Regular Article

CLINICAL TRIALS AND OBSERVATIONS

Prognostic impact of *NPM1* and *FLT3* mutations in patients with AML in first remission treated with oral azacitidine

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KEY POINTS

- Patients in remission treated with Oral-AZA had better OS and RFS vs placebo regardless of *NPM1* or *FLT3* mutational status at AML diagnosis.

- Oral-AZA conferred OS and/or RFS benefits in patients with favorable (*NPM1*^mut, no MRD) or adverse (*FLT3*^mut, MRD^+) prognostic AML features.

The randomized, placebo-controlled, phase 3 QUAZAR AML-001 trial (ClinicalTrials.gov identifier: NCT01757535) evaluated oral azacitidine (Oral-AZA) in patients with acute myeloid leukemia (AML) in first remission after intensive chemotherapy (IC) who were not candidates for hematopoietic stem cell transplantation. Eligible patients were randomized 1:1 to Oral-AZA 300 mg or placebo for 14 days per 28-day cycle. We evaluated relapse-free survival (RFS) and overall survival (OS) in patient subgroups defined by *NPM1* and *FLT3* mutational status at AML diagnosis and whether survival outcomes in these subgroups were influenced by presence of post-IC measurable residual disease (MRD). Gene mutations at diagnosis were collected from patient case report forms; MRD was determined centrally by multiparameter flow cytometry. Overall, 469 of 472 randomized patients (99.4%) had available mutational data; 137 patients (29.2%) had *NPM1* mutations (*NPM1*^mut), 66 patients (14.1%) had *FLT3* mutations (*FLT3*^mut; with internal tandem duplications [ITD], tyrosine kinase domain mutations [TKD^mut], or both), and 30 patients (6.4%) had *NPM1*^mut and *FLT3*-ITD at diagnosis. Among patients with *NPM1*^mut, OS and RFS were improved with Oral-AZA by 37% (hazard ratio [HR], 0.63; 95% confidence interval [CI], 0.41-0.98) and 45% (HR, 0.55; 95% CI, 0.35-0.84), respectively, vs placebo. Median OS was improved numerically with Oral-AZA among patients with *NPM1*^mut whether without MRD (48.6 months vs 31.4 months with placebo) or with MRD (46.1 months vs 10.0 months with placebo) post-IC. Among patients with *FLT3*^mut, Oral-AZA improved OS and RFS by 37% (HR, 0.63; 95% CI, 0.35-1.12) and 49% (HR, 0.51; 95% CI, 0.27-0.95), respectively, vs placebo. Median OS with Oral-AZA vs placebo was 28.2 months vs 16.2 months, respectively, for patients with *FLT3*^mut and without MRD and 24.0 months vs 8.0 months for patients with *FLT3*^mut and MRD. In multivariate analyses, Oral-AZA significantly improved survival independent of *NPM1* or *FLT3* mutational status, cytogenetic risk, or post-IC MRD status.

Introduction

A wide variety of cytogenetic and molecular abnormalities are implicated in the pathogenesis of acute myeloid leukemia (AML).[1-3] Among the most common gene mutations in patients with AML are alterations in nucleophosmin 1 (*NPM1*) and fms-related tyrosine kinase 3 (*FLT3*) genes, both of which have been shown to be prognostic of therapeutic outcomes and survival.[1,4] NPM1 proteins have a variety of cellular functions, including activity related to RNA expression and maturation; DNA replication, transcription, and repair; and preventing protein misfolding and aggregation (molecular chaperoning) for

histones and other proteins.[5,6] *NPM1* mutations (*NPM1*[mut]) occur in approximately one-third of younger adult patients at AML diagnosis and decrease in frequency with older age.[1,7-10] *NPM1*[mut] AML is recognized as a distinct clinical entity by the World Health Organization.[11] Functional FLT3 proteins are expressed by myeloid progenitor cells and play an important role in proliferation, differentiation, and survival of multipotent stem cells.[12] *FLT3* mutations (*FLT3*[mut]) are observed in ~30% of patients at AML diagnosis,[1] also decreasing with older age,[12] and manifesting as internal tandem duplications (ITD) in ~15% to 30% of patients, most often located in the juxtamembrane domain of the gene, and as point mutations in the tyrosine kinase domain (TKD) in ~8% of patients.[1,13] FLT3 is overexpressed in AML blasts, and *FLT3*[mut] can lead to constitutive phosphorylation of the FLT3 receptor in the absence of FLT3 ligand and activate downstream signaling pathways.[14]

Current guidelines for AML ascribe disease risk, in part, based on *NPM1* and *FLT3* mutational status[15]; these mutations frequently co-occur in patients with AML, implying molecular synergisms that promote AML development.[1,6] *NPM1*[mut] are particularly sensitive to intensive chemotherapy (IC) and typically are associated with favorable prognosis when no co-occurring *FLT3*-ITD mutation is present or when *FLT3*-ITD is present at a low allelic ratio (<0.5).[4,13,15-17] Conversely, *FLT3*-ITD alterations generally confer a poor prognosis in the absence of co-occurring *NPM1*[mut], or at a high allelic ratio (≥0.5) when accompanying an *NPM1*[mut].[9,13,15] The prognostic implication of *FLT3*-TKD mutations seems to depend on co-occurring mutational status; although these point mutations generally confer negative outcomes, prognosis mainly is favorable when *FLT3*-TKD mutations are accompanied by *NPM1*[mut] or core-binding factor AML.[18-20]

Although 40% to 80% of patients with AML can attain complete remission (CR) with IC, most patients eventually relapse because of regrowth of existing (or development of new) leukemic clones.[15,21] The presence of measurable residual disease (MRD) after IC is strongly prognostic of poorer overall survival (OS) and relapse-free survival (RFS) for patients with AML in remission.[15,21-28] An ongoing need exists for treatments that can suppress regrowth of leukemic cells to maintain remission and prolong OS.[29] Oral azacitidine (Oral-AZA [CC-486]) is a hypomethylating agent currently approved in the United States, the European Union, Switzerland, the United Kingdom, and Canada for patients with AML who achieve CR or CR with incomplete blood recovery (CRi) after IC and who are not eligible for curative therapy (eg, hematopoietic stem cell transplantation). Oral administration of azacitidine allows for extended dosing schedules that are not practical with the injectable regimen. In the randomized, phase 3 QUAZAR AML-001 trial (ClinicalTrials.gov identifier: NCT01757535), Oral-AZA significantly improved both OS and RFS from the time of randomization in older patients in first remission after IC compared with placebo.[30] Moreover, both OS and RFS were prolonged with Oral-AZA vs placebo regardless of whether patients had post-IC MRD at study entry.[31]

We performed post hoc analyses of data from the QUAZAR AML-001 trial to understand better the effects of Oral-AZA vs placebo in patients with prognostic mutations at AML diagnosis, specifically *NPM1*[mut] and/or *FLT3*[mut], and whether survival outcomes for patients in these gene mutation subgroups were influenced by post-IC MRD status at baseline.

