Impact of *FLT3* Mutation on Outcomes after Venetoclax and Azacitidine for Patients with Treatment-Naïve Acute Myeloid Leukemia

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ABSTRACT

Purpose: To evaluate efficacy and safety of venetoclax + azacitidine among treatment-naïve patients with *FLT3*-mutant acute myeloid leukemia.

Patients and Methods: Data were pooled from patients enrolled in a phase III study (NCT02993523) that compared patients treated with venetoclax + azacitidine or placebo + azacitidine and a prior phase Ib study (NCT02203773) where patients were treated with venetoclax + azacitidine. Enrolled patients were ineligible for intensive therapy due to age ≥75 years and/or comorbidities. Patients on venetoclax + azacitidine received venetoclax 400 mg orally (days 1–28) and azacitidine (75 mg/m^2; days 1–7/28-day cycle). *FLT3* mutation was analyzed centrally on pretreatment bone marrow aspirates.

Results: In the biomarker evaluable population, *FLT3* mutation was detected in 42 (15%) and 22 (19%) patients in the venetoclax + azacitidine and azacitidine groups. Composite complete remission [CRc; complete remission (CR) + CR with incomplete hematologic recovery (CRi)] rates (venetoclax + azacitidine/azacitidine) for *FLT3*-mutant patients were 67%/36%, median duration of remission (DoR) was 17.3/5.0 months, and median OS was 12.5/8.6 months. The CRc rates among *FLT3* wild-type patients were 67%/25%, median DoR 18.4/13.4 months, and median OS 14.7/10.1 months. In patients treated with venetoclax + azacitidine, CRc in patients with *FLT3-ITD* and *FLT3-TKD* was 63% and 77% and median OS was 9.9 and 19.2 months, and in comutated *FLT3-ITD* + *NPM1* patients, CRc was 70%, median DoR was not reached, and median OS was 9.1 months. There were no unexpected toxicities in the venetoclax + azacitidine group.

Conclusions: When treated with venetoclax + azacitidine, patients with *FLT3* mutations and *FLT3* wild-type had similar outcomes. Future analyses in larger patient populations may further define the impact of venetoclax + azacitidine in patients harboring *FLT3-ITD*.

See related commentary by Perl and Vyas, p. 2719

Introduction

Acute myeloid leukemia (AML) is a highly heterogeneous disease with genomic abnormalities, including *NPM1, TP53, FLT3*, and *IDH1/2*, that are predictors of treatment outcomes (1). The *fms*-like tyrosine kinase 3 (*FLT3*) gene is mutated in approximately 20% of patients with acute myeloid leukemia (AML) ≥70 years (2). Both *FLT3-internal tandem duplication (ITD)* and *FLT3-tyrosine kinase domain* (*TKD)* mutations are associated with AML proliferation and potentially targetable with small molecule inhibitors (3, 4). The presence of *FLT3-ITD* mutation correlates with a high leukemic burden with increased risk of relapse and is recognized to be a driver mutation in patients with AML (5). In particular, high (>0.5) mutant-to-wild-type (WT) allelic ratios (AR) in the *FLT3-ITD* gene are associated with inferior prognosis (6, 7). In patients with a normal cytogenetic profile, AML with *NPM1* mutation has a favorable prognosis, but in coexistence with *FLT3-ITD*, the risk level of AML depends on the AR of *FLT3-ITD* (8). NPM1 mutation with low AR of *FLT3-ITD* is considered as a favorable-risk group, but when combined with high AR, it is classified as an intermediate-risk group (9). Despite a low or high AR of *FLT3-ITD*, patients with a comutation of *FLT3-ITD* and *NPM1* belong to the intermediate-risk category according to the recent National Comprehensive Cancer Network (NCCN) guidelines (10). Overall, there is limited evidence on the prognostic implications of *FLT3* and *NPM1* mutations among older patients.

For treatment-naïve AML patients with *FLT3* mutation, the current standard treatment entails induction with intensive chemotherapy in physically fit or younger patients and in combination with the *FLT3* inhibitor, midostaurin (11). Other *FLT3*-inhibitors have induced modest single-agent responses with short-lived remissions among patients with relapsed/refractory disease, while combination trials with intensive chemotherapy in first-line fit patients are ongoing (12–14). Currently, there is no approved targeted therapy option for treatment-naïve patients with AML harboring a *FLT3* mutation ineligible for intensive therapy. Preliminary results from a phase III randomized trial (NCT02752035) failed to show a survival benefit with the addition of gilteritinib to

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

The *fms*-like tyrosine kinase 3 (*FLT3)* gene is mutated in approximately 20% of patients with acute myeloid leukemia (AML) ≥70 years and correlates with high leukemic burden and increased risk of relapse. The results of the phase III VIALE-A trial showed that patients treated with venetoclax and azacitidine had higher remission rates and more prolonged overall survival (OS) as compared with patients treated with azacitidine alone. Herein, we further evaluated the efficacy and safety of the combination and reported that the remission rates and OS among patients with or without *FLT3* mutation were similar, suggesting that venetoclax and azacitidine may be used for treatment-naïve AML patients who are ineligible for intensive chemotherapy regimens irrespective of *FLT3* mutations. Although high remission rates were observed among patients exhibiting both *FLT3-ITD* and *TKD* mutations, the impact on OS warrants confirmation in larger datasets.

azacitidine in patients with *FLT3*-mutant AML ineligible for intensive chemotherapy (15).

