Supplemental Material

Midostaurin plus Intensive Chemotherapy for Younger and Older Patients with AML and *FLT3* Internal Tandem Duplications

Short title: Midostaurin in patients with *FLT3*-ITD

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AMLSG 16-10 trial

The original enrollment goal was 142 patients. The trial was amended in June 2013 with a doubling of the sample size to 284 patients to better define the effect of midostaurin in patients 61 to 70 years of age. This amendment also included a dose reduction of midostaurin to 25 mg every other day in case of co-medication with strong CYP3A4 inhibitors. This dose reduction became necessary based on data from pharmacokinetic modeling of drug-drug interactions showing an approximately 10-fold increase of midostaurin plasma concentrations in case of co-medication with the potent CYP3A4 inhibitor ketoconazole.[1] An additional amendment in October 2016 increased the sample size to 440 patients to ensure sufficient statistical power for evaluation of OS as key secondary endpoint, and a further amendment in June 2017 again omitted midostaurin dose reduction in case of CYP3A4 inhibitory co-medication based on new pharmacokinetic data provided by Novartis.

Treatment schedule. All patients received one induction cycle with daunorubicin (60 mg/m^2, d1-3) and cytarabine (200 mg/m^2, continuous intravenous infusion d1-7). Midostaurin was administered orally in a dose of 50 mg twice daily starting on day 8, thereafter continuous dosing until 48 hours before start of the subsequent chemotherapy cycle. Patients achieving partial remission (PR) could receive an optional second cycle of induction therapy identical in timing and dosages to the first induction therapy. Response assessment was scheduled between day 21 and day 28; blood counts were documented at the time of bone marrow assessment.

Patients who achieved complete remission (CR) or CR with incomplete hematological recovery (CRi) following induction therapy received post-remission therapy. All patients were assigned to allogeneic hematopoietic-cell transplantation (HCT) from a matched related or unrelated donor (one consolidation cycle before allogeneic HCT was optional). Patients not considered eligible for allogeneic HCT received up to 4 cycles of high-dose cytarabine (3 g/m^2/q12 hrs, d1,3,5; patients >65 yrs cytarabine 1 g/m^2/q12 hrs, d1,3,5). Midostaurin was administered orally in a dose of 50 mg twice daily starting on day 6, thereafter continuous dosing until 48 hours before start of conditioning therapy for allogeneic HCT or 48 hours before start of the subsequent cycle of consolidation chemotherapy.

Maintenance therapy with midostaurin was intended in all patients. Midostaurin was administered orally in a dose of 50 mg twice daily for 365 days. After consolidation therapy midostaurin was started immediately after the last consolidation cycle. Following allogeneic HCT, start of midostaurin was intended at the earliest 30 days after transplantation.

Assumptions for sample size calculation

Based on the data from the historical control group, we considered for the primary endpoint event-free survival (EFS) an increase in the overall 2-year EFS rate from 25% to 37.5% (18-

60 yrs, 28% to 42%; 61-70 yrs, 14% to 26%), corresponding to a hazard ratio of 0.7, as a clinically relevant improvement. For the key secondary endpoint overall survival (OS) an increase in the overall 2-year OS rate from 38% to 46% (18-60 yrs, 43% to 53%; 61-70 yrs, 17% to 31%), corresponding to a hazard ratio of approximately 0.8, was considered clinically relevant.

Statistical analysis

Primary and key secondary endpoints, EFS/OS, in the AMLSG 16-10 study population were compared to a historical cohort. EFS, OS, relapse-free survival (RFS), cumulative incidence of relapse (CIR) and death (CID) were defined according to 2017 European LeukemiaNet (ELN) recommendations.[2] To reduce confounding bias originating from structural differences in the two cohorts concerning prognostic factors, a double-robust adjustment strategy was utilized to account for age (as continuous variable), sex, log10 white blood cell (WBC) count, bone marrow (BM) blasts, *NPM1* mutational status, and *FLT3*-ITD allelic ratio as potential confounders. More specifically, these clinical variables were included as covariates in a (weighted) Cox proportional hazards model as well as for the calculation of propensity score weights via a logistic regression model with the "treatment group" (AMLSG 16-10 vs. historical control) as a dependent variable and the above mentioned covariates as explanatory variables.

Missing values of the covariates were addressed via multiple imputation by chained equations.[3] All covariates (confounders plus study population [AMLSG 16-10 vs. historical]) as well as the endpoint information were used for imputation of missing values. For time-to-event endpoints, endpoint information amounts to the cumulative hazard and the survival status,[4] for binary endpoints, the response variable itself was included and for competing risk analyses, the cumulative hazards for both competing events along with the event indicator have been used (cf. Resche-Rigon and others [2012] described in ref 5). Imputation of binary variables was based on logistic regression (*NPM1* and *FLT3*-ITD allelic ratio), whereas continuous variables were imputed using predictive mean matching (log10 WBC and BM blasts). Separate Cox models were then fitted on each of the 10 imputed data sets and results were combined using Rubin's rule.

The same imputation strategy was also used to fit the propensity models, and propensity scores were derived by averaging over the predicted probabilities for all imputed data sets, obtained based on the pooled results of the 10 logistic models.

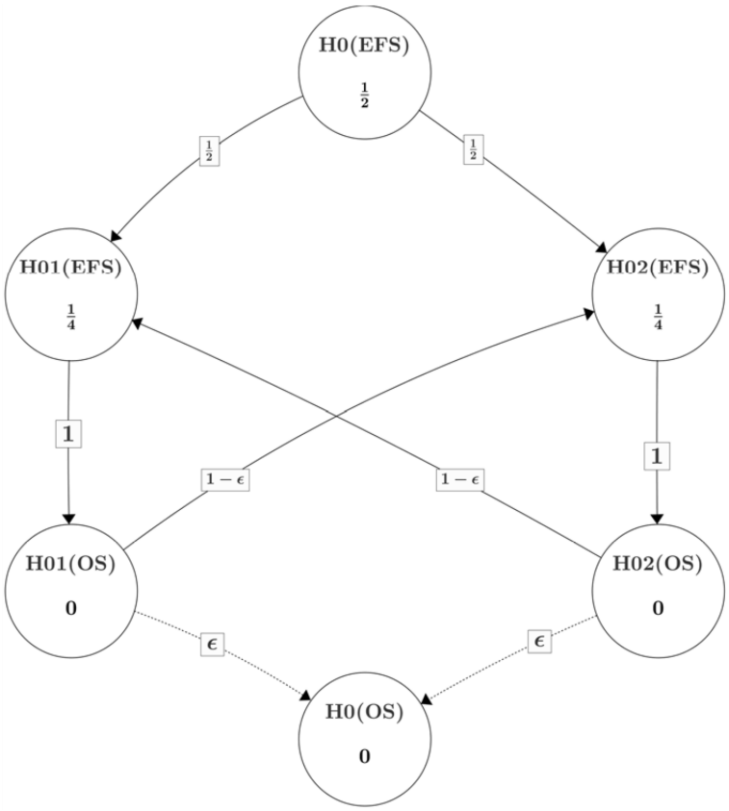
The Wald test statistics for the treatment group comparison resulting from these double-robust adjusted regression models were used to address the null hypotheses of "no treatment effect", whereby treatments compared were midostaurin in addition to standard chemotherapy (AMLSG 16-10) versus standard chemotherapy without midostaurin (historical control population).

