Midostaurin in addition to intensive chemotherapy in acute myeloid leukemia with t(8;21) and *KIT* and/or *FLT3*-ITD mutations: results of the SAL MIDOKIT trial

Acute myeloid leukemia (AML) with t(8;21)(q22;q22.1); *RUNX1::RUNX1T1*, along with AML with inv(16)(p13.1q22)/t(16;16)(p13.1;q22);*CBFB::MYH11* considered as core binding factor (CBF)-AML, is known to confer a favorable prognosis.[1-3] However, a considerable proportion of patients with CBF-AML, especially those with t(8;21) AML, still experience relapse, emphasizing the need of novel therapeutic approaches.[4,5] Several studies have identified additional molecular alterations, such as *KIT* or *FLT3* mutations, as risk factors for relapse and impaired survival in CBF-AML. Here, in particular the prognosis of patients with t(8;21) AML seems to be negatively impacted by these additional mutations.[6-9] Thus, there is a molecular rationale to implement KIT/FLT3 inhibitors into treatment of patients with AML with t(8;21).

Accordingly, we conducted a prospective, single-arm, multi-center, phase II trial MIDOKIT (*clinicaltrials gov. Identifier: NCT01830361*) to evaluate the molecular guided addition of midostaurin, an oral multi-kinase inhibitor with activity on KIT and FLT3, to standard daunorubicin/cytarabine (DA)-based intensive chemotherapy (IC) in adult patients with newly diagnosed AML with t(8;21) with evidence of *KIT* and/or *FLT3* internal tandem duplication (*FLT3*-ITD) mutations. Patients aged 18-65 years with previously untreated AML according to the World Health Organization (WHO) classification were eligible for molecular prescreening. Those with evidence of t(8;21)(q22;q22.1); *RUNX1::RUNX1T1* with additional *KIT* and/or *FLT3*-ITD mutations and response to one course of DA induction chemotherapy (IT) performed outside the trial could be enrolled. Inclusion and exclusion criteria are summarized in the *Online Supplementary Table S1*.

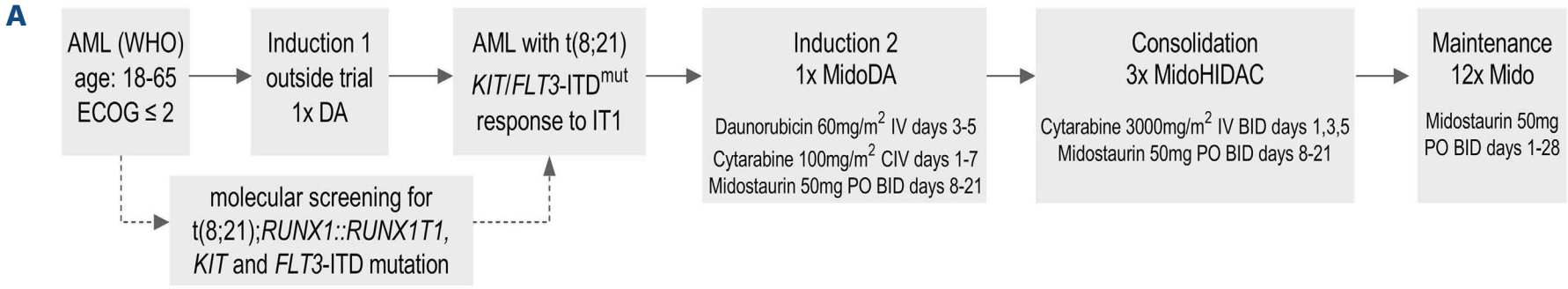
The trial design and treatment algorithm is shown in Figure 1A. Enrolled patients were scheduled to receive a second course of DA induction chemotherapy (cytarabine 100 mg/m^2/day continuously intravenously, days 1-7 and daunorubicin 60 mg/m^2/day intravenously, days 3-5) and three courses of high-dose cytarabine consolidation (cytarabine 3000 mg/m^2/ intravenously twice daily, days 1, 3, 5) each followed by midostaurin 50 mg orally twice daily on days 8-21, and subsequent midostaurin maintenance for 12 months.

