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Umbilical Cord Blood Transplantation in Adults: Results of the Prospective Cord Blood Transplantation (COBLT)

Kenneth Cornetta,[1] Mary Laughlin,[2] Shelly Carter,[3] Donna Wall,[4] Joel Weinthal,[5] Colleen Delaney,[6] John Wagner,[7] Robert Sweetman,[8] Philip McCarthy,[9] Nelson Chao[10]

[1]Indiana University, Indianapolis, Indiana; [2]Case Western Reserve, Cleveland, Ohio; [3]The EMMES Corporation, Rockville, Maryland; [4]Texas Transplant Institute, San Antonio, Texas; [5]North Texas Hospital for Children, Dallas, Texas; [6]Fred Hutchinson Cancer Research Center, Seattle, Washington; [7]University of Minnesota, Minneapolis, Minnesota; [8]City of Hope, Duarte, California; [9]Roswell Park Cancer Institute, Buffalo, New York; [10]Duke University, Durham, North Carolina

Correspondence and reprint requests: Kenneth Cornetta, MD, Department of Medical and Molecular Genetics, Indiana University School of Medicine, 975 W. Walnut St., Indianapolis, IN 46202 (e-mail: kcornett@iupui.edu).

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ABSTRACT

The Cord Blood Transplantation study group conducted a prospective study of unrelated cord blood transplantation (CBT) to better define the role of this stem cell source for subjects requiring unrelated allogeneic transplantation. We report on 1 stratum of the study designated for adult subjects. The primary end point of the study was survival at 180 days. Secondary end points included engraftment, graft-versus-host disease, relapse, and long-term survival. Eligibility criteria for malignant and nonmalignant diseases were specified. Subjects with active central nervous system disease, Karnofsky performance status <70%, grade 3 or 4 or primary myelofibrosis, or suitable related donors were excluded. Enrollment required a single cord blood unit containing $>10^7$ nucleated cells per kilogram of recipient weight and matched at ≥4 HLA-A and -B (low or intermediate resolution) and -DRB1 (high resolution) types. Thirty-four subjects were entered, with a median age of 34.5 years (range, 18.2-55 years). Most subjects (n = 23) had a 4 of 6 match, 10 subjects had a 5 of 6 match, and 1 subject had a 6 of 6 match. Diagnoses at transplantation included acute myelogenous leukemia (n = 19), acute lymphoblastic leukemia (n = 9), chronic myelogenous leukemia (n = 3), myelodysplastic syndrome (n = 1), paroxysmal nocturnal hemoglobinuria (PNH) (n = 1), and non-Hodgkin lymphoma (n = 1); 94% were classified as poor risk according to National Marrow Donor Program criteria. Subjects received total body irradiation/cyclophosphamide (n = 27) or busulfan/melphalan (n = 7) conditioning regimens. Four subjects died before CBT and are described here but are not included in the main analysis. The cumulative incidence rates and median times to neutrophil (500/μL) and platelet (>20 000/μL) engraftment were 0.66 by day 42 (median, 31 days) and 0.35 by day 180 (median, 117 days). The cumulative incidence rate for grade II-IV GVHD was 0.34 by day 100. For the primary end point, survival at 180 days, Kaplan-Meier survival estimates were 0.30 (95% confidence interval, 0.14-0.46) by day 180 after transplantation. To date there are 2 survivors, and both are >36 months from enrollment. A retrospective analysis was performed by using high-resolution HLA-A and -B typing, which revealed that approximately one third of subjects had 1 or more additional HLA mismatches compared with results of low- or intermediate-resolution HLA typing. The findings of high treatment-related mortality and slow engraftment kinetics indicate that CBT should continue to be performed in specialized centers with a research focus on cord blood cells.

KEY WORDS

Cord blood • Hematopoietic stem cell transplantation • COBLT

INTRODUCTION

Since the first umbilical cord blood transplantation (CBT) in 1988, increasing clinical experience has documented cord blood as a viable source for allogeneic transplantation [1]. CBTs have the potential advantage of rapid availability, a lower risk of viral contamination, and a lower risk of graft-versus-host

disease (GVHD), thus permitting less stringent HLA matching and thereby resulting in an increase in the pool of potential donors [2-4]. Disadvantages of CBT include slower engraftment, inability to obtain additional cells, and the possibility of a decrease in the graft-versus-leukemia effect [5-7]. Because the feasibility of unrelated CBT is now well established [6,8-13], the challenge is to define its role in relationship to other sources of allogeneic stem cells. Because most studies to date are single-institution studies with a variety of preparative regimens and eligibility criteria, the Cord Blood Transplantation (COBLT) study group initiated a prospective study of CBT in subjects requiring unrelated allogeneic transplantation. Because most enrollees were anticipated to be pediatric subjects, the study was designed to assess individuals older than 18 years as a specific subgroup. This distinction was due to a variety of factors suggesting that the outcomes in adults may differ from those in children [10]. For example, the larger size of adults amplifies the low cell dose associated with most cord blood units (CBUs). Lower cell dose has been reported to increase the risk of graft failure and to contribute to early mortality in all ages, although adults seem to be at increased risk. Adults are known to be at increased risk of GVHD in allogeneic bone marrow and peripheral blood transplantation, although the risk of GVHD after CBT has not been precisely defined. Also, the indications for transplantation in adults are distinctly different from those in children. Therefore, treatment of older subjects as a unique subset was prospectively designed into the COBLT study.

METHODS

The COBLT study group is a multi-institutional trial of CBT sponsored by the National Heart, Lung, and Blood Institute/National Institutes of Health. The transplantation protocol was approved at the institutional review board of each of the 9 participating institutions. All subjects or their legal guardians were required to give written informed consent before enrollment. Enrollment in the adult stratum began in 1999 and was stopped in 2002 after the accrual target of 34 subjects was met.

