Characteristics and outcome of patients with core binding factor acute myeloid leukemia and FLT3-ITD: results from an international collaborative study


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Running title: Outcome of CBF-AML with FLT3-ITD

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References: 46

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ABSTRACT

To evaluate the prognostic impact of FLT3-ITD in core-binding factor acute myeloid leukemia in an international, multicenter survey on 97 patients (52%, t(8;21)(q22;q22); 48% inv(16)(p13q22)/t(16;16)(p13;q22)). Median age was 53 (range, 19-81) years. Complete remission (CR) after anthracycline-based induction (n=86) and non-intensive therapy (n=11) was achieved in 97% and 36% of the patients. Median follow-up was 4.43 years (95%-CI, 3.35-7.39 years). Median survival after intensive and non-intensive treatment was not reached and 0.96 years, respectively. In intensively treated patients, inv(16) with trisomy 22 (n=11) was associated with a favorable 4-year relapse-free survival rate of 80% (95%-CI, 59-100%) as compared to 38% (95%-CI, 27-54%; P=0.02) in all other CBF-AML/FLT3-ITD positive patients (n=75). Overall, 24 patients underwent allogeneic hematopoietic cell transplantation (allo-HCT), 12 in first CR and 12 after relapse. Allo-HCT in first CR was not beneficial (P=0.60); however, allo-HCT seems to improve median survival in relapsed patients compared to chemotherapy (not reached versus 0.6 years; P=0.002). Excluding inv(16) with trisomy 22, our data indicate that the outcome of CBF-AML patients with FLT3-ITD seems to be inferior compared to published data on those without FLT3-ITD, suggesting that prognostically these patients should not be classified as favorable-risk. FLT3-inhibitors may improve outcome in those patients.
INTRODUCTION

Core binding factor acute myeloid leukemia (CBF-AML) is defined by the presence of either t(8;21)(q22;q22)/RUNX1/RUNX1T1 or inv(16)(p13.1q22)/t(16;16)(p13.1;q22)/MYH11-CBFB and is recognized by the World Health Organization classification as a separate entity within the category “AML with recurrent genetic abnormalities”. Both aberrations result in formation of novel chimeric fusions involving genes of the CBF complex, a master regulator of definitive hematopoiesis. CBF-AML is associated with a favorable outcome, particularly if treated with repetitive cycles of high-dose cytarabine as post-remission therapy. Long-term 10-year overall survival (OS) rate was reported with 58% in FLT3 internal tandem duplication (FLT3-ITD) negative patients. Thus, CBF-AML is categorized into the favorable-risk group according to the National Comprehensive Cancer Network guidelines as well as the European Leukemia Net recommendations regardless of the FLT3-ITD mutational status. Nevertheless, 30-40% CBF-AML patients experience relapse.

FLT3-ITD mutations occur in roughly 5-10% of adult CBF-AML. In a murine transplantation model the co-transduction of FLT3-ITD with RUNX1-RUNX1T1 or CBFB-MYH11 promoted progression to AML, indicating the cooperative nature of FLT3 aberrations. However, their prognostic relevance in CBF-AML is still controversial. In a study on 176 patients with inv(16) AML, those with FLT3 mutations (n=30) were associated with inferior OS as compared to FLT3 wild type patients. Of note, in the same analysis trisomy 22 was a favorable prognostic marker irrespective of concomitant FLT3 mutations, confirming previous reports in which trisomy 22 was associated with a lower cumulative incidence of relapse (CIR) as compared to those patients with sole inv(16) (estimated long-term CIR rates of 42% and 66%,
respectively; \( P = 0.02 \)^3 and an excellent relapse-free survival (RFS) of 82%.\(^{10}\) Two other studies were in line with this finding.\(^{15,16}\)

