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FLT3 inhibitor based induction and allogeneic stem cell transplant in CR1 improves outcomes in patients with newly diagnosed AML with very low FLT3 allelic burden

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Keywords

FLT3 mutated AML; low allelic frequency; FLT3 inhibitors; stem cell transplant; *NPM1*

To the editor:

Internal tandem duplication (ITD) mutations of the FMS-like tyrosine kinase (*FLT3*) gene are seen in approximately 25% of the patients with acute myeloid leukemia (AML) and are associated with a high risk of relapse and poor survival [1]. However, *FLT3*-ITD allelic ratio (AR), calculated as the ratio of mutated *FLT3*-ITD divided by wild type *FLT3* alleles (using DNA fragment analysis), less than 0.5, especially with concurrent *NPM1* mutations, have been categorized as a lower-risk disease by the European Leukemia Network (2017) guidelines [2]. The use of FLT3 inhibitors (FLT3i), such as sorafenib, midostaurin, gilteritinib, and quizartinib, has improved survival in patients with *FLT3*-mutated AML in the frontline, relapsed/refractory (R/R), and post-transplant maintenance settings. FLT3i's appear to be effective irrespective of the *FLT3*-ITD ARs, but recent analysis have suggested a clearer benefit for second-generation FLT3i's compared with salvage chemotherapy in patients with R/R AML who had higher FLT3 ARs [3]. Furthermore, the phase III FLT3i trials, RATIFY and ADMIRAL, excluded patients with *FLT3* AR <0.05 [4, 5]. The role and impact of FLT3i's, allogeneic stem cell transplantation (ASCT) and *NPM1* co-mutation, in

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patients with very low allelic burden *FLT3* mutations is unclear, often debated, and is the focus of this analysis.

In our institution, *FLT3* assays are reported as allelic frequency (AF) rather than AR. AF is calculated as the ratio of mutated *FLT3*-ITD divided by wild type *plus* mutated *FLT3*. In this study, baseline *FLT3*-ITD AFs ≤ 0.1 (equivalent to *FLT3* ARs ≤ 0.11) were defined as very low level. We retrospectively reviewed patients who received therapy for newly diagnosed *FLT3*-mutated AML (excluding core-binding factor AML and acute promyelocytic leukemia) between 2012–2020 at our institution. We identified 50 patients with *FLT3*-ITD AF ≤ 0.1 (at diagnosis). A polymerase chain reaction (PCR) based DNA analysis followed by capillary electrophoresis was utilized to detect *FLT3*-ITD and codon 835/836 point mutation at diagnosis and relapse as described previously. This study excluded patients with isolated *FLT3*-D835 mutations at diagnosis. All patients were treated with intensive (cytarabine and anthracycline-based) or low-intensity (hypomethylating agents or low-dose cytarabine based) regimens with or without a *FLT3i*.

Patients were analyzed in two cohorts depending on receipt of a *FLT3i* with frontline chemotherapy. Categorical variables were compared for statistical significance using the chi-square or Fisher's exact test, and continuous variables were compared using the Mann-Whitney U test. Kaplan-Meier analysis was performed to calculate the probability of overall survival (OS) and the log-rank test was used to compare OS between cohorts. Statistical analyses were performed in SPSS© (version 26). Of the 50 patients, 30 received frontline chemotherapy without a *FLT3i* (no *FLT3i* cohort), and 20 received induction with a *FLT3i* (*FLT3i* cohort). Baseline characteristics, treatment regimens, and outcomes of patients treated with or without a *FLT3i*-based therapy are summarized in Table 1. Patients in the *FLT3i* cohort were younger than the no *FLT3i* cohort (median age 58 vs. 67 years old, $p=0.03$), and more often were treated with an intensive induction (80% vs 43%, $p=0.01$).

Overall, a total of 37 patients achieved a CR/CRi, and 12 (32%) relapsed with a median follow-up of 42 months (range 30–53 months) (CONSORT diagram: Supplemental (S.) Figure 1). Of the 12 relapses, 8 (8/30, 27%) and 4 (4/20, 20%) were among patients treated without and with *FLT3i*-based regimens, respectively. The overall incidence of *FLT3*-ITD positivity at relapse was 42% (5 of 12 relapsed patients). However, all of the five *FLT3*-ITD positive relapses were observed in the no *FLT3i* cohort (5 of 8), and no *FLT3* positive relapses were seen in the *FLT3i* cohort (0 of 4), 63% versus 0%, respectively ($p=0.038$) (S. Figure 2A - D).

