Unrelated cord blood transplant in children with high-risk acute lymphoblastic leukemia: a long-term follow-up

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ABSTRACT

Background and Objectives

The aim of this single center study was to assess the impact of pre-transplant factors on long-term follow-up in young patients affected by high-risk acute lymphoblastic leukemia (ALL) who underwent an unrelated cord blood transplant (CBT). The conditioning regimens, graft-versus-host disease (GVHD) prophylaxis and supportive policies were uniform for all patients.

Design and Methods

We analyzed the results of CBT performed in 30 patients, aged <18 years, affected by high risk ALL. As conditioning regimen, all patients received 12 Gy fractionated total body irradiation, etoposide, cyclophosphamide and horse anti-lymphocyte globulin. GVHD prophylaxis consisted of 6-methylprednisolone and cyclosporine A.

Results

The cumulative incidence of engraftment was 93% (95% CI:0.85-0.93). The cumulative incidence of grade III-IV acute and chronic GVHD was 7% (95% CI:0.01-0.19) and 33% (95% CI: 0.17-0.64), respectively. The 9-year cumulative incidence of transplant-related mortality and relapse was 34% (95% CI:0.13-0.45) and 31% (95% CI:0.16-0.61), respectively. The 9-year overall survival, leukemia-free survival and event-free survival were 42% (95% CI:0.52-0.93), 47% (95% CI:0.25-0.61) and 46% (95% CI:0.33-0.61), respectively. A number of CFU-GM $<1\times10^4$/Kg of recipient body weight was the only factor that negatively affected all outcome parameters both in univariate and multivariate analyses.

Interpretation and Conclusions

The infused cell dose expressed as *in vitro* progenitor cell growth represents the most important pre-transplant factor affecting the long-term outcome after an unrelated CBT in young patients with high risk ALL. The number of CFU-GM should thus be considered in the selection process of cord blood units for transplant.

Key words: cord blood transplant, leukemia, acute lymphoblastic leukemia, unrelated HSC transplant, prognostic factors.

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Acute lymphoblastic leukemia (ALL) is the most common malignancy in childhood.[1] Treatment of children with ALL has become more effective over the past three decades.[2] However, despite a steady improvement over the years, 20-30% of children still relapse and conventional intensive chemotherapy can cure up to 30% of children who have relapsed.[2-5] In this setting, allogeneic hematopoietic stem cell transplant (HSCT) seems to be the best therapeutic option particularly for early relapses.[6-9] Transplantation in first complete remission (CR) remains controversial even though patients with very high risk criteria at diagnosis are commonly treated with allogeneic HSCT at this disease phase.[10-14] A broad application of allogeneic HSCT is often limited by the absence of a suitable identical sibling donor. Transplants from an HLA-matched unrelated donor have become a feasible procedure for the cure of a significant proportion of children with ALL lacking an HLA identical family donor.[15-18] Nevertheless, despite the increasing number of bone marrow donor registries, some patients cannot be transplanted because a donor is not available or because the time to identify a donor is too long. Cord blood represents the most recent source of HSCT used in the unrelated transplant setting for patients who lack a related or unrelated HLA compatible donor[19-31] and comparative studies have shown that unrelated cord blood transplant (CBT) is an acceptable alternative to unrelated bone marrow transplant in children.[31-32] However, these studies were mainly retrospective, involved multicenter series of patients with non-homogeneous clinical characteristics and were based on a variety of transplant procedures.

Herein, we report the results of the long-term follow-up of a single center study performed on 30 consecutive young patients with high-risk ALL who underwent an unrelated CBT and who were treated with the same conditioning regimen, graft-versus-host disease (GVHD) prophylaxis and supportive therapy. One of the major aims of this study was the prospective analysis of pre-transplant factors capable of influencing the outcome parameters.