Eligibility, Treatment, and Assessments

Eligible subjects were those with a defined series of malignant and nonmalignant diseases suitable for allogeneic transplantation. Of note, subjects with malignant diseases refractory to therapy, including refractory acute leukemias and chronic myeloid leukemia (CML) blast crisis, were eligible for enrollment. Subjects with active central nervous system disease, human immunodeficiency virus seropositivity, Karnofsky performance status $<70\%$, age >55 years, primary myelofibrosis, or suitable related (5/6 or 6/6 matched) donors and subjects who were pregnant or breastfeeding were ineligible. Subjects with prior allogeneic stem cell transplantation within 12 months or autologous transplantation within 6 months were excluded, as were individuals with uncontrolled bacterial, viral, or fungal infections. Multiorgan assessment, performed before enrollment, was required to demonstrate the following: serum creatinine normal for age or a creatinine clearance $>50\%$ of the lower limits of normal for age; aspartate aminotransferase <5 times the upper limits of normal and total serum bilirubin <2.5 mg/dL; cardiac status asymptomatic or left ventricular ejection fraction $>40\%$ that improved with exercise; and asymptomatic pulmonary function, or forced expiratory volume in 1 second and diffusion capacity $>45\%$ of predicted (corrected for hemoglobin). The selected CBU was required to provide a minimum of 1×10^7 total nucleated cells (TNC; before cryopreservation) per kilogram of recipient weight.

Only subjects receiving 1 CBU were eligible. For initial enrollment, the CBU's and subject's HLA match were determined by low- or intermediate-resolution molecular typing for class I HLA-A and HLA-B alleles and by high-resolution molecular typing for HLA-DRB1 alleles (subsequently referred to as "original HLA typing"). Eligibility criteria required the availability of a cryopreserved CBU with a minimum 4 of 6 HLA match. Initially CBUs had to be obtained from the COBLT cord blood banks [14]. This criterion was subsequently expanded to allow units obtained from the New York Blood Center, National Marrow Donor Program (NMDP)–approved cord blood banks or US banks meeting Netcord/Foundation for the Accreditation of Cellular Therapy standards. If more than 1 suitable unit was identified, the selection of the CBU used for transplantation was at the discretion of the transplant center.

Two conditioning regimens were defined for subjects with malignant disease. Subjects with refractory acute leukemia, third or more medullary relapse, or CML in blast crisis and subjects unable to tolerate total body irradiation (TBI) because of prior dose-limiting irradiation or cardiac toxicity received busulfan/melphalan. Busulfan was given on day -8 through day -5 either orally (1 mg/kg) or by intravenous infusion (0.8 mg/kg) every 6 hours for 16 doses. Busulfan/doses were adjusted to a target concentration at steady state of 600 to 900 ng/mL. Melphalan (45 mg/m^2 for 3 doses) was given on day -4 through day -2. Antithymocyte globulin (30 mg/kg/d) was given on day -3 through day -1. All other subjects, including those with primary induction failure, received 9 fractions (150 cGy) of TBI given up to twice daily

(BID) on days −8 through −4. Cyclophosphamide 60 mg/kg was administered on days −3 and −2. Both induction regimens included methylprednisolone 1 mg/kg before each dose of antithymocyte globulin (equine) given at 15 mg/kg BID on days −3 through −1. On day 0, subjects received 2 infusions of methylprednisolone 1 mg/kg; 1 dose was given just before infusion of the CBU. GVHD prophylaxis included methylprednisolone 0.5 mg/kg BID days +1 thru +4. The dose was 1 mg/kg BID beginning on day +5 and was continued until day +19 or until the first day the absolute neutrophil count (ANC) reached 500/μL, at which time a steroid taper of 0.2 mg/kg/wk was begun. Cyclosporine was begun on day −3 and continued to at least day 50; it was then tapered at 5% of the initial dose per week if subjects had no evidence of GVHD. Transplantations were performed in a high-efficiency particulate air–filtered or laminar airflow room. Infection prophylaxis was administered according to institutional practice, but timing was defined: *Pneumocystis carinii* prophylaxis was continued for 1 year or until 3 months past discontinuation of immunosuppression, and herpes simplex prophylaxis was continued until day 30 for seropositive individuals. Fungal prophylaxis and immunoglobulin administration was performed according to institutional practice. Surveillance for cytomegalovirus (CMV) was performed according to institutional policy, but subjects who were CMV antibody negative before CBT were given CMV-seronegative or leukocyte-depleted blood products. Although this was not mandatory, CMV antibody–positive subjects were permitted to begin CMV prophylaxis when the ANC recovered to >750/μL for 2 consecutive days, and this was continued until day +100.

Clinically significant infections were reported after transplantation by site of infection, organism, and severity of infection. Readmissions after the initial discharge for the transplantation were reported by date and primary and secondary reasons for discharge. The staging of acute GVHD followed NMDP guidelines and included weekly capture of symptoms through day 100 and at days 120 and 150, with characterization of alternative causes. The grading of acute GVHD followed the GVHD consensus grading scheme. An algorithm calculated the maximum GVHD clinical grade according to the weekly organ staging in skin, upper and lower gastrointestinal tract, and liver. This calculated organ stage was decreased by 1 stage if a listed specific differential diagnosis was reported for either gastrointestinal tract or liver. An independent panel reviewed weekly records and assigned each subject a final maximum grade; this is similar to the methods described in Weisdorf et al. [15].

Study End Points and Statistical Analysis

The primary end point of the study was survival at 180 days after transplantation. The secondary end points included engraftment (neutrophil and platelet), acute and chronic GVHD, disease-free survival, long-term survival, relapse, and regimen-related toxicities.

Neutrophil engraftment was defined as achieving an ANC of at least 500/μL for 3 consecutive measurements on different days and demonstrated donor chimerism >90%. Primary graft failure was defined as failure to attain neutrophil engraftment by day 42. Subjects who underwent transplantation, survived to day 14, and died before neutrophil engraftment were classified as having primary graft failures. Secondary graft failure was defined as a loss of neutrophil engraftment. Platelet engraftment to >20 000/μL or ≥50 000/μL was defined as the first day of a minimum of 3 consecutive measurements on different days that the subject achieved the target platelet count and was also platelet transfusion independent for a minimum of 7 days. The time to neutrophil or platelet engraftment was defined as the time from transplantation to the first day of engraftment.

Primary causes of death were reported according to the hierarchy developed for the Unrelated Donor Marrow Transplantation Trial (http://spitfire.emmes.com/study/tcd/). The hierarchy was developed by an expert panel on the basis of refinements to the NMDP hierarchy for reporting causes of death. The hierarchy requires that graft failure, relapse, or GVHD (if occurring) be reported as the primary cause of death rather than causes such as infection, organ failure, or hemorrhage and also provides rules for reporting secondary causes of death.

