



Pediatric

## Single-Unit versus Double-Unit Umbilical Cord Blood Transplantation in Children and Young Adults with Residual Leukemic Disease



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### A B S T R A C T

We previously reported in a French prospective randomized study that transplantation of 2 unrelated cord blood (UCB) units instead of 1 unit does not decrease the risk of transplantation failure but may enhance alloreactivity. Here we evaluated the influence of pretransplantation minimal residual disease (MRD) on leukemia relapse and survival after single- versus double-UCB transplantation (UCBT). Among 137 children and young adults who underwent UCBT in this randomized study, 115 had available data on MRD assessment done immediately before initiation of the pretransplantation conditioning regimen. MRD was considered positive at a level of  $\geq 10^{-4}$ , which was the case of 43 out of 115 patients. Overall, the mean 3-year survival probability was  $69.1 \pm 4.4\%$ , and it was not significantly influenced by the MRD level:  $70.7 \pm 5.4\%$  in MRD-negative ( $< 10^{-4}$ ) patients ( $n = 72$ ),  $71.1 \pm 9.4\%$  in MRD-positive patients with  $10^{-4} \leq \text{MRD} < 10^{-3}$  ( $n = 26$ ) and  $58.8 \pm 11.9\%$  in MRD-positive patients with  $\geq 10^{-3}$  ( $n = 17$ ). In the MRD-positive group, the mean risk of relapse was significantly lower in the double-UCBT arm compared with the single-UCBT arm ( $10.5 \pm 7.2\%$  versus  $41.7 \pm 10.4\%$ ;  $P = .025$ ) leading to a higher mean 3-year survival rate ( $82.6 \pm 9.3\%$  versus  $53.6 \pm 10.3\%$ ;  $P = .031$ ). This difference was observed only in patients who had not received antithymocyte globulin during their conditioning regimen. In the MRD-negative group, there was no difference between the single- and the double-UCBT arms. We conclude that even in cases of positive pretransplantation MRD, UCBT in children and young adults with acute leukemia yields a high cure rate, and that a double-unit strategy may enhance the graft-versus-leukemia effect and survival in these patients.

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### INTRODUCTION

Cryopreserved unrelated cord blood (UCB) can serve as a stem cell source for patients with acute leukemia (AL) who need hematopoietic stem cell transplantation (HSCT) and lack

an HLA-identical donor [1]. Historically, it was hypothesized that the greater the number of hematopoietic stem cells in UCB units, the better the outcome [2]. In this context, some retrospective analyses suggested a lower relapse rate after double-unit UCBT compared with single-unit UCBT [3–5]. However, in 2 prospective randomized studies conducted in the United States and France, the double-unit strategy proved ineffective at improving the overall outcome of UCBT in children and young adults with AL when a single-UCB unit had an adequate cell dose [6,7]. Although graft-versus-host disease (GVHD) was more frequent after double-unit transplantation in both studies, no enhanced graft-versus-leukemia (GVL) effect was seen in the US study, and in the French study, the GVL effect was strictly limited to patients who had not received any antithymocyte globulin (ATG) in their conditioning regimen. Of note, neither of those studies analyzed the results of randomization according to pretransplantation minimal residual leukemia disease (MRD).

The presence of MRD before transplantation is a major prognostic factor in the context of non-cord blood HSCT for AL [8–14]. Interestingly, a recently published large retrospective, single-center study reported that among patients with positive pretransplantation MRD, the probability of relapse was lower after UCBT than after HLA-matched or HLA-mismatched unrelated donor transplantation. In the UCBT group, which included a large majority of patients receiving a double-unit transplantation, the overall survival and relapse rate were similar between patients with MRD and those without MRD [15].

Here we present the results of the French single-unit versus double-unit UCBT prospective and randomized study according to pretransplantation MRD status. The main aims of this additional study were (1) to examine whether the promising results of UCBT, reported retrospectively in a single-center study of MRD-positive patients, are reproduced in our prospective and multicenter study, and (2) to investigate a potentially different effect of the single-unit versus double-unit randomization according to pretransplantation MRD status.

## METHODS

### Inclusion Criteria

In the prospective, randomized single-unit versus double-unit French study, patients were eligible if they met all of the following criteria: (1) age <35 years, (2) AL in complete remission or myelodysplastic syndrome with <20% bone marrow blasts, (3) requiring unrelated HSCT, and (4) absence of unrelated donor considered acceptable on the basis of HLA compatibility and donor availability. In addition, eligible patients had to have at least 2 UCB units that were 4/6 HLA-identical to the patient and between them, and that contained  $>3 \times 10^7$  total nucleated cells per kilogram of recipient body weight for the first UCB unit and  $>1.5 \times 10^7$  total nucleated cells/kg for the second UCB unit. The children and young adults with AL who were enrolled in that study and underwent assessment for MRD immediately before conditioning are included in the present report. This randomized study was approved by the French National Program for Clinical Research and the French National Cancer Institute and was registered at [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) (NCT01067300). All patients (or a parent/legal guardian) provided written informed consent.