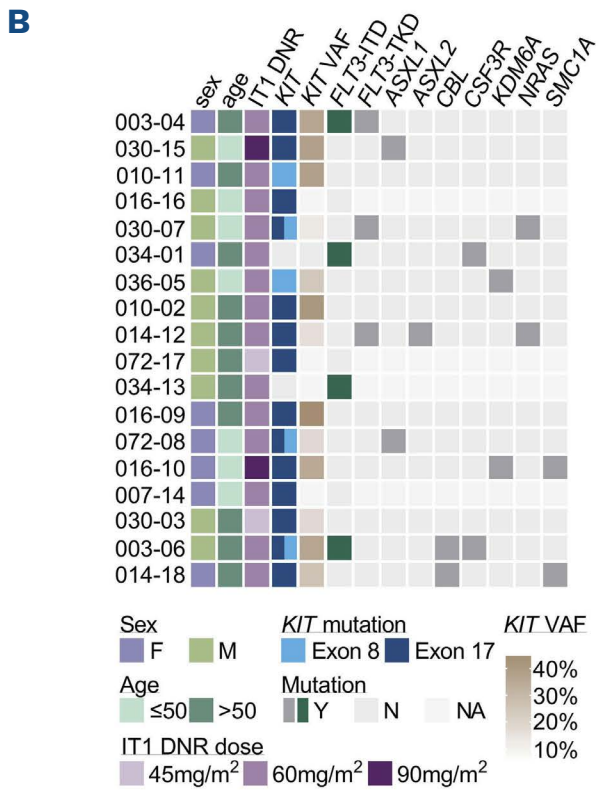
The primary endpoint of the study was 2-year event-free survival (EFS). The primary objective was to improve 2-year EFS from a historical benchmark of 50% to 80%. The historical benchmark was based on data of 103 patients with AML with t(8;21) treated within the AML96 trial (*clinicaltrials gov. Identifier: NCT00180115*), the AML2003 trial (*clinicaltrials gov. Identifier: NCT00180102*) and the AML60+ trial (*clinicaltrials gov. Identifier: NCT00180167*), hereof 32 patients - matched for sex, age and number of courses of induction and consolidation chemotherapy - with evidence of *KIT* and/or *FLT3*-ITD mutations (28.1% of patients harboring *FLT3*-ITD mutations) showing chemo-responsiveness after IT1. Secondary endpoints included composite complete remission (CR) rate, relapse-free survival (RFS), overall survival (OS), incidence of treatment-emergent adverse events (AE) and measurable residual disease (MRD) kinetics.

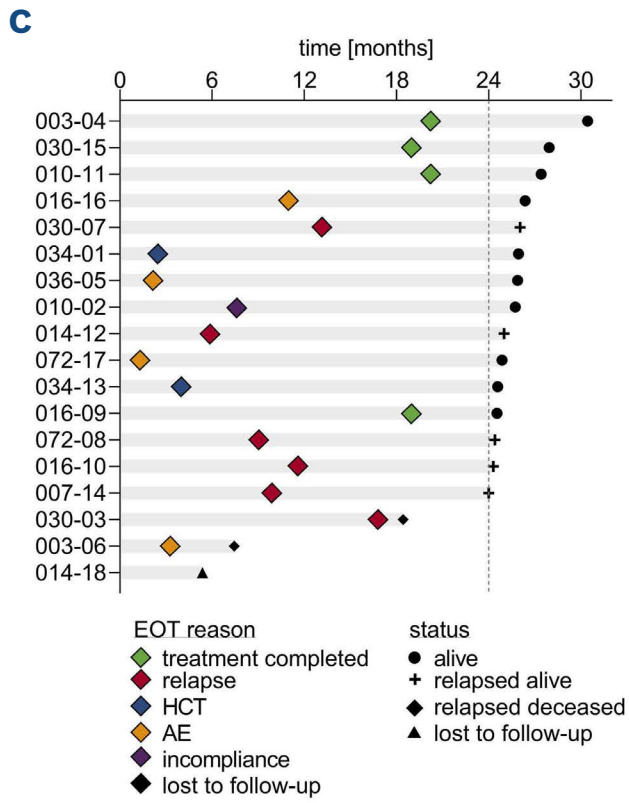
For prospective molecular screening the presence of AML with t(8;21)(q22;q22.1); *RUNX1::RUNX1T1* was assessed via reverse transcription polymerase chain reaction (RT-PCR) (evidence of *RUNX1::RUNX1T1*) and fluorescence *in situ* hybridization (FISH) (evidence of t(8;21)(q22;q22.1)), results were validated in one of the SAL reference laboratories. Patients were evaluated for *FLT3*-ITD and *KIT* mutations via fragment length analysis (limit of detection [LOD] 0.05 allelic ratio) and Sanger sequencing (LOD 10% variant allele frequency [VAF]), respectively.[10] In addition, patients with available samples were retrospectively assessed via amplicon-based next-generation sequencing (NGS; MiSeq, Illumina, Inc; DHS-003Z human myeloid neoplasms panel + custom AML panel, Qiagen N.V.; LOD 1% VAF). Quantitative bone-marrow *RUNX1::RUNX1T1* MRD was assessed via RT-PCR according to the Europe Against Cancer (EAC) initiative guidelines.[11]

