($>2.5\times10^7$ total nucleated cells (TNCs) per kilogram).[4]

Unfortunately, adults receiving CB transplantation are at high risk of early mortality (as high as 40–60% day 100 mortality) due to delayed engraftment and higher rates of

engraftment failure leading to increased rate of infections. Not surprisingly, grafts with a higher number of TNC[5,6] and higher numbers of CD34+ cell dose[7] have been reported to result in higher probabilities of survival. Strategies to improve outcomes have included the use of two units for transplantation,[8] and the co-transplantation of CD34 or CD133-selected peripheral blood stem cells from a third party donor and one unrelated donor CB unit.[9]

To try and overcome the problem of low progenitor cell dose, *ex vivo* expansion of CB-derived cells has been attempted. The true test of this method is whether an expansion technology will be able to provide a reliable, reproducible increase in the number of progenitor cells available from a single unit of CB, resulting in superior rates of engraftment and overall survival in adult patients. A significant hurdle of presently available methods for graft production is the ability to generate an expanded population of committed hematopoietic progenitor cells without compromising the numbers of less differentiated progenitor cells (CD34+CD38− or CD34+Lin− cells), which are important functional hematopoietic repopulating cells. It was previously demonstrated that introducing a differentiation inhibitor into an expansion culture augmented the expansion potential of CB-derived progenitor cells. The molecule used was the copper chelator tetraethylenepentamine (TEPA), based on findings that cellular copper concentrations modulate cellular differentiation.[10,11] Adding the polyamine copper chelator TEPA to an early acting cytokines cocktail (thrombopoietin (TPO), interleukin-6 (IL-6), FLT-3 ligand (FLT3) and SCF) resulted in both proliferation of mature, committed CB cells (CD34+Lin+) and colony-forming units cells (CFUc) as well as robust expansion of the CD34+CD38− and CD34+Lin− subpopulations.[11] Most importantly, the percentage of engrafted human progenitor cells as well as that of myeloid and lymphoid cells was reproducibly and significantly superior in SCID mice transplanted with TEPA-mediated *ex vivo* expanded cells than mice transplanted with similar numbers of cells expanded with the cytokine cocktail without TEPA, or with the corresponding cell fraction before expansion.[12,13]

Based on these preclinical studies, we conducted a phase I/II clinical trial to test the safety of StemEx, a product containing an expanded portion of a CB unit, cultured for 21 days with early acting cytokines and the copper chelator TEPA, in combination with the remaining, unmanipulated portion of the same unit.

Patients, materials and methods

Study objectives

We studied the transplantation of StemEx in combination with the remaining unmanipulated CB portion, as hematopoietic support for patients with acute leukemia and lymphoma receiving high-dose therapy. The study objectives were to evaluate the safety and tolerability of StemEx transfusion, along with the rate and durability of hematopoietic reconstitution, incidence of acute and chronic GVHD, disease-free and overall survival following this approach.

Definitions

Neutrophil engraftment was defined as the first of three consecutive days with an ANC $\geqslant 0.5 \times 10^9$/l. Platelet engraftment was defined as the first of seven consecutive days in which a sustained transfusion-independent platelet count $\geqslant 20 \times 10^9$/l was documented. Engraftment failure was defined as failure to achieve an ANC $\geqslant 0.5 \times 10^9$/l by transplant day 42, with $<10\%$ cellularity on the bone marrow biopsy, and without evidence of disease relapse. Acute and chronic GVHD were scored according to standard criteria.[14]

Overall survival was measured from the first day of CB infusion (day 0) until death from any cause, with censoring performed at the date of last contact. Death of any cause other than relapse or disease progression was scored as non-relapse mortality. Deaths occurring during the first 30 days with aplastic marrow and/or pancytopenia were labeled 'early deaths' and scored as nonrelapse mortality. Toxicity was scored using the modified National Cancer Institute criteria.[15]

Adverse events, blood chemistry and hematologic parameters were monitored daily during the initial hospitalization and then at gradually increasing intervals up to day +180. Subsequently, disease status and survival were followed at least quarterly during the first year, as a post-study follow-up per routine standard of care.

Determination of chimerism

Peripheral blood or bone marrow donor–recipient chimerism was evaluated by analysis of DNA microsatellite polymorphisms by PCR with D6S264, D3S1282, D18S62 and D3S1300 fluorescence-labeled primers, and then analyzed using GeneScan software in all cases. Mixed chimerism was defined as the presence of any detectable percentage of recipient DNA.

