

# Clinicopathologic and genetic characterization of nonacute *NPM1*-mutated myeloid neoplasms

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## Key Points

- Nonacute *NPM1*-mutated myeloid neoplasms are biologically distinct from nonacute *NPM1* wild-type myeloid neoplasms.
- Nonacute *NPM1*-mutated myeloid neoplasms are associated with poorer survival compared with *NPM1*-mutated AML and *NPM1*-WT myeloid neoplasms.

*NPM1*-mutated myeloid neoplasms (*NPM1*<sup>+</sup> MNs) with <20% blood or bone marrow blasts are rare and have been previously shown in limited case series to exhibit an aggressive clinical course. We assembled the largest cohort of *NPM1*<sup>+</sup> MN cases to date (n = 45) and compared it with *NPM1*<sup>-</sup> MN (n = 95) and *NPM1*<sup>+</sup> de novo acute myeloid leukemia (AML; n = 119) patients. Compared with *NPM1*<sup>-</sup> MN, *NPM1*<sup>+</sup> MN were associated with younger age (*P* = .007), a normal karyotype (*P* < .0001), more frequent mutations involving *DNMT3A* (*P* = .01) and *PTPN11* (*P* = .03), and fewer involving *ASXL1* (*P* = .003), *RUNX1* (*P* = .0004), and *TP53* (*P* = .02). Mutations involving *IDH1* or *IDH2* (*IDH1/2*) (*P* = .007) and *FLT3* (internal tandem duplication, *P* < .0001; noninternal tandem duplication, *P* = .01) were less frequent in *NPM1*<sup>+</sup> MN than in *NPM1*<sup>+</sup> AML. In multivariable analyses performed in patients with myelodysplastic syndrome only, total mutation count (hazard ratio [HR], 1.3; *P* = .05), *NPM1* mutation (HR, 3.6; *P* = .02), *TP53* mutation (HR, 5.2; *P* = .01), and higher International Prognostic Scoring System-R score (HR, 1.7; *P* = .0003) were independently associated with shorter overall survival, whereas stem cell transplant conferred a favorable effect (HR, 0.1; *P* < .0001). These data suggest that *NPM1*<sup>+</sup> MN are biologically distinct from *NPM1*<sup>-</sup> MN. Similar to *NPM1*<sup>+</sup> AML, patients with *NPM1*-mutated myelodysplastic syndrome may benefit from more intensive therapeutic regimens.

## Introduction

Carboxy-terminal insertion mutations in the nucleophosmin-1 (*NPM1*) gene represent the most common somatic mutations identified in de novo acute myeloid leukemia (AML), and are typically associated with a favorable prognosis in the absence of unfavorable clinical parameters or *FLT3* internal tandem duplication (*FLT3*-ITD) comutation.<sup>1-6</sup> Previous studies have reported *NPM1* mutations in 5% to 17% of secondary AML and 1% to 5% of myelodysplastic syndrome (MDS) and/or MDS/myeloproliferative (MDS/MPN) neoplasms.<sup>7-16</sup> In these small series, the presence of an *NPM1* mutation in the setting of a myeloid neoplasm with fewer than 20% blasts (*NPM1*<sup>+</sup> MN) has been associated with aggressive disease and a relatively rapid progression to overt AML. However, most of these studies have interrogated for *NPM1* mutations using limited single-gene assays and have therefore been unable to comprehensively compare the genetic profiles of *NPM1*<sup>+</sup> MN with myeloid neoplasms lacking *NPM1* mutation (*NPM1*<sup>-</sup> MN).