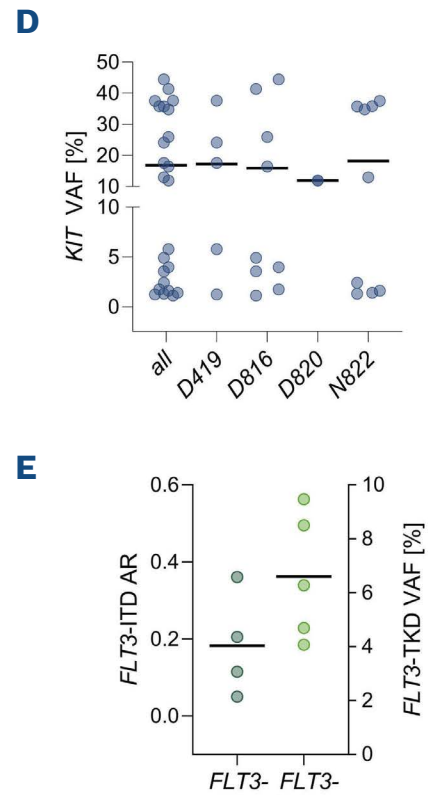
The trial was conducted in accordance with the principles of the Declaration of Helsinki; the trial protocol was approved by the Ethics Committee of the TU Dresden. Written informed consent was provided by all patients before screening.

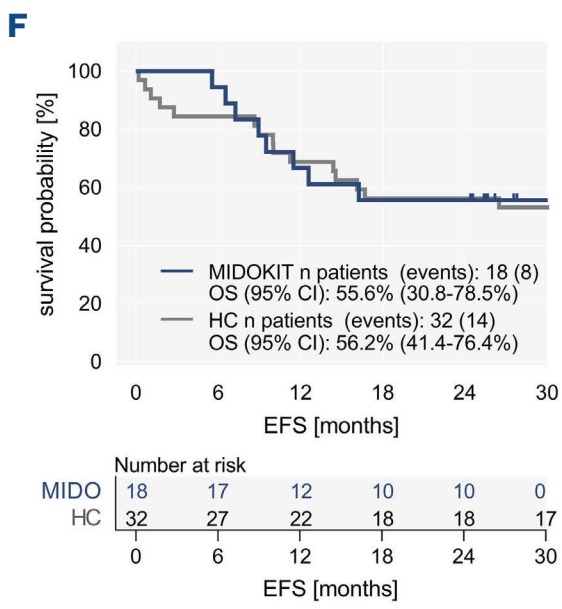
Between Mar 13, 2013 and Dec 15, 2017 a total of 53 patients were diagnosed with t(8;21) AML at 11 participating centers, of those, 23 patients were screened and 18 patients with AML with t(8;21) and evidence of *KIT* and/or *FLT3*-ITD mutations were enrolled. All 18 patients had received DA for IT1 outside the trial (most patients received daunorubicin at a dose of 60 mg/m^2) and had shown chemo-responsiveness. Table 1 and Figure 1B, C show the baseline characteristics, molecular features, and clinical course of the enrolled patients. Patient disposition is provided in the *Online Supplementary Figure S1A*. The median age was 50 years (range, 27-65 years), ten patients (55.6%) were male. Sixteen patients were diagnosed with *de novo* AML. A total of 24 *KIT* mutations were found in 16 patients (88.9%), some patients harbored several (subclonal) vari-

