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Engraftment kinetics and graft failure after single umbilical cord blood transplantation using myeloablative conditioning regimen

Running title: Engraftment kinetics after single UCBT

Annalisa Ruggeri [1,2,3], Myriam Labopin[2], Maria Pia Sormani[4], Guillermo Sanz[5], Jaime Sanz[5], Fernanda Volt[1], Gerard Michel[6], Franco Locatelli[7], Cristina Diaz De Heredia[8], Tracey O'Brien[9], William Arcese[10], Anna Paola Iori[11], Sergi Querol[12], Gesine Kogler[13], Lucilla Lecchi[14], Fabienne Pouthier[15], Federico Garnier[16], Cristina Navarrete[17], Etienne Baudoux[18], Juliana Fernandes[1], Chantal Kenzey[1], Mary Eapen[19], Eliane Gluckman*[1, 20], Vanderson Rocha*[1,21] and Riccardo Saccardi*[1,22] on behalf of Eurocord, Cord Blood Committee EBMT and Netcord

1. Eurocord, Hospital Saint Louis, AP-HP, and IUH University Paris VII, Paris, France
2. Hospital Saint Antoine, Service d'Hématologie et Thérapie Cellulaire, AP-HP, UPMC Univ Paris 06, UMR-S 938, CEREST-TC EBMT, Paris, France
3. Cord Blood Committee EBMT
4. Biostatistics Unit, Department of Health Sciences (DISSAL), University of Genova, Italy
5. Hospital Universitario y Politécnico La Fe, Valencia, Spain
6. Hôpital d'Enfants de la Timone, Marseille, France
7. Dipartimento di Oncoematologia Pediatrica, Ospedale Bambino Gesù, IRCSS, Roma, Università di Pavia, Italia
8. Servicio de Hematologia y Oncologia Pediátricas, Hospital Universitario Vall d'Hebron, Barcelona, Spain Servicio de Hematologia y Oncologia Pediátricas, Hospital Universitario Vall d'Hebron, Barcelona, Spain
9. Sydney Children's Hospital, Sydney, Australia
10. Rome Transplant Network, University Tor Vergata, Rome, Italy
11. Università La Sapienza, Dip. Biotecnologie Cellulari ed Ematologia, Rome, Italy
12. Barcelona Cord Blood Bank, Barcelona, Spain
13. Dusseldorf Cord Blood Bank, University of Dusseldorf, Dusseldorf, Germany
14. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano Italia
15. Besançon Cord Blood Bank of the Etablissement Français du Sang, Besançon, France
16. Agence de la Biomedecine, Paris, France
17. NHS-Cord Blood Bank, NHSBT, Colindale Ave,, and University College London London, UK
18. Liege Cord Blood Bank, University of Liege, Belgium
19. Department of Medicine, Medical College of Wisconsin, Milwaukee, WI, USA
20. Monacord, Centre scientifique de Monaco, Monaco
21. Churchill Hospital, Oxford University Hospitals, Oxford, United Kingdom
22. Careggi University Hospital, Firenze, Italy

EG, VR and RS are co-senior authors

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Correspondence

Annalisa Ruggeri, MD, Eurocord, Hospital Saint Louis 1 av Claude Vellefaux,

75010 Paris France. Email: annalisa.ruggeri@sls.aphp.fr

Abstract

Umbilical cord blood transplantation recipients are exposed to an increased risk of graft failure, a complication leading to higher transplantation-related mortality. The decision and timing to offer a second transplant after graft failure is challenging. With the aim of addressing this issue, we analyzed engraftment kinetics and outcomes of 1268 patients (73% children) with acute leukemia (64% acute lymphoblastic leukemia, 36% acute myeloid leukemia) in remission who received single-unit umbilical cord blood transplantation after myeloablative conditioning regimen. Median follow-up was 31 months. Overall survival) at 3-year was 47%; 100-day cumulative incidence of transplant related mortality was 16%. Longer time to engraftment was associated with increased transplant related mortality and lower overall survival. Cumulative incidence of neutrophil engraftment at day-60 was 86%, median time 24 days. Probability density analysis showed that the likelihood of engraftment after umbilical cord blood transplantation increased after day+10, peaked on day+21 and slowly decreased to 21% on day+31. Beyond day+31, the probability of engraftment dropped rapidly, and the residual probability to engraft after day+42 was 5%. Graft failure was reported in 166 patients, and 66 of them received a second graft (allogeneic, n=45). Rescue actions, such as the search for another graft, should be considered starting after day+21; diagnosis of Graft failure can be established for patients not achieving neutrophil recovery by day+42. Moreover, subsequent transplants should not be postponed after day+42.

Introduction

Umbilical cord blood (UCB) is an alternative option to standard graft sources for hematopoietic stem cell transplantation (HSCT), and it has been successfully used in both children and adults. Several studies (1-3) comparing results of unrelated cord blood transplantation (UCBT) and either bone marrow (BM) or peripheral blood (PB) stem cell transplant, showed similar results in terms of overall survival (OS) and leukemia free survival (LFS), in spite of a slower hematopoietic recovery and a higher incidence of graft failure (GF) for UCBT recipients. Possible reasons for delayed/failed engraftment include the low stem cell content of UCB units, a higher degree of HLA disparity in the donor/recipient pair and poor T-cell function after UCBT, leading to a high rate of infections in the early post-transplant period. GF is a life-threatening complication of all kinds of HSCT and it occurs more frequently after UCBT than with other standard graft sources.(3) Some authors have reported an overall incidence of GF after UCBT between 10 and 20%.(3, 4) GF increases transplant-related mortality (TRM) due to the prolonged period of aplasia when the recipient is at a higher risk of infection and hemorrhage. Importantly, treatment of GF is not standardized.(5, 6) Either autologous rescue or second HSCT from a related or unrelated donor (UD) can be considered, depending on the availability of the additional graft and the needs of the individual patient. While autologous rescue is immediately available, allogeneic graft procurement takes time; therefore the decision and timing to initiate the search for the new graft and to proceed with the second transplant is of critical importance. A delayed response on the part of the treating physician to initiate donor search in anticipation of second transplantation may result in the occurrence of fatal complications. Conversely, proceeding to second transplantation too early will counteract the residual chances for engraftment from the primary transplant. Second HSCT performed as rescue of GF is associated with poor prognosis.(7, 8) The timing of rescue transplantation varies between transplant centers and the transplanting physician's experience and possibly bias. Therefore the availability of an evidence-based strategy based on the probability of engraftment at various time points after UCBT is desirable to determine optimal timing of second transplantation. The probability of engraftment after transplantation follows the distribution of a sinusoid curve. Further, delayed and/or lower engraftment probabilities are associated with higher TRM. In order to develop an evidence-based strategy to facilitate decision-making and timing of second transplantation, we analyzed engraftment kinetics and clinical outcomes of patients who underwent unrelated UCBT after a myeloablative conditioning regimen (MAC).

