# Unrelated Cord Blood Transplantation: Outcomes After Single-Unit Intrabone Injection Compared With Double-Unit Intravenous Injection in Patients With Hematological Malignancies

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**Background.** Unrelated cord blood transplantation (UCBT) is associated with delayed hematopoietic recovery. Intrabone injection of cord blood cells (IB-UCBT) and double-UCBT (dUCBT) are designed to circumvent this problem.
**Methods.** In a retrospective registry-based analysis, we compared outcomes of 87 IB-UCBT with 149 dUCBT recipients, after myeloablative conditioning regimen adjusting for the differences between the two groups. Median-infused total nucleated cells were $2.5\times10^7$/kg for IB-UCBT and $3.9\times10^7$/kg for dUCBT ($P<0.001$).
**Results.** At day +30, cumulative incidence (CI) of neutrophil recovery was 76% and 62% ($P=0.014$) with a median time to engraftment of 23 and 28 days ($P=0.001$), after IB-UCBT and dUCBT, respectively. At day +180, CI of platelets recovery was 74% after IB-UCBT, and 64%, after dUCBT ($P=0.003$). In multivariate analysis, IB-UCBT was associated with neutrophil and platelets recovery and lower acute graft versus host disease (II–IV) ($P<0.01$). At 2 years, CI of nonrelapse mortality and relapse incidence were 30% and 25% after IB-UCBT and 34% and 29% after dUCBT, and disease-free survival was 45% and 37%, respectively. However, after landmark analysis at 4.7 months from transplantation, in multivariate analysis, relapse incidence was reduced ($P=0.03$), and there was a trend for better disease-free survival after IB-UCBT ($P=0.09$).
**Conclusion.** Both approaches expand the possibility of offering UCBT to patients with hematopoietic malignancies; IB-UCBT is associated with faster myeloid and platelet recovery and lower acute graft versus host disease and may reduce the total cost. However, studies on cost effectiveness are needed to compare both strategies.

**Keywords:** Double cord blood transplantation, Single-unit intrabone injection, Myeloablative conditioning regimen.

(*Transplantation* 2013;95: 1284–1291)

This work was supported by grants from the Associazione Italiana Ricerca contro il Cancro (AIRC) (F.F.), Compagnia di San Paolo Torino (F.F.), Progetto CARIGE Cellule Staminali (FF), Progetto LIMONTE (F.F., M.P., G.M.S.), Ministero della Salute (Ricerca Finalizzata Ministeriale 2005) (F.F.), Progetto Ministero, and Progetto Ricerca Sanitaria Regionale—Regione Liguria 2009 (F.F.). This work was funded in part by the INSERM grant TGIR0805 (V.R., E.G.).
The authors declare no conflicts of interest.

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V.R., A.R. participated in making the research design, writing the paper, and analyzing the data. M.L. participated in making the research design and analyzing the data. M.P., A.G., F.B., F.M.S.-G., M.R., G.S., I.B., Ma.M., E.D., A.B., and Mo.M. provided cases for the study and edited the paper. E.G. participated in writing the paper. F.F. participated in making the research design and in writing the paper.
Received 15 October 2012. Revision requested 10 November 2012.
Accepted 18 January 2013.

ISSN: 0041-1337/13/9510-1284
DOI: 10.1097/TP.0b013e318288ca4d

Allogeneic hematopoietic stem-cell transplantation (HSCT) is a consolidated therapeutic option offered to patients with hematological diseases. The field of HSCT is rapidly evolving, and hematopoietic stem cells from different sources are routinely used, including umbilical cord blood unit (CBU). Umbilical cord blood transplantation (UCBT) offers some advantages: there is no risk for the donor, and the time to transplantation is short once a UCBT is planned because the unit is already available in the cord blood banks. Moreover, there is the possibility of performing a successful transplantation with higher degree of human leukocyte antigen (HLA) incompatibility than allowed with other stem-cell sources. Nonetheless, UCBT in adult patients has remained as a small proportion of HSCT because finding a CBU with high cell count and less than 1 to 2/6 HLA mismatches has often been a problem. Most clinicians feel that the risk of delay/failure of engraftment is too high and are reluctant to recommend the procedure. Different articles (*1–3*) comparing UCBT with matched unrelated donor transplantation showed no significant difference in outcome. The use of two CBUs to overcome the issue of the low content of hematopoietic stem cells in cord blood (CB) was received with enthusiasm, and it is now current practice in several centers (*4–6*). Nevertheless, the cost of double CBU is a concern, and engraftment failure is still approximately 15% as reported in different series (*7, 8*).