Methods

Patients and assessments

The international, randomized, double-blind, placebo-controlled QUAZAR AML-001 trial was conducted in accordance with the principles outlined in the Declaration of Helsinki. All patients provided written informed consent before study participation. Study design, eligibility criteria, primary and secondary efficacy outcomes, and safety for all enrolled patients have been reported in detail.[30] Briefly, eligible patients were 55 years of age or older with newly diagnosed de novo or secondary AML (World Health Organization 2008 classification[32]) and intermediate- or poor-risk cytogenetic findings at diagnosis (per National Comprehensive Cancer Network 2011 criteria[33]), had achieved first remission (CR or CRi) after IC (induction chemotherapy with or without subsequent consolidation) and were not candidates for hematopoietic stem cell transplantation. Within 4 months (±7 days) of first CR or CRi, patients were randomized 1:1 to receive Oral-AZA 300 mg or placebo once daily for 14 days of repeated 28-day treatment cycles.

Cytogenetic and molecular gene mutation assessments were performed locally at AML diagnosis (ie, before study screening). Molecular profiling was performed according to institutional practices using standard metaphase cytogenetics and targeted molecular sequencing methods, with no protocol-specified methodology requirements or central confirmation. Mutations in specific genes present at diagnosis were captured on electronic case report forms completed for each patient at study screening. The gene mutation analyses typically were performed using polymerase chain reaction-based or next-generation sequencing methods. *FLT3*-ITD mutations typically were determined using polymerase chain reaction and fragment analyses. Any variant allele frequency data were reported rarely.

The primary and key secondary trial end points were OS and RFS, respectively, both measured from the time of randomization. OS was assessed until death by any cause, and RFS was the time to relapse (ie, ≥5% blasts in bone marrow) or death, whichever occurred first. To determine the impact of *NPM1*[mut] and *FLT3*[mut] at AML diagnosis on survival end points for these patients in remission after IC, OS and RFS results were compared within each treatment arm for patients with vs without each mutation, using the placebo arm as the key indicator of prognosis in the absence of active maintenance therapy. The effect of each mutation as a biomarker for survival was evaluated by comparing OS and RFS between the Oral-AZA and placebo arms.[34]

The influence of post-IC MRD status at screening on survival outcomes also was assessed in patients with *NPM1*[mut] or *FLT3*[mut]. MRD status was assessed prospectively by multiparameter flow cytometry (Münchner Leukämielabor, Munich, Germany) on samples collected at screening (baseline), on day 1 of cycles 3, 6, 9, 12, 15, 18, 21, 24, 30, and 36, and as clinically indicated using a different-from-normal approach and an MRD positivity cutoff of ≥0.1%, which has been shown to have prognostic relevance.[35-38] MRD-related end points included

patients with MRD evaluations at baseline and at ≥1 post-baseline visit. The rate of conversion from MRD-positivity at baseline to an MRD⁻ state during treatment (ie, MRD response) was assessed in subgroups defined by $NPM1^{mut}$ status at diagnosis. An MRD response required conversion to MRD⁻ status at 2 or more consecutive MRD assessments. For all patients with $NPM1^{mut}$, MRD⁻ duration during treatment was computed as the total duration of MRD⁻ status excluding intervals of MRD⁺ status.

Statistical methods

Baseline characteristics were summarized in patient subgroups defined by $NPM1$ and $FLT3$ mutational status using descriptive statistics and compared using χ^2 and Fisher exact tests as appropriate. OS and RFS were estimated using Kaplan–Meier methods and were compared within and between treatment arms in mutation-based subgroups with hazard ratios (HR) and 95% confidence intervals (CI) from exploratory Cox proportional hazards regression models and nominal P values from log-rank tests. These analyses were not powered prospectively to detect statistically significant differences in OS or RFS between treatment arms for patient subgroups defined by mutational status. Except when stated otherwise, the $FLT3^{mut}$ subgroup includes all patients with an $FLT3$-ITD and/or $FLT3$-TKD mutation.

Multivariate Cox regression analyses were conducted to evaluate treatment effects of Oral-AZA vs placebo on OS and RFS while adjusting for the influence of multiple prognostic variables simultaneously. The covariates in the model were $NPM1$ mutational status at diagnosis ($NPM1^{mut}$ vs $NPM1$ wild-type [$NPM1^{wt}$]), $FLT3$ mutational status at diagnosis ($FLT3^{mut}$ vs $FLT3^{wt}$), cytogenetic risk at diagnosis (intermediate vs poor), post-IC MRD status at baseline (MRD⁺ vs MRD⁻), and randomized treatment arm (Oral-AZA vs placebo).

Statistical analyses were performed using R statistical software[39] version 4.0.3 and the survival package version 3.2-7 (R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism software version 8.0 (GraphPad Software, San Diego, CA).

Results

Patients

In all, 472 patients were enrolled in QUAZAR AML-001 and 469 patients (99.4%) had mutational data available at AML diagnosis recorded on electronic case report forms (supplemental Figure 1, available on the *Blood* website). $NPM1^{mut}$ and $FLT3^{mut}$ were found in 137 patients (29.2%) and 66 patients (14.1%), respectively (Table 1).

NPM1

Compared with $NPM1^{wt}$ (n = 332), patients with $NPM1^{mut}$ (n = 137) were significantly more likely to be female (P = .033), to have intermediate-risk cytogenetic findings (P < .001) and co-occurring $FLT3^{mut}$ (P < .001) at diagnosis, to have received consolidation chemotherapy after induction (P = .011), and to be without MRD after IC (P = .014) (supplemental Table 1). Baseline characteristics for patients with $NPM1^{mut}$ at AML diagnosis were comparable between the Oral-AZA and placebo arms (supplemental Table 2).

Table 1. *NPM1* and *FLT3* co-mutational statuses at AML diagnosis

|	Biomarker cohort* (N = 469)		
	Oral-AZA (n = 236)	Placebo (n = 233)	All patients (N = 469)
Gene mutation, n (%)			
$NPM1^{mut}$	66 (28.0)	71 (30.5)	137 (29.2)
$FLT3^{mut}$	30 (12.7)	36 (15.5)	66 (14.1)
$FLT3$-ITD⁺	21 (8.9)	25 (10.7)	46 (9.8)
$FLT3$-TKD^{mut}	11 (4.7)	13 (5.6)	24 (5.1)
$FLT3$-ITD⁺ and $FLT3$-TKD^{mut}	2 (0.8)	2 (0.9)	4 (0.9)
$NPM1$/$FLT3$-ITD co-mutation status, n (%)			
$NPM1^{mut}$ + $FLT3$-ITD⁺	12 (5.1)	18 (7.7)	30 (6.4)
$NPM1^{mut}$ + $FLT3$-ITD⁻	54 (22.9)	53 (22.7)	107 (22.8)
$NPM1^{wt}$ + $FLT3$-ITD⁺	9 (3.8)	7 (3.0)	16 (3.4)
$NPM1^{wt}$ + $FLT3$-ITD⁻	161 (68.2)	155 (66.5)	316 (67.4)
$NPM1$/$FLT3$-TKD co-mutation status, n (%)			
$NPM1^{mut}$ + $FLT3$-TKD^{mut}	9 (3.8)	8 (3.4)	17 (3.6)
$NPM1^{mut}$ + $FLT3$-TKD^{wt}	57 (24.2)	63 (27.0)	120 (25.6)
$NPM1^{wt}$ + $FLT3$-TKD^{mut}	2 (0.8)	5 (2.1)	7 (1.5)
$NPM1^{wt}$ + $FLT3$-TKD^{wt}	168 (71.2)	157 (67.4)	325 (69.3)

*Patients with available mutation data at diagnosis on electronic case report forms.

