

# Clinical outcomes associated with *NPM1* mutations in patients with relapsed or refractory AML

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## Key Points

- In relapsed or refractory AML, mutated *NPM1* has no impact on the risk of relapse or death.
- The addition of venetoclax to salvage treatment for *NPM1*-mutated AML is associated with improved outcomes.

Mutations in *Nucleophosmin 1* (*NPM1*) are associated with a favorable prognosis in newly diagnosed acute myeloid leukemia (AML), however, their prognostic impact in relapsed/refractory (R/R) settings are unknown. In a retrospective analysis, we identified 206 patients (12%) with mutated *NPM1* (*NPM1c*) and compared their outcomes to 1516 patients (88%) with *NPM1* wild-type (*NPM1<sup>wt</sup>*). *NPM1c* was associated with higher rates of complete remission or complete remission with incomplete count recovery compared with *NPM1<sup>wt</sup>* following each line of salvage therapy (first salvage, 56% vs 37%;  $P < .0001$ ; second salvage, 33% vs 22%;  $P = .02$ ; third salvage, 24% vs 14%;  $P = .02$ ). However, *NPM1* mutations had no impact on relapse-free survival (RFS) and overall survival (OS) with each salvage therapy with a median OS following salvage 1, 2 or 3 therapies in *NPM1c* vs *NPM1<sup>wt</sup>* of 7.8 vs 6.0; 5.3 vs 4.1; and 3.5 vs 3.6 months, respectively. Notably, the addition of venetoclax to salvage regimens in patients with *NPM1c* improved RFS and OS (median RFS, 15.8 vs 4.6 months;  $P = .05$ ; median OS, 14.7 vs 5.9 months;  $P = .02$ ). In conclusion, *NPM1* mutational status has a minimal impact on prognosis in relapsed or refractory AML; therefore, novel treatment strategies are required to improve outcomes in this entity.

## Introduction

Mutations in the *Nucleophosmin 1* (*NPM1*) gene are the most common genetic alterations in acute myeloid leukemia (AML), occurring in 20% to 30% of adults with this disease.<sup>1,2</sup> AML with mutated *NPM1* is considered a distinct entity according to the World Health Organization classification and included in the European LeukemiaNet (ELN) 2017 classification owing to its biological and prognostic significance.<sup>3,4</sup> These mutations frequently occur in exon 12 of *NPM1*, causing truncation of the protein and disruption of shuttling between the cytoplasm and nucleus, thereby leading to persistence of *NPM1* in the cytoplasm (thus termed *NPM1c*).<sup>5</sup> *NPM1c* frequently co-occur with *FMS*-like tyrosine kinase 3 (*FLT3*), isocitrate dehydrogenase (*IDH*) 1 and *IDH2* or DNA methyltransferase 3 alpha (*DNMT3A*) mutations.<sup>6</sup> In newly diagnosed patients, AML with *NPM1c* without a *FLT3*-ITD mutation is associated with high response rates and a favorable prognosis.<sup>7,8</sup> However, this prognosis is significantly affected

Submitted 24 June 2022; accepted 14 September 2022; prepublished online on *Blood Advances* First Edition 2 November 2022; final version published online 16 March 2023. <https://doi.org/10.1182/bloodadvances.2022008316>.

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The full-text version of this article contains a data supplement.

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by the presence of co-occurring mutations.<sup>1,4,6,9</sup> Given that *NPM1c* is a leukemia initiating event, multiple studies have demonstrated the value of detecting *NPM1c* as a measurable disease marker, albeit growing evidence that these mutations can be lost at relapse.<sup>10-12</sup> Despite better understanding of the disease course associated with *NPM1c* following first-line treatment, very little is known about the prognostic impact and response to various therapies in the relapsed or refractory (R/R) settings.

We conducted a retrospective analysis of patients with R/R AML and *NPM1c* to characterize the clinical presentation, prognosis, and response to various lines of therapy.

## Methods

### Study design and patient selection

We screened 1722 adult patients with R/R AML treated at The University of Texas MD Anderson Cancer Center between 1 September 2012 and 1 December 2020. Targeted next-generation sequencing was performed using panels of genes recurrently mutated in hematologic malignancies (panels of either 28, 53, or 81 genes were used at our center during this time as described in previous publications).<sup>13</sup> Measurable residual disease (MRD) assessment was performed on bone marrow samples using multicolor flow cytometry (sensitivity  $10^{-4}$  to  $10^{-5}$ ) as previously described by our group.<sup>14</sup>

Various treatment strategies were used, depending on factors such as age, performance status, comorbidities, and comutations (supplemental Table 1). The treatment consisted of either high- or low-intensity regimens based on age and comorbidities. High-intensity (HI) regimens included combinations of cytarabine and idarubicin with or without the addition of a nucleoside analog (ie, cladribine, fludarabine, or clofarabine). Low-intensity (LI) regimens included either hypomethylating agents (5-azacitidine or decitabine) or low-dose cytarabine, with the addition of venetoclax more recently (starting in 2018) or investigational agents. Targeted therapies (ie, FLT3, IDH1, and IDH2 inhibitors) were used as single agents or in combination, as indicated. This study was approved by the institutional review board and was performed in accordance with the Declaration of Helsinki.

### Statistical methods

Patient characteristics were summarized using medians and ranges for continuous variables, and frequencies or percentages for categorical variables. Continuous variables were compared using the Wilcoxon rank-sum test for pairwise comparisons and Kruskal-Wallis test for multiple comparisons. Categorical variables were compared using the Fisher exact test. Responses were defined according to the International Working Group recommendations.<sup>15</sup> Overall survival (OS) was calculated from the treatment start date in patients with relapsed disease to the time of death or the last follow-up. Relapse-free survival (RFS) was calculated from the time of complete remission (CR)/complete remission with incomplete count recovery (CRi) until relapse or death and censored if the patient was alive at the last follow-up. The Kaplan-Meier method was used to estimate the probability of OS or RFS and was compared using the log-rank test. We assessed the independent effect of variables on prognosis in a multivariate analysis, where all variables with  $P < .1$  in the univariate

analysis were included. Analyses were performed using GraphPad Prism version 8.0 and SPSS statistics version 26.0.

## Results

### Baseline characteristics

We identified 1722 patients with R/R AML treated between 2012 and 2020, of whom 206 (12%) had *NPM1c*. The baseline characteristics of the patients are summarized in Table 1. Most patients (63%) in this cohort received their first salvage therapy (S1) at our institution, whereas the remaining patients received 2 or more previous lines of therapy (S2+). The median number of therapy lines administered in this cohort was 2 (range, 2 to 15 lines of therapy). The median age of all patients was 64 years (range, 16 to 91 years).