Other investigators have proposed an alternative route of administration consisting into delivery of CBU directly into the bone marrow space (*9*) (intrabone injection of cord blood cells [IB-UCBT]), with the aim of enhancing its seeding efficiency. In a rodent model (*10*), the seeding efficiency accounts for only 10% of the cells when administered intravenously (IV).

In this retrospective analysis, we compared the results of patients who received double cord blood transplant (dUCBT) with those who received IB-UCBT after a myeloablative conditioning regimen to assess the outcomes.

## RESULTS

### Patients Characteristics

Two-hundred thirty-six adults underwent either dUCBT (n=149) or IB-UCBT (n=87). Most patients had acute leukemia. Table 1 compares patients and disease and transplantation characteristics by treatment groups. IB-UCBT patients were older ($P<0.001$) and had a previous autologous graft ($P<0.001$) and positive cytomegalovirus (CMV) serology ($P<0.001$), and were ABO compatible ($P< 0.004$) more frequently than dUCBT patients. Moreover, IB-UCBT patients had more advanced disease at transplantation ($P=0.04$). The median time from diagnosis to transplantation for patients transplanted in first remission was 194 (range, 84–453) days for dUCBT and 227 (range, 140–464) days for IB-UCBT, respectively ($P=0.10$).

### Transplantation Characteristics

The median number of prefreeze total nucleated cells (TNC) was $5\times10^7$/kg (range, 2.3–27) in dUCBT and $3\times10^7$/kg (range, 1.4–8.4) in IB-UCBT ($P<0.001$). The median number of postthaw TNC was $3.9\times10^7$/kg (range, 1.14–22) in dUCBT and $2.4\times10^7$/kg (range, 0.9–7.72) in IB-UCBT ($P<0.001$). The recovery of TNC was approximately 80% postthawing, and 70% of grafts were HLA 4/6 matched (Class I-A and I-B antigen and class II-DRB1 allele matching) in both groups. Graft–versus–host disease (GvHD) prophylaxis was based on cyclosporine and mycophenolate mofetil (CsA+MMF) in 62% of dUCBT cases and 100% of IB-UCBT cases. Antithymocyte globulin (ATG) was used in all IB-UCBT and 40% of dUCBT ($P<0.0001$). All patients received myeloablative conditioning regimen. Total body irradiation was used for 75% of dUCBT versus 83% of IB-UCBT.

### Outcomes

#### Neutrophil and Platelet Recovery

At day +30, cumulative incidence (CI) of neutrophil recovery was 62% after dUCBT and 76% after IB-UCBT ($P=0.014$), and at day +60, it was 91% after dUCBT and 83% after IB-UCBT ($P=0.62$); the median time to reach an absolute nucleated cell count greater than $0.5\times10^9$/L was 28 and 23 days after dUCBT and IB-UCBT ($P=0.001$), respectively (Fig. 1A). At day +180, CI of platelets recovery greater than $20\times10^9$/L was 64% after dUCBT and 74% after IB-UCBT ($P=0.003$) with a median time to reach greater than $20\times10^9$/L of 49 and 36 days, respectively ($P=0.002$) (Fig. 1B). The CI of platelets recovery ($>50\times10^9$/L) was 69% after dUCBT and 74% after IB-UCBT at day +180 ($P<0.006$). Table 2 reports patients who died before neutrophil recovery in both groups. Eight deaths occurred in the IB-UCBT group (5 acute myeloid leukemia, 2 non-Hodgkin's disease, 1 acute lymphoblastic leukemia), in patients with advanced disease (3 intermediate and 5 advanced status) with comorbidities before transplantation.

We evaluated whether the TNC had an impact on the speed of recovery. We did not find a cutoff value of the number of TNC after dUCBT or IB-UCBT, which could be associated with outcomes.

Chimerism analysis at day +100 was available for 122 patients transplanted with dUCBT and 76 of those transplanted with IB-UCBT. Among dUCBT recipients, 107 (88%) had complete chimerism and 9 (7%) had mixed chimerism. Autologous reconstitution was reported in six cases. In recipients of IB-UCBT, 71 (93%) had complete chimerism, 1 had mixed chimerism, and 4 had autologous recovery (Table 2).

