Unrelated cord blood transplants in adults with hematologic malignancies

William Arcese
Vanderson Rocha
Myriam Labopin
Guillermo Sanz
Anna Paola Iori
Marcos de Lima
Anne Sirvent
Alessandro Busca
Shigheta Asano
Irina Ionescu
Peter Wernet
Eliane Gluckman
*on behalf of Eurocord-Netcord Transplant group**

Background and Objectives. We analyzed outcomes and risk factors after unrelated cord blood transplantation (CBT) in adults with hematologic malignancies.

Design and Methods. One hundred and seventy-one patients were transplanted after 1997. Their median age was 29 years (15-55), and the median follow-up time was 18 months (1-71). Most patients had acute or chronic leukemia (n=142, 83%), 91 (53%) were transplanted in advanced phase and an autologous transplant had failed in 32 (19%). Most patients (87%) received an HLA-mismatched cord blood unit with 1–2 HLA disparities. At infusion, the median number of nucleated cells and CD34$^+$ cells was 2.1×10^7/kg and 1×10^5/kg, respectively

Results. The cumulative incidence of neutrophil recovery at day 60 was 72±3% with a median of 28 days (11-57). A higher neutrophil count and use of hematopoietic growth factors were independently associated with faster neutrophil recovery. The cumulative incidence of grade II-IV acute graft-versus-host disease was 32±4% and this complication was not associated with the number of HLA mismatches. The 2-year cumulative incidence of chronic graft-versus-host disease, transplant related-mortality and relapse were 36±10%, 51±4% and 22±4%, respectively. At 2-years, disease-free-survival for patients transplanted in early, intermediate and advanced phases of disease was 41±9%, 34±10% and 18±4%, respectively. In multivariate analyses, advanced disease status was an adverse factor for relapse and disease-free survival.

Interpretation and Conclusions. Unrelated CBT is a clear alternative for adults with hematological malignancies lacking an HLA-matched related or unrelated donor. The choice of units containing a higher neutrophil count and a policy of earlier transplantation are likely to provide better results.

Key words: unrelated cord blood transplants, adults, hematologic malignancies.

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All authors from the Eurocord-Netcord.
**Other members of Eurocord-Netcord registry are listed in the Appendix.*

Correspondence:
Eliane Gluckman, MD, FRCP, Hospital Saint Louis, Hematology Bone Marrow Transplant Department, 1, Avenue Claude Vellefaux, 75475 Paris cedex 10, France. E-mail: eliane.gluckman@sls.ap-hop-paris.fr

Umbilical cord blood from unrelated donors represents a clear alternative source of hematopoietic progenitor cells to bone marrow for children lacking an HLA identical sibling.[1-9] The lower risk of graft-versus-host disease (GVHD) in the cord blood transplant setting than in bone marrow transplants permits less stringent criteria for donor-recipient HLA matching.[10] Moreover, cord blood units are acquired faster than bone marrow from unrelated donors,[11] which is particularly relevant for patients who require an urgent transplant.[12,13] However, despite the high proliferative potential of cord blood hematopoietic progenitors,[14,15] the low number of nucleated and CD34$^+$ cells in a single cord blood unit has represented the major limitation to the use of unrelated cord blood transplantation (CBT) in adults, due to concern about the risk of graft failure and delayed engraftment. However, pilot studies, including small series of adults transplanted from unrelated, mostly HLA mismatched, cord blood donors, reported encouraging results in terms of engraftment, incidence of GVHD, risk of relapse and event-free survival.[16-19] As CBT has been increasingly used for adults, two independent studies have recently compared the results of unrelated CBT and bone marrow transplantation in adult patients with acute leukemia.[20-21] Despite some differences between the two analyses, in both studies the main outcomes (relapse, transplant-related mortality, leukemia-free survival) were similar in the patients receiving the two different types of transplants.

Herein, we report a retrospective analysis of results and risk factors for different outcomes in 171 unrelated cord blood transplants performed between January 1998 and January 2003 in adults with hematologic malignancies and reported by 63 centers to the Eurocord, a European co-operative clinical-trial group for CBT of the European Blood and Marrow Transplantation (EBMT). Out of 171 patients, 45 with acute myeloblastic leukemia and 53 with acute lymphoblastic leukemia were included in a previously published study produced by Eurocord.[21]

Design and Methods

Data collection and selection of patients

Eurocord is an international registry operating on behalf of the EBMT. Participation is open to both European and non-European centers conducting CBT. Eurocord works in

close collaboration with Netcord banks. This retrospective analysis is based on data reported to the Eurocord Registry from European and non-European centers through a standardized questionnaire concerning the characteristics of patients, donors, diseases and grafts as well as transplant outcomes, reviewed by two physicians and checked for computerized errors to ensure data quality. The study included patients undergoing unrelated CBT between January 1998 and January 2003 who met the following criteria: (i) age ≥15 years old, (ii) diagnosis of a hematologic malignancy, (iii) conditioning with a myeloablative regimen, (iv) transplant with a single, non *ex vivo* expanded cord blood unit and (v) not having received a previous allogeneic transplant. All patients gave informed consent for CBT according to the Declaration of Helsinki. The present study was approved by the Eurocord institutional review board.