Methods

The study included all patients (n=1268) with a diagnosis of acute leukemia in complete remission, transplanted with a single, unrelated UCB unit following a MAC between 1994 and 2011 at EBMT centers and reported to Eurocord. The Institutional Review Boards of the Eurocord-Netcord scientific committee approved this study.

Definitions and Endpoints

Adults were defined patients with 18 years of age or more. MAC was defined as a regimen containing either total body irradiation (TBI) with a dose greater than 6 Gy, a dose of oral busulfan greater than 8 mg/kg, or a dose of intravenous busulfan greater than 6.4 mg/kg. Neutrophil engraftment was defined as an absolute neutrophil count (ANC) greater than 0.5 x 10^9/L for three consecutive days. HLA compatibility was defined at antigen level for HLA-A and -B loci and at allelic level for HLA-DRB1 locus. Full donor chimerism was defined as >95% of donor cells and mixed chimera between 5% and 95% of donor cells. Methods of chimerism analysis varied among transplant centres. Graft failure was defined as failure to achieve an ANC greater than 0.5 x 10^9/L or as achievement of ANC greater than 0.5 x 10^9/L without evidence autologous reconstitution. Transplant-related mortality (TRM) was defined as death in remission, and it was considered the competing event for engraftment. OS was defined as the probability being alive, regardless of disease status, at any time point; surviving patients were censored at last follow-up, while only death was considered an event. Leukemia-free survival (LFS) was defined as the probability of being alive and disease free at any time point; both death and relapse were considered events, and patients who were alive and leukemia-free were censored at last follow-up.

Statistical analysis

The probabilities of OS and LFS were calculated using the Kaplan-Meier method and the log-rank test for univariate comparisons.(9)

The probability of neutrophil engraftment was investigated through both the conditional probability and the probability density. The probability density function for neutrophil engraftment was estimated differentiating the cumulative incidence (CI) engraftment curve, therefore describing the probability to engraft at each time point from UCBT, and taking in consideration competing events, such as early deaths. The conditional probability is the probability of neutrophil engraftment at each time point from UCBT, on condition of having still not engrafted at that specific time point, and it is estimated as the ratio between engrafted patients within each time interval and patients at risk entering that interval. In this study, time intervals of five days were chosen. The overall incidence of GF and TRM were calculated with the CI estimator.

The following variables were tested in univariate analyses: age at UCBT, type of leukemia, disease status, year of UCBT, CMV sero status, HLA compatibility, ABO compatibility, total nucleated cell (TNC) count at cryopreservation, use of TBI, and use of ATG. The time to engraftment was used as time-dependent covariate for TRM. TNC count was analysed as continuous variable given the proportional increase of TNC count with the age of the recipient.(10, 11).

Multivariate analyses adjusted were performed using Cox proportional hazards regression model. All factors associated with a p value less than 0.10 by univariate analysis were included in the model. Then a stepwise backward procedure was used with a cut-off significance level of 0.05 for deleting factors in the model. All tests are two-sided. The type I error rate was fixed at 0.05 for determination of factors associated with time to event outcomes. Statistical analyses were performed with SPSS 19 (SPSS Inc./IBM, Armonk, NY) and R 2.13.2 (R Development Core Team, Vienna, Austria) software packages.

Results

Patients and transplant characteristics are summarized in Table 1; 813 patients were transplanted for acute lymphoblastic leukemia (ALL) and 455 for acute myeloid leukemia (AML). Twelve percent of patients were HLA-matched to the UCB unit, 45% were mismatched at one HLA antigen, 40% at 2 antigens and 3% at greater than 2 antigens. The median TNC at cryopreservation was $5.2x10^7$/Kg (range 1.1-34.8). All patients received a MAC regimen, 50% TBI based and 50% Busulfan based and 87% received anti-thymocyte globulin (ATG) before the UCBT.

Engraftment and risk factor

The CI of engraftment was 64% and 86% at 30 and 60 days after UCBT, respectively. Overall 1102 patients engrafted at a median time of 24 days (range 10-116). The median time to engraft was 25 days (range 11-108) for children and 23 days (11-116) for adult recipients (p=0.6). For patients who engrafted, chimerism analysis within 100 days after UCBT confirmed full donor chimerism for 98% of patients and mixed for 2%. In the multivariate analysis (Table 2), factors independently associated with greater neutrophil engraftment were higher TNC count at cryopreservation (p<0.001), age at UCBT (p=0.001), and year at UCBT (after 2006) (p=0.002).

The conditional probability of engraftment increased starting as 8.1% by 10-15 days after transplantation, reached its peak at 33.2% by 25-30 days and thereafter rapidly declined after day 40 to 6.1%, 50-55 days after transplantation. The probability density analysis (Figure 1) shows the likelihood of engraftment after a UCBT increased after day 10, reached its peak at day 21 and decreased slowly until day 31. The likelihood of engraftment beyond day 31 was only 21% with rapid decline such that the residual probability of engraftment after day 42 was only 5%. The conditional probability of engraftment was not different for children and adult.

Graft Failure

The CI of GF at day 60 was 12%, and it was 11% for children and 12% for adults (p=0.64). One-hundred-sixty-six patients were reported to have experienced GF: of these patients 13 (0.8%) are alive with autologous reconstitution at a median of 45 months after UCBT, while 87 died without receiving any treatment for GF, (26 died before day 24 after UCBT, and 61 died untreated at a median of 51 days after UCBT (TRM, n=43; relapse, n=17)). The remaining 66 patients who experienced GF received a second graft.

