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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 ($NPM1^{wt}$). *NPM1c* was associated with higher rates of complete remission or complete remission with incomplete count recovery compared with $NPM1^{wt}$ 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 $NPM1^{wt}$ 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.[1,2] 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.[3,4] 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).[5] *NPM1c* frequently co-occur with *FMS*-like *tyrosine kinase 3* (*FLT3*), *isocitrate dehydrogenase* (*IDH) 1* and *IDH2* or *DNA methyltransferase 3 alpha (DNMT3A)* mutations.[6] In newly diagnosed patients, AML with *NPM1c* without a *FLT3*-ITD mutation is associated with high response rates and a favorable prognosis.[7,8] However, this prognosis is significantly affected

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

by the presence of co-occurring mutations.[1,4,6,9] 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.[10-12] 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).[13] 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.[14]

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.[15] 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*^{wt}) (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*^{wt} 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*^{wt} (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.[16,17] 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.[1] In this R/R cohort, *NPM1c* more commonly co-occurred with *DNMT3A* (50% vs 17%; $P < .0001$), *FLT3*-ITD

Table 1. Baseline characteristics

	NPM1c	*NPM1*^{wt}	*P*
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 10^9/L (range)	8.1 (0.1-227)	3.6 (0.1-339)	<.0001
Platelet count, median x 10^9/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
Cytogenetics (194/1451)			<.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)	
Mutations			
DNMT3A (%)	98/195 (50)	245/1412 (17)	<.0001
FLT3-ITD (%)	99/201 (49)	162/1451 (11)	<.0001
TET2 (%)	50/159 (31)	760/1174 (65)	<.0001
IDH1 (%)	40/196 (20)	102/1457 (7)	<.0001
IDH2 (%)	40/198 (20)	160/1455 (11)	.0003
KRAS/NRAS (%)	36/195 (19)	279/1461 (19)	1.0
WT1 (%)	30/156 (19)	111/1065 (10)	.009
FLT3-D835 (%)	25/198 (13)	64/1454 (4)	<.0001
ASXL1 (%)	10/154 (6)	242/1116 (22)	<.0001
TP53 (%)	12/189 (6)	354/1432 (25)	<.0001
RUNX1 (%)	7/155 (5)	244/1102 (22)	<.0001
Lines of therapy			
S1 (%)	132 (64)	953 (63)	.8
S2 (%)	32 (15)	277 (18)	.3
≥S3 (%)	42 (21)	287 (19)	.5
Duration of first remission			
≤ 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*^{wt} (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*^{wt} 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, *NPM1*c 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