The median OS was not statistically different in the *FLT3i* cohort compared with no *FLT3i* cohort; not reached (N=20) versus 16.9 months (N=30), ($p=0.31$) (Figure 1A). We also investigated the impact of *NPM1* co-mutations on OS. Although statistically not significant, *NPM1* co-mutated patients had median OS of 32 months compared with 17 months in *NPM1* negative patients ($p=0.18$) (S. Figure 3A). The 5-year OS, not censored for ASCT, in patients with *NPM1* treated with *FLT3i* (n=12), *NPM1* without *FLT3i* (n=9), no *NPM1* with *FLT3i* (n=8), no *NPM1* without *FLT3i* (n=19) were 58%, 40%, 50%, and 22% months, ($p=0.38$) respectively (S. Figure 3B).

Overall, 14 patients underwent ASCT (6 FLT3i and 8 no FLT3i cohort) in the first remission with a median time to ASCT of 114 days (range 79–230 days). A landmark analysis (at 3-month post ASCT) showed that 5-year OS was significantly superior in patients who underwent ASCT (N=14) compared with those who did not (N=22), 85% vs. 34%, respectively (p=0.01) (Figure 1B). Given the heterogeneity of induction therapies used (intensive versus low-intensity chemotherapies), we performed landmark analysis in patients treated with intensive (N=25) or low-intensity regimens (n=11). When analyzed separately, the statistical significance of ASCT in CR1 was lost. However, there still was a trend for superior OS in transplant recipients regardless of chemotherapy intensity (S. Figure 4A-B).

Although the numbers were small, patients who received FLT3i-based induction and underwent ASCT achieved the longest OS in this very low FLT3 burden population. The 5-year OS rates in patients who received a FLT3i with induction followed by ASCT in CR1 (n=6), did not receive a FLT3i with induction followed by ASCT in CR1 (n=8), received a FLT3i with induction but did not undergo ASCT (n=10), and did not receive a FLT3i with induction and did not undergo ASCT (n=12) were 100%, 71%, 48%, and 27%, respectively (p=0.08) (S. Figure 5). The 5-year OS rates only in patients who received FLT3i and ASCT in CR1 (n=6) versus those who received neither FLT3i nor ASCT in CR1 were 100% vs 27% (n=12) (p=0.02). Among 14 ASCT recipients, 2 died; 1 due to relapse (*FLT3* negative) and 1 death in remission.

Interestingly, even in this very low FLT3 burden population ASCT was associated with favorable OS irrespective of the baseline *NPM1* co-mutation status. Among the 34 patients (2 with no baseline *NPM1* testing excluded), the 5-year OS in patients with *NPM1* with ASCT (n=7), no *NPM1* with ASCT (n=5), *NPM1* without ASCT (n=11), and no *NPM1* without ASCT (n=11) were 86%, 80%, 34%, and 38%, (p=0.15) respectively (Figure 1C).

Midostaurin was approved by the Food and Drug Administration and European Medicines Agency for patients with newly diagnosed *FLT3*-mutated AML based on improved survival in a randomized phase III study of induction chemotherapy with or without midostaurin (RATIFY) [4]. In a subsequent analysis, the addition of midostaurin was demonstrated to consistently improve survival in patients with low and high *FLT3*-ITD ARs supporting the use of midostaurin irrespective of the ITD AR [6]. However, this pivotal clinical trial excluded patients with *FLT3*-ITD AR < 0.05, and there remained a debate regarding the biological and clinical impact of using FLT3i's and/or ASCT in very low *FLT3* burden. In our study, the median *FLT3*-ITD AF was 0.03 for the entire group, and we demonstrated that the use of a FLT3i (sorafenib in 95% of the cases) non-significantly improved CR/CRi rates (85% versus 67%), reduced the incidence of *FLT3* positive relapses (0% vs 63%) with a trend to improved OS. Given the high rate of *FLT3* positive relapses even among patients with very low *FLT3*-ITD burden, we believe that future clinical trials investigating FLT3 inhibitors in AML should not exclude these patients.