Twenty one (32%) of these received an autologous back-up at a median of 45 days (range 28-88) from the first UCBT; among them, 8 engrafted and 5 were alive at the last follow-up. The remaining forty-five patients (68%) underwent a second allogeneic transplantation at a median of 52 (range 21-152) days and information on donor source was available for 42 of 45 patients. Rescue strategies included the following: 1) peripheral blood stem cells (PBSC) from haploidentical family members (n=17); 2) second unrelated UCB unit (n=17); 3) PBSC from adult unrelated donors (n=6); 4) PBSC from a matched sibling (n=1); 5) second UCB unit + PBSC from a haploidentical family member (n=1). Most of those patients received a fludarabine-based reduced intensity conditioning. Thirty-one patients (71%) engrafted at a median time of 15 days and 8 patients experienced grade II-IV aGVHD. Fifteen patients are alive at a median of 12 months after the second HSCT. The probability of OS after the second HSCT was 37±10% and 29±7% after autologous and allogeneic HSCT, respectively. No secondary graft failure was reported in our series.

TRM

Cumulative incidence of TRM was 16%, 23%, 29% and 33% at 100 days, 6 months, 12 months and 36 months, respectively. Main cause of TRM was infection (46%), followed by GvHD (22%), organ failure (16%), rejection (5%), hemorrhage (5%), interstitial pneumonia (4%) and other (2%).

In the multivariate analysis (Table 2), TRM was higher for adults (p=0.004), for those with a pre-transplant CMV positive serology (p=0.02), and for those transplanted in second or subsequent CR (p=0.001).

Impact of time to engraftment on TRM

Patients who achieved engraftment were divided in 4 categories according to the interval to engraftment as following: within 21 days, between 22 and 30 days, between 31 and 42 days and beyond 42 days from UCBT. Cumulative incidence of TRM according to time to engraftment is shown in Figure 2a. Engraftment beyond day+42 was associated with significantly higher TRM; the incidence rate of TRM was 25% for patients engrafting within 21 days, 29% for patients engrafting between 22-30 days, 30% for those engrafting between 31-42 days, and 37% beyond 42 days (p=0.07).

Relapse, LFS and OS

Median follow up was 31 months (range 3-186 months). The CI of relapse at 3 years was 30%, it was 24% for patients with AML and 37% for ALL (p=0.004). In multivariate analysis (Table 2) CR1 at time of UCBT was the only factor associated with lower incidence of relapse (p<0.001).

The probability of OS at 3 years was 47+2%, while that of LFS was 43+2%. In multivariate analysis, (Table 2) diagnosis of ALL (p=0.002), disease status ≥CR2 (p<0.001), age at UCBT (p<0.001), and pre-transplant CMV positive serology (p=0.02) were independently associated with decreased LFS.

Impact of time to engraftment on OS

For patients who achieved engraftment, engraftment beyond day 42 resulted in a lower probability of OS (Figure 2b) (OS at 3-years was 51% for patients engrafting within 21 days, 52% for patients engrafting between 22-30 days, 49% for those engrafting between 31-42 days, and 44% beyond 42 days; p=0.13).

Discussion

Cord blood transplantation is associated with delayed engraftment and GF. However, the clinical work-up including timing and the decision to initiate a donor search in anticipation of a second transplant remains an open question (12). Some transplant centers have adopted day 21 to initiate donor search for second transplants(13), others reserve a second CB unit when the first CB unit is selected for transplantation, or collect an autologous back-up,(14) and there are some who advocate the use of haploidentical donor transplantation. In this study, the median times to infuse an autologous or allogeneic second graft were 45 (28-88) and 52 (21-152) days, respectively. This information suggests the need for more robust data to define the engraftment kinetics and recommendations on optimal timing for donor search. With this aim, we conducted a registry-based study in a large cohort of over 1000 patients. We observed that the probability of engraftment peaked at 21 days after UCBT, decreased gradually until day 31 and rather rapidly thereafter, with the likelihood of engraftment being very unlikely after day 42. One could argue that engraftment kinetics may be different for children and adults, however, we calculated the probability density to engraftment separately for children and adults and there were not differences, being 5% the residual probability for engraftment after day +42 for both groups. Taken together, our data support initiating a full work-up during the 4^{th} week after UCBT, including search for viral infection such as HHV6 (15), chimerism and bone marrow examination for patients who did not engraft by that time. These results will enable physicians to counsel patients and their families and if warranted, take the next step which is to initiate a second donor search in preparation for second transplantation. For patients who fail to engraft, but achieve autologous recovery, time for performing the subsequent transplant can be more lenient, once patients are not neutropenic, and, consequently, at high risk for NRM. However, due to the high risk of disease relapse in this type of population, delaying the transplant much further need to be carefully considered.

In this study we analyzed the engraftment kinetics through the assessment of the conditional probability and the probability density. The two methods provided similar results in terms of probability trend to neutrophil engraftment. However, the conditional probability is affected by the low number of subjects at risk in the later stages of the engraftment curve; therefore probability density gives a more accurate estimation of the engraftment kinetics at the time points that are the most relevant for the present analysis. There are a number of factors that may be considered when selecting the UCB unit for the primary transplant. In our analysis, TNC dose at freezing was associated with engraftment. This variable is a major determinant for outcomes in UCBT, and Eurocord and others have demonstrated that a minimum

cryopreserved TNC dose greater than 2.5-3x10^7/Kg is required to optimize results of UCBT. One limitation of this retrospective multicenter registry based study is the lack of standardization of the chimerism methods used to evaluate engraftment. However, the difference in chimerism methods among centers would probably not impact on the engraftment definition. Among the other factors in the multivariate analysis, disease status at UCBT and the year of UCBT were both independently associated with engraftment. Advances in UCB unit selection taking into account the current knowledge about the importance of high TNC dose and better HLA matching (16, 17) (including HLA C locus and high resolution typing), and the selection of the most appropriate conditioning regimen in MAC(18) or RIC(19, 20) may account for the improvement of the results over the years. Once and if high resolution typing and HLA C locus matching became standard practice, their impact of HLA on engraftment kinetics should be analyzed in further studies.

The presence of anti-HLA antibodies in the recipient is a known contributing factor for non-engraftment after UCBT. Different studies reported an increased risk of graft failure in the presence of donor specific anti-HLA antibodies in both single and double UCBT after MAC (21) and RIC.(22) However, due the retrospective character of our study, we were not able to analyze the impact of anti-HLA antibodies in this series of UCBT recipients.