**Table 1. Characteristics of *NPM1*<sup>-</sup> MN, *NPM1*<sup>+</sup> MN, and *NPM1*<sup>+</sup> AML cohorts**

	<i>NPM1</i> <sup>-</sup> MN (n = 95)	<i>NPM1</i> <sup>+</sup> MN (n = 45)	<i>NPM1</i> <sup>+</sup> AML (n = 119)
<b>Patient characteristics</b>			
Median age (range), y	68 (38-84)*	63 (36-96)	61 (15-85)
Male:female	1.9	1.0	0.75
<b>Clinical parameters</b>			
Hemoglobin, median (range), g/dL	9.7 (4.8-15.9)	9.0 (6.1-12.7)	9.0 (5.7-15)
WBC, median (range), ×10 <sup>9</sup> /L	3.5 (0.6-69.4)	3.3 (1.2-225)	21 (0.69-340)*
Platelet count, median (range), ×10 <sup>9</sup> /L	84 (15-808)	79 (15-607)	72 (10-356)
Median of BM cellularity (range), %	70 (10-95)	80 (10-100)	90 (30-98)*
Median of BM blasts (range), %	8 (1-18)	10 (1-19)	73 (21-96)*
<b>Diagnosis, n (%)</b>			
MDS non-EB	5 (5)	2 (4)	NA
MDS-EB	55 (58)	24 (53)	NA
CMMML	16 (17)	9 (20)	NA
MDS/MPN (non-CMML)	8 (8)	5 (11)	NA
t-MN	11 (12)	5 (11)	NA
AML	NA	NA	119 (100)
IPSS-R scores (MDS cases only), median (range)	5.0 (1.0-10.0)	5.0 (1.5-7.0)	NA
<b>Outcome</b>			
Median follow-up time (range), mo	19.4 (0.3-57)	10 (0.07-70)	24 (0.13-125)
Alive at last follow-up, n (%)	53 (56)	29 (64)	67 (56)
Progression to AML, n (%)	30 (32)	20 (44)	NA
Median time to progression (range), mo	6.3 (1.7-43)	5.2 (0.4-17.5)	NA
Received upfront HMA therapy, n (%)	55 (58)	33 (73)	5 (4)
Received upfront induction chemotherapy, n (%)	0 (0)	3 (7)	113 (95)
Received SCT at any time, n (%)	44 (46)	19 (42)	67 (56)

BM, bone marrow; EB, excess blasts; NA, not available; t-MN, therapy-related myeloid neoplasm.  
\**P* < .05 for difference in comparison with *NPM1*<sup>+</sup> MN group (Mann-Whitney *U* test).

De novo AML with mutated *NPM1* (*NPM1*<sup>+</sup> AML) is classically associated with a normal karyotype and frequent mutations in *DNMT3A*, *IDH1/IDH2*, and *RAS* pathway genes, notably *FLT3*.<sup>1,6,17-20</sup> Interestingly, morphologic dysplasia can be seen in *NPM1*<sup>+</sup> AML, although this finding carries no prognostic significance and should not invoke a diagnosis of AML with myelodysplasia-related changes, as mandated by the 2017 World Health Organization (WHO) classification.<sup>1,21</sup> Because it is unknown if *NPM1*<sup>+</sup> MN and *NPM1*<sup>+</sup> AML groups share genetic features, it is conceivable that morphologic dysplasia could be uniformly disregarded in the context of *NPM1*-mutated disease if they are related; moreover, *NPM1*<sup>+</sup> MN may simply represent *NPM1*<sup>+</sup> AML “caught early.”

MDS is the most common type of non-AML myeloid neoplasm, presents at a median age of 70 years, and is often associated with karyotypic abnormalities and/or 1 or more somatic mutations classically involving the “DTA” genes (*DNTM3A*, *TET2*, *ASXL1*), the splicing factor genes *SRSF2* and *SF3B1*, and/or the transcription factor *RUNX1*.<sup>22</sup> Risk stratification in MDS is performed using the revised International Prognostic Scoring System (IPSS-R) with the primary goal of identifying patients who may benefit from hypomethylating agent (HMA) therapy and/or stem cell transplantation (SCT).<sup>23</sup> Whether or not this approach to prognostication and management is appropriate

for *NPM1*<sup>+</sup> MN remains unclear; although *NPM1*-mutated AML has been shown to be sensitive to induction chemotherapy, intensive therapy is not standard treatment of MDS and other non-AML MN.<sup>24,25</sup>

In this study, we assembled the largest known cohort of *NPM1*<sup>+</sup> MN and compared its clinicopathologic and genetic profile with 2 control groups: *NPM1*<sup>-</sup> MN and *NPM1*<sup>+</sup> AML.