Design and Methods

Eligibility criteria

A search for an unrelated hematopoetic stem cell donor was carried out for patients aged ≤18 years affected by ALL in second or subsequent CR or in relapse who lacked a related HLA compatible donor. A search was also conducted for ALL patients in first CR if they had at least one of the following poor prognosis features: failure to achieve CR with the primary induction regimen; prednisone poor response and t(9;22) translocation or Bcr/Abl positive transcript; prednisone poor response and t(4;11) or MLL/AF4 positive transcript; presence of minimal residual disease (MRD) by polymerase chain reaction (PCR) analysis $\geq 10^{-3}$ on day +78 after induction therapy (time 2 according to van Dongen *et al)*.[33] Patients in first or second CR at transplantation were considered to have high-risk disease, while patients in relapse (with with <30% of blasts in the marrow) or > second CR were considered to have very high-risk disease.

HLA typing was determined by molecular definition for both class I (HLA-A, B, C) and class II (HLA-DR, DQ) antigens; DRB1 alleles were defined by high resolution methods. A cord blood unit was considered suitable for transplantation when ≤2 HLA antigen (HLA-A, B, DRB1) differences with the recipient were detected and its content of nucleated cells (NC) was at least 2×10^7/Kg of the recipient body weight (bw). All patients had an autologous cryopreserved unit of hematopoietic stem cells collected from marrow or peripheral blood as back-up. Informed consent was required from the patient and/or the guardian before the CBT. Between July 1995 and September 2005, 30 consecutive patients with high risk ALL underwent a CBT from an unrelated donor at "La Sapienza" University of Rome; 12 of them have already been reported.[27]

Transplant procedures

Cord blood units were provided by the Cord blood banks of GRACE, Italy (n=15); New York, USA (n=6); Düsseldorf, Germany (n=6); and Barcelona, Spain (n=3). The cryopreserved cord blood unit was shipped in an appropriate container cooled by liquid nitrogen in vapor phase. The unit was thawed and washed according to previously published methods.[34] After thawing, the following tests were performed on the CB: NC count, quantification of $CD34^+$ cells by cytofluorimetric analysis, clonogenic assays, cell viability tests and microbiological cultures.

As conditioning regimen, all patients received 12 Gy fractionated total body irradiation (TBI) by linear accelerator (200 cGy twice a day for six doses, given over 3 consecutive days starting on day -7), etoposide (20 mg/Kg by continuous i.v. infusion over 24 hours on day -4), cyclophosphamide (60 mg/Kg, once daily i.v. on days -3 and -2, total dose 120 mg/Kg), horse anti-lymphocyte globulin (Lymphoglobuline, Sangstat, Lyon, France) (600 IU/Kg once daily by i.v. infusion over 16 hours for 4 consecutive days starting on day -6, for a total of four doses) and 6-methylprednisolone (2 mg/Kg from day -6 through day -1).

GVHD prophylaxis and treatment

For GVHD prophylaxis, all patients received i.v. 6-methylprednisolone at a dose of 1 mg/Kg daily from day 0 and i.v. cyclosporine-A, administered from day -1 at the dose of 3 mg/Kg, with drug levels maintained between 200-400 ng/mL. The dose of 6-methylprednisolone was slowly tapered starting from day +30, while cyclosporine A was tapered by 5% every week from day 50. Acute GVHD was scored by standard criteria[35] and initially treated by increasing doses of 6-methylprednisolone (3 mg/Kg). Patients surviving in remission at least 100 days after transplantation with full donor chimerism were considered evaluable for chronic GVHD, graded according to established criteria.[36]

Supportive care

All patients were nursed in HEPA filtered single rooms and received the same supportive care, anti-microbial prophylaxis and transfusion policy, as described elsewhere.[24] Growth factors were intentionally not used in the first 25 patients, who received granulocyte colony-stimulating factor (G-CSF, Lenograstim, Aventis-Pharma) at the dose of 5 μg/Kg daily if the neutrophil count was $<0.2\times10^9$/L and the marrow cellularity <5% on day +25 after transplant. Reinfusion of autologous cryopreserved hematopoietic stem cells was utilized for severely neutropenic patients failing to reach a sustained neutrophil count $>0.5\times10^9$/L after 10 days of therapy with G-CSF. In the last 5 patients, the protocol was modified and G-CSF at a dose of 5 μg/Kg daily was administered from day 7 after transplant until the neutrophil count was $>1\times10^9$/L.

