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Regular Article

MYELOID NEOPLASIA

Impact of *NPM1*/*FLT3*-ITD genotypes defined by the 2017 European LeukemiaNet in patients with acute myeloid leukemia

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KEY POINTS

- **OS of patients with *FLT3*-ITD differs significantly when categorized by the 2017 ELN risk stratification.**
- **In a multivariate Cox model for OS, there is a consistent beneficial effect of midostaurin across the 3 2017 ELN risk groups.**

Patients with acute myeloid leukemia (AML) harboring *FLT3* internal tandem duplications (ITDs) have poor outcomes, in particular AML with a high (≥0.5) mutant/wild-type allelic ratio (AR). The 2017 European LeukemiaNet (ELN) recommendations defined 4 distinct *FLT3*-ITD genotypes based on the ITD AR and the *NPM1* mutational status. In this retrospective exploratory study, we investigated the prognostic and predictive impact of the *NPM1*/*FLT3*-ITD genotypes categorized according to the 2017 ELN risk groups in patients randomized within the RATIFY trial, which evaluated the addition of midostaurin to standard chemotherapy. The 4 *NPM1*/*FLT3*-ITD genotypes differed significantly with regard to clinical and concurrent genetic features. Complete ELN risk categorization could be done in 318 of 549 trial patients with *FLT3*-ITD AML. Significant factors for response after 1 or 2 induction cycles were ELN risk group and white blood cell (WBC) counts; treatment with midostaurin had no influence. Overall survival (OS) differed significantly among ELN risk groups, with estimated 5-year OS probabilities of 0.63, 0.43, and 0.33 for favorable-, intermediate-, and adverse-risk groups, respectively ($P < .001$). A multivariate Cox model for OS using allogeneic hematopoietic cell transplantation (HCT) in first complete remission as a time-dependent variable revealed treatment with midostaurin, allogeneic HCT, ELN favorable-risk group, and lower WBC counts as significant favorable factors. In this model, there was a consistent beneficial effect of midostaurin across ELN risk groups. (*Blood*. 2020;135(5):371-380)

Introduction

Activating mutations of *FLT3* are among the most common mutations in patients with acute myeloid leukemia (AML).[1-3] There are 2 major types of mutations, internal tandem duplications (ITDs), and mutations within the activation loop of the second tyrosine kinase domain.[4] ITDs are in-frame duplications that involve different functional domains of the receptor, most commonly the juxtamembrane domain, and lead to constitutive activation of the receptor.[4] *FLT3*-ITD has consistently been associated with higher white blood cell (WBC) counts, higher percentages of bone marrow (BM) blast cells, an increased risk for relapse, and inferior survival.[5-7]

Factors that have been shown to influence the prognostic impact of *FLT3*-ITDs are the mutational context, in particular the mutational

Table 1. Patient and disease characteristics as well as incidence of 2017 ELN high-risk molecular markers by the 4 *NPM1*/*FLT3*-ITD genotypes

	NPM1^{mut}/*FLT3*-ITD^{low}	*NPM1*^{mut}/*FLT3*-ITD^{high}	*NPM1*^{wt}/*FLT3*-ITD^{low}	*NPM1*^{wt}/*FLT3*-ITD^{high}	*P*
Patients, n (%)	85 (19.9)	159 (37.2)	74 (17.3)	109 (25.5)	
Age, median, y	50.6	48.1	47.2	45.7	.05
Female, %	64.7	66.0	47.3	48.6	.0065
WBCs, median (range), $\times 10^9$/L	23.6 (1.4-253.2)	45.3 (1.4-329.8)	23.6 (0.6-207.4)	44.6 (0.8-236.0)	<.0001
Median BM blasts, %	72	80	72	77	.0019
Incidence of 2017 ELN high-risk mutations, n (%)					
RUNX1	1/73 (1.4)	1/140 (0.7)	16/53 (30.2)	24/92 (26.1)	.0005
ASXL1	4/73 (5.5)	9/140 (6.4)	6/53 (11.3)	12/92 (13.0)	.23
TP53	0/73 (0)	1/140 (0.7)	1/53 (1.9)	0/92 (0)	.50
Karyotype, n (%)					
Normal	52 (89.7)	101 (90.2)	32 (52.5)	54 (58.1)	.0005
Abnormal	6 (10.3)	11 (9.8)	29 (47.5)	39 (41.9)	
Missing	27	47	13	16	

The Fisher's exact test was used for categorical variables, and the Kruskal-Wallis test was used for continuous variables.

status of *NPM1*,[8-10] the insertion site of the ITD,[11-13] and, importantly, the allelic ratio (AR),[14-16] which is most commonly assessed by DNA fragment analysis using a polymerase chain reaction (PCR)-based method combined with capillary electrophoresis. Recent studies have indicated that patients with *NPM1* mutation (*NPM1*^{mut}) and concurrent *FLT3*-ITD with a low (<0.5) AR (*FLT3*-ITD^{low}) have a favorable outcome that is similar to patients with *NPM1*^{mut} and wild-type *FLT3* (*FLT3*^{wt}).[8,9,15,16] In contrast, patients with wild-type *NPM1* (*NPM1*^{wt}) and *FLT3*-ITD with a high (≥0.5) AR (*FLT3*-ITD^{high}) have a poor outcome.[9,17]

Another important observation relates to the impact of allogeneic hematopoietic cell transplantation (HCT) in patients with these different genotypes. Several groups have shown that patients with the more favorable genotype *NPM1*^{mut}/*FLT3*-ITD^{low} may not derive benefit from allogeneic HCT as first-line treatment,[10,15,18] although this effect has not been observed in all studies.[19,20] The preponderant evidence for the prognostic significance of the *FLT3*-ITD AR is now reflected in the 2017 European LeukemiaNet (ELN) recommendations that distinguish prognostic *NPM1*/*FLT3*-ITD genotypes by accounting for the *FLT3*-ITD AR.[21]

The natural course of AML with *FLT3* mutation may change with the use of FLT3 inhibitors, and the prognostic impact of the above genotypes will need to be revisited.[22] Midostaurin, a multikinase inhibitor, is a first-generation FLT3 inhibitor.[23] Based on the results of the international randomized CALGB 10603/RATIFY study, midostaurin was approved, in combination with intensive chemotherapy, by the US Food and Drug Administration and by the European Medicines Agency; in addition, it was approved as maintenance therapy for adult patients with AML exhibiting an activating *FLT3* mutation by the European Medicines Agency.[24] Further evidence for the efficacy of midostaurin in patients with *FLT3*-ITD$^+$ AML comes from the AMLSG 16-10 trial, which also included older patients aged 60 to 70 years.[25]

The objectives of this study were to validate the prognostic impact of the *NPM1*/*FLT3*-ITD genotypes, as defined by the 2017 ELN recommendations, and to evaluate the potential predictive impact of these genotypes for response to midostaurin in randomized patients from the RATIFY trial.