## Methods

### Case selection

The pathology archives of 7 major academic medical centers (Brigham and Women’s Hospital/Dana-Farber Cancer Institute, Massachusetts General Hospital, University of Pennsylvania, Vanderbilt University School of Medicine, Weill Cornell Medical College, Memorial Sloan Kettering Cancer Center, and Yale) were queried for cases of *NPM1*<sup>+</sup> MN, defined by the presence of <20% blood or bone marrow blasts in conjunction with a C-terminal insertion mutation involving *NPM1*. Separately, the pathology archive at Brigham and Women’s Hospital/Dana-Farber Cancer Institute was queried to generate a control group of *NPM1*<sup>-</sup> MN patients diagnosed between 2014 and 2018 that were randomly selected to match the WHO Classification subtypes of *NPM1*<sup>+</sup> MN (Table 1).

“Pure” MPNs, defined by the presence of either *JAK2*, *CALR*, or *MPL* mutations in isolation, were excluded. The control cohort was unselected for patient age or other specific mutational profiles. A previously examined cohort of *NPM1*<sup>+</sup> AML was also retrieved for comparison as a second control group.<sup>21</sup> Cytogenetic data were available for all cases. Flow cytometric and cytomorphic data were reexamined in a subset of cases.

### Next-generation sequencing studies

Next-generation sequencing was performed on either diagnostic blood or bone marrow samples, and data were available for all cases. All sequencing panels (n = 8) included the following common set of genes: *DNMT3A*, *IDH1*, *IDH2*, *TET2*, *ASXL1*, *KRAS*, *NRAS*, *FLT3*, *PTPN11*, *RUNX1*, *SF3B1*, and *TP53*. A large subset of *NPM1*<sup>+</sup> MN (40/45), and all *NPM1*<sup>-</sup> MN and *NPM1*<sup>+</sup> AML cases were also evaluated for mutations involving *SRSF2*.

### Statistical analyses

Continuous variables including patient age, white blood cell count (WBC), bone marrow blast percentage, IPSS-R scores, total mutation number at diagnosis, and median time to leukemic transformation were compared between cohorts using the Mann-Whitney *U* test. Rates for abnormal karyotype and specific somatic mutations were compared using Fisher's exact test. Statistical significance was set at *P* < .05.

We evaluated patients' overall survival (OS) as previously reported.<sup>26</sup> Briefly, OS was defined as the time in months from the date of initial diagnosis to last follow-up, or death. Outcome profiles of the study cohorts were compared using Kaplan-Meier curves. Multivariable analyses were performed using a cross-cohort subset of only those patients diagnosed with subtypes of MDS (26 *NPM1*<sup>+</sup>, 60 *NPM1*<sup>-</sup>; n = 86). Backward elimination was performed on the following variables until only those associated with *P* < .05 remained in the model: age >60 years, IPSS-R score (continuous), total mutations at diagnosis (continuous), upfront HMA therapy, receipt of SCT at any time, presence of mutations in *NPM1*, *DNMT3A*, *TET2*, *ASXL1*, *SRSF2*, *RUNX1*, and *TP53* (each found at >10% of the MDS subgroup).

Statistical analyses were performed using XLSTAT (v2018.7) and Prism 8.0c (GraphPad) software packages.