Human leukocyte antigen typing

HLA typing for class I antigens was performed using standard serological techniques. Class II alleles (HLA-DRB1, -DQB1) were resolved with low-resolution molecular typing using sequence-specific oligonucleotide primers for hybridization of patient-derived amplified DNA, followed by high-resolution molecular typing in all patients.

Eligibility

Eligible patients were no older than 55 years old, with adequate major organ system function as demonstrated by left ventricular ejection fraction of at least 40%, pulmonary function test demonstrating a corrected CO_2 diffusion capacity of at least 50% predicted, serum creatinine <1.6 mg per 100 ml, SGPT and bilirubin $\leqslant 2.0 \times$ normal and functional performance score (Karnofsky/Lansky) $\geqslant 70$.

Allowed diagnosis and disease stages included acute myelogenous leukemia (AML) and acute lymphoblastic

leukemia (ALL) in second complete remission, induction failure, or second relapse or high-risk first complete remission (CR) (AML with deletion of chromosomes 7 and 5; ALL with the Philadelphia chromosome or t(4;11)). Patients with non-Hodgkin's lymphoma (NHL) or Hodgkin's disease (HD) in relapse or beyond first remission were also eligible. Twenty-one or more days had to have elapsed since the patient's last radiation or chemotherapy prior to initiation of the preparative regimen (exceptions were Hydrea, Gleevec and intrathecal therapy).

Patients should lack an HLA-matched sibling donor or a matched unrelated donor (defined as a donor matching in class I A, B and C loci, and class II HLA-DRB1 and -DQB1 loci, by high-resolution allele level typing). Informed consent was obtained from patients or from the parents or legal guardians of pediatric patients. Patients who were HIV+, had evidence of central nervous system disease or active infection or had prior allogeneic transplant were not eligible. The institutional review board of MD Anderson Cancer Center approved this phase I/II study, and all patients signed informed consent.

Autologous hematopoietic stem cell backup
All patients without overt bone marrow disease were submitted to granulocyte colony-stimulating factor (G-CSF) mobilization and peripheral blood stem cell collection or bone marrow harvesting. These cells were to be used in case of engraftment failure. In cases where autologous harvesting was not an option, a haploidentical family member was identified and formally consented to be a potential donor for a haploidentical transplant in case of engraftment failure.

Cord blood unit selection
Units had to be stored frozen in two fractions (80 of 20, 60 of 40 or 50 of 50%) and provide at least 1×10^7 TNC per kilogram in the unexpanded fraction (pre-thaw). CB units and recipients should match at least to four HLA classes I (HLA-A and -B; serological) and II (DRB1; high resolution) loci. CB units were obtained from public CB banks only.

Expansion of cord blood cells ex vivo
Frozen UCB units were transported to MD Anderson Cancer Center in liquid nitrogen in vapor phase and kept in the vapor phase of liquid nitrogen in a freezer at the cell processing laboratory. The two fractions were separated, and the smaller (when frozen in 80 of 20 or 60 of 40) or equal (when frozen in 50 of 50) fraction was thawed on day −20, and washed with 10%w/v Gentran (Baxter, Deerfield, IL, USA) and 5% w/v human serum albumin (Baxter or Aventis, Bridgewater, NJ, USA). The cells were incubated with 0.15% w/v intravenous immunoglobulin (IvIg, Baxter) for 10 min at room temperature before centrifugation, and then resuspended in phosphate-buffered saline (PBS) containing 0.4% sodium citrate solution, 1% human serum albumin and 0.2-mg/ml rHu-DNase (Genentech, San Francisco, CA, USA). Subsequently, the cells were labeled with Miltenyi's CD133 CliniMACS reagent (Miltenyi Biotech, Auburn, CA, USA) and separated by CliniMACS (according to the manufacturer's instructions). Following selection, cells were stained with Trypan blue, counted, assayed for CFUc and immunophenotyped to determine purity. Purified CD133+ cells were cultured in culture bags (American Fluoroseal Co., Gaithersburg, MD, USA) at a concentration of 1×10^4 cells per milliliter in minimum essential medium-α (MEMα) and 10% fetal calf serum (FCS, Hyclone, Logan, UT, USA) containing the following human recombinant cytokines: TPO, IL-6, FLT3 and SCF, each at a final concentration of 50 ng/ml (R&D Systems Minneapolis, MN, USA) and 5 μM TEPA (Sigma, St Louis, MD, USA and NovaSep, Boothwyn, PA, USA). Expansion was performed in 72 ml VueLife Teflon PEP cell culture bags (American Fluoroseal Co.) if the number of cells recovered following the enrichment procedure was up to 19×10^4, and in 270 ml VueLife Teflon PEP cell culture bags (American Fluoroseal Co.) if the number of cells were between 20 and 70×10^4.