Currently, it is unclear whether patients with CBF-AML and KIT or FLT3 mutations may benefit from allogeneic hematopoietic stem cell transplantation (allo-HCT) in first complete remission (CR1). A meta-analysis of several prospective trials evaluating the impact of allo-HCT for AML in CR1 did not show a benefit of allo-HCT on RFS and OS for favorable-risk AML (n=547) as compared to non-allo-HCT therapies including post-remission chemotherapy, autologous HCT, or both.\(^{17}\) In line with these findings, Burnett et al. reported no survival benefit in CBF-AML patients who underwent allo-HCT in CR1 as compared to patients not receiving HCT.\(^{18}\) Another retrospective analysis of younger (< 60 years) AML patients with t(8;21) compared the outcomes of 118 patients who received allo-HCT from a matched-related donor with 132 patients treated with cytarabine-based chemotherapy.\(^{19}\) After allo-HCT, the risk of relapse was significantly lower (hazard ratio (HR) 0.47; \( P = 0.014 \)), but the treatment-related mortality (TRM) was significantly higher (HR 6.76; \( P < 0.001 \)) as compared to chemotherapy.\(^{19}\) No benefit regarding RFS and OS was found for allo-HCT in the entire study cohort. Two other studies compared the results of allo- versus autologous HCT in CBF-AML.\(^{20,21}\) Gorin et al reported a significantly higher relapse risk after autologous as compared to allo-HCT in patients with t(8;21) (\( P = 0.03 \); 28% vs 15%) but not in patients with inv(16) AML.\(^{20}\) Again, TRM after allo-HCT was significantly higher in both t(8;21) (\( P = 0.003 \); 24% vs 6%) and inv(16) (\( P = 0.003 \); 14% vs 2%) patients as compared to autologous HCT, but the type of transplant did not affect leukemia-free survival.\(^{20}\) A Japanese study also showed comparable results for OS after allo- and autologous HCT in CR1 for both t(8;21) and
inv(16) AML. Nevertheless, in none of these reports the mutational status of FLT3-ITD was taken into account.

Taken together, the prognostic impact of FLT3-ITD in CBF-AML is still a matter of debate. Objectives of our study were to characterize CBF-AML patients with FLT3-ITD within an international, multicenter cohort study and compare outcomes according to treatment strategies, with a specific focus on the impact of allo-HCT as compared to conventional chemotherapy on survival.

**Methods**

**Patients and treatment**

Information on 97 adult patients with CBF-AML diagnosed between 1996 and 2019 (prior to 2000, n=7; 2000-2010, n=39; after 2010, n=51) was collected from eight study groups/institutions in the US and Europe. Participating centers were chosen upon network relationships of the first and last author. Detailed case report forms (including information on baseline characteristics, chemotherapy, allo-HCT, response, and survival) were collected from all participating centers. Inclusion criteria were adult CBF-AML patients with FLT3-ITD and all patients who fulfilled these criteria were included by the participating groups/institutions, respectively. Diagnosis of AML was based on French-American-British Cooperative Group criteria, and, after 2003, on revised International Working Group criteria. Chromosome banding was performed using standard techniques, and karyotypes were described according to the International System for Human Cytogenetic Nomenclature. FLT3 mutation screening for ITDs and point mutations within the tyrosine kinase domain (TKD) was carried out at each institution as previously described. Data collection and analysis were approved by the Institutional Review Boards of the participating centers.
Treatment

Eighty-six (89%) of the 97 patients received intensive induction treatment either within clinical trials (n=30) or according to local institutional standards (n=56). Treatment protocols included the Study Alliance Leukemia (SAL) AML60+ (n=2),27 AML96 (n=18)28 and AML2003 (n=9)29 as well as the CALGB/Ratify trial (n=1).30 Induction therapy of the 86 patients consisted of the anthracycline/cytarabine based „7+3“ regimen (n=62) or comparable intensive treatment (n=24); additionally, five of the intensively treated patients received midostaurin and four patients gemtuzumab ozogamicin, respectively.

Eleven (11%) of the 97 patients were treated non-intensively. Of those, five received azacitidine with (venetoclax, n=2; sorafenib, n=1) or without (n=2) combination therapy, four patients received fludarabine and low-dose cytarabine, one patient was treated with tipifarnib and etoposide within a clinical trial and one patient was treated with hydroxyurea only.

Response was assessed according to International Working Group recommendations.23 All clinical studies were approved by the institutional review boards of the participating centers. All patients provided written informed consent for participation in one of the treatment trials or for therapy according to local standards.