## Results

### Patient characteristics

We identified 45 *NPM1*<sup>+</sup> MN, 95 *NPM1*<sup>-</sup> MN, and 119 *NPM1*<sup>+</sup> AML. Clinicopathologic features of these cohorts are shown in Table 1. Compared with *NPM1*<sup>-</sup> MN, *NPM1*<sup>+</sup> MN were associated with younger patient age (*P* = .007), and most (73%) received upfront HMA therapy. There were no statistically significant differences between *NPM1*<sup>-</sup> MN and *NPM1*<sup>+</sup> MN cohorts with respect to other variables shown, including rates of leukemic progression and median time to progression (*P* > .05). Compared with *NPM1*<sup>+</sup> AML, *NPM1*<sup>+</sup> MN were associated with lower WBC (*P* < .0001) and bone marrow cellularity (*P* = .005) at diagnosis, and only 3/45 patients received upfront induction chemotherapy.

**Table 2. Genetic features of *NPM1*<sup>-</sup> MN, *NPM1*<sup>+</sup> MN, and *NPM1*<sup>+</sup> AML cohorts**

	<i>NPM1</i> <sup>-</sup> MN (n = 95)	<i>NPM1</i> <sup>+</sup> MN (n = 45)	<i>NPM1</i> <sup>+</sup> AML (n = 119)
<b>Comutations, by pathway, n (%)</b>			
<i>DNMT3A</i>	14 (15)*	15 (33)	57 (48)
<i>IDH1</i>	22 (2)	4 (9)*	25 (21)*
<i>IDH2</i>	5 (5)	2 (4)*	16 (13)*
<i>TET2</i>	29 (31)	7 (16)	32 (27)
<i>ASXL1</i>	30 (32)*	4 (9)	3 (3)
<i>RUNX1</i>	20 (21)*	0 (0)	1 (0.8)
<i>KRAS</i>	3 (3)	1 (2)†	4 (3)†
<i>NRAS</i>	8 (8)	4 (9)†	27 (23)†
<i>FLT3</i> (non-ITD)	1 (1)	3 (7)	29 (24)‡
<i>FLT3</i> -ITD	1 (1)	1 (2)	36 (30)‡
<i>PTPN11</i>	2 (2)*	6 (13)	25 (21)
<i>SRSF2</i>	20 (21)	3 (8)	9 (8)
<i>SF3B1</i>	9 (9)	4 (9)	1 (0.8)
<i>TP53</i>	15 (16)*	1 (2)	0 (0)
Mutation count, median (range)	2 (0-8)	2 (1-7)	4 (1-8)§
Abnormal karyotype, n (%)	57 (61)*	5 (12)	18 (16)

\**IDH1* and *IDH2* are grouped for the analysis comparing *NPM1*<sup>+</sup> MN with *NPM1*<sup>+</sup> AML (Fisher's exact test).

†*KRAS* and *NRAS* are grouped for the analysis comparing *NPM1*<sup>+</sup> MN with *NPM1*<sup>+</sup> AML (Fisher's exact test).

‡Significantly different compared with *NPM1*<sup>+</sup> MN (Fisher's exact test).

