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

Sabine Kayser, Michael Kramer, David Martínez-Cuadrón, Justin Grenet, Klaus H. Metzeler, Zuzana Sustkova, Marilise R. Luskin, Andrew M. Brunner, Michelle A. Elliott, Cristina Gil, Sandra Casal Mariño, Zdeněk Ráčil, Petr Cetkovský, Jan Noval, Alexander E. Perl, Uwe Platzbecker, Friedrich Stölzel, Anthony D. Ho, Christian Thiede, Richard M. Stone, Christoph Röllig, Pau Montesinos, Richard F. Schlenk and Mark J. Levis

The aim of this study was to evaluate the prognostic impact of FLT3-ITD in core-binding factor acute myeloid leukemia (CBF-AML) in an international, multicenter survey of 97 patients of whom 52% had t(8;21)(q22;q22) and 48% had inv(16)(p13q22)/t(16;16)(p13;q22). The median age of the patients was 53 years (range, 19-81). Complete remission after anthracycline-based induction (n=86) and non-intensive therapy (n=11) was achieved in 97% and 36% of the patients, respectively. The median follow-up was 4.43 years (95% confidence interval [95% CI]: 3.35-7.39 years). The median survival after intensive and non-intensive treatment was not reached and 0.96 years, respectively. Among 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 patients with CBF-AML/FLT3-ITD (n=75). Overall, 24 patients underwent allogeneic hematopoietic cell transplantation (HCT), 12 in first complete remission and 12 after relapse. Allogeneic HCT in first complete remission was not beneficial (P=0.60); however, allogeneic HCT seemed to improve median survival in relapsed patients compared to that of patients treated with chemotherapy (not reached vs. 0.6 years, respectively; P=0.002). Excluding patients with inv(16) with trisomy 22, our data indicate that...
the outcome of CBF-AML patients with FLT3-ITD may be inferior to that of patients without FLT3-ITD (based on previously published data), suggesting that prognostically CBF-AML patients with FLT3-ITD should not be classified as favorable-risk. FLT3-inhibitors may improve the outcome of these 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-CBF 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 repeated cycles of high-dose cytarabine as post-remission therapy. The 10-year overall survival (OS) rate was reported to be 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 LeukemiaNet recommendations, regardless of the FLT3-ITD mutational status. Nevertheless, 30-40% of patients with CBF-AML experience relapse.

FLT3-ITD mutations occur in roughly 5-10% of adults with 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, the prognostic relevance of such aberrations in CBF-AML is still controversial. In a study of 176 patients with inv(16) AML, those with FLT3 mutations (n=30) had an inferior OS compared to those with wild-type FLT3. Of note, in the same analysis trisomy 22 was a favorable prognostic marker irrespective of concomitant FLT3 mutations, confirming the findings of previous studies, in which trisomy 22 was associated with a lower cumulative incidence of relapse (CIR) than that in patients with only inv(16) (estimated long-term CIR rates of 42% and 66%, respectively; \( P = 0.02 \)) and an excellent relapse-free survival (RFS) of 82%.

Currently, it is unclear whether patients with CBF-AML and KIT or FLT3 mutations may benefit from allogeneic hematopoietic stem cell transplantation (HCT) in first complete remission (CR1). A meta-analysis of several prospective trials evaluating the impact of allogeneic HCT for AML in CR1 did not show a benefit of allogeneic HCT on RFS or OS for favorable-risk AML (n=547) as compared to non-allogeneic HCT therapies including post-remission chemotherapy, autologous HCT, or both. In line with these findings, Burnett et al. reported that CBF-AML patients who underwent allo-HCT in CR1 had no survival benefit compared to patients not receiving HCT. Another retrospective analysis of younger (<60 years) AML patients with t(8;21) compared the outcomes of 118 patients who received allogeneic HCT from a matched-related donor with the outcomes of 152 patients treated with cytarabine-based chemotheraphy. After allogeneic HCT, the risk of relapse was significantly lower (hazard ratio=0.47; \( P = 0.014 \)), but the treatment-related mortality was significantly higher (hazard ratio=6.76; \( P < 0.001 \)) than after chemotherapy. No benefit regarding RFS and OS was found for allogeneic HCT in the entire study cohort. Two other studies compared the results of allogeneic versus autologous HCT in CBF-AML. Gorin et al. reported a significantly higher relapse risk after autologous HCT than after allogeneic HCT in patients with t(8;21) (28% vs. 15%, \( P = 0.05 \)) but not in patients with inv(16) AML. Again, treatment-related mortality was significantly higher after allogeneic HCT than after autologous HCT in both t(8;21) patients (24% vs. 6%, \( P = 0.008 \)) and in those with inv(16) (14% vs. 2%, \( P = 0.003 \)) but the type of transplant did not affect leukemia-free survival. A Japanese study also showed comparable results for OS after allogeneic and autologous HCT in CR1 for both t(8;21) and inv(16) AML.