Survival estimates were calculated by using the Kaplan-Meier method [16,17]. Testing for differences in survival between groups in the univariate analysis used the log-rank test. Hazard estimates were computed using both the cumulative incidence and the complement of the Kaplan-Meier methods. The complement of the Kaplan-Meier method is defined as 1-Kaplan-Meier estimate. Cumulative incidence curves were used for engraftment and GVHD end points, with death as a competing risk [18,19]. No imputation was used for any missing data. Frequency data in cross-tabulation tables were compared by using the Kruskal-Wallis test. All analyses were performed with SAS software, version 8.2 (SAS Institute Inc., Cary, NC). Baseline characteristics are described for all 34 subjects. The primary analysis of graft failure was conducted on subjects who survived at least 14 days. Twenty-nine subjects were included in engraftment analysis after excluding 2 subjects who died before beginning conditioning, 2 subjects who died during conditioning before CBT, and 1 subject who died early (day 6) after CBT. All additional analyses are restricted to the 30 subjects who received a transplant.

Retrospective HLA Typing

High-resolution molecular typing for HLA-A and -B became available after the study was initiated. To determine the effect of this typing information on subject outcome, high-resolution typing for HLA-A, HLA-B, and HLA-DRB1 alleles was performed for 28 donor/recipient pairs (subsequently referred to as "retrospective HLA typing"). The results of statistical analysis with this information were compared with analyses in which HLA-A and -B typing was performed by using the original HLA typing results.

Compassionate Use Arm

An expanded access protocol was developed as part of the COBLT project. The expanded access protocol was used as a compassionate use protocol for subjects not eligible for the main study or for subjects who met criteria for a stratum that had been closed to accrual. The only eligibility criteria were consenting to an institutional review board–approved alternative CBT protocol and receipt of a COBLT CBU. The transplantation induction regimen was not specified, and subjects could receive multiple CBUs or units treated in ex vivo expansion protocols.

RESULTS

Subject Characteristics

In the COBLT study, the adult stratum was designed for enrollment of subjects aged 18 to 55 years. Enrollment was closed after the accrual goal of 34 subjects was met. There were 9 participating centers. One center enrolled almost half of the subjects (47% at Duke University Medical Center; 10 of these have been reported elsewhere) [20]. Baseline characteristics of the study subjects are listed in Table 1. Participants had a median age of 34.5 years (range, 18.2-55.0 years), were primarily white (76%), were balanced in sex, and were all diagnosed with malignant disease. With use of low- or intermediate-resolution typing for HLA-A and -B and high-resolution typing for HLA-DRB1, most subjects (68%) matched at 4 of 6 types. The median precryopreservation TNC count was 23.3 $\times$ 10^6/kg (range, 13.8-54.8 $\times$ 10^6/kg).

TBI with cyclophosphamide was used in most subjects (79%). Busulfan/melphalan was used in the remaining 21% of subjects. The maximum Bearman toxicity grade across all organ systems reported by day 42 was analyzed, and no significant difference was seen between conditioning regimens. Most subjects (32 of 34) were poor risks for transplantation (with NMDP criteria). Twenty-seven of the 30 subjects who received cord blood cell infusion underwent transplantation for leukemia, of which 2 subjects with CML were in accelerated phase, 5 had acute leukemia in relapse, and 6 had primary refractory acute leukemia. The poor-risk population was also reflected in the death of 4 subjects before transplantation and in 1

Table 1. *Baseline Characteristics*

Variable	Data
No. subjects	34
Age (yr)	
Median	34.5
Range	18.2-55.0
Weight (kg)	
Median	72.9
Range	53.6-112.0
Precryopreservation $CD34^+$ cell dose (10^5 cells/kg)	
n	27
Median	6.6 or .66
Range	0.18-3.18
Precryopreservation total nucleated cell dose (10^6 cells/kg)	
Median	23.3
Range	13.8-54.8
Recipient ethnicity, n (%)	
White	26 (76)
Black	4 (12)
Hispanic	1 (3)
Asian	1 (3)
Mixed	1 (3)
Unknown	1 (3)
Donor ethnicity, n (%)	
White	14 (41)
Hispanic	3 (9)
Asian	1 (3)
Mixed	3 (9)
Unknown	13 (38)
Sex, n (%)	
Male	17 (50)
Female	17 (50)
Karnofsky performance status, n (%)	
100%	10 (29)
90%	10 (29)
80%	10 (29)
70%	4 (12)
Original HLA match, n (%)*	
6 of 6	1 (3)
5 of 6	10 (29)
4 of 6	23 (68)
Retrospective HLA match, n (%)†	
5 of 6	6 (18)
4 of 6	17 (50)
3 of 6	5 (15)
Untyped	6 (18)
Primary disease, n (%)	
Acute Myelogenous Leukemia	19 (56)
Acute lymphoblastic leukemia	9 (26)
Chronic myelogenous leukemia	3 (9)
Nonlymphoblastic non-Hodgkin lymphomas	1 (3)
Myelodysplastic syndrome	2 (6)
Risk status, n (%)	
Good risk	2 (6)
Poor risk	32 (94)
Transplant center, n (%)	
Duke	16 (47)
Other	18 (53)

*A and B at low/intermediate and DRB1 at high resolution.
†A, B, and DRB1 at high resolution.

subject's death 6 days after transplantation. For the 30 subjects infused with cord blood, there have been 2 unexpected events reported as of the data cutoff. One was reported as moderate tachycardia, and the other was documented as fatal cardiogenic shock. For this analysis, all 30 subjects who received cord blood cell infusion were used in survival analysis, and 29 subjects who were alive on day 14 were used in the engraftment analysis.

Neutrophil Engraftment

Of the 29 evaluable subjects, 19 were considered to have neutrophil engraftment and did so in an estimated median time of 31 days (range, 13-55 days; 95% confidence interval [CI], 27-48 days), and 17 obtained engraftment at or before day 42. Figure 1A shows the cumulative incidence and 1-Kaplan-Meier estimate of neutrophil engraftment. The cumulative incidence was 0.66 (95% CI, 0.48-0.79) by day 42 and was 0.76 (95% CI, 0.55-0.86) by day 100. The 1-Kaplan-Meier estimate was 0.74 (95% CI, 0.56-0.92) by day 42 and was 0.87 (95% CI, 0.72-1.00) by day 100. Baseline characteristics such as age, weight, sex, race, disease, performance status, HLA match, conditioning regimen, CMV serostatus, cell dose, and transplant center were not associated with differential rates of neutrophil engraftment.