Overexpression of BCL-2 is a predictor of poor response to chemotherapy and can lead to therapeutic resistance in AML (16). Mutations in *FLT3* lead to subsequent constitutive activation of *FLT3* kinase and its downstream proliferative signaling pathways, including the Ras/MAPK kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway and PI3K/Akt pathway (17). *FLT3-ITD* is also known to activate the STAT5 pathway (18). STAT5 induces its target genes such as cyclin D1, c-myc, and the antiapoptotic gene p21, which are essential for cell growth (19–21), and also increased BCL-X_L and MCL1 protein expression (22). Azacitidine can induce expression of the BH3-only sensitizing/neutralizing NOXA and PUMA proteins, which inhibit MCL-1 and BCL-X_L, increasing the dependence of the malignant cell on BCL-2. Combining venetoclax with azacitidine has been shown to induce apoptosis in malignant myeloid cells (23) and to be synergistic to overcome antiapoptotic signals downstream of an activated *FLT3* pathway (24, 25). **Figure 1** represents a simplified schematic of the mechanism of venetoclax and azacitidine action in the downstream *FLT3* signaling pathway.

In a prior phase Ib study, treatment with venetoclax and a hypomethylating agent (azacitidine or decitabine) demonstrated a 72% composite complete remission rate [CRc defined by complete remission (CR) plus CR with incomplete count recovery (CRi)] among patients with *FLT3* mutation with a median duration of remission (DoR) of 11.0 [6.5–not evaluable (NE)] months. These results were further validated by the phase III VIALE-A study where patients with *FLT3* mutation, when treated with venetoclax and azacitidine, had significantly higher CRc rates (72.4% vs. 36.4%) and longer median overall survival (OS; 13.6 months vs. 8.6 months) as compared with azacitidine alone (26, 27). Herein, we further detail the efficacy and safety of venetoclax and azacitidine among treatment-naïve AML patients with comorbidities and/or age ≥75 years, ineligible for intensive treatment and harboring a *FLT3* mutation.

Patients and Methods

Patients and treatment

Data were pooled from patients enrolled in an ongoing randomized phase III VIALE-A study (NCT02993523) comparing patients treated with venetoclax combined with azacitidine or placebo with azacitidine and a prior phase Ib study (NCT02203773) where a subset of patients was treated with venetoclax and azacitidine. Patients enrolled were ≥18 years with a confirmed diagnosis of AML by the World Health Organization criteria. Both studies required that the patients be ineligible for standard induction chemotherapy either due to age ≥75 years or due to the presence of comorbidities. Patients were excluded if they had white blood cell (WBC) count >25 × 10^9/L. Additional eligibility criteria have been previously published (26, 28). This analysis included patients treated with venetoclax at 400 mg orally on days 1 to 28 and azacitidine at 75 mg/m^2 intravenously or subcutaneously on days 1 to 7 every 28-day cycle. Patients treated with azacitidine alone received the same standard dose of azacitidine as above.

Both study protocols and related documents were approved by the applicable regional review boards or ethics committees and were conducted in accordance with the International Conference on Harmonization, Good Clinical Practice guidelines, and the Declaration of Helsinki. All patients provided written informed consent.

Assessment of outcomes

Disease responses were evaluated per modified International Working Group (IWG) response criteria for AML and as previously described (26, 29, 30). Efficacy was assessed as CRc (CR + CRi), DoR among responders, and OS. CR was defined as absolute neutrophil count >10^3/μL, platelets >10^5/μL, and red cell transfusion independence for at least 56 days between the first and last day of treatment, and bone marrow with <5% blasts. CRi was defined as all criteria for CR, except for neutropenia ≤10^3/μL or thrombocytopenia ≤10^5/μL. Duration of CRc was defined as the number of days from the date of first response (CR or CRi) per the modified IWG criteria for AML to the earliest evidence of confirmed morphologic relapse, confirmed progressive disease, or death due to disease progression. OS was defined as the time from randomization to the date of death from any cause. Response assessments were performed at screening, end of cycle 1, and every three cycles thereafter. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.0 (31).

Assessment of molecular data

Deoxyribose nucleic acid (DNA) was isolated from bone marrow (BM) aspirates collected from patients prior to the first dose of the study drug and analyzed centrally. For the VIALE-A study, Leukostrat CDx *FLT3* mutation assay panel (Invivoscribe) was used to detect *FLT3-ITD* or *FLT3-TKD*. The next-generation sequencing (NGS) MyAML gene assay (Invivoscribe) was used to detect *NPM1* comutation and was also used to detect *FLT3-ITD* or *FLT3-TKD* in 22 patients with insufficient material for the Leukostrat CDx FLT3 assay. For the phase Ib study, *FLT3* and *NPM1* mutations were detected by the MyAML gene assay. The Leukostrat CDx is a polymerase chain reaction (PCR) based assay designed to detect *ITD* and *TKD* mutation in D835 and I836 in the *FLT3* gene, whereas MyAML is a next-generation targeted sequencing assay capable of detecting variants in the entire coding region of the *FLT3* gene. Given these differences, the *FLT3* mutations as determined by MyAML were limited to those recognizable by the Leukostrat CDx assay (*FLT3-ITD* or *TKD* in D835 or I836 with a variant allelic frequency ≥ 2.5%, which corresponds with a mutant to wild-type signal ratio ≥0.05). Concordance between the two methodologies was assessed. Patients with positive test results for *FLT3* were counted as mutation "detected"; patients with a negative test result