The prognostic impact of FLT3-ITD in CBF-AML is still a matter of debate. The 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 allogeneic 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 USA and Europe. Participating centers were chosen upon network relationships of the first and last authors. Detailed case report forms (including information on baseline characteristics, chemotherapy, allogeneic 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. The 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 ITD 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 AML60+ (n=2), AML96 (n=18) and AML2003 (n=9) as well as the CALGB/Rafitry trial (n=1). Induction therapy of the 86 patients consisted of the anthracycline/cytarabine based +8-8 regimen (n=62) or compa-
rable intensive treatment (n=24); additionally, five of the intensively treated patients received gemtuzumab ozogamicin.

Eleven (11%) of the 97 patients were treated non-intensively. Of these, five received azacitidine, either alone (n=2) or in combination with venetoclax (n=2) or sorafenib (n=1); 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. All clinical studies were approved by the institutional review boards of the participating centers. All patients provided written informed consent to participation in one of the treatment trials or to 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. Patients’ characteristics were compared with the Kruskal-Wallis rank sum test for continuous variables and Fisher exact test for categorical variables. The median follow-up time was computed using the reverse Kaplan-Meier estimate. The Kaplan-Meier method was used to estimate the distribution of RFS and OS. Confidence interval (CI) estimates for survival curves were based on the cumulative hazard function using the Greenwood formula for variance estimation. Log-rank 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 allogeneic HCT on OS as a time-dependent intervening event was tested using the Mantel-Byar method. The method of Simon and Makuch was applied 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 allogeneic HCT, they were censored at this time point in the chemotherapy group and further followed up within the allogeneic 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

Demographic and clinical data were collected from 97 patients (Study Alliance Leukemia, n=46; Spanish Programa Español de Tratamientos en Hematología [PETHEMA], 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; and Mayo Clinic Rochester, n=2) diagnosed with CBF-AML between 1996 and 2019. The median age of the patients was 53 years (range, 19-81 years) and 45 patients (46%) were female. The patients’ baseline characteristics are summarized in Table 1. Median white blood cell (WBC): count was higher in patients with inv(16)/t(16;16) than in patients with t(8;21). In addition, median WBC count in patients with inv(16)/t(16;16) was lower in those with trisomy 22 (n=11; median WBC 28.8x10^9/L; range, 3.9-186.7x10^9/L) than in those without trisomy 22 (n=56; median WBC 54.8x10^9/L; range, 2.7-298x10^9/L; P=0.18).

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

| Characteristic                  | All patients (n=97) | Inv(16) (n=47) | t(8;21) (n=50) | P-value |
|--------------------------------|---------------------|----------------|----------------|---------|
| Median age, years (range)      | 53 (19-81)          | 50 (19-81)     | 53,5 (22-77)   | 0.95    |
| Female, n. (%)                 | 45 (46)             | 28 (56)        | 17 (36)        | 0.07    |
| Median WBC, 10^9/L (range)     | 20.5 (1.8-298)      | 43.4 (1.8-153.4) | 14.3 (1.8-153.4) | <0.001 |
| Missing                        | 2                   | 2              | 2              |         |
| Median Hb, g/dL (range)        | 8.6 (4.6-14.3)      | 8.7 (5.6-14.3) | 8.4 (4.6-12.0) | 0.1     |
| Missing                        | 5                   | 2              | 3              |         |
| Median platelets, 10^9/L (range)| 33 (7.372)          | 33 (7.261)     | 33 (7.373)     | 0.92    |
| Missing                        | 5                   | 2              | 3              |         |
| Median BM blasts, % (range)    | 60 (0.98)           | 61 (0.98)      | 58 (17.96)     | 0.49    |
| Missing                        | 8                   | 2              | 6              |         |
| Cytogenetics, n. (%)           | CBF as sole abn    | 41 (42)        | 26 (55)        | 15 (30) | 0.01    |
|                               | CBF + other abn    | 56 (58)        | 21 (45)        | 35 (70) |         |
| Trisomy 22                     | 12 (12)             | 11 (23)        | 1 (2)          | 0.002   |
| Trisomy 8                      | 7 (7)               | 5 (11)         | 2 (4)          | 0.26    |
| Disease type, n. (%)           | De novo AML        | 87 (90)        | 42 (89)        | 45 (90) | 0.99    |
| Secondary AML                  | 2 (2)               | 1 (2)          | 1 (2)          |         |
| Therapy-related AML            | 8 (8)               | 4 (9)          | 4 (8)          |         |
| Median FLT3-ITD allelic ratio (range) | 0.35               | 0.32           | 0.35           | 0.99    |
|                               | 0.003-50            | 0.003-50       | 0.003-34       |
| Missing                        | 20                  | 9              | 11             |         |
| FLT3-TKD N. (%)                | 10 (21)             | 8 (29)         | 2 (10)         | 0.16    |
| Missing                        | 49                  | 19             | 30             |         |