Patient, donor and transplant characteristics

The main patient, disease, donor and transplant characteristics are reported in Table 1. A total of 171 patients reported from 63 centers in 13 countries (see Appendix) met the selection criteria. At the time of CBT, 32 patients (19%) (19 with acute leukemia [AL]; 3 with chronic myeloid leukemia [CML]; 7 with non-Hodgkin's lymphoma [NHL]; and 3 with myelodysplastic syndrome [MDS]) had previously undergone an autologous transplant. Ninety-one patients (53%) were transplanted in an advanced phase of the disease (defined as AL in third or subsequent complete remission or refractory AL [n=43], MDS [n=16], CML in blast crisis [n=6], or lymphoma in partial remission or in resistant relapse [n=9]). The median time from diagnosis to CBT was 13.4 months (range, 2.6-247), and median time from attainment of last complete remission to CBT in patients with AL transplanted in remission (n=75) was 129 days (range, 9-701). The median follow-up for survivors after transplant was 18.1 months (range, 1.5-70.7).

Donor-recipient compatibility for HLA-A and -B antigens was defined by serology, whereas the HLA-DRB1 match was defined at the antigen level by low-resolution DNA techniques and at the allelic level by high resolution DNA techniques. HLA-DRB1 high resolution data were missing for only four (2.3%) donor-recipient pairs. The degree of matching was classified by the number (0, 1, 2 or 3) of HLA antigens or allelic disparities (Table 1). The HLA incompatibility involved antigens of class I in 65 pairs, class II antigens in 29 and both class I and class II antigens in 55 donor-recipient pairs. Most cord blood units (n=157, 92%) came from Netcord banks which followed the FACT-Netcord standards for collecting, freezing and storing cord blood units;[10] the procedures for thawing and washing cryopreserved cord blood usually followed the method described by Rubinstein *et al.*[22]

The median number of nucleated cells counted at the time of cord blood collection or freezing and infused are reported in Table 1. After thawing, there was a median loss of 24% (range: 4-32) of the nucleated cells CD34$^+$ cell quantification at the time of infusion, calculated through non standardized methods among centers and reported for only 124 cord blood units, was 1.0×10^5/kg (range: 0.02-15). Conditioning regimens differed according to centers, type of disease and disease status. An irradiation-based regimen with total body irradiation or total lymphoid irradiation was administered to 110 (64%) patients, while 61 patients were conditioned with combined chemotherapy only. In 129 (75%) cases the preparative regimen included an anti-lymphocyte or anti-thymocyte globulin or a monoclonal anti-T-cell antibody. Most patients (n=117, 68%)

Table 1. Patient-, disease- donor and transplant-related characteristics.

Characteristics (n=patients available)	*Number of patients (%) or median (min-max)*
Age, years (range) (n=171)	29 (15-55)
Male gender (n=171)	84 (49%)
Weight, kg (range) (n=169)	62 (38-110)
Diagnosis (n=171)	
acute myeloid leukemia	46 (27%)
acute lymphoblastic leukemia	53 (31%)
secondary acute leukemia	11 (6%)
chronic leukemia	32 (19%)
lymphomas	13 (8%)
myelodysplasia	16 (9%)
Disease status at transplant (n=171)°	
early	35 (21%)
intermediate	45 (26%)
advanced	91 (53%)
Previous autologous transplant (n=169)	32 (19%)
Graft after 2000	101(59%)
Positive CMV serology (n=161)	106(66%)
ABO compatibility (n=164)	64(39%)/36(22%)/64(39%)
Identical/minor/major disparities	
HLA compatibility (n=169)	
6 out of 6	9 (5%)
5 out of 6	77 (46%)
4 out of 6	68 (41%)
3 out of 6	13 (8%)
Number of cells at freezing	
nucleated cells, 10^7/kg (n=169)	2.7 (1.1-9.5)
Number of infused cells	
Nucleated cells, 10^7/kg (n=159)	2.1 (0.8-7.3)
CD34, 10^5/kg (n=124)	1.0 (0.2-15.0)
Conditioning (n=171)	
Irradiation based	110 (64%)
TBI+CY±VP16	60
TBI+CY+ARA-C	14
others (2 TLI)	36
Busulfan based	61 (36%)
BU+CY±Thiotepa	51
others	10
Anti-T in the conditioning (ATG/ALG/MoAb)	129 (78%)
GvHD prophylaxis (n=171)	
CsA alone	11 (6%)
CsA + Prednisone	117 (68%)
CsA+MTX±Prednisone	18 (11%)
FK+MTX	13 (8%)
others	12 (7%)
Early hematopoietic growth factors (n=132)$^+$	105 (80%)