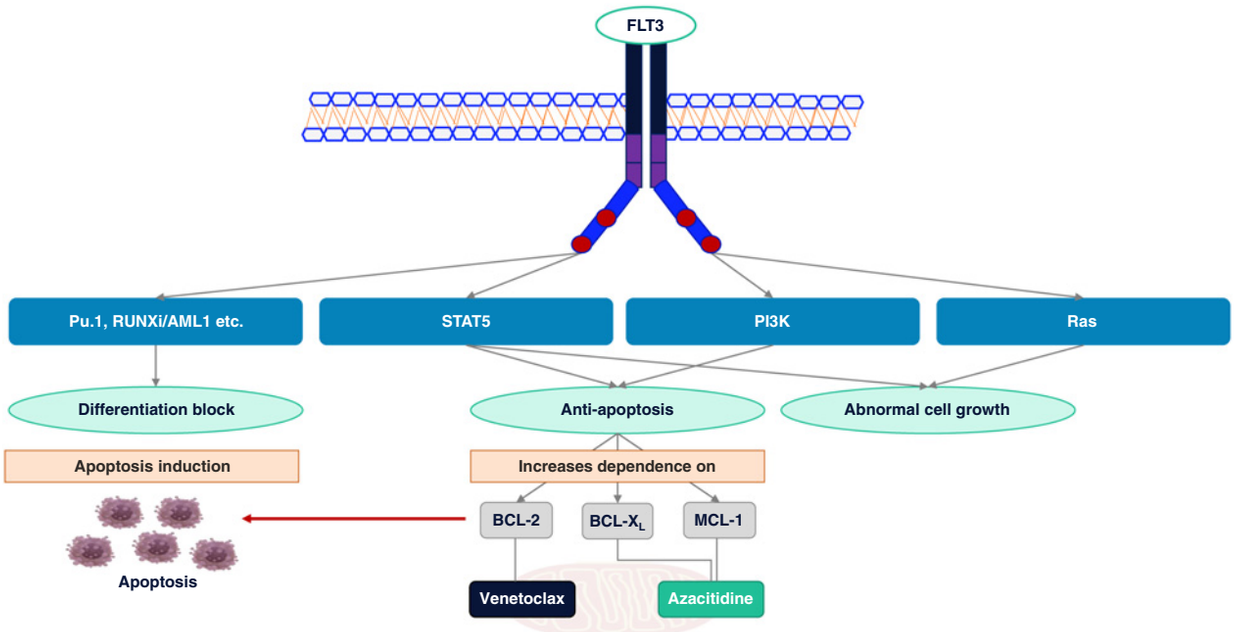

Figure 1.
Venetoclax and azacitidine work synergistically to overcome antiapoptotic signals downstream of an activated *FLT3* pathway.

were counted as mutation "not detected," and patients without a result either due to an inconclusive test or missing specimen were counted as missing or indeterminate.

Statistical analysis

Demographics were summarized by descriptive statistics. Remission rates were summarized in counts and proportions, and CIs were estimated using the exact binomial method. OS and DoR were evaluated by the Kaplan–Meier methodology. The hazard ratios (HR) and 95% confidence interval (CI) between treatment groups were estimated using the Cox proportional hazards model.

Concordance between Leukostrat CDx and MyAML assays for *FLT3* mutation was evaluated using positive percent agreement and negative percent agreement instead of sensitivity and specificity as both assays have the potential for false-negative tests.

Data sharing statement

This clinical trial data can be requested by any qualified researchers who engage in rigorous, independent scientific research and will be provided following the review and approval of a research proposal and Statistical Analysis Plan (SAP) and execution of a Data Sharing Agreement (DSA). Data requests can be submitted at any time, and the data will be accessible for 12 months, with possible extensions considered. For more information on the process or to submit a request, visit the following link: https://www.abbvie.com/our-science/clinical-trials/clinical-trials-data-and-information-sharing/data-and-information-sharing-with-qualified-researchers.html.

Results

Patient disposition and baseline characteristics

The data cut-off dates were January 4, 2020, for the phase III VIALE-A study, and July 19, 2019, for the phase Ib study. In the pooled analysis, there were 353 patients in the venetoclax and azacitidine group (VIALE-A, $n = 286$; phase Ib, $n = 67$) and 145 patients in the azacitidine group.

In the biomarker evaluable population (venetoclax and azacitidine group, $n = 280$; azacitidine group, $n = 117$), *FLT3* mutations were detected (venetoclax and azacitidine group vs. azacitidine group) in 15% (42/280) versus 19% (22/117), and 85% (238/280) versus 81% (95/117) were *FLT3* WT, respectively. The study design and overview of the molecular categorization of patients are shown in **Fig. 2** and Supplementary Table S1. The key demographic and clinical characteristics are shown in **Table 1**. In *FLT3*-mutated patients, higher rates of poor-risk cytogenetics (14.3% vs. 4.5%) and secondary AML (21.4% vs. 13.6%) were observed in the venetoclax and azacitidine versus azacitidine group, respectively.

Agreement between the methods of assessing *FLT3* mutation

The concordance study of 293 specimens from VIALE-A with results from both assays demonstrated 100% positive percent agreement and 90% negative percent agreement for *FLT3* detected by CDx and MyAML assays (Supplementary Table S2). Twenty-four results were discordant between the two assays, of which seven were discordant as MyAML identified a FLT3 mutation type that was not applicable to the *FLT3* CDx assay, 16 were detected by MyAML assay below the level of detection for the *FLT3* CDx assay, and for one, the reason for discordance was unknown. The 51 patients positive by CDx and the 13 patients positive by MyAML with a *FLT3* mutation type detectable by the CDx assay were included as *FLT3* mutation detected (*ITD* or *TKD*).