Among patients with R/R AML, *NPM1c* occurred more commonly in women than in *NPM1* wild-type (*NPM1<sup>wt</sup>*) (58% vs 38%;  $P < .0001$ ), was associated with a higher white blood cell count at presentation ( $P < .0001$ ), and higher percentages of circulating blasts ( $P < .0001$ ), and bone marrow blasts ( $P < .0001$ ). This was likely owing to the significantly higher co-occurrence of *FLT3* mutations in patients with *NPM1c*. Similar to what has been previously described in the newly diagnosed setting, patients with R/R *NPM1c* AML more commonly had a diploid karyotype than *NPM1<sup>wt</sup>* patients (61% vs 31%;  $P < .0001$ ). At relapse, patients with *NPM1c* had a significantly lower incidence of therapy-related AML (t-AML) or AML secondary to an antecedent hematological malignancy (s-AML) than those with *NPM1<sup>wt</sup>* (8% vs 15%,  $P = .007$ ; 3% vs 16%,  $P < .0001$ , respectively). The proportion of patients with early vs late first relapse did not differ according to the *NPM1* mutational status (Table 1).

### Mutational landscape

Consistent with the favorable prognosis associated with *NPM1c* at AML diagnosis, 76% of the evaluable patients showed remission following first-line therapy (Figure 1A). Among the remaining patients with relapsed disease (24%), there was an overall increase in ELN risk, reflecting cytogenetic and mutational changes at relapse. There was an increase in the ELN intermediate risk proportion from 10% at diagnosis to 16% at relapse, and an increase in the ELN adverse risk proportion from 12% to 25% ( $P = .01$ ) (Figure 1A). Mutations in *NPM1*, *DNMT3A*, and *FLT3* were stable at relapse (detected both at baseline and relapse), reflecting the frequent persistence at relapse of leukemia clones and subclones detected at diagnosis (Figure 1B). However, we identified *NPM1c* loss at relapse in 6 of 212 evaluable patients (3%) and *FLT3* mutation loss in 2 of 31 patients (6%) with *FLT3* mutation at diagnosis (Figure 1B). Notably, mutations in the *WT1* gene were gained in 7 of 65 evaluable patients (11%), a pattern previously identified in *FLT3* mutated AML relapse.<sup>16,17</sup> In addition, 4 patients acquired mutations in *TET2* at relapse, 3 acquired mutations in *IDH1* or 2, and 2 acquired mutations in *TP53*. A full list of mutations gained or lost at relapse is provided in the supplement (supplemental Tables 2 and 3).

Mutational co-occurrence patterns at relapse of AML with *NPM1c* were mostly similar to patterns described in the frontline setting with co-occurrence of *NPM1c* with *DNMT3A*, *FLT3*, *IDH1*, and *IDH2* mutations.<sup>1</sup> In this R/R cohort, *NPM1c* more commonly co-occurred with *DNMT3A* (50% vs 17%;  $P < .0001$ ), *FLT3*-ITD

**Table 1. Baseline characteristics**

	<i>NPM1c</i>	<i>NPM1<sup>wt</sup></i>	<i>P</i>
Patients, n (%)	206 (12)	1516 (88)	
Median age, y (range)	64 (17-91)	64 (16-90)	.6
Male, n (%)	85 (42)	938 (62)	<.0001
Hemoglobin, median g/dL (range)	9.3 (6-15)	9.1 (4-18)	.1
WBC, median x 10 <sup>9</sup> /L (range)	8.1 (0.1-227)	3.6 (0.1-339)	<.0001
Platelet count, median x 10 <sup>9</sup> /L (range)	45 (4-624)	43 (1-1552)	.007
Peripheral blast %, median (range)	37 (0-100)	8 (0-100)	<.0001
BM blast %, median (range)	60 (0-99)	30 (0-98)	<.0001
t-AML, n (%)	16 (8)	221 (15)	.007
s-AML, n (%)	6 (3)	236 (16)	<.0001
<b>Cytogenetics (194/1451)</b>			<.0001
Diploid, n (%)	118/194 (61)	445/1451 (31)	
Complex, -5, -7, n (%)	15/194 (8)	554/1451 (38)	
Other, n (%)	61/194 (31)	452/1451 (31)	
<b>Mutations</b>			
<i>DNMT3A</i> (%)	98/195 (50)	245/1412 (17)	<.0001
<i>FLT3-ITD</i> (%)	99/201 (49)	162/1451 (11)	<.0001
<i>TET2</i> (%)	50/159 (31)	760/1174 (65)	<.0001
<i>IDH1</i> (%)	40/196 (20)	102/1457 (7)	<.0001
<i>IDH2</i> (%)	40/198 (20)	160/1455 (11)	.0003
<i>KRAS/NRAS</i> (%)	36/195 (19)	279/1461 (19)	1.0
<i>WT1</i> (%)	30/156 (19)	111/1065 (10)	.009
<i>FLT3-D835</i> (%)	25/198 (13)	64/1454 (4)	<.0001
<i>ASXL1</i> (%)	10/154 (6)	242/1116 (22)	<.0001
<i>TP53</i> (%)	12/189 (6)	354/1432 (25)	<.0001
<i>RUNX1</i> (%)	7/155 (5)	244/1102 (22)	<.0001
<b>Lines of therapy</b>			
S1 (%)	132 (64)	953 (63)	.8
S2 (%)	32 (15)	277 (18)	.3
≥S3 (%)	42 (21)	287 (19)	.5
<b>Duration of first remission</b>			
≤ 6 mo	18/44 (41%)	98/242 (40%)	1.0
Between 6 and 12 mo	12/44 (27%)	61/242 (25%)	.9
≥ 12 mo	14/44 (32%)	83/242 (34%)	.9

Cytogenetics, mutations and duration of first remission values are mutated/evaluable (%). BM, bone marrow; s-AML, AML secondary to antecedent hematologic neoplasm; S1, salvage 1; S2, salvage 2; S3, salvage 3; t-AML, therapy-related AML; WBC, white blood cell.

(49% vs 11%;  $P < .0001$ ), *IDH1/2* (20% vs 9%;  $P < .0001$ ), and *WT1* (19% vs 10%;  $P = .009$ ) mutations compared with *NPM1<sup>wt</sup>* (Table 1). Most *FLT3-ITD* cases (71%) were detected at a high allelic ratio (AR) ( $AR > 0.5$ ). In contrast, mutations in *ASXL1*, *RUNX1*, *TET2*, and *TP53* co-occurred less commonly with *NPM1c* than with *NPM1<sup>wt</sup>* patients (5% to 31% vs 22% to 65%;  $P < .0001$ ).