**for continuous variables, number of patients available are reported. + early means between day 0 to day +7 after UCBT; °IBMTR classification: early: first complete remission (acute leukemia or lymphoma) or first chronic phase of chronic myeloid leukemia; intermediate: second or subsequent complete remission (acute leukemia or lymphoma) or accelerated phase of chronic myeloid leukemia; advanced: refractory disease or relapse or partial response (acute leukemia or lymphoma) or blastic crisis of chronic myeloid leukemia or other malignancies. CR: complete remission, CP: chronic phase, CMV: cytomegalovirus, CsA: cyclosporine A, ALG: anti lymphocyte globulin, ATG: anti-thymocyte globulin, MoAb: monoclonal antibody, MTX: methotrexate, FK: FK506, TBI: total body irradiation, TLI: total lymphoid irradiation, BU: busulfan, CY: cyclophosphamide, ARA-C: Aracytine, VP16: Vepeside, GvHD: graft-versus-host disease.*

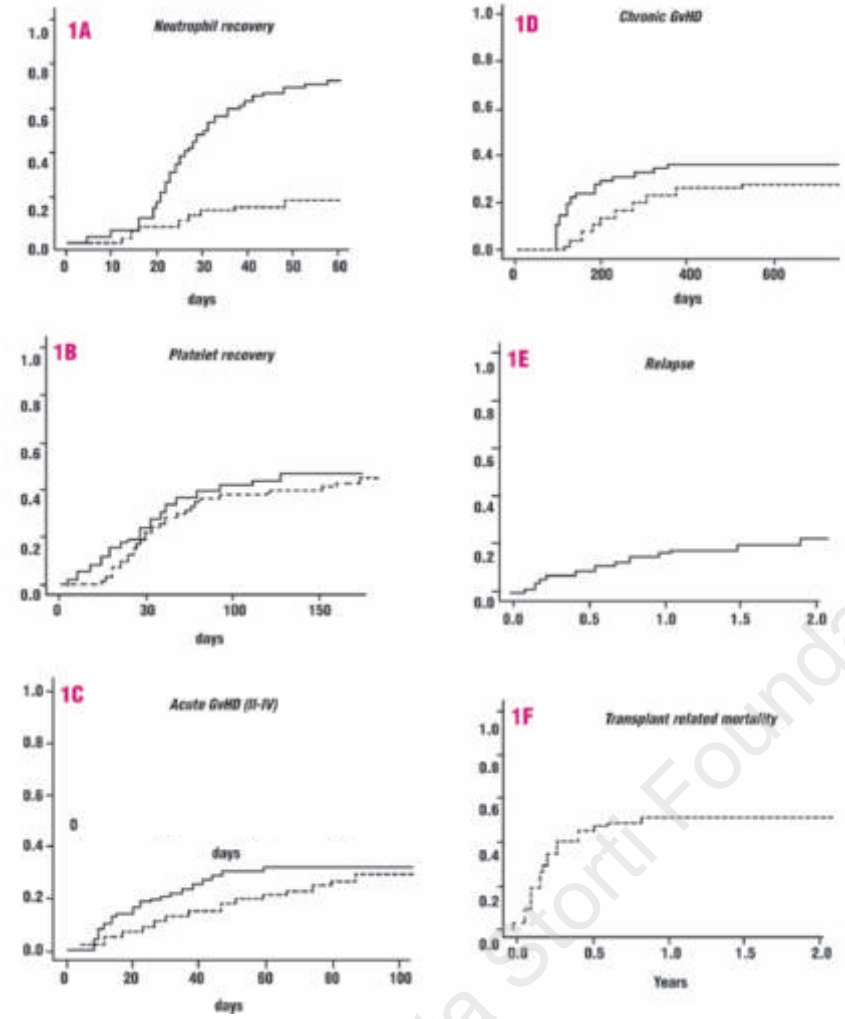

Figure 1. Cumulative incidence of outcomes of CBT in adults with hematologic malignancies. Death is used as a competing event and represented by a dotted line (- - -).

received cyclosporine combined with steroids as prophylaxis against GVHD (Table 1).

Definitions of end-points

Neutrophil recovery. Myeloid engraftment was defined as a recovery of an absolute neutrophil count of at least 500/mm^3 on 3 consecutive days. Platelet recovery was defined as the time needed to reach a sustained platelet count of at least 20,000/mm^3 without transfusion support for 7 consecutive days. Absence of hematopoietic recovery at day 60, second transplantation or autologous hematopoietic reconstitution was considered as failure of engraftment.

Graft-versus-host disease. Starting on day 1, acute GVHD was scored according to the standard criteria[23] and counted only for grades ≥II. Patients surviving more than 100 days after transplant with sustained donor hematopoiesis were considered at risk for the development of chronic GVHD.[24]

Relapse. Time to relapse was measured from the date of CBT to the date of disease recurrence as defined by morphological evidence of the neoplastic clone in either the bone marrow or in any extramedullary site. Patients who died in complete remission were censored at the date of death.

Transplant-related mortality. This was defined as all causes of death occurring at any time after CBT and not related to the underlying malignant disease.

Overall survival. Overall survival was measured as the time interval between the date of CBT and the date of death of any cause or the date of the last follow-up for survivors.

Disease-free survival. This was defined as the time interval between the date of CBT and the date of relapse or death in complete remission, whichever occurred first.