Hematopoietic recovery, chimerism and engraftment

Peripheral blood counts were performed daily and hematopoietic recovery was defined on the basis of the first of 3 consecutive days with an absolute peripheral blood count $>0.5\times10^9$/L for neutrophils and on the first of 7 consecutive days with $>20\times10^9$/L for platelets in the absence of transfusional support. Chimerism status after transplant was evaluated, on bone marrow and peripheral blood cells, by standard cytogenetics or fluorescence *in situ* hybridization (FISH) for transplant of opposite sex and by DNA polymorphism analysis, detected by PCR amplification of variable number tandem repeat (VNTR) regions, at days 20, 35, 60, 100, 180, 270 and 365 after transplant. CFU-GM and CFU-GEMM were evaluated on bone marrow cells at the same time points after transplantation by clonogenic assays in complete methylcellulose medium (MethoCult, Stem Cell, Canada). Engraftment with full chimerism required myeloid hematopoietic recovery with all cells in the recipient of donor origin, while the simultaneous presence of donor and recipient cells was defined as engraftment with mixed chimerism. The absence of hematopoietic recovery at 90 days after transplant and autologous hematopoietic reconstitution was considered as an engraftment failure.

Statistical analysis

Data were collected in an XLS database and imported into SAS for the statistical analysis. The close-out date for analysis was June, 2006. The end-points were engraftment, acute and chronic GVHD, relapse, transplant-related mortality (TRM), overall survival (OS), leukemia-free survival (LFS) and event-free survival (EFS). Relapse was indicated by morphological evidence of leukemia in the bone marrow or in any other extramedullary site or by cytogenetic recurrence of the neoplastic clone. TRM was defined as death due to non-leukemic causes. OS was calculated from transplantation to either death or last observation. LFS was defined as the time from transplantation to leukemia relapse, death or to the last observation. EFS denotes the time from transplantation to graft failure, leukemia relapse, development of lymphoproliferative disorders, death or to the last observation. The actuarial curves of OS, LFS and EFS were prepared according to the Kaplan-Meier method. Cumulative incidence rates, calculated taking into account the appropriate competing risks,[37] were used to estimate the probabilities of hematopoietic recovery, engraftment, GVHD, relapse and TRM. The log-rank test was employed to compare hazards and two-sided p-values ≤0.05 were considered to represent statistical significance. The correlation between number of total NC, $CD34^+$ cells and CFU-GM infused was calculated using Spearman's rank-order correlation coefficient. Variables considered in univariate analysis were: age, sex, weight, degree of HLA matching, donor-recipient sex combination, ABO compatibility, duration of first CR, interval between start of search and transplantation, cytomegalovirus (CMV) serology before transplant, disease phase at transplant and cell dose infused in terms of total NC, $CD34^+$ cells, CFU-GEMM and CFU-GM. Variables with a p value <0.1 for each end-point were tested in multivariate analysis using the Cox proportional hazard regression model. Ninety-five percent confidence intervals (CI) are reported for the main summary statistics.

Results

Patients' characteristics

The patients' characteristics are reported in Table 1. All patients had high-risk ALL. Twenty patients in first or second CR were defined as having *high-risk disease* and ten patients in > second CR or relapse as having *very high-risk disease*. Of the four patients transplanted in first CR, three had been resistant to first induction therapy and one presented MRD $\geq10^{-3}$ at day +72 after induction therapy. Of the 16 patients transplanted in second CR, eight had had a very early relapse (<18 months from diagnosis to relapse) and four an early relapse (<30 months from diagnosis to relapse), while four patients were in late relapse. Of these last four patients, one had a T-cell ALL relapse and three showed combined marrow and extramedullary relapse. The median time from the start of the search for a cord blood unit to transplant was 91 days (range 49-204).

Within the series of 30 patients, the cord blood unit was mismatched with the recipient for one (n=16) or two (n=12) HLA loci. Two patients showed a complete HLA match with their donors. Fourteen patients were sex-mismatched and 20 pairs were major (n=12) or minor (n=8) ABO mismatched. All cord blood units were CMV negative, while 18 patients were serologically CMV positive.