Primary graft failures were defined prospectively as those that failed to attain neutrophil engraftment by day 42. Overall, 10 (34%) of 29 subjects were considered to have primary graft failures. Five of the 10 died between days 14 and 42: 3 of primary graft failure and 2 of disease relapse. Of the remaining 5 subjects, 2 engrafted after day 42, and 3 had ANC recovery, but chimerism tests were not performed, and the subjects were classified as having primary graft failure according to protocol.

Platelet Engraftment

The median time to platelet engraftment (20 000/μL) was 117 days. As shown in Figure 1B, the cumulative incidence of engraftment by day 100 was 0.17 (95% CI, 0.07-0.31) and was 0.35 (95% CI, 0.17-0.52) by day 180. Competing risk was an important factor, as reflected by the complement of Kaplan-Meier of 20 000/μL engraftment of 0.33 (95% CI, 0.09-0.56) at day 100 and 0.79 (95% CI, 0.54-1.00) at day 180. Analysis of 50 000/μL platelet engraftment revealed a cumulative incidence of 0.07 (95% CI, 0.00-0.17) by day 100 and 0.21 (95% CI, 0.07-0.35) by day 180. The corresponding complement of Kaplan-Meier was 0.13 (95% CI, 0.00-0.31) at day 100 and 0.46 (95% CI, 0.18-0.73) at day 180. Baseline characteristics such as age, weight, sex, race, disease, performance status, conditioning regimen, CMV serology, cell dose, and transplant center did not suggest a significant increase in platelet engraftment.

Graft-versus-Host Disease

Table 2 shows frequencies and percentages by reviewer grade among the 29 subjects with any acute GVHD assessments. As shown in Figure 1C, by day 100 after transplantation, the cumulative incidence of grade II to IV acute GVHD was 0.34 (95% CI, 0.21-0.52), and the complement of Kaplan-Meier was 0.48 (95% CI, 0.25-0.71). By day 100, chronic GVHD developed in 2 subjects: 1 with a limited maximum grade (localized skin involvement, hepatic dysfunction, or both) and 1 with an extensive maximum grade. Four other subjects developed chronic GVHD after day 100 (2 limited and 2 extensive). At 1 year after transplantation, the cumulative incidence of chronic GVHD was 0.21 (95% CI, 0.07-0.38), and the complement of Kaplan-Meier was 0.61 (95% CI, 0.24-0.97), as shown in Figure 1D.

Subject Survival

The primary end point of this study was survival at day 180, and the survival curves for the 30 subjects who were infused with cord blood are displayed by original HLA match (Figure 2A). For these 30 subjects, the survival probability was 0.47 (95% CI, 0.29-0.65), 0.30 (95% CI, 0.14-0.46), and 0.17 (95% CI, 0.03-0.30) at day 100, day 180, and 1 year, respectively. The stated accrual goal of 34 adult subjects was met, and enrollment was suspended after analysis of day +180 survival suggested inadequate survival. When subjects were analyzed on the basis of original HLA match, there was not a significant difference in survival. Table 3 shows the number of deaths, survival probability, and 95% CI at day 180 and 1 year by different characteristics.

The summary for primary and secondary causes of death is shown in Table 4. Thirty-two of the 34 subjects died. Of the 4 subjects who died before transplantation, 2 died of relapse before the start of conditioning, and 2 died after initiation of conditioning therapy (one of cyclophosphamide-induced cardiac toxicity and the other of bacterial infection). Among the 30 subjects who underwent transplantation, GVHD was the leading cause of death (acute, n = 7; chronic, n = 3). Disease recurrence was another significant cause of death and occurred in 7 of the 30 subjects who underwent transplantation. Figure 1D shows the cumulative incidence and complement of Kaplan-Meier of relapse. At 1 year after transplantation, the cumulative incidence of relapse was 0.17 (95% CI, 0.13-0.23). When the influence of other early mortality factors is considered, the relapse rate is predicted to be significantly higher, as reflected in the complement of Kaplan-Meier of 0.46 (95% CI, 0.15-

