

# Long-term outcome after autologous *BCR::ABL1*-negative peripheral blood stem cell transplantation in adults with Philadelphia-positive acute lymphoblastic leukemia: a comparative study

Outcome of Philadelphia-positive acute lymphoblastic leukemia (Ph<sup>+</sup> ALL) improved significantly with the introduction of tyrosine kinase inhibitors (TKI) in combination with chemotherapy. Although autologous stem cell transplantation (auto-SCT) has never been considered as a standard of care in this setting, it has been widely used by several groups.<sup>1-3</sup> In the setting of first molecular remission of Ph<sup>+</sup> ALL, outcome after auto-SCT has been reported as similar compared to allogeneic SCT (allo-SCT), as well as in a large retrospective registry study<sup>4</sup> as in at least two prospective clinical trials,<sup>2,3</sup> all in the TKI era. Recent targeted immunotherapies, namely blinatumomab and anti-CD19 chimeric antigen receptor T cells (CAR T cells) have improved patients' outcome, rendering the role of SCT as the optimal curative therapy for Ph<sup>+</sup> ALL in first remission to be re-evaluated.<sup>5-7</sup> However, auto- or allo-SCT may be considered after relapse in case of CD19 loss of expression where blinatumomab and CAR T cells cannot be considered. In case of a CD19-positive relapse, blinatumomab in combination with TKI and/or chemotherapy may be used to reach a further molecular remission and possibly bridge to auto- or allo-SCT.

We hypothesized that minimal residual disease (MRD) evaluation by *BCR::ABL1* transcript quantification within mobilized peripheral blood stem cells (PBSC) collected from patients with Ph<sup>+</sup> ALL in molecular remission would predict the risk of relapse after auto-SCT. We thus compared the outcome of 34 patients receiving *BCR::ABL1*-negative PBSC with a control group of 35 patients receiving PBSC that were not monitored for putative *BCR::ABL1* contamination. After matching the two cohorts on patient age at time of auto-SCT, the cumulative incidence of relapse (CIR) at 5 years was significantly lower in patients receiving MRD-negative PBSC as compared to the control group, which translated into a significant advantage in the probability of disease-free survival (DFS). Written informed consent was obtained from all patients.