As expected, interval from transplant to engraftment influenced TRM, this being higher with increasing time to engraftment. TRM is a leading cause of treatment failure after transplantation, especially when GF occurs. In this study, of the 166 recipients who failed to engraft only 40% received a subsequent transplant. While we do not know the reasons for not offering a second transplant, we speculate clinical conditions including life-threatening infections could have been a major limitation coupled with the transplant centers having lost the optimal window to search for another donor. It is important to note that, in our series, second transplants were performed after a median time of 45 (range 28-88) (autologous) and 52 (range 21-152) (allogeneic) days after the first UCBT, indicating that a significant number of patients received a rescue procedure beyond 42 days from their first UCBT.

Survival after second transplant as rescue for primary GF ranges from 10 to 30% in different reports.(23-26) The CIBMTR (23) reported 11% survival in a large series of patients transplanted using a second unrelated donor (BM or PBSC). The median time between first and second HSCT was of 48 days. Guardiola(24) et al reported 3 year OS of 30% in 82 patients with hematological diseases. They showed that a longer time interval between GF and second HSCT was associated with lower engraftment rate and probability of survival. McCann (25) reported that a delay superior to 60 days between first and second HSCT negatively impacted the outcomes of 41 patients with aplastic anemia. Despite these results, the optimal timing to perform a second HSCT for patients with GF, has not yet been defined.

A number of strategies have been proposed to reduce the risk of graft failure after UCBT, including the use of multiple units(27), the intrabone infusion of the UCB unit(28), the co-infusion of purified stem cells from

an haploidentical family donor(29, 30), the administration of molecules facilitating stem cells homing(31), and the co-infusion of ex-vivo expanded progenitor cells(32, 33) or mesenchymal stromal cells. All the above strategies were reported to have promising results, but so far no definitive conclusion can be raised on their long-term outcome, or their reproducibility. Physicians involved in UCBT programs are frequently confronted with difficult clinical decision making for patients experiencing delayed engraftment. Some of the patients with GF will eventually recover their autologous cells, but for those who do not, the only treatment option is an additional transplant or autologous rescue.(34)

To our knowledge, this is the first study looking at the probability density of engraftment with the objective of identifying when engraftment is more likely to occur, and determining the ideal time period to perform a subsequent transplant in patients experiencing delayed engraftment or GF. The results of this study will help the transplant physician make a faster, evidence-based decision regarding the treatment of early GF and the timing of initiating a further donor search. How this result will apply in the setting of reduced intensity conditioning regimen or double UCBT need to be addressed in a different study.

In the case of autologous rescues, when cells have been previously cryopreserved, the physician may choose to proceed with the rescue as soon as the patient approaches the time window where the likelihood of engraftment is low. For patients needing a further donor, the search can be initiated early in order to proceed quickly to the subsequent transplant. The selection of the optimal donor source for a second HSCT is challenging. UCB and haploidentical donors, both offer the possibility of shortening the delay of donor procurement. In our series, the wide distribution of graft sources for the second transplant in a relatively small number of patients prevents any indications about the optimal graft source.

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References

1. Rocha V, Labopin M, Sanz G, Arcese W, Schwerdtfeger R, Bosi A, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. N Engl J Med. 2004;351(22):2276-85.
2. Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med. 2004;351(22):2265-75.

3. Eapen M, Rocha V, Sanz G, Scaradavou A, Zhang MJ, Arcese W, et al. Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. Lancet Oncol. 2010;11(7):653-60.

4. Barker JN, Scaradavou A, Stevens CE. Combined effect of total nucleated cell dose and HLA match on transplantation outcome in 1061 cord blood recipients with hematologic malignancies. Blood. 2010;115(9):1843-9.

5. Remberger M, Ringden O, Ljungman P, Hagglund H, Winiarski J, Lonnqvist B, et al. Booster marrow or blood cells for graft failure after allogeneic bone marrow transplantation. Bone Marrow Transplantation. 1998;22(1):73-8.
6. Weisdorf DJ, Verfaillie CM, Davies SM, Filipovich AH, Wagner JE, Jr., Miller JS, et al. Hematopoietic growth factors for graft failure after bone marrow transplantation: a randomized trial of granulocyte-macrophage colony-stimulating factor (GM-CSF) versus sequential GM-CSF plus granulocyte-CSF. Blood. 1995;85(12):3452-6.
7. Jabbour E, Rondon G, Anderlini P, Giralt SA, Couriel DR, Champlin RE, et al. Treatment of donor graft failure with nonmyeloablative conditioning of fludarabine, antithymocyte globulin and a second allogeneic hematopoietic transplantation. Bone Marrow Transplantation. 2007;40(5):431-5.
8. Fernandes J, Rocha V, Robin M, de Latour RP, Traineau R, Devergie A, et al. Second transplant with two unrelated cord blood units for early graft failure after haematopoietic stem cell transplantation. British Journal of Haematology. 2007;137(3):248-51.
9. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association. 1958;53 (282):457- 81.
10. Cox DR. Regression Models and Life-Tables. Journal of the Royal Statistical Society. 1972;34(2):187-220.
11. R.J. FJPG. A proportional hazards model for the subdistribution of a competing risk. Journal of the American Statistical Association. 1999;94(446):496-9.
12. Moscardo F, Sanz J, Senent L, Cantero S, de la Rubia J, Montesinos P, et al. Impact of hematopoietic chimerism at day +14 on engraftment after unrelated donor umbilical cord blood transplantation for hematologic malignancies. Haematologica. 2009;94(6):827-32.
13. Barker JN, Byam C, Scaradavou A. How I treat: the selection and acquisition of unrelated cord blood grafts. Blood. 2011;117(8):2332-9.
14. Arcese W, Rocha V, Labopin M, Sanz G, Iori AP, de Lima M, et al. Unrelated cord blood transplants in adults with hematologic malignancies. Haematologica. 2006;91(2):223-30.
15. Chevallier P, Hebia-Fellah I, Planche L, Guillaume T, Bressolette-Bodin C, Coste-Burel M, et al. Human herpes virus 6 infection is a hallmark of cord blood transplant in adults and may participate to delayed engraftment: a comparison with matched unrelated donors as stem cell source. Bone Marrow Transplantation. 2010;45(7):1204-11.
16. Eapen M, Klein JP, Sanz GF, Spellman S, Ruggeri A, Anasetti C, et al. Effect of donor-recipient HLA matching at HLA A, B, C, and DRB1 on outcomes after umbilical-cord blood transplantation for leukaemia and myelodysplastic syndrome: a retrospective analysis. Lancet Oncol. 2011;12(13):1214-21.