### Impact of comutations on outcomes in *NPM1c* R/R AML

Given that the favorable prognosis associated with *NPM1c* is context dependent and particularly influenced by the co-occurrence

of *FLT3-ITD* mutations, we examined the responses corresponding to these specific comutations following salvage 1 (supplemental Table 4). We observed that response rates were similar in patients with *NPM1c* with or without co-occurring *FLT3-ITD* mutations (CR/CRi, 55% vs 58% respectively;  $P = .9$ ), with a trend for an improved OS in patients with *NPM1c* and wild-type *FLT3* compared with *NPM1c* and *FLT3-ITD* comutations (median OS, 8.6 vs 5.8 months;  $P = .05$ ) (supplemental Figure 1).

Similarly, *NPM1c* with co-occurring mutations in *DNMT3A*, *IDH1*, *TET2*, or *RAS* had similar response rates compared with *NPM1c* and the corresponding wild-type genes. However, *NPM1c* and *IDH2* comutations was associated with a higher CR rate compared with *NPM1c* and *IDH2* wild-type (CR rates, 50% vs 27%;  $P = .03$ ) in addition to an improved OS (median OS, 14.5 vs 5.8 months, respectively;  $P = .04$ ) (supplemental Table 4, supplemental Figure 1). *TET2* comutations with *NPM1c* was associated with worse OS compared with *NPM1c* and wild-type *TET2* (median OS, 5.1 vs 8.3 months, respectively;  $P = .01$ ) (supplemental Table 4, supplemental Figure 1).

### *NPM1c* loss at relapse

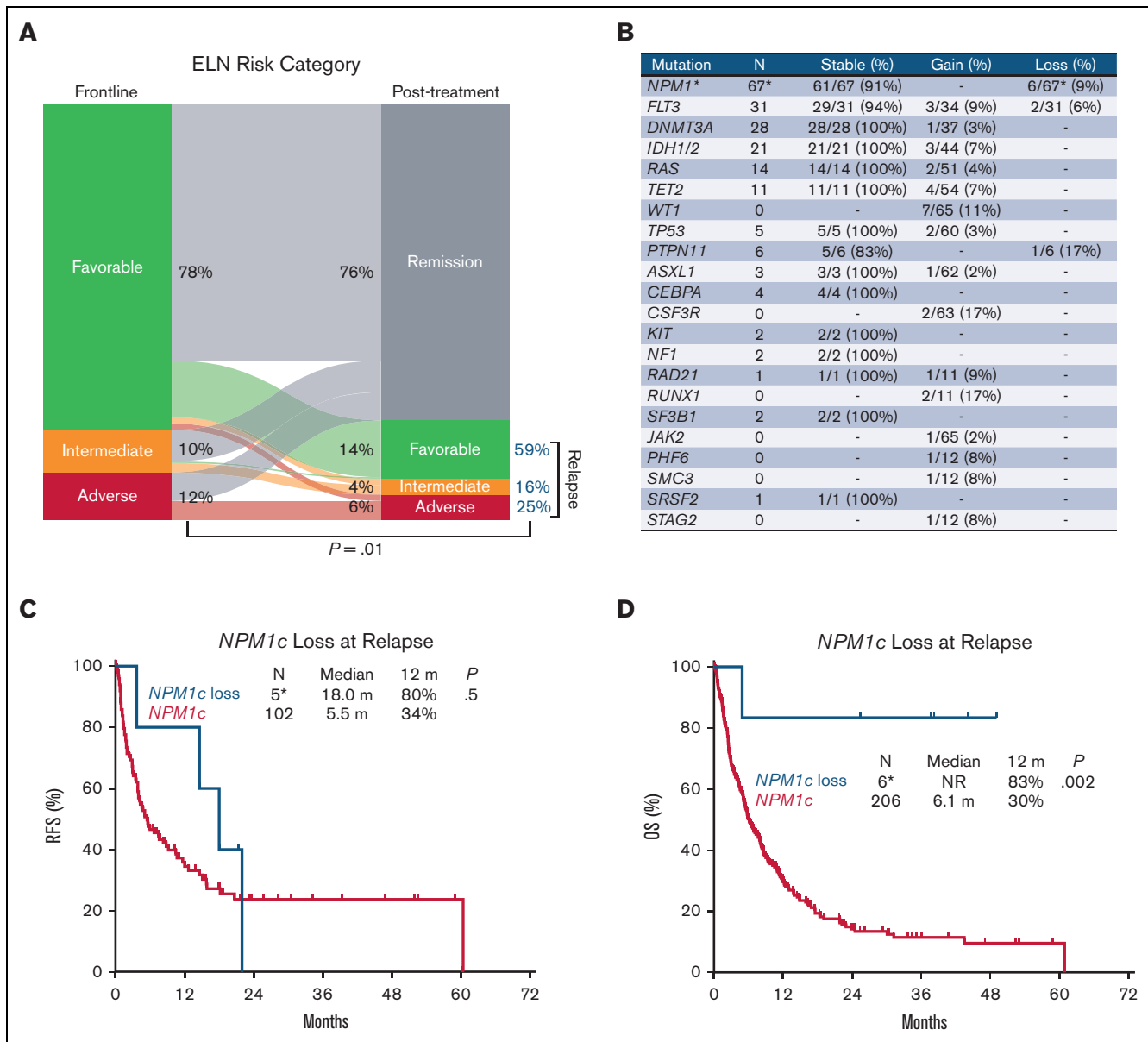
*NPM1c* is considered a founding leukemia event that is stable throughout the disease process and therefore has been used as a surrogate for MRD.<sup>18</sup> However, in a small fraction of cases, *NPM1c* is lost at relapse.<sup>11,12</sup> Among patients with newly diagnosed AML with *NPM1c*, 3% (6 of 212 patients) relapsed without *NPM1c*. *NPM1c* loss at relapse was associated with improved RFS and OS compared with persistence of *NPM1c*, although the numbers were small for this comparison ( $n = 6$  vs  $n = 206$ ) (1-year RFS, 80% vs 34%;  $P = .5$ ; median OS, NR vs 6.1 months; 1-year OS, 83% vs 30%;  $P = .002$ ) (Figure 1C,D). This highlights the possibility that *NPM1c* loss at relapse may represent a de novo leukemia.<sup>11</sup>

### Outcomes by line of therapy in *NPM1c* R/R AML

As expected, the response rates decreased sequentially with each line of salvage therapy for all patients with R/R AML. Patients with *NPM1c* had higher response rates compared with those with *NPM1<sup>wt</sup>* following S1 with a CR/CRi rate of 56% vs 37%, respectively ( $P < .0001$ ), and a significant but less pronounced difference with subsequent lines of therapy (S2, 33% vs 22%;  $P = .02$ ; ≥S3, 24% vs 14%;  $P = .02$ ) (Table 2). There was no significant difference in 30-day mortality between *NPM1c* and *NPM1<sup>wt</sup>*, regardless of salvage regimen (9% each in S1, 17% vs 13% in S2, and 13 vs 14% in ≥S3).

