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The improved prognosis of *FLT3*-internal tandem duplication but not tyrosine kinase domain mutations in acute myeloid leukemia in the era of targeted therapy: a real-world study using large-scale electronic health record data

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Contributions: MS, SH, DW, JHC, and TYZ extracted the data. MS, EB, and GR analyzed the data. MS wrote the manuscript. GM, TYZ, JHC, EB, and RM supervised the study.

Data sharing statement: The data used for this study are available upon request by emailing Tian Yi Zhang (tzhang8@stanford.edu).

Mutations in *FLT3* occur in 20-30% of adults with acute myeloid leukemia (AML) and have historically been associated with a high relapse rate and less favorable prognosis[1]. Multiple *FLT3* inhibitors have received United States Food and Drug Administration (FDA) approval, but their impact on overall survival of patients in real-world settings remains uncharacterized. Novel therapies can have differing effects than the original clinical trials showed[2], and *FLT3* inhibitors can be used both at diagnosis and relapse during a patient's course[3,4]. Additionally, not all *FLT3* inhibitors are approved for *FLT3* tyrosine kinase domain (TKD) mutations, and those that are may produce less benefit in TKD compared to internal tandem duplication (ITD) mutations[5]. Therefore, we hypothesized that the relative prognosis of patients with *FLT3*-mutated AML improved significantly after the FDA approved midostaurin, the approved first *FLT3*-inhibitor for AML. Because multiple *FLT3* inhibitors have been approved since 2017, we also hypothesized that the aggregate effect size would be greater than that seen in the original phase III trial studying midostaurin[3]. Finally, we explored whether *FLT3* TKD mutations are associated with benefit in the era of targeted therapy.

We gathered data primarily from our institution's electronic health record (EHR), except for overall survival (OS) data, which was supplemented with external databases, including the Social Security Death Index. Patients were included if they had AML, were at least 18 years old, and were diagnosed between March 2008 and September 2022. They were excluded if they had acute promyelocytic leukemia, or they received gilteritinib, sorafenib, or midostaurin before the FDA approval date for midostaurin. This retrospective study was approved by the Institutional Review Board at Stanford University (IRB 62692). Baseline characteristics of patients are shown in Table 1. Diagnosis dates came from the Stanford Cancer Registry. EHR data were extracted using the OMOP (Observational Medical Outcomes Partnership) common data model, a commonly used EHR model that has been previously used for oncology[6]. Patients were included if both *FLT3*-ITD and *FLT3* D835 TKD mutations were tested within 14 days of AML diagnosis at the Stanford Cancer Center. Testing consisted of a rapid turn-around polymerase chain reaction (PCR) assay developed internally, which also include testing of *NPM1*, a frequently co-mutated gene. This assay has been used since 2008, except for 3/2012-7/2013, when *FLT3* was tested by a commercial assay. *FLT3* mutation results were extracted from molecular pathology reports, converting phrases from pathology reports (e.g. "Negative for FLT3-ITD mutation") into binary variables using rule-based systems in Python. Allelic ratio was unavailable for the *FLT3*-ITD mutations, and I836 mutations were not specifically identified by the PCR assay.

The FDA approval of midostaurin in April 2017 served as a real-world, post-market experiment illustrating the impact of *FLT3* inhibitors' availability on the prognosis of *FLT3*-mutated AML. We first explored the relative survival of *FLT3*-mutated AML before and after the approval of midostaurin (Figures 1A and 1B). Although OS did not significantly differ between *FLT3*-mutated and wildtype cases, their relationship reversed, with *FLT3* relatively unfavorable before midostaurin's approval (Figure 1A) and *FLT3* relatively favorable after midostaurin's approval (Figure 1B).

We then quantified this change in the difference in relative survival associated with *FLT3* mutation. First, we explored whether one-year OS changed for *FLT3*-mutated vs. wildtype cases. While one-year survival increased from 36% to 67% for *FLT3*-mutated cases (an absolute

difference of 31%), it increased only from 49% to 54% (difference of 5%) for *FLT3*-wildtype (Figure 1C), with an absolute difference in these differences of 26%.