Patients and methods

Patients

Overall, 717 patients with AML and activating *FLT3* mutations (ITD and tyrosine kinase domain mutations) were included in the CALGB 10603/RATIFY trial.[24] This post hoc exploratory analysis focuses on the subset of patients with *FLT3*-ITD.

Data on the 4 *NPM1*/*FLT3*-ITD genotypes, considering the *FLT3*-ITD AR (low, 0.05 to <0.5; high, ≥ 0.5), were available in 427 of 549 patients with *FLT3*-ITD AML. Table 1 shows the characteristics of these patients. The 2017 ELN high-risk markers *RUNX1*, *ASXL1*, and *TP53* could be assessed in 358 of these patients who gave informed consent for further molecular studies and for whom DNA was still available. Table 1 shows how these high-risk markers segregated among the 4 *NPM1*/*FLT3*-ITD genotypes. The study was approved by the Institutional Review Board of Ulm University.

Complete 2017 ELN risk categorization could be done for 318 of 549 patients. Table 2 provides the clinical and genetic characteristics of these 318 patients by risk group. Baseline characteristics between the clinical trial cohort of all *FLT3*-ITD$^+$ patients (n = 549) and the ELN biomarker cohort (n = 318) were balanced, as were complete remission (CR) rates and overall survival (OS) times (supplemental Table 1; supplemental Figure 1, available on the *Blood* Web site).

Table 2. Patient and disease characteristics and response to therapy by 2017 ELN risk groups

	Favorable risk*	Intermediate risk†	Adverse risk‡	*P*
Patients, n (%)	85 (26.7)	111 (34.9)	122 (38.4)	
Age, median, y	50.6	47.9	47.0	.07
Female, %	64.7	64.9	49.2	.04
WBC count, median (range), $\times 10^9$/L	23.6 (1.4-253.2)	42.6 (0.6-329.8)	38.0 (0.8-236.0)	.0019
Median BM blasts, %	72	78	76	.33
Treatment, n (%)				
Placebo	47 (55.3)	57 (51.4)	54 (44.3)	.27
Midostaurin	38 (44.7)	54 (48.6)	68 (55.7)	
Allogeneic HCT in CR1	24 (28.2)	31 (27.9)	36 (29.5)	.95
Concurrent gene mutations, n (%)				
RUNX1	1/73 (1.4)	0/110 (0)	31/106 (29.2)	.0005
ASXL1	4/73 (5.5)	0/110 (0)	21/106 (19.8)	.0005
TP53	0/73 (0)	0/110 (0)	2/106 (1.9)	.19
Karyotype, n (%)				
Normal	52 (89.7)	98 (88.3)	68 (57.1)	.0005
Abnormal	6 (10.3)	13 (11.7)	51 (42.9)	
Missing	27	1	3	
	All patients	**Midostaurin**	**Placebo**	
Response to induction therapy, %§				
Favorable	69.4	71.1	68.1	.82
Intermediate	63.1	66.7	59.6	.56
Adverse	51.6	57.4	44.4	.20

For definition of risk groups, see also supplemental Table 2.

*$NPM1^{mut}$/*FLT3*-ITD^{low} AML cases, irrespective of additional high-risk gene mutation or additional chromosomal abnormalities.

†$NPM1^{mut}$/*FLT3*-ITD^{high} AML (n = 93) and $NPM1^{wt}$/*FLT3*-ITD^{low} AML (n = 18), both subgroups without the concurrent presence of the high-risk molecular markers *RUNX1*, *ASXL1*, and *TP53*. Also, $NPM1^{wt}$/*FLT3*-ITD^{low} AML without adverse-risk cytogenetics.

‡$NPM1^{wt}$/*FLT3*-ITD^{high} AML (n = 92), $NPM1^{mut}$/*FLT3*-ITD^{high} AML exhibiting high-risk molecular markers (n = 8), and $NPM1^{wt}$/*FLT3*-ITD^{low} AML with high-risk molecular markers and/or adverse-risk cytogenetics (n = 22).

§CRs achieved during induction therapy (cycles 1 and 2).

Genetic analyses

FLT3-ITD mutation analysis was performed as described.[24] Testing was done in 9 reference laboratories in 6 countries using a harmonized PCR method based on capillary electrophoresis detection. PCR was done in triplicate, and the mean values of these measurements were reported. To ensure consistency among laboratories, a cross-validation quality control procedure was performed every 6 months.[26] Patients were eligible for the clinical trial when exceeding the diagnostic cutoff for the *FLT3*-ITD AR (≥0.05). Randomization to midostaurin vs placebo was stratified by the ITD AR (low, 0.05-0.7; high, >0.7).[6] For this analysis, we chose a cutoff for the AR of 0.5 (low, >0.05 to <0.5; high, ≥0.5), because this cutoff value has been shown to better discriminate and has also been adopted in the 2017 ELN risk classification.[10,15,21] Semiquantitative assessment of *FLT3*-ITD AR (using DNA fragment analysis) was determined as the area under the curve "*FLT3*-ITD" divided by the area under the curve "$FLT3^{wt}$."[21]

NPM1 mutational status was assessed as previously described.[27] Data on *RUNX1*, *ASXL1*, and *TP53* mutation status were available from a comprehensive targeted sequencing study performed on pretreatment specimens from the trial.[28]

Statistical analysis

CR was defined by standard criteria[21]; responses included all CRs achieved during induction cycles 1 and 2. The definition of OS, event-free survival (EFS), cumulative incidence of relapse (CIR), and cumulative incidence of death (CID) were based on recommended criteria.[21] Survival times were calculated from the date of randomization. The median follow-up for survival was calculated using the reverse Kaplan-Meier estimate.[29] Logistic regression and Cox proportional hazards models were used to identify prognostic variables for CR, OS, and CIR.[30] Additional covariates in multivariate analysis were age, BM blast counts, and WBC counts as continuous variables and sex and treatment (midostaurin vs placebo) as dichotomous variables; allogeneic HCT was included as a time-dependent variable. The subgroup results of proportional hazards models were summarized in forest plots. Comparisons between the *NPM1*/*FLT3*-ITD genotypes and the 2017 ELN risk groups with respect to quantitative variables were performed using the Kruskal-Wallis test. Survival distributions were estimated using the Kaplan-Meier method,[31] and differences between groups were analyzed using 2-sided log-rank tests.

