Prospective long-term minimal residual disease monitoring using RQ-PCR in RUNX1-RUNX1T1-positive acute myeloid leukemia: results of the French CBF-2006 trial

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Prospective long-term minimal residual disease monitoring using RQ-PCR in RUNX1-RUNX1T1-positive acute myeloid leukemia: results of the French CBF-2006 trial.

Christophe Willekens (1); Odile Blanchet (2); Aline Renneville (3); Pascale Cornillet-Lefebvre (4); Cécile Pautas (5); Romain Guieze (6); Norbert Ifrah (7); Hervé Dombret (8); Eric Jourdan (9); Claude Preudhomme (3) and Nicolas Boissel (8) on behalf of the French AML Intergroup.

(1) Maladie du Sang, Hôpital Claude Huriez, Lille, France;
(2) Département Hématologie-Immunologie CHU Angers - Tumor Bank CHU-ICO - CRB-CHU Angers France BB-0033-00038 - UMR Inserm 892 CNRS 6299 CRCNA - Université d'Angers, France;
(3) Laboratoire d’hématologie, Centre de Biologie-Pathologie, CHRU de Lille; Equipe 3 INSERM U837, JPARC Lille, France;
(4) Laboratoire d'Hématologie, Centre Hospitalier Universitaire de Reims, Reims, France;
(5) Hématologie Clinique, Centre Hospitalier Henri Mondor, Créteil, France;
(6) Hématologie Clinique, Centre Hospitalier Universitaire, Clermont-Ferrand, France;
(7) Hématologie Clinique, Centre Hospitalier Universitaire, Angers, France;
(8) Département d'Hématologie, Hôpital Saint-Louis, EA3518, Institut Universitaire d'Hématologie, Université Paris 7, Paris, Paris, France;
(9) Service d'Hématologie, Centre Hospitalier Universitaire de Nîmes, Nîmes, France;

Running title: RUNX1-RUNX1T1 MRD should be evaluated on blood.

Correspondence: Nicolas Boissel, MD PhD, Hematology Department, Hôpital Saint-Louis, 1 avenue Claude Vellefaux, 75010, Paris, France; telephone number: +33 1 42 49 96 43; fax number: +33 1 42 49 40 10. E-mail: nicolas.boissel@sls.aphp.fr
**Trial registration**
This study has been part of the CBF 2006 trial referred in EudraCT #2006-005163-26 and ClinicalTrials.gov ID #NCT00428558.

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Abstract

In t(8;21)(q22;q22) acute myeloid leukemia, prognostic value of early minimal residual disease assessed with real-time quantitative polymerase chain reaction is the most important prognostic factor, but how long-term minimal residual disease monitoring may contribute to drive individual patient decisions remained poorly investigated. In the multicenter CBF-2006 study, a prospective monitoring of peripheral blood and bone marrow samples was performed every 3 months and every year, respectively, for 2 years after intensive chemotherapy in 94 patients in first complete remission. A complete molecular remission was defined as a \((RUNX1-RUNX1T1/ABL1) \times 100 \leq 0.001\%\). After completion of consolidation therapy, a bone marrow complete molecular remission was observed in 30\% of the patients, but was not predictive of subsequent relapse. Indeed, 8 patients (9\%) presented a positive bone marrow minimal residual disease up to 2 years of follow-up while still remaining in complete remission. Conversely, a peripheral blood complete molecular remission was statistically associated with a lower risk of relapse whatever the time-point considered after completion of consolidation therapy. During the 2-year follow-up, the persistence of peripheral blood complete molecular remission was associated with a lower risk of relapse (4-year cumulative incidence, 8.2\%), while molecular relapse confirmed on a following peripheral blood sample predicted hematological relapse (4-year cumulative incidence, 86.9\%) within a median time interval of 3.9 months. In t(8;21)(q22;q22) acute myeloid leukemia, minimal residual disease monitoring on peripheral blood every 3 months allows to predict hematological relapse and to identify patients who could potentially benefit from intervention therapy (ClinicalTrials.gov ID #NCT00428558).
Introduction