Next, we formally estimated this survival difference using a difference-in-differences (DiD) approach. DiD is a regression-based approach for estimating a treatment effect by comparing the before-after difference in outcomes in a treated group (one difference) relative to an untreated group (second difference). The DiD is captured using an interaction effect between binary variables, in this case between whether the AML had a *FLT3* mutation and whether the case was diagnosed after midostaurin's approval. A DiD approach is advantageous because variables unrelated to *FLT3* mutation status would not affect the *relative* improvement in prognosis of *FLT3*. For example, new mutation-agnostic treatments (e.g. venetoclax) would not benefit *FLT3* specifically unless they specifically benefited cases with *FLT3* mutation. However, because of the potential for demographic shifts or class imbalance to affect the result, we adjusted each regression for *NPM1* mutation, whether a patient received an allogenic hematopoietic stem cell transplant, patient age at diagnosis, and the interaction between age and diagnosis post-midostaurin approval. Given that a major change before and after midostaurin's approval is the introduction of *FLT3* inhibitors, we interpret the interaction effect to primarily reflect the introduction of *FLT3* inhibitors. This assumption is supported by the fact that 89% of *FLT3*-positive cases getting intensive chemotherapy after approval received midostaurin. We built two regression models in R: a Cox proportional hazards regression predicting overall survival (Table 2), which is the primary DiD regression, and a logistic regression model predicting one-year survival. Cox proportional hazards regression was used to make results comparable to clinical trial hazard ratios, and logistic regression was used to validate the trends of Cox regression in the absence of the proportional hazards assumption.

The prognosis of *FLT3*-ITD mutations significantly improved following the approval of midostaurin, based on the multiple Cox regression DiD model (hazard ratio [HR] 0.39, 95% confidence interval [CI] 0.22-0.68, p = 0.001, Table 2). However, this effect was not seen for *FLT3* TKD mutations (HR 0.82, CI 0.22-3.1, p = 0.77). Similar results were also observed using logistic regression, where ITD mutations had a substantially better one-year survival in the era of targeted therapy (odds ratio [OR] of death 0.14, CI 0.04-0.46, p = 0.001), but TKD mutations did not (OR 1.2, CI 0.11-13, p = 0.87).

For ITD mutations, comparable hazard ratios were seen in subset analyses (with smaller sizes) of younger patients (age < 60, HR 0.47, CI 0.13-1.7), older patients (age ≥ 60, HR 0.40, CI 0.20-0.80), and those receiving intensive chemotherapy (HR 0.44, CI 0.18-1.1) or non-intensive chemotherapy (HR 0.47, CI 0.11-1.9). This occurred despite older patients receiving different treatment and potentially having different disease biology[7]. The benefit for *FLT3*-ITD mutations result was also not explained by a change in the use of allogeneic transplant among patients with *FLT3* mutations because the use of transplant was adjusted for in the DiD regression. The proportion of patients with *FLT3*-ITD mutations receiving transplant also did not significantly change pre- vs. post-midostaurin (27% vs. 35%, Fisher's exact test p = 0.53). Although our analysis excluded 37 patients who received *FLT3* inhibitors (23 received only sorafenib) before midostaurin's approval, a regression including this subset showed similar improvement in *FLT3*-ITD prognosis (HR 0.41, CI 0.20-0.82). In our dataset, the relative prognosis of *FLT3*-ITD mutation was exceptionally poor in 2011 and 2012 for unclear reasons, so we also performed

regressions after eliminating those two years of data. We still found that *FLT3*-ITD mutations had a significantly improved prognosis in the era of targeted therapies (HR 0.47, CI 0.26-0.86).