Statistical analysis

Variables related to the patients and donors (age, sex, sex match, cytomegalovirus serology, ABO compatibility, number of HLA disparities), disease- (acute and chronic leukemia versus other diseases, status at transplant, previous autologous transplant), and transplant (n. of nucleated cells at freezing and infused per kg, conditioning regimen, GVHD prevention) variables were analyzed for their potential prognostic value on each of the aforementioned endpoints. Univariate and multivariate proportional hazards

Table 2. Multivariate analyses of risk factors for the main outcomes after CBT for adults with hematopoietic malignancies.

Outcomes and unfavorable risk factors*	*Hazard ratio (95% confidence interval)*	*p value*
Neutrophil recovery		
Number of nucleated cells collected ≥2.6×10^7/kg	2.02 (1.30-3.15)	0.009
Prophylactic hematopoietic growth factor	2.13 (1.31-3.47)	0.002
Relapse		
Diseases other than CML	10.98 (1.45-81.3)	0.02
Advanced status of the disease	1.47 (1.03-2.10)	0.04
Transplant-related mortality at 2 years		
Age ≥29 years	1.74 (1.08-2.82)	0.02
Female recipient	1.78 (1.13-2.79)	0.01
Survival		
Female recipient	0.59 (0.40-0.91)	0.01
Major ABO incompatibility	0.63 (0.42-0.95)	0.03
Disease-free survival		
Advanced status at the disease	0.59 (0.39-0.90)	0.015
Major ABO incompatibility	0.65 (0.43-0.95)	0.03
Female recipient	0.68 (0.46-0.99)	0.05

Risk factors for acute GVHD and platelets were not selected in the Cox model. CML chronic myeloid leukemia , GVHD (graft versus host disease).

regression models were used to identify independent risk factors for death and disease-free survival by means of log-rank tests and Cox proportional hazards models.[25] For assessment of factors predicting neutrophil and platelet recovery, acute GVHD (grade II-IV), chronic GVHD, relapse and transplant-related mortality, a similar methodology was used in a competing risks setting, death being treated as a competing event.[26] Univariate and multivariate analyses were then performed using Gray's test and the proportional sub-distribution hazard regression model of Fine and Gray.[27] A stepwise backward procedure was used to construct a set of independent predictors for each end-point. All predictors with a *p*-value below 0.10 were considered, and sequentially removed if the *p*-value in the multiple model was above 0.05. All tests were two-sided. The type I error rate was fixed at 0.05 for factors potentially associated with time-to-event outcomes. Since we found that the sex of the recipient was selected as a prognostic factor for transplant-related mortality and survival in a multivariate analysis without a clinical explanation, we also performed an analysis comparing recipient-, donor-, disease- and transplant-related factors according to recipient's gender. All analyses were carried out using the cmprsk package (developed by Gray, June 2001) on Splus 2000 software and SPSS software.

Results

Neutrophil and platelet recovery

The cumulative incidence of neutrophil recovery at day 60 was 72±3% and the median time to reach an absolute neutrophil count of at least 500/mm^3 was 28 days (range, 11-57) (Figure 1A). Of the 13 patients who did not recover neutrophil counts at day 60, four engrafted later between day 66 and 80 after transplant, five underwent a second transplant, three experienced autologous hematopoietic reconstitution and one died in aplasia. Twenty-six patients died early after transplant and before the median date of engraftment in the overall series (between days 4 and 28), thus the graft failure rate was 9% (13 out of 145 evaluable cases). In univariate analysis, a number of nucleated cells greater than 2.7×10^7/Kg (81±5% vs 65±5%; *p*=0.02) at the time of freezing and early status of disease at transplant (89±6% for early vs 62±7% for intermediate vs 70±5% for advanced; *p*=0.03) were the two factors favorably affecting the probability of neutrophil recovery. In multivariate analysis, factors found to be significantly associated with neutrophil recovery were the number of nucleated cells at collection or freezing (relative risk [RR] 2.02, 95% confidence interval [95%CI]: 1.30 to 3.15; *p*=0.002) and the use of hematopoietic growth factors within one week after transplant (RR 2.13, 95% CI: 1.31 to 3.47; *p*=0.002) (Table 2). The cumulative incidence of platelet recovery at day 180 was 46±4% and the median time to reach a platelet count of at least 20,000/mm^3 was 84 days (range, 22-176) (Figure 1B). None of the analyzed factors was associated with the probability of platelet recovery in either univariate or multivariate analysis.

Acute and chronic graft-versus-host disease

Acute GVHD was counted as absent or grade I in 36 (21%) patients, grade II in 27 (16%), grade III in 15 (9%) and grade IV in 13 (7%). The cumulative incidence of acute GVHD at 100 days after CBT was 32±4% (Figure 1C). In univariate analysis, acute GVHD greater or equal to grade II was found to be more frequently associated with a diagnosis of CML than with AL or other malignant diseases (respectively, 50±8% vs 25±2% vs 36±7%, *p*=0.015). No other variables, including number and class distribution of the HLA disparities, were statistically associated with the incidence or severity of acute GVHD. In multivariate analysis, no factor was found to significantly influence the development or severity of acute GVHD. Chronic GVHD was observed in 34 of 92 patients at risk (limited in 18 and extensive in 16) with a cumulative incidence of chronic GVHD at 2 years of 36±10% (Figure 1D). In multivariate analysis, no factor showed a significant association with the occurrence of chronic GVHD.

