ORIGINAL ARTICLE

Phase II study of unrelated cord blood transplantation for adults with high-risk hematologic malignancies

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Cell dose is a critical determinant of outcomes in unrelated cord blood (CB) transplantation. We investigated a strategy in which CB units should contain at least 2×10^7 total nucleated cells/kg of recipient weight, otherwise a second unit had to be added. We report the results of a study that was prematurely closed owing to toxicity. Patients with advanced hematologic malignancies without a human leukocyte antigen-matched sibling or unrelated donor were eligible. Conditioning regimen consisted of fludarabine and 12 Gy of total body irradiation ($n = 11$), or melphalan ($n = 4$), with antithymocyte globulin. Graft-versus-host disease prophylaxis was tacrolimus and methotrexate. Fifteen patients with acute leukemia ($n = 9$), chronic myelogenous leukemia ($n = 2$), multiple myeloma ($n = 2$) and lymphoma ($n = 2$) were treated; 60% had relapsed disease at transplantation. Three patients received double CB transplants. The 100-day and 1-year treatment-related mortality rates were 40 and 53%, respectively. Median time to neutrophil and platelet engraftment was 22 days ($n = 10$) and 37 days ($n = 10$), respectively. One patient had secondary graft failure and five patients failed to engraft. Two patients are alive and disease free; 4-year actuarial survival is 33 versus 0% for patients transplanted in remission versus in relapse. We concluded that disease status was the main determinant of treatment failure in this study.
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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is the treatment of choice for a variety of hematologic malignancies, but only 20–30% of the patients in need of HSCT are expected to have a suitable donor in the family.[1] Conversely, the probability of success of a bone marrow- or peripheral blood-matched unrelated donor search is related to ethnicity, and is limited by stringent matching criteria. Furthermore, delays intrinsic to the search process may impact decidedly in the outcome of transplantation for diseases at high risk of relapse or progression. Using mismatched unrelated donors may increase the donor pool, but this type of transplant carries a high risk of graft-versus-host disease (GVHD) and severely impaired immune recovery.

Umbilical cord blood (UCB) provides an alternative to mismatched hematopoietic grafts.[2] Cord blood (CB) procurement is fast and there are reports indicating a reduced incidence of severe GVHD.[3–5] Interestingly, lymphokine-activated killer (LAK) cells from CB have been shown to have higher apoptotic-mediated cytotoxicity *in vitro*, when compared to bone marrow-derived LAK cells. This provides one of the potential explanations for preserved graft-versus-leukemia effect with this type of graft,[6,7] which contains a small number of lymphocytes.

The major limitation of the use of UCB transplantation for adults is the relatively small number of hematopoietic stem cells. This problem is associated with high rates of early treatment-related mortality (TRM) owing to delayed engraftment. In order to overcome this barrier, we investigated a strategy by which patients without higher priority donors were to be transplanted with an unrelated CB unit that should contain at least 2×10^7 total nucleated cells (TNCs)/kg of recipient weight. If one unit containing that cell number was not available, a second unit had to be added in order to achieve that target. Here, we report the results of a phase II trial that enrolled heavily pretreated patients with high-risk hematologic malignancies that was prematurely interrupted owing to high rates of engraftment failure and excessive mortality.

Methods

Eligibility criteria

The upper age limit for participation was 55 years. Patients should have high-risk hematologic malignancies. Up to

three human leukocyte antigen (HLA) mismatches between CB units and recipients were allowed (low resolution typing at HLA-A and -B loci, and high-resolution typing at HLA-DRB1). If one unit providing at least 2×10^7 TNC/kg was not available, pooled units (up to three CB units) were to be used in order to achieve that target. This cell dose was arbitrarily chosen in order to maximize the speed of engraftment. Patients with adequate organ function, capable of providing written informed consent were eligible. Patients were not eligible if they had an HLA-compatible related or unrelated donor, defined as an unrelated donor that matched on HLA-A, -B and DRB1, or a related donor that either matched on HLA-A, -B and -DRB1 or had one mismatch in these loci. The Institutional Review Board of the MD Anderson Cancer Center approved the study protocol, and all patients gave informed consent upon enrollment.