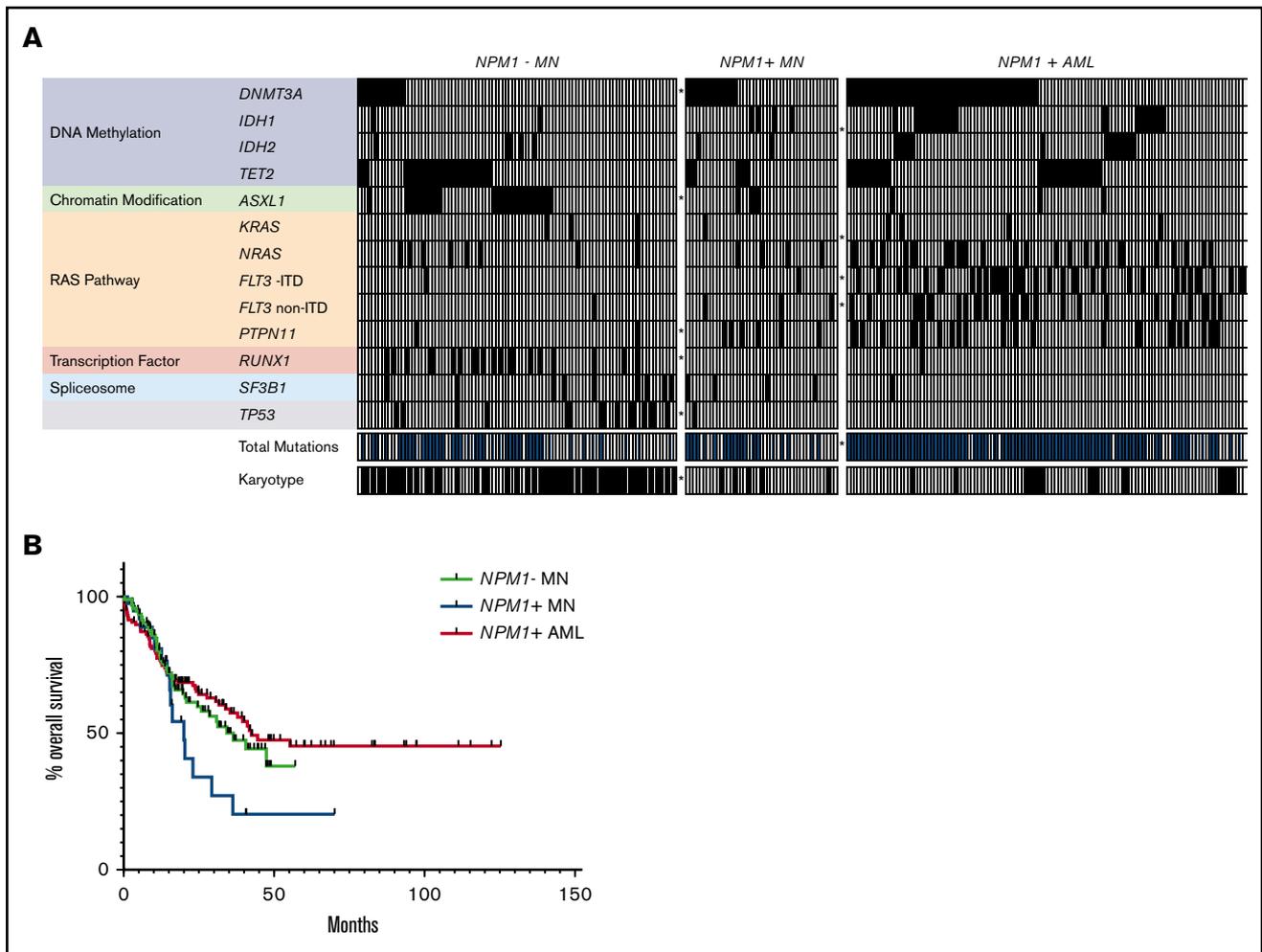
§*P* < .05 for difference compared with *NPM1*<sup>+</sup> MN (Mann-Whitney *U* test).

### Immunophenotypic findings

Data from flow cytometric immunophenotyping performed on bone marrow aspirate material at the time of diagnosis were reexamined for a subset of the study cohort (n = 13), specifically with respect to CD34 expression on myeloid blasts. Out of this subset, CD34 was positive in 5 cases, negative in 5, and variable in 1. One case had <1% myeloid blasts, but an increased population of immunophenotypically abnormal immature monocytes (CD16<sup>+</sup>CD64<sup>+</sup>CD56<sup>+</sup>). The median blast count was 8% in 5 cases with CD34<sup>+</sup> blasts and 10% in 5 cases that were CD34<sup>-</sup>.