In young adult patients with core binding factor acute myeloid leukemia (CBF-AML, i.e. with t(8;21)(q22;q22) [CBFA-AML] or inv(16)(p13q22)/t(16;16)(p13;q22) [CBFB-AML]), complete remission (CR) is reached in more than 90% of cases. However, around 20-30% patients will experience hematological relapse.1,2 In CBFA-AML, many disease-related factors have been correlated to the risk of relapse including extramedullary disease,3 hyperleucocytosis,4 CD56 expression,5 additional cytogenetic aberrations6 and gene mutations such as KIT and FLT3-ITD.7,8 Quantification of the leukemia associated fusion gene RUNX1-RUNX1T1 (formerly AML1-ETO) by RQ-PCR provides a perfect target for minimal residual disease (MRD) assessment. In the French CBF-2006 study, we previously showed that early reduction in MRD level (>3 logs) during consolidation treatment in AML with t(8;21) was the most powerful marker to predict relapse in multivariable analysis.2

During longer-term post-consolidation follow-up, retrospective studies reported that MRD detection was associated with an increased risk of relapse in these patients.9,10 On the other hand, several studies also observed that a bone marrow positive MRD could be detectable in patients with long-term persistent CR.1,10,11 Recently, the Medical Research Council (MRC) reported that MRD positivity at a rate of >500 RUNX1-RUNX1T1 copies in bone marrow (BM) and >100 copies in peripheral blood (PB) during follow-up was predictive of hematological relapse.1

We report here the results of a prospective assessment of BM and PB MRD levels during the follow-up of 94 CBFA-AML patients enrolled into the French CBF-2006 study.
Methods

Patients and Treatment Protocol

Diagnosis of CBFA-AML was defined by the presence of either the t(8;21) translocation by karyotype and/or fluorescence in situ hybridization analysis and/or evidence of RUNX1-RUNX1T1 fusion transcript. Ninety-seven patients aged 18-60 years and with newly diagnosed CBA-AML were enrolled at 35 French centers between July 2007 and November 2010 in the CBF-2006 trial.

The CBF-2006 trial (EudraCT #2006-005163-26; ClinicalTrials.gov ID #NCT00428558) compared two intensive induction regimens in CBF leukemias. After induction, complete remission (CR) was obtained in 96 CBF-AML patients (1 early death). Patients received 3 monthly consolidation cycles with cytarabine at 3,000 mg/sqm/12 h by 2-hour IV infusion on days 1, 3, and 5, followed by lenograstim granulocyte colony-stimulating factor starting at day 8 until neutrophil recovery.

The study was approved by the ethics committee of Nimes University Hospital and by the Institutional Review Board of the French Regulatory Agency and was conducted in accordance with the Declaration of Helsinki. All samples were collected as part of the treatment protocol. Clinicians were prospectively informed of the MRD results. According to the protocol, patients with molecular recurrence defined by an MRD ratio increase of more than 1-log on two successive samples were eligible to participate to the phase II clinical trial DasaCBF. Thus, 5 patients were pre-emptively treated with dasatinib 140mg once daily. All 5 patients rapidly presented a haematological relapse within a median of 1.8 months. Another patient was pre-emptively treated with high-dose chemotherapy (MIDAM; intermediate dose cytarabine, mitoxantrone and gemtuzumab ozogamycin) after a molecular relapse confirmed on a following sample without haematological relapse. This patient was censored at the time of allogeneic transplant.
Samples and MRD evaluation

Bone marrow and PB samples were requested at diagnosis and then on therapy after induction chemotherapy and before the second and third consolidation chemotherapy. Results of early MRD evaluation have already been published. At the end of treatment, MRD was assessed on PB and BM again. During post-consolidation follow-up, PB samples were monitored every 3 months for 2 years and BM samples every year up to 2 years. Among the 96 CR patients, 2 patients had no MRD monitoring during follow-up. Long-term MRD level monitoring was thus analyzed on 94 patients (Figure 1).

MRD levels were monitored for $RUNX1$-$RUNX1T1$ transcript by RQ-PCR in 2 central laboratories (Angers, Lille), as described previously. Calibration curves were performed using Ipsogen plasmids (Ipsogen SA, Marseille, France) and $ABL1$ was amplified concomitantly as internal reference. Results were expressed as a $[RUNX1$-$RUNX1T1/ABL1] \times 100$ transcript ratio. The sensitivity of this quantification was 0.001%. A complete molecular response (CMR) was thus defined as a transcript ratio $\leq 0.001\%$, providing that at least 20 000 copies of the ABL1 control gene had been amplified. Molecular relapse was arbitrarily defined as a positive MRD occurring after having reached CMR.