Our analysis supports the use of FLT3i and ASCT in CR1 in patients with very low *FLT3*-ITD AF (< 0.1), irrespective of *NPM1* co-occurrence. Patients who received a FLT3i with induction and underwent ASCT in CR1 had a 5-year OS of 100%, compared with 71% in patients who did not receive a FLT3i with induction but underwent ASCT in CR1,

and only 27% in patients who neither received a FLT3i nor underwent ASCT in CR1. In addition, ASCT appeared to improve outcomes regardless of *NPM1* co-mutation status among this low *FLT3* burden population; the 5-year OS rates were 86% and 80% in patients with *NPM1* positive or negative, respectively, who went to ASCT compared with 30–40% in *NPM1* positive or negative patients who did not go to ASCT in CR1 (Figure 1C). We do acknowledge that the decision for ASCT needs to be further individualized for patients in this group based on co-existing factors including patient fitness, donor availability, MRD positivity, and co-occurring mutations.

Small cohort sizes and differences among baseline clinical characteristics in FLT3i and no FLT3i cohorts limit our ability to make definitive conclusions. Our findings require confirmation by studies with a larger patient size. However, in our study,, we demonstrated that the use of FLT3i reduced the incidence of *FLT3* positive relapses in AML patients with very low *FLT3*-ITD AFs (AF = 0.1). Our data suggests that eligible patients should be considered to receive a FLT3i based therapy and be considered for ASCT in CR1 irrespective of their baseline *FLT3* AF and *NPM1* co-mutation status, after weighing all patient and disease specific factors for individual patients. ASCT non-eligible patients should be encouraged to enroll in novel maintenance clinical trials. To better understand the impact of very low *FLT3* burden, future clinical trials investigating FLT3i based therapies should include all allelic burden *FLT3*-mutated patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of interest:

MY reports research funding from Pfizer and Daiichi Sankyo.

TK reports personal fees from Novartis, grants and personal fees from Pfizer, grants from BMS, grants and personal fees from Abbvie, grants and personal fees from Genentech, grants and personal fees from JAZZ, grants from Amgen, grants from AstraZeneca; Celgene: research grant; Incyte: research grant; Ascentage: research grant.

CD reports grants and personal fees from Abbvie, grants and personal fees from Agios, grants and personal fees from Novartis, grants and personal fees from ImmuneOnc, grants and personal fees from Daiichi Sankyo, grants and personal fees from Celgene, personal fees from Jazz, personal fees from Notable Labs, personal fees from MedImmune, grants from Calithera, personal fees from Bayer.

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Other authors reported no relevant COIs.

Data Availability Statement:

Research Data is not shared

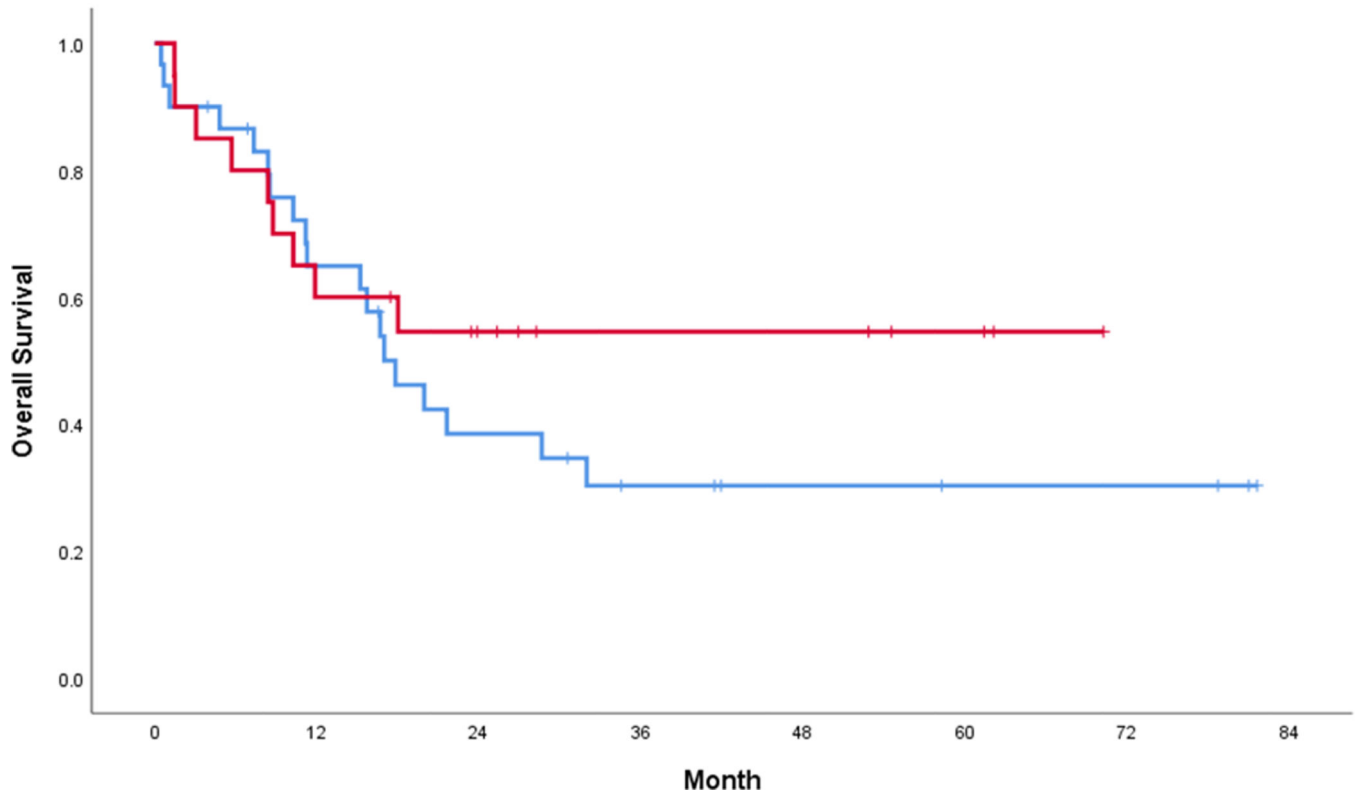
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A.