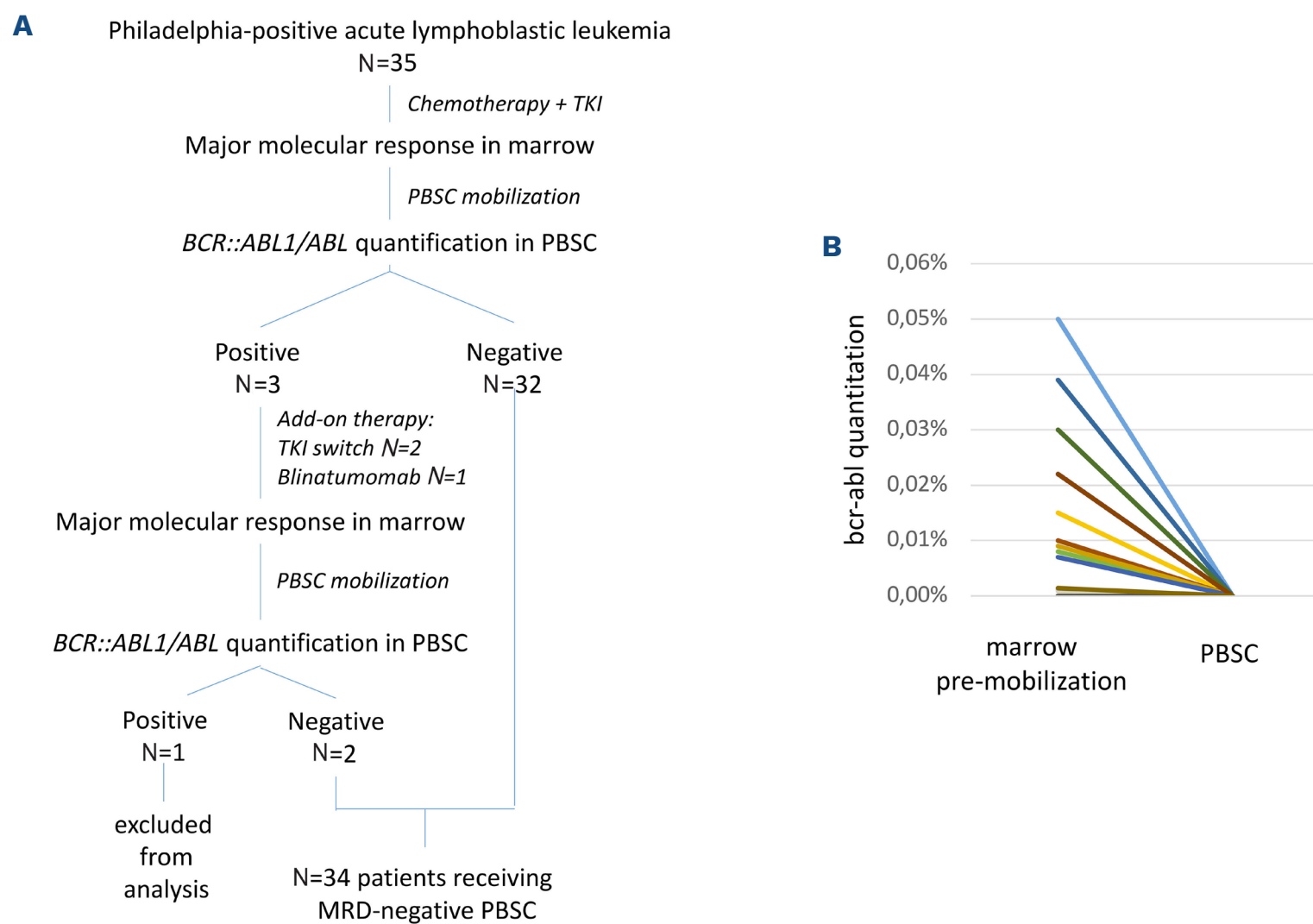
Between March 2009 and September 2022, we mobilized consecutively 35 patients diagnosed with Ph<sup>+</sup> ALL in order to collect PBSC before auto-SCT. *BCR::ABL1* transcript levels by quantitative reverse-transcription polymerase chain reaction (qRT-PCR) using standardized methods with international scale were used to monitor MRD.<sup>8,9</sup> As shown in study flow-chart (Figure 1A), *BCR::ABL1* quantification in PBSC was found negative in 32 patients. In