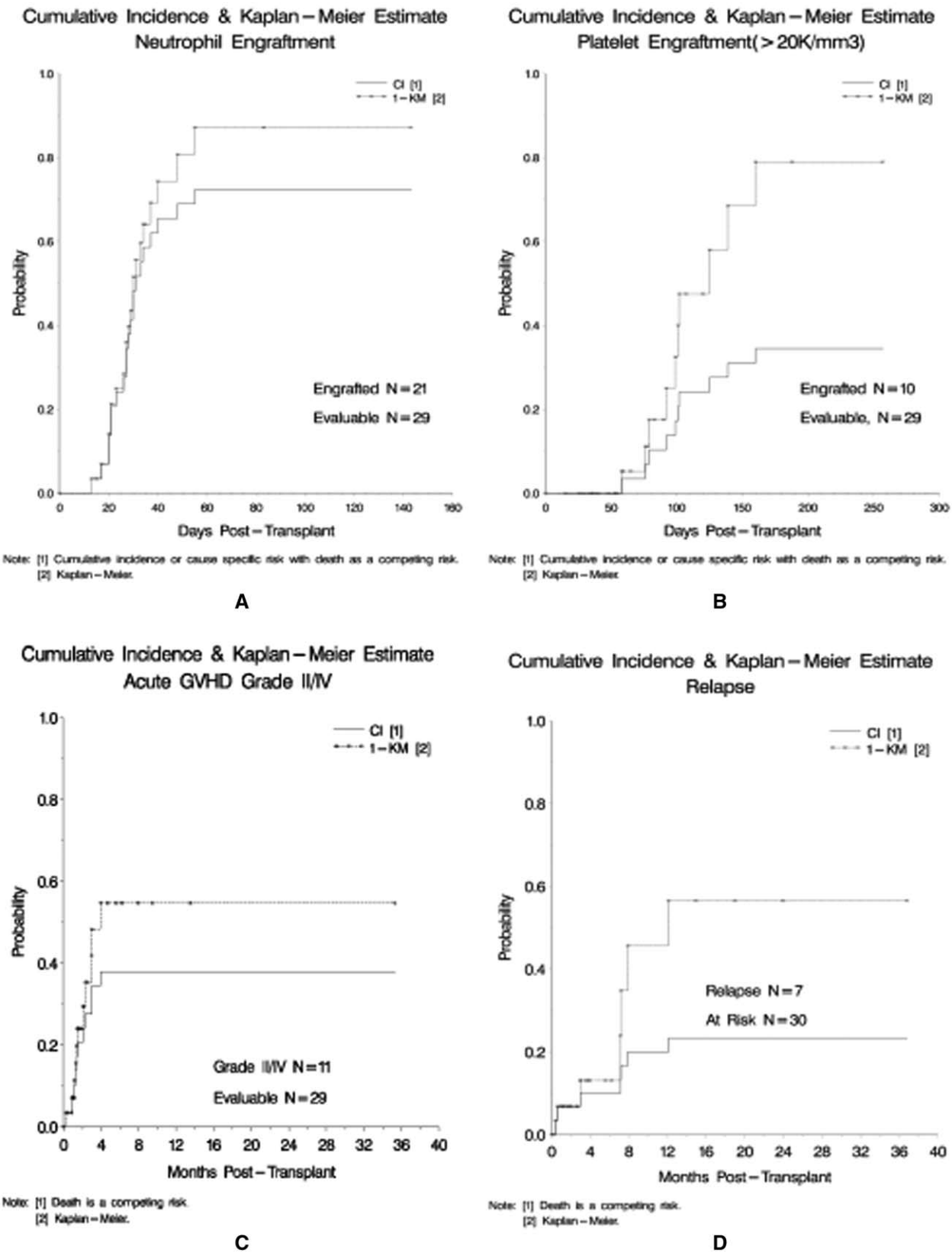

Figure 1. Cumulative incidence and Kaplan-Meier estimates for neutrophil engraftment, platelet engraftment, graft-versus-host disease, and relapse. A, For neutrophil engraftment by day 42 after transplantation, the cumulative incidence was 0.66 (95% CI, 0.48-0.79), and the complement of Kaplan-Meier was 0.74 (95% CI, 0.56-0.92). By day 100, the cumulative incidence was 0.76 (95% CI, 0.55-0.86), and the complement of Kaplan-Meier was 0.87 (95% CI, 0.72-1.00). The median time to engraftment was 31 days (95% CI, 27-48). B, By day 100, the cumulative incidence of platelet engraftment was 0.17 (95% CI, 0.07-0.31), and the complement of Kaplan-Meier was 0.33 (95% CI, 0.09-0.56).

Table 2. *Acute GVHD Reviewer Grade*

	Grade, n (%)					
Grading Method	**0**	**I**	**II**	**III**	**IV**	**Total***
Reviewer grade	**13 (45)**	**5 (17)**	**6 (21)**	**5 (17)**	**0 (0)**	**29**

*Five patients did not submit GVHD forms (the 4 who died before transplantation and the 1 who died on day 6).

0.76). This estimate is consistent with the high-risk population of subjects enrolled on this study. Other reported primary causes of death included graft failure (n = 3), infection (n = 7), and graft rejection (n = 1).

As part of this protocol, detailed data were collected and audited to provide insight into the infectious complications associated with CBT. As shown in Table 5, most subjects (90%) had infections, and two thirds reported 3 or more infections during the first 6 months after CBT. The vast majority of infections were rated severe, life-threatening, or fatal. Fatal infections were noted in 15 (50%) of the 30 subjects who underwent CBT and were classified as the primary cause of death in 8 subjects according to the Unrelated Donor Marrow Transplantation Trial hierarchy for cause of death. Although bacterial infections were the most common (n = 39), fungal infections were nearly as common (n = 28), and fungal infections were the most commonly listed organism contributing to fatal infections.

Retrospective Analysis of HLA Matching

With the subsequent availability of high-resolution typing for HLA-A and -B types, a retrospective analysis of HLA typing was performed on 28 subjects, 24 of whom underwent transplantation. As shown in Table 1, whereas 11 subjects were classified as ≥5 of 6 matches by low- or intermediate-resolution HLA-A and -B typing (original HLA match), only 6 subjects remained classified as ≥5 of 6 after high-resolution typing (retrospective HLA match). Of the 19 subjects originally typed as 4 of 6 who underwent retrospective typing, 5 were reclassified as 3 of 6. Of the 9 subjects originally typed as 5 of 6 who underwent retrospective typing, 3 were reclassified as 4 of 6. Therefore, 8 (29%) of 28 subjects were reclassified on the basis of high-resolution typing.

HLA matching by retrospective typing was compared with survival probability (Figure 2B). Although only 4 subjects were matched for 5 of 6 HLA types by using high-resolution typing, their probably of survival at day +180 was 0.75 (95% CI, 0.33-1.00), compared with subjects who were less matched and had a day +180 survival probability of 0.45 (95% CI, 0.23-0.67). Retrospective HLA matching did not significantly affect survival, neutrophil or platelet engraftment, or acute GVHD; however, this may be an artifact of the small number of subjects.

Compassionate Use Stratum

Subjects who did not meet the eligibility criteria of the study or who could not be included in this adult stratum because of its closure were permitted to enroll in a compassionate stratum. Subjects enrolled in the compassionate use stratum were not required to receive the preparative regimen defined in the COBLT study, and multiple cord units were permitted. Follow-up for primary and secondary end points was performed, but at fewer time points than for subjects in the COBLT trial. There are 37 adult subjects on the compassionate use stratum. Among the 37 subjects, 1 died before CBT, and 4 subjects were still waiting for transplantation at the time of this analysis. For the remaining 32 subjects, the survival probabilities were 0.54 (95% CI, 0.36-0.72), 0.37 (95% CI, 0.19-0.56), and 0.24 (95% CI, 0.07-0.41) at day 100, day 180, and 1 year, respectively. There was no significant survival difference (P = .30) for the adult subjects enrolled on the study compared with the adult subjects on the compassionate use stratum.

DISCUSSION

The COBLT study group is the first multicenter prospective study of adult CBT. The COBLT study was designed to study the toxicity and engraftment of CBT in pediatric and adult subjects. As such, the primary end point was defined as survival at day 180. After the accrual goal of 34 subjects was reached, subsequent individuals seeking CBT were offered a compassionate use protocol.