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NPM1 mutational status at AML diagnosis was highly prognostic of survival within each treatment arm. In the placebo arm, OS was improved significantly for patients with *NPM1*^mut (n = 71) vs *NPM1*^wt (n = 162; median, 15.9 months vs 14.6 months, respectively) (Figure 1), correlating with a 31% reduced risk of death (OS: HR, 0.69; 95% CI, 0.49-0.97; *P* = .032) (supplemental Table 3). Within the Oral-AZA arm, patients with *NPM1*^mut (n = 66) had longer median OS than patients with *NPM1*^wt (n = 170; 47.2 months vs 19.6 months, respectively) and a 48% reduced risk of death (OS: HR, 0.52; 95% CI, 0.36-0.75; *P* < .001). Similarly, patients with *NPM1*^mut at diagnosis had significantly longer median RFS compared with patients with *NPM1*^wt in both the placebo (6.9 months vs 4.6 months) and Oral-AZA (23.2 months vs 7.7 months) arms (Figure 1), reflecting a 35% RFS improvement within the placebo arm (HR, 0.65; 95% CI, 0.47-0.91; *P* = .011) and a 54% improvement in RFS within the Oral-AZA arm (HR, 0.46; 95% CI, 0.31-0.66; *P* < .001) (supplemental Table 3).

NPM1 mutational status also was predictive of a survival benefit with Oral-AZA compared with placebo. In the *NPM1*^mut subgroup, median OS was substantially longer in the Oral-AZA arm

Figure 1. OS and RFS from randomization by *NPM1* mutational status at AML diagnosis and randomized treatment arm. (A) OS. (B) RFS.
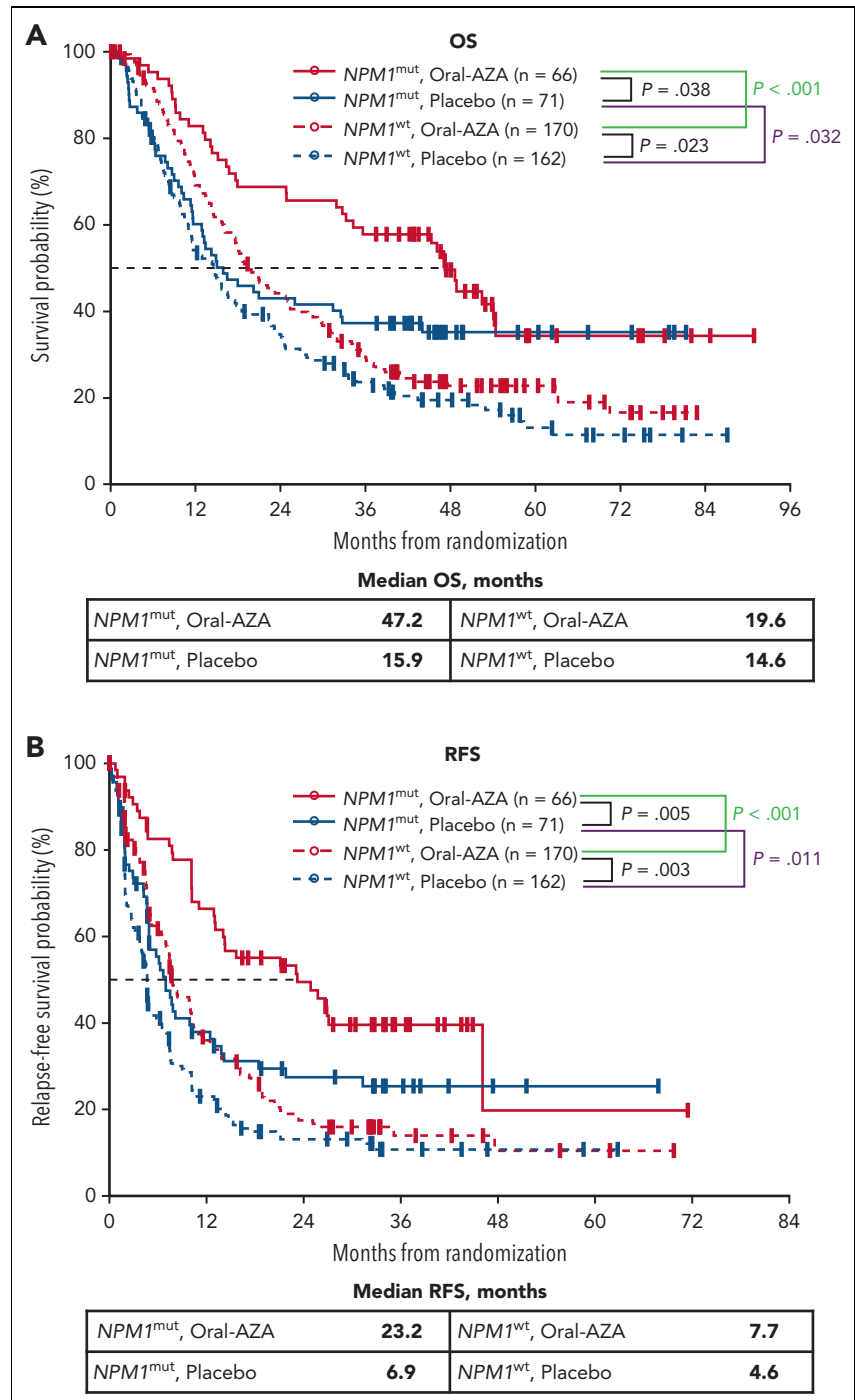
than in the placebo arm: 47.2 months vs 15.9 months, respectively (HR, 0.63; 95% CI, 0.41-0.98; P = .038) (Figure 1; supplemental Table 4). Median RFS in this subgroup was 23.2 months with Oral-AZA vs 6.9 months with placebo (HR, 0.55; 95% CI, 0.35-0.84; P = .005). Among patients with $NPM1^{wt}$, Oral-AZA also was associated with significant improvements vs placebo in both OS (median, 19.6 months vs 14.6 months, respectively; P = .023) and RFS (median, 7.7 months vs 4.6 months; P = .003) (Figure 1).