The procedure described above (Cox model with double-robust adjustment and multiple imputation) was applied to test the local null hypotheses concerning the primary and key secondary endpoints of the study, EFS and OS:

1. *H*0(EFS): “no treatment effect” for the entire efficacy population
2. *H*01(EFS): “no treatment effect” for the subset of patients 18-60 years
3. *H*02(EFS): “no treatment effect” for the subset of patients 61-70 years
4. *H*0(OS): “no treatment effect” for the entire efficacy population
5. *H*01(OS): “no treatment effect” for the subset of patients 18-60 years
6. *H*02(OS): “no treatment effect” for the subset of patients 61-70 years

The 6 null hypotheses were tested using a gatekeeping procedure based on the graphical approach to sequentially rejective multiple test procedures proposed by Bretz et al 2009.[6] Significance levels $\alpha_i = w_i \alpha$, i = 1, . . . , 6, were initially defined such that they sum up to $\alpha = 0.05$. The primary hypothesis H1=H0(EFS) and 2 key secondary hypotheses, H2=H01(EFS) and H3=H02(EFS), were allocated the initial levels $\alpha_1 = 1/2\ \alpha$, $\alpha_2 = 1/4\ \alpha$, $\alpha_3 = 1/4\ \alpha$ where $\alpha = 0.05$ (two-sided). The levels of the 3 OS related hypotheses (H4-H6) were all 0 initially.

The procedure then was defined as follows: Test the hypotheses Hi, i = 1, 2, 3, 4, 5, 6, each at its local significance level α_i. If a hypothesis Hi can be rejected, remove the rejected hypothesis from the graph, reallocate its local significance level to the other hypotheses according to the weights of the directed edges given in the graph.[6] The testing step for the remaining, non-rejected hypotheses is repeated with the updated local significance levels. This possibly leads to further rejected null hypotheses with associated reallocation of the local significance levels. The procedure is repeated until no further hypothesis can be rejected. The reallocation of the local alpha levels is fully determined by the initial graph given below.



Adjusted p-values were calculated by dividing the raw p-values by the corresponding weights $w_i = {\alpha_i}/{\alpha}$ (with local significance levels after reallocation during the testing sequence). If the ratio was smaller than the adjusted p-value for the previous hypothesis, the adjusted p-value for the present hypothesis was defined as the adjusted p-value for the previous hypothesis such that the series of adjusted p-values was monotonically increasing. Confidence intervals are Wald type intervals matching the adjusted local significance levels.

In addition to the primary analysis, sensitivity analyses for EFS and OS were conducted by censoring patients proceeding to allogeneic HCT in first CR/CRi at the date of transplant. This was implemented in a competing risks setting using cause-specific proportional hazards models with allogeneic HCT as competing event. Alternatively, allogeneic HCT was included in the Cox model as a time-dependent covariate in order to adjust the effect of midostaurin for a potential effect of allogeneic HCT.

Since patients of the historical control were treated about one decade earlier, we also performed multivariate analysis for OS using the placebo arm of the CALGB 10603/RATIFY study as a reference (Supplementary Table 7; Supplementary Figure 1 A). Due to differences in response assessment between the trials, we restricted this comparison to the analysis of OS. Supplementary Figure 1 in addition provides Kaplan-Meier plots comparing OS of AMLSG 16-10 *versus* midostaurin arm of CALGB 10603/RATIFY (panel B) and comparing AMLSG historical controls *versus* placebo arm of CALGB 10603/RATIFY. In order to be included in this analysis, patients had to meet all eligibility criteria of both trials. More explicitly, this means that

all patients of aged ≥60 years, with *FLT3*-TKD mutation only, with therapy-related AML, secondary AML with prior cytotoxic therapy, or CBF-AML had to be excluded. This resulted in 300 patients of the AMLSG 16-10 trial, 342 patients of the historical control cohort, and 272/273 patients of the midostaurin/placebo arm of the CALGB 10603/RATIFY study.

Secondary endpoints were analyzed analogously to the primary and key secondary endpoints, that is, using (cause-specific) Cox models with double-robust adjustment on multiple imputed data sets (for relapse-free survival [RFS], cumulative incidence of relapse [CIR] and death [CID]) as well as logistic regression with double-robust adjustment on multiple imputed data sets (for CR/CRi). Analyses for RFS, CIR and CID are based on the subset of patients with CR/CRi. The effect of allogeneic HCT on EFS itself was analyzed based on patients of the AMLSG 16-10 trial only since the decision to assign a patient to allogeneic HCT in the historical cohort followed a more conservative approach. Within the AMLSG 16-10 cohort, the decision of whether a patient was transplanted was again not random and thus, analyses had to be adjusted appropriately in order to reduce confounding bias. To this aim, another set of propensity score weights was calculated using logistic regression with allogeneic HCT as the dependent variable and age (as continuous variable), log10 WBC count, *NPM1* mutational status, and *FLT3*-ITD allelic ratio as explanatory variables. The effect of allogeneic HCT was then investigated as a time-dependent covariate in a weighted Cox model using double-robust adjustment - the same covariates that were used for the calculation of propensity score weights were added as covariates in the model. The prognostic effect of allogeneic HCT itself was analyzed in two ways, overall and according to donor type (matched related donor [MRD] vs. matched unrelated donor [MUD]).

Unplanned interim analysis for EFS

An unplanned interim analysis comparing EFS to historical controls has previously been conducted based on the first two cohorts of the trial (n=284).[7] Since the analysis took place 6 months after end of enrollment, trial conduct was not affected. The propensity score adjusted comparison between the AMLSG 16-10 population and the historical controls showed a significantly improved EFS for the overall population as well as in both subgroups of younger and older patients.

Supplementary Table 1: Historical control patients.

Trial	Accrual period	No. of pts in historical cohort	Reference
AMLHD93	1993 - 1998	n=29 (7.0%)	8
AMLHD98A	1998 - 2004	n=121 (29.2%)	9
AMLHD98B	1997 - 2004	n=23 (5.5%)	10
AMLSG 06-04	2004 - 2008	n=39 (9.4%)	11
AMLSG 07-04	2004 - 2009	n=203 (48.9%)	12

The historical control population comprised all AML cases with *FLT3*-ITD from 5 previous AMLSG trials, excluding patients >70 years, acute promyelocytic leukemia, and core-binding factor AML.

Induction therapy in all trials consisted of idarubicin, standard-dose cytarabine and etoposide, followed by consolidation with up to 4 cycles of high-dose cytarabine (single dose 3 g/m^2); allogeneic hematopoietic cell transplantation was performed at the investigators' discretion.

Supplementary Table 2: Patient baseline characteristics according to study cohort and age group.