The median number of NC/Kg of recipient bw was 4.34×10^7 (range 1.56-11.3) at collection and 3.73×10^7 (range 1.52-10.8) at infusion. The median proportion of NC lost after thawing was 10% (range 0-44%). The median number of infused $CD34^+$ cells$\times10^5$/Kg of recipient bw was 3.47 (range 0.25-8.9), the median number of infused CFU-GEMM and CFU-GM$\times10^4$Kg of recipient bw was, respec-

Table 1. Patients' characteristics.

N. of patients	*30*
Sex: male/female	16 / 14
Median age, years (range)	9.5 (4-18)
Median body weight, Kg (range)	39.5 (13-77)
Disease status at transplant	
First CR	4
Second CR	16
Months from diagnosis to 1st relapse:	
Median	18
Range	(3-69)
>Second CR	5
Relapse	5
HLA compatibility	
4/6	12
5/6	16
6/6	2
Gender	
M/F	8
F/F	8
F/M	6
M/M	8
ABO incompatibility	
Major	12
Minor	8
CMV serology	
Negative	12
Positive	18

Table 2. Groups of cell dose.

	CFU-GM $\times 10^4$/kg	*CD34* $\times 10^6$/kg	*NC* $\times 10^7$/kg	*Infused NC* $\times 10^7$/kg	*CFU-Tot* $\times 10^4$/kg	*CFU-GEMM x* $\times 10^4$/kg
Low dose (10 patients)	<1	<2	<3.5	<3.2	<3.5	<1
Intermediate dose (10 patients)	≥1 ≤2	≥2 ≤5	≥3.5≤5	≥3.2≤4,7	≥3.5≤7	≥1≤2.2
High dose (10 patients)	>2	>5	>5	>4,7	>7	>2.2

Table 3. Univariate analysis of pre-transplant variables affecting the outcome parameters.

Variable	*Engraftment*	*TRM*	*Relapse*	*OS*	*LFS*	*EFS*
	p	*p*	*p*	*P*	*P*	*p*
Sex	0.48	0.53	0.28	0.15	0.18	0.11
Age	0.46	0.16	0.20	0.91	0.58	0.43
Weight	0.55	0.66	0.17	0.63	0.34	0.52
Sex mismatch	0.65	0.80	0.77	0.59	0.53	0.58
HLA	0.38	0.78	0.67	0.78	0.70	0.83
ABO	**0.005**	0.63	0.66	0.99	0.93	0.85
CMV	**0.09**	0.54	0.11	0.13	0.18	0.05
Disease stage	0.26	0.31	**0.01**	0.43	0.28	0.40
NC	0.4	0.27	0.57	0.25	0.31	0.10
Infused NC	0.58	0.14	0.85	0.19	0.15	0.43
$CD34^+$	0.26	0.32	0.20	0.87	0.92	0.95
CFU-GEMM	0.36	0.54	0.69	0.46	0.97	0.89
CFU-GM	**0.01**	0.20	0.33	**0.01**	**0.03**	**0.04**

tively, 1.15 (range 0.16-4.47) and 1.78 (range 0.19-9.8). Based on our previous experience,[40] we stratified all patients into three groups according to the infused cell dose: low, intermediate and high. Each group contained ten patients (Table 2).