However, despite the relatively higher response rates associated with *NPM1c* AML, there was no significant difference in RFS or OS compared with *NPM1<sup>wt</sup>* in the aggregate population, with a median RFS of 5.5 vs 5.6 months ( $P = .4$ ) and a median OS of 6.1 vs 5.5 months respectively ( $P = .07$ ) (Figure 2A,B). Albeit a trend for an improved RFS and OS associated with *NPM1c* following salvage 1 (median RFS, 8.3 vs 5.7 months;  $P = .2$ ; median OS, 7.8 vs 6.0 months;  $P = .05$ ), survival outcomes were similar with subsequent salvage lines of therapy (median RFS, 3.3 vs 5.1 months;  $P = .08$ ; median OS, 5.3 vs 4.1 months;  $P = .4$ ) in salvage 2, and (median RFS, 4.0 vs 5.4 months;  $P = .9$ ; median OS, 3.5 vs 3.6 months;  $P = .7$ ) in salvage 3 (Figure 3).

When restricting the analysis to patients with R/R AML and a diploid karyotype only, there was no difference in RFS or OS according to *NPM1* mutational status. The median OS for diploid



**Figure 1. Clonal architecture of AML with mutated *NPM1* at relapse.** (A) Change in the ELN risk classification at relapse. (B) Mutational evolution, including stability, gain, or loss of mutations at relapse. N is the number of patients with the corresponding mutation at diagnosis among those evaluable by mutational analysis performed at diagnosis and at the time of relapse. The percentages for stability and loss were calculated as the number of patients with mutations that persisted or were lost at relapse divided by patients with mutations in the corresponding gene present at diagnosis. Percentage gain was calculated as the number of patients with mutations acquired at relapse divided by the number of patients without mutations in the corresponding gene at diagnosis. (C-D) Impact of *NPM1c* loss at relapse on relapse-free survival and overall survival. \*Two of the 6 patients who lost *NPM1c* at relapse underwent mutational analysis at diagnosis before referral to our center.

R/R *NPM1c* AML was 8.0 months vs 7.9 months in those with diploid R/R *NPM1<sup>wt</sup>* AML ( $P = .2$ ) (supplemental Figure 2). Similarly, there was no difference in RFS or OS according to *NPM1* mutational status in the subgroup of patients with R/R AML below the age of 60 years or in the subgroup above this age cut-off (supplemental Figure 3).

### Outcomes by type of therapy in *NPM1c* R/R AML

**Combinations with Venetoclax.** Patients with R/R *NPM1c* AML treated with HI regimens had higher response rates than

those with *NPM1<sup>wt</sup>*, with a CR/CRi rate of 63% vs 37%, respectively ( $P < .0001$ ) (Table 2). Conversely, there was no impact of the *NPM1* mutational status on response rates when LI regimens were used (CR/CRi, 34% with *NPM1c* vs 26% with *NPM1<sup>wt</sup>*;  $P = .1$ ) (Table 2). However, the addition of venetoclax to LI regimens used in salvage therapy led to an improved response rate in patients with *NPM1c*, with a CR/CRi rate of 71% vs 32% in those with *NPM1<sup>wt</sup>* ( $P = .02$ ). This in turn led to improved RFS with a median of 15.8 months for *NPM1c* patients who received venetoclax vs 4.6 months for *NPM1<sup>wt</sup>* ( $P = .05$ ), and an improved OS with a median

**Table 2. Response rates by line and type of salvage therapy**

	All therapies		HI		LI		LI + venetoclax	
	<i>NPM1c</i>	<i>NPM1wt</i>	<i>NPM1c</i>	<i>NPM1wt</i>	<i>NPM1c</i>	<i>NPM1wt</i>	<i>NPM1c</i>	<i>NPM1wt</i>
<b>All lines (N)</b>	206	1516	68	459	109	762	24	201
CR (%)	49 (24)*	224 (15)	32 (47)†	95 (21)	7 (6)	87 (11)	7 (29)	34 (12)
CRi (%)	53 (26)*	272 (18)	11 (16)	73 (16)	31 (28)*	117 (15)	10 (42)	56 (20)
CR/CRi (%)	102 (50)†	496 (33)	43 (63)†	168 (37)	38 (34)	194 (26)	17 (71)*	90 (32)
<b>S1 (N)</b>	132	953	52	313	63	443	13	140
CR (%)	42 (32)*	178 (19)	28 (48)†	82 (26)	6 (10)	56 (13)	5 (38)	27 (19)
CRi (%)	32 (24)	175 (18)	8 (19)	49 (16)	18 (29)*	71 (18)	5 (38)	37 (26)
CR/CRi (%)	74 (56)†	353 (37)	36 (67)*	131 (42)	24 (38)	127 (29)	10 (76)*	64 (45)
<b>S2 (N)</b>	85	707	20	193	52	396	9	87
CR (%)	12 (14)	68 (10)	6 (30)	27 (14)	4 (8)	28 (7)	1 (11)	10 (11)
CRi (%)	16 (19)	84 (12)	2 (10)	26 (13)	11 (21)*	42 (11)	3 (33)	12 (14)
CR/CRi (%)	28 (33)*	152 (22)	8 (40)	53 (27)	15 (29)*	70 (18)	4 (44)	22 (25)
<b>≥S3 (N)</b>	83	615	18	161	48	358	14	60
CR (%)	6 (7)	23 (4)	2 (11)	10 (62)	1 (2)	9 (3)	3 (21)*	2 (3)
CRi (%)	14 (17)	62 (10)	3 (17)	18 (11)	6 (13)	24 (7)	5 (36)	10 (17)
CR/CRi (%)	20 (24)*	85 (14)	5 (28)	28 (17)	7 (15)	33 (9)	8 (57)*	12 (20)