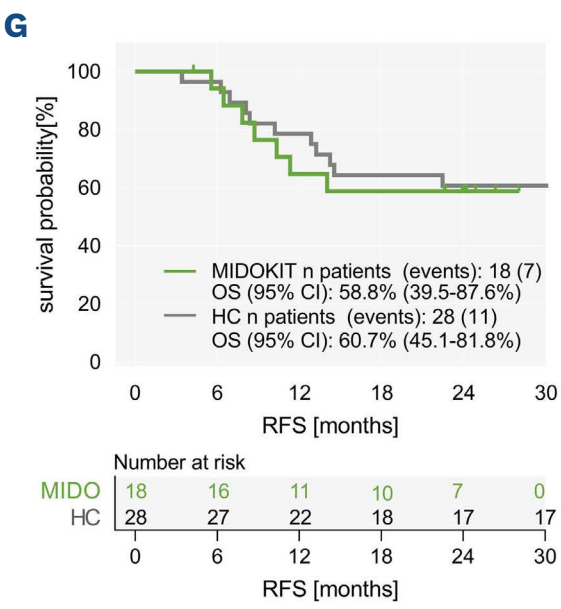


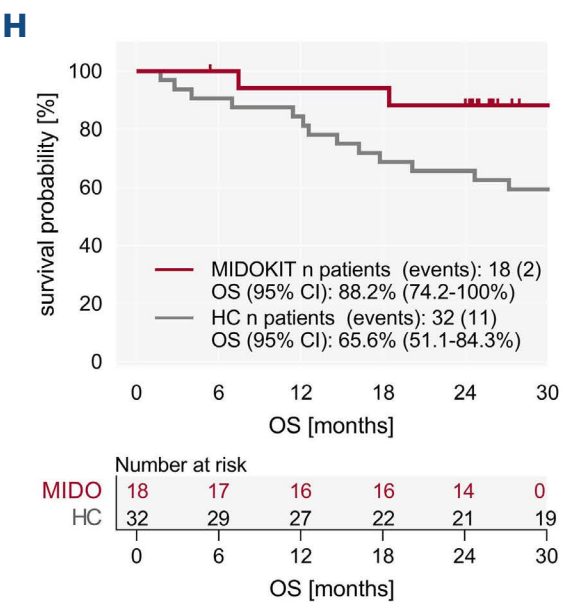


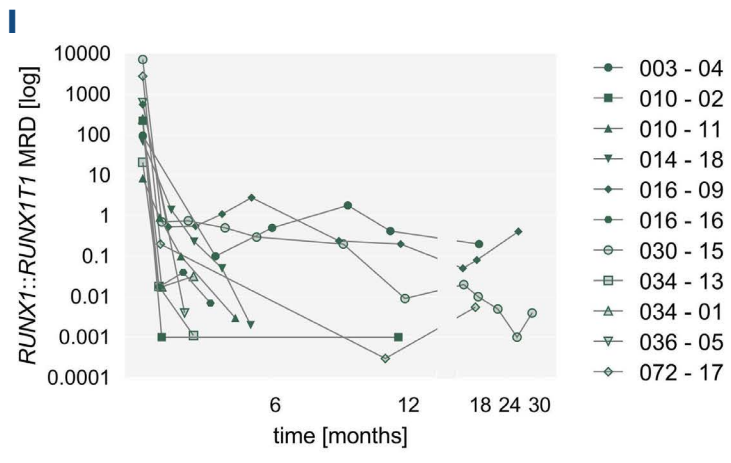












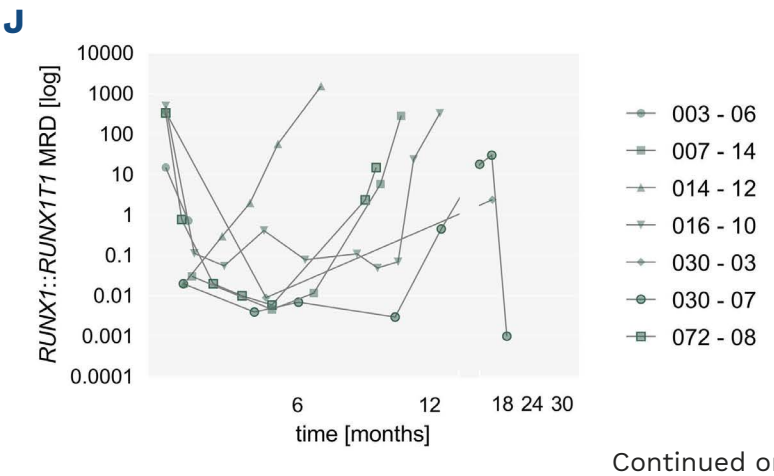


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Figure 1. Trial design, treatment algorithm, patient characteristics, molecular features, outcome and *RUNX1::RUNX1T1* measurable residual disease kinetics. (A) MIDOKIT trial design and treatment algorithm. (B) Patient clinical characteristics and molecular features, each row represents 1 unique patient, columns represent clinical features or molecular alterations (only recurring variants/variants of special interest [*ASXL2*] considered pathogenic/likely pathogenic are shown). (C) Swimmer plot showing individual patient outcomes. (D) *KIT* variant alle frequency (VAF) according to *KIT* variants. (E) *FLT3*-ITD AR and *FLT3*-TKD VAF. (F-H) Event-free survival, relapse-free survival and overall survival in MIDOKIT patients as compared to historical controls (patients with acute myeloid leukemia [AML] with t(8;21) with *KIT* and/or *FLT3*-ITD mutations treated with intensive chemotherapy (IC) without midostaurin within the AML96 trial (*clinicaltrails gov. Identifier: NCT00180115*), the AML2003 trial (*clinicaltrails gov. Identifier: NCT00180102*) and the AML60+ trial (*clinicaltrails gov. Identifier: NCT00180167*). (I and J) Quantitative bone-marrow *RUNX1::RUNX1T1* measurable residual disease (MRD), 11 patients achieved persistent hematologic complete remission (CR) (left panel), 7 patients experienced subsequent molecular and/or hematologic relapse (right panel). AE: adverse event; AR: allelic ratio; CI: confidence interval; DNR: daunorubicin; EOT: end of treatment; F: female; HCT: hematopoietic cell transplantation; HC: historical control; IT1: induction therapy 1; M: male; MRD: minimal residual disease; N: no/mutation absent; NA: not assessed ; RT-PCR: reverse transcription polymerase chain reaction; Y: yes/mutation present.