FLT3

Of the 66 patients with $FLT3^{mut}$ at diagnosis, 46 patients (9.8% of all patients) had $FLT3$-ITD, 24 patients (5.1%) had $FLT3$-TKD^{mut}, and 4 patients (0.9%) had both $FLT3$-ITD and $FLT3$-TKD^{mut} (Table 1).

Patients with $FLT3^{mut}$ at diagnosis were significantly younger than those with $FLT3^{wt}$ and significantly more likely to have intermediate-risk cytogenetic findings, a co-occurring $NPM1^{mut}$, and higher Eastern Cooperative Oncology Group performance status score (supplemental Table 5). $FLT3$ mutational status was not correlated with post-IC MRD status at baseline. Baseline characteristics for patients with $FLT3^{mut}$ at diagnosis generally were balanced between the Oral-AZA and placebo arms (supplemental Table 6).

Presence of $FLT3^{mut}$ seemed to confer a negative, but nonsignificant, prognostic effect for patients who received placebo: median OS for patients with $FLT3^{mut}$ (n = 36) and $FLT3^{wt}$ (n = 197) in this arm was 9.7 months vs 15.2 months, respectively (Figure 2), and risk of death was increased by 25% for patients with $FLT3^{mut}$ vs $FLT3^{wt}$ status (HR, 1.25; 95% CI, 0.83-1.89; P = .280) (supplemental Table 3). In contrast to the placebo arm, median OS within the Oral-AZA arm was similar in the $FLT3^{mut}$ subgroup (n = 30) and the $FLT3^{wt}$ subgroup (n = 206), with median OS of 28.2 and 24.7 months, respectively (HR, 0.96; 95% CI, 0.60-1.54; P = .871).

Maintenance therapy with Oral-AZA was associated with nominally prolonged median OS vs placebo in patients with $FLT3^{mut}$ at diagnosis (28.2 months vs 9.7 months, respectively; P = .114) and significantly improved median OS for patients with $FLT3^{wt}$ (24.7 months vs 15.2 months; P = .013) (Figure 2; supplemental Table 4). RFS also was improved substantially with Oral-AZA vs placebo in the $FLT3^{mut}$ subgroup, with median RFS of 23.1 months vs 4.6 months in the Oral-AZA and placebo arms, respectively (HR, 0.51; 95% CI, 0.27-0.95; P = .032) (supplemental Table 4). Median RFS with Oral-AZA vs placebo in the $FLT3^{wt}$ subgroup was 10.2 months vs 4.9 months, respectively (P = .001) (Figure 2).

NPM1 and FLT3-ITD co-mutations

At diagnosis, 30 patients (6.4%) had co-occurring $NPM1^{mut}$ and $FLT3$-ITD, 107 patients (22.8%) had $NPM1^{mut}$ but no $FLT3$-ITD, and 16 patients (3.4%) had $NPM1^{wt}$ and $FLT3$-ITD (Table 1); the small number of patients in the latter subgroup precluded meaningful comparison of survival outcomes between the Oral-AZA (n = 9) and placebo (n = 7) arms.

Co-occurrence of $NPM1^{mut}$ and $FLT3$-ITD at diagnosis showed a generally negative prognostic trend, as indicated by

differences in median OS within the placebo arm: 18.0 months for patients with $NPM1^{mut}$ and no $FLT3$-ITD vs 11.5 months in patients with both mutations (Figure 3). In contrast, within the Oral-AZA arm, the presence or absence of co-occurring $FLT3$-ITD in patients with $NPM1^{mut}$ did not impact survival meaningfully: median OS was 46.1 months for patients with co-occurring $FLT3$-ITD and 48.6 months for those with no $FLT3$-ITD. Oral-AZA nominally prolonged OS vs placebo in patients with $NPM1^{mut}$ with or without co-occurring $FLT3$-ITD (Figure 3).

NPM1/FLT3 mutations and post-IC MRD status

Overall, the MRD-evaluable cohort included 463 patients (98.1%; Oral-AZA, n = 236; placebo, n = 227). The rate of post-IC MRD negativity at screening was 61.7% (82/133 patients) for patients with $NPM1^{mut}$ at diagnosis, vs 48.9% (160/327 patients) for patients with $NPM1^{wt}$ (P = .014).

In patients with $NPM1^{mut}$, MRD status after IC was prognostic of survival in the placebo arm, with median OS of 31.4 months for patients without MRD at screening vs 10.0 months for those with MRD (P = .024). In contrast, median OS was not influenced meaningfully by MRD status at screening in patients with $NPM1^{mut}$ receiving Oral-AZA (median, 48.6 months vs 46.1 months for patients without MRD and with MRD, respectively; P = .366) (Figure 4). Median RFS for patients with $NPM1^{mut}$ at diagnosis who were MRD$^-$ or MRD$^+$ at screening within the placebo arm was 9.9 and 4.9 months, respectively (P = .079), and within the Oral-AZA arm was 25.7 and 15.6 months, respectively (P = .324) (Figure 4).

Similar to findings in the overall study population,[31] median OS was improved numerically with Oral-AZA vs placebo in patients with $NPM1^{mut}$ AML, regardless of MRD status at study entry. Median OS for patients without MRD but with $NPM1^{mut}$ in the Oral-AZA and placebo arms was 48.6 months vs 31.4 months, respectively (P = .182), and was 46.1 months vs 10.0 months (P = .033) for patients with MRD at study entry (Figure 4). Thus, patients with MRD at baseline who received Oral-AZA had nominally longer median OS than patients without MRD who received placebo. Oral-AZA also prolonged RFS relative to placebo in the $NPM1^{mut}$ subgroup regardless of MRD status at baseline (Figure 4).

Among patients with $NPM1^{mut}$ at diagnosis, maintenance therapy with Oral-AZA after remission was associated with a higher rate of MRD response (conversion from MRD at baseline to no MRD on study) compared with placebo (63% [17/27] vs 33% [8/24], respectively; P = .051). For all patients with $NPM1^{mut}$ at diagnosis, duration of no MRD was significantly longer in the Oral-AZA arm vs the placebo arm (15.6 months vs 7.1 months; P = .006).