three others with *BCR::ABL1*-positive detection in PBSC, we aimed to further improve their molecular response in order to collect PBSC without detectable residual disease. To that aim, one patient received blinatumomab while the two others were switched from nilotinib to ponatinib in combination with chemotherapy, which led to a deeper molecular response in the three patients (*BCR::ABL1/ABL* ratio of  $\leq 0.01\%$  in marrow). All three were mobilized again in these better conditions. *BCR::ABL1* quantification in PBSC was found negative in two but still positive in one who was thus excluded from the final analysis. Consequently, 34 patients (17 females, 17 males; median age 58 years; range, 17-76) received *in vivo*-purged ASCT in CR1 (N=32) or CR2 (N=2) at a median interval of 6 months (range, 4-66) after diagnosis of ALL. One patient had initial central nervous system (CNS) leukemic disease. Transcripts types were m-*BCR* and M-*BCR* in 28 and six patients, respectively. All patients had previously received a first-line treatment combining chemotherapy with TKI, namely imatinib (16), nilotinib (7), dasatinib (10) or ponatinib (1). Response was evaluated after induction/consolidation courses by conventional morphologic criteria together with MRD evaluation in bone marrow. Major molecular response (MMoR) was defined as a *BCR::ABL1/ABL* ratio of  $\leq 0.1\%$  in the bone marrow, and molecular complete remission (CR) was defined by the absence of detectable MRD. As recommended by the French-Belgian-Swiss Group for Research on adult acute lymphoblastic leukemia (GRAALL), patients in MMoR after induction/consolidation were eligible for auto-SCT using myeloablative conditioning regimen, particularly when they lacked an HLA-matched sibling or unrelated donor. Figure 1B parallels *BCR::ABL1* quantification in i) bone marrow at the last time point before mobilization (median 0.008%; range, 0-0.05%) and ii) collected PBSC in which no MRD was detected in any of the 34 patients. The median infused CD34<sup>+</sup> cell dose was  $6.0 \times 10^6/\text{kg}$  (range, 2.6-18.0). Maintenance therapy was given after autologous SCT in all patients with imatinib (11), nilotinib (6), dasatinib (7), ponatinib (2), bosutinib (1), unknown (2) or TKI successive combinations (5). TKI could be finally interrupted in ten of the 23 patients (43%) who survived disease-free at 2 years, at a median time of 55 months (range, 25-77) after auto-SCT as their long term MRD remained negative by *BCR::ABL1* quantification in PB every 1 to 3 months. In order to analyze the impact of using MRD-negative

PBSC, we compared the outcome of this cohort with a control group of patients aged 18 to 59 years who were included in the GRAAPH-2005 trial of the GRAALL group and received auto-SCT in first CR.<sup>3</sup> In this trial, mobilized PBSC were not planned to be analyzed for the presence of residual *BCR::ABL1* contamination and the recommended conditioning regimen before auto-SCT combined cyclophosphamide and fractionated total body irradiation (8 to 12 Gy). The main difference between our cohort and the GRAAPH-2005 control group relied on patient age at the time of auto-SCT that was significantly higher in patients receiving residual disease-negative PBSC (Table 1).

After a median follow-up of 6.1 and 4.1 years, the cumulative incidence of relapse (CIR) and of non-relapse mortality (NRM) at 5 years post-transplant were 36% (95% confidence interval [CI]: 20-53) versus 51% (95% CI: 32-67;  $P=0.17$ ) and 9% (95% CI: 2-22) versus 6% (95% CI: 1-19;

$P=0.7$ ) in patients receiving residual disease-negative PBSC and control group, respectively (Figure 2). In these same groups, the probabilities of disease-free survival (DFS) and overall survival (OS) at 5 years post-transplant were 55% (95% CI: 36-70) versus 43% (95% CI: 25-59;  $P=0.25$ ; Figure 1B) and 67% (95% CI: 48-80) versus 54% (95% CI: 35-69;  $P=0.6$ ), respectively. Causes of non-relapse deaths in both cohorts are given in *Online Supplementary Appendix*. Among patients receiving residual disease-negative PBSC who survived disease-free at 2 years, probabilities of CIR and DFS at 5 years tended to be better when TKI could be interrupted following SCT versus not (23% [95% CI: 5-47] vs. 0%;  $P=0.11$  and 100% vs. 61% [95% CI: 31-0 81];  $P=0.03$ , respectively; *Online Supplementary Figure S1*). As patient age was the main factor differing between the two cohorts, we estimated these same probabilities in a subgroup of patients aged 59 years or less as 59 years was