### MRD Quantification and Stratification

Methods of MRD quantification differed according to primary disease and to the transplantation center's protocol. In patients with acute lymphoblastic leukemia (ALL), most pretransplantation MRD assessment was performed in bone marrow aspirates using real-time quantitative RT-PCR (RQ-PCR) of T cell receptor (TCR) and IgH gene rearrangements, with multicolor multiparameter flow cytometry assessment also used in 4 centers. In patients with acute myelogenous leukemia (AML), MRD was evaluated by RQ-PCR of leukemia-specific fusion transcripts or flow cytometry if available, or by Wilms tumor gene (*WT1*) expression, using the standardized European LeukemiaNet method [16].

The MRD level was considered positive when  $\geq 0.01\%$  (1 leukemic cell among 10,000 bone marrow mononucleated cells) and negative when undetectable or  $<0.01\%$  for RQ-PCR of TCR and IgH gene rearrangements, leukemia-

specific fusion transcripts, and flow cytometry. *WT1* expression was considered a positive MRD with  $>1$  *WT1* RNA copy for 100 abl in peripheral blood or  $>2$  *WT1* RNA copies for 100 abl in bone marrow.

### Transplantation Procedures

The transplantation procedures have been described in detail previously [7]. In brief, all patients received a myeloablative conditioning regimen with either busulfan, cyclophosphamide, and anti-thymocyte globulin (Bu-Cy-ATG) if they were age <4 years or fludarabine, 12 Gy total body irradiation (TBI) and cyclophosphamide (Flu-TBI-Cy) if older. Bu-Cy-ATG was mandatory for children age <4 years and also could be used in older patients with AML, according to the policy of the transplantation center. Donor-recipient HLA matching was assessed at low/intermediate-resolution level molecular typing for HLA-A and -B loci and at high-resolution genotyping for the HLA-DRB1 locus. HLA compatibility was expressed as the number of identical loci out of 6. All selected UCB units had to fulfill the foregoing eligibility criteria. The GVHD prophylaxis consisted of cyclosporine A and steroids after Bu-Cy-ATG and cyclosporine A and mycophenolate mofetil after Flu-TBI-Cy.

### Study Endpoints and Statistical Methods

The main outcomes investigated here were relapse risk, disease-free survival (DFS), and overall survival (OS) at 3 years. Relapse rates were calculated using the cumulative incidence function, with nonrelapse mortality as the competing risk [17]. Comparisons were made using the Fine and Gray model [18]. The probabilities of DFS and OS were calculated using the Kaplan-Meier estimator and compared using the log-rank test [19]. For this analysis, we used February 2017 as the reference date (ie, the month that patients' outcomes were locked). All probabilities were given at 3 years and are provided with 95% confidence intervals.

## RESULTS

### Study Cohort

The study flow chart is presented in Figure 1. Among 137 patients who underwent transplantation in the single-unit versus double-unit UCBT study, 115 had AL and adequate MRD assessment before initiation of conditioning (79 out of 80 patients with ALL and 36 out of 57 with AML). All of these patients were included in the analysis presented here and represent the study cohort. Among the remaining 22 patients who lacked adequate pretransplantation MRD data, 5 had myelodysplastic syndrome, 16 had AML, and 1 had ALL. Seventy-four out of the 115 patients (64 with ALL and 10 with AML) received Flu-TBI-Cy without ATG as a conditioning regimen, and the other 41 (26 with AML and 15 with ALL) received Bu-Cy-ATG. Fifty-six of the 115 patients were in the single-unit UCBT arm and 59 were in the double-unit UCBT arm.

### Pretransplantation MRD Groups

Pretransplantation MRD was estimated molecularly in 72 of the 79 patients with ALL and by flow cytometry in the remaining 7 patients. In the patients with AML, pretransplantation MRD was performed using leukemia-specific fusion transcript in 27 patients, *WT1* expression in 5 patients, and flow cytometry in 4 patients. Forty-three patients had positive pretransplantation MRD, including 4 with an MRD load  $\geq 10^{-2}$ , 13 with an MRD load  $\geq 10^{-3}$  and  $<10^{-2}$ , and 26 with an MRD load  $\geq 10^{-4}$  and  $<10^{-3}$ . The other 72 patients were considered MRD-negative, including 58 with undetectable pretransplantation MRD and 14 with a detectable but  $<10^{-4}$  MRD load. As shown in Table 1, the 2 MRD groups were well balanced in terms of sex, age at transplantation, diagnosis, hematologic pretransplantation status, conditioning regimen, transplanted cell dose, graft HLA compatibility, and allocation to the single-unit or the double-unit arm of the randomized study.