Median OS was similar within the Oral-AZA arm in patients with $FLT3^{mut}$ at diagnosis regardless of whether patients were without MRD after IC at baseline (n = 14; 28.2 months) or with MRD after IC at baseline (n = 16; 24.0 months); whereas within the placebo arm, median OS for patients with $FLT3^{mut}$ who were without MRD at baseline (n = 18; 16.2 months) was twice that of patients who were with MRD at baseline (n = 17; 8.0 months) (Figure 5). The proportion of patients with $FLT3^{mut}$ who converted from having MRD at baseline to being without

Figure 2. OS and RFS from randomization by *FLT3* mutational status at AML diagnosis and randomized treatment arm. (A) OS. (B) RFS. *FLT3*^mut includes both *FLT3*-ITD and *FLT3*-TKD mutations.
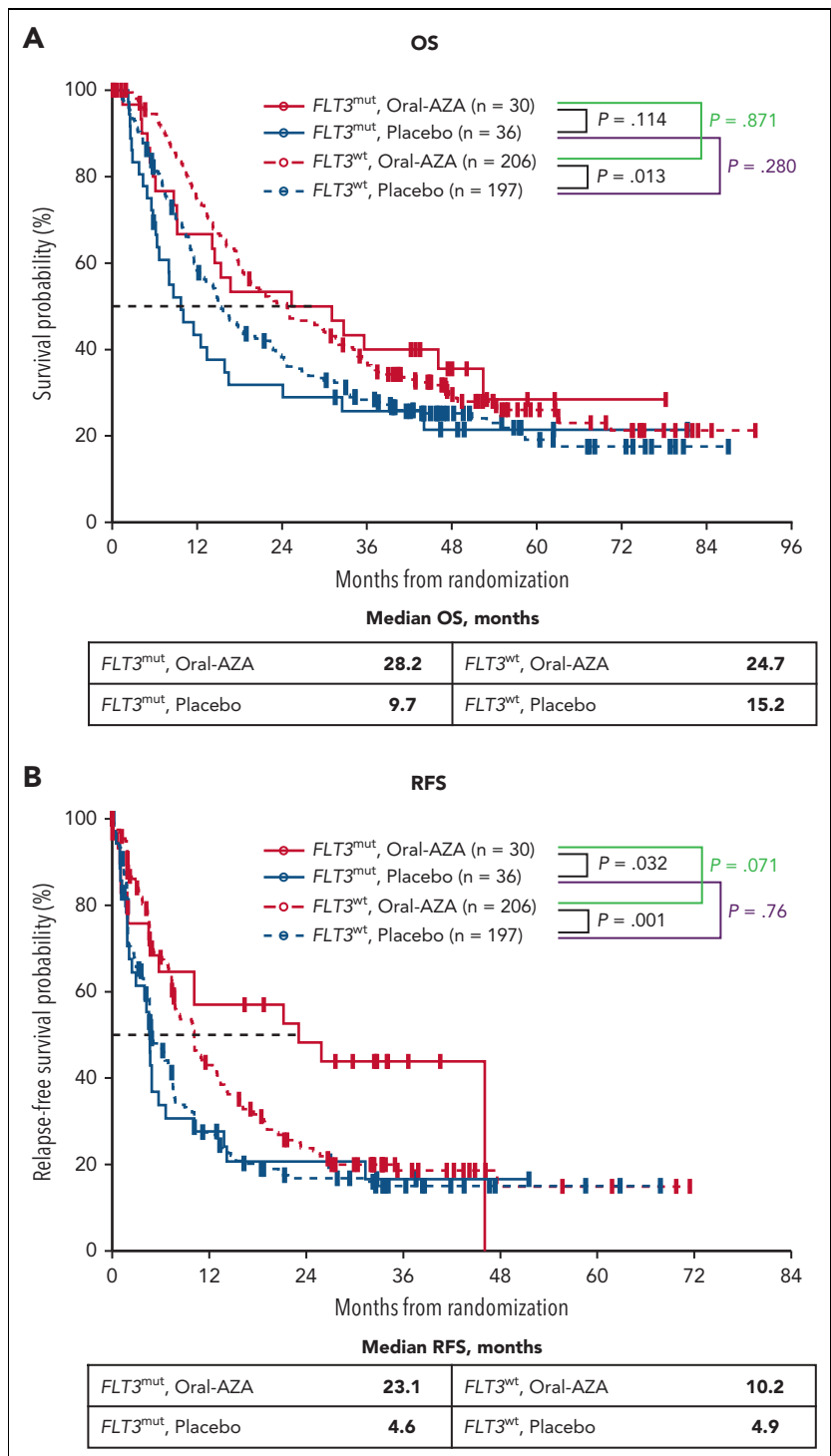
MRD during treatment (MRD responders) was 50% (8/16 patients) in the Oral-AZA arm, compared with 18% (3/17 patients) in the placebo arm.

In treatment comparisons, median OS in patients with *FLT3*^mut at diagnosis and without MRD at baseline was 28.2 months vs 16.2 months (*P* = .495) in the Oral-AZA and placebo arms, respectively, and in patients with *FLT3*^mut and MRD at baseline was 24.0 months vs 8.0 months, respectively (*P* = .158) (Figure 5).

Multivariate analysis

In multivariate analyses, treatment with Oral-AZA (vs placebo) was an independent prognostic factor of improved OS (*P* = .004) and RFS (*P* < .001) after controlling for *NPM1* mutational status, *FLT3* mutational status, and cytogenetic risk at AML diagnosis and for post-IC MRD status at baseline (Table 2). Each other covariate in the model also was independently predictive of OS: *NPM1* (*P* = .001), *FLT3* (*P* = .036), cytogenetic risk (*P* < .001), and MRD status (*P* < .001). All covariates in the

A

OS, *NPM1*^{mut} + *FLT3*-ITD⁻ (n = 107)

Legend:
- *NPM1*^{mut} + *FLT3*-ITD⁻, Oral-AZA (n = 54)
- *NPM1*^{mut} + *FLT3*-ITD⁻, Placebo (n = 53)
- Other, Oral-AZA (n = 182)
- Other, Placebo (n = 180)

Y-axis: Survival probability (%)
X-axis: Months from randomization

Median OS, months

NPM1^{mut} and *FLT3*-ITD⁻, Oral-AZA	**48.6**	Other, Oral-AZA	**20.2**
NPM1^{mut} and *FLT3*-ITD⁻, Placebo	**18.0**	Other, Placebo	**14.6**

B

OS, *NPM1*^{mut} + *FLT3*-ITD⁺ (n = 30)

Legend:
- *NPM1*^{mut} + *FLT3*-ITD⁺, Oral-AZA (n = 12)
- *NPM1*^{mut} + *FLT3*-ITD⁺, Placebo (n = 18)
- Other, Oral-AZA (n = 224)
- Other, Placebo (n = 215)