Statistical analyses

Survival endpoints including OS, RFS, CIR and cumulative incidence of death in CR (CID) were defined according to the revised recommendations of the International Working Group.23 Comparisons of patient characteristics were performed with the Kruskal-Wallis rank sum test for continuous variables and Fisher’s exact test for categorical variables. The median follow-up time was computed using the reverse
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Kaplan-Meier estimate. The Kaplan-Meier method was used to estimate the distribution of RFS and OS. Confidence interval (CI) estimation for survival curves was based on the cumulative hazard function using Greenwood’s formula for variance estimation. Logrank tests were employed to compare survival curves between groups. A Cox proportional hazards regression model was used to identify prognostic variables for RFS. CIR and CID and their standard errors were computed according to the method described by Gray and included only patients attaining CR. The effect of allo-HCT on OS as a time-dependent intervening event was tested by using the Mantel-Byar method. The method of Simon and Makuch was used to estimate survival distributions with respect to time-dependent interventions. The individuals at risk were initially all represented in the chemotherapy group. If patients received an allo-HCT, they were censored at this time point in the chemotherapy group and further followed up within the allo-HCT group.

All statistical analyses were performed with the statistical software environment R, version 3.3.1, using the R packages prodlim, version 1.5.7, and survival, version 2.39-5.

RESULTS

Study cohort

Overall demographic and clinical data were collected from 97 patients (SAL, n=46; Spanish PETHEMA (Programa Español de Tratamientos en Hematología), n=20, Johns Hopkins University, Baltimore, n=8; Perelman School of Medicine at the University of Pennsylvania, n=6; University of Munich, n=6; Czech Leukemia Centers, n=6; Dana-Faber Cancer Institute and Massachusetts General Hospital, Boston, n=3; Mayo Clinic Rochester, n=2) diagnosed with CBF-AML between 1996
and 2019. Median age was 53 years (range, 19-81 years) and 45 patients (46%) were female; baseline characteristics are summarized in Table 1. Median white blood cell (WBC) was higher in patients with inv(16)/t(16;16) as compared to patients with t(8;21). In addition, median WBC count in patients with inv(16)/t(16;16) and trisomy 22 was lower (n=11; median WBC 28.8 x 10^9/L; range, 3.9-186.7 x 10^9/L) as compared to patients with inv(16)/t(16;16) without trisomy 22 (n=36; median WBC 54.8 x 10^9/L; range, 2.7-298 x 10^9/L; P=0.18).

**Cytogenetic and molecular analyses**

The balanced translocation t(8;21)(q22;q22) was present in fifty (52%) of the 97 patients. It occurred as a sole abnormality in 15 (30%) patients, while additional cytogenetic abnormalities were present in 35 (70%) patients, most frequently loss of the sex chromosome (n=26; loss of X/Y n=13, each), ≥3 abnormalities (n=10) and deletion of the long arm of chromosome 9 (del(9q), n=6). Of the del(9q), all but one co-occurred within a karyotype with ≥3 abnormalities.

An inv(16)(p13q22) (n=46) or t(16;16)(p13;q22) (n=1) was detected in 47 (48%) patients. It was the sole abnormality in 25 (53%) patients, while concurrent cytogenetic abnormalities were present in 22 (47%) of the 47 patients, most frequently trisomy 22 (n=11), ≥3 abnormalities (n=7), trisomy 8 (n=8; all except one within a karyotype with ≥3 abnormalities) as well as monosomy 7 or deletion of the long arm of chromosome 7 (del(7q); n=4; all within ≥3 abnormalities).

The FLT3-ITD allelic ratio was available in 77 (79%) patients and the median allelic ratio was 0.35 (range, 0.003-50). Median WBC count was higher in patients with high as compared to those with low allelic ratio (31.7 vs. 16 x 10^9/L, P=0.02).

The median FLT3-ITD size and number of ITD clones were available in 29 (30%) patients. The median FLT3-ITD size was 39 (range, 3-120) base-pairs and most of
the patients harbored one clone (one clone, n=24; two clones, n=4, three clones, n=1). Besides the FLT3-ITD, ten (21%) of 48 patients with available data also harbored a FLT3-TKD (Table 1).