**Figure 1. Study flow-chart based on *BCR::ABL1* quantitation in marrow premobilization and in mobilized peripheral blood stem cells.** (A) Study flow-chart. Major molecular response (MMoR) was defined as a *BCR::ABL1/ABL* ratio of  $\leq 0.1\%$  in the bone marrow. (B) *BCR::ABL1* quantitation in i) marrow at the last time point before mobilization and ii) collected peripheral blood stem cells (PBSC). For *BCR::ABL1* quantitation, mononuclear cells from peripheral blood, bone marrow and PBSC were isolated by density gradient centrifugation (Lymphocytes separation medium, Eurobio® or Abcys®), lysed in TriZol (ThermoFisher®) and RNA extracted according to the manufacturer's instructions. *MBCR::ABL1* transcripts were quantified on the international scale (IS) using the automated GeneXpert® platform (Cepheid®, Sunnyvale, CA, USA) with a detection limit of 0.003%. *mBCR::ABL1* transcripts were quantified using the EAC standardized polymerase chain reaction protocol and plasmid standards from Qiagen (Courtaboeuf, France) (Gabert *et al.*,<sup>8</sup> PMID: 14562125). When *mBCR::ABL1* was undetectable, the limit of detection was expressed in percentage as  $100/(\text{ABL1 transcript copies})$ . The average background signal was quantified using 19 *mBCR::ABL1*-negative RNA samples. A Ct threshold corresponding to the average background Ct value minus two times the standard deviation of the background Ct values was calculated and found at 37.1. Any sample with *mBCR::ABL1* Ct >37.1 was reported as undetectable. TKI: tyrosine kinase inhibitor; MRD: minimal residual disease.

the upper limit of age to be included in the GRAAPH-2005 trial control group. In this subgroup, the probability of disease-free survival (DFS) at 5 years post-transplant was higher in patients receiving residual disease-negative PBSC (65% [95% CI: 40-81] vs. 42% [95% CI: 24-59] in the control group;  $P=0.047$ ; Figure 2). This difference correlated with the CIR which was also lower in patients receiving residual disease-negative PBSC (25% [95% CI: 9-45] vs. 51% [95% CI: 32-68] in the control group;  $P=0.038$ ) while NRM did not differ between the two groups. Within patients receiving residual disease-negative PBSC, the CIR at 5 years thus reduced from 36% in the whole cohort to 25% in patients aged 59 years or less. This may be related to a significantly higher use of intensive conditioning regimen before auto-SCT in the latter population. Indeed, the use of TBI or of 4-days busulfan was more frequent in patients aged 59 years or less versus older ones within the group receiving residual disease-negative PBSC ( $P=0.01$ ).

There are at least two limitations in our study. The first one concerns the definition of the control group. As PBSC within this group of patients were not analyzed for their