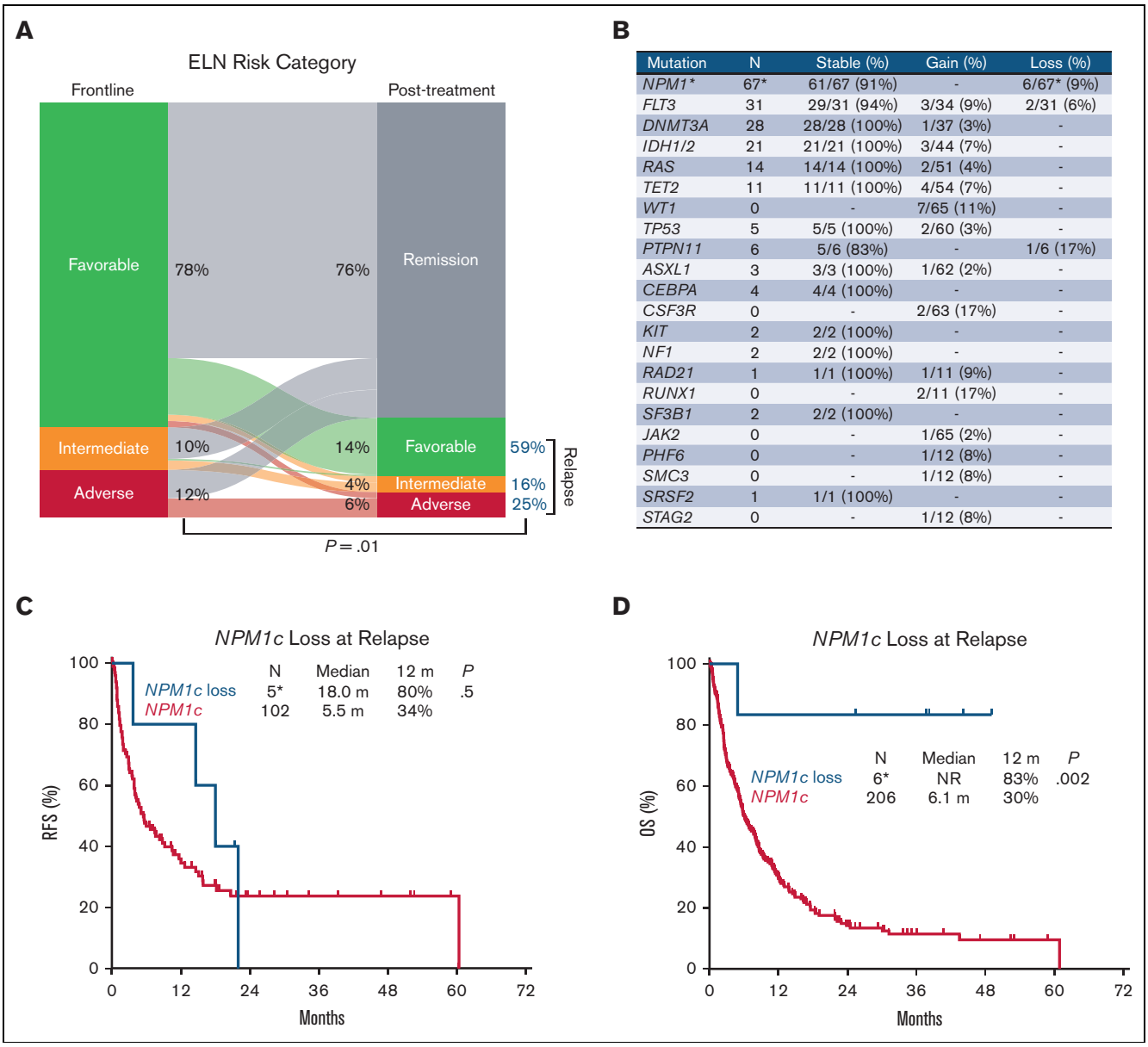
NPM1c is considered a founding leukemia event that is stable throughout the disease process and therefore has been used as a surrogate for MRD.[18] However, in a small fraction of cases, *NPM1c* is lost at relapse.[11,12] Among patients with newly diagnosed AML with *NPM1c*, 3% (6 of 212 patients) relapsed 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.[11]

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*^{wt} 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*^{wt}, 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*^{wt} 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



Mutation	N	Stable (%)	Gain (%)	Loss (%)
*NPM1**	67*	61/67 (91%)	-	6/67* (9%)
FLT3	31	29/31 (94%)	3/34 (9%)	2/31 (6%)
DNMT3A	28	28/28 (100%)	1/37 (3%)	-
IDH1/2	21	21/21 (100%)	3/44 (7%)	-
RAS	14	14/14 (100%)	2/51 (4%)	-
TET2	11	11/11 (100%)	4/54 (7%)	-
WT1	0	-	7/65 (11%)	-
TP53	5	5/5 (100%)	2/60 (3%)	-
PTPN11	6	5/6 (83%)	-	1/6 (17%)
ASXL1	3	3/3 (100%)	1/62 (2%)	-
CEBPA	4	4/4 (100%)	-	-
CSF3R	0	-	2/63 (17%)	-
KIT	2	2/2 (100%)	-	-
NF1	2	2/2 (100%)	-	-
RAD21	1	1/1 (100%)	1/11 (9%)	-
RUNX1	0	-	2/11 (17%)	-
SF3B1	2	2/2 (100%)	-	-
JAK2	0	-	1/65 (2%)	-
PHF6	0	-	1/12 (8%)	-
SMC3	0	-	1/12 (8%)	-
SRSF2	1	1/1 (100%)	-	-
STAG2	0	-	1/12 (8%)	-

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*^{wt} 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*^{wt}, 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*^{wt}; $P = .1$) (Table 2). However, the addition of venetolax 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*^{wt} ($P = .02$). 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*^{wt} ($P = .05$), and an improved OS with a median

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

	All therapies		HI		LI		LI + venetoclax	
	NPM1c	*NPM1*^{wt}	*NPM1c*	*NPM1*^{wt}	*NPM1c*	*NPM1*^{wt}	*NPM1c*	*NPM1*^{wt}
All lines (N)	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)
S1 (N)	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)
S2 (N)	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)
≥S3 (N)	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