In multivariate analysis adjusting for statistical differences between treatment groups (such as disease status at transplantation, age, CMV, previous transplantations, GvHD prophylaxis), recipients of IB-UCBT had faster neutrophil recovery at day +30 (hazards ratio [HR], 1.5; 95% confidence interval [CI], 1.04–2.17; $P=0.03$) and platelet recovery greater than $20\times10^9$/L at day 180 (HR, 1.97; 95% CI, 1.35–2.29; $P=0.0004$) compared with dUCBT recipients.

#### Acute and Chronic GvHD

At day 100, CI of acute GvHD (aGvHD) (II–IV) was 47% and 19% ($P<0.0001$) (Fig. 2), and chronic GvHD was 35% and 38% ($P$=NS), respectively, for dUCBT and IB-UCBT.

The statistical difference in the incidence of aGvHD remained (38% vs. 19%, $P=0.03$) in a subgroup analysis considering only patients who received ATG in both treatment groups, because only 40% in the dUCBT group received ATG, compared with all patients in the IB-UCBT group. In multivariate analysis, IB-UCBT recipients had a lower incidence/severity of aGvHD (HR, 0.31; 95% CI, 0.16–0.62; $P<0.0008$) compared with dUCBT recipients.

**TABLE 1.** Patient and graft characteristics of patients transplanted with dUCBT and IB-UCBT

| | dUCBT (n=149) | IB-UCBT (n=87) | *P* |
|---|---|---|---|
| Follow-up, median (range), mo | 16 (1.6–53) | 18 (1–44) | |
| Age at transplantation, y | 30 (3.8–69) | 36 (1–59) | 0.003 |
| Weight, median (range), kg | 67 (24–110) | 70 (26–114) | 0.31 |
| Transplantation year (date) | February 2008 (6–9) | July 2008 (6–10) | 0.07 |
| Diagnosis, % (n) | | | |
| ALL | 44 (66) | 26 (23) | |
| AML | 36 (53) | 47 (40) | |
| MDS | 9 (14) | 9 (8) | |
| CML | 3 (4) | 8 (7) | |
| CLL | 0 (0) | 1 (1) | |
| NHL | 7 (10) | 7 (6) | |
| HD | 1 (2) | 2 (2) | |
| Disease status at transplantation, % (n) | | | 0.04 |
| Early disease | 36 (54) | 25% (22) | |
| Intermediate | 40 (59) | 36 (31) | |
| Advanced | 24 (36) | 39 (34) | |
| Previous transplantation, % (n) | | | |
| Previous allotransplantation | 3 (4) | 7 (6) | 0.1 |
| Previous autotransplantation | 5 (8) | 27 (23) | 0.001 |
| Recipient CMV status, % (n) | | | <0.0001 |
| Negative | 48 (72) | 17 (12) | |
| Positive | 52 (77) | 83 (60) | |
| ABO matching, % (n) | | | Global, *P*=0.004; Match vs. no, *P*=0.001 |
| Matched | 16 (22) | 37 (31) | |
| Minor mismatch | 26 (34) | 20 (17) | |
| Major mismatch | 58 (76) | 43 (37) | |
| HLA matching, % (n) | | | 0.15 |
| 6/6 and 5/6 | 28 (39) | 24 (20) | |
| 4/6 | 72 (98) | 76 (66) | |
| Infused TNC×107/kg, mean (range), n | 3.9 (1.14–22.2), 134 | 2.5 (0.9–7.72), 69 | <0.0001 |
| Conditioning regimen, % (n) | | | |
| Busulfan based | 21 (31) | 17 (15) | |
| TBI based | 75 (111) | 83 (72) | |
| Others | 4 (7) | 0 (0) | |
| GvHD prophylaxis, % (n) | | | |
| MMF based (including CsA+MMF) | 62 (92) | 100 (85) | |
| CsA (alone or combined w/ PDN) | 31 (45) | 0 (0) | |
| CsA+others | 7 (11) | 0 (0) | |
| Use of ATG before day 0, % (n) | | | <0.0001 |
| No | 60 (80) | 0 (0) | |
| Yes | 40 (53) | 100 (86) | |

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ; ATG, antithymocyte globulin CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CMV, cytomegalovirus; CsA, cyclosporine A; dUCBT, double cord blood administered intravenously; GvHD, graft versus host disease; HD, Hodgkin's disease; IB-UCBT, cord blood administered intrabone; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; NHL, non-Hodgkin's disease; PDN, prednisone; TBI, total body irradiation; TNC, total nucleated cells.