### Overall Results According to Pretransplantation MRD Quantification

The 3-year survival probability was  $69.1 \pm 4.4\%$  for the 115 patients in our study cohort. (It was  $69.7 \pm 4\%$  for all

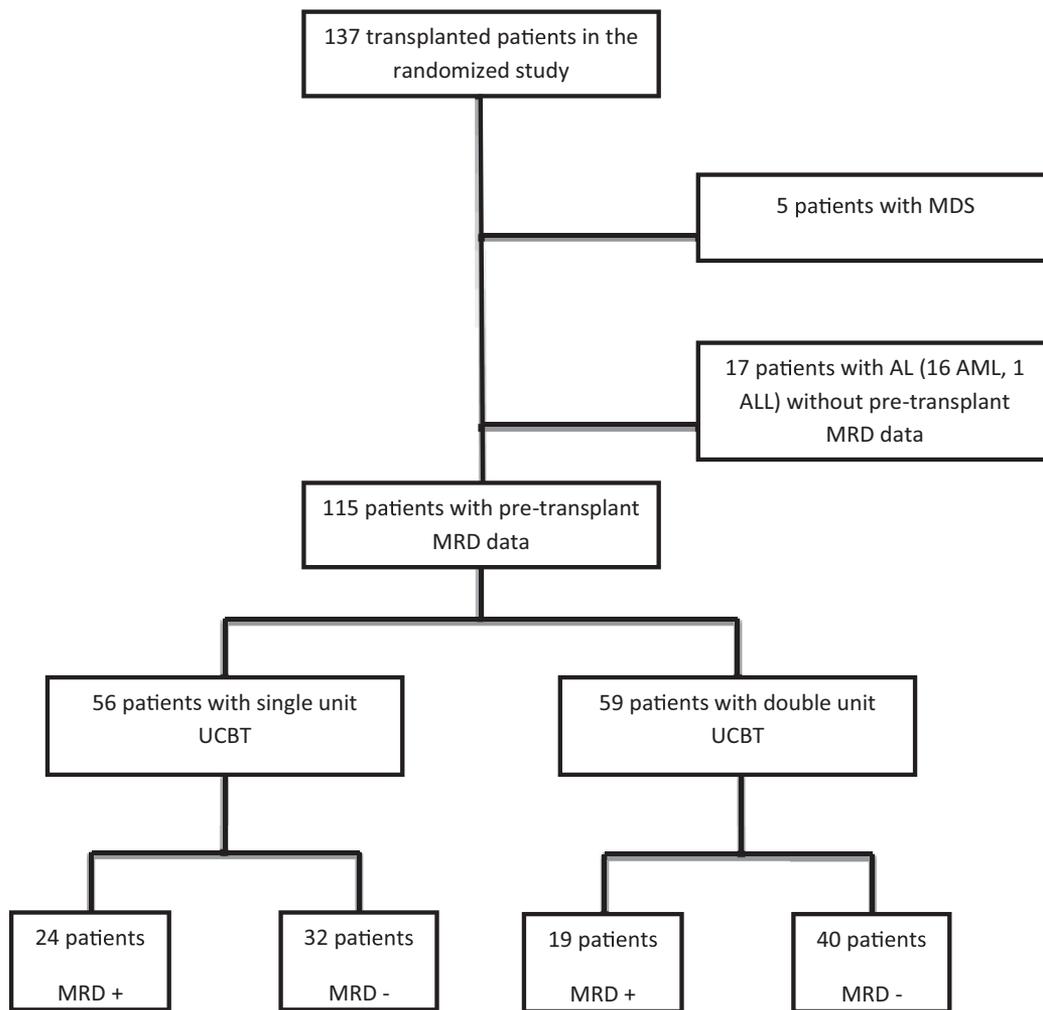


Figure 1. Flow chart.

137 patients in the French single-unit versus double-unit UCBT study.) We did not detect any significant influence of pretransplantation MRD status on post-transplantation outcomes in our study cohort (Figure 2). The mean 3-year OS was  $70.7 \pm 5.4\%$  in the MRD-negative ( $<10^{-4}$ ) patients,  $71.1 \pm 9.4\%$  in the MRD-positive patients with  $<10^{-3}$  and  $\geq 10^{-4}$ , and  $58.8 \pm 11.9\%$  in the MRD-positive patients with  $\geq 10^{-3}$  ( $P = .593$ ). Although a nonsignificant trend for a better 3-year OS in MRD-negative patients compared with MRD-positive patients was observed in the single-unit arm ( $78.1 \pm 7.3\%$  versus  $53.6 \pm 10.3\%$ ;  $P = .071$ ), this was not detectable in the double-unit arm ( $64.8 \pm 7.6\%$  versus  $82.6 \pm 9.3\%$ ;  $P = .113$ ) (Figure 3A and B).

#### Results of the Single-Unit versus Double-Unit Randomization in MRD-Positive Patients

In the MRD-positive group, the mean 3-year OS was significantly higher after double-unit UCBT than after single-unit UCBT ( $82.6 \pm 9.3\%$  versus  $53.6 \pm 10.3\%$ ;  $P = .031$ ). A statistically significant difference was also seen in mean 3-year DFS ( $82.6 \pm 9.3\%$  versus  $53 \pm 10.5\%$ ;  $P = .028$ ) and in the mean 3-year relapse rate ( $10.5 \pm 7.2\%$  versus  $41.7 \pm 10.4\%$ ;  $P = .025$ ) (Figure 4C and D).