Hematopoietic recovery and engraftment

Two patients died at day 23 and 66 without evidence of hematopoietic recovery. The remaining 28 patients recovered hematopoiesis at a median time of 29 days (range 13-73) for neutrophils and 66 days (range 23-133) for platelets >20×10^9/L. For the 25 patients who did not receive programmed G-CSF, the median time to hematopoietic recovery was 30 days (range 17-73) for neutrophils and 66 days (range 23-180) for platelets >20×10^9/L. Ten of them were given the growth factor because the neutrophil count was <0.2×10^9/L at day 25 and all but one recovered hematopoiesis. The latter patient was reinfused with autologous cryopreserved marrow at day 38 after transplantation; after this maneuver, acute GVHD developed and engraftment of full donor origin was recorded at day 73. At the time of autologous marrow infusion, the patient showed mixed chimerism in the blood and an absence of donor cells in the bone marrow.[28] Three of 25 (12%) patients who did not receive programmed G-CSF experienced autologous hematopoietic reconstitution which occurred spontaneously at days 27, 33 and 37 after transplantation. The five patients who received G-CSF from day 7 after transplantation, according to the modified protocol, recovered full donor hematopoiesis at a median of 14 days (range 13-29) after their transplant for neutrophils and at a median of 48 days (range 23-125) for platelets. The difference in the speed of myeloid engraftment for the two groups of patients was statistically significant (p=0.0004). For all patients, the cumulative incidence of myeloid recovery and engraftment at day 90 after transplant was 93% (95% CI:0.86-0.93) and 86% (95% CI: 0.78-0.86), respectively (Figure 1).

In univariate analysis, the factors which negatively affected myeloid engraftment were a low dose of CFU-GM (low dose CFU-GM 77% of engraftment vs intermediate dose 88% vs high dose 90%; p=0.01) and ABO incompatibility (ABO incompatibility 86% vs ABO compatibility 100%; p=0.005). A trend for better engraftment was found for patients who were CMV serologically negative at transplantation (CMV negative 100% vs CMV positive 70%; p=0.09) (Table 3). CFU-GM dose, ABO incompatibility and CMV status at transplantation had a significant impact in multivariate analysis: CFU-GM p=0.05; ABO p=0.005; CMV p=0.004. The cumulative incidence of platelet recovery >20×10^9/L and >50×10^9/L by day 180 after transplant was 87% (95% CI: 0.79-0.95) and 76% (95%CI: 0.62-0.91), respectively. In univariate analysis, HLA compatibility (<two loci 78% vs two loci 50%; p=0.04) significantly affected platelet engraftment. The small number of patients does not allow an evaluation of the role of GFU-GM on platelet engraftment.

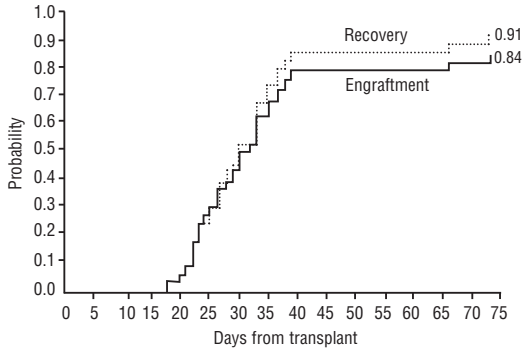

Figure 1. Cumulative incidence of PMN recovery and engraftment at day 90 after transplant.

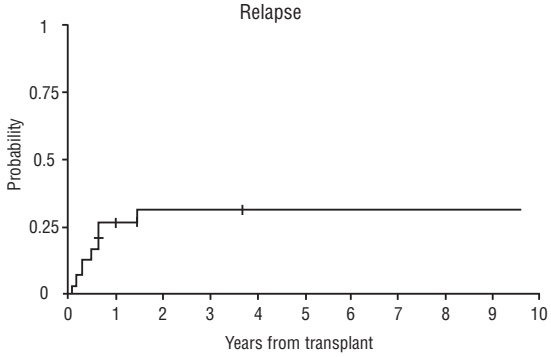

Figure 2. Cumulative incidence of relapse.

Graft-versus-host disease

Acute GVHD was observed in 16 patients (53%) at a median time of 26 days (range 9-71) after transplantation. Seven (23%) patients had grade I, seven (23%) grade II and two (6%) grade IV acute GVHD. One of the last two patients had grade IV acute GVHD following discontinuation of cyclosporine because of autologous hemopoietic reconstitution. The cumulative incidence of grade II-IV and III-IV acute GVHD by day 100 after transplantation was 30% (95% CI: 0.16-0.63) and 7% (95% CI: 0.07-0.31), respectively. In univariate analysis, we found no significant factor that affected acute GVHD. Among the 20 patients who survived in CR with full donor chimerism for more than 100 days after transplant, five developed chronic GVHD at a median of 203 days (range 121-240), for a cumulative incidence of 33% (95% CI: 0.17-0.64). Chronic GVHD was limited in four (23%) patients and extensive in one (6%). We found no factor that significantly affected chronic GVHD.