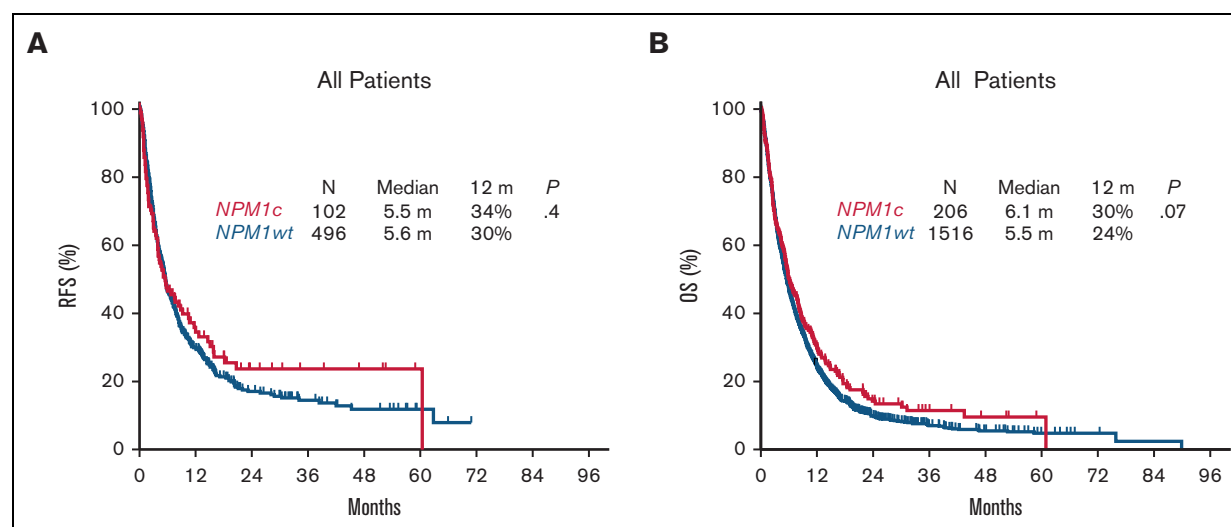
N, number of evaluable patients in each line of therapy.

\* $P < .05$ ;

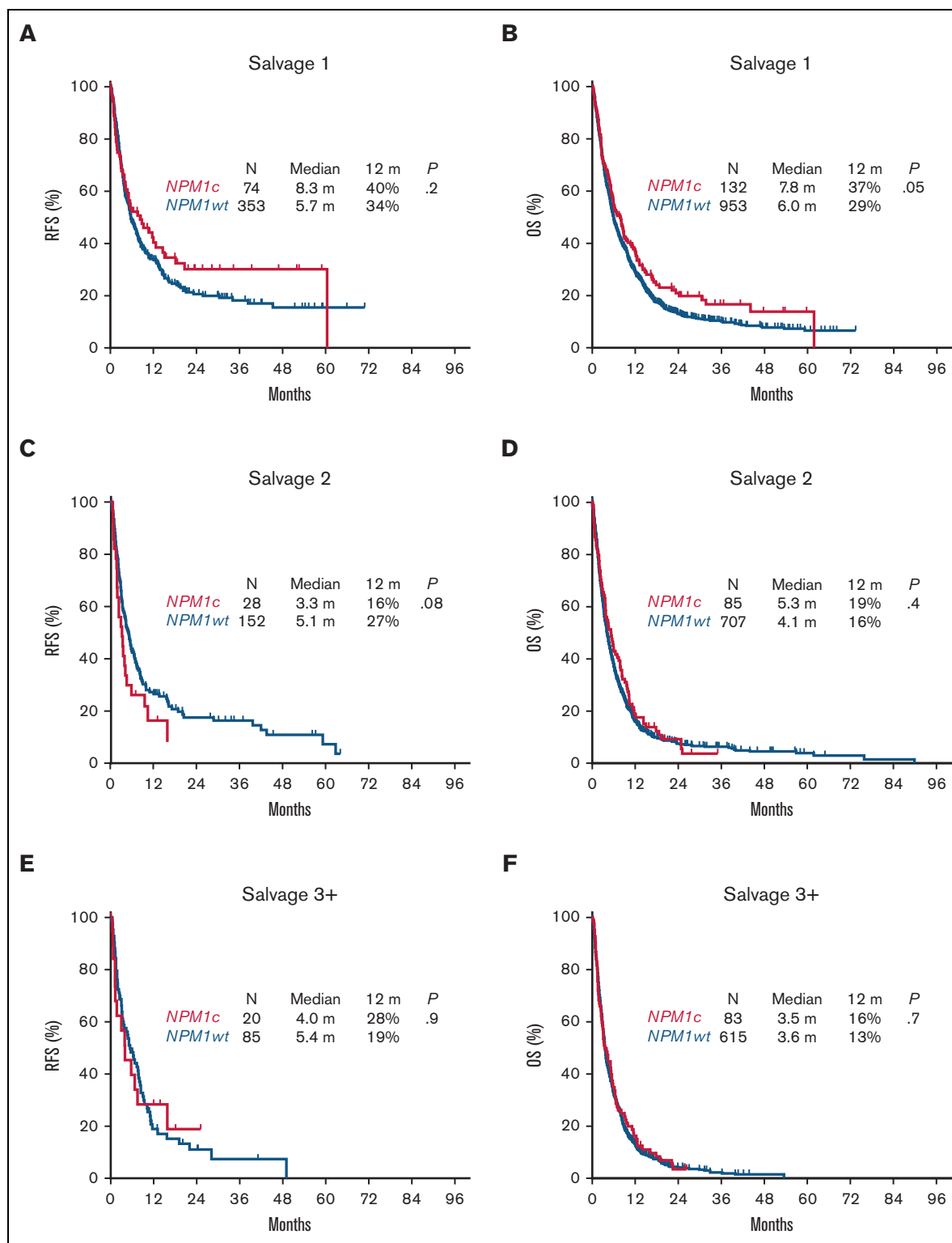
† $P < .001$ .

of 14.7 months vs 5.9 months, respectively ( $P = .02$ ) (Figure 4). These outcomes in patients with *NPM1c* associated with LI and venetoclax matched those associated with standard HI regimens with a median OS of 14.5 months vs 8.1 months respectively ( $P = .4$ ) (supplemental Figure 4). Only 5 patients received HI regimens with the addition of venetoclax for salvage at the time of this analysis, thereby limiting the comparison of outcomes.