Statistical analysis

Spearman rank correlation coefficient and Pearson correlation test were used to calculate correlation between transcript ratio in BM and PB. The rate of PB-MRD increase was calculated in patients with an available PB-MRD at relapse as $\log_{10}(PB$-MRD$_R/PB$-MRD$_{befR})/\Delta T$, where PB-MRD$_R$ is the MRD at relapse, PB-MRD$_{befR}$ is the MRD on the previous PB sample before relapse, and DT the time between both assessments. Outcomes were updated as of August 2013, with a median follow-up of 44.7 months.
Overall survival (OS) was estimated by the Kaplan-Meier method and compared by the log-rank test. Cumulative incidence of relapse (CIR) was estimated taking into account death in first CR for competing risk and compared by cause-specific hazard Cox models. Patients were censored at allogeneic stem cell transplant in first complete remission. Specific hazards of relapse (SHRs) and HRs are given with 95% confidence interval (CI). To evaluate the impact of time to CMR or time to molecular relapse, outcome data were estimated by the Mantel-Byar method, considering CMR or molecular relapse as time-dependent covariate. The method described by Simon and Makuch was applied for appropriate graphical representation of CMR and molecular relapse impact on OS and CIR. All statistical tests were performed with the Stata/IC 12.1 software (StataCorp, College Station, TX).
Results

A total of 479 BM samples and 800 PB samples were collected, corresponding respectively to 71.3% and 64.1% of the samples planned by the protocol. Seventy-four BM samples and 74 PB samples were assessed at the end of treatment time-point (after the third consolidation cycle) and 78 BM samples and 399 PB samples during longer follow-up. During the 2 years of follow-up, a median number of 5 PB samples per patient (range 0-9) were collected. Seventy-nine BM and 79 PB samples not planned by the protocol, corresponding to control of previous MRD results or relapse time-points, were also included in the analysis.

Correlation between MRD results obtained on BM and PB

To evaluate the correlation between PB- and BM-MRD levels, 525 paired PB and BM samples were compared at diagnosis, after induction, before each consolidation course, at the end of treatment and during the follow-up. Analyses were restricted to paired samples with comparable ABL Ct values. With a threshold of 0.001%, a positive MRD was detected on BM but not on PB in 134 paired samples (25.5%). Conversely, only 8 (1.5%) of the paired samples presented a detectable MRD on PB but not on BM (Figure 2).

A comparison of the 268 (51.0%) paired samples with both PB- and BM-MRD levels >0.001% was performed. This analysis was split into two groups of MRD levels lower or higher than 1%, corresponding to samples collected in CR or at diagnosis/relapse respectively. A significant correlation was observed between BM and PB MRD levels, irrespective of the MRD subgroup (Table 1). However, low PB- and BM-MRD values (<1%) were less closely correlated than more elevated values collected at diagnosis or at hematological relapse. The median value of BM-MRD in patients with persistent CHR was 0.03% (range: 0.001-125) and 70.8% (range: 0.8-383) in case of haematological relapse.
The corresponding PB-MRD levels were 0.001% (range: 0.001-27) and 64.6% (range: 0.1-368) respectively.

Peripheral blood versus bone marrow MRD prognostic value

Clinical characteristics of the 94 patients included in this MRD study are shown in Table 2. All patients were in first CR after induction chemotherapy. Median follow-up was 44.7 months. For this entire cohort, the 4-year estimated CIR was 33.3% (95%CI: 24.4-44.4) and 4-year estimated OS was 83.4% (95%CI: 74.0-89.7). Of note, 4 hematological relapses were observed after the 2 years of MRD follow-up planned by protocol.

At the end of consolidation therapy, MRD (called MRD4) was assessed on PB and BM within a median time of 39 days after the third consolidation (range: 15-100). On 74 patients evaluated at the end of treatment, 52 (70%) obtained a PB complete molecular response (CMR) compared to 22 (30%) on BM. The persistence of a detectable BM-MRD was not associated with an increased risk of relapse (4-year CIR, 33.8% versus 28.2%; SHR 1.20, p= 0.71; Figure 3A) or death (4-year OS, 87.7% versus 86.4%; HR 0.95, p=0.94; Figure 3B). Conversely, detectable PB-MRD at the end of consolidation was significantly associated with both a higher risk of relapse (4-year CIR, 50.9% versus 23.6%; SHR= 2.97, p=.01; Figure 3C) and a shorter survival (4-year OS, 63.6% versus 96.0%; HR= 6.8, p=.005; Figure 3D).