As detailed in Table 2, these differences were particularly marked in the subgroup of pretransplantation MRD-positive patients who received Flu-TBI-Cy conditioning and

cyclosporine A plus mycophenolate mofetil without ATG as GVHD prophylaxis. In that subgroup, the mean 3-year OS was  $91.7 \pm 8\%$  after double-unit UCBT versus  $57.8 \pm 12.2\%$  after single-unit UCBT ( $P = .027$ ).

Conversely, there was no statistically significant difference between the 2 UCBT arms was found in the Bu-Cy-ATG MRD-positive subgroup who received cyclosporine A, steroids, and pretransplantation ATG for GVHD prophylaxis.

We also analyzed the results of randomization in MRD-positive patients according to their diagnosis (supplementary Table), and found significantly higher OS and DFS and a lower relapse rate in the double-unit UCBT arm compared with the single-unit arm in MRD-positive patients with ALL, but no difference between the arms in MRD-positive patients with AML.

#### Results of the Single-Unit versus Double-Unit Randomization in MRD-Negative Patients

In the MRD-negative group, there was no difference between the 2 UCBT arms in terms of risk of relapse, DFS, or survival for the entire group as well as for both conditioning regimen subgroups (Figure 4C and D and Table 2).

#### DISCUSSION

This additional analysis of the French prospective single-UCBT versus double-UCBT trial shows that UCBT offers a good

**Table 1**  
Patient Characteristics According to Pretransplantation MRD Status

Characteristic	MRD-Positive (n = 43)	MRD-Negative (n = 72)	P Value
Sex, n (%)			
Male	25 (58.1)	45 (62.5)	.64
Female	18 (41.9)	27 (37.5)	
Age, yr, n (%)			
<18	36 (83.7)	57 (79.2)	.55
≥18	7 (16.3)	15 (20.8)	
Diagnosis, n (%)			
ALL	30 (69.8)	49 (68.1)	.85
AML	13 (30.2)	23 (31.9)	
Hematologic status, n (%)			
First complete remission	19 (44.2)	40 (55.6)	.24
Second or more complete remission	24 (55.8)	32 (44.4)	
Conditioning regimen, n (%)			
Flu-TBI-Cy	30 (69.8)	44 (61.1)	.35
Bu-Cy-ATG	13 (30.2)	28 (38.9)	
Number of UCB units, n (%)			
1	24 (55.8)	32 (44.4)	.24
2	19 (44.2)	40 (55.6)	
TNCs infused, × 10 <sup>7</sup> /kg, mean ± SEM			
1-unit UCBT	4.93 ± .66	4.23 ± .37	.36
2-unit UCBT	7.57 ± .89	9.36 ± 1.29	.26
HLA compatibility, n (%)			
1-unit UCBT			
4/6	4 (16.7)	7 (21.9)	.74
5-6/6	20 (83.3)	25 (78.1)	
2-unit UCBT			
4/6	6 (31.6)	14 (35)	.80
5-6/6	13 (68.4)	26 (65)	

TNC indicates total nucleated cells.

chance of cure for children and young adults with AL who need HSCT, even in those positive for pretransplantation MRD. The 3-year OS, which was  $69.1 \pm 4.4\%$  for the whole cohort, remained at  $71.1 \pm 9.4\%$  in MRD-positive patients at a level  $\geq 10^{-4}$  but  $< 10^{-3}$  and at  $58.8 \pm 11.9\%$  in MRD-positive patients at a level  $> 10^{-3}$ . These results confirm, in a prospective and multicenter cohort, those reported by Milano et al. [15]. Their 4-year OS was 71% after UCBT in patients with AL and myelodysplastic syndrome, with no significant difference between patients with positive and undetectable pretransplantation MRD. However, 2 other retrospective studies reported less favorable results in patients with ALL who were MRD-positive before UCBT. In the Eurocord pediatric study [20], a positive MRD before single-unit UCBT was associated with a significantly higher rate of relapse, lower DFS, and lower OS; 4-year OS was only 41% in MRD-positive patients, compared with 58% in MRD-negative patients. Another group also reported 40% survival in a small retrospective cohort (n = 10) of MRD-positive children and adults [21].

Interestingly, our analysis suggests that double-unit UCBT may offer a better GVL effect than single-unit UCBT in MRD-positive patients, resulting in a significantly lower risk of relapse after double-unit UCBT (10.5% versus 41.7%) and leading to a significantly better survival rate (82.6% versus 53.6%). This strong double-UCBT-related GVL effect has been previously reported in several retrospective studies. Verneris et al. [3] reported a significantly lower risk of relapse after double-unit UCBT than after single-unit UCBT (19% versus 34%) in 177 patients with ALL or AML. Similarly, a European Society for Blood and Marrow Transplantation study compared single-unit and double-unit UCBT in 134 adults with AL [4] and found a significantly lower risk of relapse and a higher leukemia-free survival and OS after double-unit UCBT (21% versus 42% for relapse risk and 62% versus 42% for 2-year survival). Finally, a Eurocord analysis reported a lower relapse risk in a double-

unit UCBT group compared with a single-unit UCBT group (18% versus 26%) among 591 adult patients with AL [5].