Y-axis: Survival probability (%)
X-axis: Months from randomization

Median OS, months

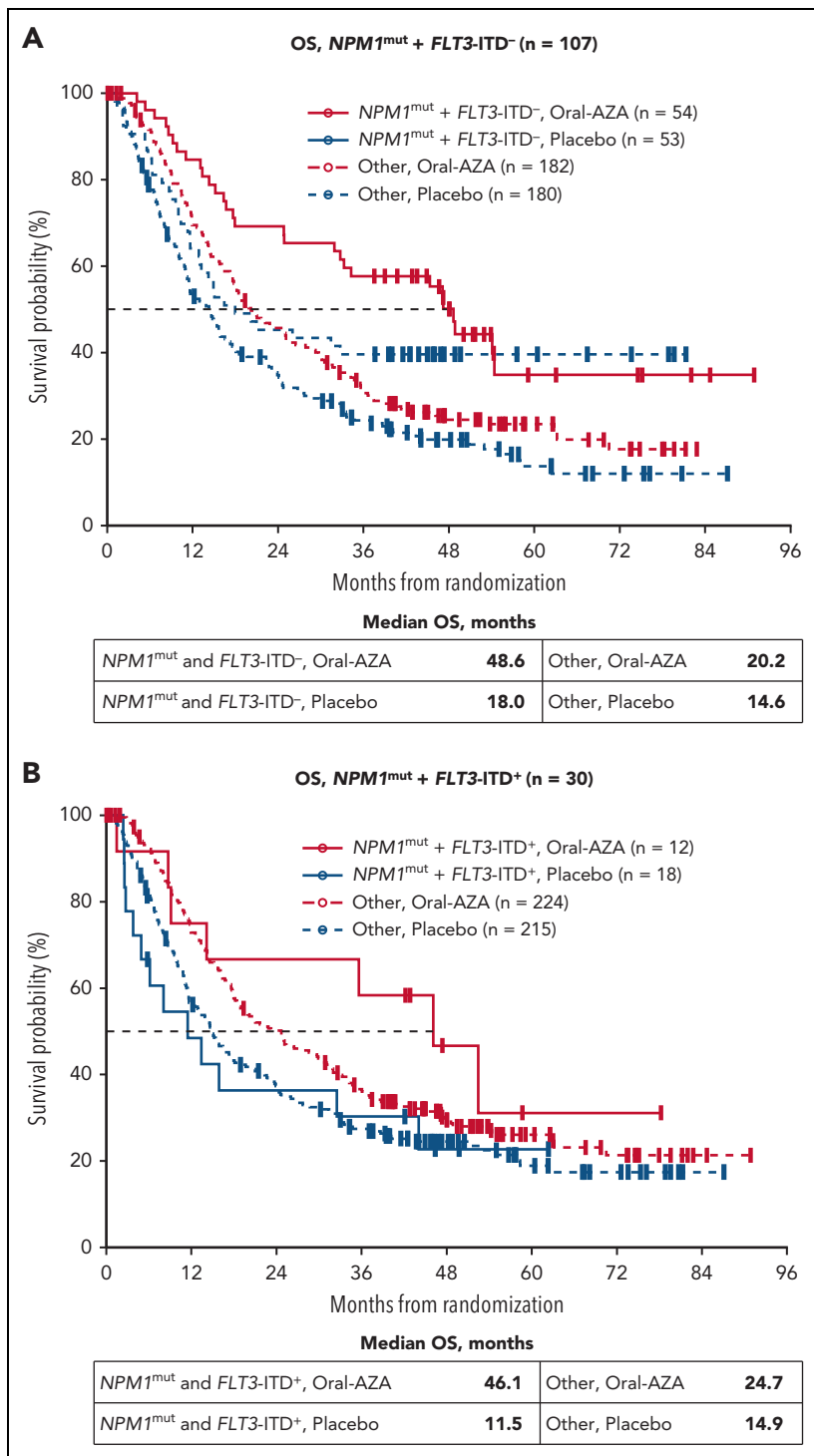|			
NPM1^{mut} and *FLT3*-ITD⁺, Oral-AZA	**46.1**	Other, Oral-AZA	**24.7**
NPM1^{mut} and *FLT3*-ITD⁺, Placebo	**11.5**	Other, Placebo	**14.9**

Figure 3. OS from randomization in patients with *NPM1*^{mut}, with or without co-occurring *FLT3*-ITD, at AML diagnosis. (A) *NPM1*^{mut} without co-occurring *FLT3*-ITD. (B) *NPM1*^{mut} with co-occurring *FLT3*-ITD. "Other" includes study patients without the specific genetic status at diagnosis.
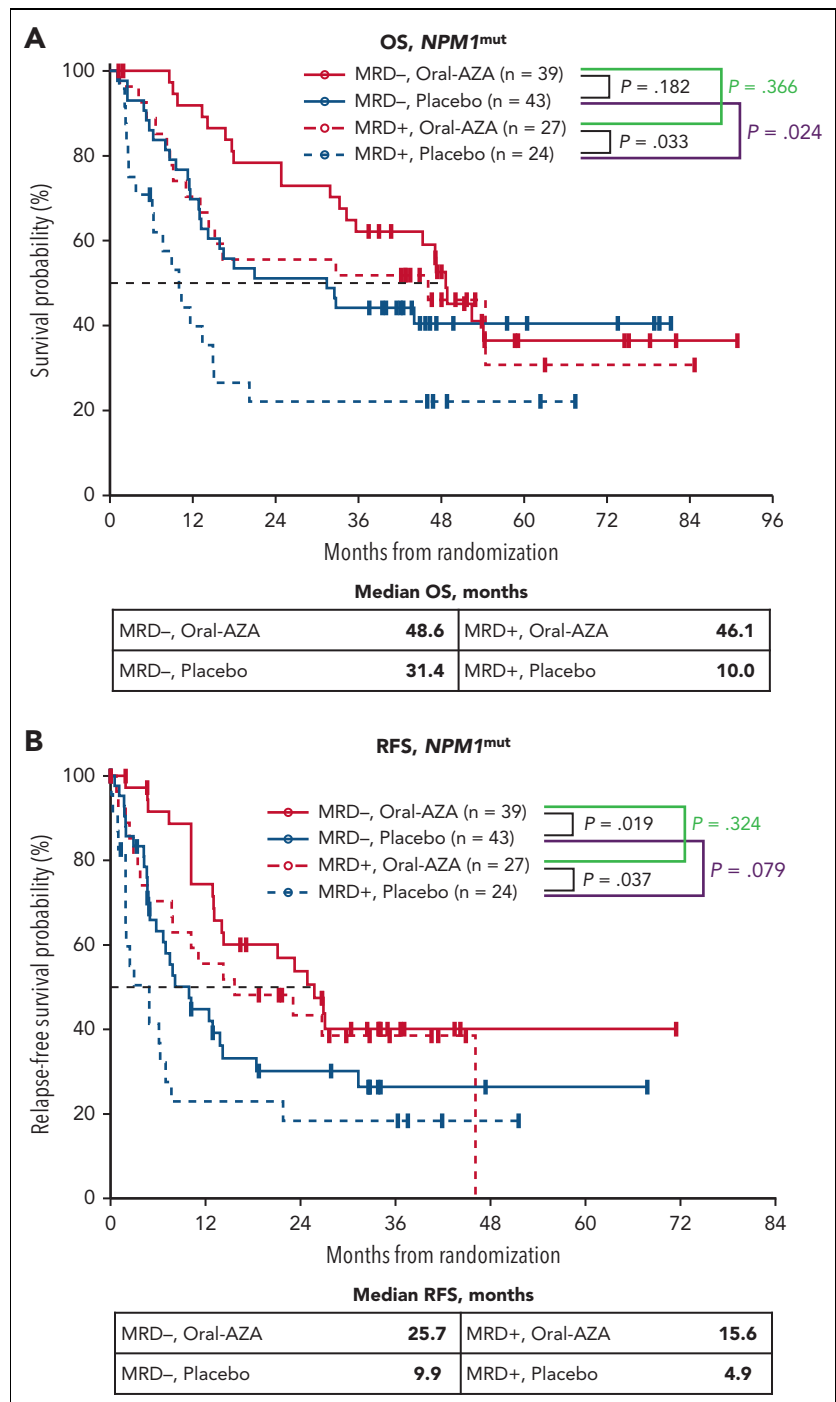
model, except *FLT3* mutational status at diagnosis (P = .851), were independently predictive of RFS.