The cultures were incubated for 3 weeks at 37 °C in a humidified atmosphere of 5% CO_2 in air. Cultures were topped weekly with the same volume of fresh medium, FCS, growth factors and TEPA. At the termination of the expansion, cells were washed and then suspended in 100 ml of a PBS/EDTA/HSA infusion buffer. A sample of the cells in the infusion buffer was counted following staining with Trypan blue, assayed for CFUc and immunophenotyped for surface antigen analysis (CD34, CD38 and CD133). Cells were administered to the patient 24 h following administration of the unmanipulated fraction of the unit. Aliquots from the cell suspension were tested throughout the process and at its completion for mycoplasma, endotoxin, sterility and Gram's staining. All infusions were given via a central catheter. Patients were monitored closely during transplantation for any toxicity.

The first three products were processed at the GMP laboratory of the Baylor College of Medicine, and the subsequent seven expansions were performed at the MD Anderson Stem Cell Transplant and Cellular Therapy Laboratory.

Conditioning regimens, graft-versus-host disease prophylaxis and supportive care
Patients with ALL, NHL and HD received melphalan 140 mg/m^2 on day −8, thiotepa 10 mg/kg on day −7 and fludarabine 40 mg/m^2 from day −6 to −3. Patients with AML received a test dose of busulfan (BU) 32 mg/m^2 on day −8. Based on the pharmacokinetics of this test dose, BU dose was calculated to achieve an area under the curve of 6500 μM/min in a single daily dose from day −6 to −3. Fludarabine 40 mg/m^2 was also administered from day −6 to −3. Rabbit anti-thymocyte globulin was given to all patients on days −4 (1.25 mg/kg) and −3 (1.75 mg/kg).

GVHD prophylaxis consisted of tacrolimus and 'mini'-methotrexate (5 mg/m^2) on transplant days +2, +4 and +7.[16] This methotrexate (MTX) schedule reflected the fact that donor cells were infused over 2 days, that is, day 0 and +1. Tacrolimus was administered in a starting dose of 0.03 mg/kg (ideal body weight) as a 24 h continuous infusion daily, changed to oral dosing when tolerated, after engraftment. Tacrolimus was to be tapered beginning transplant day 180, if no GVHD was present.

G-CSF was administered at a dose of 5 mcg/kg per day subcutaneously beginning on day 0, and continued until the absolute neutrophil count was greater than 2.5×10^9/l. Antibiotic prophylaxis was given according to the institution's standard regimen and as clinically indicated. Blood products and other supportive care measures were used as per institutional routines. Patients who developed grade 2 or greater acute GVHD received methylprednisolone 2 mg/kg per day as primary treatment.

Study design and statistical analysis

This was a phase I/II study, in which development of infusional toxicity, delayed time to neutrophil engraftment (beyond 42 days), and engraftment failure would evoke study stopping rules. The trial proceeded to enroll the intended 10 patients, since no major toxicity related to the expansion was documented.

Probability of event-free or overall survival was estimated using the Kaplan–Meier method. Statistical analyses were performed by i3 Statprobe. All analyses were carried out using SAS Version 6.12 in a Windows NT environment.

Results

Patient and disease characteristics

Patients were enrolled from March 2003 to July 2004. Ten patients with a median age of 21 years (range, 7–53 years), and a median weight of 68.5 kg (range, 30.9–82.2 kg) were treated. Six patients were not in remission at transplantation, and nine were beyond first remission (Table 1).

Preparative regimens and donor–recipient HLA matching

All patients received mismatched units. Eight patients received CB units that were mismatched in two loci, while two patients were treated with units mismatched at a single locus. The degree of donor–recipient HLA matching is noted in Table 1. Melphalan, thiotepa and fludarabine constituted the preparative regimen for eight patients with lymphoid malignancies, while two patients with myeloid diseases were treated with intravenous BU and fludarabine.