Among the 94 patients studied, 8 patients (9%) had a detectable BM-MRD up to 2 years of follow-up (BM-MRD<0.1%; range: 0.002-0.076%) while still remaining in first CR and in PB-CMR (Figure 4). Notably, the 4 patients that were found to have both positive PB- and BM-MRD at 2 years relapsed. After consolidation completion, whatever the time-point, BM-MRD failed to predict subsequent outcome (data not shown). For these reasons, further analyses were performed using PB-MRD.
Prognostic impact of complete molecular response and time to complete molecular response

During the follow-up, 77 patients (81.9%) reached a PB-CMR. The median time from CR to PB-CMR was 2.5 months. Notably, PB-CMR was achieved up to 16 months after CR (Figure S1A). Among these 77 patients, 18 experienced a hematological relapse. Among the 17 patients that never reached PB-CMR, eleven were in relapse within 13 months after CR.

In time-dependent Mantel-Byar analysis, patients who achieved PB-CMR had a significant reduced incidence of relapse (4-year CIR, 26.6% versus 51.2%; SHR .25, p=.001; Figure 5A) and a significant better overall survival (4-year OS, 89.8% versus 56.1%; HR 0.23, p=.008; Figure 5B). In this favorable subgroup of patients, the presence of FLT3-ITD was associated with a trend towards a longer time to achieve PB-CMR (7.8 months versus 2.4 months in FLT3-ITDpos and FLT3-ITDneg, respectively; p=.11, Figure S1D) while RAS gene mutation was associated with a significantly shorter time to PB-CMR (1.8 vs 2.59 months in RAS mutated and RAS wild-type, respectively; p=.05, Figure S1C). None of the other patient- or disease-related characteristics (i.e. age, WBC, bone marrow blast %, del(9q), loss of sexual chromosome, KIT and FLT3-TKD mutations) significantly impacted time to CMR.

The prognostic impact of time to achieve CMR was investigated considering CMR as a time-dependent variable. Time to CMR was not predictive of outcome, neither when considered as continuous (not shown) nor as categorical variable (Figure 5C).

Prognostic impact of loss of complete molecular response

Among the 77 patients who achieved PB-CMR, 23 patients presented a molecular relapse (MR), arbitrarily defined as MRD>0.001% on one PB sample. Among these 23 patients, 1 patient immediately received a salvage therapy, 2 patients (9%) were
simultaneously in hematological relapse, 13 patients (57%) presented a confirmed positive MRD on the following sample and 7 patients (30%) had a negative MRD on the following sample. Median time between PB-CMR and MR was 6.9 months (95%CI: 3.3-25.7). Median MRD level in patients with a MR not confirmed on a following sample was 0.007% (range 0.003%-0.06%) compared to 0.04% (range: 0.02%-1.55%) in patients with confirmed MR (p=.07).

In time-dependent Mantel-Byar analysis, a molecular relapse was associated with a higher cumulative incidence of hematological relapse when compared to persistent PB-CMR (4-year CIR, 74.5% versus 8.2%; SHR= 16.5, p< 0.001; Figure 6A). This excess of relapse translated into a shorter OS (4-year OS, 78.6% versus 94.2%; HR=5.9, p=0.019; Figure 6B). The outcome of patients that presented a confirmed positive MRD was compared to those with a negative MRD on the following sample. Patients with a confirmed molecular relapse had a higher cumulative incidence of molecular relapse (4-year CIR, 86.9% versus 23.4%; SHR= 5.7, p=0.026; Figure 6C) but a similar survival (4-year OS, 77.8% versus 77.8%; HR=0.6, p=0.604; Figure 6D). The median time from confirmed detectable PCR positivity to hematological relapse was 3.9 months (IQ, 3.3-6.9). Of note, in case of loss of CMR with a PB-MRD $\geq$0.5%, hematological relapse was systematically observed within median time of 28 days (range, 10-99 days).