Therapy	N=50	Median OS:	5-yr OS
with FLT3i	20	not reached	55%
without FLT3i	30	16.9 m	30%

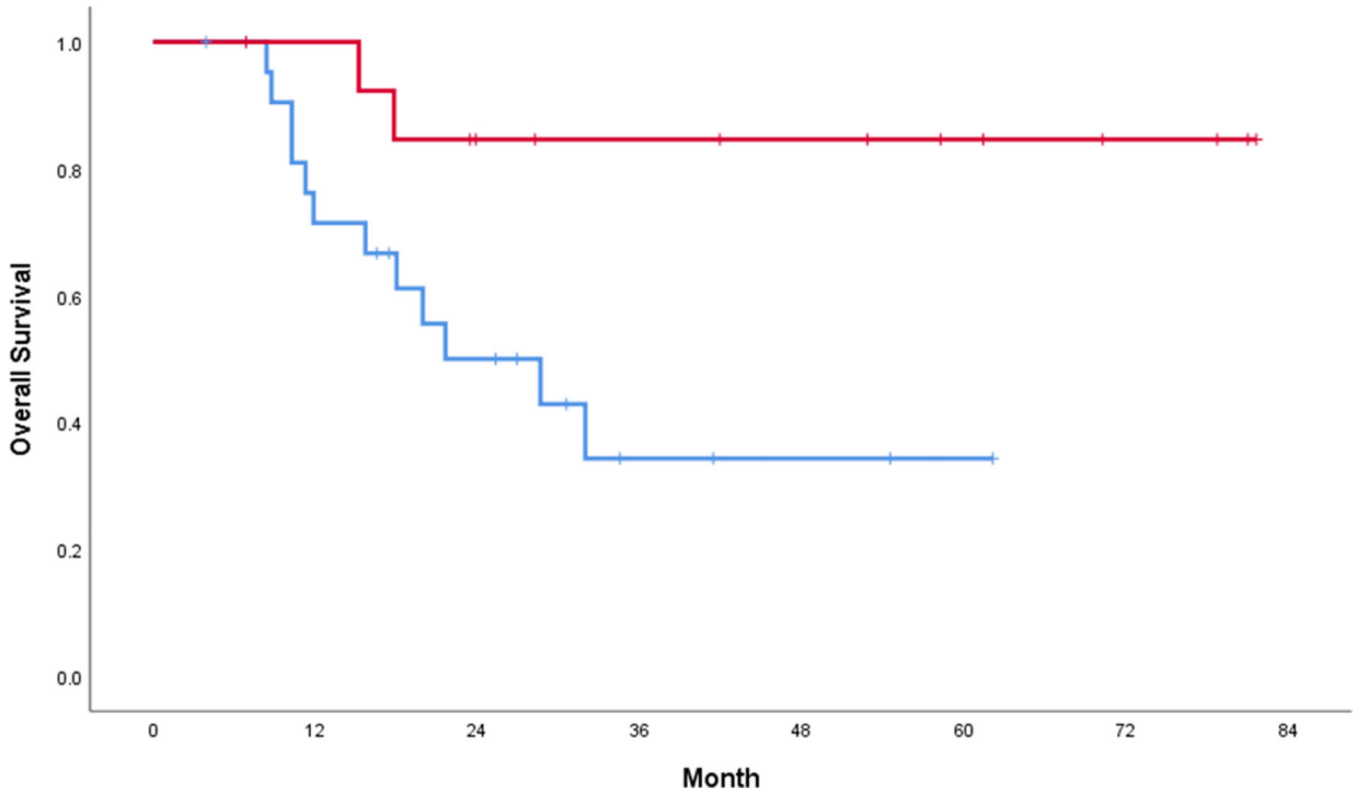
P=0.311



B.

Therapy	N=36	Median OS:	5-yr OS