abn: aberration; allo: allogeneic; AML: acute myeloid leukemia; BM: bone marrow; CBF: core-binding factor; FLT3: imatinib-resistant tyrosine kinase 3; Hb: hemoglobin; TKD: tyrosine kinase domain; WBC: white blood cell count. Results may not add up to 100 due to rounding.
The FLT3-ITD allelic ratio was available in 77 (79%) patients and the median allelic ratio was 0.85 (range, 0.003-50). Median WBC count was higher in patients with a high allelic ratio than in those with a low allelic ratio (31.7x10^9/L vs. 16x10^9/L, P=0.02).

The median FLT3-ITD size and number of ITD clones were available for 29 (50%) patients. The median FLT3-ITD size was 39 (range, 3-120) base-pairs and most of the patients harbored one clone (1 clone, n=24; 2 clones, n=4, 3 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 for 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 1 g cytarabine every 12 h on 4 days as well as mitoxantrone 12 mg/day on 3 days. Early death occurred in two (2%) patients; none of the intensively treated patients had refractory disease. All patients, who received “7+3” treatment, either with midostaurin (n=5) or gilteritinib ozoegamicin (n=4), achieved CR.

Eleven patients were treated less intensively because of their older age (median: 72 years; range, 40-81 years) or comorbidities. Of these 11 less intensively treated patients, four (36%) achieved CR, one had a partial remission, four were refractory and two patients died early. The four patients who achieved CR had been treated with a hypomethylating agent (n=1), venetoclax + azacitidine (n=1) and fludarabine + low-dose cytarabine (n=2).

Further therapy including intensive consolidation and allogeneic hematopoietic cell transplantation

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 for 54 patients. Of those, ten patients received four cycles of consolidation, 14 patients received three cycles, seven received two cycles and 23 patients received one cycle. For analysis, we compared patients who received one or two consolidation cycles with those who received more than two cycles of consolidation. There was no difference in CIR between patients who received one or two cycles and those who received more than two cycles (P=0.97). One of the transplanted patients received maintenance with gilteritinib for 2 years after allogenic HCT within a randomized trial. In addition, one patient with intensive chemotherapy consolidation received maintenance with midostaurin.

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

Among the patients consolidated with chemotherapy, relapses occurred in 31 and there were six treatment-related deaths after consolidation. In patients consolidated with allogenic HCT in CR1 three patients relapsed and there was one treatment-related death. In those relapsing after chemotherapy, allogeneic HCT was performed in 12 patients: eight in second CR (CR2) and four with active disease.

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

Characteristics of patients undergoing allogeneic hematopoietic cell transplantation

Overall, an allogeneic 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 in eight patients; additionally eight patients received reduced-intensity conditioning (missing, n=5). The stem cell source was a matched related donor in 11 cases, a matched unrelated donor in ten cases, a haplo-identical donor in two, and unknown in one of the 24 patients.

Cumulative incidences of relapse and death in complete remission, and survival

The median follow-up of the entire cohort was 4.43 years (95% CI: 3.35-7.39 years). The 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%), respectively.

In intensively treated patients RFS and OS were not different between patients with inv(16) and those with t(8;21) (P=0.70 and P=0.80, respectively) (Figure 1). Furthermore, CIR (P=0.26) (Figure 2A) and CID (P=0.96) (Figure 2B) were comparable in patients proceeding to allogeneic HCT in CR1 or not. However, in relapsed patients survival was dismal without allogeneic 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 allogeneic HCT either in CR2 or with active disease the median survival was not reached and survival at 4 years was 53% (95% CI: 30-94% Figure 3). In a Mantel-Byar analysis including allogenic HCT performed after relapse as a time-dependent event, survival after relapse was significantly improved by allogeneic HCT (P=0.002).