Discussion

Of the several aberrant genes detected in patients with AML, *NPM1*^{mut} and *FLT3*^{mut} are among the most frequent and among the few molecular genetic abnormalities with established (albeit context-dependent) prognostic implications in AML.[1,15,40,41] The

proportion of patients with *NPM1* mutations at diagnosis in this study (29.2%) was approximately equal to historical rates in other AML populations,[1,3] which would be expected, because these patients generally tend to respond well to IC.[1,42] Consistent with findings in other populations of older patients with newly diagnosed AML,[1,42,43] the presence of *NPM1*^{mut} at diagnosis in this cohort of patients was associated with longer remission duration and prolonged survival compared with those with *NPM1*^{wt} within each treatment arm. Notably, however, treatment with

Figure 4. OS and RFS from randomization for patients with *NPM1*^mut at AML diagnosis by MRD status at baseline (after chemotherapy) and randomized treatment arm. (A) OS. (B) RFS. MRD was determined centrally at study entry by multiparameter flow cytometry using a different-from-normal method with a 0.1% positivity threshold.

A — OS, *NPM1*^mut

Legend:
- MRD–, Oral-AZA (n = 39)
- MRD–, Placebo (n = 43)
- MRD+, Oral-AZA (n = 27)
- MRD+, Placebo (n = 24)

P = .182
P = .366
P = .033
P = .024

Y-axis: Survival probability (%)
X-axis: Months from randomization

Median OS, months

MRD–, Oral-AZA	**48.6**	MRD+, Oral-AZA	**46.1**
MRD–, Placebo	**31.4**	MRD+, Placebo	**10.0**

B — RFS, *NPM1*^mut

Legend:
- MRD–, Oral-AZA (n = 39)
- MRD–, Placebo (n = 43)
- MRD+, Oral-AZA (n = 27)
- MRD+, Placebo (n = 24)

P = .019
P = .324
P = .037
P = .079

Y-axis: Relapse-free survival probability (%)
X-axis: Months from randomization

Median RFS, months

MRD–, Oral-AZA	**25.7**	MRD+, Oral-AZA	**15.6**
MRD–, Placebo	**9.9**	MRD+, Placebo	**4.9**

Oral-AZA maintenance therapy was associated with further improvements in both OS and RFS vs placebo in patients with *NPM1*^mut, and even patients treated with Oral-AZA with *NPM1*^wt at diagnosis showed longer median OS and median RFS than patients with *NPM1*^mut who received placebo.

Up to 90% of all patients with AML in remission with detectable MRD will experience morphologic relapse in the absence of post-remission therapy, typically within 6 to 12 months.[4] In the subgroup of patients with *NPM1*^mut who had MRD post-IC at baseline in this study, approximately twice as many patients in

the Oral-AZA arm achieved an MRD response (converted to MRD^– state) compared with the placebo arm, and duration of MRD negativity overall in the Oral-AZA arm was more than twice as long. Among patients with *NPM1*^mut at diagnosis who had no MRD at screening, Oral-AZA prolonged median RFS by approximately 15 months compared with placebo, suggesting that even patients with particularly favorable prognostic disease features can experience extended remission with Oral-AZA maintenance therapy. Multivariate analysis confirmed the significant independent prognostic benefit of Oral-AZA maintenance therapy vs placebo on OS and RFS,
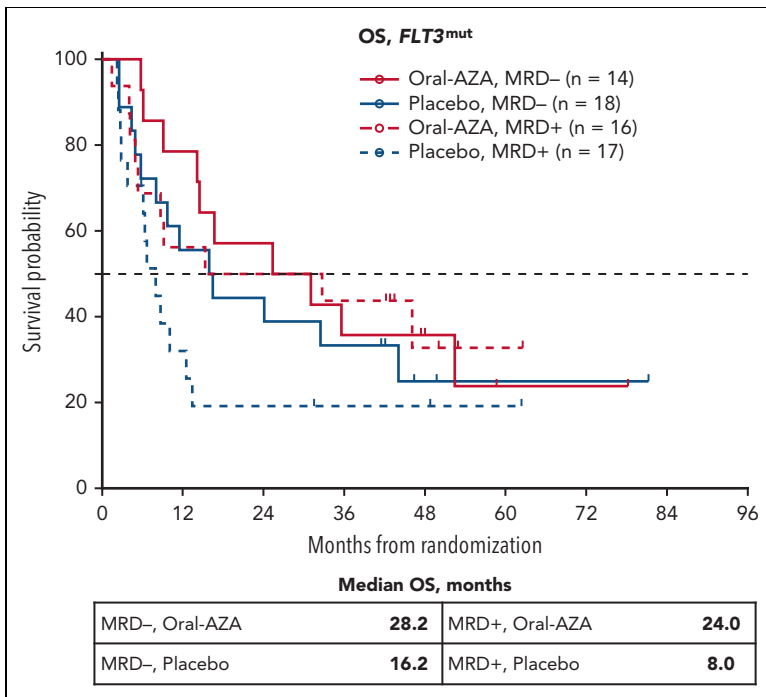
OS, *FLT3*^{mut}

— Oral-AZA, MRD– (n = 14)
— Placebo, MRD– (n = 18)
-o- Oral-AZA, MRD+ (n = 16)
-o- Placebo, MRD+ (n = 17)

Median OS, months

MRD–, Oral-AZA	**28.2**	MRD+, Oral-AZA	**24.0**
MRD–, Placebo	**16.2**	MRD+, Placebo	**8.0**

Figure 5. OS from randomization for patients with *FLT3*^{mut} at AML diagnosis by MRD status at baseline (after chemotherapy) and randomized treatment arm. MRD was determined at study entry by multiparameter flow cytometry using a different-from-normal method with a 0.1% positivity threshold. *FLT3*^{mut} includes both *FLT3*-ITD and *FLT3*-TKD mutations.

regardless of *NPM1* mutational status at diagnosis and post-IC MRD status.