One advantage of CBT is the time required to identify suitable donors: a median time as short as 2 weeks has been reported [2,6]. This contrasts with unrelated donor transplantation from international

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By day 180, the cumulative incidence was 0.35 (95% CI, 0.17-0.52), and the 1-Kaplan-Meier estimate was 0.79 (95% CI, 0.54-1.00). C, By day 100, the cumulative incidence of acute GVHD (grades II-IV) was 0.34 (95% CI, 0.21-0.52), and the 1-Kaplan-Meier estimate was 0.48 (95% CI, 0.25-0.71). D, By >1 year, the cumulative incidence of relapse was 0.17 (95% CI, 0.13-0.23), and the >1-Kaplan-Meier estimate was 0.46 (95% CI, 0.15-0.46). 1-KM indicates Kaplan-Meier.

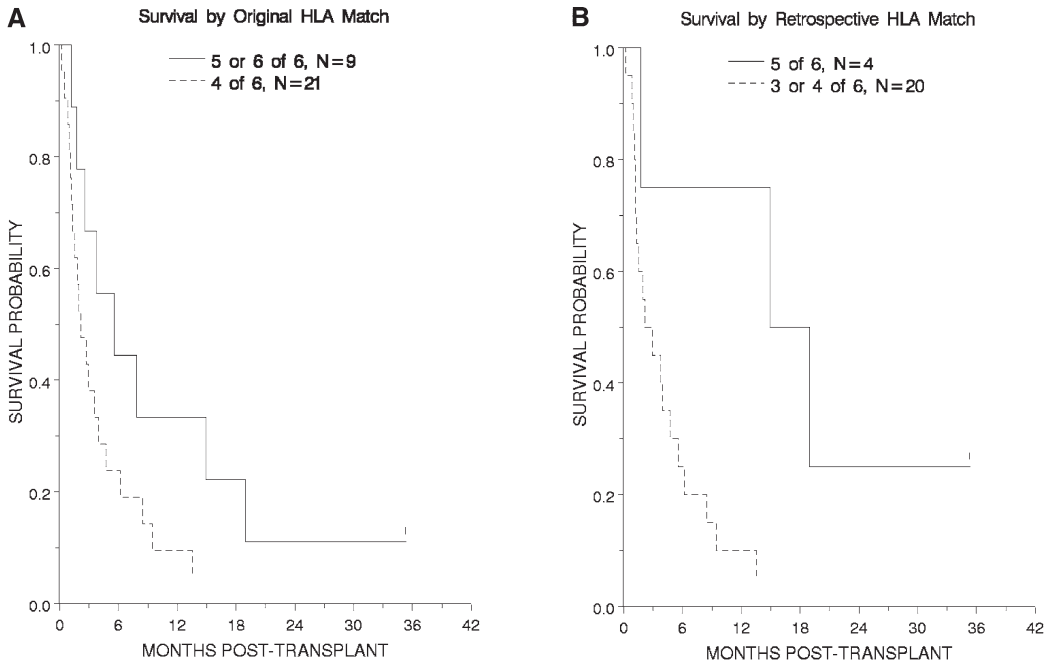

Figure 2. Survival curves with original and retrospective HLA matching. A, Survival curve by original HLA match (P = .13). By day 100, the survival probability for a 5 or 6 of 6 original HLA match is 0.67 (95% CI, 0.36-0.97), and for a 4 of 6 match it is 0.38 (95% CI, 0.17-0.59). By day 180, the survival probability for a 5 or 6 of 6 original HLA match is 0.44 (95% CI, 0.12-0.77), and for a 4 of 6 match it is 0.24 (95% CI, 0.06-0.42). B, Survival curve by retrospective HLA match (P = .09). By day 100, the survival probability for a 5 of 6 retrospective HLA match is 0.75 (95% CI, 0.33-1.00); for a 4 of 6 match, it is 0.44 (95% CI, 0.19-0.68); and for a 2 or 3 of 6 match, it is 0.29 (95% CI, 0-0.62). By day 180, the survival probability for a 5 of 6 original HLA match is 0.75 (95% CI, 0.33-1.00); for a 4 of 6 match, it is 0.25 (95% CI, 0.04-0.46); and for a 2 or 3 of 6 match, it is 0.14 (95% CI, 0-0.40).

registries that generally involve a lag time of approximately 3 to 4 months [2,6,21]. A shorter interval may allow CBT to be performed in subjects with a lower disease burden or fewer prior chemotherapy regimens. The shortened time also allows CBT to be offered to individuals who might not survive the delay associated with unrelated donor identification and acquisition. The inclusion of such subjects has the potential of biasing the outcome statistics by including subjects with an extremely poor prognosis. The latter seems to be a significant concern for this study. The study was open to subjects with nonmalignant conditions and good- and poor-risk malignancies, yet the vast majority (94%) enrolled were poor risk by NMDP criteria. Half (13 of 26) of the subjects with leukemia who underwent CBT had active disease at the time of transplantation. This unanticipated predominance of poor-risk subjects may reflect physician/subject concern regarding enrollment of good-risk subjects onto investigational protocols, as well as an underestimation of the pool of poor-risk leukemia subjects. Further understanding of these factors will be important in the design of future multi-institutional CBT protocols. The high number of subjects in relapse also indicates that the population accrued is likely to differ from many previously published trials of allogeneic transplantation with unrelated marrow and peripheral blood stem cell donors.