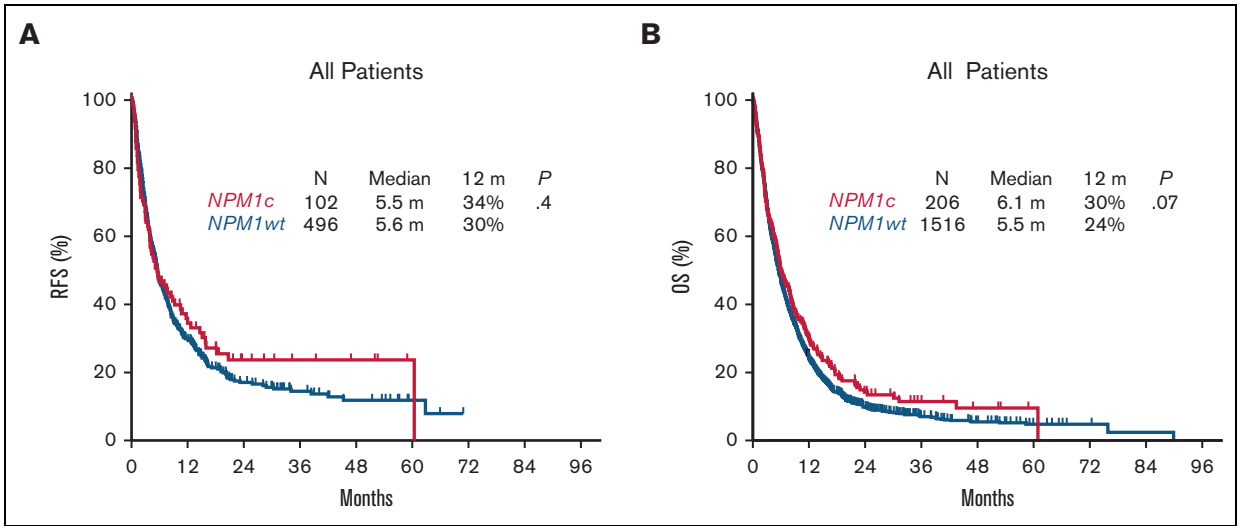


Figure 2. Survival associated with *NPM1* mutational status at AML relapse.

