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

Leo Caillot¹, Mathieu Leclerc², Emmanuel Jacques Raphaël Sleiman¹, Ivan Sloma³, Oianne Wagner-Ballon⁴, Alexis Claudel⁵, Florence Beckerich⁶, Rabah Redjoul⁷, Christine Robin⁸, Vincent Parinet⁹, Cécile Pautas², Dehbia Menouche², Selwa Bouledroua⁴, Ludovic Cabanne¹, Yakout Nait-Sidenas³, Eric Gautier³, Hélène Rouard³, Ingrid Lafon¹, Yves Chalandon⁵, Nicolas Boissel⁵, Denis Caillot¹ and Sébastien Maury², for the Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL).

¹Hematology Department, CHU François Mitterrand, Dijon, France
²AP-HP, UPEC University, Henri Mondor Hospital, Hematology Department, Créteil, F-94010, France
³Etablissement Français du Sang, Île de France, UPEC University, Créteil, F-94010, France
⁴Division of Hematology, Department of Medical Specialties, University Hospital and University of Geneva, Geneva, Switzerland
⁵AP-HP, Paris Cité University, Unité d'Hématologie Adolescents et Jeunes Adultes, Hôpital Saint Louis, Paris, France.

Author contribution: 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.

Disclosures: none for any author
Data-sharing statement: original data can be obtained by contacting the corresponding author.

Corresponding author: Pr. Sébastien Maury, Assistance Publique – Hôpitaux de Paris, Hôpital Henri Mondor, 51 avenue du Mal de Lattre de Tassigny, 94010 Créteil cedex, France. Email: sebastien.maury@aphp.fr
Outcome of Philadelphia-positive acute lymphoblastic leukemia (Ph+ 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. In the setting of first molecular remission of Ph+ 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 as in at least two prospective clinical trials, 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+ ALL in first remission to be reevaluated. 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 MRD evaluation by BCR::ABL1 transcript quantification within mobilized peripheral blood stem cells (PBSC) collected from patients with Ph+ 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+ 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. As shown in study flow-chart (Figure 1A), BCR::ABL1 quantification in PBSC was found negative in 32 patients. In 3 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 3 patients (BCR::ABL1/ABL ratio of ≤ 0.01% in marrow). All 3 were mobilized again in these better conditions. BCR::ABL1 quantification in PBSC was found
negative in 2 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 CNS leukemic disease. Transcripts types were BCR and M-BCR in 28 and 6 patients, respectively. All patients had previously received a first-line treatment combining chemotherapy with tyrosine kinase inhibitors (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 (MMolR) was defined as a BCR::ABL1/ABL ratio of ≤0.1% in the bone marrow, and molecular 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 MMolR 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) in bone marrow at the last time-point before mobilization (median 0.008%, range 0%-0.05%) and ii) in collected PBSC in which no MRD was detected in any of the 34 patients. The median infused CD34+ cell dose was 6.0 x 10^6/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 (9). TKI could be finally interrupted in 10 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.

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. 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% CI, 20 to 53] vs. 51% [95% CI, 32 to 67] (p=0.17) and 9% [95% CI, 2 to 22] vs. 6% [95% CI, 1 to 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 to 70] vs. 43% [95% CI, 25 to 59] (p = 0.25, Figure 1B) and 67% [95% CI, 48 to 80] vs. 54% [95% CI, 35 to 69] (p = 0.6), respectively. Causes of non-relapse deaths in both cohorts are given in supplemental data. 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 vs. not (23% [95% CI, 5 to 47] vs. 0%, p = 0.11 and 100% vs. 61% [95% CI, 31 to 81], p = 0.03, respectively, supplemental Figure 1).

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 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 to 81] vs. 42% [95% CI, 24 to 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 to 45] vs. 51% [95% CI, 32 to 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 vs. older ones within the group receiving residual disease-negative PBSC (p = 0.03).

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 6 out of 34 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 6 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 \( BCR::ABL1 \). 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+ ALL, the
detection of Ig/TCR gene rearrangements within mobilized PBSC also correlated with DFS.12

In conclusion, our study indicates that the absence of MRD contamination in autologous PBSC correlates with a better outcome after auto-SCT in Ph+ ALL. This reinforces the need to mobilize patients in the situation of profound molecular remission. In case of contaminated PBSC, the option to re-mobilize 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.
References


Table 1. Characteristics of patients receiving autologous SCT for Ph-positive ALL