**Outcomes with other targeted therapies.** The advent of therapies targeting specific mutations has increased the treatment arsenal for AML, particularly in patients with *NPM1c*, where mutations in *FLT3* or *IDH* frequently co-occur. The use of an *FLT3* inhibitor, either alone or in combination, was associated with a CR/CRi rate of 57% (29/56 patients) in patients with *NPM1c* and *FLT3* comutations (CR/CRi of 43% with *FLT3* inhibitor alone and



**Figure 2. Survival associated with *NPM1* mutational status at AML relapse.**



**Figure 3. Relapse-free survival and overall survival for patients with relapsed or refractory AML with *NPM1c* by line of therapy.** (A) Relapse-free survival following S1. (B) Overall survival following S1. (C) Relapse-free survival following S2. (D) Overall survival following S2. (E) Relapse-free survival following S3+. (F) Overall survival following S3+.

59% with FLT3 inhibitor combinations) (supplemental Tables 5 and 6). In contrast, IDH inhibitor-based therapies had an associated CR/CRi rate of 33% (2/6 patients) in patients with *NPM1c* and

*IDH* mutations. Only 2 patients with *NPM1c* received gemtuzumab ozogamicin, 1 of them achieved CRi (supplemental Tables 5 and 6).

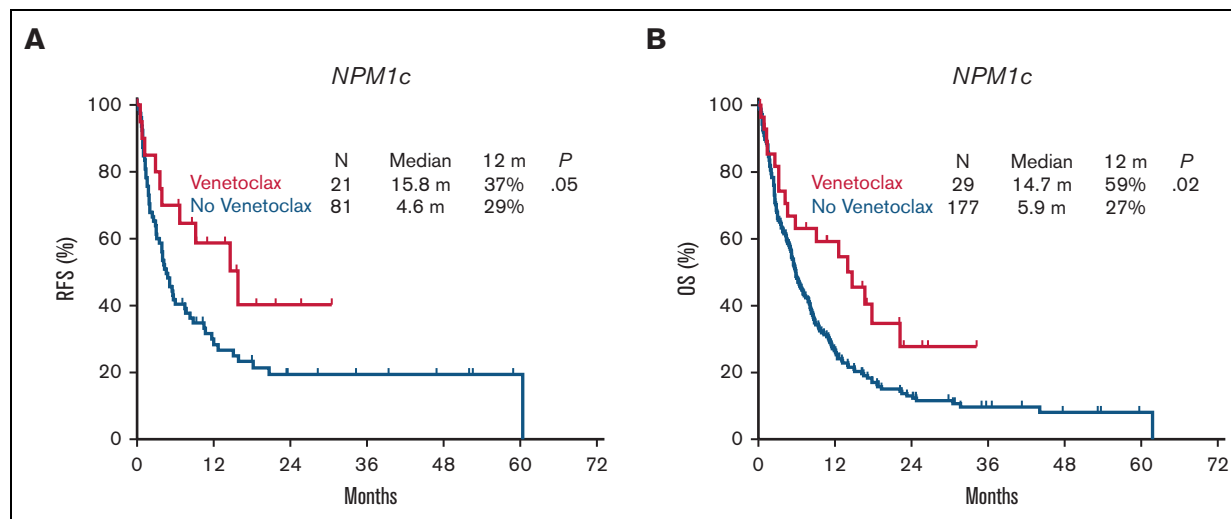


Figure 4. Impact of the addition of venetoclax on survival in relapsed or refractory AML with *NPM1c*.

### Impact of HSCT in *NPM1c* R/R AML

Among patients evaluated in this analysis, 211 (12%), including 197 with CR or CRi and 14 with other responses, received an allogeneic hematopoietic stem cell transplant (HSCT). Among them, 149 (70%) underwent transplantation after achieving remission following their first relapse. The proportion of patients who received an allo-HSCT was similar in the *NPM1c* and *NPM1<sup>wt</sup>* groups (all salvage, 16% vs 12%;  $P = .1$ ; S1, 19% vs 13%,  $P = .06$ ; S  $\geq 2$ , 11% vs 9%,  $P = .6$ ). The median time from the start of therapy to HSCT was 2.8 months (range, 0.5 to 14.3).

In a landmark analysis, HSCT was associated with improved RFS and OS in patients with R/R AML with *NPM1c*, regardless of the salvage line status. The median RFS for patients with *NPM1c* and HSCT was 20.7 months compared with 4.0 months for those with *NPM1c* who did not have HSCT ( $P < .0001$ ), whereas the median OS was 22.2 months vs 8.6 months, respectively ( $P < .0001$ ) (supplemental Figure 5).

### Multivariate analysis

To assess the independent prognostic effect of various factors in this group of patients with R/R AML (both *NPM1c* and *NPM1<sup>wt</sup>*), we performed univariate and multivariate analyses, including baseline characteristics, mutational and cytogenetic status, duration of first remission, and type of therapy received. The *NPM1* mutational status had no effect on OS in this analysis (supplemental Table 7). The only independent factors identified that predicted worse OS in this group of patients with R/R AML included older age, mutated *TP53*, and duration of first remission of less than 6 months (supplemental Table 7).

### Discussion

In this study, we found that AML with *NPM1c* at relapse had similar survival outcomes to those with the wild-type gene. Despite a marginal increase in response rates following salvage therapy, *NPM1c* was not associated with an improved RFS or OS when all therapies were considered. In a limited analysis, we found significantly improved response rates when venetoclax was added to

therapy, leading to a decreased risk of relapse and an improved overall survival. However, a longer follow-up with larger cohorts of patients is needed to validate this finding. It is unclear whether this susceptibility is related to comutations with *IDH*, an established vulnerability to BCL2 inhibition, or is broadly applicable to all patients with mutated *NPM1*.<sup>19</sup>

The proportion of comutations in AML with *NPM1c* at relapse seemed mostly similar to the previously described genomic composition at diagnosis, albeit a relatively higher frequency of *FLT3*-ITD (49% vs 39% in Papaemmanuil et al).<sup>1</sup> Previous analyses have shown an increase in high risk copy number alterations at relapse of AML with *NPM1c* when using methods with an improved resolution compared with conventional cytogenetics available in our analysis.<sup>11</sup> In addition, gain of distinct *FLT3*-ITD clones at relapse, despite relatively preserved mutational proportions compared with what is seen at diagnosis, or selection of inherently resistant leukemia cells following induction therapy (*NPM1c* forms 30% to 35% of cases at diagnosis vs 12% at relapse), could explain the observed resistance and poor outcomes in this setting.<sup>10</sup> This pattern of mutations at relapse differs from what is expected when all patients with *FLT3* mutations receive a frontline *FLT3* inhibitor, which leads to loss of these mutations at relapse in some. Notably, this cohort also included *FLT3* wild-type and older or unfit patients with AML who received frontline LI therapies without the addition of a *FLT3* inhibitor.<sup>17,20</sup>

Interestingly, we found that *NPM1c* loss at relapse (seen in about 3% of patients) was associated with relatively improved outcomes similar to what has been previously described, further justifying the concept that these leukemias could be arising from a de novo clone rather than persistence and evolution of the original founding clone.<sup>10-12</sup> This could affect use of *NPM1c* for MRD monitoring, therefore addition of phenotypic MRD assays such as multicolor flow cytometry would be complimentary. It remains unclear if this rare occurrence justifies the need to confirm *NPM1* mutational status for trials investigating *NPM1c* directed therapies in the R/R setting but could be justified for registrational studies.