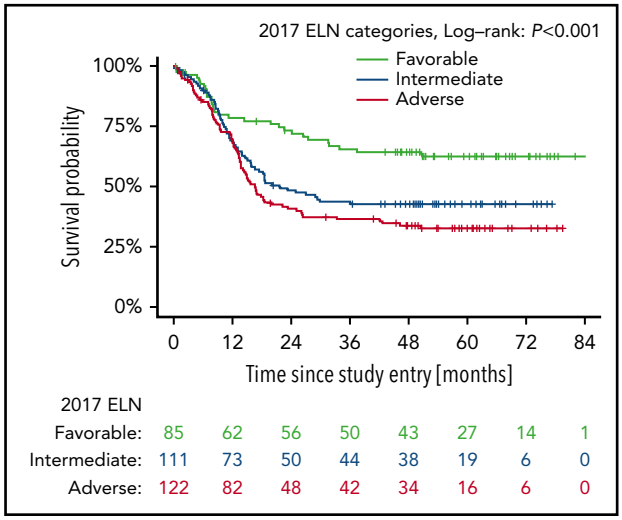

Figure 1. Prognostic effect on overall survival of patients with the different *NPM1*/*FLT3*-ITD genotypes categorized according to 2017 ELN genetic risk groups. The *P* values for the log-rank tests comparing favorable vs intermediate and intermediate vs adverse are $P = .007$ and $P = .20$, respectively.

To estimate survival probabilities considering the effect of allogeneic HCT in first CR (CR1), the Simon-Makuch method was used with clock-back correction, according to Bernasconi et al.[32] Simon-Makuch estimates show the survival probabilities for fictional patients who either never receive an allogeneic HCT or have received an allogeneic HCT at t = 0. To examine the effect of allogeneic HCT, univariate and multivariate Cox models, with allogeneic HCT as a time-dependent intervening event, were applied.[33] An effect was considered significant if its *P* value was <5%. The analyses were not adjusted for multiple testing.

Leave-1-out cross-validated prediction errors were used to evaluate the prognostic value of the 2017 ELN risk classification, in which the prediction error is defined using Brier's score as a function of time.[34] To account for allogeneic HCT in CR1 as a time-dependent intervention, prediction errors were calculated using multistate models.[35] The "reference" model is the Aalen-Johansen estimator.[36] For ordinary (single-event) survival this reduces to the Kaplan-Meier estimate. All statistical analyses were performed with statistical software (R 3.5.1).

Results

Categorization of patients

The 427 patients, for whom data on *FLT3*-ITD AR and *NPM1* mutational status were available, were first categorized to 1 of the 4 *NPM1*/*FLT3*-ITD genotypes: *NPM1*^{mut}/*FLT3*-ITD^{low} (n = 85, 19.9%), *NPM1*^{mut}/*FLT3*-ITD^{high} (n = 159, 37.2%), *NPM1*^{wt}/*FLT3*-ITD^{low} (n = 74, 17.4%), and *NPM1*^{wt}/*FLT3*-ITD^{high} (n = 109, 25.5%). Patient and disease characteristics according to these genotypes are given in Table 1. Patients with concurrent *NPM1*^{mut} were older and more frequently female; patients with high *FLT3*-ITD AR had higher WBC counts and higher BM blast counts. Patients with *NPM1*^{mut} AML more frequently had a normal karyotype compared with patients with *NPM1*^{wt} AML. With regard to the concurrent presence of 2017 ELN high-risk markers, *NPM1*^{mut} was almost mutually exclusive with *RUNX1* mutations, whereas 30.2% and 26.1% of the *NPM1*^{wt}/*FLT3*-ITD^{low} and *NPM1*^{wt}/*FLT3*-ITD^{high} genotypes, respectively, had *RUNX1* mutations; *ASXL1* mutations were distributed more equally, and *TP53* mutations were only found in 2 cases.

We subsequently categorized patients according to the 2017 ELN risk groups (supplemental Table 2). Complete categorization could be done for 318 patients ("ELN biomarker cohort"): (1) favorable risk (n = 85, ie, *NPM1*^{mut}/*FLT3*-ITD^{low} AML), (2) intermediate risk (n = 111, ie, *NPM1*^{mut}/*FLT3*-ITD^{high} AML [n = 93] and *NPM1*^{wt}/*FLT3*-ITD^{low} AML [n = 18], both subgroups without the concurrent presence of the high-risk molecular markers *RUNX1*, *ASXL1*, *TP53*, as well as *NPM1*^{wt}/*FLT3*-ITD^{low} AML without adverse-risk cytogenetics; and (3) adverse risk (n = 122, *NPM1*^{wt}/*FLT3*-ITD^{high} AML [n = 92], *NPM1*^{mut}/*FLT3*-ITD^{high} AML [n = 8] exhibiting high-risk molecular markers, and *NPM1*^{wt}/*FLT3*-ITD^{low} AML [n = 22] with high-risk molecular markers and/or adverse-risk cytogenetics). Patient and disease characteristics are given in Table 2.

Response to induction therapy

We assessed response to therapy by ELN risk group and by treatment arm (midostaurin vs placebo). Responses included all CRs achieved during induction cycles 1 and 2 (Table 2). Responses in patients with favorable, intermediate, and adverse risk were as follows: with placebo, 68.1% vs 59.6% vs 44.4%, respectively ($P = .05$); with midostaurin, 71.1% vs 66.7% vs 57.4%, respectively ($P = .34$). There was no significant difference in response between treatment arms in the 3 ELN risk groups.

In multivariable logistic regression analysis, factors for lower CR rates were ELN adverse vs favorable risk (odds ratio [OR], 0.54; 95% confidence interval [CI], 0.29-0.99; $P = .052$) and higher WBC (10-fold) (OR, 0.62; 95% CI, 0.39-0.97; $P = .039$). Age (difference of 10 years; OR, 1.07, 95% CI, 0.85-1.34; $P = .55$), sex (female vs male; OR, 1.00; 95% CI, 0.62-1.63; $P = .99$), treatment (midostaurin vs placebo; OR, 1.26; 95% CI, 0.78-2.03; $P = .35$), and BM blasts (twofold) (OR, 0.96; 95% CI, 0.66-1.38; $P = .84$) did not have a significant influence.

Outcomes

The estimated median follow-up of the 318 patients was 57.5 months (95% CI, 55.2-61.2). Median OS and 5-year OS rate were 26.3 months (95% CI, 18.6-50.7) and 0.44 (95% CI, 0.39-0.50); median EFS and 5-year EFS rate were 4.70 months (95% CI, 1.97-7.49) and 0.24 (95% CI, 0.20-0.29). Median OS and 5-year OS rates in the placebo and midostaurin arms were 16.6 months (95% CI, 13.9-23.2) and 0.34 (95% CI, 0.27-0.43) and not reached (95% CI, 29.8 months-not reached) and 0.53 (95% CI, 0.46-0.61), respectively.