ants, median *KIT* VAF was 11.94% (range, 1.13-44.47%), and most patients had exon 17 mutations (Figure 1B, D). Four patients had evidence of an *FLT3*-ITD mutation, the median *FLT3*-ITD allelic ratio was 0.16 (range, 0.05-0.36); three patients harbored *FLT3*-TKD mutations. (Figure 1B, E). Two patients were positive for both, *KIT* and *FLT3*-ITD. The mutational landscape was in line with previous reports, none of the patients harbored *CEPBA* or *NPM1* mutations. Sixteen patients (88.8%) completed IT2, 12 patients (66.6%) completed consolidation therapy and started midostaurin maintenance and four patients (22.2%) completed midostaurin maintenance (Figure 1C; *Online Supplementary Figure S1A*). Allogeneic hematopoietic cell transplantation (HCT) was performed in seven patients (38.9%), of whom three patients were in first CR and four patients received salvage HCT after relapse. Of note, one patient was diagnosed with colorectal cancer (CRC) AJCC stage IVC after IT2, he was withdrawn from the trial and died 5 months later (UPN 003-06). Another patient was withdrawn from the trial due to non-compliance (UPN 010-02) and one patient was lost to follow-up during consolidation (UPN 014-18) (Figure 1C).

After IT2 CR rate was 100%, 16 patients (88.9%) achieved CR and two patients achieved CR with incomplete neutrophil recovery (11.1%). After a minimum follow-up of 2 years, seven patients suffered from relapse (of which 2 were molecular and 5 were hematologic relapses) and two patients died after preceding relapse. Here, patients with low *KIT* VAF seem to more frequently experience relapse. Detailed information on salvage therapies for patients experiencing relapse is provided in the *Online Supplementary Table S2*. One patient was lost to follow-up (Figure 1C). In the intention-to-treat analysis, this results in eight events and a 2-year EFS of 55.6% (95% confidence interval [CI]: 30.8-78.5), accordingly the alternate hypothesis of a 2-year EFS ≥80% was not met (Figure 1F). The median EFS, RFS and OS were not reached. At 2 years, rates of RFS and OS were 58.8% (95% CI: 39.5-87.6) and 88.2% (95% CI: 74.2-100), respectively (Figure 1G, H). No differences in EFS and RFS between patients treated within MIDOKIT and historic controls were seen. However, we ob-

Table 1. Patient characteristics.