	AMLSG 16-10			Historical Controls		
	All patients (n=440)	**Younger, 18 to 60 yrs (n=312)**	**Older, 61 to 70 yrs (n=128)**	**All patients (n=415)**	**Younger, 18 to 60 yrs (n=352)**	**Older, 61 to 70 yrs (n=63)**
Age, years						
Median (range)	54.1 (18-70)	50.3 (18-60)	65.2 (61-70)	50.5 (18-70)	47.2 (18-60)	66.3 (61-70)
Sex, n (%)						
Male	191 (43)	129 (41)	62 (48)	193 (46)	157 (45)	36 (57)
Female	249 (57)	183 (59)	66 (52)	222 (54)	195 (55)	27 (43)
ECOG PS, n (%)						
0	169 (38)	126 (40)	43 (34)	92 (22)	81 (23)	11 (18)
1	218 (50)	147 (47)	71 (55)	255 (61)	217 (62)	38 (60)
2	53 (12)	39 (13)	14 (11)	68 (16)	54 (15)	14 (22)
WBC, 10^9/L						
Median (range)	41.8 (0.3-420)	41.8 (0.3-420)	42.8 (0.5-333)	44.8 (0.2-439)	41.5 (0.2-427)	66.5 (1.2-439)
Missing	3	3	-	3	2	1
Hemoglobin, g/dL						
Median (range)	9.0 (4.1-18.1)	8.9 (4.1-18.1)	9.3 (5.4-15.0)	9.0 (3.1-16.6)	9.0 (3.1-14.6)	9.4 (7.0-13.0)
Missing	4	3	1	3	2	
Platelets, 10^9/L						
Median (range)	59 (5-681)	55 (5-681)	64 (5-352)	58 (6-734)	58 (8-734)	65 (6-358)
Missing	3	3	-	2	2	-
BM blasts, %						
Median (range)	80 (0-100)	80 (0-100)	80 (7-100)	85 (2-100)	84 (2-100)	90 (10-100)
Missing	46	32	14	25	20	5
PB blasts, %						
Median (range)	52 (0-100)	54 (0-100)	47 (0-98)	60 (0-100)	58 (0-100)	72 (0-100)
Missing	30	22	8	20	17	3

AML type, n (%)						
De novo	390 (89)	288 (92)	102 (80)	396 (96)	338 (96)	58 (94)
Secondary	31 (7)	13 (4)	18 (14)	6 (1)	4 (1)	2 (3)
Therapy-related	19 (4)	11 (4)	8 (6)	12 (3)	10 (3)	2 (3)
Missing	-	-	-	1	-	1
Cytogenetics,* n (%)						
Intermediate-I	285 (69)	207 (70)	78 (66)	321 (77)	276 (78)	45 (71)
Intermediate-II	101 (25)	73 (25)	28 (24)	72 (17)	55 (16)	17 (27)
Adverse	26 (6)	14 (5)	12 (10)	22 (5)	21 (6)	1 (2)
Missing	28	18	10	0	0	0
***FLT3*-ITD, n (%)**						
Allelic ratio <0.5	196 (45)	137 (44)	59 (46)	129 (44)	123 (44)	6 (35)
Allelic ratio ≥0.5	242 (55)	173 (56)	69 (54)	165 (56)	154 (56)	11 (65)
Missing	2	2	-	121	75	46
***FLT3*-TKD,** n (%)**						
Yes	16 (4)	12 (4)	4 (3)	16 (4)	15 (4)	1 (2)
No	424 (96)	300 (96)	124 (97)	377 (96)	316 (96)	61 (98)
Missing	-	-	-	22	21	
Mutated *NPM1*, n (%)						
Yes	266 (60)	191 (61)	75 (59)	229 (56)	195 (57)	34 (54)
No	174 (40)	121 (39)	53 (41)	178 (44)	149 (43)	29 (46)
Missing	-	-	-	8	8	-

Abbreviations: BM, bone marrow; ECOG PS, Eastern Cooperative Oncology Group performance status; ITD, internal tandem duplication; PB, peripheral blood; TKD, tyrosine kinase domain; WBC, white blood cells

* Cytogenetics categorization according to 2010 European LeukemiaNet (ELN) categories.[12]

** *FLT3*-TKD mutation concurrent to a *FLT3*-ITD

Supplementary Table 3: Extent of exposure to the study drug midostaurin during maintenance therapy.

	All patients (n=163)	Patients 18-60 yrs (n=114)	Patients 61-70 yrs (n=49)
Patients assigned to maintenance	**237**	**170**	**67**
Reasons for not starting maintenance, n (%)			
Adverse events	26 (35)	19 (34)	7 (39)
Patient wish	9 (12)	5 (9)	4 (22)
Relapse	7 (9)	6 (11)	1 (6)
Death	7 (9)	5 (9)	2 (11)
Withdrawal of Informed Consent	1 (1)	1 (2)	0 (0)
Other reasons	24 (32)	20 (36)	4 (22)
Duration of treatment, days			
Median, range	225 (2-464)	207 (2-464)	259 (3-400)
Days on treatment, n (%)			
≤93	58 (36)	39 (34)	19 (39)
94 - 186	18 (11)	16 (14)	2 (4)
187 - 279	14 (9)	9 (8)	5 (10)
>279			
Cumulative dose, mg			
Median, range	14.825 (50-46.300)	14.350 (75-46.350)	16.300 (50-38.100)
Early termination, n (%)	98 (60)	67 (59)	31 (63)
Adverse events	46 (47)	33 (49)	13 (42)
Relapse	21 (21)	12 (18)	9 (29)
Patient wish	14 (14)	13 (19)	1 (3)
Withdrawal of Informed Consent	3 (3)	2 (3)	1 (3)
Death	3 (3)	2 (3)	1 (3)
Other reasons	11 (11)	5 (7)	6 (19)
Dose reductions, n (%)	141 (87)	101 (89)	40 (82)
Toxicity*	105 (74)	804 (79)	25 (63)
Patient wish	0	0	0
Other reasons	36 (26)	21 (21)	15 (38)
*Comedication	31 (22)	22 (22)	9 (23)
Missing, n	0	0	0
Dose interruptions, n (%)	76 (47)	58 (51)	18 (37)

Toxicity*	58 (82)	45 (85)	13 (72)
Patient wish	0	0	0
Other reasons	13 (18)	8 (15)	5 (28)
*Comedication	2 (3)	2 (3)	0 (0)
Missing	5	5	0
Duration of dose interruptions, days			
Median, range	21 (1-243)	21 (1-243)	24 (1-173)

***** Dose reductions contributed by co-medication (e.g. strong CYP3A4 inhibitors)

Supplementary Table 4: One- to 5-year efficacy outcomes.