Remission rates

Among patients with *FLT3* mutation (venetoclax and azacitidine group vs. azacitidine group), the median number of treatment cycles delivered was 7.0 (range: 1.0–31.0) versus 5.0 (1.0–21.0). The CRc rates

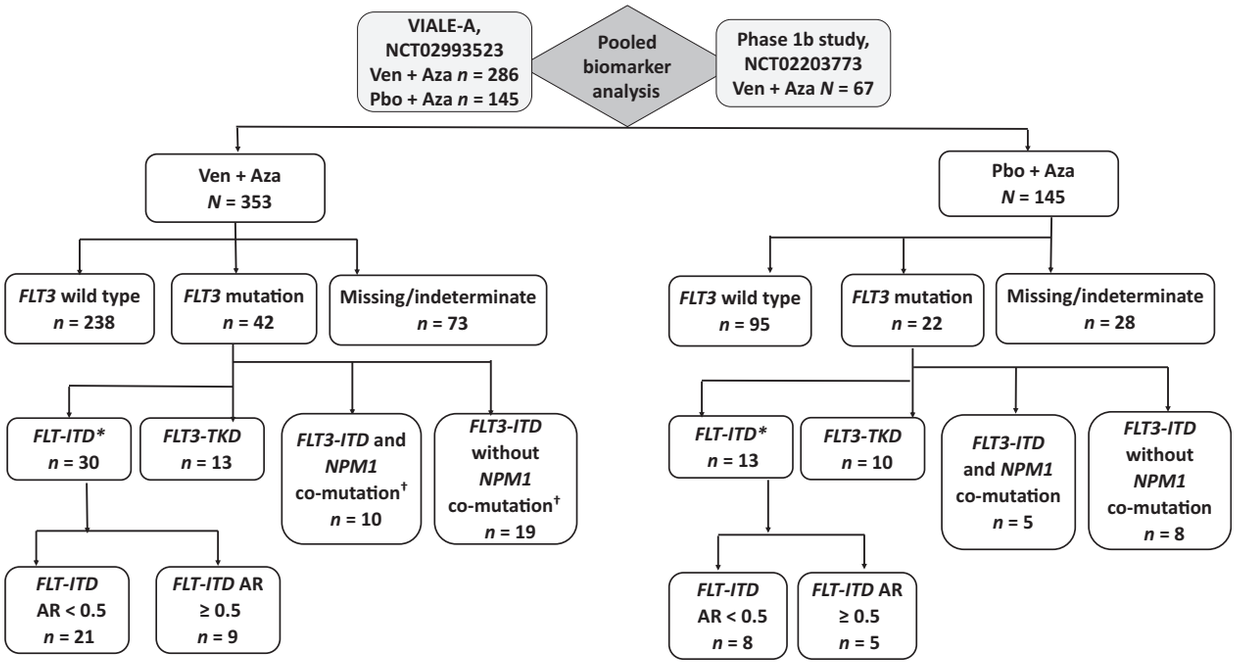

Figure 2.

Study design and molecular classification. *One patient in the venetoclax and azacitidine group and one patient in the azacitidine group had both *FLT3-ITD* and *TKD* mutation; †One patient in the venetoclax and azacitidine group was indeterminate for *NPM1* comutation status.

were higher in the venetoclax and azacitidine group as compared to the azacitidine group [67% (n = 28) vs. 36% (n = 8)], the median time to first response of CR or CRi was 1.2 (95% CI, 0.8–7.7) versus 2.8 (1.0–11.2) months, and the median DoR was 17.3 (95% CI, 10.1–NE) versus 5.0 (1.0–15.9) months. In patients with *FLT3* WT (venetoclax and azacitidine group vs. azacitidine group), the CRc rates were 67% (n = 159) versus 25% (n = 24); the median time to first response for CR or CRi was 1.3 (range: 0.7–9.9) versus 2.9 (1.0–13.2) months, and the median DoR was 18.4 (95% CI: 15.1–NE) versus 13.4 (6.7–15.6) months (**Fig. 3**).

In patients treated with venetoclax and azacitidine combination, high remission rates were observed in *FLT3-ITD* and *FLT3-TKD* subgroups. In patients with *FLT3-ITD*, the CRc rate was 63% (n = 19); median time to first response for CR or CRi was 1.2 (range: 0.8–4.8) months, and the median DoR was 17.3 (95% CI: 4.6–NE) months. In patients with *FLT3-TKD*, the CRc rate was 77% (n = 10); median time to first response to CR or CRi was 1.2 (range: 1.0–7.7) months, and the median DoR was 15.9 (95% CI: 2.8–NE) months.

In patients who had a comutation of *FLT3-ITD* and *NPM1*, the treatment with venetoclax and azacitidine resulted in a CRc rate of 70% (n = 7). The median time to first response for CR or CRi was 1.2 (range: 1.0–1.9) months. The median DoR was not reached (95% CI: 4.6–NE) and the estimated 12-month in-remission rate was 66.7% (95% CI: 19.5%–90.4%). The patients with *FLT3-ITD* and *NPM1* WT, when treated with venetoclax and azacitidine, attained a CRc rate of 58% (n = 11). The median time to first response for CR or CRi was 1.0 (range: 0.8–4.8) months, and the median DoR was 10.1 (95% CI: 1.8–NE) months.