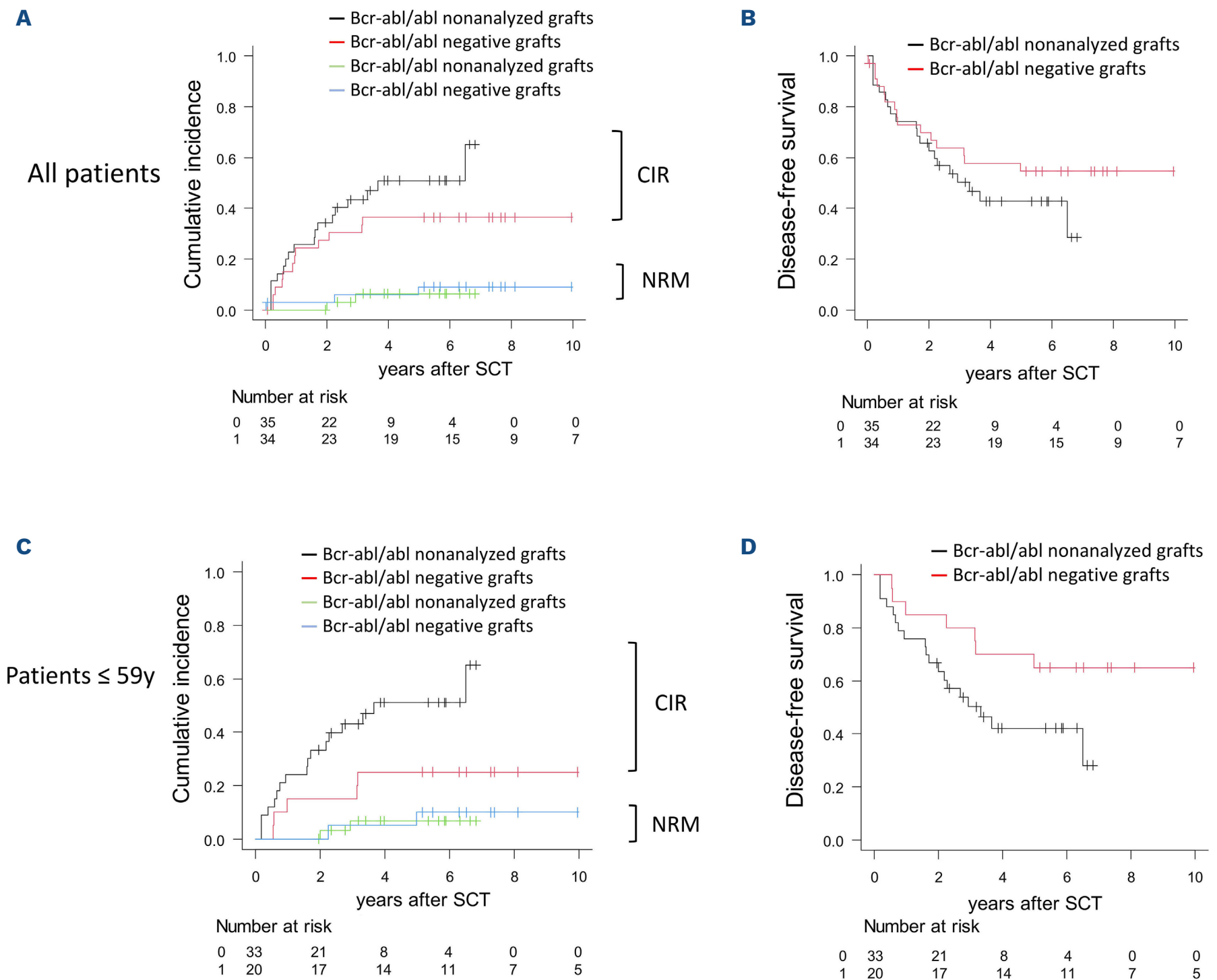
putative MRD contamination, one cannot exclude that some of those may be MRD-negative. In case, this may have lowered the difference in outcome between the two groups. As six of 35 patients (17%) within this control group did not reach MMoLR at time of mobilization, MRD contamination of PBSC may have been more frequent in this group as compared to our cohort in which all patients were in MMoLR at time of mobilization. However, this was probably not the sole explanation since differences in outcome between the two cohorts were not modified when excluding these six patients from the comparison. Second, MRD monitoring in bone marrow and in PBSC was based on quantification of *BCR::ABL1* fusion transcripts but not of immunoglobulin/T-cell receptor (Ig/TCR) gene rearrangements. In MRD longitudinal monitoring, the overall concordance between the two methods is 70% to 80% with significantly higher positivity by *BCR::ABL1*.<sup>10,11</sup> In that sense, *BCR::ABL1* may be more sensitive than Ig/TCR monitoring for prediction of relapse. Of note, in a previous study of 32 ALL patients receiving auto-SCT including 12 patients with Ph<sup>+</sup> ALL, the detection of Ig/TCR gene rearrangements within mobilized PBSC also

**Table 1.** Characteristics of patients receiving autologous stem cell transplantation for Philadelphia-positive acute lymphoblastic leukemia.