	AMLSG 16-10			Historical Controls		
	All (n=440)	18-60 yrs (n=312)	61-70 yrs (n=128)	All (n=415)	18-60 yrs (n=352)	61-70 yrs (n=63)
Event-free survival (EFS)						
Median EFS, mo	13.6 (10.4, 17.9)	14.5 (10.5, 23.1)	11.7 (8.5, 17.7)	5.3 (4.4, 6.7)	6.03 (5.03, 7.1)	2.53 (0.03, 5.0)
1-yr EFS rate	0.52 (0.48, 0.57)	0.53 (0.48, 0.59)	0.49 (0.41, 0.59)	0.29 (0.25, 0.34)	0.31 (0.27, 0.36)	0.16 (0.09, 0.28)
2-yr EFS rate	0.41 (0.36, 0.46)	0.43 (0.38, 0.49)	0.34 (0.27, 0.44)	0.21 (0.17, 0.25)	0.23 (0.19, 0.28)	0.10 (0.04, 0.20)
3-yr EFS rate	0.37 (0.32, 0.42)	0.39 (0.34, 0.45)	0.30 (0.23, 0.40)	0.19 (0.15, 0.23)	0.21 (0.17, 0.26)	0.06 (0.02, 0.16)
4-yr EFS rate	0.34 (0.29, 0.38)	0.37 (0.32, 0.43)	0.26 (0.19, 0.36)	0.18 (0.15, 0.22)	0.20 (0.16, 0.25)	0.06 (0.02, 0.16)
5-yr EFS rate	0.33 (0.28, 0.38)	0.36 (0.3, 0.43)	0.26 (0.19, 0.36)	0.17 (0.14, 0.21)	0.19 (0.15, 0.24)	0.06 (0.02, 0.16)
Overall survival (OS)						
Median OS, mo	36.2 (24.6, 57.3)	57.3 (28.4, NA)	22.7 (14.7, 36.7)	13.2 (11.9, 15.7)	14.9 (12.9, 18.2)	8.4 (7.1, 11.7)
1-yr OS rate	0.7 (0.66, 0.74)	0.74 (0.69, 0.79)	0.59 (0.51, 0.69)	0.54 (0.49, 0.59)	0.58 (0.53, 0.63)	0.32 (0.22, 0.46)
2-yr OS rate	0.55 (0.50, 0.60)	0.57 (0.52, 0.63)	0.50 (0.41, 0.59)	0.38 (0.33, 0.43)	0.41 (0.36,0.47)	0.18 (0.10, 0.31)
3-yr OS rate	0.50 (0.45, 0.55)	0.54 (0.49, 0.60)	0.40 (0.32, 0.50)	0.33 (0.28, 0.37)	0.36 (0.32, 0.42)	0.10 (0.05, 0.22)
4-yr OS rate	0.47 (0.43, 0.53)	0.52 (0.47, 0.59)	0.36 (0.28, 0.47)	0.31 (0.27, 0.36)	0.35 (0.30, 0.40)	0.10 (0.05, 0.22)
5-yr OS rate	0.44 (0.38, 0.5)	0.49 (0.43, 0.56)	0.31 (0.23, 0.43)	0.29 (0.25, 0.34)	0.33 (0.28, 0.38)	0.08 (0.03, 0.2)
Cumulative incidence of relapse (CIR)						
1-yr CIR	0.19 (0.15-0.23)	0.18 (0.13-0.23)	0.22 (0.14-0.31)	0.50 (0.44, 0.56)	0.48 (0.42-0.54)	0.65 (0.49-0.81)
2-yr CIR	0.28 (0.23, 0.33)	0.24 (0.19, 0.3)	0.37 (0.27, 0.47)	0.57 (0.51, 0.63)	0.54 (0.48, 0.61)	0.74 (0.59, 0.88)
3-yr CIR	0.32 (0.26, 0.37)	0.28 (0.22, 0.34)	0.4 (0.3, 0.51)	0.59 (0.53, 0.65)	0.57 (0.5, 0.63)	0.74 (0.59, 0.88)
4-yr CIR	0.34 (0.28, 0.40)	0.31 (0.24, 0.37)	0.42 (0.31, 0.53)	0.60 (0.54, 0.65)	0.57 (0.51, 0.64)	0.74 (0.59, 0.88)
5-yr CIR	0.34 (0.28, 0.4)	0.31 (0.24, 0.37)	0.42 (0.31, 0.53)	0.60 (0.54, 0.66)	0.58 (0.52, 0.64)	0.74 (0.59, 0.88)
Cumulative incidence of death (CID)						
1-yr CID	0.15 (0.11, 0.19)	0.15 (0.1, 0.2)	0.15 (0.07, 0.22)	0.09 (0.05, 0.12)	0.09 (0.05, 0.13)	0.06 (0.0, 0.14)
2-yr CID	0.20 (0.16, 0.25)	0.20 (0.15, 0.26)	0.19 (0.11, 0.28)	0.12 (0.08, 0.15)	0.12 (0.07, 0.16)	0.12 (0.01, 0.23)
3-yr CID	0.21 (0.16, 0.25)	0.21 (0.16, 0.27)	0.19 (0.11, 0.28)	0.12 (0.08, 0.16)	0.12 (0.08, 0.16)	0.15 (0.03, 0.28)
4-yr CID	0.23 (0.18, 0.28)	0.22 (0.16, 0.28)	0.24 (0.14, 0.33)	0.13 (0.09, 0.17)	0.13 (0.08, 0.17)	0.15 (0.03, 0.28)
5-yr CID	0.24 (0.18, 0.29)	0.24 (0.17, 0.31)	0.24 (0.14, 0.33)	0.14 (0.1, 0.18)	0.14 (0.09, 0.18)	0.15 (0.03, 0.28)

Supplementary Table 5: Results of gatekeeping procedure including adjusted p-values and 95% confidence intervals (underlying models based on the full analysis set)

Testing order	Endpoint, set	HR	95% CI (adj)	p-Value (adj)
1.	EFS	0.55	0.47, 0.65	<.001
2.	EFS 18-60 yrs	0.59	0.49, 0.71	<.001
3.	OS 18-60 yrs	0.59	0.47, 0.73	<.001
4.	EFS 61-70 yrs	0.41	0.29, 0.59	<.001
5.	OS 61-70 yrs	0.47	0.33, 0.67	<.001
6.	OS	0.56	0.47, 0.68	<.001

Abbreviations: adj, adjusted; CI, confidence interval; EFS, event-free survival; HR, hazard ratio; OS, overall survival

Supplementary Table 6: Results of multivariate analysis for event-free (EFS) and overall survival (OS) with allogeneic hematopoietic-cell transplantation (HCT) used as a competing event in the full analysis set.

	Event-free survival			Overall survival		
Variable	**HR**	**95%CI**	***P*-value**	**HR**	**95%CI**	***P*-value**
AMLSG 16-10	0.63	0.52, 0.77	<0.001	0.72	0.57, 0.90	0.005
Age (10 yrs increase)	1.16	1.07, 1.25	<0.001	1.35	1.22, 1.48	<0.001
Female sex	0.87	0.73, 1.05	0.157	0.81	0.65, 1.00	0.055
***NPM1*-mutated**	0.46	0.38, 0.56	<0.001	0.83	0.66, 1.03	0.089
WBC (log10)	1.29	1.08, 1.53	0.005	1.37	1.11, 1.68	0.003
BM blasts	0.77	0.47, 1.26	0.295	1.02	0.58, 1.79	0.936
***FLT3*-ITD^{high}**	1.31	1.03, 1.65	0.027	1.28	0.99, 1.65	0.063

Abbreviations: BM, bone marrow; CI, confidence interval; HR, hazard ratio; ITD, internal tandem duplication; WBC, white blood cells

Supplementary Table 7: Results from multivariate analysis for OS comparing AMLSG 16-10 (younger patient cohort) with the placebo arm of the CALGB 10603/RATIFY trial as reference; with matched eligibility criteria.

	Overall survival		
Variable	**HR**	**95%CI**	***P*-value**
AMLSG 16-10	0.71	0.56, 0.90	0.005
Age (10 yrs increase)	1.09	1.03, 1.15	0.002
Female sex	0.73	0.57, 0.93	0.011
***NPM1*-mutated**	0.66	0.50, 0.86	0.002
WBC (log10)	1.20	0.96, 1.49	0.112
BM blasts	1.10	0.62, 1.95	0.751
***FLT3*-ITD^{high}**	1.18	0.91, 1.52	0.207

Model is based on 300 patients (134 events) of the AMLSG 16-10 trial and 273 patients (150 events) of the placebo arm of the CALGB 10603/RATIFY study.