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

The median survival of non-intensively treated patients was 0.96 years (95% CI: 0.24-not reached) and none of these patients survived beyond 3 years.

Exploratory subset analysis revealed trisomy 22 in patients with inv(16) as a significant prognostic factor for RFS (n=11; P=0.02) (Figure 4A); the outcome of these 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 4A). In addition, OS tended to be higher in patients with inv(16) and trisomy 22 (P=0.10) than in those with all other CBF-AML (Figure 4B).

Other relevant prognostic factors, such as type of CBF-
AML, older age (≥60 years), WBC count, platelet count, 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 variable with a significant effect on the RFS endpoint (hazard ratio=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 allogeneic HCT, as compared to conventional chemotherapy, on survival.

Secondary chromosome aberrations can be detected in more than 60% of AML cases with t(8;21) and in 35% to 40% of those with inv(16). In line with published data, 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) than in those with t(8;21).

In contrast to previous reports there was no impact of WBC count, older age (≥60 years) or loss of the sex chromosome on outcome.

---

**Figure 1.** Kaplan-Meier plots of survival in intensively treated patients according to type of core-binding factor acute myeloid leukemia. (A) Relapse-free survival. (B) Overall survival.

**Figure 2.** Plots of cumulative incidence of relapse and cumulative incidence of death according to treatment strategy in first complete remission. (A) Cumulative incidence of relapse. (B) Cumulative incidence of death. Only patients attaining complete remission are included. Treatment strategy is divided into consolidation chemotherapy or allogeneic hematopoietic stem cell transplantation (allo-HCT) in first complete remission (CR1).
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 chemosensitive regardless of a concurrent FLT3-ITD. We confirmed 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 with an inv(16) and trisomy 22 so rarely relapse after intensive induction and consolidation therapy. Obviously, leukemic clones harboring both abnormalities are very chemosensitive. Our study adds to previous knowledge that despite the proliferative signal induced by a FLT3-ITD and the chemoresistance induced by high FLT3-ITD allelic ratios patients with inv(16) and trisomy 22 remain extremely chemosensitive. The underlying pathogenetic mechanism by which trisomy 22 exerts its prognostic impact remains elusive.

Regarding the outcome of intensively treated CBF patients exhibiting a FLT3-ITD without trisomy 22, results were dismal with a RFS rate of 38% after 4 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 was 51% after 4 years in FLT3-ITD-positive patients as compared to 58% after 10 years in those with wild-type FLT3. In addition, the outcome of intensively treated patients was not affected by CBF subtype, inv(16) and t(8;21), or CR1 consolidation approach (chemotherapy or transplantation). These results might argue for the benefit of repeated 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 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 not treated 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 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 favor-
able-risk category. Rather, these patients might be 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 a few patients were treated with either midostaurin or gemtuzumab ozogamicin, so the effect of these drugs on outcome could not be evaluated. The impact of midostaurin in combination with gemtuzumab ozogamicin on outcome is currently being evaluated within a phase II/I trial (ClinicalTrials.gov Identifier: NCT04385290).

The survival of relapsed patients was dismal without allogeneic 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 allogeneic HCT after relapse, either in CR2 or with active disease, the median survival had not been reached and survival at 4 years was 55%, arguing that allogeneic HCT should be the preferred approach in relapsed patients. However, we would like to emphasize that retrospectively collected data have serious limitations regarding the factors for allocating patients to allogeneic HCT, such as co-morbidities, individual assessment of the treating physician, choice of conditioning, and availability of a donor, remain unknown and these factors need to be taken into account when evaluating the value of allogeneic HCT in our series.

In conclusion, despite a high remission rate, patients with FLT3-ITD had an inferior outcome compared to those without FLT3-ITD, based on previously published data on CBF-AML. Thus, CBF-AML with FLT3-ITD should not be classified within the favorable-risk category. Our data suggest that allogeneic 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).

Disclosures
No conflicts of interest to disclose.

Contributions
SK and RFS were responsible for the concept of this paper, contributed to the literature search data collection, analyzed and interpreted data, and wrote the manuscript. MJL was responsible for the concept of this paper, contributed to the literature search data collection, contributed patients, analyzed and interpreted data, and critically revised the manuscript. CTH performed research and critically reviewed the manuscript. MK, DM-C, JC, KHM, ZS, AM, MB, MAE, CG, SCM, ZR, PC, JN, AEP, FS, ADH, UP, RMS, CR, and PM contributed patients and critically revised the manuscript. All authors reviewed and approved the final manuscript.