Graft characteristics and ex vivo *expansion*

All infused expanded fractions were negative for bacterial and fungal contamination and were endotoxin-free. Patients received a median number of 1.8×10^7 TNC per kilogram and 1.5×10^5 CD34+ cells per kilogram.

The median number of viable cells seeded for culture at day −20 was 78×10^4 (range, 15.6–264×10^4), with a mean viability of 71.1% ($n = 10$). The median and mean amounts of cells harvested at the end of the expansion process were 68.5×10^6 and 255×10^6 (range, 2.15–1638×10^6) respectively, resulting in a median and mean fold expansion of 161 (range, 2–620) and 219, respectively. The median and mean infused TNC dose in the expanded fraction was 0.9×10^6 and 4.4×10^6 cells per kilogram (range, 0.1–29.8×10^6 cells per kilogram), respectively. The mean viability of the expanded cells was 93%.

To conserve cells for the patient, CD34 analysis was not performed on the selected progenitor fraction prior to expansion, but was observed for the unmanipulated fraction of the same unit. The contributions of the unmanipulated and expanded fractions to the final CB graft CD34+ cell dose are summarized in Table 2. The median CD34+ cell dose infused with the combined fractions (unmanipulated as well as expanded) was 1.5×10^5 cells per kilogram (range, 0.5–46.3×10^5 cells per kilogram). This corresponded to a mean sixfold (median 2.26-fold) increase of CD34+ cells delivered over the hypothetical calculated value of CD34+ cells in the entire CB unit before expansion (as measured by FACS analysis of the unmanipulated fraction after thaw and extrapolated for the entire CB unit). The CD34+ cell population represented a median of 12.8% (range, 2.7–18.1%) of the total cell number in the manipulated fraction. The median and mean CD34+CD38− cell numbers contributed by the expanded portion were 4.0×10^5 and 43.1×10^5 (range, 0–321.5×10^5 cells), respectively (Table 2). The median fold increase for CFUc was 37.8 (range, 1.6–85.9). Data on the expansion cultures are provided in Table 3.

Table 1 Patient, disease and treatment characteristics

Subject[a]	*Age (years)*	*Weight (kg)*	*Gender*	*Race*	*Diagnosis*	*CMV status*	*Preparative regimen*[a]	*HLA match*[b]	*Fractions*[c]	*Infused TNC/kg ($\times 10^7$/kg)*
1	21	50.0	Female	Caucasian	AML, induction failure	Negative	FB	5 of 6	60/40	1.87
2	17	71.3	Male	Hispanic	ALL, CR2	Positive	MTF	4 of 6	50/50	1.89
3	24	79.0	Male	Caucasian	HD, second relapse	Positive	MTF	4 of 6	60/40	1.15
4	13	53.2	Male	East Indian	ALL, CR1 Ph+	Positive	MTF	4 of 6	60/40	1.80
5	20	74.0	Male	Hispanic	HD, second relapse	Negative	MTF	4 of 6	80/20	1.90
6	44	82.2	Male	Caucasian	ALL, second relapse	Positive	MTF	5 of 6	80/20	1.12
7	53	63.6	Female	Caucasian	AML, CR2	Negative	FB	4 of 6	60/40	1.83
8	12	55.0	Male	Hispanic	ALL, PIF	Positive	MTF	4 of 6	50/50	5.63
9	7	30.9	Male	Caucasian	ALL, CR2	Negative	MTF	4 of 6	60/40	3.86
10	51	78.2	Female	Hispanic	NHL, third relapse	Positive	MTF	4 of 6	50/50	1.27

Abbreviations: ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia; CR = complete remission; FB = fludarabine and busulfan; HD = Hodgkin's disease; MTF = melphalan, thiotepa and fludarabine; NHL = non-Hodgkin's lymphoma; PIF = primary induction failure.
[a]All patients received rabbit ATG.
[b]Serologic typing for HLA-A and -B; high-resolution typing for HLA-DRB1.
[c]Units were frozen in two fractions; numbers reflect the proportion of volume contained in each fraction.