Survival analysis by ELN risk groups Figure 1 shows OS according to the 2017 ELN risk groups. Patients with favorable-risk AML had the best outcome (5-year OS probability 0.62; 95% CI, 0.53-0.74), followed by patients with intermediate-risk AML (0.43; 95% CI, 0.34-0.53) and patients with adverse-risk AML (0.33; 95% CI, 0.25-0.42). The difference between the curves was statistically significant ($P < .001$). Supplemental Figure 2A shows the prediction error curve for the 2017 ELN risk classification in comparison with the marginal Kaplan-Meier reference. An advantage of the ELN 2017 risk classifier is observed from 1-year follow-up onward.

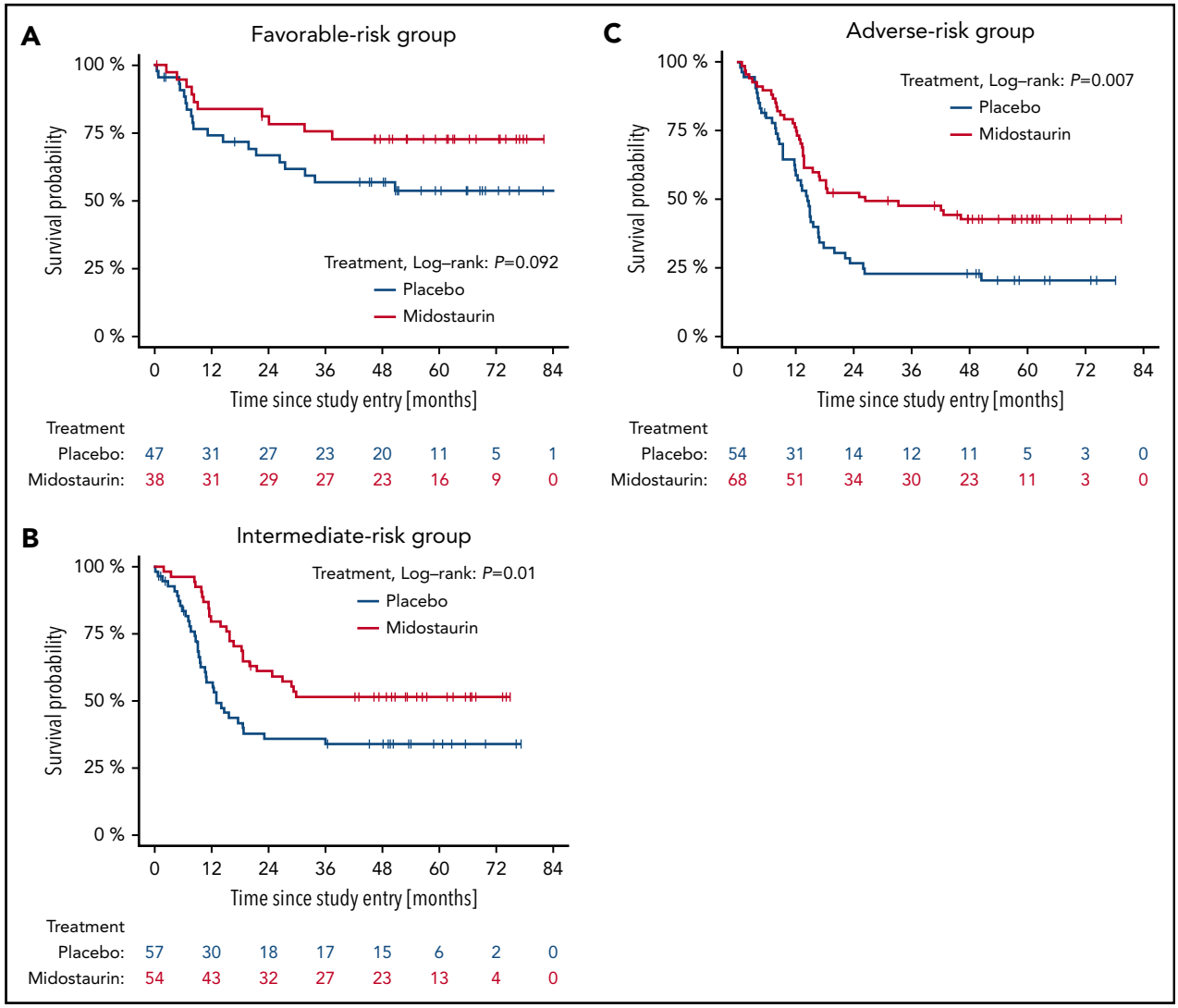

Figure 2. OS of patients with the different *NPM1/FLT3*-ITD genotypes by 2017 ELN risk group and by treatment. (A) Favorable-risk group. (B). Intermediate-risk group. (C) Adverse-risk group.

For illustration purposes, we included supplemental Figure 3, which shows OS according to the 4 *NPM1/FLT3*-ITD genotypes not categorized according to the ELN risk groups. Supplemental Figure 4 shows the forest plot of hazard ratios (HRs) from the treatment arm (midostaurin vs placebo), derived from univariate Cox models, by *NPM1/FLT3*-ITD genotypes.

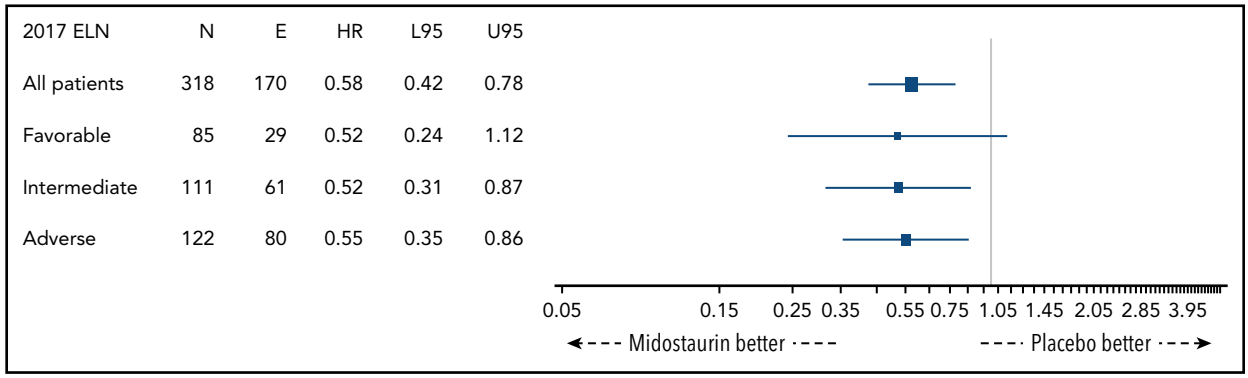

Figure 3. Forest plot of HRs of treatment arm (midostaurin vs placebo) derived from univariate Cox models by 2017 ELN risk groups. E, number of events; L95, lower 95% CI; N, number of patients; U95, upper 95% CI.