We then performed regressions adjusting for other variables to ensure we did not miss important mediators, and we found comparable HR when adjusting for venetoclax, white blood cell count, and performance status (HR 0.34-0.40, Supplementary Table 1). However, when adjusting for the use of FLT3 inhibitors, the improved prognosis of *FLT3*-ITD was substantially attenuated (HR 0.62 CI 0.30-1.3), suggesting that these inhibitors at least partially mediated this change in prognosis. However, we noticed that in the era of targeted therapies, *FLT3*-ITD mutations did not reach the level of a favorable mutation, *NPM1*, which in *FLT3*-ITD-negative cases still had better survival than *FLT3*-ITD-positive (Figure 1D).

Thus, we found that *FLT3*-ITD mutations are associated with a relatively more favorable prognosis in the era of targeted therapy. The HR of approximately 0.4 was also better than what was reported in clinical trials of midostaurin and gilteritinib (0.78 and 0.64, respectively)[3,4], although the 95% confidence interval still included the HR for gilteritinib, and the prognosis was still worse than a favorable *NPM1* mutation. To our surprise, we did not appreciate an improved prognosis for *FLT3* TKD mutations. This could be in part related to the relatively small sample size of the TKD cases (29 cases), variable prognostic significance of TKD mutations with heterogenous co-mutations, and/or variable efficacy of midostaurin on TKD mutations[8]. However, these results support prior literature that OS has not improved with therapy that ostensibly targets TKD mutations[5], implying that there is room for improvement in targeting these mutations. Our analysis also showed that the relative improvement of *FLT3*-ITD was at least partially mediated by *FLT3* inhibitor use, but we cannot totally exclude the possibility that factors other than *FLT3* inhibitors contributed to this improved prognosis.

A weakness of this study is that some variables could not be accounted for, such as other molecular changes that are now known to be high risk[9] but were not previously included in AML guidelines. However, given that many changes in AML diagnostics and care are agnostic to *FLT3* status, other advancements in AML are unlikely to invalidate the finding that *FLT3* inhibitors have produced substantial real-world benefit. Several additional regressions also did not support that other variables mediated this relatively improved prognosis.

Importantly, our study also serves as a framework for similar future studies. With the ability to rapidly generate large longitudinal clinical datasets from the EHR, our approach can facilitate data mobilization from other institutions and support large-scale analyses as additional therapies are used for AML. Such studies are crucial for confirming the benefits of novel therapies for all AML patients, including patients that are excluded from clinical trials. Our analysis indicates that the prognosis of *FLT3*-ITD AML has markedly improved since 2017, and that the benefit of *FLT3* inhibitors may be better in aggregate than is suggested by individual clinical trials.

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Table 1: Baseline patient characteristics for patients before and after midostaurin's approval. Continuous variables are shown as median and range, and others as total cases and percentage. *For a minority of patients, whether they received intensive vs. non-intensive induction was unclear from the EHR database, so the percentages use a different denominator. † If the patient cohort had included those who received *FLT3* inhibitors prior to midostaurin's approval, then the pre-midostaurin group increases by 37 patients, 23 of whom received only sorafenib. After this adjustment to the pre-midostaurin cohort, 23% had *FLT3* internal tandem duplication (ITD) mutations and 6% had *FLT3* tyrosine kinase domains (TKD) mutations (see text for addition analysis on this group).