### Relapse, Nonrelapse Mortality, Overall Survival, and Disease-Free Survival

Unadjusted CI of nonrelapse mortality (NRM) and relapse incidence (RI) at 2 years were 34% and 29% after dUCBT and 30% and 25% after IB-UCBT, respectively (*P*=NS). Unadjusted 2-year disease-free survival (DFS) estimation was 37% after dUCBT and 45% after IB-UCBT (*P*=NS). Overall survival (OS) at 2 years was 45% after dUCBT and 47% after IB-UCBT (*P*=NS). The DFS and RI curves of dUCBT and IB-UCBT crossed at 4.7 months. Therefore, we performed a landmark analysis at 4.7 months after transplantation; 2-year RI was 29% after dUCBT and 19% after IB-UCBT (*P*=0.18), and 2-year DFS was 53% after dUCBT and 65% after IB-UCBT (*P*=0.31) (Fig. 3). In the multivariate analysis, RI was reduced after IB-UCBT compared with dUCBT (HR, 0.36; 95% CI, 0.14–0.93; *P*=0.03). There was

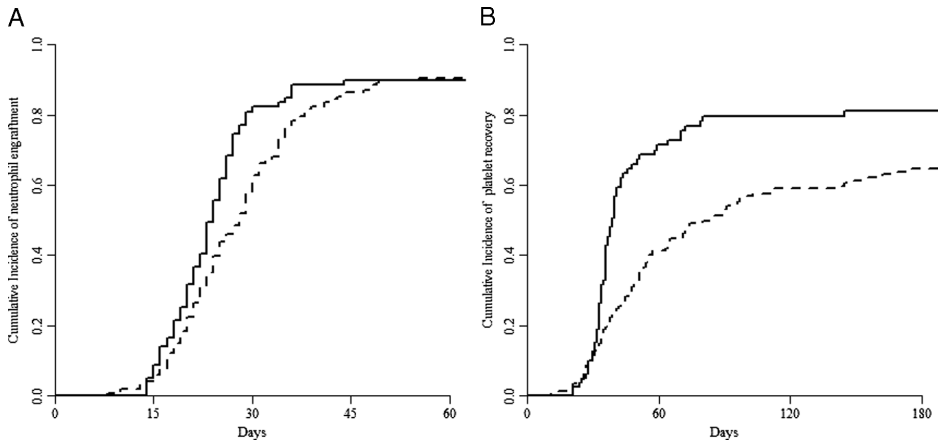


**FIGURE 1.** The cumulative incidence of engraftment in patients receiving cord blood administered intrabone (___) and double cord blood administered intravenously (– – –). A, neutrophil. B, platelet.

a trend for better DFS after IB-UCBT (HR, 1.92; 95% CI, 0.91–4.05; *P*=0.09).

According to disease status at transplant (early, intermediate, and advanced), unadjusted 2-year DFS was 45%, 31%, and 29% versus 57%, 40%, and 41% for dUCBT and IB-UCBT, respectively (*P*=NS).

One-hundred fourteen patients died after UCBT; 72 patients died of nonrelapse causes and 42 died of relapse. The causes of death were similar between the two groups and are shown in Table 3.

## DISCUSSION

The main problem of UCBT in adults compared with other hematopoietic cell sources (*3*) is delayed engraftment and graft failure. Correlation between the TNC, CD34, or colony forming unit-granulocyte/macrophage infused and