Table 3. Multivariate time-dependent Cox model for OS

	HR (95% CI)	P
All patients (n = 318)		
2017 ELN intermediate-risk group*	1.75 (1.11-2.76)	.017
2017 ELN adverse-risk group*	2.64 (1.69-4.13)	<.001
Allogeneic HCT	0.57 (0.42-0.94)	.021
Age	1.01 (0.99-1.02)	.709
WBC count (log_{10})	1.51 (1.11-2.00)	.009
Female vs male	0.77 (0.54-0.99)	.045
Treatment (midostaurin vs placebo)	0.55 (0.43-0.83)	<.002
BM blasts (log_2)	1.03 (0.84-1.37)	.603
Favorable-risk group (n = 85)		
Allogeneic HCT	0.78 (0.28-2.13)	.621
Age	1.02 (0.97-1.06)	.515
WBC count (log_{10})	0.89 (0.44-1.82)	.750
Female vs male	0.53 (0.24-1.14)	.102
Treatment (midostaurin vs placebo)	0.48 (0.20-1.11)	.086
BM blasts (log_2)	0.98 (0.62-1.54)	.916
Intermediate-risk group (n = 111)		
Allogeneic HCT	0.81 (0.41-1.58)	.535
Age	1.02 (0.99-1.05)	.178
WBC count (log_{10})	2.03 (1.16-3.55)	.013
Female vs male	1.06 (0.61-1.82)	.846
Treatment (midostaurin vs placebo)	0.53 (0.31-0.89)	.018
BM blasts (log_2)	1.17 (0.68-2.01)	.761
Adverse-risk group (n = 122)		
Allogeneic HCT	0.39 (0.21-0.73)	.003
Age	1.00 (0.98-1.02)	.867
WBC count (log_{10})	1.52 (0.97-2.38)	.068
Female vs male	0.66 (0.42-1.06)	.085
Treatment (midostaurin vs placebo)	0.51 (0.31-0.82)	.006
BM blasts (log_2)	0.98 (0.65-1.48)	.928

*Favorable-risk group was used as reference.

Outcome analysis by ELN risk group and by treatment arm Figure 2 shows OS by ELN risk group and by treatment arm (midostaurin vs placebo). Five-year OS rates of patients on the midostaurin and the placebo arm were 0.73 (95% CI, 0.60-0.89) and 0.53 (95% CI, 0.40-0.72), 0.52 (95% CI, 0.40-0.67) and 0.34 (95% CI, 0.23-0.49), and 0.43 (95% CI, 0.32-0.56) and 0.20 (95% CI, 0.12-0.35) in the favorable-, intermediate-, and adverse-risk groups, respectively. The corresponding forest plot of HRs for OS is shown in Figure 3. A beneficial effect of midostaurin was observed across all 3 risk groups. No treatment effect modification by the 2017 ELN risk classification was found.

A multivariate Cox model for OS of the entire cohort, including the covariates ELN risk groups, age, WBC count, BM blasts, sex, allogeneic HCT in CR1, and treatment, identified the 2017 ELN risk classification, treatment (midostaurin vs placebo), allogeneic HCT in CR1, and log_{10} WBC count as significant prognostic variables (Table 3). Leave-1-out cross-validated prediction error curves, according to Spitoni et al,[35] demonstrate the added value of the 2017 ELN classifier for 1-year follow-up and beyond, comparing the models with and without the 2017 ELN risk classification (supplemental Figure 2B).

CIR by ELN risk group, as well as by ELN risk group and by treatment arm, is shown in supplemental Figures 5A and 6, respectively. Five-year CIR rates for patients on the midostaurin and the placebo arms were 0.22 (95% CI, 0.07-0.38) and 0.35 (95% CI, 0.19-0.52), 0.36 (95% CI, 0.20-0.52) and 0.62 (95% CI, 0.45-0.78), and 0.58 (95% CI, 0.42-0.74) and 0.61 (95% CI, 0.41-0.81) in the favorable-, intermediate-, and adverse-risk groups, respectively. Supplemental Figures 5B and 7 show CID by ELN risk group and by treatment arm. In the adverse-risk group, there was a significantly lower CID rate with midostaurin (0.05; 95% CI, 0-0.12) vs placebo (0.26; 95% CI, 0.08-0.44; $P = .024$), providing an explanation for the discrepancy between improved OS on the midostaurin arm without decreasing the relapse rate. A multivariate Cox model for CIR identified ELN risk classification, treatment with midostaurin, allogeneic HCT in CR1, and WBC count as independent prognostic factors (supplemental Table 3). Supplemental Figure 8 displays the leave-1-out cross-validated prediction error curves according to Spitoni et al,[35] indicating the advantage of the 2017 ELN classifier, comparing the models with and without the 2017 ELN risk classification.

EFS by ELN risk group and treatment arm is shown in supplemental Figure 9.

Impact of allogeneic HCT in the ELN risk groups To illustrate the effect of allogeneic HCT in CR1 on OS, the Simon-Makuch method was used to estimate survival probabilities with respect to time-dependent interventions.[32] Of note, there was a consistent beneficial effect of midostaurin across all 3 risk groups, whereas this was not evident for allogeneic HCT. A strong beneficial effect of allogeneic HCT was only found in the adverse-risk group, with an HR of 0.39 (95% CI; 0.21-0.73; $P = .003$). Table 3 summarizes results from the multivariate Cox model for OS in the entire cohort, as well as in the 3 ELN risk groups. Figure 4 provides Simon-Makuch plots illustrating OS by 2017 ELN risk group, type of postremission therapy (conventional consolidation vs allogeneic HCT in CR1), and treatment arm (placebo vs midostaurin). Supplemental Figure 10 shows the corresponding Simon-Makuch plots by 2017 ELN risk group and by type of postremission therapy only.

Discussion

The results from this retrospective explorative analysis of the RATIFY trial confirm the prognostic value of the 2017 ELN genetic risk classification in patients with *FLT3*-ITD$^+$ AML. Furthermore, the data show that midostaurin exerts a beneficial effect in these patients across all 3 ELN risk groups.