The remission rates by *FLT3-ITD* ARs are summarized in **Table 2**. In addition, the comparisons of remission rates by treatment groups are shown in the Supplementary Table S3 and should be interpreted with caution due to the small number of patients that warrant further investigation. The remission rates and DoR among patients who achieved CR plus CR with a partial hematologic response (CRh) with venetoclax and azacitidine are shown in Supplementary Table S4.

Overall survival

The median OS in patients with *FLT3* mutation was 12.5 (95% CI: 7.3–19.2) versus 8.6 (95% CI: 5.9–14.7) months, HR: 0.63 (95% CI: 0.35–1.13) in the venetoclax and azacitidine group versus azacitidine group, respectively (**Fig. 4A**). In patients with *FLT3* WT (**Fig. 4B**), the median OS was 14.7 (95% CI: 11.3–19.4) months versus 10.1 (95% CI: 6.8–12.7) months, HR: 0.61 (95% CI: 0.46–0.81) in the venetoclax and azacitidine group versus azacitidine group, respectively.

In patients treated with venetoclax and azacitidine, the median OS was longer in patients with *FLT3-TKD* as compared with those with *FLT3-ITD*. In patients with *FLT3-TKD*, the median OS was 19.2 (95% CI: 1.8– NE) months as compared with 9.9 (95% CI: 5.3–17.6) months in patients with *FLT3-ITD* (**Fig. 4C**).

Patients with a comutation of *FLT3-ITD* and *NPM1* had a median OS of 9.1 (95% CI: 1.3–NE) months when treated with venetoclax and azacitidine, and the patients with *FLT3-ITD* and *NPM1* WT had a median OS of 10.6 (95% CI: 2.8–17.2) months (**Fig. 4D**).

The median OS of patients with *FLT3-ITD*, *FLT3-TKD*, *FLT3-ITD* + *NPM1* mutated, and *FLT3-ITD* + *NPM1* WT compared by treatment groups are presented in Supplementary Fig. S1.

Safety

Predominant ≥3 grade hematologic adverse events in patients with versus without *FLT3* mutations when treated with venetoclax and azacitidine (≥20% in either group) were febrile neutropenia (38% vs.

Table 1. Baseline characteristics of patients.

	Venetoclax + Azacitidine		Azacitidine	
	***FLT3* mutated (*n* = 42)[a]**	***FLT3* wild type (*n* = 238)**	***FLT3* mutated (*n* = 22)**	***FLT3* wild type (*n* = 95)**
Age, median (range)	75.0 (49.0–91.0)	77.0 (53.0–90.0)	75.0 (65.0–85.0)	76.0 (60.0–90.0)
Age category - *n* (%)				
<65	3 (7.1)	7 (2.9)	0	4 (4.2)
65–<75	17 (40.5)	75 (31.5)	9 (40.9)	30 (31.6)
≥75	22 (52.4)	156 (65.5)	13 (59.1)	61 (64.2)
Gender - *n* (%)				
Female	18 (42.9)	97 (40.8)	6 (27.3)	39 (41.1)
Male	24 (57.1)	141 (59.2)	16 (72.7)	56 (58.9)
ECOG performance status - *n* (%)				
0–1	17 (40.5)	148 (62.2)	11 (50.0)	59 (62.1)
2–3	25 (59.5)	90 (37.8)	11 (50.0)	36 (37.9)
Cytogenetics - *n* (%)				
Intermediate	36 (85.7)	141 (59.2)	21 (95.5)	48 (50.5)
Poor	6 (14.3)	97 (40.8)	1 (4.5)	47 (49.5)
Bone marrow blast count - *n* (%)				
<30%	7 (16.7)	76 (31.9)	3 (13.6)	28 (29.5)
≥30%–<50%	4 (9.5)	59 (24.8)	4 (18.2)	23 (24.2)
≥50%	31 (73.8)	103 (43.3)	15 (68.2)	44 (46.3)
Type of AML - *n* (%)				
De novo AML	33 (78.6)	175 (73.5)	19 (86.4)	67 (70.5)
Secondary AML	9 (21.4)	63 (26.5)	3 (13.6)	28 (29.5)
AML-MRC - *n* (%)	8 (19.0)	81 (34.0)	7 (31.8)	36 (37.9)
RBC or platelet transfusion within 8 weeks prior to the first dose of study drug or randomization - *n* (%)	24 (57.1)	132 (55.5)	17 (77.3)	53 (55.8)
FLT3-ITD AR - *n* (%)				
<0.5	21 (70.0)	—	8 (61.5)	—
≥0.5	9 (30.0)	—	5 (38.5)	—
Molecular mutations				
IDH1 or IDH2[b]	10 (23.8)	64 (27.6)	2 (10.0)	22 (23.4)
NPM1[c]	15 (35.7)	30 (12.6)	8 (36.4)	11 (11.6)

Abbreviations: AML-MRC, AML with myelodysplasia-related changes; ECOG, Eastern Cooperative Oncology Group.
[a]*FLT3* was detected by CDx assay in 40 patients and by MyAML assay in 2 patients. *FLT3-ITD* was detected by CDx assay in 28 patients and by MyAML assay in 2 patients.
[b]*IDH1* or *IDH2 was* detected by CDx assay.
[c]NPM1 was detected by MyAML assay.