Fourteen percent of patients in this study carried *FLT3*^{mut} at diagnosis, proportionately less than what has been reported historically,[1,3] which may reflect the observed lower incidence of these mutations in older patients or low rates of CR or CRi achieved with IC in patients with these mutations.[44] Consistent with other studies,[1,23,41] in this study, the presence of a *FLT3*^{mut} at diagnosis in the placebo arm was associated with a negative prognosis, whereas median OS for patients with *FLT3*^{mut} within the Oral-AZA arm was comparable with that of patients with

FLT3^{wt}. Similar to the findings in the *NPM1* analyses, patients with *FLT3*^{mut} at diagnosis treated with Oral-AZA showed better OS and RFS than patients with *FLT3*^{wt} in the placebo arm, and multivariate analysis demonstrated the significant prognostic benefit of Oral-AZA treatment vs placebo regardless of *FLT3* mutational status.

OS and RFS outcomes for patients with *FLT3*^{mut} at diagnosis in these analyses may be confounded because this subgroup included patients with *FLT3*-ITD and/or *FLT3*-TKD mutations, which have differing prognostic implications.[13,18,19,45] The number of patients with *FLT3*-TKD mutations in these analyses

Table 2. Multivariate analysis of effects of select prognostic variables and randomized treatment on overall survival and relapse-free survival on study

Survival	HR	95% CI	*P* value
Overall			
Randomized treatment: Oral-AZA vs placebo	0.732	0.5898-0.9074	.004
NPM1 mutation status at diagnosis: *NPM1*^{mut} vs *NPM1*^{wt}	0.624	0.469-0.829	.001
FLT3 mutation status at diagnosis: *FLT3*^{mut} (ITD/TKD) vs *FLT3*^{wt}	1.444	1.023-2.039	.036
Cytogenetic risk at diagnosis: poor vs intermediate	1.853	1.376-2.495	<.001
MRD status at screening: MRD⁺ vs MRD[–]	1.716	1.380-2.133	<.001
Relapse-free			
Randomized treatment: Oral-AZA vs placebo	0.631	0.509-0.782	<.001
NPM1 mutation status at diagnosis: *NPM1*^{mut} vs *NPM1*^{wt}	0.614	0.467-0.808	<.001
FLT3 mutation status at diagnosis: *FLT3*^{mut} (ITD/TKD) vs *FLT3*^{wt}	1.034	0.727-1.472	.851
Cytogenetic risk at diagnosis: poor vs intermediate	1.753	1.294-2.375	<.001
MRD status at screening: MRD⁺ vs MRD[–]	1.984	1.596-2.466	<.001

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was too small to make meaningful comparisons within or between treatment arms. For patients with NPM1^{mut}, the median OS in the Oral-AZA arm was approximately 4 years, regardless of whether patients had a co-occurring FLT3-ITD mutation, and more than 2 years longer than the median OS in patients without co-occurring FLT3-ITD in the placebo arm. However, these subgroups were small and further investigation of Oral-AZA effects in patients with co-occurring NPM1^{mut} and FLT3-ITD is required.

The mechanisms by which Oral-AZA therapy augments clinical benefits in patients with NPM1^{mut} and may attenuate the negative prognostic effect of FLT3^{mut} are not clear. In animal models and leukemic cells from patients with AML, FLT3-ITD mutations can collaborate with mutations in epigenetic regulating genes (eg, TET2, IDH1/2), leading to DNA hypermethylation, altered gene expression, and impaired cellular differentiation.[46] It is possible that DNMT1 inhibition and hypomethylating activity with Oral-AZA[47,48] ameliorates DNA hypermethylation and restores gene expression and downstream gene signaling pathways in leukemic cells of patients with AML with NPM1^{mut} or FLT3-ITD, but this remains to be determined.

These analyses have some limitations, most notably that mutational assessments were conducted locally at the time of patient diagnosis, before AML treatment with IC and before entry into the QUAZAR AML-001 trial. Although mutational data were collected for almost all patients on the screening electronic case report form for this study, individual mutation calls may have been influenced by a variety of testing approaches with differing gene panels, assay sensitivities, and methodologies performed by institutions worldwide. Further, over the duration of patient enrollment in this study, methodologic advancements in genetic testing and understanding of the genetic landscape of AML were evolving rapidly. Before initiation of this study, the World Health Organization AML diagnostic recommendations included testing for NPM1^{mut} and FLT3^{mut} (at least in patients with normal cytogenetic findings), so it is likely that the mutational subgroups evaluated here included most patients with these mutations. Diagnostic mutational data were binary; that is, the mutation was present or not, and the variant allele frequencies of individual mutations were unknown, limiting interpretation of outcomes in the FLT3-ITD subgroup (or 2017 European LeukemiaNet risk segments) because of the prognostic importance of the FLT3-ITD allelic ratio. Moreover, FLT3-ITD mutations may have been cleared by the time of study entry, after IC. Thus, we cannot be certain about whether or to what extent the positive prognostic effect of co-occurring NPM1^{mut} may have influenced survival outcomes for patients with FLT3-ITD. Finally, patients in this study obtained remission from treatment with IC; it is unknown how Oral-AZA maintenance therapy might influence outcomes of patients who obtain remission by other means, such as treatment with CPX-351 or a venetoclax-based regimen. Despite these limitations, the double-blind, randomized design of the QUAZAR AML-001 trial allows for cross-treatment comparisons of Oral-AZA vs placebo because these shortcomings should apply broadly to both treatment arms.

Patients' post-IC mutational status at study screening and during treatment in QUAZAR AML-001 are of high interest, and these data currently are under investigation. Because patients entered the trial in morphologic remission with <5% bone marrow blasts, quantitating post-IC mutations with widely different detection thresholds in a small number of leukemic blasts at baseline has been a challenge. Deep sequencing methods are being refined to track gene mutations during the course of Oral-AZA treatment, particularly the types of mutations occurring at relapse.

These data suggest that Oral-AZA maintenance therapy can benefit substantially patients with AML in remission who are not candidates for hematopoietic stem cell transplantation and who have poor prognostic disease features (FLT3^{mut} at diagnosis, MRD after IC) and further can improve survival outcomes for patients with more favorable prognostic disease characteristics (NPM1^{mut} at diagnosis, no MRD after IC) compared with placebo.

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Authorship

Contribution: The sponsors collected and analyzed data in conjunction with all authors. H. Döhner and D.L.d.M. wrote the first draft of the manuscript; the data were reviewed and verified by H. Döhner, B.S., W.L.S., M.U., A.R., E.T.C., A.T., C.L.B., and D.L.d.M.; and all authors revised the manuscript and reviewed and approved the final version.

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