Randomization in the RATIFY trial was stratified by the *FLT3*-ITD AR with a cutoff > 0.7 vs 0.05 to 0.7.[24] This cutoff was chosen based on the initial study reported by Thiede et al.[6] More recent studies found an ITD AR of 0.5 to be a better discriminator for prognosis.[10,15] Based on these more recent studies, the 2017 ELN recommendations put forward the current *NPM1*/*FLT3*-ITD risk categories that are based on an ITD AR cutoff of 0.5 and the *NPM1* mutational status.[21] An important aspect relates to the diagnostic assay for assessment of the AR. In the RATIFY trial, testing for *FLT3*-ITD was done in 9 reference laboratories in 6 countries using a harmonized PCR method based on capillary electrophoresis detection. To ensure a high degree of consistency among laboratories, a cross-validation quality control procedure was performed

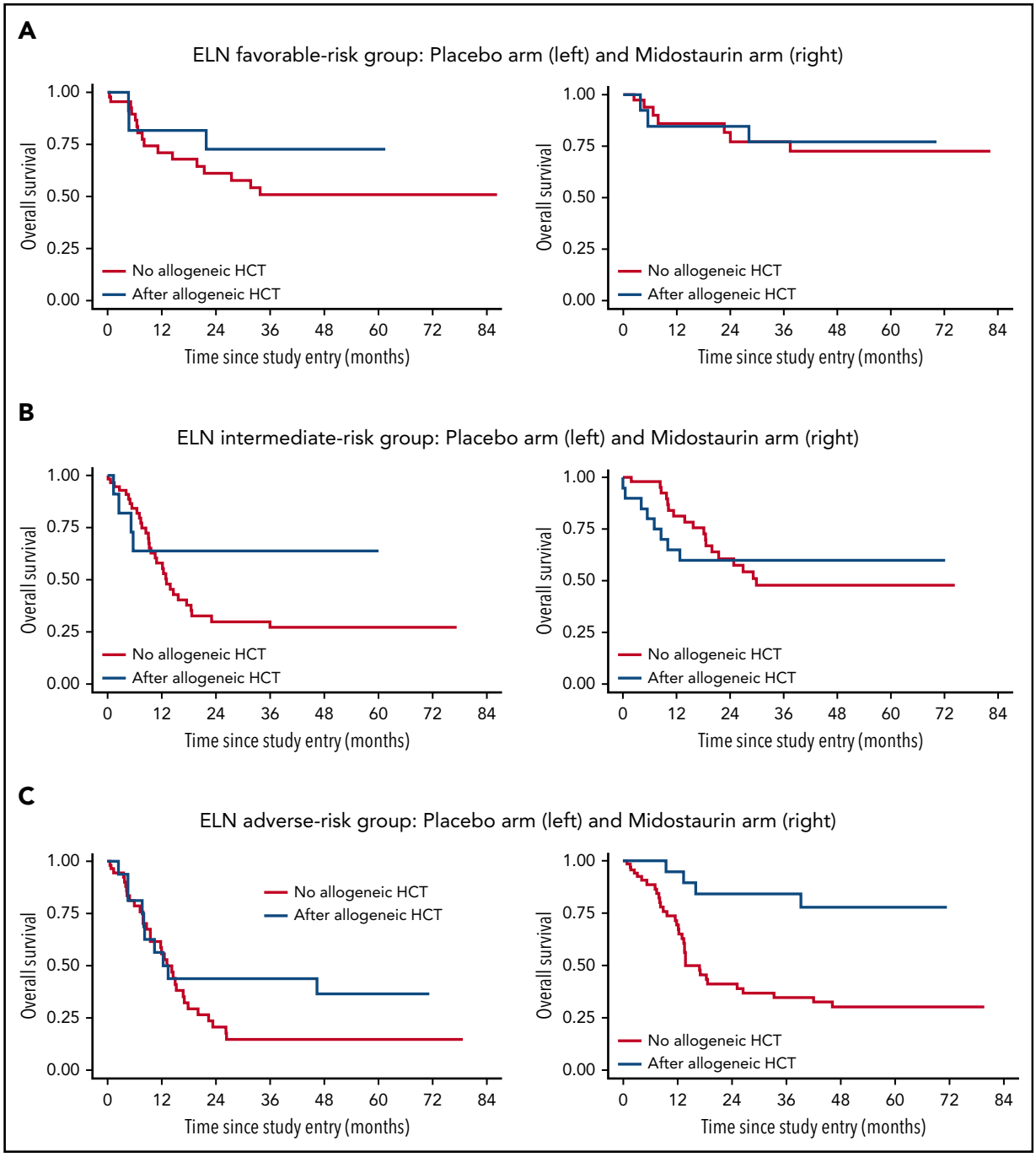

Figure 4. OS by 2017 ELN risk group, by type of postremission therapy (conventional consolidation vs allogeneic HCT in CR1), and by treatment arm (placebo vs midostaurin). Simon-Makuch plots illustrating the influence of allogeneic HCT as a time-dependent variable: patients who receive an allogeneic HCT move from the red to the blue curve at the time that the allogeneic HCT is performed. (A) ELN favorable-risk group: placebo arm (left panel) and midostaurin arm (right panel). (B) ELN intermediate-risk group: placebo arm (left panel) and midostaurin arm (right panel). (C) ELN adverse-risk group: placebo arm (left panel) and midostaurin arm (right panel).

every 6 months. The assessment of the ITD AR showed variability, which could be reduced by using a triplicate analysis.[26] Nevertheless, similar to many other diagnostic assays, there is a need for further harmonization and standardization of the testing.

The 4 *NPM1*/*FLT3*-ITD genotypes were associated with significant differences in clinical and concurrent genetic features. As previously shown,[27] compared with patients with *NPM1*^{wt}, more *FLT3*-ITD patients with concurrent *NPM1*^{mut} were female and had a normal karyotype. Patients with a high *FLT3*-ITD allelic burden had significantly higher WBC counts and higher BM blast numbers. The genotypes also differed significantly with regard to the concurrent presence of the 2017 ELN high-risk molecular markers *RUNX1*, *ASXL1*, and *TP53*. *RUNX1* mutations were almost mutually exclusive of *NPM1*^{mut}. The highest frequencies of *RUNX1* mutations were found in *NPM1*^{wt}/*FLT3*-ITD^{low} AML (30.2%), moving these cases from the intermediate-risk group to the adverse-risk group, as well as in *NPM1*^{wt}/*FLT3*-ITD^{high} AML (26.1%). *ASXL1* mutations

were distributed more equally among the 4 *NPM1*/*FLT3*-ITD genotypes, and *TP53* mutations were only found in 2 AML patients.