Abbreviations: BM, bone marrow; CI, confidence interval; HR, hazard ratio; ITD, internal tandem duplication; WBC, white blood cells

Supplementary Table 8: Results from logistic regression model for response to induction therapy in the full analysis set.

	Response (CR/CRi)		
Variable	**OR**	**95%CI**	***P*-value**
AMLSG 16-10	1.70	1.24, 2.33	<0.001
Age (10 yrs increase)	0.85	0.75, 0.97	0.013
Female sex	1.06	0.78, 1.45	0.707
***NPM1*-mutated**	3.76	2.73, 5.18	<0.001
WBC (log10)	0.68	0.51, 0.91	0.009
BM blasts	1.28	0.57, 2.91	0.550
***FLT3*-ITD^{high}**	0.81	0.55, 1.20	0.290

Abbreviations: BM, bone marrow; CI, confidence interval; CR, complete remission; CRi, CR with incomplete hematologic recovery; ITD, internal tandem duplication; OR, odds ratio; WBC, white blood cells

Supplementary Table 9: Results from multivariate analysis for relapse-free survival in the full analysis set.

	Relapse-free survival		
Variable	**HR**	**95%CI**	***P*-value**
AMLSG 16-10	0.50	0.41, 0.62	<0.001
Age (10 yrs increase)	1.20	1.10, 1.31	<0.001
Female sex	0.88	0.71, 1.08	0.219
***NPM1*-mutated**	0.63	0.50, 0.78	<0.001
WBC (log10)	1.14	0.94, 1.37	0.177
BM blasts	1.01	0.60, 1.71	0.976
***FLT3*-ITD^{high}**	1.22	0.96, 1.56	0.104

Model is based on 328 patients (165 events) of the AMLSG 16-10 trial and 268 patients (199 events) of the historical control cohort having achieved CR/CRi.

Abbreviations: BM, bone marrow; CI, confidence interval; HR, hazard ratio; ITD, internal tandem duplication; WBC, white blood cells

Supplementary Table 10: Multivariate analyses for cumulative incidence of relapse (CIR) and death (CID) in the full analysis set.

	CIR			CID		
Variable	**HR**	**95%CI**	***P*-value**	**HR**	**95%CI**	***P*-value**
Entire analysis cohort						
AMLSG 16-10	0.37	0.29, 0.48	<0.001	1.10	0.72, 1.68	0.645
Age (10-yr increase)	1.14	1.02, 1.26	0.016	1.39	1.16, 1.67	<0.001
Female sex	0.80	0.62, 1.02	0.075	1.14	0.76, 1.72	0.529
***NPM1*-mutated**	0.61	0.47, 0.79	<0.001	0.67	0.44, 1.02	0.064
WBC (log10)	1.23	0.99, 1.54	0.066	0.92	0.66, 1.30	0.650
BM blasts	0.73	0.39, 1.34	0.306	2.23	0.73, 6.79	0.157
***FLT3*-ITD^{high}**	1.27	0.96, 1.70	0.097	1.10	0.71, 1.69	0.671

Models are based on 328 patients (165 events) of the AMLSG 16-10 trial and 268 patients (199 events) of the historical control cohort having achieved CR/CRi.

Abbreviations: BM, bone marrow; CI, confidence interval; CID, cumulative incidence of death; CIR, cumulative incidence of relapse; HR, hazard ratio; ITD, internal tandem duplication; WBC, white blood cells

Supplementary Table 11: Results from multivariate regression model to investigate the effect of allogeneic hematopoietic-cell transplantation (HCT) (included as a time-dependent covariate, model uses multiple imputation and doubly robust adjustment) on event-free survival within the AMLSG 16-10 trial.

	Overall				**By donor type**		
Variable	**HR**	**95%CI**	***P*-value**	**Variable**	**HR**	**95%CI**	***P*-value**
Age (10 yrs increase)	1.04	0.94, 1.16	0.401	**Age (10 yrs increase)**	1.04	0.93, 1.15	0.500
Female sex	1.06	0.83, 1.35	0.632	**Female sex**	1.04	0.82, 1.33	0.726
***NPM1*-mutated**	0.40	0.31, 0.51	<0.001	***NPM1*-mutated**	0.40	0.31, 0.51	<0.001
WBC (log10)	0.94	0.75, 1.16	0.545	**WBC (log10)**	0.94	0.76, 1.17	0.572
BM blasts	1.31	0.69, 2.46	0.405	**BM blasts**	1.30	0.69, 2.46	0.412
***FLT3*-ITD^{high}**	1.19	0.92, 1.54	0.195	***FLT3*-ITD^{high}**	1.18	0.91, 1.53	0.206
HCT	0.49	0.35, 0.70	<0.001	**HCT MRD**	0.39	0.22, 0.67	<0.001
				HCT MUD	0.52	0.36, 0.75	<0.001

Abbreviations: BM, bone marrow; CI, confidence interval; HR, hazard ratio; ITD, internal tandem duplication; MRD, matched-related; MUD, matched-unrelated; WBC, white blood cells

Supplementary Table 12: Extent of exposure to the study drug midostaurin during the entire study.

	All patients (n=432)	**Patients 18-60 yrs (n=309)**	**Patients 61-70 yrs (n=123)**
Duration of treatment, days			
Median, range	61 (1-557)	59 (1-557)	71 (1-519)
Cumulative dose, mg			
Median, range	5312 (50-55.600)	5.225 (50-55.600)	5.725 (100-47.500)
Dose reductions, n (%)	365 (84)	261 (84)	104 (85)
Toxicity*	268 (74)	194 (75)	74 (72)
Patient wish	13 (4)	10 (4)	3 (3)
Other reasons	80 (22)	54 (21)	26 (25)
*Co-medication	69 (19)	43 (16)	26 (25)
Missing, n	4	3	1
Dose interruptions, n (%)	171 (40)	123 (40)	48 (39)
Toxicity*	114 (73)	87 (75)	27 (66)
Patient wish	0	0	0
Other reasons	43 (27)	29 (25)	14 (34)
*Co-medication	9 (5)	5 (4)	4 (8)
Missing	1	1	0
Duration of dose interruptions, days			
Median, range	13 (1-243)	14 (1-243)	12 (1-176)

***** Dose reductions contributed by co-medication (e.g. strong CYP3A4 inhibitors)

Supplementary Table 13: Adverse events CTCAE grade ≥3 occurring in >10% of patients during study treatment within the AMLSG 16-10 trial according to age group.