Response to induction therapy
Data on response to induction therapy were available in all 97 patients. Of the intensively treated patients (n=86), CR after induction therapy was achieved in 84 (98%), including one patient who achieved CR after salvage therapy with 1g cytarabine every 12 hours on 4 days as well as mitoxantrone 12 mg/day on 3 days. Early death (ED) occurred in two (2%) patients; none of the intensively-treated patients had refractory disease. All patients, who received 7+3 either with midostaurin (n=5) or gemtuzumab ozogamicin (n=4) achieved CR.

Eleven patients were treated less intensively due to higher age (median: 72 years; range, 40-81 years) or comorbidities. In less intensively treated patients (n=11) CR was achieved in four (36%; hypomethylating agent, n=1; venetoclax+azacitidine, n=1; fludarabine+low-dose cytarabine, n=2) and a partial remission in one patient; four were refractory and two patients died early.

Further therapy including intensive consolidation and allo-HCT
Seventy-two (86%) of 84 intensively treated patients in CR1 received intensive chemotherapy consolidation consisting of high-dose cytarabine with or without additional chemotherapy (mitoxantrone and/or amsacrine, n=25). Precise information on applied consolidation cycles was available in 54 patients. Of those, 10 patients received 4 consolidation cycles, 14 patients 3 cycles, 7 patients 2 cycles and 23 patients 1 cycle. For analysis, we compared patients who received ≤ 2 consolidation
cycles vs. those who received > 2 consolidation cycles. There was no difference in CIR in those patients who received ≤ consolidation 2 cycles vs. those who received > 2 cycles (P=0.97). One of the transplanted patients received maintenance with gilteritinib vs. placebo within a randomized trial post-allo-SCT for two years. In addition, one patient with intensive chemotherapy consolidation received maintenance with midostaurin.

Twelve (14%) patients proceeded to allo-HCT in CR1 with five of the transplanted patients receiving some consolidation chemotherapy prior to transplant. There was no difference in baseline characteristics in patients proceeding to allo-HCT in CR1 as compared to patients with consolidation chemotherapy, such as median white blood cell (WBC) count, median age and median FLT3-ITD allelic (data not shown).

In patients consolidated with chemotherapy, relapses occurred in 31 patients and six patients experienced TRM after consolidation. In patients consolidated with allo-HCT in CR1 three patients relapsed and one died of TRM. In those relapsing after chemotherapy, allo-HCT was performed in 12 patients: 8 in CR2 and 4 with active disease.

FLT3 mutational status was available in 17 (50%) of 34 relapsed patients with intensive treatment. Of those, eight (47%) were still FLT3-ITD positive. Interestingly, one of the ITD positive patients developed a new FLT3-TKD mutation.

**Characteristics of patients undergoing allo-HCT**

Overall, an allo-HCT was performed in 24 (25%) of the 97 patients, either in CR1 (n=12; inv(16), n=4; t(8;21), n=8) or CR2 (n=8; inv(16), n=5; t(8;21), n=3), or with active disease (n=4; inv(16), n=2; t(8;21), n=2).
Thirteen patients received myeloablative conditioning, including total body irradiation (TBI) in eight patients; additionally eight patients received reduced-intensity conditioning (missing, n=3). Source of donor was matched related in 11, matched unrelated in 10, haplo-identical in two, and unknown in one of the 24 patients.

**CIR, CID and survival**

The median follow-up of the entire cohort was 4.43 years (95%-CI, 3.35-7.39 years). Median and 4-year OS of the entire cohort were 4.48 years (95%-CI, 2.48-not reached), and 51% (95%-CI, 41-64%).