Complete categorization of the *NPM1*/*FLT3*-ITD genotypes according to the 2017 ELN classification could be done for 318 of the 549 trial patients with *FLT3*-ITD^{+} AML. The first important finding of this study is that the data confirm the high prognostic value of the 2017 ELN risk categorization, also among patients with *FLT3*-ITD^{+} AML (Figure 1). In particular, the data provide further evidence for the favorable prognosis of patients with *NPM1*^{mut}/*FLT3*-ITD^{low} AML. Five-year OS for patients in the 2 treatment arms combined was 62.5%; it was 73.0% in patients treated on the midostaurin arm, which is comparable to the outcome of patients with the other more favorable-risk AML, such as core-binding factor AML[37,38] and AML with biallelic *CEBPA* mutations.[39] Five-year OS for patients in the ELN adverse-risk group was 32.7%; it was 43.0% on the midostaurin arm. Compared with historical controls, outcomes for the adverse-risk patient group appear to be significantly improved by the addition of midostaurin and by allogeneic HCT.

The second important finding is that midostaurin had a beneficial effect on OS in *FLT3*-ITD AML across all 3 ELN risk groups (Figures 2 and 3). Thus, midostaurin appears to be active in *FLT3*-ITD^{+} AML, irrespective of the allelic burden of the ITD, and, importantly, as well as on different mutational backgrounds (eg, with or without *NPM1*^{mut}) and with or without selected adverse-risk genetic features. The effect on CIR appeared to be most pronounced in the ELN intermediate-risk group (supplemental Figure 6). The fact that midostaurin has a beneficial effect on OS, independent of the ITD allelic burden and across various underlying genetic signatures, raises the question of whether the therapeutic effect is primarily mediated through its FLT3-inhibitory effect or through other antileukemic effects of this multikinase inhibitor.[40]

Finally, an important point of discussion in the past has been the value of allogeneic HCT in patients with the different *NPM1*/*FLT3*-ITD genotypes.[10,15,17-19] To address the impact of allogeneic HCT in CR1, we used the Simon-Makuch method to estimate survival distributions of time-dependent interventions.[32] Multivariate analysis for OS using the Mantel-Byar test in the entire patient cohort identified treatment with midostaurin, allogeneic HCT, 2017 ELN favorable risk, and lower WBC counts as significant favorable factors for OS (Table 3). The same variables were identified in the multivariate model for cause-specific hazard of relapse (supplemental Table 3). Next, we performed multivariate analysis for OS within the 3 ELN risk groups. Of note, the only variable that showed a consistent favorable effect across all risk groups was treatment with midostaurin. For allogeneic HCT, in contrast, a strong beneficial effect was only observed in the adverse-risk group. Figure 4 provides Simon-Makuch plots illustrating the influence of allogeneic HCT vs conventional consolidation on OS in the 3 risk groups and by treatment arm (placebo vs midostaurin). These results need to be interpreted with caution because the clinical trial was not powered to show statistically significant differences in these genetic subgroups with regard to allogeneic HCT and with regard to treatment with midostaurin vs placebo. Nevertheless, the data provide further evidence that conventional consolidation plus midostaurin is a postremission treatment option for patients with *FLT3*-ITD, ELN favorable-risk AML, and allogeneic HCT may be delayed until first relapse in this patient population. Importantly, in these patients, *NPM1*^{mut} provides a solid target for the monitoring of measurable residual disease, allowing for further refinement of the prognostic assessment, which taken all together, will inform the most appropriate postremission therapy.[41,42] Based on the results of this study, one could also envision a similar treatment strategy for many patients with *FLT3*-ITD, ELN intermediate-risk AML, the majority of whom also carry concurrent *NPM1*^{mut}. This treatment algorithm should be explored in future studies.

Of note, the RATIFY trial only recruited patients 18 to 60 years of age; thus, the data provided by this retrospective exploratory study cannot be automatically extrapolated to patients older than 60 years. Nevertheless, the AMLSG 16-10 trial, evaluating midostaurin in patients 18 to 70 years of age, reported very encouraging results in patients 60 to 70 years of age, as well.[25]

In conclusion, the data from this study provide further support for the high prognostic value of the 2017 ELN risk categorization in patients with *FLT3*-ITD. A complete work-up according to 2017 ELN recommendations, including assessment of the ITD allelic burden, should be mandatory for all newly diagnosed *FLT3*-ITD patients eligible for intensive therapy. The multikinase inhibitor midostaurin showed a beneficial effect across all risk groups and independent of allogeneic HCT. This study further stresses the beneficial impact of allogeneic HCT in patients with *FLT3*-ITD, ELN adverse-risk AML.

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Authorship

Contribution: K.D. and C.T. designed the study, performed research, collected, assembled, analyzed, and interpreted data, and wrote the manuscript; N.J., E.P., T.W.P., D.J., M.H., T.O., J.F.N., and J.H.J. performed molecular analyses and analyzed data; A. Gambietz, S.J.M., I.G., and A.B. performed statistical analyses; R.A.L., G.M., R.B.K., A.H.W., J.S., M.A.S., J.M.B., T.d.W., D.N., F.R.A., B.C.M., M.S.T., R.F.S., A. Ganser, H.S., G.E., S.A., and C.P. collected, assembled, analyzed, and interpreted data; J.K. and M.T.V. collected and assembled data, performed molecular analyses, and interpreted and analyzed data; and R.M.S., H.D., and C.D.B. designed the study, collected, assembled, analyzed and interpreted data, and wrote the manuscript. All authors approved the final version of the manuscript.

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Footnotes

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REFERENCES

1. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med*. 2016; 374(23):2209-2221.

2. Grimwade D, Ivey A, Huntly BJ. Molecular landscape of acute myeloid leukemia in younger adults and its clinical relevance. *Blood*. 2016;127(1):29-41.

3. Bullinger L, Döhner K, Döhner H. Genomics of acute myeloid leukemia diagnosis and pathways. *J Clin Oncol*. 2017;35(9):934-946.

4. Leick MB, Levis MJ. The future of targeting FLT3 activation in AML. *Curr Hematol Malig Rep*. 2017;12(3):153-167.

5. Kottaridis PD, Gale RE, Frew ME, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood*. 2001;98(6):1752-1759.

6. Thiede C, Steudel C, Mohr B, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. *Blood*. 2002; 99(12):4326-4335.

7. Fröhling S, Schlenk RF, Breitruck J, et al; AML Study Group Ulm. Acute myeloid leukemia. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. *Blood*. 2002;100(13):4372-4380.

8. Schlenk RF, Döhner K, Krauter J, et al; German-Austrian Acute Myeloid Leukemia Study Group. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *N Engl J Med*. 2008; 358(18):1909-1918.

9. Gale RE, Green C, Allen C, et al; Medical Research Council Adult Leukaemia Working Party. The impact of *FLT3* internal tandem duplication mutant level, number, size, and interaction with *NPM1* mutations in a large cohort of young adult patients with acute myeloid leukemia. *Blood*. 2008;111(5):2776-2784.

