Published in final edited form as:

Am J Hematol. 2017 October ; 92(10): E614–E618. doi:10.1002/ajh.24861.

Nucleophosmin 1 (*NPM1*) Mutations in Chronic Myelomonocytic Leukemia and Their Prognostic Relevance

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Keywords

NPM1; CMML; Prognosis

To the Editor

Chronic myelomonocytic leukemia (CMML) is an aggressive hematologic malignancy with overlapping features of myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN).[1, 2] Patients with CMML, display between 10-15 mutations per kilobase of coding DNA regions; with majority of these (>90%) involving epigenetic regulator genes (*TET2* 60%, *ASXL1* 40%), splicing machinery (*SRSF2* 40%) and cell signalling (oncogenic *RAS* pathway 30%).[3-5] Nucleophosmin 1 (*NPM1*, chromosome 5q35) is a gene encoding for the NPM1 protein that primarily resides in the nucleus, but has the ability to shuttle between the nucleus and the cytoplasm.[6] *NPM1* is required for critical cellular functions including; ribosome biogenesis, regulation of genomic stability, p53/MDM2 (mouse double minute 2 homolog) dependant stress response and the modulation of growth suppressive pathways.[6] *NPM1* mutations are seen in $\approx$ 30% of patients with acute myeloid leukemia (AML) with normal cytogenetics, with common phenotypic associations being; high bone marrow (BM) blasts, high white blood cell counts, reduced CD34 expression and the presence of extramedullary manifestations.[6, 7] The presence of *NPM1* mutations in the absence of *FLT3-ITD* (fms related tyrosine kinase 3- internal tandem duplications) in cytogenetically normal AML is associated with favorable outcomes. [8] *NPM1* mutations are infrequent in CMML (<5%) and MDS (<5%) and have an unclear prognostic impact.[9-11] We carried out this study to assess the i) frequency and clinical correlates, ii) prognostic impact and iii) survival outcomes related to *NPM1* mutations in CMML.

Patients were selected according to the 2016 World Health Organization (WHO) diagnostic criteria for CMML and identified from the institutional database.[1] All patients had BM

biopsies and cytogenetic studies performed at diagnosis. A 29 gene panel next generation sequencing assay was carried out on BM DNA specimens obtained at diagnosis for the following genes; *TET2*, *DNMT3A*, *IDH1*, *IDH2*, *ASXL1*, *EZH2*, *SUZ12*, *SRSF2*, *SF3B1*, *ZRSR2*, *U2AF1*, *PTPN11*, *Tp53*, *SH2B3*, *RUNX1*, *CBL*, *NRAS*, *KRAS*, *JAK2*, *CSF3R*, *FLT3*, *KIT*, *CALR*, *MPL*, *NPM1*, *CEBPA*, *IKZF1*, *ETNK1* and *SETBP1*, by previously described methods.[5, 11] Statistical analyses considered parameters obtained at time of CMML diagnosis. Differences in the distribution of continuous variables between categories were analyzed by either Mann-Whitney or Kruskal-Wallis tests. Patient groups with nominal variables were compared by chi-square test. Over-all survival (OS) was calculated from the date of first referral to date of death or last contact. Leukemia-free survival (LFS) was calculated from the date of first referral to date of leukemic transformation or death/last contact. OS and LFS curves were prepared by the Kaplan-Meier method and compared by the log-rank test. Cox proportional hazard regression model was used for multivariable analysis.

A total of 373 WHO-defined CMML patients were included in the study; median age 71 years (range, 20-95), 66% males. *NPM1* mutations were identified in 8 (2%) patients (table 1). All (100%) *NPM1* mutations occurred in patients with normal cytogenetics, involved exon 12, with 4 of 8 (50%) being the classical type A *NPM1* mutations resulting in a duplication of TCTG (nucleotides 956-959) creating a 4 base pair insertion at position 960. Morphological subtypes included; CMML-0 2 (25%), CMML-1 3 (38%) and CMML-2 3 (38%). Risk stratification by the Mayo Molecular Model included; intermediate-1 4 (50%) and high risk 4 (50%). [4] In comparison to their wild type counterparts, *NPM1* mutant CMML patients were more likely to be anemic ($p=0.02$), have a “dysplastic CMML subtype” (87%, $p=0.04$), have an increased incidence of *DNMT3A* (50%, $p<0.0001$), *FLT3* ITD (13%, $p=0.04$) and a lower frequency of *TET2* (13%, $p=0.03$) and *ASXL1* (13%, $p=0.03$) mutations.