In intensively treated patients RFS and OS were not different between inv(16) and t(8;21) (p=0.70 and p=0.80, respectively; Figure 1). Furthermore, CIR (P=0.26; Figure 2, Panel A) and CID (P=0.96; Figure 2, Panel B) were comparable in patients proceeding to allo-HCT in CR1 or not. However, in relapsed patients survival was dismal without allo-HCT (n=22) irrespective of CBF-AML type with a median survival of 0.6 years after relapse (95%-CI, 0.31-1.11 years) and none of the patients survived beyond 2 years. In contrast, in relapsed patients proceeding to allo-HCT either in CR2 or with active disease median survival was not reached and survival at 4 years was 53% (95%-CI, 30-94% Figure 3). In a Mantel-Byar analysis including allo-HCT performed after relapse as a time dependent event, survival after relapse was significantly improved by allo-HCT (P=0.002).

Since supportive care might have impacted outcome, we performed a Cox regression analysis. This analysis revealed no impact of date of diagnosis either as a continuous (P=0.92) or as dichotomized variable (on the year 2010; P=0.23).

In non-intensively treated patients, median survival was 0.96 years (95%-CI, 0.24-not reached) and none of the patients survived beyond 3 years.
Explorative subset analysis revealed trisomy 22 in patients exhibiting an inv(16) as a significant prognostic factor for RFS (n=11; P=0.02; Figure 4, Panel A); outcome of those patients was favorable with a 4-year RFS rate of 80% (95%-CI, 59-100%), whereas all other CBF patients had a high relapse rate resulting in a 4-year RFS rate of 38% (95%-CI, 27-54%, P=0.02; Figure 4, Panel A). In addition, OS was in trend higher in patients with inv(16) and trisomy 22 (P=0.10; Figure 4, Panel B) as compared to all other CBF patients.

Other relevant prognostic factors, such as type of CBF-AML, higher age (≥60 years), WBC count, platelet counts, trisomy 8, complex karyotype, and high FLT3-ITD allelic ratio (≥0.5) were not identified as significant variables either for RFS or OS (Table 2). In addition, loss of the Y-chromosome in patients with t(8;21) had no impact on outcome (RFS, P=0.7; OS, P=0.3). Trisomy 22 was the only significant variable on the endpoint RFS HR, 0.22; P=0.04; Table 2).

DISCUSSION

The focus of our study was to characterize adult CBF-AML patients with FLT3-ITD in an international, multicenter cohort study and compare outcomes according to treatment strategies, with a specific focus on the impact of allo-HCT as compared to conventional chemotherapy on survival.

Secondary chromosome aberrations can be detected in more than 60% of t(8;21) and in 35% to 40% of inv(16) AML cases. In line with published data,10,38 the most frequent secondary chromosome aberration in our cohort of t(8;21) AML patients was loss of a sex chromosome, whereas the most frequent secondary chromosome
aberration in inv(16) AML was trisomy 22. In addition, we found a higher WBC count in patients with inv(16) as compared to those with t(8;21). In contrast to previous reports there was no impact of WBC count, higher age (≥60 years) or loss of the sex chromosome on outcome.

In our cohort, remission rate after intensive treatment was very high as was reported in CBF-AML without FLT3-ITD, suggesting that CBF-AML is highly chemo-sensitive regardless of a concurrent FLT3-ITD. We confirm the excellent prognosis of patients with inv(16) and trisomy 22, despite the additional presence of a FLT3-ITD. To date, it is unclear why patients exhibiting an inv(16) and trisomy 22 so rarely relapse after intensive induction and consolidation therapy. Obviously, leukemic clones harboring both abnormalities are very chemo-sensitive. Our study adds to previous knowledge that despite the proliferative signal induced by a FLT3-ITD and the chemo-resistance induced by high FLT3-ITD allelic ratios patients exhibiting an inv(16) and trisomy 22 remain extremely chemo-sensitive. The underlying pathogenetic mechanism by which trisomy 22 exerts its prognostic impact, however, remains elusive.

Regarding outcome of intensively treated CBF patients exhibiting a FLT3-ITD without trisomy 22 results are dismal with a RFS rate of 38% after four years. The relapse rate in these patients was high and confirmed findings from previous studies. In comparison, an OS rate of 58% after 10 years was reported in FLT3-ITD negative patients. In our cohort OS after four years in FLT3-ITD positive patients was 51% as compared to 58% after 10-years in those with wild-type of FLT3. In addition, outcome of intensively treated patients was not affected by CBF subtype inv(16) and t(8;21) or CR1 consolidation approach (chemotherapy or transplant). These results might argue for the benefit of repetitive cycles of intensive chemotherapy as post-
remission treatment, i.e. high-dose cytarabine in this subgroup of patients, although we would like to emphasize that this finding needs to be validated in a larger cohort.