Pretransplantation conditioning

Patients eligible for a myeloablative preparative regimen were treated with a total of 3 Gy of total body irradiation (TBI) daily on days −9 to −6. Fludarabine ($30 \, \text{mg/m}^2$) was given on days −5 to −2 daily, and transplant was performed on day 0. Patients with multiple myeloma or those not eligible for the TBI-based regimen owing to co-morbid conditions were given fludarabine at the same dose, and melphalan $180 \, \text{mg/m}^2$. Filgrastim ($5 \, \mu\text{g/kg/day}$) was given from day +7 until the absolute neutrophil count (ANC) reached $1000/\mu\text{l}$ for at least 3 days. GVHD prophylaxis consisted of tacrolimus (targeting levels of 5–15 ng/ml) and methotrexate ($5 \, \text{mg/m}^2$) on transplant days 1, 3 and 6. All patients received rabbit antithymocyte globulin (ATG) 3 mg/kg, administered on transplant days −5, −4, −3 and −2. Supportive care including prophylactic antibiotics and transfusion of blood products followed institutional standards. Cytomegalovirus antigenemia was monitored twice weekly, and pre-emptive treatment with foscarnet started in case of any positive result. Whenever possible, autologous stem cells were collected and stored as a back up in case of graft failure.

Engraftment, GVHD and chimerism

Engraftment was defined as the first of three consecutive days with an ANC greater than $0.5. \times 10^9/\text{l}$. Platelet engraftment was defined as the first of seven consecutive days when the platelet count exceeded $20 \times 10^9/\text{l}$ without transfusion support. Failure to engraft neutrophils by day 42 was considered as primary graft failure. GVHD was scored as per consensus criteria.[8] Chimerism was monitored using fluorescent in situ hybridization studies in sex-mismatched cases for Y chromosome, and by DNA microsatellite polymorphisms by polymerase chain reaction with D6S264, D3S1282, D18S62 and D3S1300 fluorescence-labeled primers.

Statistical considerations

This was a phase II trial that used a Bayesian sequential monitoring design for single-arm clinical trials with multiple outcomes, as proposed by Thall et al.[9] Patients were scored as 'success' if they were alive, engrafted and

without GVHD on day +100. The trial was prematurely stopped in January 2003 owing to excessive number of early failures.

Results

Patients and preparative regimens

Fifteen patients with a median age of 34 years (range, 19–56 years) and a median weight of 71 kg (range, 49–99 kg) received a median TNC of 3.2×10^7 (range, 1.9×10^7– 5.2×10^7) from one or two cords. Three donor–recipient pairs had only one HLA mismatch, whereas all others had more than one HLA mismatch. Patient characteristics are shown in Table 1. Only six patients (40%) were in complete remission at the time of transplantation.

We used the melphalan-based conditioning regimen to treat four patients, with multiple myeloma ($n = 2$), non-Hodgkin's lymphoma ($n = 1$) and acute lymphocytic leukemia ($n = 1$). Eleven patients received the TBI-based preparative regimen.

Engraftment

Ten patients engrafted neutrophils at a median of 22 days (range, 15–38 days). Autologous peripheral blood stem cells were used to rescue a patient in sepsis and delayed pancytopenia (UPIN 6). Another patient had recovery of autologous hemopoiesis. Three additional patients had neither engraftment nor autologous hematopoietic reconstitution. Platelet engraftment was documented in 10 cases at a median of 37 days (range, 23–60 days). Six patients achieved a platelet count greater than $50\,000/\text{mm}^3$, and three patients reached a count greater than $100\,000/\text{mm}^3$ without transfusions. Red cell transfusion independence was achieved in seven cases.