At last follow up (median, 14 months), 249 (67%) deaths and 69 (18%) leukemic transformations were documented. Median survival in *NPM1* mutated CMML patients was 12.5 months vs. 20.5 months in their wild type counterparts ($p=0.12$, figure 1A). Five of 8 (62%) *NPM1* mutated CMML patients underwent blast transformation (BT), at a median of 5 months (range, 1-16). In 2 (of 5) evaluable patients, *NPM1* mutations were detected at both, CMML diagnosis and at BT, without additional cytogenetic or molecular clonal evolution. Four (of 5) patients with BT were treated with AML-like induction chemotherapy, with 2 patients subsequently undergoing allogeneic stem cell transplantation (HCT). Median survival after BT was 3 months (range, 1-12), with only one patient that underwent HCT being alive at last follow up. In univariate analysis, LFS was adversely impacted by age ($p<0.0001$), low hemoglobin ($p=0.0009$), high absolute lymphocyte count ($p=0.02$), higher serum LDH (lactate dehydrogenase) levels ($p=0.023$), PB blast % ($p<0.0001$), BM blast % ($p<0.0001$), 2016 WHO morphological subtype CMML-2 ($p<0.0001$), abnormal cytogenetics ($p=0.002$), Mayo-French cytogenetic risk stratification ($p=0.01$), [12] *NPM1* ($p=0.0004$) and *Tp53* ($p=0.002$) mutations. *ASXL1*, *DNMT3A*, *TET2* and *FLT3* ITD mutations did not impact LFS; in multivariable analysis that included the aforementioned significant variables, only PB blasts % ($p<0.0001$, HR 1.2, 95% CI 1.13-1.3), *NPM1*

mutations ($p=0.03$, HR 4.7, 95% CI 1.7-12.8) and *Tp53* mutations ($p=0.001$, HR 5.4, 95% CI 1.6-18.4) remained significant.

The current study confirms the infrequent occurrence of *NPM1* mutations in CMML; also, the number of informative cases was large enough to allow demonstration of clustering of *NPM1* mutations in CMML with normal cytogenetics, “dysplastic CMML phenotype” and *DNMT3A* mutations. More importantly, we show a significantly higher risk of blast transformation in patients with CMML harboring *NPM1* mutations.

Acknowledgments

Current publication is supported in part by grants from the “The Henry J. Predolin Foundation for Research in Leukemia, Mayo Clinic, Rochester, MN, USA”.

This publication was supported by CTSA Grant Number KL2 TR000136 from the National Center for Advancing Translational Science (NCATS). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

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Figure 1A.

Overall survival of 373 patients with chronic myelomonocytic leukemia stratified by their *NPM1* mutational status.

Figure 1B.

Leukemia-free survival of 373 patients with chronic myelomonocytic leukemia stratified by their *NPM1* mutational status.

Table 1
Comparison of clinical and laboratory characteristics of 373 chronic myelomonocytic leukemia (CMML) patients obtained at time of diagnosis, stratified by the presence or absence of *NPM1* mutations

Variables	All patients with CMML (<i>n</i> =373)	CMML Patients with <i>NPM1</i> mutations (<i>n</i> =8)	CMML patients without <i>NPM1</i> mutation (<i>n</i> =365)	P-value
Age in years; median (range)	71 (20-95)	76 (48-87)	71 (20-95)	0.8
Males; <i>n</i> (%)	246 (66%)	4 (50%)	242 (66%)	0.34
Hemoglobin, g/dL; median (range)	10.6 (1.4-17)	9.5 (7.4-10.6)	10.6 (1.4-17)	0.02
WBC $\times 10^9$ /L; median (range)	12.2 (1.3-265)	11 (3.7-186)	12 (1.3-265)	0.56
ANC $\times 10^9$ /L; median (range)	5.8 (0-151)	5.6 (2.9-143)	5.8 (0-151)	0.63
ALC $\times 10^9$ /L; median (range)	1.8 (0-22)	3.2 (0.4-6)	1.7 (0-22)	0.3
AMC $\times 10^9$ /L; median (range)	2.5 (1.0-84)	1.7 (1.2-30)	2.5 (1.0-84)	0.6
Platelets $\times 10^9$ /L; median (range)	96 (7-1184)	80 (23-293)	96 (7-1184)	0.62
Presence of circulating immature myeloid cells; <i>n</i> (%)	155 (42%)	3 (38%)	152 (42%)	0.8
PB blast %; median (range)	0 (0-18)	1 (0-12)	0 (0-18)	0.19
BM blast % ; median (range)	2 (0-21)	5 (0-15)	2 (0-21)	0.47
Lactate dehydrogenase levels IU/ml; median (range)	225 (84-1824)	285 (153-696)	225 (84-1824)	0.41
Cytogenetics (<i>n</i> =344) Abnormal; <i>n</i> (%)	105 (32%)	0 (0%)	105 (31%)	0.06
FAB; <i>n</i> (%)				
Proliferative	176 (47%)	1 (13%)	175 (48%)	0.047
Dysplastic	197 (53%)	7 (87%)	190 (52%)	
Next generation sequencing analysis; <i>n</i> (%)				
1. Epigenetic regulators				
<i>TET2</i>	116 (51%)	1 (13%)	115	0.03
<i>DNMT3A</i>	15 (7%)	4 (50%)	11 (5%)	<0.0001
<i>IDH1</i>	3 (1%)	0	3 (1%)	0.74
<i>IDH2</i>	13 (6%)	0	13 (6%)	0.5
2. Chromatin regulators				
<i>ASXL1</i>	113 (50%)	1 (13%)	112 (51%)	0.03
<i>EZH2</i>	6 (3%)	0	6 (3%)	0.63
3. Transcription factors				
<i>RUNX1</i>	21 (9%)	0	21 (10%)	0.4