The study is limited by the retrospective nature of the analysis, genomic and biological heterogeneity of the subsets included, and the use of various types of therapy in a single center. Therefore, these results must be interpreted within the context of these limitations.

There is relatively no data on the outcomes of relapsed AML with *NPM1c*. To the best of our knowledge, this is the first study to examine the outcomes of these patients with each line of therapy. The median OS of AML with *NPM1c* following S1 was 7.8 months, with a decrease to 5.3 months following S2, and 3.5 months following S3 and beyond. These dismal outcomes indicate the unmet need for novel therapeutic strategies. Among salvage therapies for AML with *NPM1c*, HI treatment regimens and the addition of venetoclax to LI regimens appeared to be the most advantageous. However, comparison across these therapy options is limited by considerations such as age and fitness. Nevertheless, HI regimens or venetoclax combinations are likely to be the preferred backbone for the addition of novel agents to this entity. Notable examples include menin or spleen tyrosine kinase (SYK) inhibitors.<sup>21,22</sup> Early results from the ongoing phase I trials investigating menin inhibition in this population are encouraging (NCT04065399; NCT04067336; NCT04752163; NCT04811560; NCT05153330; NCT04811560).<sup>23,24</sup>

In conclusion, AML with *NPM1c* is associated with poor outcomes at relapse. The use of HI regimens and/or the addition of venetoclax to salvage therapy was associated with improved outcomes in patients with *NPM1c* in this setting. Combination strategies incorporating emerging novel therapies should be rapidly evaluated to further improve outcomes and long-term survival.

## Acknowledgment

G.C.I. received funding through the K12 Paul Calabresi Clinical Scholarship Award (NIH/NCI K12 CA088084).

## Authorship

Contribution: G.C.I., A.B., S.V., and N.D. designed the study and wrote the manuscript; S.V. and A.B. analyzed the data; M.K., C.D.D., T.M.K., G.B., E.J., N.P., M.Y., N.J.S., A.M., K.S., L.M., S.P., K.T., G.T., S.L., K.P., M.A., K.B., G.G.M., F.R., and H.K. provided suggestions and revised the manuscript; G.C.I. and N.D. supervised the analysis; and all authors read and approved the final version of the manuscript.

Conflict-of-interest disclosure: G.C.I. reports research funding from Celgene, Kura Oncology, Syndax, and Novartis, and received consultancy fees from Novartis and Kura Oncology. M.K. reports grant support from Agios, Rafael Pharmaceuticals, Cellectis, Ascentage, AstraZeneca, Sanofi, Ablynx, Genentech, Forty Seven, AbbVie, F Hoffmann-La Roche, and Calithera; research funding from Agios, Rafael Pharmaceuticals, KisoJi, Eli Lilly, Ascentage, AstraZeneca, Sanofi, Ablynx, Genentech, Forty Seven, AbbVie, Stemline Therapeutics, and Calithera; intellectual property rights from Eli Lilly, Novartis, and Reata Pharmaceuticals; current stock options in Reata Pharmaceuticals; consultancy for Genentech, F Hoffmann-La Roche, and AbbVie; and honoraria from Genentech, F Hoffmann-La Roche, and AbbVie. C.D.D. reports membership on an entity's Board of Directors or advisory committees from GlaxoSmithKline

and Notable Labs; current stock options in a privately-held company in Notable Labs; honoraria from Immune-Onc, Bristol Myers Squibb, Agios/Servier, Takeda, Novartis, Foghorn, Celgene, a Bristol Myers Squibb company, and Forma; research funding from Immune-Onc, Bristol Myers Squibb, AbbVie, Agios/Servier, Foghorn, Celgene, a Bristol Myers Squibb company, and Forma; and consultancy from AbbVie and Agios/Servier. T.M.K. reports consultancy from AbbVie, Sanofi-Aventis, Liberum, Jazz, Genentech, Daiichi Sankyo, Novartis, Pfizer, and Agios; grant or research support from AbbVie, Genentech, Bristol Myers Squibb, and Amgen; speaker's bureau fees from Cure; and research support from Genfleet, Cellonkos, Astellas, AstraZeneca, Ascentage, and Pulmotech. G.B. reports consultancy fees from Protagonist Therapeutics, Novartis, and GlaxoSmithKline (GSK); research funding from Ryvu and Astex; and membership to an entity's Board of Directors or advisory committees from Takeda, and ArgenX. E.J. reports research grants from AbbVie, Adaptive Biotechnologies, Amgen, Pfizer, and Takeda, and consultancy fees from AbbVie, Adaptive Biotechnologies, Amgen, Bristol Myers Squibb, Genentech, Incyte, Novartis, Pfizer, and Takeda. N.P. reports consultancy from DAVA Oncology, LFB Biotechnologies, MustangBio, Stemline Therapeutics, Affymetrix, Roche Diagnostics, Blueprint Medicines, Clearview Healthcare Partners, Novartis Pharmaceuticals, Celgene Corporation, Incyte, Protagonist Therapeutics, AbbVie Pharmaceuticals, Aptitude Health, Bristol Myers Squibb, ImmunoGen, Pacylex Pharmaceuticals, CareDx, Daiichi Sankyo, Springer Science + Business Media, Samus, Plexicon, and Cellectis. N.J.S. reports consultancy for Takeda Oncology, Jazz Pharmaceuticals, NGMBio, AstraZeneca, and Amgen; research funding from Takeda Oncology and Astellas; honoraria from Amgen and Novartis. K.S. reports consultancy and research funding from Novartis, and membership on the Board of Directors or advisory committees of Pfizer and Daiichi-Sankyo. K.T. reports consultancy for Novartis, Celgene/Bristol Myers Squibb, GSK, and Symbio Pharmaceuticals; and membership on Symbio Pharmaceuticals, Board of Directors or advisory committee. M.A. reports consultancy from Daiichi Sankyo, Inc., Amgen, and AstraZeneca; patents licensed, royalty bearing and research funding from Jazz Pharmaceuticals; equity ownership with Aptose, Eutropics, Senti Bio, Oncolyze, BrooklynITX, SAB, Chimerix, and Reata; membership with SAB; research funding from the Breast Cancer Research Foundation; and membership with BiolineRx on an entity's Board of Directors or advisory committees. F.R. reports honoraria from Astex, Taiho, Bristol Myers Squibb, Xencor, Agios, AstraZeneca, Novartis, AbbVie, Celgene, Jazz, Syros Pharmaceuticals, and Amgen; research funding from Astex, Taiho, Bristol Myers Squibb, Xencor, Agios, Prelude, AbbVie, Celgene, Jazz, Syros Pharmaceuticals, and Amgen; membership on the Board of Directors or advisory committees of Bristol Myers Squibb and Celgene; and consultancy for Syros Pharmaceuticals. H.K. reports research funding from Ariad, Astex, Bristol Myers Squibb, Cyclacel, Daiichi-Sankyo, Pfizer, Immunogen, Jazz, Novartis, and honoraria from Pfizer, Immunogen, Actinium, and Takeda. N.D. reports consultancy for Novartis, Trovogene, AbbVie, Genentech, Amgen, Pfizer, Bristol Myers Squibb, Astellas, Daiichi Sankyo, Sevier, ImmunoGen, Gilead Sciences, Trillium, Dava Oncology (Arog), Celgene, Syndax, Shattuck Labs, Agios, Kite Pharmaceuticals, SOBI, STAR Therapeutics, and Jazz Pharmaceuticals; is a member of the Data Monitoring Committee for Jazz Pharmaceuticals; and research funding from Trovogene, Genentech, FATE Therapeutics, Hanmi, Amgen, Pfizer, Bristol Myers Squibb, Novimmune, Astellas, Daiichi Sankyo, Sevier, ImmunoGen,