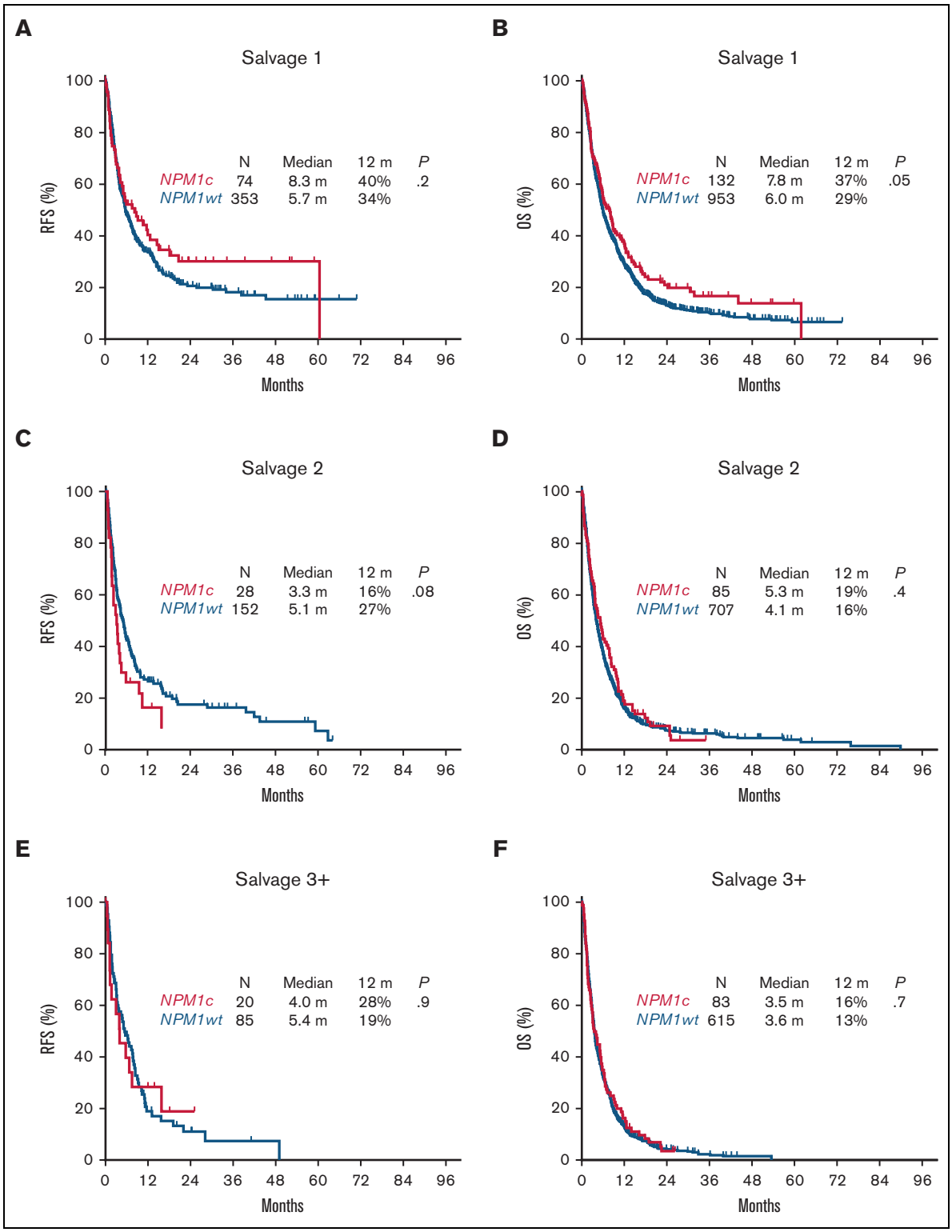


Figure 3. Relapse-free survival and overall survival for patients with relapsed or refractory AML with *NPM1c* by line of therapy. (A) Relapse-free survival following S1. (B) Overall survival following S1. (C) Relapse-free survival following S2. (D) Overall survival following S2. (E) Relapse-free survival 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* comutations. Only 2 patients with *NPM1c* received gemtuzumab ozogamicin, 1 of them achieved CRi (supplemental Tables 5 and 6).

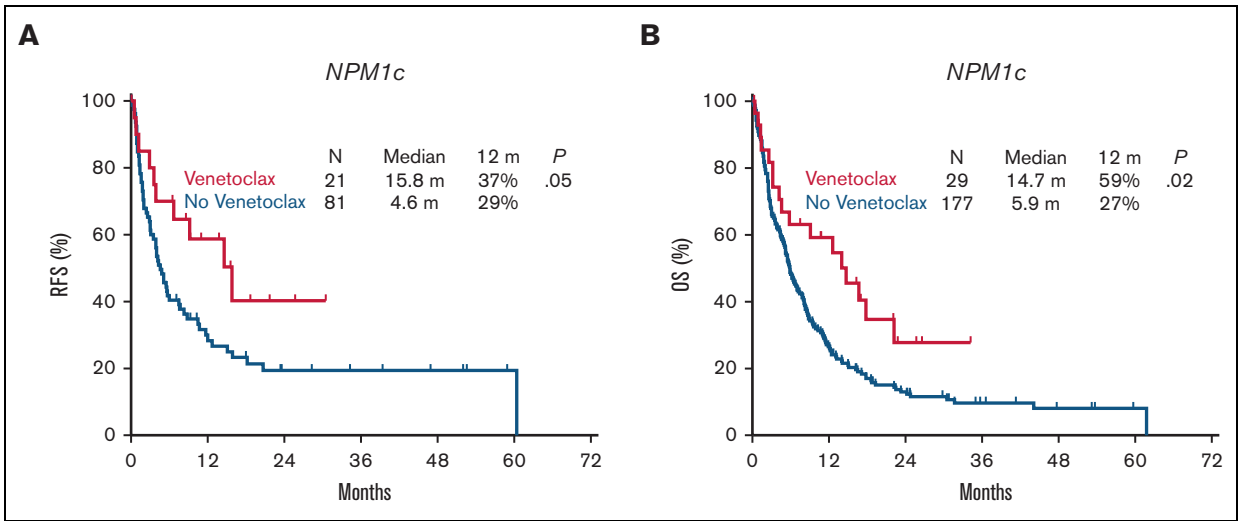


Figure 4. Impact of the addition of venetoclax on survival in relapsed or refractory AML with *NPM1c*.

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*^{wt} 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*^{wt}), 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 venetolcax 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*.[19]

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).[1] 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.[11] 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.[10] 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.[17,20]

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.[10-12] 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.[21,22] Early results from the ongoing phase I trials investigating menin inhibition in this population are encouraging (NCT04065399; NCT04067336; NCT04752163; NCT04811560; NCT05153330; NCT04811560).[23,24]

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.

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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.

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