Table 2 Contributions of StemEx and the unmanipulated cord blood unit portion to the total number of infused CD34+ cells

Patient no.	*Unit partition (%)*	*CD34 cells/kg ($\times 10^5$)*				*Ratio: actual CD34 cells infused/total in 100% CBU B/A*
		Total CBU without expansion[a] (A)	*StemEx*	*Unmanipulated CBU fraction*	*Total infused: StemEx+ unmanipulated fraction (B)*	
1	40/60	0.53	0.16	0.32	0.48	0.90
2	50/50	1.97	3.03	0.99	4.01	2.04
3	40/60	0.32	0.39	0.19	0.58	1.84
4	40/60	0.69	3.42	0.42	3.83	5.56
5	20/80	0.63	1.05	0.50	1.55	2.49
6	20/81	0.13	1.34	0.10	1.44	11.43
7	40/60	0.70	10.00	0.42	10.42	14.84
8	50/50	2.41	45.04	1.21	46.24	19.18
9	40/60	1.67	0.13	1.00	1.13	0.67
10	50/50	0.66	0.57	0.33	0.90	1.38
Mean		0.97	6.51	0.55	7.06	6.03
Median		0.67	1.20	0.42	1.50	2.26

Abbreviations: CBU = cord blood unit; UMN = Unmanipulated.
[a]The count for the total CBU without expansion was calculated based on the FACS measurement in a pre-infusion aliquot from the unmanipulated CBU and the partition of the CBU (CD34+ in total CBU = CD34+ in UMN × 100% per percent large portion).

Table 3 Expansion results

Patient no.	*No. of viable cells seeded ($\times 10^4$)*	*% Viability*	*% Purity of CD133+ cells*	*No. of CFUc per 1000 cells*	*No. of viable cells harvested ($\times 10^6$)*	*% Viability*	*No. of CFUc per 1000 harvested cells*	*No. of harvested CD34+ cells ($\times 10^6$)*	*No. of harvested CD34+CD38− cells ($\times 10^5$)*	*TNC FE*	*CFUc FE*
1	40.5	54.8	65.8	76	30	100	17.1	0.8	0	74	16.7
2	35.2	17.4	94.8	245	148	94.9	26	21.5	5.0	420.5	44.6
3	15.6	53.8	73.4	130	31	100	23.5	3.1	2.6	231	35.9
4	59	57	68.4	86.7	126	91	18.9	18.1	6.9	214	46.6
5	112	98	75.6	54	69	100	18	7.8	1.7	62	20.5
6	63.1	86	38.4	49	68	94	14.7	11	3.1	108	32.3
7	93	90	79.7	59	393	79	12	64	321.5	423	85.9
8	264	93	76.6	98	1638	94	11.1	247.7	83.5	620	70.3
9	110	85	58.9	3	2.15	86	2.4	0.39	0.8	2	1.6
10	130	76	78.6	70	48	89	45.3	4.48	6.5	37	23.9
Mean	92.2	71.1	71	87.1	255.3	92.8	18.9	37.9	43.16	219.2	37.8
Median	78	80.5	74.5	73	68.5	94	17.6	9.4	4.05	161	34.1

Abbreviations: CFUc = colony forming units cells; TNC = total nucleated cell; FE = fold expansion.

Infusion of unmanipulated cord blood fraction and ex vivo *expanded cells*

All patients received the *ex vivo* expanded cultured product (StemEx) on day +1. The infusion of the expanded cells was well tolerated by all recipients. There were no infusion-related toxicities such as allergic reactions, respiratory distress or hypertensive responses. Mean duration of expanded CB cell infusion was 0.46 h (range, 0.3–0.9 h). Infusion of unmanipulated cells was also uneventful.

Engraftment and chimerism

All but one patient had donor cell engraftment. As described above, one patient with primary refractory AML recovered autologous hematopoiesis without remission at transplant day +33. Eight patients had evidence of myeloid engraftment before transplant day +42, while one patient engrafted at day +46, after what was considered sulfamethoxazole–trimethoprim-induced engraftment delay. Median time to neutrophil engraftment was 30 days ($n = 9$; range, 16–46 days). Six patients achieved platelet transfusion independence at a median of 48 days (range, 35–105). Three patients that survived beyond transplant day +180 did not achieve platelet transfusion independence. All engrafted patients had 100% donor cell chimerism. T-lymphocyte and myeloid cell subpopulation chimerism data were available in eight cases; all showed 100% donor chimerism (Table 4). Figure 1 shows cumulative incidences of neutrophil and platelet engraftment.