**TABLE 2.** Outcomes of patients transplanted with dUCBT and IB-UCBT

| | dUCBT (n=149) | IB-UCBT (n=87) | *P* |
|---|---|---|---|
| Neutrophil engraftment | | | |
| Events, % (n) | 91 (136) | 84 (73) | |
| No. patients who died before neutrophil engraftment at day +20, % (n) | 0.7 (1) | 9 (8) | |
| ANC, $\geq 500\times10^9$/L (day 30), mean (SD), % | 62 (4) | 76 (2) | 0.014 |
| ANC recovery, median (range), d | 28 (8–151) | 23 (14–106) | 0.001 |
| ANC, $\geq 500\times10^9$/L (day 60) , mean (SD), % | 91 (4) | 83 (2) | 0.62 |
| Platelet engraftment | | | |
| PLT, $>20\times10^9$/L (day 180), mean (SD), % | 64 (4) | 74 (5) | 0.003 |
| PLT, $>50\times10^9$/L (day 180), mean (SD), % | 69 (5) | 74 (5) | 0.006 |
| PLT recovery, median (range), d | 49 (11–193) | 36 (21–405) | 0.002 |
| Chimerism analysis at day 100, n | 122 | 76 | 0.48 |
| Full donor, % (n) | 88 (107) | 93 (71) | |
| Mixed, % (n) | 7 (9) | 1 (1) | |
| Autologous reconstitution, % (n) | 5 (6) | 6 (4) | |
| Day 100 aGvHD grade, ≥2, mean (SD), % | 47 (4) | 19 (5) | <0.0001 |
| aGvHD, % (n) | | | <0.0001 |
| Grade 0–1 | 50 (71) | 80 (61) | |
| Grade 2 | 32 (46) | 19 (15) | |
| Grade 3 | 15 (23) | 1 (1) | |
| Grade 4 | 3 (4) | 0 (0) | |
| 2-yr cGvHD, mean (SD), % | 35 (5) | 38 (6) | 0.44 |
| 2-yr relapse incidence, mean (SD), % | 29 (4) | 25 (5) | 0.72 |
| 2-yr NRM, mean (SD), % | 34 (−5) | 30 (5) | 0.97 |
| 2-yr DFS, mean (SD), % | 37 (5) | 45 (6) | 0.89 |
| 2-yr OS, mean (SD), % | 45 (5) | 47 (6) | 0.84 |

aGvHD, acute graft versus host disease; ANC, absolute neutrophil count; cGvHD, chronic graft versus host disease; DFS, disease-free survival; dUCBT, double cord blood administered intravenously; IB-UCBT, cord blood administered intrabone; NRM, nonrelapse mortality; OS, overall survival; PLT, platelet.

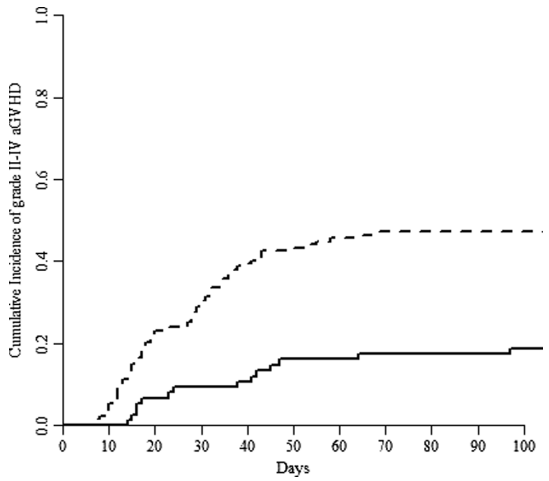


**FIGURE 2.** The cumulative incidence of day-100 acute graft versus host disease (aGvHD) in patients receiving cord blood administered intrabone (___) and double cord blood administered intravenously (– – –).

speed of myeloid recovery has been shown in several studies (*11–13*). To overcome the cell dose limit, different approaches have been developed including ex vivo expansion (*14*) and the use of two CBU (*6*).

Although the possibility of increasing the cell dose by summing two units was attractive, the comparison of engraftment between single and double UCBT showed that using two units did not substantially modify the speed and rate of engraftment (*7, 15*). Compared with single UCBT injected IV, dUCBT showed a higher rate of GvHD and a lower relapse rate (*7, 15*). The kinetics of dUCBT is still obscure: typically, dUCBT results in a period of mixed chimerism but, beyond 3 to 4 months from transplantation, the hematopoiesis is sustained by only one of the two transplanted units. The mechanism for one unit prevailing over the other remains elusive and, consequently, so does the criteria

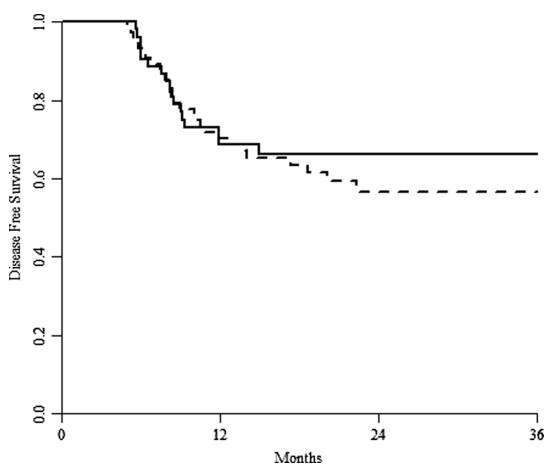


**FIGURE 3.** Landmark analysis of disease-free survival 4.7 months after transplantation in patients receiving cord blood administered intrabone (___) and double cord blood administered intravenously (- - -).