10. Pratcorona M, Brunet S, Nomdedéu J, et al; Grupo Cooperativo Para el Estudio y Tratamiento de las Leucemias Agudas Mieloblásticas. Favorable outcome of patients with acute myeloid leukemia harboring a low-allelic burden FLT3-ITD mutation and concomitant NPM1 mutation: relevance to post-remission therapy. *Blood*. 2013;121(14):2734-2738.

11. Breitenbuecher F, Schnittger S, Grundler R, et al. Identification of a novel type of ITD mutations located in nonjuxtamembrane domains of the FLT3 tyrosine kinase receptor. *Blood*. 2009;113(17):4074-4077.

12. Kayser S, Schlenk RF, Londono MC, et al; German-Austrian AML Study Group (AMLSG). Insertion of FLT3 internal tandem duplication in the tyrosine kinase domain-1 is

associated with resistance to chemotherapy and inferior outcome. *Blood*. 2009;114(12):2386-2392.

13. Rücker F, Du L, Blätte TJ, et al. Prognostic impact of insertion site in acute myeloid leukemia (AML) with FLT3 internal tandem duplication: results from the RATIFY study (Alliance 10603). *Blood*. 2018;132(suppl 1):435.

14. Whitman SP, Archer KJ, Feng L, et al. Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of *FLT3*: a cancer and leukemia group B study. *Cancer Res*. 2001;61(19):7233-7239.

15. Schlenk RF, Kayser S, Bullinger L, et al; German-Austrian AML Study Group. Differential impact of allelic ratio and insertion site in FLT3-ITD-positive AML with respect to allogeneic transplantation. *Blood*. 2014;124(23):3441-3449.

16. Linch DC, Hills RK, Burnett AK, Khwaja A, Gale RE. Impact of FLT3(ITD) mutant allele level on relapse risk in intermediate-risk acute myeloid leukemia. *Blood*. 2014;124(2):273-276.

17. Versluis J, In 't Hout FE, Devillier R, et al. Comparative value of post-remission treatment in cytogenetically normal AML subclassified by NPM1 and FLT3-ITD allelic ratio. *Leukemia*. 2017;31(1):26-33.

18. Ho AD, Schetelig J, Bochtler T, et al; Study Alliance Leukemia. *Allogeneic stem cell transplantation improves* survival in patients with acute myeloid leukemia characterized by a high allelic ratio of mutant FLT3-ITD. *Biol Blood Marrow Transplant*. 2016;22(3):462-469.

19. Oran B, Cortes J, Beitinjaneh A, et al. Allogeneic transplantation in first remission improves outcomes irrespective of FLT3-ITD allelic ratio in FLT3-ITD-positive acute myelogenous leukemia. *Biol Blood Marrow Transplant*. 2016;22(7):1218-1226.

20. Sakaguchi M, Yamaguchi H, Najima Y, et al. Prognostic impact of low allelic ratio *FLT3*-ITD and *NPM1* mutation in acute myeloid leukemia. *Blood Adv*. 2018;2(20):2744-2754.

21. 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.

22. Stone RM. What FLT3 inhibitor holds the greatest promise? *Best Pract Res Clin Haematol*. 2018;31(4):401-404.

23. Stone RM, Manley PW, Larson RA, Capdeville R. Midostaurin: its odyssey from discovery to approval for treating acute myeloid leukemia and advanced systemic mastocytosis [published correction appears in *Blood Adv*. 2018;2(7):787]. *Blood Adv*. 2018;2(4):444-453.

24. 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.

25. Schlenk RF, Weber D, Fiedler W, et al; German-Austrian AML Study Group. Midostaurin added to chemotherapy and continued single-agent maintenance therapy in acute myeloid leukemia with *FLT3*-ITD. *Blood*. 2019;133(8):840-851.

26. Thiede C, Prior TW, Lavorgna S, et al. FLT3 mutation assay laboratory cross validation: Results from the CALGB 10603/Ratify trial in patients with newly diagnosed FLT3-mutated acute myeloid leukemia (AML). *Blood*. 2018;132(suppl 1):2800.

27. Döhner K, Schlenk RF, Habdank M, et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. *Blood*. 2005;106(12):3740-3746.

28. Jahn N, Panina E, Bullinger L, et al. Comprehensive molecular profiling of FLT3-mutated acute myeloid leukemia (AML) patients treated within the Ratify trial (Alliance C10603). *Blood*. 2018;132(suppl 1):1534.

29. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. *Control Clin Trials*. 1996;17(4):343-346.

30. Cox DR. Regression models and life-tables. *J R Stat Soc Series B Stat Methodol*. 1972;34(2):187-220.

31. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53(282):457-481.

32. Bernasconi DP, Rebora P, Iacobelli S, Valsecchi MG, Antolini L. Survival probabilities with time-dependent treatment indicator: quantities and non-parametric estimators. *Stat Med*. 2016;35(7):1032-1048.

33. Therneau T, Crowson C, Atkinson E. Using time dependent covariates and time dependent coefficients in the Cox model. https://cran.r-project.org/web/packages/survival/vignettes/timedep.pdf. Accessed 15 October 2019.

34. Gerds TA, Schumacher M. Consistent estimation of the expected Brier score in general survival models with right-censored event times. *Biom J*. 2006;48(6):1029-1040.

35. Spitoni C, Lammens V, Putter H. Prediction errors for state occupation and transition probabilities in multi-state models. *Biom J*. 2018;60(1):34-48.

36. Aalen OO, Johansen S. An empirical transition matrix for non-homogeneous Markov chains based on censored observations. *Scand J Stat*. 1978;5:141-150.

37. Marcucci 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.

38. 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.

39. Taskesen E, Bullinger L, Corbacioglu A, et al. Prognostic impact, concurrent genetic mutations, and gene expression features of AML with CEBPA mutations in a cohort of 1182 cytogenetically normal AML patients: further evidence for CEBPA double mutant AML as a distinctive disease entity. *Blood*. 2011;117(8):2469-2475.

40. Manley PW, Caravatti G, Furet P, et al. Comparison of the kinase profile of midostaurin (Rydapt) with that of its predominant metabolites and the potential relevance of some newly identified targets to leukemia therapy. *Biochemistry*. 2018;57(38):5576-5590.

41. Krönke J, Schlenk RF, Jensen KO, et al. Monitoring of minimal residual disease in NPM1-mutated acute myeloid leukemia: a study from the German-Austrian acute myeloid leukemia study group. *J Clin Oncol*. 2011;29(19):2709-2716.

42. Schuurhuis GJ, Heuser M, Freeman S, et al. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. *Blood*. 2018;131(12):1275-1291.