*Peripheral blood kinetic of molecular relapse*

In 22 out of the 29 patients who experienced a relapse, a PB-MRD was assessed at the time of relapse. Among these 22 patients, 15 patients had a previous positive PB-MRD assessment within the last 3 months. In these 15 patients, the median rate in PB-MRD increase was 1.25 log$_{10}$/month (range: -0.14,3.58), which is in line with the 3.9 months of median time from confirmed detectable PCR positivity to hematological relapse observed above. Individual PB-MRD evolutions for the 22 patients are shown in Figure S2.
Discussion

In CBF-AML, recent reports have indicated that RUNX1-RUNX1T1 MRD reduction in the BM after 1 or 2 consolidation courses was the most powerful prognostic factor of relapse.\(^1,2,16\) However, early MRD response to treatment does not allow to predict all subsequent events, and patients with satisfying early response will still experience relapse in around 20% of cases.\(^2\) In this supplementary analysis, we showed that PB monitoring on a 3 monthly basis up to 24 months after the end of treatment allowed to detect molecular relapse and to anticipate hematological relapse in RUNX1-RUNX1T1 positive AML patients.

This study is the first report of systematic prospective MRD monitoring in CBFA-AML patients homogeneously treated in a phase III study. In CBFA-AML patients, persistent detection of RUNX1-RUNX1T1 transcripts have been reported in BM and/or PB in patients with prolonged CR.\(^1,10,11,17\) Thus, no real consensus has emerged concerning the source of sample to monitor MRD after the end of chemotherapy. We confirm here the good correlation between BM and PB MRD levels in a large number of samples.\(^18\) Despite the use of a quantitative RT-PCR assay with a sensitivity of 0.001%, we observed a significant amount of positive BM-MRD with negative PB-MRD samples (25.5%). Difference in MRD kinetics upon therapy partially accounts for this disparity between PB and BM samples. However, as previously reported, the persistence of a positive BM MRD at 2 years was detected in 9% of patients who remained in long-term CR. The persistence of RUNX1-RUNX1T1 positive non-leukemic cells (hematopoietic stem cells, B-cells, monocytes and mast cells) in the BM has been suggested to explain this persistence of positive BM signal.\(^19,20\) This probably contributes to the lack of prognostic impact of post-consolidation BM MRD and strongly supports the use of PB MRD to monitor RUNX1-RUNX1T1 positive AML patients.
Out of the 29 hematological relapses observed in our cohort, 11 (38%) occurred early before 13 months of follow-up in the context of persistent PB-MRD positivity, 2 (7%) were diagnosed at the same time than molecular relapse, 2 (7%) after molecular relapse with a negative MRD on following sample, 10 (34%) after molecular relapse confirmed on a following sample. Among the 4 patients (14%) who relapsed without previous molecular relapse, one patient had a late relapse occurring after the last negative PB-MRD assessed at 2 years, and the three others were not monitored as it was scheduled. In 21 patients, the relapse was thus predicted either by the persistence of PB-MRD positivity or by a confirmed molecular relapse. Complete molecular response on PB occurred in 81.9% of patients and was associated with a reduced risk of relapse and a longer survival. However, our data do not suggest a better time-point to consider persistent PB MRD positivity for intervention, a positive PB-MRD at 3, 6 or 9 months after the end of treatment being associated with a 4-year CIR of around 60% (data not shown). Consistent with previous reports, a molecular relapse was highly correlated to subsequent risk of hematological relapse. Indeed, Ommen et al. reported on 42 patients that a MRD > 10^{-4} on PB or BM was highly predictive of hematological relapse within 3 months. In a more recent study, Yin JA et al. reported that MRD positivity at a rate of >500 \textit{RUNX1-RUNX1T1} copies on BM and >100 \textit{RUNX1-RUNX1T1} copies on PB were highly correlated to the risk of hematological relapse and to survival. In this study, median times between detectable PCR positivity (10^{-5} sensitivity, normalized to \textit{ABL1}) to hematological relapse were 4.9 months and 4.5 months in BM and PB, respectively. All patients with loss of CMR and PB MRD ≥ 0.5% relapsed but we were not able to define a best threshold to predict hematological relapse. With a median time to hematological relapse of 3.9 months, it seems reasonable to recommend confirming a molecular relapse on a second sample before to consider any therapeutic intervention.
Nowadays, there are no recommendations concerning patients with CBF-AML in molecular relapse. Gemtuzumab ozogamicin has been suggested to be a drug of interest in CBF-AML therapy, frontline and as part of salvage therapy, but it has been shown to be associated with increased liver toxicity.\textsuperscript{21,22} Allogeneic stem cell transplant in second CR is a standard recommendation in young adult with AML but remains subject of discussion in CBF-AML.\textsuperscript{22,23}

In conclusion, we suggest that MRD level monitoring by quantitative RT-PCR during follow-up after high-dose cytarabine-based therapy in CBFA-AML should be evaluated in PB every 3 months up to two years after consolidation completion. In case of persistent positive PB MRD after treatment, patients should be closely monitored. These data also suggest that the reappearance of a detectable MRD in PB should be confirmed in a second PB sample to more accurately predict hematological relapse. Further studies will be mandatory to define the best strategy in terms of therapeutic attitude according to molecular relapse.