Variables	All patients with CMML (n=373)	CMML Patients with NPM1 mutations (n=8)	CMML patients without NPM1 mutation (n=365)	P-value
4. Spliceosome components				
<i>SF3B1</i>	11 (5%)	1 (13%)	10 (5%)	0.3
<i>SRSF2</i>	98 (43%)	2 (25%)	97 (44%)	0.3
<i>U2AF1</i>	18 (8%)	1 (13%)	17 (8%)	0.62
<i>ZRSR2</i>	4 (2%)	0	4 (2%)	0.7
5. Cell signaling				
<i>JAK2 V617F</i>	18 (7%)	0	18 (7%)	0.43
<i>MPL</i>	2 (1%)	0	2 (1%)	0.79
<i>CBL</i>	29 (13%)	0	29 (13%)	0.27
<i>NRAS</i>	31 (14%)	2 (25%)	29 (13%)	0.33
<i>KRAS</i>	12 (5%)	0	11 (5%)	0.52
<i>PTPN11</i>	7 (3%)	0	6 (3%)	0.64
<i>CSF3R</i>	3 (1%)	0	3 (1%)	0.74
<i>C-KIT</i>	10 (4%)	0	10 (5%)	0.54
<i>FLT3</i>	5 (2%)	1 (13%)	4 (2%)	0.04
<i>CALR</i>	1 (0.5%)	0	1 (0.5%)	0.85
6. Tumor suppressor genes				
<i>Tp53</i>	6 (3%)	0	6 (3%)	0.64
7. Others				
<i>SETBP1</i>	28 (12%)	0	28 (13%)	0.28
<i>ETNK1</i>	1 (0.5%)	0	1 (0.5%)	0.85
Spanish Cytogenetic risk stratification; n (%) (n=343)				
Low	242 (71%)	8 (100%)	235 (70%)	0.18
Intermediate	69 (20%)	0	69 (21%)	
High	32 (9%)	0	31 (9%)	
Mayo-French cytogenetic risk stratification; n (%) (n=343)				
Low	242 (71%)	8 (100%)	235 (70%)	0.19
Intermediate	80 (23%)	0	79 (24%)	
High	21 (6%)	0	21 (6%)	
Mayo prognostic model (n=372)				
Low	91 (24%)	2 (25%)	89 (24%)	0.76
Intermediate	136 (36%)	2 (25%)	134 (37%)	
High	145 (40%)	4 (50%)	141 (39%)	
MDAPS (n=372)				
Low	159 (43%)	1 (13%)	159 (44%)	0.17
Intermediate-1	113 (30%)	4 (50%)	109 (30%)	
Intermediate-2	88 (24%)	2 (25%)	85 (23%)	

Variables	All patients with CMML (<i>n</i> =373)	CMML Patients with <i>NPM1</i> mutations (<i>n</i> =8)	CMML patients without <i>NPM1</i> mutation (<i>n</i> =365)	P-value
High	12 (3%)	1 (13%)	11 (3%)	
GFM CMML prognostic model (<i>n</i> =371)				
Low	174 (47%)	6 (74%)	168 (46%)	0.24
Intermediate	143 (38%)	1 (13%)	142 (39%)	
High	54 (15%)	1 (13%)	53 (15%)	
MMM (<i>n</i> =371)				
Low	36 (10%)	0	36 (10%)	0.16
Intermediate-1	121 (33%)	4 (50%)	117 (32%)	
Intermediate-2	107 (29%)	0	107 (29%)	
High	107 (29%)	4 (50%)	103 (28%)	
Leukemic transformations; <i>n</i> (%)	69 (18%)	5 (63%)	64 (18%)	0.0012
Deaths; <i>n</i> (%)	249 (67%)	6 (75%)	243 (67%)	0.62
Median follow up in months; median (range)	13.6 (0-169)	9.4 (0.3-41)	13.8 (0-169)	0.22

Key: CMML- chronic myelomonocytic leukemia, *NPM1* nucleophosmin 1, WBC, white blood cell count; ANC- absolute neutrophil count, ALC- absolute lymphocyte count, AMC, absolute monocyte count; PB, peripheral blood; BM, bone marrow; FAB- French American British Classification, EM, extramedullary involvement; MDAPS, MD Anderson Prognostic Scoring System; GFM- Groupe Français des Myélodysplasies, MMM- Mayo Molecular Model.