As suggested by Milano et al. [15], alloreactivity after double-unit UCBT could be even higher than after transplantation from other stem cell sources. They found a significantly lower risk of relapse after UCBT (the UCBT group contained a large majority of double-unit UCBTs; 89%) than after transplantation from a matched unrelated donor (MUD) or a mismatched unrelated donor (MMUD), when pretransplantation MRD was positive. This difference was smaller and not statistically significant among the 3 stem cell source groups (UCB, MUD, and MMUD) in the subgroup of pretransplantation MRD-negative patients. Comparing different stem cell sources (matched sibling donor [MSD], MUD, MMUD, and double-unit UCB) in 536 patients with hematologic malignancies, Brunstein et al. [22] reported a lower risk of relapse in the double-unit UCBT group (15%) compared with the other 3 groups (43% for MSD, 37% for MUD and 35% for MMUD). Other retrospective cohorts have shown comparable or better results with UCBT compared with transplantation with other stem cell sources [23–25].

In both the US and the French randomized studies comparing single-unit and double-unit UCBT, GVHD was more frequent in the double-unit arm [6,7], confirming the findings of several previous retrospective reports [3–5,26]. On the other hand, GVHD has been repeatedly associated with a lower risk of relapse after UCBT [27,28]. Taken together, these data support the hypothesis of a higher alloreactivity with double-unit UCBT, which may lead to a greater GVL effect and potentially a lower risk of relapse. In the US randomized study, however, despite a higher incidence of GVHD, an enhanced GVL effect was not seen after double-unit UCBT, but it is worth noting that the reported rates of relapse at 1 year after single-unit and double-unit UCBT were remarkably low, at 12% and 14%, respectively. This low risk of relapse may be explained by a

