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

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ABSTRACT

Umbilical cord blood transplant recipients are exposed to an increased risk of graft failure, a complication leading to a higher rate of transplant-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 underwent single-unit umbilical cord blood transplantation after a myeloablative conditioning regimen. The median follow-up was 31 months. The overall survival rate at 3 years was 47%; the 100-day cumulative incidence of transplant-related mortality was 16%. Longer time to engraftment was associated with increased transplant-related mortality and shorter overall survival. The cumulative incidence of neutrophil engraftment at day 60 was 86%, while the median time to achieve engraftment was 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% by day 31. Beyond day 31, the probability of engraftment dropped rapidly, and the residual probability of engrafting 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. A diagnosis of graft failure can be established in patients who have not achieved 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 comparing results of unrelated cord blood transplantation (UCBT) and either bone marrow or peripheral blood stem cell transplantation showed similar results in terms of overall survival and leukemia-free survival, in spite of slower hematopoietic recovery and a higher incidence of graft failure for UCB transplant recipients.[1-3] 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.

Graft failure is a life-threatening complication of all kinds of HSCT and occurs more frequently after UCBT than after transplants using other standard graft sources.[3] Some authors have reported that the overall incidence of graft failure after UCBT is between 10% and 20%.[3,4] Graft failure increases transplant-related mortality because of the prolonged period of aplasia when the recipient is at a higher risk of infection and hemorrhage. Importantly, the treatment of graft failure is not standardized.[5,6] Either autologous rescue or second HSCT from a related or unrelated donor can be considered, depending on the availability of the additional graft and the needs of the individual patient. While autologous rescue is immediately available, procurement of an allogeneic graft takes time; therefore, the decision to initiate the search for a new graft, and the timing of doing so and proceeding with the second transplant are of critical importance. A delay by the treating physician to initiate the

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search for a donor in anticipation of a second transplant may result in fatal complications. Conversely, proceeding to second transplant too early will counteract the residual chances of engraftment from the primary transplant. Second HSCT performed as rescue of graft failure is associated with a poor prognosis.[7,8] The timing of rescue transplantation varies between transplant centers and the transplanting physicians' experience and, possibly, bias. It is, therefore, desirable to have an evidence-based strategy to determine the optimal timing of the second transplant; such a strategy should be based on the probability of engraftment at various time points after UCBT. The probability of engraftment after transplantation follows the distribution of a sinusoid curve. Further, delayed and/or lower engraftment probabilities are associated with higher transplant-related mortality. 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.

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 myeloablative conditioning regimen 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 as patients 18 years of age or more. The conditioning regimen was defined as myeloablative when it contained total body irradiation at 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 greater than $0.5x10^9$/L for three consecutive days. HLA compatibility was determined at the antigen level for HLA-A and -B loci and at the allelic level for the HLA-DRB1 locus. Full donor chimerism was defined as >95% of donor cells and mixed chimerism as between 5% and 95% of donor cells. The methods of analyzing chimerism varied among transplant centers. Graft failure was defined as failure to achieve an absolute neutrophil count greater than $0.5x10^9$/L or as achievement of an absolute neutrophil count greater than $0.5x10^9$/L without evidence of autologous reconstitution. Transplant-related mortality was defined as death in remission, and was considered as a competing event for engraftment. Overall survival was defined as the probability of being alive, regardless of disease status, at any time point; surviving patients were censored at last follow-up, and death was considered an event. Leukemia-free survival 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 overall survival and leukemia-free survival 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 conditional probability and probability density. The probability density function for neutrophil engraftment was estimated differentiating the cumulative incidence engraftment curve, therefore describing the probability of engrafting at each time point after UCBT, and taking into consideration competing events, such as early deaths. The conditional probability is the probability of neutrophil engraftment at each time point after 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 5 days were chosen. The overall incidences of graft failure and transplant-related mortality were calculated with the cumulative incidence estimator.