**TABLE 3.** Causes of death in patients transplanted with dUCBT and IB-UCBT

| | dUCBT (n=149) | IB-UCBT (n=87) |
|---|---|---|
| Total events, n | 72 | 42 |
| Relapse, % (n) | 40 (28) | 37 (14) |
| GvHD, % (n) | 11 (8) | 5 (2) |
| Infection, % (n) | 24 (19) | 34 (15) |
| Rejection, % (n) | 6 (4) | 0 (0) |
| VOD, % (n) | 3 (2) | 0 (0) |
| Hemorrhage, % (n) | 3 (2) | 0 (0) |
| Cardiac failure, % (n) | 1 (1) | 5 (3) |
| MOF, % (n) | 9 (6) | 16 (6) |
| Others, % (n) | 3 (2) | 3 (2) |

dUCBT, double cord blood administered intravenously; GvHD, graft versus host disease; IB-UCBT, cord blood administered intrabone; MOF, multiorgan failure; VOD, venoocclusive disease.

of donor selection (*16*). dUCBT is now widely used especially in older patients with hematological malignancies and is often in association with reduced intensity conditioning regimens (*7, 8*). However, the cost for the acquisition of two CBUs may represent some concerns (*17*).

Direct intrabone transplantation of a single unit was pioneered by the Genova group with interesting results in a phase I/II study (*9*). The rationale for using intrabone infusion of a single unmanipulated CBU derives from experiments performed in animal models (*18, 19*). These studies showed that, when given IV, most of the cells are trapped in peripheral organs, and fewer than 10% reach the bone marrow niche. Conversely, when cells are injected directly intrabone, a proportion of cells remains in the bone marrow spaces, and the cells that eventually leave the marrow and enter the circulation are able to seed into the remote bone much more efficiently than those injected into the vein. The direct interaction between hematopoietic stem cell and the marrow environment might modify the homing capacity (*20*). A phase I/II trial showed the feasibility of this procedure using a single cord blood unit injected intrabone in 32 patients with acute leukemia (*9*).

With the objective of comparing outcomes after two different approaches currently used to circumvent the engraftment problem of UCBT, we conducted a retrospective registry-based study analyzing patients transplanted with cord blood cells injected intrabone and patients receiving regular (IV) dUCBT in Europe from 2006 to 2010.

We are aware that, even using statistical tools for comparisons (adjustments for statistical differences and risk factors), as it has been done in previous studies of our group (*1, 3*), we cannot balance for all the differences between the groups, and this is a limitation of our study. Importantly, both new technologies try to circumvent the problem of engraftment in the UCBT field; further prospective studies comparing outcomes are needed.

There were some major differences between the two groups: IB-UCBT recipients were transplanted in fewer transplantation centers, had worse prognostic factors (older age, more advanced phase of disease, and positive CMV serology), and received lower number of cells, and their conditioning

regimen and GvHD prophylaxis were more homogenous than dUCBT recipients. Statistical adjustments were performed for retrospective comparison.

No center effect was demonstrated when the outcomes of centers that used IB-UCBT (n=20) were compared to those of the Genova, the center performing most of the IB-UCBT (n=67) (data not shown).

The results clearly show that patients having IB-UCBT had a faster myeloid and platelet engraftment despite receiving a median collected TNC dose significantly lower than dUCBT recipients ($2.5\times10^7$/kg and $3.9\times10^7$/kg). In our study, the difference in platelet recovery after IB-UCBT versus dUCBT was impressive (74% at 6 months compared with 64% after dUCBT), with a median time to reach greater than $20\times10^9$/L of 36 days compared with 46 days after dUCBT, reducing the need for platelet transfusion.

Interestingly, in IB-UCBT, we did not find any correlation between TNC dose and speed of polymorphonuclear leukocyte and platelet recovery, thus suggesting that the intrabone technique may be able to overcome the cell dose threshold which represents a major source of concern when considering UCBT as a useful and safe transplant option.