Relapse

Clinical relapse was detected between 34 and 531 days (median 183) after transplantation in seven patients: three in second CR, one in third CR and three in relapse at the time of transplantation, yielding a cumulative incidence of leukemia relapse of 31% (CI: 0.16-0.61) at 9 years (Figure 2). In univariate analysis, the phase of the disease at the time of the transplant was the only significant factor affecting relapse: 63% for very high-risk disease patients vs 20% for high-risk disease patients (p=0.01).

Mortality and causes of death

Sixteen patients died at a median of 282 days (range 23-1272) after their transplant. Disease recurrence was the primary cause of death in seven patients (23%). In nine patients, the causes of death were transplant-related: acute GVHD (n=2), fungal infections (n=2), multiorgan failure (n=2), thrombotic thrombocytopenic purpura (n=2) and Epstein-Barr virus (EBV)-related lymphoproliferative disorder (n=1). At 9 years, the cumulative incidence of TRM was 34% (CI: 0.13-0.45) (Figure 3). In univariate analysis, no variable statistically affected TRM, even if a lower TRM was noted for patients receiving more than 1 x 10^4/kg CFU-GM (18% vs 44%).

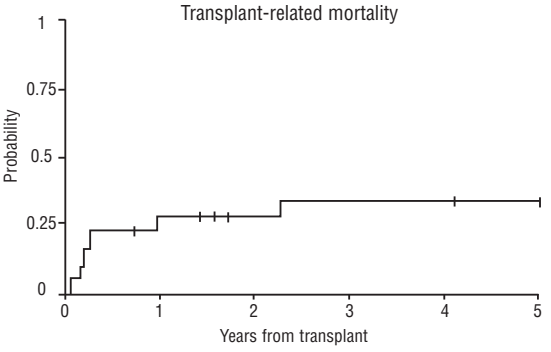

Figure 3. Cumulative incidence of transplant-related mortality.

Overall survival (OS), leukemia-free survival (LFS) and event-free survival (EFS)

The median follow-up after transplantation for the 30 patients was 19 months (range 1-124) and 58 months (range 8-124) for the 14 (47%) patients alive and disease-free with full donor chimerism. The 9-year probabilities of OS, LFS and EFS were 42% (CI: 0.52-0.93), 47% (CI: 0.25-0.61) and 46% (CI: 0.33-0.61), respectively. In univariate analysis, the only factor that favorably affected OS was the CFU-GM dose (low 16% vs intermediate 36% vs high 63%; p=0.05) (Figure 4). The CFU-GM dose also significantly influenced LFS (low 22% vs intermediate 54% vs high 64%; p=0.04) and EFS (low 22% vs intermediate 36% vs high 71%; p=0.04) (Figures 5 and 6).

Discussion

In the unrelated CBT setting, several factors have been significantly associated with patients' outcome. Most studies are, however, based on retrospective, multicenter analyses and do not detail results of allogeneic transplant for ALL

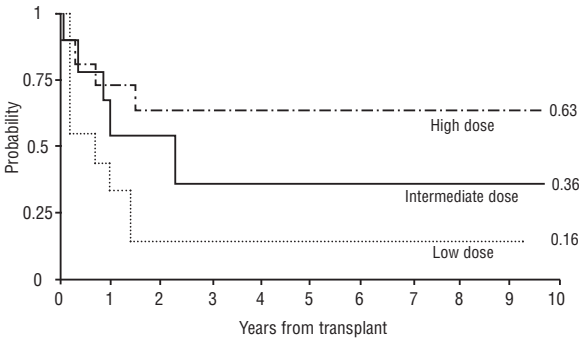

Figure 4. Overall survival according to CFU-GM dose.