Biologically, a FLT3-ITD in CBF-AML seems to impair the favorable prognosis, comparable to its negative impact in acute promyelocytic leukemia, at least in those patients without treatment with all-trans retinoic acid and arsenic trioxide. Despite the limitation that data on measurable residual disease were not available in our cohort, outcome was inferior if compared to published data in FLT3-ITD negative patients. Thus, the FLT3 mutational status should be taken into account when classifying CBF-AML; patients with FLT3-ITD should not be classified within the favorable-risk category. Those patients might be rather candidates for targeted treatment with tyrosine kinase inhibitors as well as intensive chemotherapy. In addition, there is evidence that gemtuzumab ozogamicin in combination with chemotherapy particularly benefits patients with FLT3-ITD mutations as well as patients with CBF-AML. However, in our cohort only few patients were treated with either midostaurin or gemtuzumab ozogamicin; thus, the effect on outcome could not be evaluated. Currently, the impact of midostaurin in combination with gemtuzumab ozogamicin on outcome is evaluated within a phase 1/2 trial (ClinicalTrials.gov Identifier: NCT04385290).

In relapsed patients survival was dismal without allo-HCT irrespective of CBF-AML type with a median survival of 0.6 years after relapse and none of the patients survived beyond 2 years. In contrast, in patients proceeding to allo-HCT after relapse either in CR2 or with active disease median survival was not reached and survival at 4 years was 53%, arguing that allo-HCT should be the preferred approach in relapsed patients. However, we would like to emphasize that retrospectively collected data have serious limitations since the factors for allocating patients to allo-HCT, such as co-morbidities, individual assessment of the treating physician, choice
of conditioning, and availability of a donor, remain unknown and this needs to be taken into account when evaluating the value of allo-HCT in our series.

Conclusions
Despite a high remission rate patients with FLT3-ITD had an inferior outcome as compared to previously published data on CBF-AML without FLT3-ITD. Thus, CBF-AML with FLT3-ITD should not be classified within the favorable-risk category. Our data suggest that allo-HCT should be the preferred approach in relapsed patients. CBF-AML with FLT3-ITD represents a further therapeutic target for tyrosine kinase inhibitors as well as gemtuzumab ozogamicin and should be included in combined FLT3-inhibitor/CD33-antibody trials (ClinicalTrials.gov Identifier: NCT04385290).

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Table 1: Baseline characteristics of patients with acute myeloid leukemia and core-binding factor leukemia.