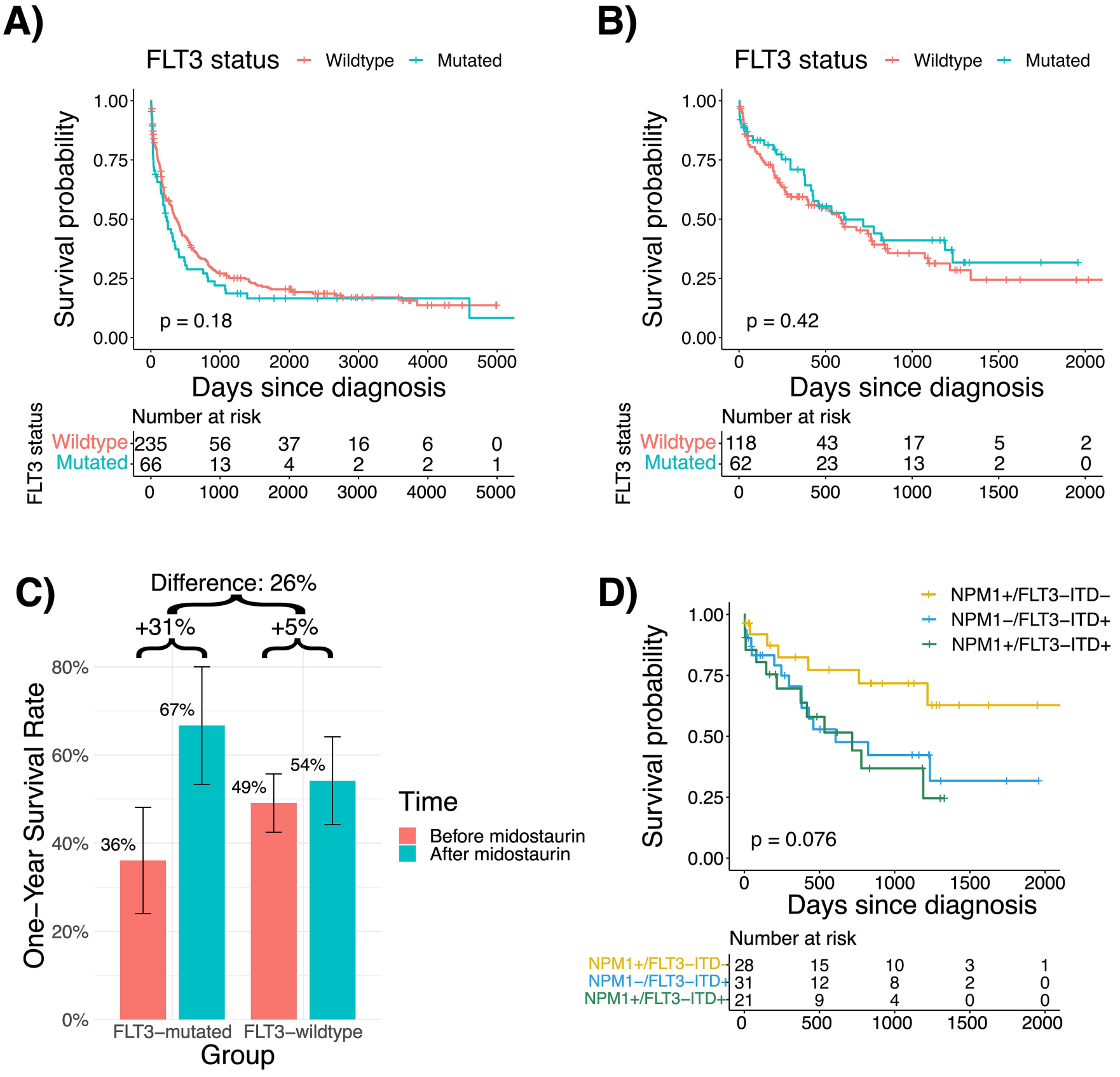
	Pre-midostaurin (n = 304)	***Post-midostaurin (n = 180)***
Age	63 (19 to >90)	64 (20 to 89)
ECOG performance status	1 (0-4)	1 (0-4)
Baseline labs:		
WBC (× 10^9/L)	7.5 (0-426)	46.5 (1-464)
Hemoglobin (g/dL)	9 (4-14)	9 (5-13)
Platelet (× 10^9/L)	56 (2-766)	50 (2-427)
NPM1 ***mutation***	55 (18%)	49 (27%)
FLT3 ***TKD mutation*** †	18 (6%)	11 (6%)
FLT3 ***ITD mutation*** †	57 (19%)	52 (29%)
Midostaurin use	0 (0%)	38 (21%)
Gilteritinib use	0 (0%)	26 (15%)
Sorafenib use	0 (0%)	5 (3%)
Intensive chemotherapy*	176 (76%)	75 (53%)
Hematopoietic stem cell transplant	87 (29%)	70 (39%)

TKD = tyrosine kinase domain, ITD = internal tandem duplication

Table 2: Differences-in-differences (DiD) model – Multiple Cox proportional hazards regression predicting overall survival. Interaction terms between *FLT3* mutations and whether the case was diagnosed after midostaurin's approval capture the change in prognosis for that *FLT3* mutation type.