Table 1 Patient and CB characteristics

Number of patients	15
Gender	8 males/7 females
Median age (years)	34 (range, 19–56)
Median weight (kg)	71 (range, 49–99)
Single versus double cords	12 versus 3
Number of infused total nucleated cells/kg ($\times 10^7$) – pre cryopreservation	3.2 (range, 1.9–5.2)
Number of CD34+ cells/kg ($\times 10^5$; pre cryopreservation)	1.0 (range, 0.4–3.7)
HLA-match (5/6 versus 4/6 versus 3/6)	4 versus 12 versus 3
Positive CMV serology before transplant	$N = 13$
ABO mismatch (minor/major/no mismatch)	5/8/2
Diagnosis	*Number of patients*
AML	2
ALL	6
NHL	2
CML	2
MM	2
NK cell leukemia	1

Abbreviations: ALL = acute lymphoblastic leukemia; AML = myeloid leukemia; CB = cord blood; CML = chronic myelogenous leukemia; CMV = cytomegalovirus; HLA = human leukocyte antigen; MM = multiple myeloma; NHL = non-Hodgkin's lymphoma; NK = natural killer.
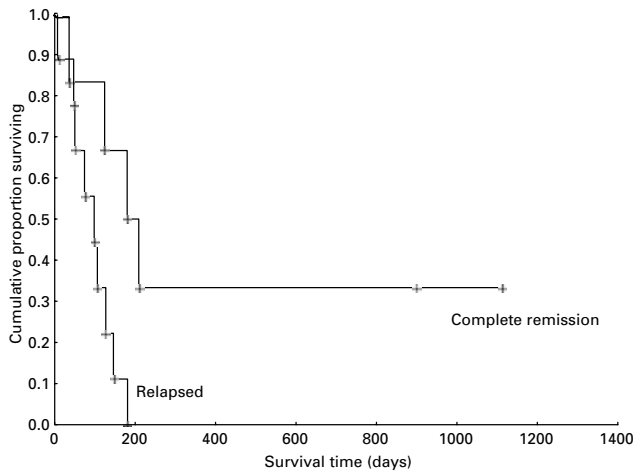
Figure 1 Survival and disease status at transplantation. Overall survival after unrelated donor CB transplantation as a function of disease status ($P = 0.04$, log rank test).

Graft-versus-host disease

Acute skin GVHD was diagnosed in three patients (incidence of 33%; grade I in one case and grade II in two cases). There were no instances of grade III or IV acute GVHD, and no cases of hepatic or gastrointestinal GVHD. One of the two patients with acute grade II GVHD went on to develop extensive chronic skin GVHD that required systemic treatment with steroids, daclizumab and PUVA. All cases of acute GVHD were responsive to steroids, but one patient died of chronic GVHD-related complications.

Survival and non-relapse mortality

Two patients transplanted in remission are alive and disease free. Median survival was 124 days. Actuarial 1- and 4-year survival rates were 13 and 13%, respectively. Kaplan–Meier estimate of survival is shown in Figure 1. The 100-day and 1-year TRM rates for the whole group were 40 and 53%, respectively. TRM rates at 100 days and 1 year after treatment were 17 and 50% for patients transplanted in remission, as opposed to 44 and 55% for those treated with active disease. Two patients died of diffuse alveolar hemorrhage and two patients developed adenovirus infections (fatal in one case). Eleven patients developed a severe fungal or viral infection, whereas GVHD-related complications were the cause of demise in one case. Four patients surviving more than 30 days did not engraft. Diagnoses were chronic myelogenous leukemia (CML) ($n = 2$), multiple myeloma ($n = 1$) and diffuse large-cell lymphoma ($n = 1$). Furthermore, one patient died early, 13 days after transplantation, without signs of engraftment. Outcomes are summarized in Table 2.

Discussion

Long-term follow-up of our small cohort of heavily pretreated patients showed a 4-year survival rate of approximately 33% for patients in remission. The early mortality and engraftment failure rates were deemed excessive and led to premature trial discontinuation.