17. Eapen M, Klein JP, Ruggeri A, Spellman S, Lee SJ, Anasetti C, et al. Impact of allele-level HLA matching on outcomes after myeloablative single unit umbilical cord blood transplantation for hematologic malignancy. Blood. 2014; 123(1):133-40
18. Ruggeri A, Sanz G, Bittencourt H, Sanz J, Rambaldi A, Volt F, et al. Comparison of outcomes after single or double cord blood transplantation in adults with acute leukemia using different types of myeloablative conditioning regimen, a retrospective study on behalf of Eurocord and the Acute Leukemia Working Party of EBMT. Leukemia. 2014;28(4):779-86.
19. Brunstein CG, Barker JN, Weisdorf DJ, DeFor TE, Miller JS, Blazar BR, et al. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease. Blood. 2007;110(8):3064-70.
20. Brunstein CG, Eapen M, Ahn KW, Appelbaum FR, Ballen KK, Champlin RE, et al. Reduced-intensity conditioning transplantation in acute leukemia: the effect of source of unrelated donor stem cells on outcomes. Blood. 2012;119(23):5591-8.
21. Takanashi M, Atsuta Y, Fujiwara K, Kodo H, Kai S, Sato H, et al. The impact of anti-HLA antibodies on unrelated cord blood transplantations. Blood. 2010;116(15):2839-46.
22. Ruggeri A, Rocha V, Masson E, Labopin M, Cunha R, Absi L, et al. Impact of donor-specific anti-HLA antibodies on graft failure and survival after reduced intensity conditioning-unrelated cord blood transplantation: a Eurocord, Societe Francophone d'Histocompatibilite et d'Immunogenetique (SFHI) and Societe Francaise de Greffe de Moelle et de Therapie Cellulaire (SFGM-TC) analysis. Haematologica. 2013;98(7):1154-60..
23. Schriber J, Agovi MA, Ho V, Ballen KK, Bacigalupo A, Lazarus HM, et al. Second unrelated donor hematopoietic cell transplantation for primary graft failure. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2010;16(8):1099-106.
24. Guardiola P, Kuentz M, Garban F, Blaise D, Reiffers J, Attal M, et al. Second early allogeneic stem cell transplantations for graft failure in acute leukaemia, chronic myeloid leukaemia and aplastic anaemia. French Society of Bone Marrow Transplantation. British journal of Haematology. 2000;111(1):292-302. Epub 2000/11/25. PubMed PMID: 11091216.
25. McCann SR, Bacigalupo A, Gluckman E, Hinterberger W, Hows J, Ljungman P, et al. Graft rejection and second bone marrow transplants for acquired aplastic anaemia: a report from the Aplastic Anaemia Working Party of the European Bone Marrow Transplant Group. Bone Marrow Transplantation. 1994;13(3):233-7.
26. Waki F, Masuoka K, Fukuda T, Kanda Y, Nakamae M, Yakushijin K, et al. Feasibility of reduced-intensity cord blood transplantation as salvage therapy for graft failure: results of a nationwide survey of adult patients. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2011;17(6):841-51.
27. Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, McGlave PB, Miller JS, et al. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood. 2005;105(3):1343-7.
28. Rocha V, Labopin M, Ruggeri A, Podesta M, Gallamini A, Bonifazi F, et al. Unrelated cord blood transplantation: outcomes after single-unit intrabone injection compared with double-unit intravenous injection in patients with hematological malignancies. Transplantation. 2013;95(10):1284-91.
29. Liu H, Rich ES, Godley L, Odenike O, Joseph L, Marino S, et al. Reduced-intensity conditioning with combined haploidentical and cord blood transplantation results in rapid engraftment, low GVHD, and durable remissions. Blood. 2011;118(24):6438-45.
30. Bautista G, Cabrera JR, Regidor C, Fores R, Garcia-Marco JA, Ojeda E, et al. Cord blood transplants supported by co-infusion of mobilized hematopoietic stem cells from a third-party donor. Bone marrow transplantation. 2009;43(5):365-73.
31. Farag SS, Srivastava S, Messina-Graham S, Schwartz J, Robertson MJ, Abonour R, et al. In vivo DPP-4 inhibition to enhance engraftment of single-unit cord blood transplants in adults with hematological malignancies. Stem Cells and Development. 2013;22(7):1007-15.
32. de Lima M, McNiece I, Robinson SN, Munsell M, Eapen M, Horowitz M, et al. Cord-blood engraftment with ex vivo mesenchymal-cell coculture. N Engl J Med. 2012;367(24):2305-15.

33. Delaney C, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RL, Bernstein ID. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. Nature medicine. 2010;16(2):232-6.
34. Stelljes M, van Biezen A, Slavin S, Olavarria E, Clark RE, Nagler A, et al. The harvest and use of autologous back-up grafts for graft failure or severe GVHD after allogeneic hematopoietic stem cell transplantation: a survey of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplantation. 2008;42(11):739-42.