Graft-versus-host disease

Grade 2 acute GVHD involving the skin was documented in four of nine engrafted patients. There were no cases of acute GVHD involving the liver or gut. Among eight patients that survived more than 100 days, chronic GVHD was diagnosed in four (Table 4). All cases responded to steroid therapy.

Table 4 Patient outcomes

Patient no.	*Acute GVHD grade*	*Chronic GVHD*	*Status at transplant day 100*	*Status at transplant day 180*
1	Not applicable	Not applicable	Autologous recovery with persistent AML, day +33	Death on day +167 (relapse)
2	2 skin	No	Engrafted, in CR	Alive, in CR
3	No	Not applicable	Death on day +94 (sepsis, in CR)	
4	No	Extensive	Engrafted, in CR	Alive, in CR
5	No	Limited	Engrafted, in CR	Alive, in CR
6	No	No	Engrafted, in CR	Death on day +122 (ARDS, in CR)
7	2 skin	Limited	Engrafted, in CR	Alive, in CR
8	2 skin	No	Engrafted, in CR	Alive, in CR
9	2 skin	Limited	Engrafted, in CR	Alive, in CR
10	No	Not applicable	Engrafted, in CR	Death on day +102 (pneumonia, in CR)

Abbreviations: ARDS = acute respiratory distress syndrome; CR = complete remission.

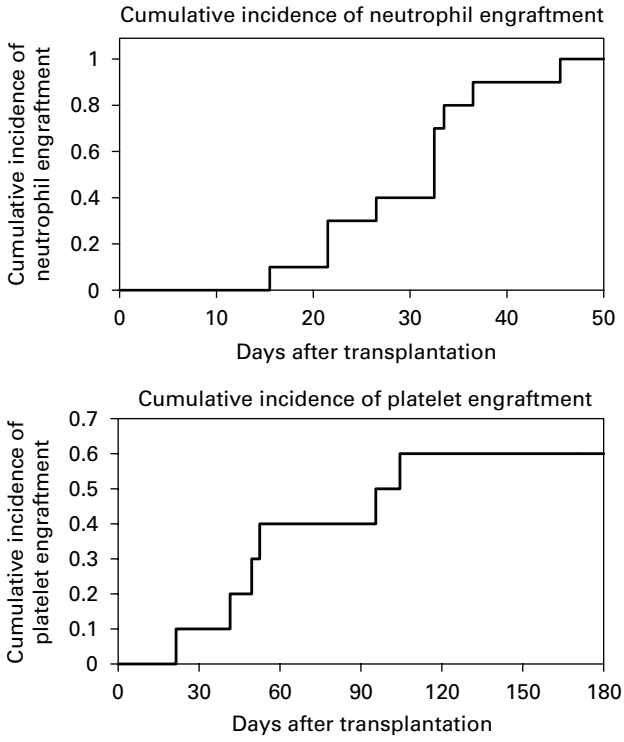

Figure 1 Cumulative incidence of neutrophil and platelet engraftment. Nine patients engrafted neutrophils, while six achieved platelet transfusion independence in a median of 48 days after transplant.

Disease relapse and mortality

One patient died during the first 100 days post transplantation, and a total of three patients died during the 180-day study period, due to infectious complications (Table 4). One patient with AML in primary induction failure did not respond to treatment, with disease progression early after transplantation, without donor cell engraftment. As a post-study follow-up, three patients are alive 21, 22 and 31 months after transplantation. Three patients died after completing the 180-day study period of disease relapse (on day +288), pneumonia (on day +221) and GVHD (on day +350).

Discussion

Transplanting a population of CD133+ CB cells which were expanded *ex vivo* for 21 days using SCF, FLT3, IL-6, TPO and the copper chelator TEPA (StemEx) was feasible. The expanded cells were well tolerated, with no infusion-related adverse events observed.