Relapse

The cumulative incidence of relapse at 2 years was 22±4% for all patients (Figure 1E). It was 24±5% for patients with AL, 5±5 % for those with CML, and 31±12% for those with MDS. Three out of 13 patients transplanted for lymphoma relapsed. According to the disease status at transplant, the cumulative incidence of relapse at 2 years was 16± 7% for patients transplanted in an early phase of the disease, 22±12% for those transplanted in intermediate status and 25±5% for those transplanted in advanced phase disease. In the Cox model, only two factors were significantly associated with an increased risk of relapse: diagnosis other than CML (RR 0.01, 95%CI: 0.01 to 0.68; *p*=0.02) and advanced disease status at the time of unrelated CBT (RR: 1.47, 95%CI: 1.03

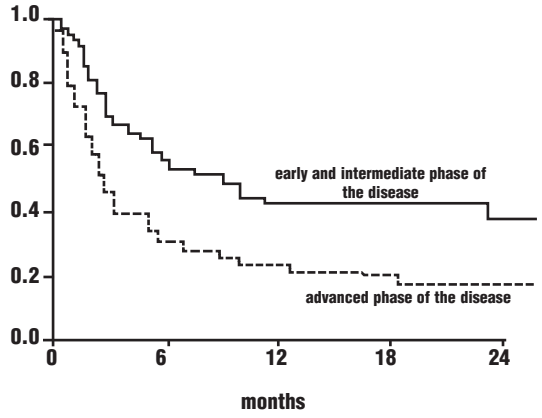

Figure 2. Disease-free survival according to disease status at transplant.

to 2.1; p=0.04).

Transplant-related mortality and causes of death

The 2-year cumulative incidence of transplant-related death was 51±4% (Figure 1F). In univariate analysis, the incidence of transplant-related mortality was significantly higher in patients older than 29 years (59±5% vs 43±6%, p=0.02), in females (60±5% vs 43±6%; p=0.01), in patients transplanted in advanced phase (57±5% vs 45±6%; p=0.04) and in those with CML compared to those with AL or with other malignant diseases (76±6% vs 44±2% vs 49±7%, respectively; p=0.006). Furthermore, there was a trend for reduced transplant-related mortality in patients receiving cord-blood units containing a higher number of nucleated cells at collection or freezing (>2.6×10^7/kg), as compared to patients receiving a lower cell dose (45±6% versus 56±5%, p=0.06). In multivariate analysis, only two factors remained significantly associated with an increased risk of transplant-related death: age >29 years (RR 1.74, 95%CI: 1.08 to 2.82; p=0.02) and female gender (RR 1.78, 95%CI: 1.13 to 2.79; p=0.01), (Table 2).

Causes of death. Of 171 patients, 110 (64%) died and 61 are alive. Of the 110 deaths, 85 (77%) were due to transplant-related causes and most of them (n=68) occurred within 100 days after transplantation. The primary causes of death were disease relapse (n=25; 23%); infection (n=40; 36%), GVHD (n=12; 11%), acute respiratory distress syndrome or interstitial pneumonitis (n=7, 6%), hemorrhage (n=5, 5%) veno-occlusive disease (n=4; 4%); cardiac toxicity (n=3, 3%) multi-organ failure (n=8; 7%) and other causes (n=6; 5%).

Overall survival and disease-free survival

For all 171 patients, the 2-year probability of overall survival and disease-free survival was 33±4% and 27 ±4%, respectively. The probability of being alive without disease at 2 years was 41±9% and 34±10% for patients transplanted in an early and intermediate phase of disease, respectively (38±7 for early and intermediate phases of the disease) and 18±4% for patients transplanted in advanced phase (p=0.001, Figure 2). Table 3 shows the results of univariate analysis for disease-free survival. In multivariate analysis, advanced disease status at the time of CBT (RR 1.69, 95%CI: 1.1 to 2.56; p=0.015), female gender (RR 1.47, 95%CI: 1.01 to 2.17; p=0.05) and ABO major incompatibility (RR 1.55, 95%CI: 1.05 to 2.29; p=0.03) were identified as significant factors unfavorably affecting disease-free survival (Table 2). The relevance of patients' gender in influencing the outcomes led us to analyze the frequency of all other variables according to the patients' sex. No variables, including patient-, donor-, disease- and transplant-related factors or causes of death were found to be statistically different when analyzed according to patients' gender (*data not shown*).