### Cytogenetic and comutational variables

*NPM1*<sup>+</sup> MN cases were significantly associated with a normal bone marrow karyotype as compared with *NPM1*<sup>-</sup> MN (88% vs 39%, *P* < .0001), at a rate similar to *NPM1*<sup>+</sup> AML (84%). Compared with the *NPM1*<sup>-</sup> MN, *NPM1*<sup>+</sup> MN more frequently harbored mutations involving *DNMT3A* (15/45 vs 14/95, *P* = .01) and *PTPN11* (5/45 vs 2/95, *P* = .03), but fewer in *ASXL1* (4/45 vs 30/95, *P* = .003), *RUNX1* (0/45 vs 20/95, *P* = .0004), and *TP53* (1/44 vs 15/95, *P* = .02) (Table 2; Figure 1A). They also exhibited a trend toward fewer *TET2* (7/45 vs 29/95, *P* = .06) and *SRSF2* (3/40 vs 20/95, *P* = .08) mutations. Of note, 2/5 *NPM1*<sup>+</sup> MN diagnosed as therapy-related myeloid neoplasms had a noncomplex abnormal karyotype, and 1/5 harbored a *TP53* mutation. Compared with *NPM1*<sup>+</sup> AML, *NPM1*<sup>+</sup> MN were associated with fewer mutations in *IDH1* or *IDH2* (*IDH1/2*) (6/45 vs 41/119, *P* = .007) and *FLT3* (ITD: 1/44 vs 36/119, *P* < .0001; non-ITD: 3/45 vs 29/119, *P* = .01), and



**Figure 1. Genetic and clinical outcome characteristics of  $NPM1^-$  MN,  $NPM1^+$  MN, and  $NPM1^+$  AML.** (A) Heatmap of commonly evaluated genes, cytogenetic findings, and total mutational counts across all cases of  $NPM1^-$  MN (n = 95),  $NPM1^+$  MN (n = 45), and  $NPM1^+$  AML (n = 119). Each column represents an individual patient. All mutations (filled squares = mutation identified) and cytogenetic findings (filled squares = identified deviation from 46,XX or 46,XY) in binary format. Total mutation count per case stratified at greater than (filled square) or less than or equal to 2, by cohort as follows:  $NPM1^-$  MN (46/95),  $NPM1^+$  MN (21/45), and  $NPM1^+$  AML (101/119). \*Statistically significant differences in proportions between flanking groups ( $P < .05$ , Fisher's exact test). (B)  $NPM1^+$  MN (n = 45) exhibit shorter median OS (20 months) than  $NPM1^-$  MN (36.6 months, n = 95), and  $NPM1^+$  AML (42.4 months, n = 119).

showed a trend toward fewer in *KRAS* or *NRAS* (*KRAS/NRAS*) (5/45 vs 31/119,  $P = .06$ ).

### Clinical outcome

Comparison of all 3 study cohorts revealed shorter OS for  $NPM1^+$  MN (20 months) as compared with  $NPM1^-$  MN (36.6 months) and  $NPM1^+$  AML (42.4 months) (Figure 1B). Of 3  $NPM1^+$  MN patients who received upfront induction chemotherapy, none progressed to AML, 2/3 were alive at last follow-up, and 1 died (OS, 20 months). Of 33  $NPM1^+$  MN patients who received upfront HMA therapy, 13 (39%) progressed to AML at a median time of 5.9 months, and 12 ultimately succumbed to their disease. Five of the 13 patients who progressed subsequently received intensive chemotherapy; 3 ultimately died (OS, 20, 23, and 36 months). All 5  $NPM1^+$  MN patients with at least 2 months of follow-up who did not receive either upfront HMA or intensive chemotherapy progressed to AML at a median time of 3 months.

In multivariable analyses performed in patients with a diagnosis of MDS only (26  $NPM1^+$  and 60  $NPM1^-$ , n = 86), total mutation count (hazard ratio [HR], 1.3;  $P = .05$ ), presence of mutations in *NPM1* (HR, 3.6;  $P = .02$ ), or *TP53* (HR, 5.2;  $P = .01$ ), and higher *IPSS-R* score (HR, 1.7;  $P = .0003$ ) were factors independently associated with shorter OS, whereas SCT status conferred a favorable effect (HR, 0.1;  $P < .0001$ ).