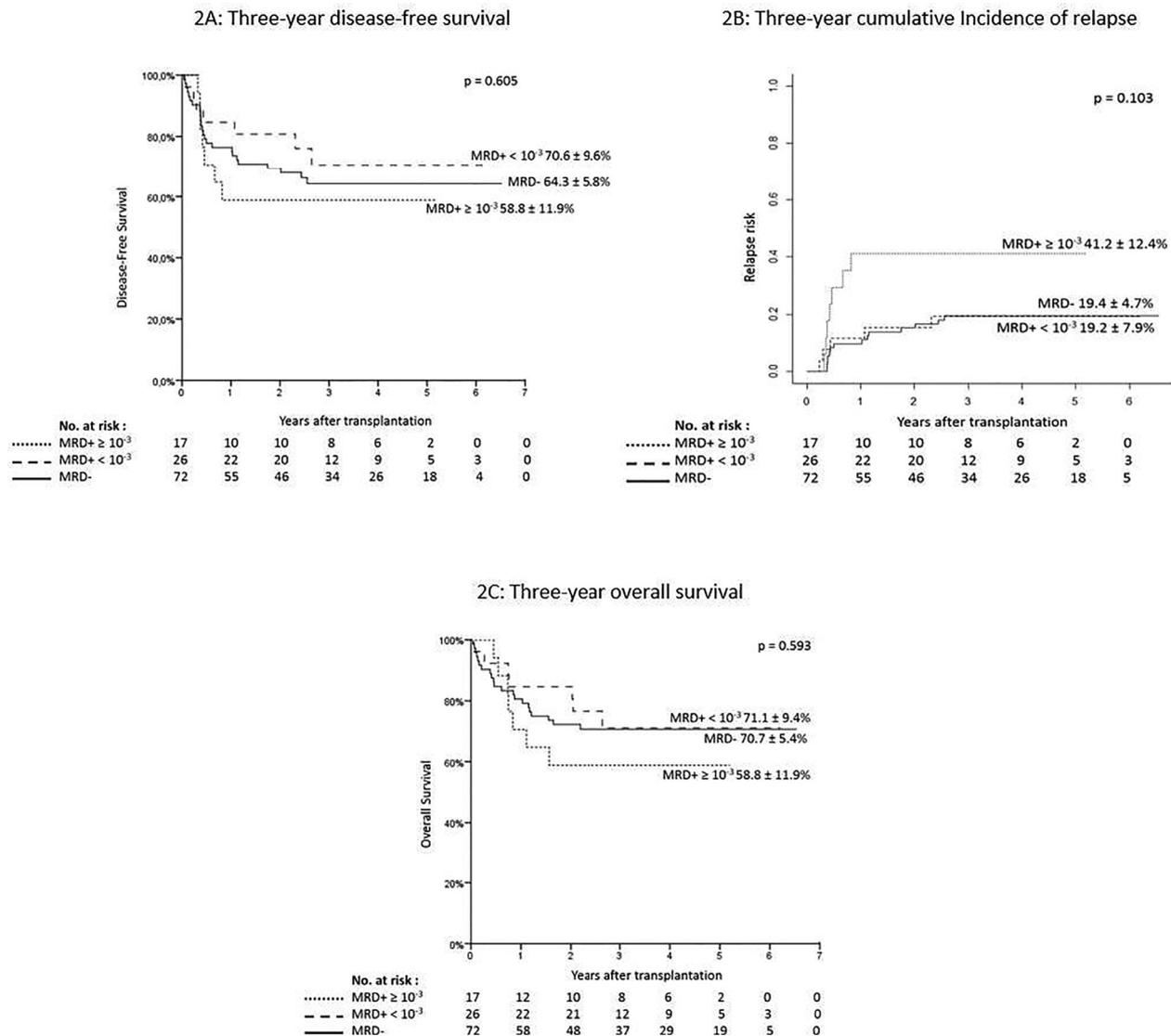


Figure 2. Overall results according to pretransplantation MRD status.

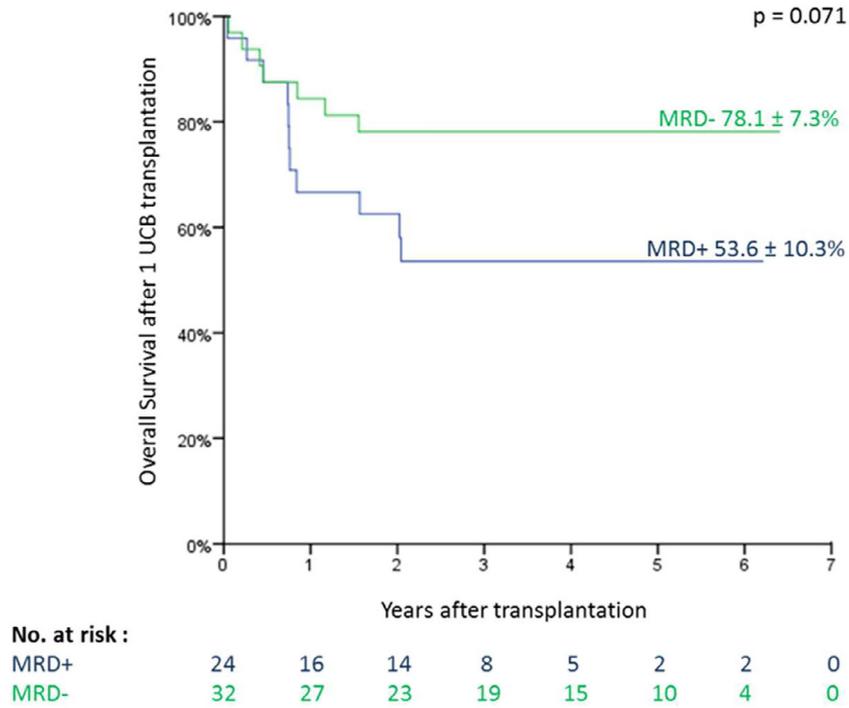
higher total TBI dose in their study compared with ours (1320 Gy versus 1200 Gy). In any case, the French results presented here suggest that the GVL effect generated by double-unit UCBTs could be more easily perceptible in the clinical setting of positive pretransplantation MRD, which is intrinsically associated with more aggressive diseases than in cases of negative pretransplantation MRD.

Interestingly, the significantly greater GVL effect that we describe after double-unit UCBT was observed only in patients who had received a Flu-TBI-Cy conditioning regimen without pretransplantation ATG. It could be argued that ATG may suppress a potentially greater GVL effect of double-unit UCBT. Several studies have demonstrated the deep impact of ATG on immune reconstitution, alloreactivity, and clinical outcomes [29–34]. Zheng et al. [33] retrospectively analyzed post-transplantation outcomes of 207 children with malignant disease who underwent UCBT according to the presence or absence of ATG in the conditioning regimen. They found a higher risk of relapse, a higher viral infection rate, and a lower DFS in those who had received ATG. Similarly, Lindemans et al. [29] compared 3 groups of children—early ATG, late ATG, and no ATG—who underwent UCBT for malignant and nonmalignant

diseases and found better immune reconstitution, a lower rate of viral infection, but a higher rate of severe acute GVHD in the no ATG group. Interestingly, Admiral et al. [30] found worse immune reconstitution, more viral infections, and more relapse in children with high ATG exposure versus those with low ATG exposure after UCBT, suggesting that ATG pharmacokinetics as an approach to better ATG management [34–36]. The impact of ATG on relapse risk has also been demonstrated in non-UCB HSCT [31,35,37].