	Hazard ratio (95% confidence interval)	***P-value***
Diagnosis after midostaurin approval	2.4 (0.82 – 7.3)	0.11
FLT3 *ITD mutation*	2.1 (1.5 – 2.9)	2.7×10^{-5}
NPM1 *mutation*	0.78 (0.59 – 1.0)	0.097
Patient age at diagnosis	1.03 (1.02 – 1.04)	5.7×10^{-10}
Hematopoietic stem cell transplant	0.34 (0.26 – 0.45)	2.3×10^{-14}
FLT3 *TKD mutation*	1.1 (0.57 – 2.0)	0.86
Post-midostaurin x* FLT3 *ITD interaction	0.39 (0.22 – 0.68)	0.001
Post-midostaurin x age interaction	0.99 (0.97 – 1)	0.088
Post-midostaurin x* FLT3 *TKD interaction	0.82 (0.22 – 3.1)	0.77

Figure 1: Overall survival for *FLT3*-mutated acute myeloid leukemia. A) Kaplan-Meier curves showing the survival of *FLT3*-mutated and wildtype cases before midostaurin's approval and B) after midostaurin's approval. C) One-year survival for *FLT3* mutated and wildtype cases before and after midostaurin was approved. The change in survival was substantially greater for *FLT3*-mutated cases. D) Prognosis of *FLT3*-ITD mutation compared to *NPM1*+/*FLT3*-ITD- after midostaurin's approval.


A)
FLT3 status
Wildtype
Mutated
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.18
0
1000
2000
3000
4000
5000
Days since diagnosis
Number at risk
FLT3 status
Wildtype 235 56 37 16 6 0
Mutated 66 13 4 2 2 1
B)
FLT3 status
Wildtype
Mutated
Survival probability
p = 0.42
0
500
1000
1500
2000
Days since diagnosis
Number at risk
FLT3 status
Wildtype 118 43 17 5 2
Mutated 62 23 13 2 0
C)
Difference: 26%
+31%
+5%
One−Year Survival Rate
80%
60%
40%
20%
0%
67%
36%
54%
49%
FLT3−mutated
FLT3−wildtype
Group
Time
Before midostaurin
After midostaurin
D)
NPM1+/FLT3−ITD−
NPM1−/FLT3−ITD+
NPM1+/FLT3−ITD+
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.076
0
500
1000
1500
2000
Days since diagnosis
Number at risk
NPM1+/FLT3−ITD− 28 15 10 3 1
NPM1−/FLT3−ITD+ 31 12 8 2 0
NPM1+/FLT3−ITD+ 21 9 4 0 0

Supplementary Table 1: Coefficient of post-midostaurin x *FLT3* ITD interaction term (the DiD term), which reflects the relative change in prognosis of *FLT3* ITD mutations, after adjusting for different variables. Each model below adjusts for the "Baseline model," which is shown in Table 2. In addition to the Baseline model, most regressions here adjust for another variable, such as patient performance status.

Variables adjusted for in model	***HR of DiD term (95% CI)***	***P-value of DiD term***
Baseline model	0.39 (0.22 – 0.68)	0.001
Baseline model + venetoclax	0.39 (0.20 – 0.77)	0.007
Baseline model + performance status	0.34 (0.16 – 0.69)	0.003
Baseline model + log(WBC + 1)	0.40 (0.23 – 0.70)	0.001
Baseline model + FLT3 inhibitor	0.62 (0.30 – 1.3)	0.19