ASCT in CR1	14	not reached	85%
No ASCT in CR1	22	21.6 m	34%

P=0.014



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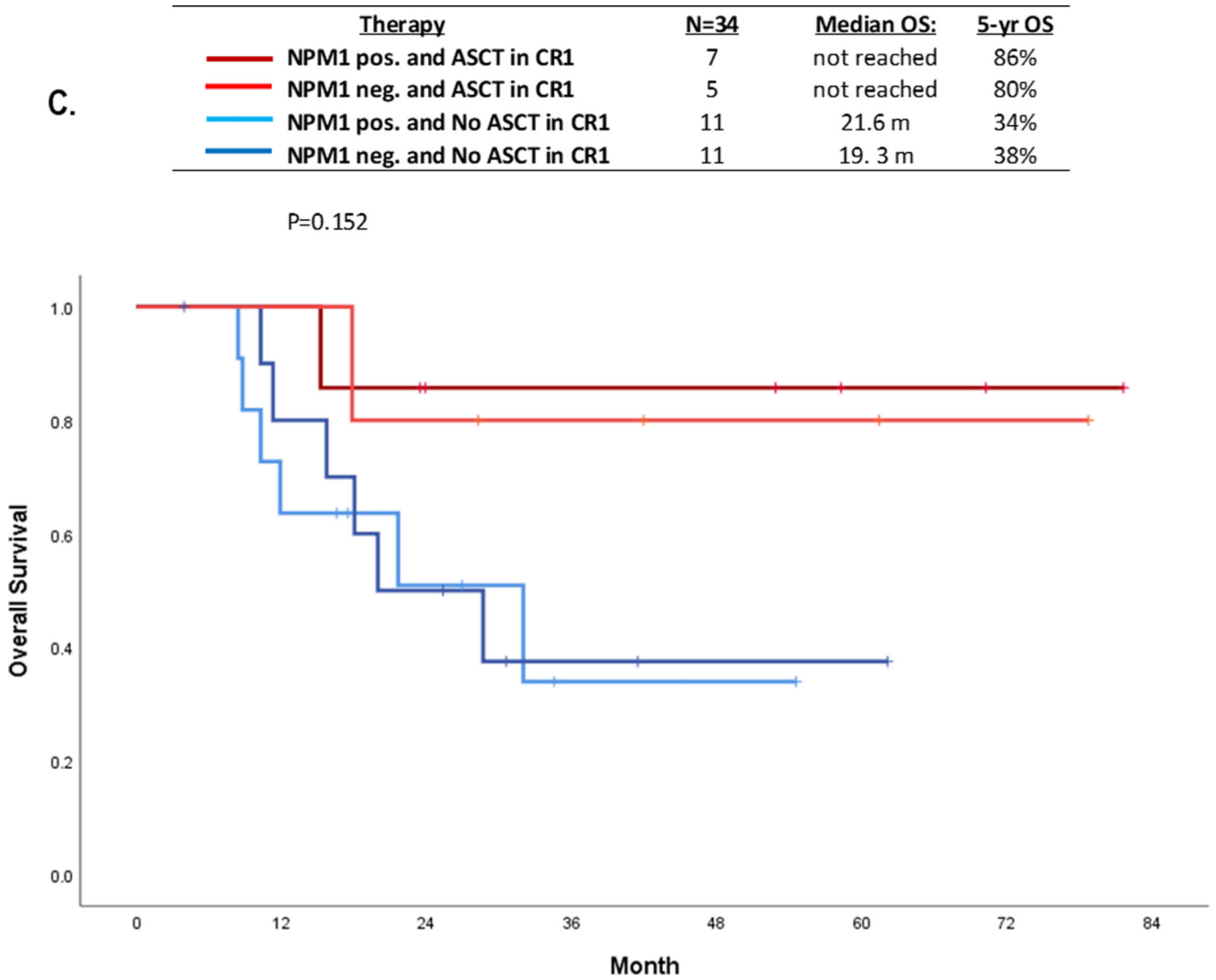