In this study, the strong GVL effect of double-unit UCBT was limited to ALL, as it was not seen in any patient with AML. This finding must be interpreted with caution, however, because a large majority (86%) of the patients who received Flu-TBI-Cy conditioning without ATG had ALL, whereas 63% of those receiving Bu-Cy-ATG had AML. Other factors, such as HLA disparity and GVHD, could have played a role, and thus we cannot conclude that it is a disease-specific effect. Another limitation of the study is that, although based on a prospective and randomized trial, the additional pretransplantation MRD analysis was not initially planned as a study endpoint. Therefore, we did not prospectively define a standard MRD assessment, and consequently, biological methods to quantify MRD differed

3A: After single-unit UCB transplant



3B: After double-unit UCB transplant

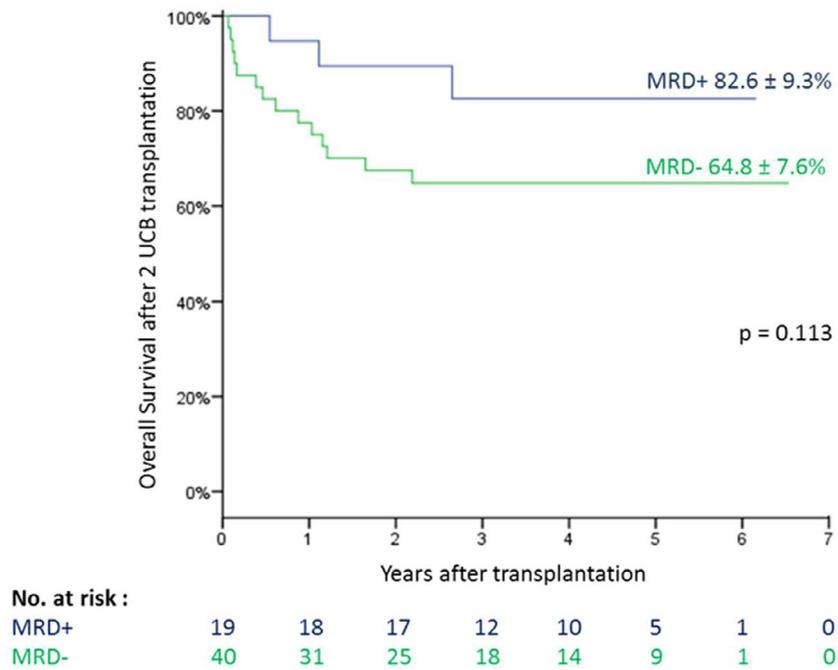
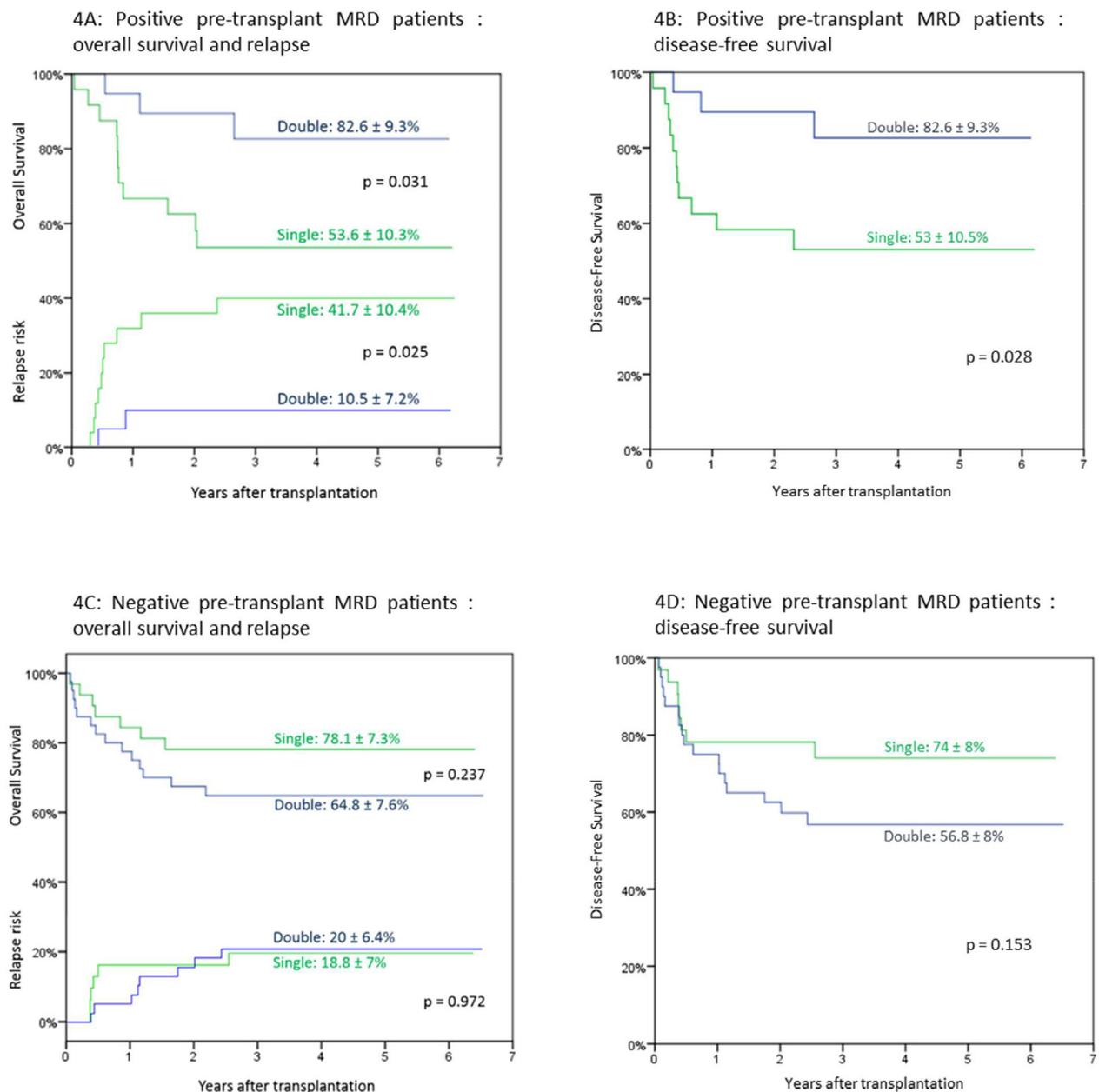