Discussion

This registry-based study confirms that cord blood represents a source of hematopoietic stem cells that can be successfully used for unrelated transplant not only in children but also in adults. Furthermore, the study provides information on outcomes and prognostic factors that should be taken into account when an unrelated cord blood graft is available for an adult patient. The reported experience on CBT in adults supports the feasibility of this strategy and very encouraging results have been obtained in single institutions.[16–19] Moreover, two recent, registry-based studies showed similar outcomes for patients with acute leukemia transplanted with cord blood or bone marrow from unrelated donors.[20–21] In the present analysis, the Eurocord Group extended the study to a large series of adult patients with different hematologic malignancies transplanted in 63 centers after 1997. In a previous study of 550 CBT recipients with malignant hematopoietic disorders, we showed that transplant-related mortality has been decreasing since 1998, due to better selection of cord blood units based on cell dose and number of HLA disparities.[28] To avoid possible biases due to the study period we decided to include only those patients transplanted after 1998 in the present analysis.

In this study the time to hematopoietic engraftment was longer after CBT than that usually reported for HLA matched unrelated bone marrow transplants, in which the median number of nucleated cells infused is about ten times higher. However, the kinetics of engraftment in adults was similar to that reported for children undergoing CBT,[2,3,4,8,9] in whom the cell dose infused is about 10-fold higher, due to their smaller size. Encouraging outcomes have been reported for adults with acute myeloblastic leukemia or MDS receiving CBT at a single center in Japan.[18,19] The lower genetic diversity and the smaller size of the Japanese population may account in part for these results. The best results were achieved in patients grafted with ≥2×10^7 nucleated cells/kg of recipient's body weight. This threshold of nucleated cord blood cells at infusion is recommended by Eurocord.[28] Although the number of CD34$^+$ cells[8,16] and the number of colony-forming cells[29,30] have been recognized to affect engraftment and patients' outcomes significantly in children and adults, in this retrospective multicenter study, the low number of evaluable patients and the absence of standardized laboratory methods prevented us from assessing the possible impact of

Table 3. Univariate analyses of disease-free survival (DFS) after CBT for adults with hematologic malignancies.

	2-year estimate of DFS (% ± SD)	*P (log rank test)*
Overall	27±4	
Diagnosis		
acute lymphoblastic leukemia	34±7	
acute myeloid leukemia	34±7	
secondary acute leukemia	22±13	
myelodysplastic syndrome	25±11	
chronic myeloid leukemia	19±7	
lymphomas*	13±10	
Disease status°		
early stage	41±9	
intermediate stage	34±10	
advanced stage	18±4	0.001
Time from diagnosis to UCBT		
<13 months	31±5	
≥13 months	24±6	0.38
No. of previous auto transplants		
0	27±5	
≥1	23±8	0.30
Age (years)		
<29 y	33±6	
≥29 y	21±5	0.02
Weight (kg)		
<60 kg	28±7	
≥60 kg	24±6	0.99
CMV serology		
negative	35±7	
positive	23±5	0.17
Recipient's gender		
male	29±6	
female	25±5	0.05
ABO compatibility		
matched+minor mismatched	32±5	
major mismatched	19±6	0.07
HLA disparities (DRB1 high resolution)		
6 out of 6	44±17	
5 out of 6	20±5	
4 out of 6	30±7	
3 out of 6	40±16	0.18
NC at freezing/kg		
$<2.7\times10^7$	28±5	
$\geq2.7\times10^7$	28±6	0.32
NC infused/kg		
$<2.1\times10^7$	25±5	
$\geq2.1\times10^7$	29±6	0.25
CD34 infused/kg		
$<1.0\times10^5$	29±6	
$\geq1.0\times10^5$	35±6	0.46
Growth factor day 0-7		
no	16±8	
yes	33±5	0.51
Irradiation		
no	29±6	
yes	26±5	0.31
Year of UCBT		
< 2000	23±5	
≥ 2000	32±5	0.44

*SD: standard deviation; CMV: cytomegalovirus; UCBT: unrelated cord blood transplant *Non -Hodgkin's lymphoma n=12; Hodgkin's lymphoma n=1. °IBMTR classification (see Table 1).*

these two parameters properly.

In multivariate analysis, the use of hematopoietic growth factors within one week after transplant was the other significant factor favorably influencing myeloid recovery. In all published series of CBT in adults, supportive care included the prophylactic use of hematopoietic growth factors, whereas contradictory results have been reported in children, for whom the use of such growth factors was identified as a factor significantly influencing engraftment in some retrospective analyses,[8,9] but not in others.[2,4] Currently, no definitive conclusion on the beneficial effect of hematopoietic growth factors can be drawn and a prospective, randomized study is needed to investigate this issue specifically.