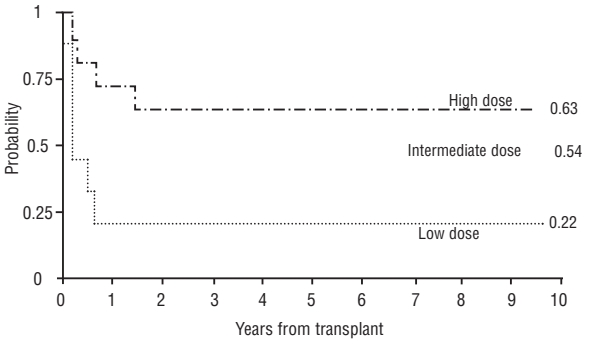

Figure 5. Leukemia-free survival according to CFU-GM infused.

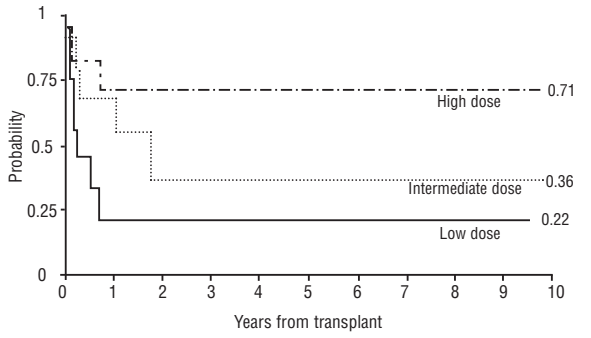

Figure 6. Event-free survival according to CFU-GM infused.

patients. More in particular, few studies[28,29,41,43] have reported results of CBT in patients with ALL and only two are pediatric, single center studies.[28,29] The results of hematopoietic recovery and engraftment in our single center series compare well with those reported from other studies. At 90 days after transplant, all but one evaluable patients showed hematopoietic recovery which was of full donor origin in 84% of cases. Patients' age and weight, leukemia type, disease status at transplant, degree of HLA mismatching and infused cell dose have been variably identified in some analyses,[19,22,23,25-27,38,39] but not in others,[20,40] as capable of influencing engraftment. In particular, the cell dose, expressed as total NC[19,22] or as CD34$^+$ cells[25] or progenitor cells[39] has emerged as the main factor correlated with a successful engraftment. Our experience shows that the content of CFU-GM infused significantly affects myeloid engraftment both in univariate and multivariate analyses, as previously observed by our group in a non-homogeneous series of patients.[027] This is in agreement with findings observed in the allogeneic and autologous bone marrow transplant setting,[44,45] as well as in two larges CBT series of patients with various hematologic malignancies.[39,46] Interestingly, it has been reported that, in the setting of double CBT, sustained hematopoiesis is usually derived from a single donor;[47,48] these studies also documented that in 93 patients who received double CBT using reduced-intensity conditioning regimens, the prevailing unit had a higher CFU-GM content regardless of the infused total number of nucleated or CD34$^+$ or CD3$^+$ cells.[47,48] It is noteworthy that all five patients treated with G-CSF starting at day +7 after the transplant recovered full donor hematopoiesis faster than the group that did not receive G-CSF (median days 14 vs 30; p=0.004). These results are in agreement with previous experiences showing the favorable effect of the prophylactic use of the hematopoietic growth factor.[26,30] However, randomized studies are required to confirm this finding conclusively. We found ABO incompatibility to represent a significant negative factor for myeloid engraftment, both in univariate and multivariate analyses, as observed in children undergoing CBT from a related donor.[49] Moreover, a CMV negative status pre-transplant favorably affected engraftment; the reduced risk of CMV-positive antigenemia in this group of patients may diminish the hematopoietic toxicity of the antiviral drugs.[50]