42%), thrombocytopenia (38% vs. 35%), neutropenia (33% vs. 33%), and anemia (31% vs. 24%). Common nonhematologic adverse event was pneumonia (21% vs. 24%; Supplementary Table S5). The most common serious adverse events (≥10% in either group) were febrile neutropenia (31% vs. 29%) and pneumonia (14% vs. 20%).

In the venetoclax and azacitidine group, there were 4 (10%) early deaths within 30 days of administration of the study drug in patients with an *FLT3* mutation and 16 (7%) deaths in patients who were *FLT3* WT. Nine (21%) and 18 (8%) patients in the *FLT3* mutated and *FLT3* WT in the venetoclax and azacitidine group used hydroxyurea during treatment. Tumor lysis syndrome was reported in one patient from each group; 1 (2.4%) versus 1 (0.4%).

Posttreatment systemic therapy was utilized by 10 (24%) patients with *FLT3* mutation after receiving venetoclax and azacitidine as compared to 7 (32%) patients after treatment with azacitidine (Supplementary Table S6).

Discussion

For older patients with AML who are ineligible for intensive chemotherapy, treatment with venetoclax and azacitidine is now recommended as the new standard of care by the NCCN clinical practice guidelines for AML regardless of mutation status (10). Historically, there have been limited treatment options for patients with AML who are ≥75 years, irrespective of *FLT3* mutation, and ineligible for intensive chemotherapy. Among such patients, treatment with the combination of venetoclax and azacitidine led to a 31% higher remission rate and a 37% reduction in the risk of death than treatment with azacitidine alone. The remission rates among patients irrespective of *FLT3* mutations were similar when treated with venetoclax and azacitidine, suggesting that the combination maybe be used as an initial treatment option for ineligible treatment-naïve patients with AML either with or without *FLT3* mutations.

Although high remission rates were observed among patients exhibiting both *FLT3-ITD* and *TKD* mutations, the survival benefit was prominent in patients with *FLT3-TKD* only. Preclinical studies have found that *FLT3-ITD* mutations may reduce BCL-2 dependence in AML cells by enhancing the expression of BCL-X_L and MCL-1 (32, 33). *FLT3-ITD* clones may expand or emerge at relapse after venetoclax-based therapy (22). Combining *FLT3* inhibitors such as midostaurin, quizartinib, or gilteritinib with venetoclax has been reported to potently and synergistically induce apoptosis in *FLT3-ITD* AML cell lines (22, 24)

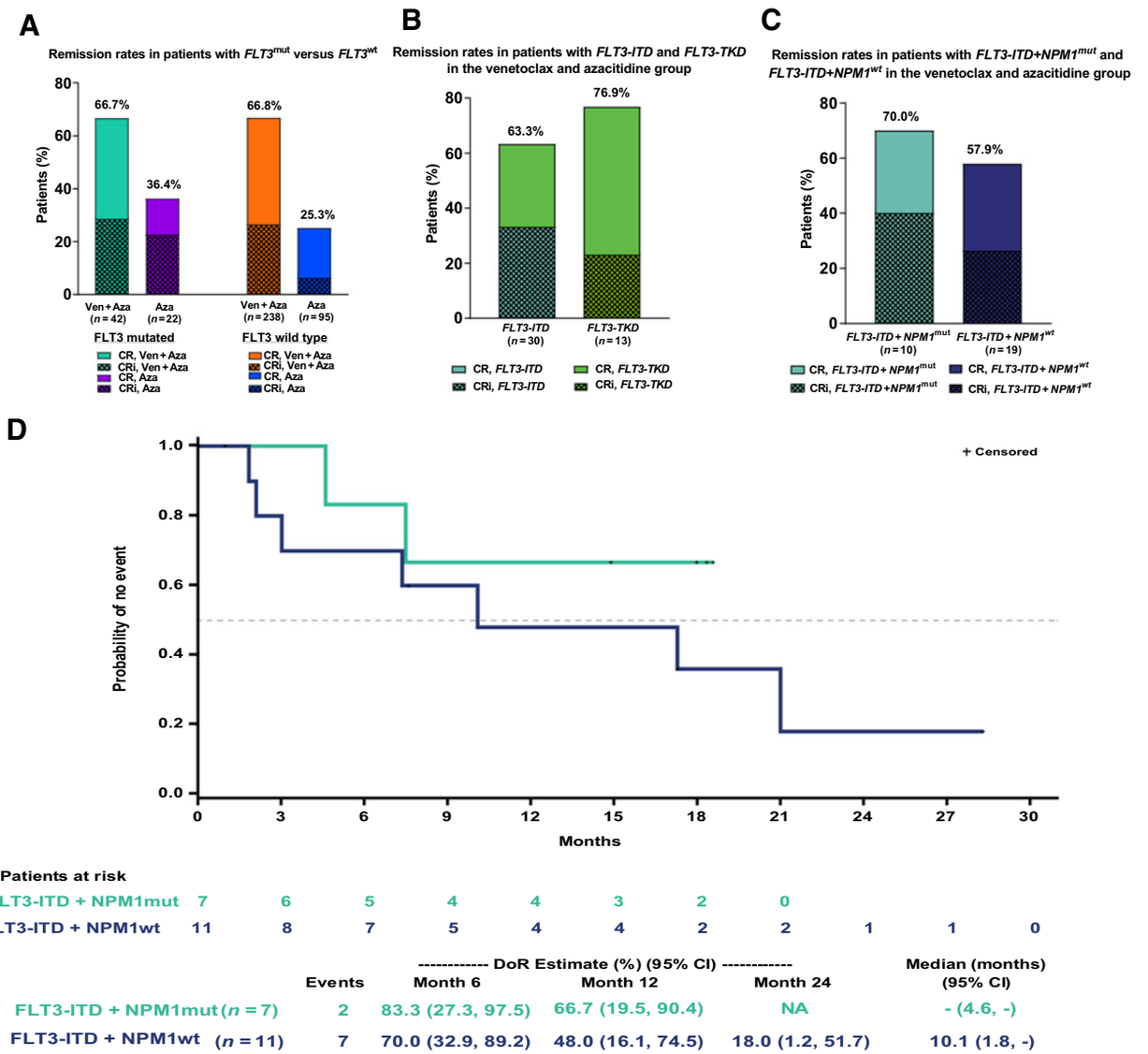

Figure 3.