Although most patients were mismatched with their cord blood donors at one (46%), two (41%) or three (8%) HLA loci, the incidence of either acute GVHD≥II or chronic GVHD was low and compares favorably with that reported in several studies on adults receiving an unrelated, HLA-matched, unmanipulated bone marrow transplant.[20,21,31-34] In a previously reported Eurocord analysis, a high number of $CD34^+$ cells at freezing and the co-existence of class I and class II HLA disparities were found to be significantly associated with severe (grade III-IV) acute GVHD.[28] As also observed by Laughlin *et al.*,[16] in the present analysis, which was conducted on a larger number of patients, no factor, including the degree of HLA disparity, was found to be significantly associated with the risk of developing GVHD. It is noteworthy that the incidence of acute GVHD in this series of adult patients was not substantially different from that observed in children.[4,6-9] The low risk of GVHD in adult recipients of an unrelated, HLA mismatched CBT confirms in the clinical setting the lower reactivity of cord blood T-lymphocytes against alloantigens observed *in vitro*, reflecting the phenotypic and functional immaturity of cord blood cells[35] and their high sensitivity to cyclosporine.[36]

Despite the low incidence of GVHD, the graft-versus-leukemia effect does not seem to be impaired after CBT. Indeed, retrospective studies in children, which compared CBT with unmanipulated or T-cell depleted unrelated bone marrow transplants, reported similar relapse rates.[6,7] The low incidence of CML relapse after CBT is comparable to that observed in other transplant settings, suggesting that the cytotoxic activity expressed by cord blood lymphocytes against CML cells is as effective as the cytotoxicity delivered by lymphocytes from other sources (peripheral blood and bone marrow). As expected, the status of advanced disease at the time of transplant significantly increased the risk of relapse.

Transplant-related mortality at 2 years was 51%, however most of the deaths (68%) occurred during the first 100 days, probably reflecting the slow hematopoietic recovery and therefore a higher incidence of early infections.[37] Immune recovery was later after CBT in adults than in children.[38] Unfortunately, no data on immune recovery was available in this retrospective analysis. In a multivariate analysis of transplant-related mortality, older age and female gender were significantly associated with higher transplant-related mortality. Patients' age was identified as a factor influencing transplant-related mortality and survival in two small single center studies,[17,30] but not

in the larger, multicenter, retrospective analysis by Laughlin *et al*., which included 68 adults.[16] The association of female gender with poorer disease-free survival was also found in a single center study including children and adults.[30] The reasons underlying this finding remain unclear. In the present series, male and female patients did not clearly differ in terms of other patient-, disease- and transplant-related characteristics (*data not shown*). ABO blood group compatibility between donor and recipient was another factor that significantly affected disease-free survival, which was better in recipients of cord blood without major ABO incompatibility. The impact of ABO compatibility on disease-free survival was also indentified in a multivariate analysis of another multicenter study which included children undergoing CBT for acute myeloblastic leukemia.[9] Finally, as expected, the outcome of patients was significantly influenced by the disease phase. Patients who were transplanted in advanced phase had unfavorable results while results were better in patients transplanted in early and intermediate phases. As reported in the two comparative studies on patients with acute leukemia,[20,21] these figures are substantially similar to those reported for patients with hematologic malignancies undergoing HLA matched or mismatched bone marrow transplants from unrelated donors.[31- 34,39,40]

The data reported herein indicate that CBT already represents a valid therapeutic approach for adult patients lacking an HLA identical donor. However, patients' outcomes could be improved simply by performing transplants with the cell dose and HLA match currently recommended by Eurocord and others.[10,28] Furthermore, different lines of research are currently being explored to extend the applications and improve the results of CBT.[41-45] Among them, the use of *ex vivo*-expanded cord blood stem cells has been shown to be clinically feasible, but in practice is expensive and time-consuming and its efficacy needs to be confirmed. Double CBT does not require any technological devices and the reported results seem very promising; however, the number of patients who have undergone double CBT is still too small to enable clear conclusions on such a transplant procedure.

Appendix

The site investigators participating in the Netcord banks and Eurocord-EBMT transplant centers were as follow: The Netcord banks: Milano – Italian Cord Blood Bank (CBB) Network, Dr. P. Rebulla and Dr. L. Lecchi, Italy; Düsseldorf CBB, Dr. P. Wernet and Dr G. Koegler, Germany; Barcelona CBB Dr. J. Garcia and Dr. S. Querol,Spain; Tokyo CBB, Dr. T. Takahashi and Dr T. Nagamura Inoue, Japan ; New York CBB, Dr. P. Rubinstein, USA; Belgium CBB, Dr. Y. Beguin, Belgium; London CBB, Dr. M. Contreras, S. Armitage, UK; Paris CBB, Dr. M. Benbunan, Paris, France; Besançon CBB, Dr. P. Herve, France; Bordeaux CBB, Dr. P. Marchand, France.