The following variables were tested in univariate analyses: age at UCBT, type of leukemia, disease status, year of UCBT, cytomegalovirus serostatus, HLA compatibility, ABO compatibility, total nucleated cell count at cryopreservation, use of total body irradiation, and use of antithymocyte globulin. The time to engraftment was used as a time-dependent covariate for transplant-related mortality. The total nucleated cell count was analyzed as a continuous variable given the proportional increase of total nucleated cell count with the age of the recipient.[10,11]

Adjusted multivariate analyses were performed using a Cox proportional hazards regression model. All factors associated with a *P* value less than 0.10 by univariate analysis were included in the model. A stepwise backward procedure was then used with a cut-off significance level of 0.05 for deleting factors in the model. All tests were 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, USA) and R 2.13.2 (R Development Core Team, Vienna, Austria) software packages.

Please refer to the the online version of this article for a *Supplementary Appendix* with a more comprehensive methods section.

Results

The characteristics of the patients and their transplants are summarized in Table 1; in brief, 813 patients were transplanted for acute lymphoblastic leukemia and 455 for acute myeloid leukemia. Twelve percent of patients were HLA-matched to the UCB unit, 45% were mismatched at one HLA antigen, 40% at two antigens and 3% at more than two antigens. The median total nucleated cell count at cryopreservation was $5.2x10^7$/Kg (range, 1.1-34.8). All patients received a myeloablative conditioning regimen, 50% based on total body irradiation and 50% based on busulfan, and 87% received antithymocyte globulin before UCBT.

Engraftment and risk factors

The cumulative incidences of engraftment were 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 engraftment was 25 days (range, 11-108) for children and 23 days (range, 11-116) for adult recipients (*P*=0.6). For patients who engrafted, chimerism analysis within 100 days after UCBT confirmed full donor chimerism in 98% of patients and showed mixed chimerism in the other 2%. In the multivariate analysis (Table 2), factors independently associated with greater neutrophil engraftment were higher total nucleated cell count at cryopreservation (*P*<0.001), age at UCBT (*P*=0.001), and year in which the UCBT was performed (after 2006) (*P*=0.002).

The conditional probability of engraftment increased, starting at 8.1% by 10-15 days after transplantation, reached its peak at 33.2% by 25-30 days and thereafter rap-

idly declined after day 40 to 6.1%, 50-55 days after transplantation. The probability density analysis (Figure 1) shows that the likelihood of engraftment after 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 a rapid decline resulting in a residual probability of engraftment after day 42 of only 5%. The conditional probability of engraftment was not different for children or adults.

Graft failure

The cumulative incidence of graft failure at day 60 was 12%, being 11% for children and 12% for adults (*P*=0.64). One-hundred and sixty-six patients were reported to have experienced graft failure: 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 graft failure (26 died before day 24 after UCBT, and 61 died untreated at a median of 51 days after UCBT (transplant-related deaths, n=43; relapse, n=17). The remaining 66 patients who experienced graft failure received a second graft. Twenty-one (32%) of these received an autologous back-up at a median of 45 days (range, 28-88) after the first UCBT; among them, eight engrafted and five were alive at the last follow-up. The remaining 45 patients (68%) underwent second allogeneic transplantation at a median of 52 days (range, 21-152). Information on donor source was available for 42 of these 45 patients. Rescue strategies included the following: (i) peripheral blood stem cells from haploidentical family members (n=17); (ii) second unrelated UCB unit (n=17); (iii) peripheral blood stem cells from adult unrelated donors (n=6); (iv) peripheral blood stem cells from a matched sibling (n=1); and (v) a second UCB unit + peripheral blood stem cells 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 eight patients experienced grade II-IV acute graft-*versus*-host disease. Fifteen patients are alive at a median of 12 months after the second HSCT. The probability of overall survival after the second HSCT was 37±10% and 29±7% after autologous and allogeneic HSCT, respectively. No secondary graft failure was recorded in our series.

Table 1. Characteristics of the patients and transplants.