Table 1. Patient and transplant characteristics

Patient and Graft Characteristics	*n=1268*
Follow-up median (range)	31 mo (3-186)
Age at Tx median (range)	9 yrs (0.3--64)
Adult (≥ 18 years)	338 (27%)
Children (<18 years)	930 (73%)
Transplant year median (range)	2006 (1994-2011)
Diagnosis	
ALL	813 (64%)
AML	455 (36%)
Recipient CMV status	
Negative	516 (44%)
Patient weight median (range)	31Kg (4--112)
Status at transplant	
1st CR	603 (48%)
2nd CR	567 (45%)
3rd CR or higher	98 (8%)
HLA disparities	
Children, 0-1 Mismatch	495 (64%)
Adult, 0-1 Mismatch	100 (35%
TNC X10^7/Kg median (range)	
Entire population	5.2 (1.1--34.8)
Adult	3.3 (1.1-- 20.4)
Children	6.4 (0.2--41.8)
Conditioning Regimen	
Bu based	669 (50%)
CY+BU	158 (13%)
CY+TBI	238 (19%)
Bu+Fluda+Thio	231 (19%)
CY+BU ± other	195 (16%)
Cy+VP16+TBI	112 (9%)
Other TBI based	96 (8%)
Cy+Fluda+TBI	76 (6%)
Cy+Thio+TBI	74 (6%)
Other Bu based	37 (3%)
Other (ncludes treosulfan)	14 (1%)
Missing information n=37	
GVHD Prophylaxis	
CsA±Pred	887 (74%)
CsA+MMF±Pred	269 (23%)
Other	40 (3%)
Missing information n=72	
Use of ATG before day+0	990 (87%)

Abbreviations: Tx means: transplant; mo,months; yrs, years; ALL, acute lymphoblastic leukemia ; AML, acute myeloid leukemia; CMV, cytomegalovirus; Kg, kilogram; CR, complete remission; HLA, human leukocyte antigen; TNC, total nucleated cells collected; BU, busulfan; CY, cyclophosphamide; Fluda, fludarabine; Thio, thiothepa; TBI, total body irradiation; CsA, cyclosporine; Pred, prednisone; MMF, mycophenolate mofetil; ATG, antithymocyte globulin.

Table 2: Multivariate analysis

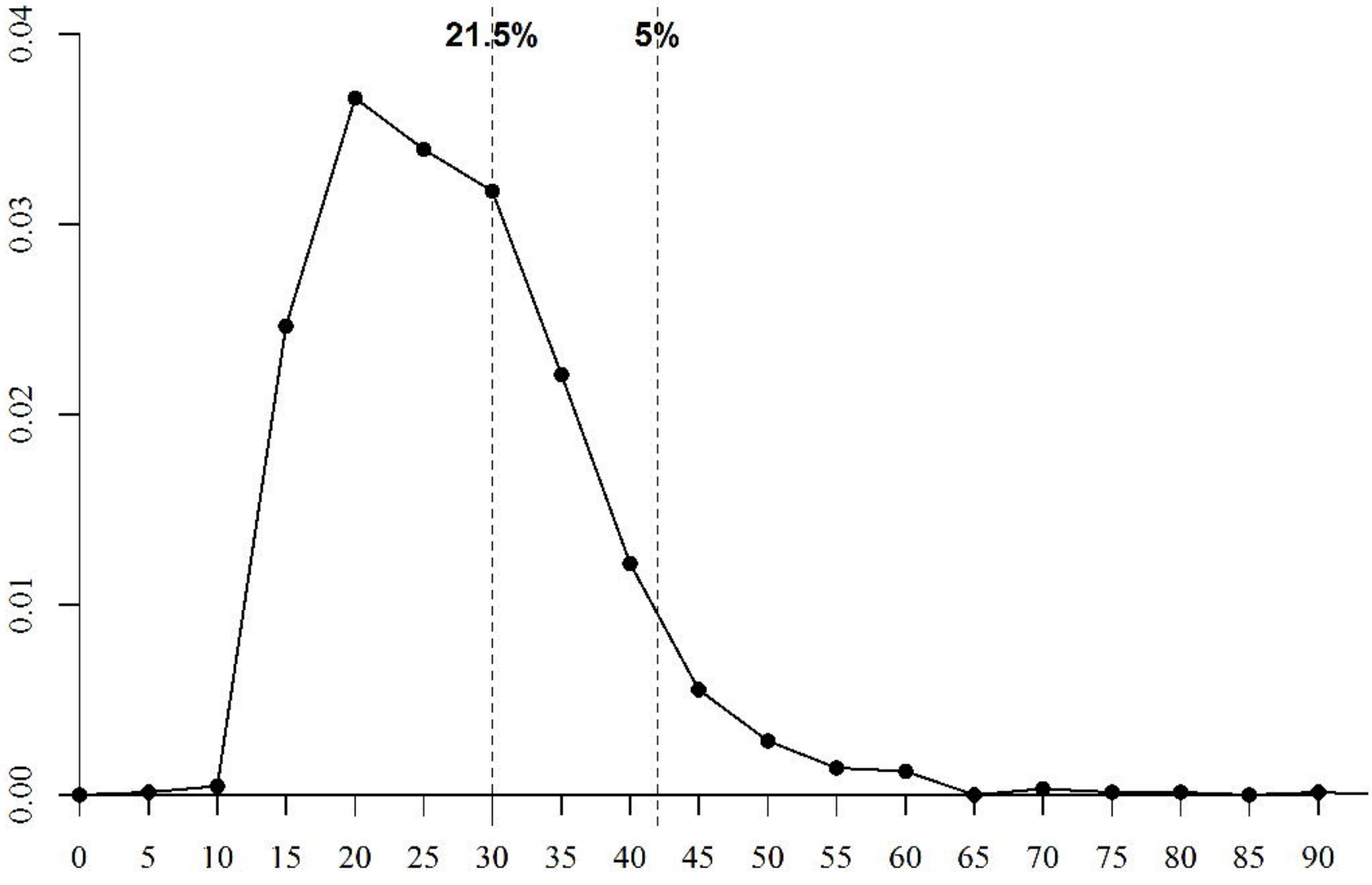
	HR	95% CI	P value
Neutrophil Engraftment			
Age at UCBT ≥ 18 years	0.75	0.63-0.89	0.001
Date of Transplantation ≥ 2006	0.97	0.95-0.99	0.002
TNC at cryopreservation ($x10^{7}$/Kg)	0.97	0.95-0.98	<0.001
TRM			
Age at UCBT ≥ 18 years	1.42	1.11-1.80	0.004
Disease status not CR1	1.47	1.17- 1.82	0.001
CMV Positive serostatus	1.28	1.03-1.60	0.02
Relapse			
Disease status CR1	0.60	0.47-0.76	<0.001
LFS			
Diagnosis of ALL	1.32	1.11- 1.58	<0.001
Disease status not CR1	1.55	1.31- 1.85	<0.001
Age at UCBT ≥ 18 years	1.40	1.16- 1.70	<0.001
CMV Positive serostatus	1.21	1.02- 1.43	0.02

Abbreviations: HR means hazard ratio; CI, confidence interval; UCBT, umbilical cord blood transplantation; TNC, total nucleated cells collected; Kg, kilogram;; CR1, first complete remission; TRM, transplant related mortality; CMV, cytomegalovirus; LFS , leukemia free survival; ALL, acute lymphoblastic leukemia.