<table>
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<td>33 (7-261)</td>
<td>33 (7-373)</td>
<td>0.92</td>
</tr>
<tr>
<td>(Range)</td>
<td>Missing</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Median BM blasts,</strong> %</td>
<td>60 (0-98)</td>
<td>61 (0-98)</td>
<td>58 (17-96)</td>
<td>0.49</td>
</tr>
<tr>
<td>(Range)</td>
<td>Missing</td>
<td>8</td>
<td>6</td>
<td></td>
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<tr>
<td><strong>Cytogenetics, No. (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBF as sole abn</td>
<td>41 (42)</td>
<td>26 (55)</td>
<td>15 (30)</td>
<td>0.01</td>
</tr>
<tr>
<td>+ additional abn</td>
<td>56 (58)</td>
<td>21 (45)</td>
<td>35 (70)</td>
<td>0.002</td>
</tr>
<tr>
<td>Trisomy 22</td>
<td>12 (12)</td>
<td>11 (23)</td>
<td>1 (2)</td>
<td>0.26</td>
</tr>
<tr>
<td>Trisomy 8</td>
<td>7 (7)</td>
<td>5 (11)</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td><strong>Disease type, No. (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>De novo AML</td>
<td>87 (90)</td>
<td>42 (89)</td>
<td>45 (90)</td>
<td>0.99</td>
</tr>
<tr>
<td>s-AML</td>
<td>2 (2)</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>t-AML</td>
<td>8 (8)</td>
<td>4 (9)</td>
<td>4 (8)</td>
<td></td>
</tr>
<tr>
<td><strong>Median FLT3-ITD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>allelic ratio</td>
<td>0.35</td>
<td>0.32</td>
<td>0.35</td>
<td>0.99</td>
</tr>
<tr>
<td>(Range)</td>
<td>0.003-50</td>
<td>0.003-50</td>
<td>0.005-34</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>20</td>
<td>9</td>
<td>11</td>
<td></td>
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<tr>
<td><strong>FLT3-TKD</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No. (%)</td>
<td>10 (21)</td>
<td>8 (29)</td>
<td>2 (10)</td>
<td>0.16</td>
</tr>
<tr>
<td>Missing</td>
<td>49</td>
<td>19</td>
<td>30</td>
<td></td>
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</tbody>
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**Abbreviations:** abn, aberration; allo, allogeneic; AML, acute myeloid leukemia; BM, bone marrow; CBF, core-binding factor; FLT3, fms-related tyrosine kinase 3; s-AML, AML after previous myelodysplastic syndrome; t-AML, therapy-related AML; TKD, tyrosine kinase domain; WBC, white blood cell count. Results may not add-up to 100 due to rounding.
Table 2: Univariable Cox models on relapse-free and overall survival.

<table>
<thead>
<tr>
<th></th>
<th>RFS</th>
<th></th>
<th></th>
<th>OS</th>
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<tr>
<td></td>
<td>HR</td>
<td>P-value</td>
<td>HR</td>
<td>P-value</td>
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<tr>
<td>Type of CBF-AML</td>
<td>0.89</td>
<td>0.72</td>
<td>0.91</td>
<td>0.80</td>
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<td>Higher age (≥60 years)</td>
<td>0.61</td>
<td>0.14</td>
<td>0.63</td>
<td>0.20</td>
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<tr>
<td>Log10(WBC)</td>
<td>0.42</td>
<td>0.22</td>
<td>1.43</td>
<td>0.34</td>
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<tr>
<td>Platelets</td>
<td>1.00</td>
<td>0.54</td>
<td>1.00</td>
<td>0.50</td>
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<tr>
<td>Complex karyotype</td>
<td>0.68</td>
<td>0.32</td>
<td>0.96</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>High FLT3-ITD allelic ratio (≥0.5)</td>
<td>1.19</td>
<td>0.63</td>
<td>1.83</td>
<td>0.13</td>
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</tr>
<tr>
<td>Trisomy 8</td>
<td>0.94</td>
<td>0.92</td>
<td>1.53</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Trisomy 22</td>
<td>0.22</td>
<td>0.04</td>
<td>0.35</td>
<td>0.15</td>
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</tr>
</tbody>
</table>

Abbreviations: AML, acute myeloid leukemia; CBF, core-binding factor; HR, hazard ratio; OS, overall survival; RFS, relapse-free survival, WBC, white blood cell count.
Figure legends

**Figure 1:** Kaplan-Meier plots on relapse-free survival and overall survival in intensively treated patients according to type of core-binding factor acute myeloid leukemia. Panel A represents the Kaplan Meier plot on relapse-free survival, Panel B on overall survival.

**Figure 2:** Cumulative incidence of relapse (CIR) and cumulative incidence of death (CID) plots according to treatment strategy (chemotherapy, allogeneic hematopoietic stem cell transplantation) in first complete remission. CIR and CID included only patients attaining complete remission. Panel A represents CIR, Panel B represents CID.

**Figure 3:** Simon Makuch plot on overall survival measured from the date of relapse in relapsed patients illustrating the impact of allogeneic hematopoietic stem cell transplantation as a time dependent event.

**Figure 4:** Kaplan Meier plot on relapse-free survival and overall survival of patients with inversion 16 and trisomy 22 as compared to all other core-binding factor acute myeloid leukemia patients. Panel A represents the Kaplan Meier plot on relapse-free survival, Panel B on overall survival.