MedDRA System Organ Class	All patients (n=440)	Younger, 18-60 yr (n=312)	Older, 61-70 yr (n=128)	*P*-Value
Blood and lymphatic system disorders, n (%)	419 (95)	297 (95)	122 (95)	1.00
Infections and infestations, n (%)	290 (66)	201 (64)	89 (70)	0.32
Gastrointestinal, n (%)	173 (39)	120 (38)	53 (41)	0.59
General disorders, n (%)	150 (34)	111 (36)	39 (30)	0.32
Investigations, n (%)	134 (30)	99 (32)	35 (27)	0.42
Metabolism and nutrition disorders, n (%)	120 (27)	72 (23)	48 (38)	0.003
Respiratory, thoracic and mediastinal, n (%)	76 (17)	47 (15)	29 (23)	0.07
Vascular, n (%)	67 (15)	40 (13)	27 (21)	0.04
Renal and urinary, n (%)	52 (12)	38 (12)	14 (11)	0.87
Nervous system, n (%)	44 (10)	32 (10)	12 (9)	0.86
Cardiac, n (%)	43 (10)	25 (8)	18 (14)	0.08
Skin and subcutaneous tissue, n (%)	43 (10)	30 (10)	13 (10)	0.86

Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; MedDRA, Medical Dictionary for Regulatory Activities

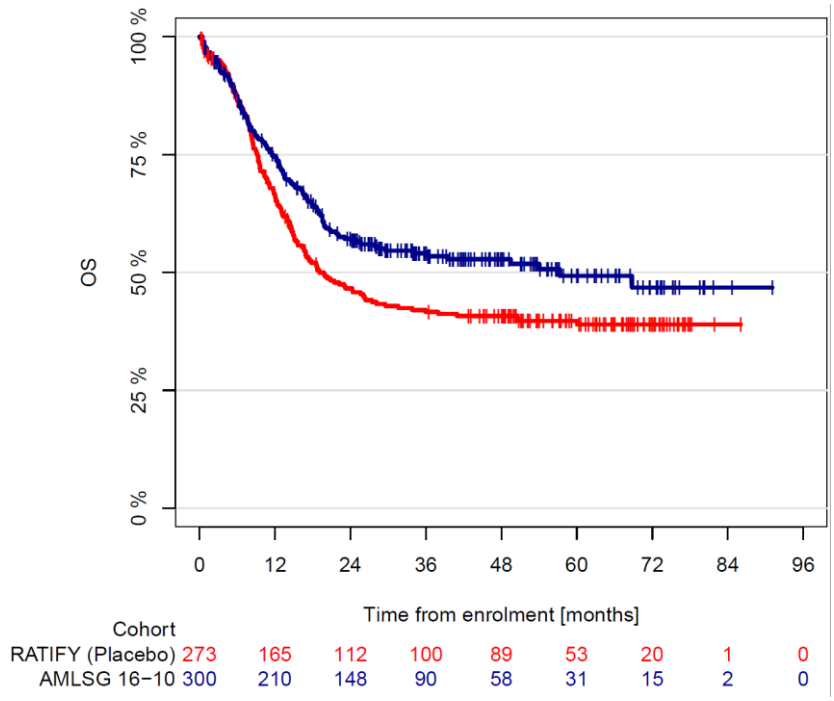
Supplementary Table 14: Adverse events CTCAE grade ≥3 occurring during maintenance treatment within the AMLSG 16-10 trial

MedDRA System Organ Class	All patients (n=163)	After allogeneic HCT, (n=128)	After conventional consolidation, (n=35)	*P*-Value
Blood and lymphatic system disorders, n (%)	45 (28)	40 (31)	5 (14)	0.06
Infections and infestations, n (%)	21 (13)	17 (13)	4 (11)	1.00
Investigations, n (%)	16 (10)	14 (11)	2 (6)	0.53
Gastrointestinal, n (%)	13 (8)	11 (9)	2 (6)	0.74
Metabolism and nutrition disorders, n (%)	13 (8)	12 (9)	1 (3)	0.30
Nervous system, n (%)	10 (6)	9 (7)	1 (3)	0.69
General disorders, n (%)	7 (4)	6 (5)	1 (3)	1.00
Vascular, n (%)	6 (4)	5 (4)	1 (3)	1.00
Renal and urinary, n (%)	6 (4)	6 (5)	0 (0)	0.34
Respiratory, thoracic and mediastinal, n (%)	3 (2)	3 (2)	0 (0)	1.00
Cardiac, n (%)	2 (1)	1 (1)	1 (3)	0.38
Skin and subcutaneous tissue, n (%)	0 (0)	0 (0)	0 (0)	-

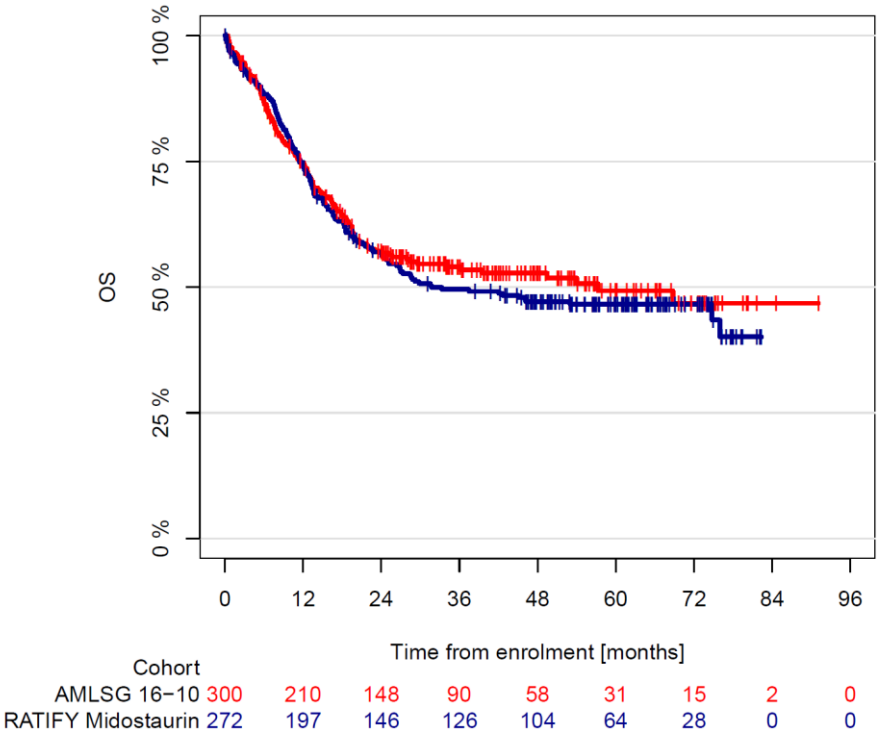
Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; HCT, hematopoietic-cell transplantation; MedDRA, Medical Dictionary for Regulatory Activities

Supplementary Figure 1: Comparison of overall survival (OS) between patients (aged 18-59 years) of the current study (AMLSG 16-10 trial / AMLSG historical controls) *versus* patients (aged 18-59 years) of the CALGB 10603/RATIFY trial; with matched eligibility criteria.