Characteristics	Patients N=18
Age in years, median (range)	50 (27-65)
Age category in years, N (%) <50 ≥50	 7 (38.9) 11 (61.1)
Male sex, N (%)	10 (55.6)
ECOG, N (%) <2 =2	 16 (88.9) 2 (11.1)
Type of AML, N (%) *De novo* Secondary / therapy-related	 16 (88.9) 2 (11.1)
Extramedullary disease, N (%)	2 (11.1)
CBC, initial diagnosis, before IT1 WBC x10^9/L, median (range) Platelet count x10^9/L, median (range) Hemoglobin mmol/L, median (range)	 9.77 (0–69.85) 32 (0-178) 8.7 (5.4–13.05)
PB blast count, initial diagnosis, before IT1, % (range)	40 (0-74)
BM blast count, initial diagnosis, before IT1, % (range)	61.5 (33-90)
Evidence of t(8;21)(q22;q22);*RUNX1::RUNX1T1*, N (%) FISH RT-PCR	 18 (100) 18 (100)
KIT mutation, N (%)	16 (88.9)
KIT mutation VAF %, median (range)	11.94 (1.13-44.47)
FLT3-ITD, N (%)	4 (22.2)
FLT3-ITD allelic ratio, median (range)	0.16 (0.05-0.36)
KIT/FLT3-ITD co-mutation, N (%)	2 (11.1)

BM: bone marrow; CBC: complete blood count; WBC: white blood cell count; ECOG: Eastern Cooperative Oncology Group performance status; FISH: fluorescence *in situ* hybridization; ITD: internal tandem duplication; IT1: induction therapy 1; PB: peripheral blood; RT-PCR: reverse transcription polymerase chain reaction; VAF: variant allele fre-

Table 2. Non-hematologic adverse events of any grade reported in >10% of patients and the corresponding frequencies of grade ≥3 events, regardless of attribution, according to CTCAE 4.03.

Adverse event	All grades N (%)	Grade ≥3 N (%)
Any AE	18 (100)	13 (72.2)
Febrile neutropenia	7 (38.9)	7 (38.9)
Mucositis oral	10 (55.6)	2 (11.1)
Nausea	13 (72.2)	1 (5.6)
Vomiting	13 (72.2)	0
Diarrhea	6 (33.3)	0
Abdominal pain	4 (22.2)	2 (11.1)
Constipation	2 (11.1)	0
Hemorrhoids	2 (11.1)	0
Fever	7 (38.9)	0
Fatigue	5 (27.8)	0
Pneumonia	5 (27.8)	3 (16.7)
Sepsis	2 (11.1)	2 (11.1)
Electrocardiogram QTc interval prolonged	3 (16.7)	2 (11.1)
Alanine aminotransferase increased	3 (16.7)	2 (11.1)
Aspartate aminotransferase increased	3 (16.7)	2 (11.1)
Lipase increased	1 (5.6)	1 (5.6)
Headache	9 (50)	0
Dizziness	4 (22.2)	0
Epistaxis	2 (11.1)	0
Cough	2 (11.1)	0
Pneumonitis	1 (5.6)	1 (5.6)
Rash*	7 (38.9)	1 (5.6)
Purpura	3 (16.7)	1 (5.6)
Dry skin	2 (11.1)	0

*Rash includes preferred terms papulopustular rash, rash pustular, rash acneiform, rash maculo-papular, and urticaria. AE: adverse event.

served a trend for an improved OS in patients treated within the MIDOKIT trial as compared to the historical controls (2-year OS 88.2%, 95% CI: 74.2-100 *vs*. 65.6%, 95% CI: 51.1–84.3; *P*=0.05), which was stable during long-term follow-up (5-year OS 73.5%%, 95% CI: 43.4-89.3 *vs*. 49.3%, 95% CI: 31.0-65.2; *P*=0.08) (Figure 1H; *Online Supplementary Figure S1B*).

Sixteen patients (88.9%) achieved complete molecular remission (CR_{MRD}-) after IT2 (≥3-log reduction of *RUNX1::RUNX1T1*). Of these, five patients experienced subsequent molecular and/or hematologic relapse, 11 patients showed persistent CR_{MRD-} (Figure 1I, J).