A, Remission rates in patients with *FLT3* mutations and *FLT3* wild type by treatment groups. **B,** Remission rates in patients with *FLT3-ITD* and *FLT3-TKD* in the venetoclax and azacitidine group. **C,** Remission rates in patients with *FLT3-ITD+NPM1*-mutated versus *FLT3-ITD+NPM1* wild type in the venetoclax and azacitidine group. **D,** Duration of remission among responders with *FLT3-ITD+NPM1* mutation versus *FLT3-ITD+ NPM1* wild type.

and suppress MCL1 overexpression (34, 35). Hence, there is a strong rationale to support clinical investigation of *FLT3* inhibitors in combination with venetoclax to treat patients with *FLT3-ITD* mutated AML, while the benefits of the combination in the *FLT3-TKD* population warrant further evaluation.

The safety and tolerability of the venetoclax and azacitidine combination among patients with *FLT3* mutation were similar to patients with wild-type *FLT3* mutation status. The toxicities were predominantly hematologic with the rate of febrile neutropenia being higher in the venetoclax and azacitidine group as compared to the azacitidine group, consistent with the safety data of the overall trial population (26, 28). The toxicities were effectively managed by the standard of care.

A key limitation of this study is the small patient numbers in several of the subgroup analyses, and the comparison and interpretation of the results warrant caution. In addition, the frequency of *FLT3* mutations seen in this patient population (15%) is lower than the overall rate of *FLT3* mutations (30%) in AML, and as such, the outcomes may not be applicable to the highly proliferative mutant *FLT3*-driven AML seen in younger adults. Per protocol, the study excluded patients with WBC >25 $\times$ 10^9/L, but hydroxyurea or leukapheresis were permitted to meet this criterion. This exclusion criterion may have resulted in the enrollment of fewer patients who had *FLT3-ITD* with high ARs. Future analyses in large datasets are required to establish further the efficacy of the combination in these subgroups.

While the data show the efficacy of venetoclax and azacitidine combination among patients with *FLT3*-mutated AML, other studies are exploring the use of triplet combinations with venetoclax, a hypomethylating agent (azacitidine, decitabine) or low-dose

Table 2. Remission rates among patients treated with venetoclax and azacitidine combination.

	FLT3 mutated (*n* = 42)	*FLT3-ITD* (*n* = 30)	*FLT3-ITD AR* < 0.5 (*n* = 21)	*FLT3-ITD AR* ≥ 0.5 (*n* = 9)	*FLT3-TKD* (*n* = 13)	*FLT3* wild type (*n* = 238)	*FLT3-ITD* and *NPM1* (*n* = 10)	*FLT3-ITD* and *NMP1* wild type (*n* = 19)
CR rate, *n* (%)	16 (38.1)	9 (30.0)	8 (38.1)	1 (11.1)	7 (53.8)	96 (40.3)	3 (30.0)	6 (31.6)
CR + CRi, *n* (%)	28 (66.7)	19 (63.3)	14 (66.7)	5 (55.6)	10 (76.9)	159 (66.8)	7 (70.0)	11 (57.9)
Duration of CR + CRi (months), median (95% CI)	17.3 (10.1–NE)	17.3 (4.6–NE)	21.0 (3.0–NE)	NR (7.4–NE)	15.9 (2.8–NE)	18.4 (15.1–NE)	NR (4.6–NE)	10.1 (1.8–NE)
Time to the 1st response, (months), median (range)	1.2 (0.8–7.7)	1.2 (0.8–4.8)	1.2 (0.9–4.6)	1.2 (0.8–4.8)	1.2 (1.0–7.7)	1.3 (0.7–9.9)	1.2 (1.0–1.9)	1.0 (0.8–4.8)
CR + CRi by initiation of C-2, *n* (%)	21 (50.0)	16 (53.3)	12 (57.1)	4 (44.4)	6 (46.2)	97 (40.8)	6 (60.0)	9 (47.4)
Post-baseline transfusion independence rate[a], n (%)	25 (59.5)	17 (56.7)	11 (52.4)	6 (66.7)	8 (61.5)	135 (56.7)	7 (70.0)	9 (47.4)

Note: CR was defined as absolute neutrophil count >10^3/μL, platelets >10^5/μL, red cell transfusion independence (TI) for at least 8 weeks, and bone marrow with <5% blasts; CRi is defined as all criteria for CR, except for neutropenia ≤10^3/μL or thrombocytopenia ≤10^5/μL.
Abbreviations: AR, allelic ratio; CR, complete remission; CRi, CR+ incomplete hematologic recovery; NE, not evaluable; NR, not reached.
[a]Post-baseline transfusion independence is defined as a period of at least 56 days with no red blood cell or platelet transfusion during the evaluation period.

cytarabine, and a targeted *FLT3* inhibitor such as with quizartinib (NCT03661307) or gilteritinib (NCT04140487). The preliminary analysis of a phase II trial (NCT03404193) has reported that triplet therapy with *FLT3* inhibitor, venetoclax, and decitabine was safe and effective in treating treatment-naïve older patients with *FLT3* mutation, with manageable cytopenias (14). If successful, these venetoclax-containing combination therapies may further improve response rates and survival outcomes among patients with *FLT3*-mutated AML.