**Authors’s disclosure of potential conflicts of interest**

The authors declare no competing financial interests.

**Author contributions**

EJ, CPr, HD, NI and NB designed the study. OB, AR and CPr collected and analyzed the samples, CW extended clinical follow-up, CW and NB analyzed the data, performed statistical analyze and wrote the manuscript, All authors initially reviewed the manuscript.
References


Table 1. Correlation between paired BM and PB MRD transcript ratio at specific time-points. Only MRD>0.001% on both PB and BM were considered.

<table>
<thead>
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<th>Pearson (r)</th>
<th>Spearman</th>
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<th>p</th>
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<tr>
<td>At diagnosis (n = 68)</td>
<td>.65</td>
<td>.68</td>
<td>&lt;.0001</td>
<td></td>
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<tr>
<td>After induction (n = 70)</td>
<td>.62</td>
<td>.55</td>
<td>&lt;.0001</td>
<td></td>
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<tr>
<td>Before 2nd consolidation course (n = 40)</td>
<td>.32</td>
<td>.30</td>
<td>.056</td>
<td></td>
</tr>
<tr>
<td>Before 3rd consolidation course (n = 29)</td>
<td>.30</td>
<td>.36</td>
<td>.052</td>
<td></td>
</tr>
<tr>
<td>At the end of treatment (n = 18)</td>
<td>.41</td>
<td>.27</td>
<td>.273</td>
<td></td>
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<tr>
<td>Total - Samples with MRD &gt; 1% (N = 89)</td>
<td>.93</td>
<td>.84</td>
<td>&lt;.0001</td>
<td></td>
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<tr>
<td>Total - Samples with MRD &lt; 1% (N = 179)</td>
<td>.79</td>
<td>.79</td>
<td>&lt;.0001</td>
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</table>

Abbreviations: MRD, minimal residual disease; BM, bone marrow; PB, peripheral blood.
Table 2. Characteristics of the 94 patients with CBFA-AML included in the CBF-2006 trial.

<table>
<thead>
<tr>
<th>Patients, n</th>
<th>94</th>
</tr>
</thead>
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<tr>
<td>Median age, y (range)</td>
<td>40.8 (18-60)</td>
</tr>
<tr>
<td>Gender (Male/Female), n</td>
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</tr>
<tr>
<td>Median WBC, $10^9$/L (range)</td>
<td>11.3 (0.72-94.5)</td>
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<tr>
<td>Median BM blasts, % (range)</td>
<td>53 (17-98)</td>
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<td>Secondary AML, % (n)</td>
<td>11% (10/94)</td>
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<tr>
<td><strong>Additional cytogenetic abnormalities, % (n)</strong></td>
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<tr>
<td>- loss of Y</td>
<td>24% (33/94)</td>
</tr>
<tr>
<td>- trisomy 8</td>
<td>2% (2/94)</td>
</tr>
<tr>
<td>- del(9q)</td>
<td>15% (14/94)</td>
</tr>
<tr>
<td>- del (7q) / monosomy 7</td>
<td>4% (4/94)</td>
</tr>
<tr>
<td><strong>Gene mutations, % (n)</strong></td>
<td></td>
</tr>
<tr>
<td>KIT mutation</td>
<td>23% (21/93)</td>
</tr>
<tr>
<td>- KIT mutation exon 8</td>
<td>5% (5/93)</td>
</tr>
<tr>
<td>- KIT mutation exon 17</td>
<td>17% (16/93)</td>
</tr>
<tr>
<td>FLT3 mutation</td>
<td>11% (10/93)</td>
</tr>
<tr>
<td>- FLT3-ITD</td>
<td>6% (6/93)</td>
</tr>
<tr>
<td>- FLT3-TKD</td>
<td>4% (4/93)</td>
</tr>
<tr>
<td>N- or K-RAS mutation</td>
<td>15% (14/93)</td>
</tr>
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</table>