	<i>BCR::ABL1</i> negative PBSC N=34	<i>BCR::ABL1</i> undetermined PBSC N=35	<i>P</i>
<b>Ph<sup>+</sup> ALL diagnosis</b>			
Sex : M/F, N	17/17	19/16	0.81
m/M <i>BCR::ABL1</i> , N	28/6	30/5	0.75
WBC $\geq 10 \times 10^9/L$ at diagnosis, N (%)	20 (59)	21 (60)	1
CNS disease at diagnosis, N (%)	1 (1)	2 (6)	1
<b>Autologous SCT</b>			
Median age at SCT in years (range)	58 (17-76)	46 (21-59)	0.001
Age > 55 years at SCT, N (%)	19 (56)	5 (14)	<0.001
CR1/CR2 at SCT, N	32/2	35/0	0.24
Median time from diagnosis to SCT in months (range)	6 (4-66)	5 (3-11)	0.03
Myeloablative conditioning regimen, N (%)		35 (100)	<0.001
TBI + Cy, 8 to 12 GY	14 (41)		
Busulfan-based +/- Mel or Cy	17 (50)		
2-days Bu	6 (18)		
3-days Bu	6 (18)		
4-days Bu	5 (14)		
Melphalan	3 (9)		
Median CD34 <sup>+</sup> cell dose, 10 <sup>6</sup> /kg (range)	6.0 (2.6-18)	ND	-
Median follow-up after SCT in years (range)	6.1 (0.02-15.1)	4.1 (0.9-10.7)	0.269

Categorical variables were compared by Fisher exact tests. Median comparisons were performed by the Mann-Whitney two-sample test. Type 1 error was fixed at the 5% level. All tests were two-tailed. Ph<sup>+</sup> ALL: Philadelphia-positive acute lymphoblastic leukemia; PBSC: peripheral blood stem cell; M: male; F: female; WBC: white blood cell count; CNS: central nervous system; SCT: stem cell transplantation; CR: complete remission; TBI: total body irradiation; BU: busulfan; Mel: melphalan; ND: not determined; Cy: cyclophosphamide.





**Figure 2. Comparison of outcome between patients receiving *BCR::ABL1*-negative peripheral blood stem cells with a control group receiving peripheral blood stem cells that were not analyzed for *BCR::ABL1* contamination.** Cumulative incidence of relapse (CIR) and of non-relapse mortality (NRM) were estimated in (A) the whole cohort as well as in (C) patients aged 59 years or less. Similarly, disease-free survival was estimated in (B) the whole cohort as well as in (D) patients aged 59 years or less. Failure time data, except for cumulative incidences, were estimated by the Kaplan-Meier method, then compared by the log-rank test, with 95% confidence intervals (CI) estimated by the Cox model. For CIR and non-relapse mortality (NRM), deaths in remission and relapses were respectively taken into account as competing risks, using the cumulative incidence curves, then compared by the Gray test while the Fine and Gray model was used to estimate sub-distribution hazard ratio. y: years.

correlated with DFS.<sup>12</sup>

In conclusion, our study indicates that the absence of MRD contamination in autologous PBSC correlates with a better outcome after auto-SCT in Ph<sup>+</sup> ALL. This reinforces the need to mobilize patients in the situation of profound molecular remission. In case of contaminated PBSC, the option to remobilize patients if deeper molecular response can be reached may be envisaged. Although no transplant is an emerging option in the era of targeted immunotherapies for B-cell precursor ALL, auto- or allo-SCT may be considered in second or further remission, particularly in case of CD19 loss of expression.

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## Disclosures

No conflicts of interest to disclose.

## Contributions

LeC, ML, DC and SM conceived the study and interpreted data. SM performed statistical analyses and drafted the initial version of the manuscript. EJRS, IS and OWB performed molecular analyses and interpreted data. AC, LuC and YNSB collected and monitored clinical data. LeC, ML, DC, FB, RR, CR, VP, CP, SB, IL, DC and SM included patients. DM was responsible for apheresis. EC and HR were responsible for cell therapy unit. YC and NB provided data of the control cohort. All authors reviewed and approved the final version of the manuscript.

## Data-sharing statement

Original data can be obtained by contacting the corresponding author.