Characteristics	n=1268
Follow-up, median (range)	31 months (3-186)
Age at transplantation, median (range)	9 years (0.3-64)
Adults (≥ 18 years)	338 (27%)
Children (<18 years)	930 (73%)
Transplant year, median (range)	2006 (1994-2011)
Diagnosis	
Acute lymphocytic leukemia	813 (64%)
Acute myeloid leukemia	455 (36%)
Recipient cytomegalovirus status	
Negative	516 (44%)
Patients' weight, median (range)	31Kg (4-112)
Status at transplant	
1st complete remission	603 (48%)
2nd complete remission	567 (45%)
3rd complete remission or beyond	98 (8%)
HLA disparities	
Children, 0-1 mismatch	495 (64%)
Adult, 0-1 mismatch	100 (35%
Total nucleated cell count x10^7/Kg, median (range)	
Entire population	5.2 (1.1-34.8)
Adults	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 (includes 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%)

*Kg: kilogram; HLA: human leukocyte antigen; BU: busulfan; CY: cyclophosphamide; Fluda: fludarabine; Thio: thiothepa; TBI: total body irradiation; CsA: cyclosporine; Pred: prednisone; MMF: mycophenolate mofetil; ATG: antithymocyte globulin; GVHD: graft-*versus*-host disease.*

Transplant-related mortality

The cumulative incidence of transplant-related mortality was 16%, 23%, 29% and 33% at 100 days, 6 months, 12 months and 36 months, respectively. The main cause of transplant-related deaths was infection (46%), followed by graft-*versus*-host disease (22%), organ failure (16%), rejection (5%), hemorrhage (5%), interstitial pneumonia (4%) and other (2%).

In the multivariate analysis (Table 2), transplant-related mortality was higher among adults (*P*=0.004), patients with pre-transplant cytomegalovirus-positive serology (*P*=0.02), and for those transplanted in second or subsequent complete remission (*P*=0.001).

Table 2. Multivariate analysis.

	HR	95% CI	P value
Neutrophil engraftment			
Age at UCBT ≥ 18 years	0.75	0.63-0.89	0.001
Year 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
Transplant-related mortality			
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
Leukemia-free survival			
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

HR: 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.

Impact of time to engraftment on transplant-related mortality

Patients who achieved engraftment were divided into four categories according to the interval to engraftment as follows: within 21 days, between 22 and 30 days, between 31 and 42 days and beyond 42 days from UCBT. The cumulative incidence of transplant-related mortality according to time to engraftment is shown in Figure 2A. Engraftment beyond day 42 was associated with significantly higher transplant-related mortality; the incidence rate of transplant-related mortality 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% for those who engrafted beyond 42 days (P=0.07).

Relapse, leukemia-free survival and overall survival

The median follow-up was 31 months (range, 3-186 months). The cumulative incidence of relapse at 3 years was 30% (24% for patients with acute myeloid leukemia and 37% for those with acute lymphocytic leukemia; P=0.004). In multivariate analysis (Table 2) first complete remission at time of UCBT was the only factor associated with a lower incidence of relapse (P<0.001).

The probability of overall survival at 3 years was 47±2%, while that of leukemia-free survival was 43±2%. In multivariate analysis (Table 2), a diagnosis of acute lymphocytic leukemia (P=0.002), disease status being second complete remission or beyond (P<0.001), age at UCBT (P<0.001), and pre-transplant cytomegalovirus-positive serology (P=0.02) were independently associated with decreased leukemia-free survival.

Impact of time to engraftment on overall survival

For patients who achieved engraftment, engraftment beyond day 42 resulted in a lower probability of overall survival (Figure 2B) (overall survival 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).