Glycomimetics, Gilead Sciences, Trillium, Karyopharm, Newave, and AbbVie. The remaining authors declare no competing financial interests.

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## References

1. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med*. 2016;374(23):2209-2221.
2. Falini B, Martelli MP, Bolli N, et al. Acute myeloid leukemia with mutated nucleophosmin (NPM1): is it a distinct entity? *Blood*. 2011;117(4):1109-1120.
3. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127(20):2375-2390.
4. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-447.
5. Falini B, Mecucci C, Tiacci E, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med*. 2005;352(3):254-266.
6. Patel JP, Gönen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med*. 2012;366(12):1079-1089.
7. Döhner K, Schlenk RF, Habdank M, et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. *Blood*. 2005;106(12):3740-3746.
8. Lachowicz CA, Loghavi S, Kadia TM, et al. Outcomes of older patients with NPM1-mutated AML: current treatments and the promise of venetoclax-based regimens. *Blood Advances*. 2020;4(7):1311-1320.
9. Bezerra MF, Lima AS, Piqué-Borràs M-R, et al. Co-occurrence of DNMT3A, NPM1, FLT3 mutations identifies a subset of acute myeloid leukemia with adverse prognosis. *Blood*. 2020;135(11):870-875.
10. Cocciardi S, Dolnik A, Kapp-Schwoerer S, et al. Clonal evolution patterns in acute myeloid leukemia with NPM1 mutation. *Nat Commun*. 2019;10(1):2031.
11. Krönke J, Bullinger L, Teleanu V, et al. Clonal evolution in relapsed NPM1-mutated acute myeloid leukemia. *Blood*. 2013;122(1):100-108.
12. Höllein A, Meggendorfer M, Dicker F, et al. NPM1 mutated AML can relapse with wild-type NPM1: persistent clonal hematopoiesis can drive relapse. *Blood Advances*. 2018;2(22):3118-3125.
13. Luthra R, Patel KP, Reddy NG, et al. Next-generation sequencing-based multigene mutational screening for acute myeloid leukemia using MiSeq: applicability for diagnostics and disease monitoring. *Haematologica*. 2014;99(3):465-473.
14. Xu J, Jorgensen JL, Wang SA. How do we use multicolor flow cytometry to detect minimal residual disease in acute myeloid leukemia? *Clin Lab Med*. 2017;37(4):787-802.
15. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the international working group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. *J Clin Oncol*. 2003;21(24):4642-4649.
16. McMahon CM, Ferng T, Canaani J, et al. Clonal selection with RAS pathway activation mediates secondary clinical resistance to selective FLT3 inhibition in acute myeloid leukemia. *Cancer Discov*. 2019;9(8):1050-1063.
17. Alotaibi AS, Yilmaz M, Kanagal-Shamanna R, et al. Patterns of resistance differ in patients with acute myeloid leukemia treated with type I versus type II FLT3 inhibitors. *Blood Cancer Discovery*. 2021;2(2):125-134.
18. Ivey A, Hills RK, Simpson MA, et al. Assessment of minimal residual disease in standard-risk AML. *N Engl J Med*. 2016;374(5):422-433.
19. Chan SM, Thomas D, Corces-Zimmerman MR, et al. Isocitrate dehydrogenase 1 and 2 mutations induce BCL-2 dependence in acute myeloid leukemia. *Nat Med*. 2015;21(2):178-184.
20. Schmalbrock LK, Dolnik A, Cocciardi S, et al. Clonal evolution of acute myeloid leukemia with FLT3-ITD mutation under treatment with midostaurin. *Blood*. 2021;137(22):3093-3104.
21. Issa GC, Ravandi F, DiNardo CD, Jabbour E, Kantarjian HM, Andreeff M. Therapeutic implications of menin inhibition in acute leukemias. *Leukemia*. 2021;35(9):2482-2495.
22. Walker AR, Byrd JC, Blachly JS, et al. Entospletinib in combination with induction chemotherapy in previously untreated acute myeloid leukemia: response and predictive significance of HOXA9 and MEIS1 expression. *Clin Cancer Res*. 2020;26(22):5852-5859.

23. Stein EM, Aldoss I, DiPersio JF, et al. Safety and efficacy of menin inhibition in patients (Pts) with MLL-rearranged and NPM1 mutant acute leukemia: a phase (Ph) 1, first-in-human study of SNDX-5613 (AUGMENT 101). *Blood*. 2021;138(Supplement 1):699.
24. Wang ES, Altman JK, Pettit K, et al. Preliminary data on a phase 1/2A first in human study of the menin-KMT2A (MLL) inhibitor KO-539 in patients with relapsed or refractory acute myeloid leukemia. *Blood*. 2020;136(Supplement 1):7-8.