The average fold expansion of TNC in the expanded fraction was 219 with a CD34+ cell mean increase of sixfold over the CD34+ cell content in the entire unit. Despite the low TNC per kilogram infused in this study (mean = 1.7×10^7 per kilogram), nine patients successfully engrafted. The median time to engraftment of neutrophils (30 days) and platelets (48 days) was similar to published reports in recipients of unmanipulated single CB units. The use of MTX for GVHD prophylaxis in our trial may have contributed to delaying neutrophil engraftment as indicated by others.[17]

Most of our patients were beyond first remission and had active disease at the time of transplant. These high-risk characteristics are typically associated with high rates of day 100 mortality (40–60%) and engraftment failure (10–60%).[18] In this small series, 9 of 10 patients engrafted, and 9 were alive at day 100 post transplant. In contrast, the multicenter prospective American Cord Blood Transplantation Study reported a day 100 survival probability of 0.47.[19]

Jaroscak *et al*.[20] used the Aastrom Replicell continuous perfusion system to expand a fraction of CB cells that were infused 12 days after the unmanipulated transplant was performed. The median fold TNC increase was 2.4 and the median expansion in the number of CD34+, lineage-negative cells was 0.5. Patients were mostly pediatric with a median weight of 17 kg and median age of 4.5 years. Myeloid engraftment occurred in 75% of the patients, with a day 100 probability of overall survival of 65%. Shpall *et al*.[21] reported data from 37 pediatric and adult patients who had a portion of their graft expanded *ex vivo* for 10 days utilizing a cytokine cocktail of SCF, G-CSF and megakaryocyte growth and development factor. TNC expansion reached a median of 56-fold, and 4.0-fold for CD34+ cells. Thirty of the patients engrafted at a median of 28 days from transplantation, while 40% of the evaluable patients developed grades 3–4 acute GVHD. In the current study, no patient developed grades 3 or 4 acute

GVHD. The comparison of GVHD incidence between these trials, however, is difficult given that a number of variables including the GVHD prophylaxis regimen were different. Both studies reported a wide range of variability in the TNC and CD34+ cell expansion results. This variability in CB units included in the current trial imposed a major challenge for quantifying and standardizing the expansion data. Although all CB units utilized in our trial met stipulated criteria including a minimum of 1×10^7 TNC per kilogram in the larger, unmanipulated fraction, the heterogeneous characteristics of each unit resulted in variability in the expansion process. These biologic phenomena are further compounded by the differences in cryopreservation processes that still exist in CB banks (for example, cryopreservation of non-volume reduced units, of which we had two in this trial). In an attempt to minimize the variability in the expansion technique, we have now performed extensive studies using units derived from US and European CB banks to further optimize and standardize the manufacturing process. These studies resulted in a more reproducible performance, including higher purities of cells after the selection procedure that in turn translates into more consistent expansion values. These changes will be implemented prior to any subsequent clinical trials.

Improved clinical outcomes have been reported with the transplantation of higher numbers of CD34+ cells in the CB graft.[7] Here, given the small sample size we were not able to determine a statistical correlation between TNC, CD34+, CFU content or other parameters and expansion performance or clinical outcome. These data will be collected in future studies of the TEPA-based expansion technology to learn whether extreme bioassay outcomes should affect any decision making in the utilization of an expanded cell product.

We determined the safety of infusing CB *ex vivo* expanded using StemEx. In addition, the 100-day non-relapse mortality observed here compares favorably with our historic experience with adult CB transplantation, and there was no indication of an increased rate of acute GVHD with this strategy.[22]

A more comprehensive study will be required to establish whether the expanded cells have an impact on rates of successful engraftment and survival of CB transplant patients. Whether increasing the numbers of CD34+ cells will result in faster times to engraftment remains to be determined in a larger trial where MTX is omitted from the GVHD prophylaxis regimen. Such a trial will be initiated in the near future.