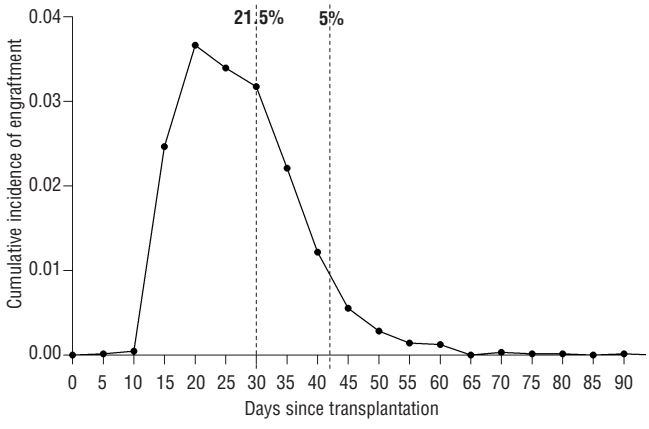

Figure 1. Probability density to engraftment. The curve shows the ratio between engrafted patients and subjects at risk for each 5-day interval from UCBT.

Discussion

Cord blood transplantation is associated with delayed engraftment and graft failure. However, the clinical work-up including the decision to initiate a donor search and the timing of doing so in anticipation of a second transplant remain unresolved issues.[12] Some transplant centers have adopted day 21 as the time to initiate the search for a donor for a second transplant,[13] others reserve a second cord blood unit when the first cord blood unit is selected for transplantation, or collect an autologous back-up,[14] and some advocate the use of haploidentical donor transplantation. In this study, the median times to infuse an autologous or allogeneic second graft were 45 days (range, 28-88) and 52 days (range, 21-152), respectively. This information suggests the need for more robust data to define engraftment kinetics and recommendations on the optimal timing for a 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 engraftment being very unlikely after day 42. One could argue that engraftment kinetics may be different for children and adults, however,

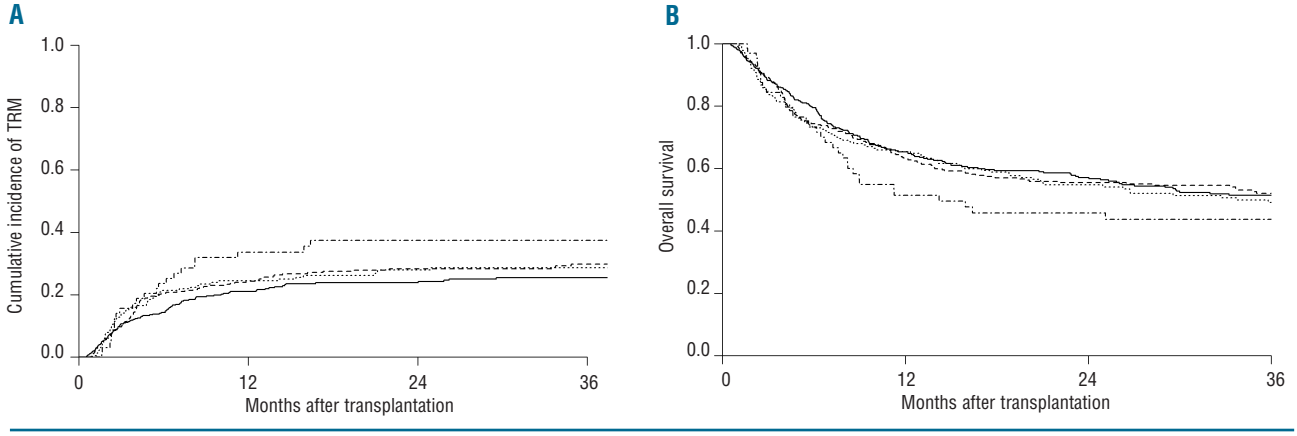

Figure 2. (A) Impact of engraftment time on transplant-related mortality (TRM); (B) Impact of engraftment time on overall survival. Solid line means engraftment within 21 days of transplantation; dashed line means engraftment between 22 and 30 days after transplantation; Dotted line means engraftment between 31 and 42 days after transplantation; Dotted-dashed line means engraftment more than 42 days after transplantation.

we calculated the probability density to engraftment separately for children and adults and did not find differences, the residual probability of engraftment after day 42 being 5% in both groups. Taken together, our data support a strategy of initiating a full work-up during the fourth week after UCBT, including a search for viral infections such as human herpes virus 6,[15] determination of the degree of chimerism and a bone marrow examination for patients who have not engrafted by that time. The results of these investigations 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 the second transplantation. For patients who fail to engraft, but achieve autologous recovery, there can be more leniency regarding the time to perform a second transplant, once patients are not neutropenic, and, consequently, at high risk of non-relapse-related death. However, due to the high risk of disease relapse in this type of population, delaying the transplant much further must be considered very carefully.