Curiously, also in dUCBT, no correlation between TNC and speed of hematopoietic recovery was found, suggesting that the kinetics of transplantation of two CBUs is complex and difficult to be dissected (*5, 13*).

We could speculate that our finding that IB-UCBT was associated with faster myeloid recovery is related to better homing of CB; however, despite the findings in mice (*18–20*), in humans, a randomized study using bone marrow cells administered via IV compared with intrabone injection has shown no advantage of this route in terms of engraftment (*21*). One may be aware that biologic properties and quantity of cord blood cells are different with those of bone marrow cells, and this could possibly be related with better homing of cord blood cells injected intrabone.

Another important finding is the decreased incidence of aGvHD after IB-UCBT compared with dUCBT, consistent with an early report of IB-UCBT (*9*). The immediate contact with osteoblasts or mesenchymal stem cells has been suggested to be a possible reason for the decreased aGvHD, but there is not yet a consensus about this issue.

A possible explanation for lower aGvHD in this study could have been that only a proportion (40%) of patients receiving dUCBT received ATG. To adjust for this, we made a separate subgroup analysis considering only patients receiving ATG, but the difference in aGvHD incidence and severity persisted. One could argue the reason that some of the dUCBT patients have received ATG in the conditioning regimen. In fact, no statistical difference was found in disease and transplant characteristics, among patients transplanted with dUCBT receiving or not receiving ATG in the conditioning regimen; therefore, we may speculate that the use or not use of ATG was mainly caused by the different transplantation protocols and transplantation center policies.

Different studies on dUCBT have reported an increase of acute grade II–IV GvHD as compared with single-unit UCBT; however, it was not found to be associated to higher mortality, but rather, to an increased graft versus leukemia because a reduced relapse incidence was registered as compared to single-unit UCBT (*22, 23*). The fact that the transplantation of two (somewhat HLA disparate) CB units is associated with a lower relapse rate is a relevant finding and deserves further attention and longer follow-up.

Some factors might contribute to the low incidence of aGvHD in the IB-UCBT recipients. First, lymphocyte trafficking is known to be a crucial factor in immunity (*24*). The possibility exists that only a proportion of transplanted T cells will reach the lymphatic organs, where they would be immediately confronted with host antigen-presenting cells, as probably occurs after IV injection. Secondly, injected T cells immediately come into contact with mesenchymal stem cells and osteoblasts in the marrow spaces (niches), which are known to have an important immunosuppressive effect (*25–28*). A decreased incidence of GvHD has also been reported in animal models in which the intrabone technique has been used compared with IV injection (*29*). Lastly, in the rat model, the hematopoietic cells transplanted directly into the bone home (in the early phase) less efficiently than when injected IV (*20*).

In this study, we found no statistical difference in overall DFS between the two approaches. However, the DFS and RI curves of dUCBT and IB-UCBT crossed at 4.7 months. Therefore, we performed a landmark analysis from 4.7 months after UCBT; we found a trend of improved DFS, related to a decreased relapse incidence after 4.7 months after IB-UCBT compared with dUCBT (Fig. 3). These results are very intriguing because we could expect a higher graft versus leukemia effect after dUCBT, but probably a lower NRM after IB-UCBT because of better platelet recovery (already described as a predictor of better survival) (*30*). We would like to emphasize that one cannot ignore that the inclusion of many patients with advanced disease in UCBT series may hide the possibility to disclose differences in outcomes when comparing different approaches, and this result should be analyzed with cautions caused by the relatively small number of patients and the still-short follow-up time. Our aim was to show the results of two different approaches to circumvent the engraftment problem of UCBT. The choice of the strategy will depend on the patient's and transplantation center's preferences.

One could argue that the total costs of IB-UCBT could be lower because of the lower price of graft acquisition and the probably lower number of platelet transfusions; however, only specific studies on costs comparing both approaches can confirm this speculation.

Both dUCBT and IB-UCBT represent valuable options in UCBT, and they expand the possibility of therapy for patients for whom the transplantation represents the only chance of survival. These approaches may open new avenues of research aimed to dissect the fate of the transplanted cells and, ultimately, substantially contribute to the understanding of the mechanism of success or failure of future cell therapies. However, only prospective studies with homogenous conditioning regimen and GvHD prophylaxis can establish which approach is associated with definitive better outcome.