Figure legend

Figure 1: Probability density to engraftment. The curve shows the ratio between engrafted patients and subjects at risk at each 5-days interval from UCBT. X axis- number of days elapsed since the transplantation, Y axis-cumulative incidence of engraftment

Figure 2a: Impact of engraftment time on TRM; **Figure 2b:** Impact of engraftment time on OS. Solid line means engraftment within 21 days of transplant; Dash line means engraftment between 22 and 30 days of transplant; Dot line means engraftment between 31 and 42 days of transplant; Dot-dash line means engraftment after 42 days of transplant


21.5%
5%
0.04
0.03
0.02
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0
5
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45
50
55
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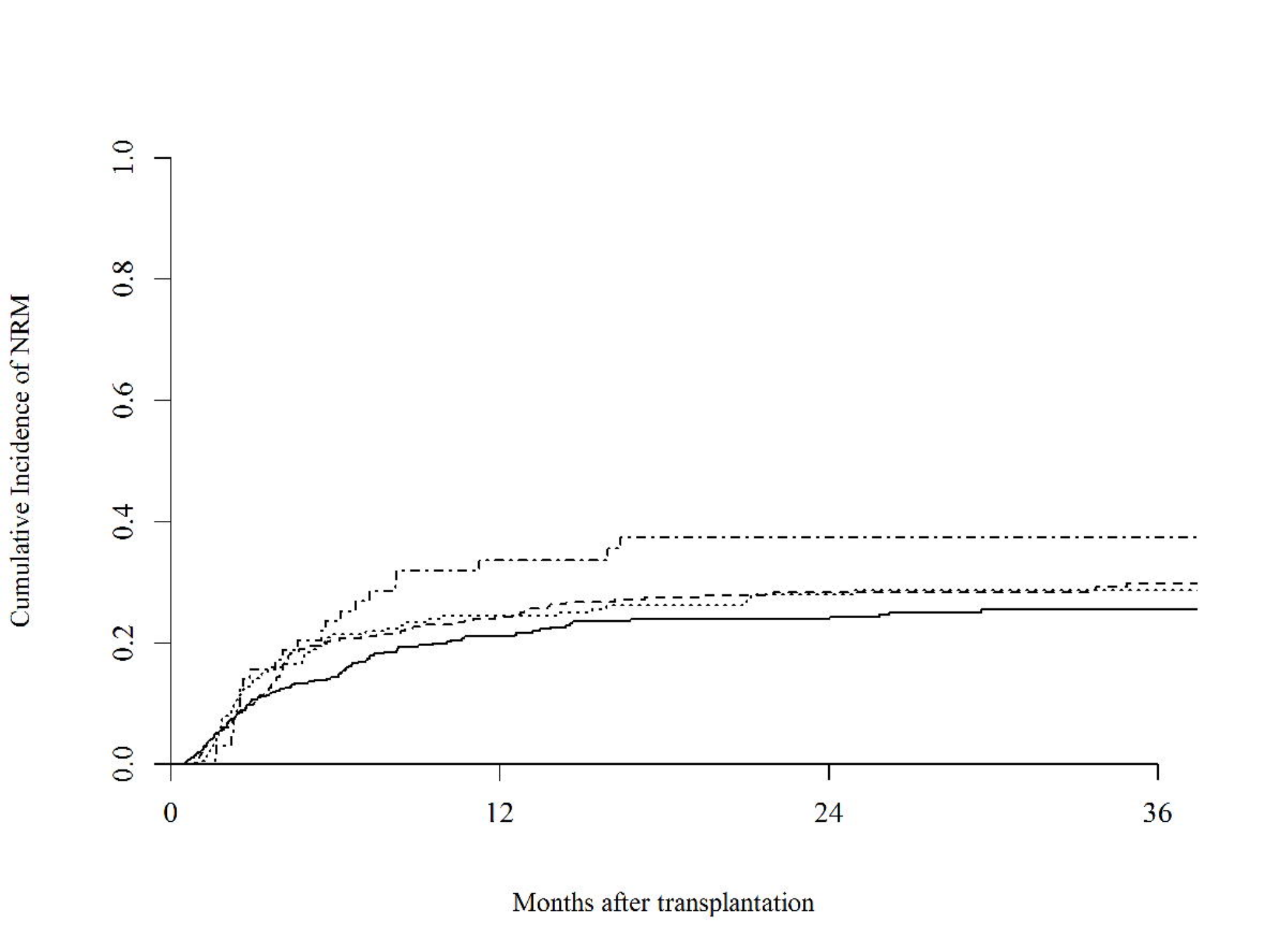
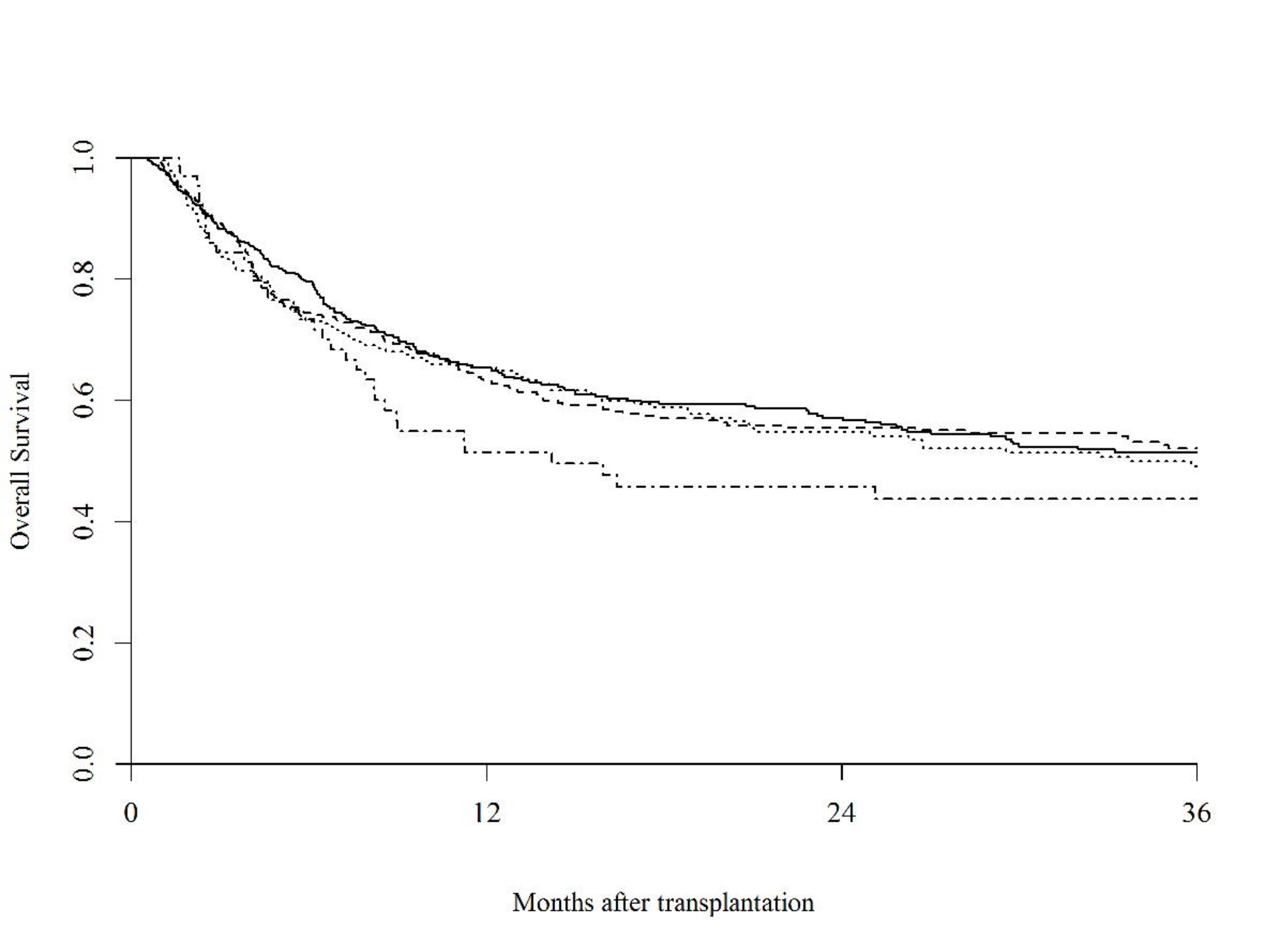
75
80
85
90