In this study we analyzed engraftment kinetics through the assessment of conditional probability and probability density. The two methods provided similar results in terms of probability trend to neutrophil engraftment. Conditional probability is, however, 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, total nucleated cell 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 total nucleated cell 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 methods used to evaluate engraftment. However, the difference in chimerism methods among centers would probably not have an impact on the definition of engraftment. Among 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 a high dose of total nucleated cells and better HLA matching[16,17] (including the HLA-C locus and high resolution typing), and the selection of the most appropriate conditioning regimen, whether myeloablative[18] or reduced intensity,[19,20] may account for the improvement in the results over the years. If and when high resolution typing and HLA-C locus matching become standard practice, the 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 to non-engraftment after UCBT. Different studies found an increased risk of graft failure in the presence of donor-specific anti-HLA antibodies in both single and double UCBT after myeloablative conditioning regimens[21] and reduced intensity conditioning.[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, the interval from transplant to engraftment influenced transplant-related mortality, this being higher with increasing time to engraftment. Transplant-related mortality is a leading cause of treatment failure after transplantation, especially when graft failure 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 that 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 days (range, 28-88) (autologous) and 52 days (range, 21-152) (allogeneic) after the first UCBT, indicating that a significant number of patients received a rescue procedure more than 42 days after their first UCBT.

The survival rate after a second transplant as rescue for primary graft failure ranges from 10% to 30% in different reports.[23-26] The CIBMTR[23] reported an 11% survival rate in a large series of patients transplanted using a second unrelated donor (bone marrow or peripheral blood stem cells). The median time between the first and second HSCT was 48 days. Guardiola *et al.*[24] reported a 3-year overall survival rate of 30% in 82 patients with hematologic diseases. They showed that a longer interval between graft failure and second HSCT was associated with a lower engraftment rate and a lower probability of survival. McCann[25] reported that a delay of more than 60 days between a first and second HSCT negatively affected the outcomes of 41 patients with aplastic anemia. Despite these results, the optimal timing to perform a second HSCT for patients with graft failure 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] intrabone infusion of the UCB unit,[28] co-infusion of purified stem cells from a haploidentical family donor,[29,30] administration of molecules facilitating stem cell 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 give promising results, but so far no definitive conclusion can be drawn on their long-term outcome or reproducibility. Physicians involved in UCBT programs must frequently make difficult clinical decisions for patients experiencing delayed engraftment. Some patients with graft failure 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 purpose 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 graft failure. The results of this study will help transplant physicians make faster, evidence-based decisions regarding the treatment of early graft failure 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 needs to be addressed in a different study.

In the case of autologous rescue, when cells have been previously cryopreserved, the physician may choose to proceed with the rescue as soon as the patient approaches the time window in which the likelihood of engraftment is low. For patients needing another 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 broad distribution of graft sources for the second transplant in a relatively small number of patients prevents us from making any indications about the optimal graft source.

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The following is a list of collaborating transplant centers (in alphabetical order of the country):

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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 Transplant. 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 Transplant. 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. Br J Haematol. 2007;137(3):248-51.
9. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958;53 (282):457-81.
10. Cox DR. Regression models and life-tables. J Royal Stat Ass. 1972;34(2):187-220.
11. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Royal Stat Ass. 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 Transplant. 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. Biol Blood Marrow Transplant. 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. Br J Haematol. 2000;111(1):292-302.
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 Transplant. 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. Biol Blood Marrow Transplant. 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 Dev. 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 Med. 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 Transplant. 2008;42(11):739-42.