## MATERIALS AND METHODS

EUROCORD is an international registry operating on behalf of European Bone Marrow Transplantation group (EBMT). Clinical outcome data are collected by questionnaires or by the electronic EBMT data management system ProMISe. All patients, receiving a myeloablative conditioning, transplanted from January 2006 to March 2010 with either an IB-UCBT or a

dUCBT, were included in this study. Stage of disease was defined according to the International Bone Marrow Transplant Registry study (*31*). One-hundred forty patients were transplanted with dUCBT, and 87, with IB-UCBT. Double UCBT was performed in 56 EBMT centers, whereas IB-UCBT, in eight EBMT centers.

## Transplantation Procedures

Double CBUs were selected, when a single CBU with an adequate number of cells was not available (*5, 11*). The selection of cord blood units, according to HLA typing, followed the current practice of low-resolution typing for HLA-A and HLA-B and high-resolution typing of HLA-DRB1. CBU had to be 4-6/6 HLA-A, HLA-B, and HLA-DRB1 matched to the recipient and to the other CBU. HLA disparities between each unit and the recipient, and between the two units, were not necessarily at the same loci (*11, 32*). CBUs were thawed and infused via the central venous access.

To perform the intrabone infusion, a single CBU was thawed in a 37°C water bath and washed to remove dimethyl sulfoxide. Cord blood cells were resuspended in 20 mL of saline solution plus dextran and albumin, and aliquoted in four 5-mL syringes. The patients were transplanted under anesthesia consisting of short propofol sedation. The entire intrabone-injection procedure lasts for 8 to 15 min. Once sedation is established, a standard needle for bone-marrow aspiration (14 gauge) is inserted a few centimeters into the superior-posterior iliac crest; an aspiration of approximately 0.5 to 1 mL is done to assess that the needle is securely inserted into the bone-marrow cavity. Subsequently, the syringe containing 4 to 5 mL of cord blood cell suspension is gently infused. This procedure is then repeated for all the remaining aliquots at a distance of approximately 2 to 3 cm from the previous injection site, across the iliac crest (*9*). All the transplantation centers performing IB-UCBT reviewed the description of methodology and used the same technique. In some patients, the procedure was done in both the right and left superior-posterior iliac crests, whereas in other patients, the injection was done in either the left or the right superior-posterior iliac crest. The latter procedure allowed the use of the controlateral superior-posterior iliac crest to document whether the hematopoietic cells had colonized the entire hematopoietic system. No side effects, such as pain, hemorrhage, or infections, were recorded.

## Endpoints

The primary endpoints were neutrophils and platelets recovery after UCBT. Secondary endpoints were aGvHD and cGvHD, RI, NRM, DFS, and OS.

Neutrophil recovery was defined as an absolute neutrophil count of greater than $500\times10^9$/L on 3 consecutive days. Complete chimerism was defined as marrow reconstitution of donor origin of 95% or more. Platelet recovery was defined as the time needed to reach a sustained platelet count of at least $20\times10^9$/L without transfusion support for 7 consecutive days. Acute GvHD was scored according to the standard criteria (*33*). The NRM was defined as patients' death with underlying disease in complete remission. Patients were censored at the time of relapse or last follow-up. OS was measured by the time interval between the date of transplantation and the date of death from any cause or the date of the last follow-up for survivors. DFS was defined by the time interval between the date of transplantation and the date of relapse or death in complete remission, whichever occurred first.

## Statistical Analysis

Patient-, disease-, and transplantation-related variables of the two groups were compared, using chi-square or Fischer exact test for categorical variables and Mann-Whitney test for continuous variables. CI functions were used to estimate RI and NRM in a competing risks setting because death and relapse are competing together. Death was also considered as a competing event for engraftment and acute and chronic GvHD. Probabilities of DFS and OS were calculated using the Kaplan-Meier estimate. Multivariate analyses were performed using Cox (*34*) proportional-hazard model for DFS and Fine and Gray (*35*) model for CI. To consider nonproportionality, we performed a landmark analysis 4.7 months after transplantation for DFS and RI. Other outcomes, namely engraftment and acute and chronic GvHD were not analyzed by a landmark analysis.

Factors differing between the two groups in terms of distribution were included in the model. All tests are two sided with type I error rate fixed at 0.05. Statistical analyses were performed with IBM SPSS Statistics version 18 (IBM Inc., Armonk, New York) and R 2.13.2 (R Development Core Team, Vienna, Austria) software packages.

## ACKNOWLEDGMENT

*The authors thank Fernanda Volt for helping with the editing of the manuscript.*

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