There are several potential explanations for these results. The most important variable was disease status at transplantation, associated with extensive prior treatment and poor tolerance to transplant-associated toxicities, factors that likely increased TRM. Here, selection of relatively large CB units with a median total infused nucleated cell count/kg of 3.2×10^7 did not impact early mortality, as observed in other series.[10,11] Use of CB units containing TNC doses below $1–1.5 \times 10^7$ is associated with high rates of engraftment failure, and accordingly, we attempted combination of units in three cases in order to optimize the cell dose. Despite our approach, we observed a high rate of primary engraftment failure. It is possible that a diagnosis of CML was an added risk factor in two of our cases as it has been suggested.[11] In the multicenter Cord Blood Transplantation (COBLT) study, 34% of the adult patients failed to achieve donor hematopoiesis.[12] Comparisons of published results are sometimes difficult to perform given that definitions of engraftment failure differ in the literature, but it is commonly accepted that lack of neutrophil recovery by day 42 indicates a very low likelihood of success. The competing risk of death before engraftment is frequently not taken into consideration, an approach that makes the interpretation of the data very difficult and subject to biases.

Our 100-day mortality rate of 40% was similar to that observed in the COBLT study, which reported a 100-day survival probability of 0.47, with approximately 17% of the patients alive after 12 months.[12] Similarly, Sanz *et al.*[13] reported a 100-day TRM of 43% in a cohort of 22 adults (median age of 29 years) with hematologic malignancies. All patients with acute leukemia were in complete remission, whereas most of the CML patients were in chronic phase, comprising a better risk group. Lower 100-day mortality has been reported by several single-center studies.[14,15] The Minnesota group transplanted 23 patients with a median age of 24 years and observed a 6-month TRM of 22%.[14] Although these results may indicate the influence of growing experience using this source of stem cells, one cannot underestimate the effect of patient selection on outcomes.

At the time our trial was initiated, we allowed the use of units with three HLA mismatches. Such degree of donor–recipient mismatch is now a well-known risk factor for poor survival.[11] Three patients were so treated (combined with another unit in one instance). Therefore, the degree of matching contributed to the poor results. Another potential criticism is our GVHD prophylaxis that employed 'mini' methotrexate. There is evidence that this drug may contribute to excess mortality, at least when used in higher doses than the ones employed here. Interestingly, the combination of tacrolimus and mini-methotrexate has been used without excessive toxicity in the pediatric setting.[16]

Fludarabine and melphalan was the preparative regimen in four cases. One patient engrafted, whereas three patients did not. In light of these results, we cannot recommend the use of this regimen in this setting. Other groups have however reported improved results using a reduced-intensity approach to reduce TRM.[17,18] High-dose ATG was part of our conditioning regimens, but its use to improve engraftment and possibly decrease the incidence of

Table 2 Patient outcome

UPIN	Diagnosis and disease status at transplantation	Age (years)	Number of cord units	Donor–recipient HLA match (mismatch location)	TNC ($\times 10^7$)	Preparative regimen	Best donor chimerism	Outcome	Cause of death	Other complications	Survival (in days)
1	NK leukemia (previous autograft) 3rd remission	21	1	5 of 6 (class I mismatch)	1.9	flu/tbi/atg	100%	Death	Systemic adenovirus infection	CMV No engraftment of platelets Torulopsis glabrata pneumonia	124
2	ALL (3rd relapse)	30	1	5 of 6 (class I mismatch)	3.6	flu/tbi/atg	99%	Death	Relapsed leukemia	Pneumonia HSV	181
3	CML (refractory myeloid blast crisis)	32	2	1st: 4 of 6 (one class I and one class II mismatch) 2nd: 4/6 (two class I mismatches)	2.4	flu/tbi/atg	100% single CB (subsequently developed secondary graft failure)	Death	Pneumonia	(aspergillus + enterococcus)	ARDS
	Multiorgan failure Graft failure CMV Possible DAH	47									
4	ALL (3rd relapse)	27	2	1st: 4/6 (one class I and one class II) 2nd: 3/6 (two class I and one class II)	3.9	flu/tbi/atg	43/57% Mixed double cord chimera[23]	Death	Recurrent ALL	Hemorrhagic cystitis CMV Renal insufficiency	146
5	ALL (2nd remission)	19	1	4/6 (one class I, one class II)	4.1	flu/mel/atg	100%	Death	Relapsed ALL	Hematuria Ascending paralysis Coma Respiratory failure Urinary tract sepsis	180
6	Myeloma (two previous autologous transplants; refractory relapse)	47	2	1st: 4/6 (class I) 2nd: 4/6 (class I)	3.2	flu/mel/atg	0%	Death	Relapsed myeloma	Graft failure (rescued with autologous hemopoiesis) Adenovirus cystitis	127
7	CLL/Richter transformation	29	1	4/6 (class I)	5.2	flu/tbi/atg	100%	Death	DAH	CMV	106
8	AML (refractory 2nd relapse)	34	1	3/6 (class I)	2.4	flu/tbi/atg	100%	Death	DAH	ARDS CMV gastritis Acute renal failure	74
9	AML (preceding RAEB-1) CR1	32	1	5/6 (class I)	3.8	flu/tbi/atg	96%	Alive		Renal failure	899 +
10	CML (refractory myeloid blast crisis)	44	1	3/6 (two class I, one class II)	2.7	flu/tbi/atg	0%	Death	Disease relapse (CML blast crisis)	Graft failure and restoration of autologous hemopoiesis Fungal pneumonia Fungal pneumonia	98
11	SLL/Richter transformation (responsive 2nd relapse)	53	1	4/6 (one class I, one class II)	2.5	flu/mel/atg	0%	Death	Disseminated Candida kruzei	Failure to engraft	50