To prevent GVHD, a standard regimen of cyclosporine A was combined with low dose 6-methylprednisolone. The cumulative incidence of grade II-IV and III-IV acute GVHD by day 100 after transplant was 30% and 7%, respectively. The overall incidence of chronic GVHD was 33%. These incidences compare favorably with those reported by other studies in unrelated CBT patients.[19,22,23,25,26,28,31,40] Although patients were mostly transplanted with an unrelated HLA mismatched cord blood unit, the incidence of GVHD was considerably lower than that usually observed in children grafted from an unrelated HLA matched bone marrow donor. We believe that our policy of cord blood selection with no more than two HLA disparities with the recipient, the use of anti-lymphocyte globulin as part of the conditioning regimen and the modality of 6-methylprednisolone administration combined with cyclosporine A, even without methotrexate, can explain the low incidence of acute GVHD in our series. Furthermore, HLA disparities in our donor-recipient combinations did not correlate with GVHD incidence, as in other experiences.[19,22,23] Relapse occurred in seven patients (five transplanted with very

high-risk disease) for a cumulative incidence of 31% at 9 years. Despite the low incidence of GVHD, the graft-versus-leukemia effect does not seem to be impaired after CBT. Indeed, case-controlled studies in children comparing unrelated CBT with unmanipulated or T-cell depleted unrelated bone marrow transplant have reported similar relapse rates.[31,32] As expected, patients with very high-risk disease at transplantation had a significantly higher rate of relapse than those with high-risk disease (63% vs 20%; p=0.01). Sixteen patients died (53%). Relapse was the cause of death in seven cases and nine patients died of transplant-related complications. In only two of them was acute GVHD the cause of death. Despite the late hematopoietic recovery, mortality due to infective complications was low (two patients died of invasive aspergillosis). Outcome parameters of these 30 patients who underwent CBT were compared to those of 40 children with high-risk ALL who received an allogeneic bone marrow transplant from their HLA identical siblings at our center from 1986. The two groups were comparable for age, diagnosis and disease stage. OS and DFS were similar in the two groups (40% vs 42% and 42% vs 44%, respectively), whereas an advantage was observed for related donor transplants in terms of TRM (16% vs 34%; p=0.07) and for CBT in terms of a lower relapse rate (35% vs 50%; p=0.02). Only few children with high-risk ALL received a transplant from an unrelated volunteer donor at our center and a comparison with other transplants was not possible. Taken together, our data confirm the role of cord blood, comparable to that of bone marrow from related and unrelated donors, as a suitable source of stem cells for the allogeneic transplantation of children with high-risk ALL.[28,31,32]

In several studies, including a single center experience in children with ALL, the NC dose represented the principal variable predicting transplant outcome.[19,20,22,25,29,39] In our analysis only the CFU-GM dose influenced all outcome parameters, despite the significant correlation between the number of $CD34^+$ cells and total NC infused. It is likely that the impact on engraftment affects survival. We were not able to demonstrate a statistically significant impact on TRM, but TRM was lower for patients who received more than 1×10^4 CFU-GM/Kg (18% vs 44%). We can argue that the standardized method used for the count of CFU-GM and the uniformity of our population enabled the proliferative potential of the CFU-GM to emerge as a prognostic factor for patients' outcome. However, it should be underlined that our study provides data on the growth of cells after thawing and that the comparison of the hematopoietic growth of cord blood units before freezing and after thawing has not been properly investigated to date.

In conclusion, our experience confirms the role of CBT for the cure of children with high-risk ALL and contributes to suggest that the principle *more is better*, referring to the quantity of total cells infused, should be extended to the graft quality estimated in terms of *in vitro* CFU-GM growth. Consequently, this indicator of proliferative potential should be considered in the selection of a cord blood unit. However, at present the limited availability of this information from the cord blood banks and the absence of standardized methods for the *in vitro* measurement of CFU-GM do not allow this parameter to be adopted in driving the cord blood search.

Authors' Contributions

API, WA, RF: conception and design of the study, analysis and interpretation of data, drafting the article, final approval of the version to be published; EC: statistical analysis; FM: acquisition, analysis and interpretation of data; GFT, WB, MLM, BL, LM, EI, RR: care of patients and data acquisition; MS, MGM, MPP, LL, SS: involvement in cord blood unit selection and article revision. All authors approved the final version submitted for publication.

Conflict of Interest

The authors reported no potential conflicts of interest.

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