Funding
SK was supported by the Olympia-Marata fellowship program from the Medical Faculty of the Heidelberg University. MJL is supported by a grant from the NCI (NCI Leukemia SPORE P50 CA100632). ZS, ZR, PC and JN were supported by the Ministry of the Czech Republic, grant n. 15-25809A. The authors also acknowledge support from Leipzig University for Open Access Publishing.

References
1. Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, Revised 4th Edition. WHO Press, Geneva, Switzerland, 2017.
2. Speck NA, Gilliland DG. Core-binding factors in haematopoiesis and leukaemia. Nat Rev Cancer 2002;2(27):902-913.
3. Marucci G, Mrózek K, Ruppert AS, et al. Prognostic factors and outcome of core binding factor acute myeloid leukemia patients with t(8;21) differ from those of patients with inv(16): a Cancer and Leukemia Group B study. J Clin Oncol. 2005;23(24):5705-5717.
4. Bloomfield CD, Berman M, Byrd JC, et al. Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. Cancer Res. 1998;58(18):4173-4177.
5. Byrd JC, Ruppert AS, Mrózek K, et al. Repetitive cycles of high-dose cytarabine benefit patients with acute myeloid leukemia and inv(16)(p13q22) or t(16;16)(p13;q22): results from CALGB 8461. J Clin Oncol. 2004;22(6):1087-1094.
6. Miyawaki S, Ohtake S, Fujisawa S, et al. A randomized comparison of 4 courses of standard-dose multiagent chemotherapy versus 5 courses of high-dose cytarabine alone in postremission therapy for acute myeloid leukemia in adults: the JALSG AML201 study. Blood. 2011;117(8):2366-2372.
7. Allen C, Hills RK, Lamb K, et al. The importance of relative mutant level for evaluating impact on outcome of KIT, FLT3 and CBL mutations in core-binding factor acute myeloid leukemia. Leukemia. 2013;27(9):1891-1899.
8. O'Donnell MR, Tallman MS, Abboud CN, et al. Acute myeloid leukemia, version 3.2017, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2017;15(5):424-447.
9. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424-447.
10. Schlenk RF, Benner A, Krauter J, et al. Individual patient data-based meta-analysis of patients aged 16 to 60 years with core binding factor acute myeloid leukemia: a survey of the German Acute Myeloid Leukemia Intergroup. J Clin Oncol. 2004;22(18):3741-3750.
11. Faber ZJ, Chen X, Gedman AL, et al. The genomic landscape of core-binding factor acute myeloid leukemias. Nat Genet. 2016;48(12):1551-1556.
12. Scheliz C, Rawlat VF, Cusan M, et al. The AML1-ETO fusion gene and the FLT3 length mutation collaborate in inducing acute leukemia in mice. J Clin Invest. 2005;115(8):2159-2168.
13. Kim HG, Kojima K, Swindle CS, et al. FLT3-ITD cooperates with inv(16) to promote progression to acute myeloid leukemia. Blood. 2009;113(5):1567-1574.
14. Paschka P, Du J, Schlenk RE, et al. Secondary genetic lesions in acute myeloid leukemia with inv(16) or t(16;16): a study of the German-Austrian AML Study Group (AMLSG). Blood. 2013;121(1):170-177.
15. Boissel N, Leroy H, Brethon B, et al. Incidence and prognostic impact of c-Kit, FLT3, and Ras gene mutations in core binding factor acute myeloid leukemia (CBF-AML). Leukemia. 2009;23(8):965-970.
16. Jones D, Yao H, Romana A, et al. Modeling interactions between leukemia-specific chromosomal changes, somatic mutations, and gene expression patterns during progression of core-binding factor leukemias. Genes Chromosomes Cancer. 2010;49(2):182-191.
17. Koreth J, Schlenk R, Kopecky KJ, et al. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials. JAMA. 2009;301(22):2349-2361.
18. Burnett AK, Goldstone A, Hills RK, et al. Curability of patients with acute myeloid leukemia who did not undergo transplantation in first remission. J Clin Oncol. 2013;31(10):1295-1301.
19. Schlenk RF, Pasquini MC, Perez WS, et al. HLA-identical sibling allogeneic transplants versus chemotherapy in acute myelogenous leukemia with t(8;21) in first complete remission: collaborative study between the German AML Intergroup and CIBMTR. Biol Blood Marrow Transplant. 2008;14(2):187-196.