The Eurocord-EBMT transplant centers: Hospital Universitario "La Fe", Dr. G. Sanz, Valencia, Spain; Hematology, Policlinico Universitario " Tor Vergata", Prof. W. Arcese, Rome, Italy; Hematology, University La Sapienza, Dr. A.P. Iori, Italy; MD Anderson Cancer Center (adults), Dr. S. Giralt, Houston, USA; The Institute of Medical Science, University of Tokyo, Dr. S. Asano, Tokyo, Japan; Hôpital Saint Louis, Prof. E. Gluckman, Dr. V. Rocha, Dr. I. Ionescu, Dr. F. Garnier, Paris, France; Hôpital Claude Huriez, Prof. J.P. Jouet, Lille, France; Hôpital de l'Archet, Dr. A. Sirvent, Nice, France; Azienda Ospedaliera S. Giovanni, Dr. A. Busca, Torino, Italy; University "Tor Vergata", St. Eugenio Hospital, Prof. S. Amadori, Rome, Italy; Hôpital Pédiatrique "La Timone", Prof. G. Michel, Marseille, France; Fairview University of Minnesota, Dr. J.Wagner, Minneapolis, USA; Hackensack University Medical Center, Dr. S. Goldberg, Hackensack, USA; Hôpital Saint Antoine, Prof. N.C. Gorin, Dr. J.P. Laporte, Paris, France; EBMT ALWP, Dr. M. Labopin, Paris, France; Institut Paoli Calmettes, Prof. D. Blaise, Marseilles, France; MD Anderson Cancer Center (Pediatrics), Dr. Ka Wah Chan , Houston, USA; Roswell Park Cancer Institute, Dr. B. Bambach, Buffalo, NY, USA; Emory University School of Medicine, Dr. A. Ogden, Atlanta, USA; University Medical Center, Dr. M. Graham, Tucson, USA; Hospital Santa Creu i Sant Pau, Dr. I. Badell Serra, Barcelona, Spain; Ospedale di Careggi, Dr. A. Bosi, Firenze, Italy; Ospedale V Cervello, Dr. R. Scimè, Palermo, Italy; Hôpital d' Enfants, Prof. P. Bordigoni, Vandoeuvre, France; University of Liege, Dr. Y. Beguin, Liege, Belgium; Tokyo Metropolitan Fuchu Hospital, Dr. H. Kodo, Fuchu, Japan; Hyogo Preferectural Adult Disease Center, Dr. T. Murakami, Kobe, Japan; University Hospitals of Cleveland, Dr. M. Laughlin, Cleveland, USA; FHCRC, Dr. E. Sievers, Seattle, USA; Medical City Dallas Hospital, RN M. Hooker, Dallas, USA; Lombardi Cancer Center, NW Washington DC, USA; FLENI, Dr. B. Diez, Buenos Aires, Argentina; Washington University School of Medicine, Dr D. Adkins, Saint Louis, USA; Pediatric Hematology/Oncology-De Vos Children's Hospitals, Dr. D. Pietryga, Grand Rapids, USA; CEMIC, Dr B. Koziner, Buenos Aires, Argentina; Cardinal Glennon Children's Hospital, St Louis, USA; The Cleveland Clinic Foundation, Cleveland, USA ; Loyola University Medical Center, Dr P.J. Stiff, Maywood, USA; City of Hope Medical School, Dr. J. Rosenthal, Duarte, USA; CETRAMOR, Dr. J. Saslavsky, Rosario, Argentina; Westmead Hospital, Haematology Department, Dr. K. Bradstock Westmead, Australia; Leiden University Hospital, Prof. R. Willemze, Leiden, The Netherlands; Hospital Clinic, Dr E. Carreras, Barcelona, Spain; Hôpital Hotel Dieu, Dr. N. Milpied, Nantes, France; Ospedale Maggiore di Milano, IRCCS, Prof. G. Lambertenghi Deliliers, Milano, Italy; University Hospital, Dr. B. Simonsson, Uppsala, Sweden; Hôpital Haut Leveque, Dr. J. Reiffers, Pessac, France ; Royal Victoria Infirmary, Dr. A. Dickinson, Newcastle-upon-Tyne, UK; Ospedale Regina Margherita, Dr. F. Fagioli, Torino, Italy; Karl-Franzens-University-Graz, Prof. W. Linkesch, Graz, Austria; Hospital M. Infantil Vall d'Hebron, Prof. J. Ortega, Barcelona, Spain; The Children's Hospital at Westmead, Dr. P. Shaw, Sydney, Australia; Clinica Puerta de Hierro, Dr. MN Fernandez, Madrid, Spain; Hospital Nino Jesus, Dr. MA Diaz, Madrid, Spain; Schneider Children's Medical Center, Dr. I. Yaniv, Petach-Tikva, IsraeI; Hospital Duran i Reynals, Dr. A. Granena, Barcelona, Spain; Saku General Hospital, Dr. S. Seki, Saku, Japan; Kyoto 1st Red Cross Hospital, Dr. H. Fujii, Kyoto, Japan; Niigata Cancer Center, Dr. J. Ogawa, Niigata, Japan; Tokyo Metropolitan Komagome Hospital, Dr. H. Sakamaki, Tokyo, Japan; Hamamatsu Med. Center, Dr. S. Yajima, Hamamatsu, Japan; Teikyo University, Dr. N. Shirafuji, Tokyo, Japan ; Hirosaki University, Dr. J. Kitazawa, Hirosaki, Japan; Ryuku University, Dr. N. Taira, Okinawa, Japan; National Cancer Center, Chuo Hospital, Dr. M. Mineishi, Tokyo, Japan.

WA, VR, ML and EG conceived the study. The study was performed in Paris at the Eurocord office under the direction of PW and EG. IO collected and checked the clinical data. GS, ML, AS, AB, SA included patients and helped WA and VR to write the manuscript. The authors declare that they hav no potential conflicts of interest. Eurocord is supported by a European Grant QLK3-CT-1999-00380.

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