Abbreviations: WBC, white blood cell count; BM, bone marrow; AML, acute myeloid leukemia; ITD, internal tandem duplication; TKD, tyrosine kinase domain.
Figure legends:

**Figure 1.** Patient flow chart.

**Figure 2.** Peripheral blood (PB) and (BM) bone marrow MRD in 525 paired samples.

**Figure 3.** Outcome according to PB- or BM-MRD at the end of consolidation therapy (MRD4). Cumulative incidence of relapse and overall survival are shown according to BM-MRD4 (A and B, respectively) or to PB-MRD4 (C and D, respectively). A MRD >.001% was considered as positive.

**Figure 4.** PB- and BM-MRD in 8 patients with persistent BM-MRD up to two years after end of treatment.

**Figure 5.** Impact of CMR achievement on patient outcome. Peripheral Blood CMR was evaluated as a time-dependent variable and Simon-Makuch representations are shown for cumulative incidence of relapse (A) and overall survival (B). Time to PB CMR was split into 4 quartiles (Q1-4) to evaluate its impact on cumulative incidence of relapse (C).

**Figure 6.** Impact of molecular relapse in patients with complete molecular remission. Simon-Makuch representations are shown for cumulative incidence of relapse (A) and overall survival (B) in patients who remain in CMR (CMR) or who experienced a molecular relapse (MRD+). Simon-Makuch representations are shown for cumulative incidence of relapse (C) and overall survival (D) in patients who experienced a molecular relapse confirmed (2 consecutive time-points, 2TP) or not confirmed (1 time-point, 1TP) on a following sample.
Figure 1

CBF-2006  
CBFA-AML  
N=96 patients

N=2 w/o MRD follow-up

N=94 patients

Complete Molecular Remission (CMR)  
N=77

Persistently CMR  
N=54

Molecular relapse (MR)  
N=23

MR confirmed  
N=13

N=1 retreated before relapse

N=4 relapses

N=10 relapses

N=2 relapses*

MR not confirmed  
N=7

N=2 relapses

Persistent MRD  
N=17

N=11 relapses

*concomitant molecular and hematological relapse
Figure 2
Figure 3

A

BM-MRD4 neg
BM-MRD4 pos
p=0.71

Number at risk
BM-MRD4 neg 22
BM-MRD4 pos 51

Time (months)
0 12 24 36 48 60

0.00 0.20 0.40 0.60 0.80 1.00

CIR (%)

B

BM-MRD4 neg
BM-MRD4 pos
p=0.94

Number at risk
BM-MRD4 neg 22
BM-MRD4 pos 51

Time (months)
0 12 24 36 48 60

0.00 0.20 0.40 0.60 0.80 1.00

OS (%)

C

PB-MRD4 neg
PB-MRD4 pos
p=0.01

Number at risk
PB-MRD4 neg 50
PB-MRD4 pos 17

Time (months)
0 12 24 36 48 60

0.00 0.20 0.40 0.60 0.80 1.00

CIR (%)

D

PB-MRD4 neg
PB-MRD4 pos
p=0.005

Number at risk
PB-MRD4 neg 50
PB-MRD4 pos 17

Time (months)
0 12 24 36 48 60

0.00 0.20 0.40 0.60 0.80 1.00

OS (%)
Figure 5

A

CMR
MRD+
p=.001

Number at risk
MRD+ 91 25 7 2 2 2 2 2 2 1 1
CMR 3 61 64 65 61 51 45 35 23 16 10

Time (months)

B

CMR
MRD+
p=.008

Number at risk
MRD+ 91 26 13 9 6 6 5 4 2 0
CMR 3 60 69 70 69 58 51 39 25 16 7

Time (months)

C

Q1
Q2
Q3
Q4
p=.496

Number at risk
Q1 91 26 13 9 6 6 5 4 2 0
Q2 3 60 69 70 69 58 51 39 25 16 7
Q3 3 60 69 70 69 58 51 39 25 16 7
Q4 3 60 69 70 69 58 51 39 25 16 7

Time (months)
Supplemental data

Supplemental figure 1. Cumulative incidence of PB-CMR (A) according to mutation profile: *KIT* mutations (B), *RAS* mutations (C), and *FLT3*-ITD (D).
Supplemental figure 2. Kinetic of PB-MRD in 22 patients with PB-MRD assessed at relapse.