FIGURE 1:

OS in patients with FLT3-ITD with AF 0.1 by: (A) Treated with or without FLT3i containing regimen; (B) with or without ASCT in CR1, and (C) impact of NPM1 co-mutation and ASCT in CR1

Table 1.

Baseline clinical characteristics of patients treated with or without a FLT3 inhibitor-based therapy

Characteristics	No FLT3i Cohort N= 30 N (%) [range]	FLT3i Cohort N= 20 N (%) [range]	<i>p</i>
Median age, years	67 [23–84]	58 [28–83]	0.03
Age >60 years old	24 (80)	8 (40)	0.04
Male gender	17 (57)	10 (50)	0.64
Type of AML			
De novo	25 (83)	20 (100)	
Secondary AML	1 (3)	0 (0)	0.16
Therapy related	4 (14)	0 (0)	
WBC, x10 ⁹ /L	5.2 [1.1–34.2]	5.8 [0.9–378.4]	0.44
Hemoglobin, g/dl	8.9 [7.7–11.6]	8.7 [5.1–11.4]	0.37
Platelets, x10 ⁹ /L	52 [9–222]	31 [10–96]	0.33
Creatinine	0.8 [0.5–1.4]	0.7 [0.5–4.6]	0.69
Total Bilirubin	0.7 [0.2–1.]	0.5 [0.3–1.3]	0.08
Peripheral blood blasts, %	24 [0–91]	19 [0–97]	0.86
Bone marrow blasts, %	64 [15–96]	72 [30–94]	0.43
Cytogenetics			
Diploid karyotype	15 (50)	12 (60)	
Adverse	7 (23)	4 (20)	0.77
others	8 (27)	4 (20)	
FLT3 mutations *			
ITD Allelic Frequencies	0.03 [0.01–0.09]	0.05 [0.01–0.09]	0.45
Other Mutations			
NPM1	9/28 (32)	12/20 (60)	0.06
RUNX1	7/21 (33)	3/19 (16)	0.20
RAS	5/27 (18)	6/20 (30)	0.36
IDH2	8/29 (28)	5/20 (25)	0.84
ASXL1	5/21 (24)	2/19 (11)	0.27
IDH1	6/29 (21)	1/19 (5)	0.12
DNMT3A	2/27 (7)	1/20 (5)	0.74
CEBPA	1/26 (4)	3/19 (16)	0.16
TP53	1/24 (4)	1/19 (5)	0.87
Treatment			
Low Intensity	17 (57)	4 (20)	0.01
High Intensity	13 (43)	16 (80)	
FLT3 inhibitors			
Sorafenib	0 (0)	19 (96)	
Quizartinib	0 (0)	1 (5)	n/a
no FLT3i	30 (100)	0 (0)	
Treatment outcome			

Characteristics	No FLT3i Cohort N= 30 N (%) [range]	FLT3i Cohort N= 20 N (%) [range]	<i>p</i>
CR + CRi	20 (67)	17 (85)	
No response	7 (23)	3 (15)	0.23
Early death	3 (10)	0 (0)	
ASCT in CR1	8 (27)	6 (20)	0.80

FLT2 inhibitor; N, number; n/a, not applicable; WBC, white blood cell; CR/CRi, complete remission/Complete remission with incomplete count recovery; ASCT, allogeneic stem cell transplant; CR1, first remission

* Only 1 patient had both ITD and D835 at baseline, others were isolated FLT-ITD

Response definitions were per international working group (IWG) criteria. Relapse is defined by the detection of blasts (>5%) in a bone marrow aspirate or by the detection of biopsy-proven extramedullary myeloid sarcoma