<table>
<thead>
<tr>
<th></th>
<th>Bcr-abl negative PBSC (n=34)</th>
<th>Bcr-abl undetermined PBSC (N=35)</th>
<th>P values</th>
</tr>
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<tbody>
<tr>
<td><strong>Ph-positive ALL diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gender, M/F</td>
<td>17/17</td>
<td>19/16</td>
<td>0.81</td>
</tr>
<tr>
<td>m/M, N</td>
<td>28/6</td>
<td>30/5</td>
<td>0.75</td>
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<tr>
<td>WBC ≥10 G/L at diagnosis, N (%)</td>
<td>20</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>CNS disease at diagnosis, N (%)</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><strong>Autologous SCT</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Median age at SCT, years (range)</td>
<td>58 (17-76)</td>
<td>46 (21-59)</td>
<td>0.001</td>
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<tr>
<td>Age &gt; 55 years at SCT (%)</td>
<td>19</td>
<td>5</td>
<td>&lt;0.001</td>
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<tr>
<td>CR1/CR2 at SCT, N</td>
<td>32/2</td>
<td>35/0</td>
<td>0.24</td>
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<tr>
<td>Median time from diagnosis to SCT, months (range)</td>
<td>6 (4-66)</td>
<td>5 (3-11)</td>
<td>0.03</td>
</tr>
<tr>
<td>Myeloablative conditioning regimen, N (%)</td>
<td>• TBI + CyI (8 to 12 Gy)</td>
<td>14 (41)</td>
<td>35 (100)</td>
</tr>
<tr>
<td></td>
<td>• Busulfan-based (+/- Mel or Cy)</td>
<td>17 (50)</td>
<td>6 (38)</td>
</tr>
<tr>
<td></td>
<td>o 2-days Bu</td>
<td>6 (38)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>o 3-days Bu</td>
<td>6 (38)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>o 4-days Bu</td>
<td>5 (34)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Melphalan</td>
<td>3 (9)</td>
<td></td>
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<tr>
<td>Median CD34+ cell dose, 10e6/kg (range)</td>
<td>6.0 (2.6-18)</td>
<td>ND</td>
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<tr>
<td>Median follow-up after SCT, years (range)</td>
<td>6.1 (0.02-15.1)</td>
<td>4.1 (0.9-10.7)</td>
<td>0.269</td>
</tr>
</tbody>
</table>

**Footnote.** Categorical variables were compared by Fisher exact tests. Median comparisons were performed by the Mann-Whitney 2-sample test. Type 1 error was fixed at the 5% level. All tests were 2-tailed.
Legends for Figures

**Figure 1.** Study flow-chart based on BCR::ABL1 quantitation in marrow pre-mobilization and in mobilized peripheral blood stem cells (A) Study flow-chart. Major molecular response (MMoIR) was defined as a BCR::ABL1 /ABL ratio of ≤0.1% in the bone marrow. PBSC, peripheral blood stem cells; TKI, tyrosine kinase inhibitor; MRD, minimal residual disease (B) BCR::ABL1 quantitation in i) marrow at the last time-point before mobilization and ii) collected 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 manufacturer 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 PCR protocol and plasmid standards from Qiagen (Courtabœuf, France) (Gabert et al. Leukemia 2003, PMID: 14562125). When mBCR-ABL1 was undetectable, the limit of detection was expressed in percentage as 100 /ABL1 transcript copies). The average background signal was quantified using 39 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.

**Figure 2.** Comparison of outcome between patients receiving BCR::ABL1 negative PBSC with a control group receiving PBSC 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 cumulative incidence of relapse (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.
Philadelphia-positive acute lymphoblastic leukemia
n=35

\textit{Chemotherapy + TKI}

Major molecular response in marrow

\textit{PBSC mobilization}

BCR::ABL1/ABL quantification in PBSC

Positive
n=3

\textit{Add-on therapy:}
\textit{TKI switch n=2}
\textit{Blinatumomab n=1}

Negative
n=32

Major molecular response in marrow

\textit{PBSC mobilization}

BCR::ABL1/ABL quantification in PBSC

Positive
n=1

excluded from analysis

Negative
n=2

n=34 patients receiving MRD-negative PBSC
Causes of non-relapse mortality

<table>
<thead>
<tr>
<th>Bcr-abl negative PBSC (n=4 deaths)</th>
<th>Bcr-abl undetermined PBSC (n=2 deaths)</th>
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<tbody>
<tr>
<td>• <em>Fusarium</em> septicemia</td>
<td>• Cerebral hemorrhage</td>
</tr>
<tr>
<td>• <em>P. aeruginosa</em> septicemia</td>
<td>• Probable cerebellum infection</td>
</tr>
<tr>
<td>• Secondary solid tumor (pulmonary)</td>
<td>(microbiologically undocumented)</td>
</tr>
<tr>
<td>• Probable cardiac failure (death at home)</td>
<td></td>
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</table>
Supplemental Figure 1.

Comparison of outcome according to TKI interruption or not after SCT within patients who received residual disease-negative PBSC and survived disease-free at 2 years. (A) Cumulative incidence of relapse (B) Disease-free survival.