Engraftment after CBT remains a major concern for developing CBT for adult subjects. This relates to the relatively small number of cells in a CBU compared with the number of cells obtained by bone marrow harvest or apheresis. This is partially offset by the higher percentage of primitive progenitor cells contained within cord blood (up to 10-fold) that allow for long-term engraftment [22-24]. In contrast, investigators have presented evidence that this enriched primitive population may contain relatively few committed progenitors [25-27], a finding that is consistent with the slow rate of engraftment after CBT. The association between engraftment and cell dose has been studied extensively [6,9-11,13,28], and doses $>1.5 \times 10^7$ TNCs per kilogram have recently been recommended [28,29]. Our study required a minimum of 1×10^7 TNCs per kilogram of recipient weight for enrollment, and the cell dose delivered was similar to prior reports of CBT in the adult population [13,30]. The time to myeloid engraftment observed in our study ranged from 13 to 55 days, a range similar to that reported in most studies of CBT [5,6,8,11-13,30]. The estimated median time to neutrophil engraftment of 31 days is similar to that in a number of studies [5,6,11,13], although other studies have reported a median time to engraftment as short as 22 to 24 days [8,12,30-32]. One difficulty in comparing the median time to engraftment is a lack of consistency in defining

Table 3. *Survival Estimates*

Characteristic	n	Deaths at Day 180	Survival (95% CI) at Day 180	Deaths at 1 Y	Survival (95% CI) at 1 Y	Deaths Overall	Median Survival, Days (95% CI)
Age (y)							
≤34.5	16	12	0.25 (0.04-0.46)	13	0.19 (0.00-0.38)	14	99 (65-143)
>34.5	14	9	0.36 (0.11-0.61)	12	0.14 (0.00-0.33)	14	54 (37-257)
Weight (kg)							
≤70	16	11	0.31 (0.09-0.54)	13	0.19 (0.00-0.38)	15	78 (35-257)
>70	14	10	0.29 (0.05-0.52)	12	0.14 (0.00-0.33)	13	99 (48-188)
Sex							
Male	15	12	0.20 (0.00-0.40)	14	0.07 (0.00-0.19)	15	78 (40-120)
Female	15	9	0.40 (0.15-0.65)	11	0.27 (0.04-0.49)	13	107 (59-288)
Recipient ethnicity							
White	23	15	0.35 (0.15-0.54)	18	0.22 (0.05-0.39)	21	90 (53-188)
Other	7	6	0.14 (0.00-0.40)	7	0.00 (0.00-0.00)	7	83 (15-114)
Donor ethnicity							
White	12	7	0.42 (0.14-0.70)	20	0.17 (0.00-0.38)	11	132 (59-257)
Other	18	14	0.22 (0.03-0.41)	5	0.17 (0.00-0.34)	17	81 (40-114)
Primary disease							
Acute leukemia	24	17	0.29 (0.11-0.47)	13	0.17 (0.02-0.32)	22	72 (48-120)
Other	6	4	0.33 (0.00-0.71)	12	0.17 (0.00-0.46)	6	156 (114-188)
Karnofsky performance status							
70% or 80%	14	10	0.29 (0.05-0.52)	19	0.07 (0.00-0.21)	14	57 (40-188)
90% or 100%	16	11	0.31 (0.09-0.54)	6	0.25 (0.04-0.46)	14	111 (65-240)
Original HLA match							
4 of 6	21	16	0.24 (0.06-0.42)	17	0.10 (0.00-0.23)	20	65 (40-120)
5/6 of 6	9	5	0.44 (0.12-0.77)	1	0.33 (0.03-0.64)	8	169 (78-454)
Retrospective HLA match							
3/4 of 6	20	15	0.25 (0.06-0.44)	18	0.10 (0.00-0.23)	19	78 (37-169)
5 of 6	4	1	0.75 (0.33-1.00)	1	0.75 (0.33-1.00)	3	516 (53-.)
Conditioning regimen							
Bu/Mel	7	5	0.29 (0.00-0.62)	17	0.14 (0.00-0.40)	7	54 (40-257)
TBI/Cy	23	16	0.30 (0.12-0.49)	8	0.17 (0.02-0.33)	21	107 (65-169)
Cytomegalovirus serostatus							
Positive	18	14	0.22 (0.03-0.41)	8	0.06 (0.00-0.16)	17	72 (48-114)
Negative	12	7	0.42 (0.14-0.70)	17	0.33 (0.07-0.60)	11	132 (53-411)
Total nucleated cell dose (×106/kg)							
0-20	10	8	0.20 (0.00-0.45)	14	0.20 (0.00-0.45)	10	96 (53-143)
>20	20	13	0.35 (0.14-0.56)	5	0.15 (0.00-0.31)	18	87 (37-240)
$CD34^+$ cell dose (×105/kg)							
0-1	17	13	0.24 (0.03-0.44)	10	0.18 (0.00-0.36)	16	65 (48-143)
>1	7	2	0.71 (0.38-1.00)	15	0.29 (0.00-0.62)	6	257 (169-454)
Transplant center							
Duke	13	8	0.38 (0.12-0.65)	10	0.23 (0.00-0.46)	12	90 (35-288)
Other	17	13	0.24 (0.03-0.44)	15	0.12 (0.00-0.27)	16	83 (54-169)

Bu indicates busulfan; CI, confidence interval; Cy, cyclophosphamide; Mel, melphalan; TBI, total body irradiation.

this parameter among published trials. In particular, there is significant variation in the censoring of subjects with early mortality and the use of direct versus Kaplan-Meier estimates of engraftment times. Nevertheless, our findings further document the slow engraftment kinetics associated with CBT. It is interesting to note that improved engraftment kinetics were not detected with increasing cell doses. The days to neutrophil and platelet engraftment were similar when the 10 subjects receiving 1 to 2×10^7 TNCs per kilogram were compared with the 19 subjects receiving $\geq 2 \times 10^7$ TNCs per kilogram. This is in contrast to an analysis of 562 subjects by Rubinstein et al. [10]. and an adult CBT study by Laughlin et al. [13], which both reported a correlation of improved engraftment with increasing cell dose. The small sample size and relatively small range of cell doses used in our study are the most likely reason for our failure to detect such a correlation.

Although increasing the cell dose has been reported to improve engraftment kinetics, it is unclear whether higher doses will increase the number of subjects who will successfully engraft. Whereas doses $<1.5 \times 10^7$ TNCs per kilogram are associated with graft failures, Rubinstein et al. [10]. did not detect a decrease in graft failures above a dose of 2.5×10^7 TNCs per kilogram, thus suggesting that there may be a threshold dose for successful engraftment. This study and previous CBT studies [9,10,13,28] indicate that despite the slow engraftment times, approximately 85% to 90% of subjects undergoing CBT will eventually engraft. In the engraftment analysis (complement of Kaplan-Meier), the observed incidence of neutrophil engraftment was 0.87 at day 100, and the

Table 4. *Primary Causes of Death*

Primary Cause of Death*	n	%
Recurrence/relapse	7	25
Acute GVHD†	7	25
Chronic GVHD‡	3	11
Infection		
Fungal§	3	11
Polymicrobial‖	1	4
Polyorganism¶	2	7
Idiopathic (pneumonia)	1	4
Graft failure	3	11
Graft rejection	1	4
Total	28	100

*Summarizes only patients who underwent transplantation.