Figure 3. Comparison of 3-year OS in MRD-positive and MRD-negative patients.



**Figure 4.** Three-year relapse rate, DFS, and OS after single-unit or double-unit UCBT according to pretransplantation MRD status.

across transplantation centers and primary diseases. Nonetheless, it should be noted that MRD assessment was performed in a large majority of patients with ALL using RQ-PCR of TCR and IgH gene rearrangements in accordance with European LeukemiaNet recommendations, aiding the standardization of results. On the other hand, the greater heterogeneity of the patients with AML might have jeopardized the reliability of our results. Finally, we defined a threshold for MRD positivity at the  $\geq 10^{-4}$  level ( $\geq 0.01\%$ ), which could be seen as controversial. However, this MRD cutoff has been shown to be relevant in several pediatric and young adult cohorts [38–43] and seems appropriate, considering the sensitivity of the various techniques used for MRD detection [42,44,45].

In conclusion, our findings demonstrate that even in cases of positive pretransplantation MRD, UCBT in children and young adults with AL is associated with a high rate of DFS, and

that a double-unit strategy may enhance GVL and survival in these patients.

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**Table 2**  
Results of Randomization According to Pretransplantation MRD and Conditioning Regimen

Parameter	Single-Unit UCBT (N = 56)	Double-Unit UCBT (N = 59)	P Value
3-year CI of relapse, %			
MRD-positive patients (n = 43)	41.7 ± 10.4	10.5 ± 7.2	<b>.025</b>
MRD-negative patients (n = 72)	18.8 ± 7	20 ± 6.4	.972
MRD+ patients, Flu-TBI-Cy (n = 30)	35.3 ± 12.1	0	<b>.019</b>
MRD-negative patients, Flu-TBI-Cy (n = 44)	15.8 ± 8.6	12 ± 6.7	<b>.669</b>
MRD+ patients, Bu-Cy-ATG (n = 13)	57.1 ± 21.1	33.3 ± 21.3	.291
MRD-negative patients, Bu-Cy-ATG (n = 28)	23.1 ± 12.4	33.3 ± 12.8	.570
3-year disease-free survival, %			
MRD-positive patients	53 ± 10.5	82.6 ± 9.3	<b>.028</b>
MRD-negative patients	74 ± 8	56.8 ± 8	.153
MRD-positive patients, Flu-TBI-Cy	56.6 ± 12.7	91.7 ± 8	<b>.025</b>
MRD-negative patients, Flu-TBI-Cy	84.2 ± 8.4	59.4 ± 9.9	.096
MRD-positive patients, Bu-Cy-ATG	42.9 ± 18.7	66.7 ± 19.2	.288
MRD-negative patients, Bu-CY-ATG	59.3 ± 14.3	53.3 ± 12.9	.721
3-year overall survival, %			
MRD-positive patients	53.6 ± 10.3	82.6 ± 9.3	<b>.031</b>
MRD-negative patients	78.1 ± 7.3	64.8 ± 7.6	.237
MRD-positive patients, Flu-TBI-Cy	57.8 ± 12.2	91.7 ± 8	<b>.027</b>
MRD-negative patients, Flu-TBI-Cy	84.2 ± 8.4	63.5 ± 9.7	.128
MRD-positive patients, Bu-Cy-ATG	42.9 ± 18.7	66.7 ± 19.2	.367
MRD-negative patients, Bu-Cy-ATG	69.2 ± 12.8	66.7 ± 12.2	.959

Data are mean ± SEM. Significant P values are in bold type.

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## SUPPLEMENTARY MATERIALS

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