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References

1 de Lima M, Shpall E. Cord blood transplantation. *Curr Opin Organ Transpl* 2002; **7**: 286–293.
2 Rocha V, Labopin M, Sanz G, Arcese W, Schwerdtfeger R, Bosi A *et al*. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med* 2004; **351**: 2276–2285.
3 Laughlin MJ, Eapen M, Rubinstein P, Wagner J, Zhang M, Champlin R *et al*. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med* 2004; **351**: 2265–2275.
4 Rubinstein P, Stevens C. An Empirical Analysis of the Probability of Finding a Well-matched Cord Blood Unit with Adequate Cell Dose [Abstract]. *Fourth Annual Cord Blood Transplantation Symposia*, Los Angeles; 2006.
5 Wagner JE, Barker JN, Defor TE, Baker K, Blazar B, Eide C *et al*. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood* 2002; **100**: 1611–1618.
6 Gluckman E, Rocha V, Boyer-Chammard A, Locatelli F, Arcese W, Pasquini R *et al*. Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. *N Engl J Med* 1997; **337**: 373–381.
7 Laughlin MJ, Barker J, Bambach B, Koc O, Rizzieri D, Wagner J *et al*. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N Engl J Med* 2001; **344**: 1815–1822.
8 Barker JN, Weisdorf D, Wagner J. Creation of a double chimera after the transplantation of umbilical-cord blood from two partially matched unrelated donors. *N Engl J Med* 2001; **344**: 1871.
9 Magro E, Regidor C, Cabrera R, Sanjuan I, Fores R, Garcia-Marco J *et al*. Early hematopoietic recovery after single unit unrelated cord blood transplantation in adults supported by co-infusion of mobilized stem cells from a third party donor. *Haematologica* 2006; **91**: 640–648.
10 Peled T, Landau E, Prus E, Treves A, Nagler A, Fibach E. Cellular copper content modulates differentiation and self-renewal in cultures of cord blood-derived CD34+ cells. *Br J Haematol* 2002; **116**: 655–661.
11 Peled T, Gluckman E, Hasson N, Adi S, Assor H, Yudin D *et al*. Chelatable cellular copper modulates differentiation and self-renewal of cord blood-derived hematopoietic progenitor cells. *Exp Hematol* 2005; **33**: 1092–1100.
12 Peled T, Mandel JL, Goudsmid R, Landor C, Hasson N, Harati T *et al*. Pre-clinical development of cord blood-derived progenitor cell graft expanded *ex vivo* with cytokines and the polyamine copper chelator tetraethylenepentamine. *Cytotherapy* 2004; **6**: 344–355.
13 Peled T, Landau E, Mandel J, Glukhman E, Goudsmid N, Nagler A *et al*. Linear polyamine copper chelator tetraethylenepentamine augments long-term *ex vivo* expansion of cord blood-derived CD34+ cells and increases their engraftment potential in NOD/SCID mice. *Exp Hematol* 2004; **32**: 547–555.
14 Przepiorka D, Weisdorf D, Martin P, Klingemann H, Beatty P, Hows J *et al*. 1994 Consensus Conference on acute GVHD grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
15 NCI. *National Cancer Institute Common Toxicity Criteria version 3* 2007. http://ctep.cancer.gov/reporting/ctc.html.
16 Przepiorka D, Ippoliti C, Khouri I, Woo M, Mehra R, Le Bhers D *et al*. Tacrolimus and minidose methotrexate for prevention of acute graft-versus-host disease after matched unrelated donor marrow transplantation. *Blood* 1996; **88**: 4383–4389.
17 Locatelli F, Rocha V, Reed W, Bernaudin F, Ertem M, Grafakos S *et al*. Related umbilical cord blood transplantation in patients with thalassemia and sickle cell disease. *Blood* 2003; **101**: 2137–2143.
18 Rubinstein P, Stevens CE. Placental blood for bone marrow replacement: the New York Blood Center's program and

clinical results. *Baillieres Best Pract Res Clin Haematol* 2000; **13**: 565–584.
19 Cornetta K, Laughlin M, Carter S, Wall D, Weinthal J, Delaney C *et al*. Umbilical cord blood transplantation in adults: results of the prospective Cord Blood Transplantation (COBLT). *Biol Blood Marrow Transplant* 2005; **11**: 149–160.
20 Jaroscak J, Goltry K, Smith A, Waters-Pick B, Martin P, Driscoll T *et al*. Augmentation of umbilical cord blood (UCB) transplantation with *ex vivo*-expanded UCB cells: results of a phase 1 trial using the Aastrom Replicell System. *Blood* 2003; **101**: 5061–5067.
21 Shpall E, Quinones R, Giller R, Zeng C, Baron A, Jones R *et al*. Transplantation of *ex vivo* expanded cord blood. *Biol Blood Marrow Transplant* 2002; **8**: 376.
22 Lekakis L, Giralt S, Couriel D, Shpall E, Hosing C, Khouri I *et al*. Phase II study of unrelated cord blood transplantation for adults with high-risk hematologic malignancies. *Bone Marrow Transplant* 2006; **38**: 421–426.