Table 2 Continued

UPIN	Diagnosis and disease status at transplantation	Age (years)	Number of cord units	Donor–recipient HLA match (mismatch location)	TNC ($\times 10^7$)	Preparative regimen	Best donor chimerism	Outcome	Cause of death	Other complications	Survival (in days)
12	Myeloma (previous autologous transplant; refractory relapse)	56	1	4/6 (two class I)	2.5	flu/mel/atg	0%	Death	Pneumonia (xanthomonas and yeast)	Progressive encephalopathy. He had not engrafted	13
13	ALL (Ph +) (1st remission)	51	1	4/6 (two class I)	3.2	flu/tbi/atg	100%	Alive		Resolved chronic GVHD	1111 +
14	ALL (Ph +) (2nd remission)	52	1	4/6 (two class I)	3.6	flu/tbi/atg	100%	Death	Chronic GVHD	Multiple infections	209
15	ALL (Ph +) (1st remission)	37	1	5/6 (one class I)	4.2	flu/tbi/atg	0%	Death	Failure to engraft	Pulmonary infections Polyoma cystitis	35

Abbreviations: ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; ARDS, acute respiratory distress syndrome; ATG = antithymocyte globulin; CLL = chronic lymphocytic leukemia; CML = chronic myelogenous leukemia; CMV = cytomegalovirus antigenemia; CR = complete response; DAH = diffuse alveolar hemorrhage; Flu = fludarabine; GVHD = graft-versus-host disease; HLA = human leukocyte antigen; HSV = herpes simplex virus; Mel = melphalan; NK = natural killer; Ph = Philadelphia chromosome; RAEB = refractory anemia with excess blast; SLL = small lymphocytic leukemia; TBI = total body irradiation; TNC = total nucleated cells.

GVHD is controversial. This drug has been reported to delay immune recovery, and we are currently using significantly reduced doses.[19]

Only three of our nine engrafted patients developed acute GVHD without visceral involvement, whereas one patient died of chronic GVHD. Again, the competing risk of early mortality owing to infections and toxicity makes this low rate difficult to interpret. In the COBLT study, GVHD was the leading cause of death.[12]

How can these results be improved? Cell dose is critical. It is unclear, however, if doubling the cell dose by combining two units, for example, will be enough to improve outcomes. At this point, there are no controlled studies comparing double versus single CB transplants. Another approach that we are currently investigating involves *ex vivo* expansion of CB progenitor cells, in an attempt to circumvent cell dose-related problems.[20–22]

CB carries the potential to extend hematopoietic transplantation to a variety of patients otherwise not eligible for this form of treatment. However, our results would indicate that high-risk patients have an increased probability of TRM even with the use of units containing higher TNC counts, and that treatment of relapsed patients should only be pursued under controlled clinical trials.

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