In conclusion, the current data demonstrated promising efficacy of venetoclax and azacitidine in *FLT3*-mutated subgroups. Future

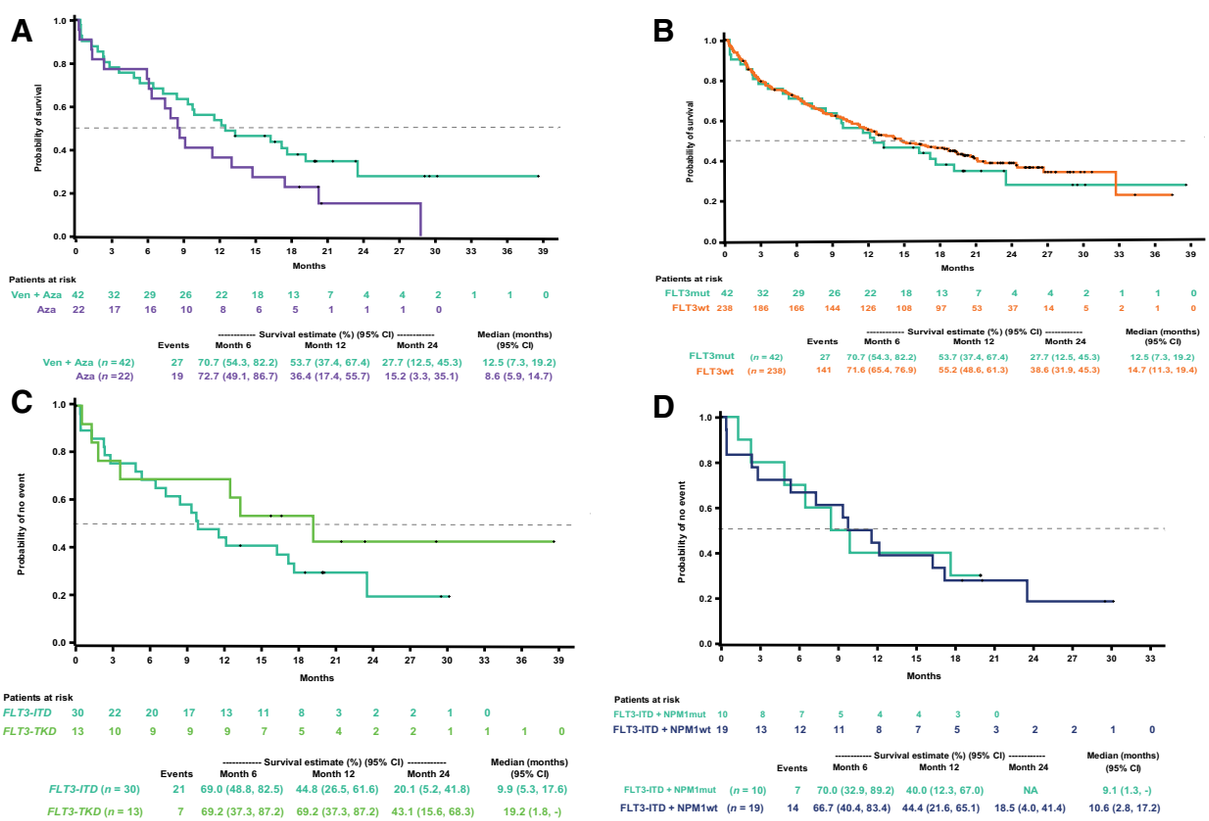

Figure 4.
Kaplan–Meier curves for OS. **A,** Patients with *FLT3* mutation by treatment groups. **B,** Patients with *FLT3* mutation or *FLT3* wild type in the venetoclax and azacitidine group. **C,** Patients with *FLT3-ITD* and FLT3-TKD in the venetoclax and azacitidine group. **D,** Patients with *FLT3-ITD*+*NPM1* mutation versus *FLT3-ITD*+ *NPM1* wild type in the venetoclax and azacitidine group.

analyses in larger patient populations are warranted to further define the risk/benefit of this combination among patients with *FLT3* mutations and establish the efficacy of combining this therapy with targeted inhibitors of *FLT3*.

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Authors' Contributions

M. Konopleva: Investigation, methodology, writing–review and editing. **M.J. Thirman:** Supervision, writing–review and editing. **K.W. Pratz:** Supervision, writing–review and editing. **J.S. Garcia:** Investigation, writing–review and editing. **C. Recher:** Supervision, writing–review and editing. **V. Pullarkat:** Supervision, writing–review and editing. **H.M. Kantarjian:** Supervision, writing–review and editing. **C.D. DiNardo:** Supervision, writing–review and editing. **M. Dail:** Methodology, writing–review and editing. **Y. Duan:** Data curation, writing–review and editing. **B. Chyla:** Data curation, methodology, writing–review and editing. **J. Potluri:** Conceptualization, supervision, methodology, writing–review and editing. **C.L. Miller:** Resources, writing–review and editing. **A.H. Wei:** Supervision, writing–review and editing.

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