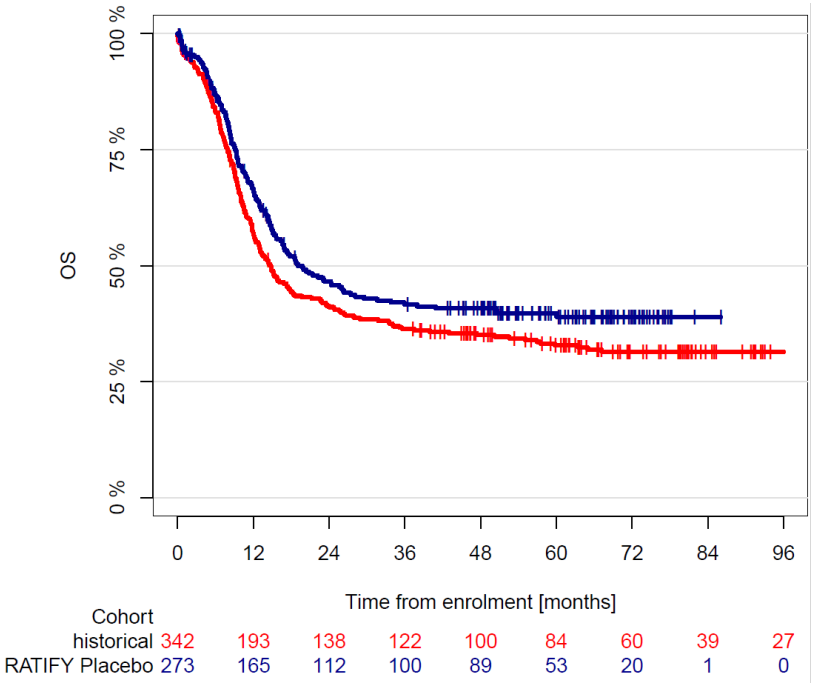
A AMLSG 16-10 trial *versus* placebo arm of CALGB 10603/RATIFY trial



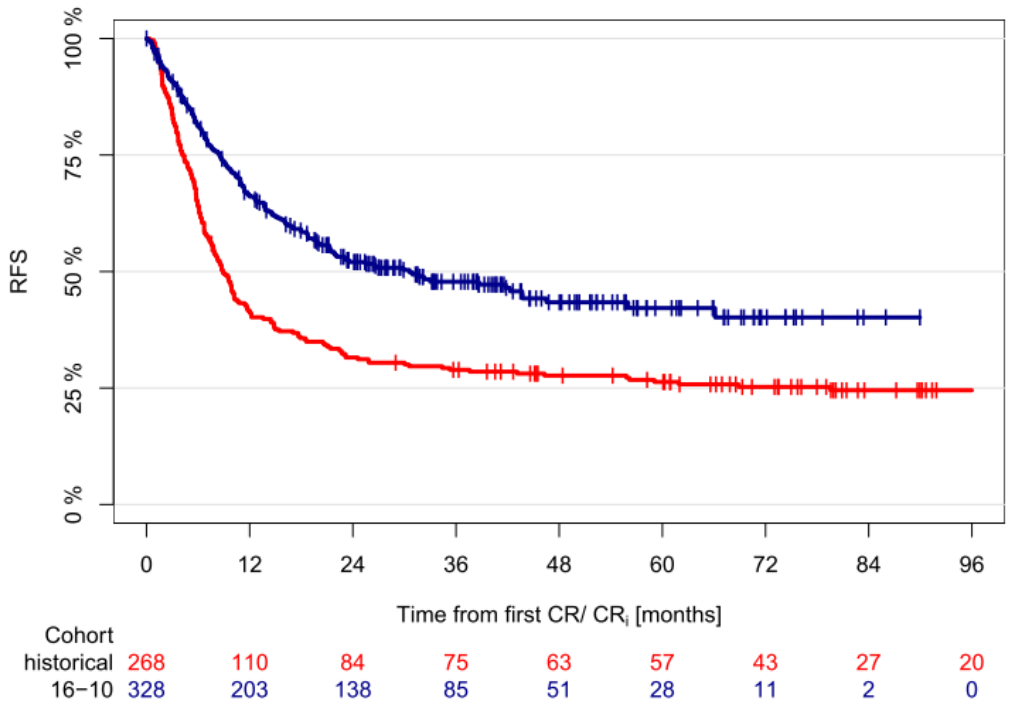
B AMLSG 16-10 trial *versus* midostaurin arm of CALGB 10603/RATIFY trial



C AMLSG historical cohort *versus* placebo arm of CALGB 10603/RATIFY trial

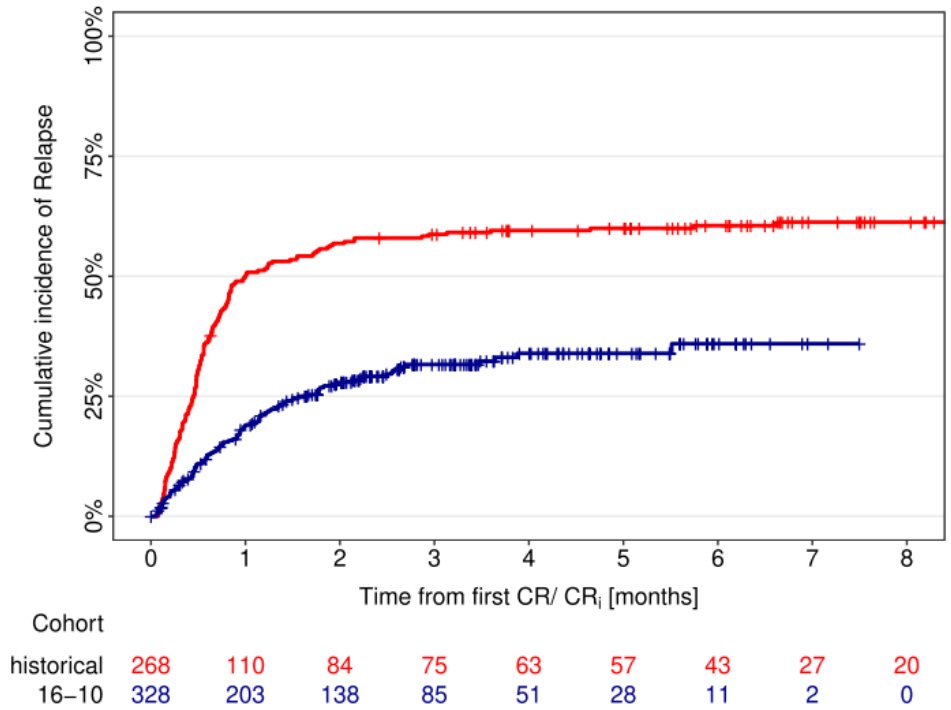


Supplementary Figure 2: Relapse-free survival (RFS) by cohort.

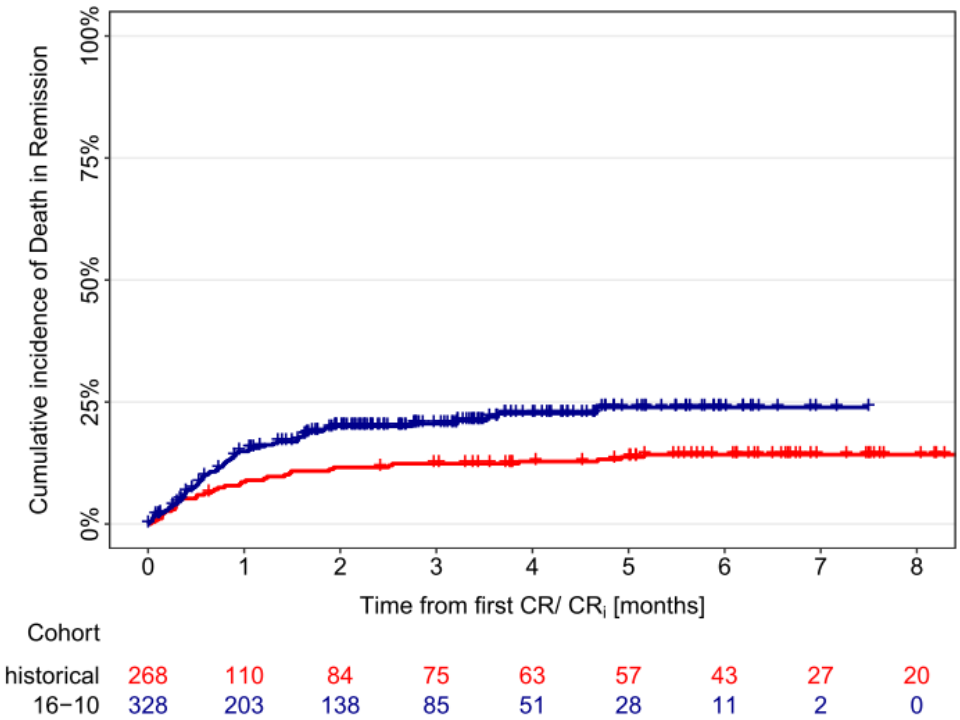


Supplementary Figure 3: Cumulative incidence of relapse (CIR) and death (CID) in complete remission (CR) / CR with incomplete hematologic recovery (CRi).