†Secondary causes of death: recurrence/relapse, infection-fungal (torulopsis), infection-fungal (yeast), infection-polyorganism (aspergillus and adenovirus), infection-polyorganism (aspergillus and enterovirus), infection-polyorganism (sepsis emboli and varicella), and no reported secondary cause of death.

‡Secondary causes of death: infection-bacterial (pneumonia; sinus), infection-fungal (aspergillus), and infection-polyorganism (aspergillus and adenovirus).

§Aspergillus (n = 3).

‖*Escherichia coli* and streptococcus.

¶Aspergillus and vancomycin-resistant enterococcus; pseudomonas and aspergillus.

incidence of platelet engraftment (20 000/μL) at day 180 was 0.79. These data are consistent with cord blood being an enriched source of primitive progenitor cells, and most CBUs containing $>1 \times 10^7$ TNCs per kilogram of recipient weight have sufficient stem cells to provide long-term engraftment. The finding that only 1 case of secondary graft failure was observed is also supportive of this hypothesis and consistent with other studies of adult CBT [13,30]. Therefore, our primary graft failure incidence of 34% (10 of 29 individuals who underwent transplantation) should be viewed with caution. Not all prior studies include donor chimerism—a stringent criterion established prospectively in our study—in their definition of engraftment. It is interesting to note that of the 4 subjects who survived longer than 42 days and were classified as having primary graft failure, 2 went on to engraft with documented donor chimerism. The remaining 2 had neutrophil recovery at day 42, but because donor chimerism was not documented, they were classified as having primary graft failure, as stipulated in the protocol. Donor engraftment beyond 42 days after CBT has been noted in a variety of other clinical trials, with rates similar to that our study (approximately 10%) [5,6,11-13,30]. This suggests that the arbitrary selection of 42 days as a indicator of primary graft failure will underestimate the true engraftment rate after CBT.

Acute and chronic GVHD were noted and, when combined, were the most common cause of death after CBT. Only 5 subjects developed grade III or IV acute GVHD, although an additional 4 subjects died of acute GVHD (3 with grade II and 1 in whom acute GVHD developed after day 150). Although GVHD was a significant cause of death, the incidence was markedly lower than that seen in transplantation of marrow or peripheral blood stem cells with similar degrees of HLA disparity. We observed an incidence of GVHD similar to rates reported in other adult CBT studies [13,30]. The retrospective use of high-resolution HLA-A and -B typing in a multicenter trial is a novel component of our study and resulted in the reclassification of approximately one third of subjects. All reclassifications were at lower matching than the original typing. It is interesting to note that HLA match was not identified as a risk factor for acute or chronic GVHD, whether the methods used low-, intermediate-, or high-resolution typing. Our findings suggest that larger studies of high-resolution typing should be pursued to confirm molecular testing as a means of improving CBT outcome.

At day 100, approximately half of the subjects had died (survival probability, 0.47; 95% CI, 0.29-0.65), and only approximately 30% and 17% were alive after 3 and 12 months, respectively. Similar to our results, treatment-related mortality (day 100) has been consistently high in CBT studies, ranging from 25% to 40% in small single-institution studies of pediatric patients to as high as 65% in 1 study reporting the experience from multiple centers [5,6,8,9,11-13,30]. Two recent studies by Ooi et al. [33,34]. report very encouraging results in adults undergoing CBT for myelodysplastic syndrome and acute myeloid leukemia. There was low treatment-related mortality, and

Table 5. *Infection Summary* for the First 6 Months in Subjects Receiving Cord Blood Transplants*

Variable	Data
No. evaluable subjects	30
No. of subjects with infection	27 (90%)
Total infections reported	96
No. infection reports	
1	7
2	3
3	5
4	3
5	4
>5	5
Maximum severity for subjects	
None	3 (10%)
Mild	1 (3%)
Moderate	3 (10%)
Severe	7 (23%)
Life threatening	1 (3%)
Fatal	15 (50%)
Infection types (infections/subjects)	
Bacterial	39/20
Fungal	28/18
Viral	23/13
Other	6/4

*The median time to first infection was 10 days (range, 0-118 days).

the probability of 2-year survival in each study was 76.2% and 76.6%, respectively. Nevertheless, these small, single-institution studies (13 and 18 subjects, respectively) differ from our study in a number of potentially important ways. Their subjects had significantly less prior chemotherapy and received a higher median cell dose; this was related to the comparatively low weight of their patients (median, 51-55 kg). Whether the preparative regimen, which used irradiation and cytosine arabinoside, is also an important factor deserves further study. Retrospective analyses suggest that cell dose and HLA matching are important factors when selecting CBUs [10,28,35], and both of these factors would affect our population, given the large number of HLA mismatches and the weight of our subjects. The cell dose issue would be particularly relevant to our study, given that the Eurocord population had a weight range of 17 to 55 kg, whereas our subjects weighed 53.6 to 112 kg [35]. Although the importance of disease status, HLA match, and prior therapy will all be important determinants in the future application of CBT, understanding the role of cell dose is key, because it is one factor that can easily be manipulated. Ongoing studies that use multiple CBUs [36-38] or ex vivo expansion of cord blood cells [39-41] may help to define the importance of cell dose for most CBT recipients.

In summary, this first prospective, multicenter study of adult CBT supports the previous observations that CBT is associated with slow engraftment kinetics and significant mortality attributable to primary graft failure. Infection rates are high and are the primary or secondary cause of death in half of CBT recipients. GVHD is the leading primary cause of death but occurred in a setting in which 23 of the 34 subjects were matched at only 4 of 6 HLA loci (with low- or intermediate-resolution typing for HLA-A and -B and high-resolution typing for HLA-DRB1). Therefore, the previous observation that CBT is associated with a relatively low rate of GVHD and graft rejection despite major HLA mismatches between recipient and cord blood donor was confirmed in this prospective study. CBT was associated with poor survival at 180 days, and the poor-risk status of most subjects was likely the most important contributing factor to early deaths after CBT, although cell dose and HLA matching may prove to be significant predictors of survival. On the basis of these findings, we believe that CBT remains a procedure that should be conducted in the setting of an approved clinical trial.

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