Cumulative Incidence of NRM
0.0
0.2
0.4
0.6
0.8
1.0
0
12
24
36
Months after transplantation


Overall Survival
0.0
0.2
0.4
0.6
0.8
1.0
0
12
24
36
Months after transplantation

Patients and methods

The study included all patients (n=1268) with a diagnosis of acute leukemia in complete remission, transplanted with a single, unrelated UCB unit following a MAC between 1994 and 2011 at EBMT centers and reported to Eurocord. Data on patient and donor characteristics and transplant outcomes were collected through EBMT and Eurocord databases. Consistency of data for this study was checked by two physicians to ensure quality. Missing data and supplemental questions specific to this study were requested directly to the transplant centers.

Definitions and Endpoints

MAC was defined as a regimen containing either total body irradiation (TBI) with a dose greater than 6 Gy, a dose of oral busulfan greater than 8 mg/kg, or a dose of intravenous busulfan greater than 6.4 mg/kg. HLA compatibility was defined at antigen level for HLA-A and -B loci and at allelic level for HLA-DRB1 locus. Neutrophil engraftment was defined as an absolute neutrophil count (ANC) greater than 0.5 x 10^9/L for three consecutive days. Full donor chimerism was defined as >95% of donor cells and mixed chimera between 5% and 95% of donor cells. Methods of chimerism analysis varied among transplant centres. Graft failure was defined as failure to achieve an ANC greater than 0.5 x 10^9/L or as achievement of ANC greater than 0.5 x 10^9/L without evidence of donor engraftment (autologous reconstitution). . Transplant-related mortality (TRM) was defined death in remission and considered the competing event for engraftment. OS was defined as the probability being alive, regardless of disease status, at any time point; surviving patients were censored at last follow-up, while only death was considered an event. Leukemia-free survival (LFS) was defined as the probability of being alive and disease free at any time point; both death and relapse were considered events, and patients who were alive and leukemia-free were censored at last follow-up.

Statistical analysis

Median values and ranges were used for continuous variables and percentages for categorical variables. For each continuous variable, the study population was initially split into quartiles and in two groups by the median. Patient-, disease-, and transplant-related variables of the groups were compared using Chi-square or Fischer exact test for categorical variables, and Mann-Whitney test for continuous variables. The probabilities of OS and LFS were calculated using the Kaplan-Meier method and the log-rank test for univariate comparisons.(9)

The probability of neutrophil engraftment was investigated through both the conditional probability and the probability density. The probability density function for neutrophil engraftment was estimated differentiating the cumulative incidence (CI) engraftment curve, therefore describing the probability to engraft at each time point from UCBT, and taking in consideration competing events,

such as early deaths. The conditional probability is the probability of neutrophil engraftment at each time point from UCBT, on condition of having still not engrafted at that specific time point, and it is estimated as the ratio between engrafted patients within each time interval and patients at risk entering that interval. In this study, time intervals of five days were chosen. The overall incidence of GF and TRM were calculated with the CI estimator.

The following variables were tested in univariate analyses: age at UCBT, type of leukemia, disease status, year of UCBT, CMV serostatus, HLA compatibility, ABO compatibility, total nucleated cell (TNC) count at cryopreservation, use of TBI, and use of ATG. The time to engraftment was used as time-dependent covariate for TRM. Multivariate analyses adjusted were performed using Cox proportional hazards regression model for LFS and OS, and Fine and Gray's proportional hazards regression model for engraftment, GvHD, NRM and relapse.(10, 11) Variables that reached a p-value of 0.15 in the univariate analysis were included in the initial models for multivariate analysis and variables were eliminated one at a time in a stepwise fashion in order to keep only those variables that reached a p-value of 0.05 or less in the final model. Statistical analyses were performed with SPSS version 19 (Inc., Chicago, IL) and Splus (MathSoft, Inc., Seattle, WA).