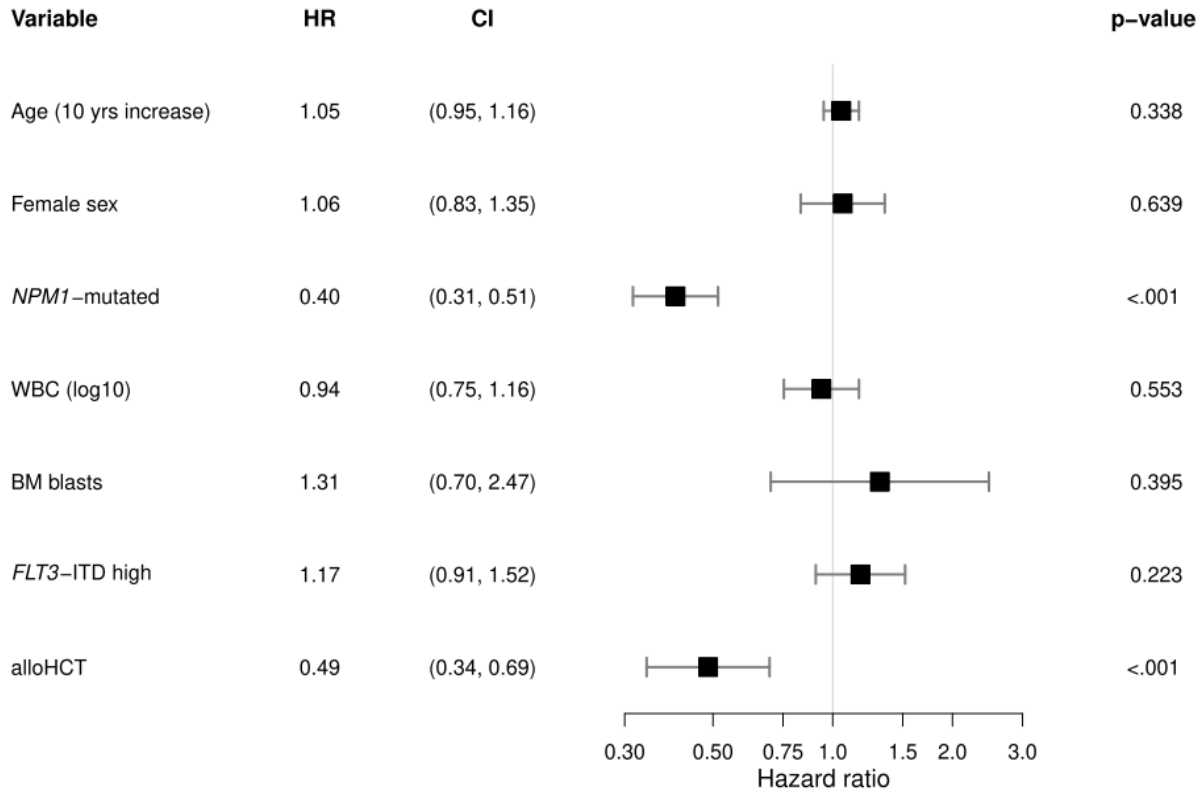
A CIR by cohort



B CID by cohort



Supplementary Figure 4: Forest plot illustrating the prognostic effect of allogeneic hematopoietic-cell transplantation (HCT) and other clinical variables in a multivariate Cox model on event-free survival (EFS), restricted to patients of the AMLSG 16-10 trial. Allogeneic HCT in first complete remission (CR) or CR with incomplete hematologic recovery entered the model as a time-dependent covariate. WBC, white blood cell count; BM, bone marrow.



References

1. Dutreix C, Munarini F, Lorenzo S, Roesel J, Wang Y. Investigation into CYP3A4-mediated drug-drug interactions on midostaurin in healthy volunteers. *Cancer Chemother Pharmacol*. 2013;72(6):1123-1234.
2. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of acute myeloid leukemia in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-447.
3. van Buuren S, Groothuis-Oudshoorn K. mice: multivariate imputation by chained equations in R. J Stat. Softw. 2011;45:1–67.
4. White IR and Royston P. Imputing missing covariate values for the Cox model. *Stat Med*. 2009;28(15):1982-1998.
5. Bartlett JW and Taylor JMG. Missing covariates in competing risks analysis. *Biostatistics*. 2016;17(4):751-763.
6. Bretz F, Posch M, Glimm E, Klinglmueller F, Maurer W, Rohmeyer K. Graphical approaches for multiple comparison procedures using weighted Bonferroni, Simes or parametric tests. *Biom J*. 2011;53(6):894-913.
7. Schlenk RF, Weber D, Fiedler W, et al. Midostaurin added to chemotherapy and continued single agent maintenance therapy in acute myeloid leukemia with FLT3-ITD. *Blood*. 2019;133(8):840-851.
8. Schlenk RF, Benner A, Hartmann F, et al; AML Study Group Ulm (AMLSG ULM). Risk-adapted postremission therapy in acute myeloid leukemia: results of the German multicenter AML HD93 treatment trial. *Leukemia*. 2003;17(8):1521-1528.
9. Schlenk RF, Döhner K, Mack S, et al. Prospective evaluation of allogeneic hematopoietic stem-cell transplantation from matched related and matched unrelated donors in younger adults with high-risk acute myeloid leukemia: German-Austrian trial AMLHD98A. *J Clin Oncol*. 2010;28(30):4642-4648.
10. Schlenk RF, Fröhling S, Hartmann F, et al. Intensive consolidation versus oral maintenance therapy in patients 61 years or older with acute myeloid leukemia in first remission: results of second randomization of the AML HD98-B treatment Trial. *Leukemia*. 2006;20(4):748-750.
11. Tassara M, Döhner K, Brossart P, et al. Valproic acid in combination with all-trans retinoic acid and intensive therapy for acute myeloid leukemia in older patients. *Blood*. 2014;123(26):4027-4036.
12. Schlenk RF, Lübbert M, Benner A, et al; German-Austrian Acute Myeloid Leukemia Study Group. All-trans retinoic acid as adjunct to intensive treatment in younger adult patients with acute myeloid leukemia: results of the randomized AMLSG 07-04 study. *Ann Hematol*. 95(12):1931-1942.
13. Döhner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an International Expert Panel, on behalf of the European LeukemiaNet. *Blood*. 2010;115(3):453-474.