20. Gorin NC, Labopin M, Frassoni F, et al. Identical outcome after autologous or allo-
geneic genoidentical hematopoietic stem-
cell transplantation in first remission of
acute myelocytic leukemia carrying inver-
sion 16 or t(8;21): a retrospective study from
the European Cooperative Group for Blood
and Marrow Transplantation. J Clin Oncol.
2008;26(19):3185-3188.
21. Kuwatsuka Y, Miyamura K, Suzuki R, et al.
Hematopoietic stem cell transplantation for
core binding factor acute myeloid leukemia:
t(8;21) and inv(16) represent different clinical
outcomes. Blood. 2009;113(9):2096-2103.
22. Bennett JM, Catovsky D, Daniel MT, et al.
Proposed revised criteria for the classifica-
tion of acute myeloid leukemia. A report of
the French-American-British Cooperative
Group. Ann Intern Med. 1985;103(4):620-
625.
23. Cheson BD, Bennett JM, Kopecky KJ, et al.
Revised recommendations of the Inter-
national Working Group for Diagnosis,
Standardization of Response Criteria,
Treatment Outcomes, and Reporting
Standards for Therapeutic Trials in Acute
Myeloid Leukemia. J Clin Oncol. 2008;26
(24):4642-4649.
24. Mitelman F. ISCN: an International System
for Human Cyogenetic Nomenclature.
Basel, Switzerland: S. Karger. 1995.
25. Yokota S, Kiyoi H, Nakao M, et al. Internal
tandem duplication of the FLT3 gene is pref-
erentially seen in acute myeloid leukemia
and myelodysplastic syndrome among vari-
ous hematological malignancies. A study on
a large series of patients and cell lines.
Leukemia. 1997;11(10):1605-1609.
26. Thiede C, Steudel C, Mohr B, et al. Analysis
of FLT3-activating mutations in 979 patients
with acute myelogenous leukemia: associa-
tion with FAB subtypes and identification of
subgroups with poor prognosis. Blood.
2002;99(12):4326-4335.
27. Röllig C, Kramer M, Gabrecht M, et al.
Intermediate-dose cytarabine plus mitox-
anthrone versus standard-dose cytarabine
plus daunorubicin for acute myeloid
leukemia in elderly patients. Ann Oncol.
2018;29(4):973-978.
28. Röllig C, Thiede C, Gramatzki M, et al. A
novel prognostic model in elderly patients
with acute myeloid leukemia: results of 909
patients entered into the prospective
AML96 trial. Blood. 2010;116(6):971-977.
29. Schaic M, Parmentier S, Kramer M, et al.
High-dose cytarabine consolidation with or
without additional ansacrine and mitox-
anthrone in acute myeloid leukemia: results
of the prospective randomized AML2003
trial. J Clin Oncol. 2013;31:2094-2102.
30. Stone RM, Mandrekar SJ, Sanford BL, et al.
Midostaurin plus chemotherapy for acute
myeloid leukemia with a FLT3 mutation.
N Engl J Med. 2017;377(5):454-464.
31. Schermer M, Smith TL. A note on quantifying
follow-up in studies of failure time.
Control Clin Trials. 1996;17(4):345-346.
32. Kaplan E, Meier P. Nonparametric estima-
tion from incomplete observations. J Am Stat
Assoc. 1958;53(282):457-481.
33. Cox DR. Regression models and life tables
(with discussion). J R Stat Soc. 1972;34(2):
187-220.
34. Gray RJ. A class of k-sample tests for com-
paring the cumulative incidence of a com-
peting risk. Ann Stat. 1988;16(3):1141-1154.
35. Mantel N, Byar D. Evaluation of response-
time data involving transient states: an illus-
tration using heart transplant data. J Am Stat
Assoc. 1974;69(345):81-86.
36. Simon R, Makuch RW. A non-parametric
graphical representation of the relationship
between survival and the occurrence of an
event: application to responder versus non-
responder bias. Stat Med. 1984;3(1):35-44.
37. R Development Core Team. R: A Language
and Environment for Statistical Computing.
Vienna, Austria, 2014.
38. Paschka P. Core binding factor acute
myeloid leukemia. Semin Oncol. 2008;35(4):
410-417.
39. Opatz S, Bamopoulos SA, Metzeler KH, et al.
The clinical mutatome of core binding factor
leukemia. Leukemia. 2020;34(6):1553-1562.