Overall, 315 non-hematologic AE - hereof 55 AE ≥ CTCAE grade 3 - and 13 serious AE (SAE) were observed (Table 2). SAE observed included sepsis, pneumonia, sinusitis and pneumonitis; no cardiac SAE were observed. Overall, four patients had an AE entailing premature end of treatment: pneumonitis and biliary tract infection in UPN 036-05, left ventricular systolic dysfunction in UPN 072-17, tremor in UPN 010-11, and diagnosis of CRC in UPN 003-06. Two patients died; deaths were not considered to be related to midostaurin. Interruptions of midostaurin administration and dose reductions were observed in five (27.7%) and eight (44.4%) patients, respectively. AE commonly associated with dose modifications were nausea, vomiting, diarrhea, and electrocardiogram QTc interval prolongation. The median number of days with midostaurin administration was 148.5 (range, 14-416 days). The median ratio of administered/scheduled cumulative dose of midostaurin was 0.34 (range, 0.04-1.06), 13 patients (72.2%) received <80% of the scheduled cumulative dose of midostaurin.

The MIDOKIT trial was the first prospective clinical trial evaluating the molecular-guided implementation of the multi-kinase inhibitor midostaurin in combination with IC in patients with newly diagnosed AML with t(8;21)(q22;q22.1); *RUNX1::RUNX1T1* with evidence of *KIT* and/or *FLT3*-ITD mutations. The addition of midostaurin to IC and single-agent maintenance therapy was tolerable and feasible, and no unexpected excess in toxicity was observed. Unfortunately, the MIDOKIT trial failed to reach the assumed primary endpoint of 80% 2-year EFS, due to - among others - early relapses in 44% of patients. However, with a composite CR rate of 100% and an improved 2-year OS of 88.2% as compared to patients with AML with t(8;21) and *KIT* and/or *FLT3*-ITD mutations treated within historic AML trials without midostaurin, the results of our trial are still promising. Our findings are comparable with two similar trials conducted by the German-Austrian AML Study Group (AMLSG) and the Cancer and Leukemia Group B (CALGB) evaluating the multi-kinase inhibitor dasatinib in a non-biomarker guided fashion in combination with IC in patients with CBF-AML (4-year OS 75%, 3-year OS 77%, and 5-year OS 73% in the AMLSG, the CALGB, and the MIDOKIT trial, respectively), arguing for further evaluation of tyrosine kinase inhibitors in combination with IC in patients with CBF-AML.[12,13] Moreover, a subgroup-analysis of the RATIFY trial found CBF rearrangements to be an independent predictor for favorable OS and EFS, again supporting the evaluation of midostaurin in a larger cohort of patients with CBF-AML.[14,15]

Of note, the MIDOKIT trial has some limitations in order to draw conclusions applicable to a larger cohort of patients, e.g., the small number of patients and the single-arm trial design. Further, non-adherence, mostly due to intolerance as well as individual protocol deviations such as allogeneic HCT in first CR, limited the time and dose of midostaurin exposure and might have contributed to the non-achievement of the primary endpoint (only 27.8% of patients received >80% of the scheduled dose of midostaurin). Moreover, the implementation of midostaurin not before IT2 might have reduced its antileukemic potential since a *post hoc* analysis of the RATIFY trial indicates the early use of midostaurin from IT1 on to be essential for its antileukemic effect.[14] Thus, our results suggest re-evaluation of molecular informed implementation of midostaurin (or novel FLT3/KIT inhibitors) in patients with CBF-AML through a larger, redesigned, randomized, controlled trial.

In conclusion, the MIDOKIT trial was the first prospective clinical trial assessing midostaurin in patients with AML with t(8;21) in a molecular guided fashion. Although MIDOKIT failed to reach the assumed primary endpoint of 80% 2-year EFS, the promising OS supports further biomarker-driven evaluation of TKI in combination with IC in CBF-AML.

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Disclosures

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Contributions

CR, GE and CT were responsible for study conception and design. CT, SH, DA and LR carried out/interpreted molecular studies. MK and LR performed statistical analyses. AH provided administrative support. LR, CR, TS, CHB, BS, KSE, SWK, MH, AR, SS, AN, JHM, JS, FS, JF, AK, RS, UP, HS, CDB, CMT, MB, GE and CT were involved in care of patients, sample procurement and/or contributed to in the data collection and interpretation. LR drafted the manuscript; and all authors agreed on the final version.

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Data-sharing statement

Original data and protocols are available upon reasonable request.

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