### Discussion

In this study, we have assembled and performed a comprehensive clinicopathologic and genetic characterization of the largest known cohort of non-AML *NPM1*-mutated myeloid neoplasms. Most of the cases in our cohort were categorized as either high-grade MDS or chronic myelomonocytic leukemia (CMML) based on current WHO criteria. Our results confirm previously reported findings from limited case series that have correlated the presence of a somatic exon 12 *NPM1* insertion mutation with relatively short

latency before leukemic transformation. Interestingly, we were unable to establish a statistically significant difference in rate of leukemic transformation, or time to transformation, between our *NPM1*<sup>+</sup> MN and *NPM1*<sup>-</sup> MN cohorts. Based on 5 patients who did not receive any upfront therapy and whose disease quickly transformed (median, 3 months), we hypothesize that upfront HMA therapy in the majority of our *NPM1*<sup>+</sup> MN patients may have altered the natural biology and time course of the underlying disease, delaying what might have been more rapid progression to overt AML.

Repeat examination of immunophenotypic findings in a subset of our *NPM1*<sup>+</sup> MN cases revealed heterogeneity in CD34 expression on myeloid blasts, without any clear correlation with the bone marrow blast count or specific WHO diagnostic subtype of disease (data not shown); in contrast, blasts in MDS are classically CD34<sup>+</sup>.<sup>1</sup> The distinct genetic features of our *NPM1*<sup>+</sup> MN group, with poor outcomes despite predominantly normal karyotypes and significantly fewer cooccurring *ASXL1* or *TP53* mutations, suggest that *NPM1*<sup>+</sup> MN are indeed biologically distinct from *NPM1*<sup>-</sup> MN. It is additionally interesting that *ASXL1* and *RUNX1* mutations, although over-represented in our *NPM1*<sup>-</sup> MN cohort, did not exhibit a statistically significant correlation with adverse outcome (data not shown), as has been reported in the context of AML.

Despite some differences from *NPM1*<sup>+</sup> AML, including fewer *IDH1/2* or *FLT3* mutations and lower WBC and bone marrow cellularity at diagnosis, *NPM1*<sup>+</sup> MN were associated with similar rates of abnormal karyotype and select gene mutations (eg, *DNTM3A*, *ASXL1*, *RUNX1*, *SRSF2*). These findings raise the possibility that patients with *NPM1*<sup>+</sup> non-AML MN may benefit from a more intensive therapeutic approach

more akin to *NPM1*<sup>+</sup> AML, when appropriate. Certainly, HMA therapy, at least when coupled with venetoclax, has shown efficacy in *NPM1*-mutated disease<sup>27</sup>; however, we observed overall poor outcomes in our *NPM1*<sup>+</sup> MN cohort, in which the majority of patients received upfront HMA therapy. Of note, 3 patients received upfront induction chemotherapy, and none of them progressed to AML.

Although we have assembled the largest known cohort of *NPM1*<sup>+</sup> non-AML MN, this study is limited by its retrospective nature and the lack of a controlled clinical trial design. Our results might suggest that upfront HMA therapy may have been inadequate in some patients; however, OS can indeed be influenced by many factors, including natural history of the disease, sequential therapy, and SCT. Therefore, confirmation of these findings in the context of a prospective study will be important.

## Authorship

Contribution: S.S.P. and O.K.W. designed the study, analyzed the data, and wrote the manuscript; C.H., R.N.P., S.S., A.B., J.T.G., M.L.X., T.P., E.F.M., A.C.S., E.A.M., D.P.S., E.S.W., W.J.W., and R.P.H. contributed patients and diagnostic data; and all authors reviewed and approved the final manuscript.

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