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Mutated Nucleophosmin-1 (*NPM1*) in patients with Acute Myeloid Leukemia (AML) in remission and relapse

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Abstract

Patients with newly diagnosed AML (n=360) including 137 (38%) with normal karyotype (NK) were evaluated. Overall, 60 (16.6%) patients including 46 of the 137 (33.5%) NK patients had *NPM1* mutation at baseline. Thirty nine patients (30 NK) had available *NPM1* status at the time of CR and all (100%) were negative for mutated *NPM1*. Among the patients with mutated *NPM1* at baseline, 10/39 overall (25%) and 7/30 NK (23%) patients relapsed. *NPM1* status was available for 8 patients (6 with NK) at the time of relapse. Among them, 7/8 overall (87%) and 5/6 NK (83%) patients had mutated *NPM1*, while 1/8 overall (12%) and 1/6 NK (16%) patients remained *NPM1* wild type. Among the 300 patients (including 91 with NK) with wild type *NPM1* at diagnosis, none acquired a mutated *NPM1* clone, either at CR or at relapse. We conclude that mutated *NPM1* is a stable and reliable prognostic marker in AML and can be used to assess MRD.

Keywords

AML; *NPM1* mutations; minimal residual disease; MRD

INTRODUCTION

The clinical course and response to therapy in patients with acute myeloid leukemia (AML) is largely dictated by the presence or absence of specific genomic aberrations and mutations.

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AUTHORSHIP

P.J. and F.R. designed the research.
J.C., S.F., T.K., G.B., N.D., N.P., H.K., and F.R. contributed patient samples.
K.P. and R.L. performed the *NPM1* mutational analysis.
P.J., S.P., and F.R. analyzed and interpreted the data.
P.J., O.B. and F.R. wrote the manuscript.
All authors reviewed and gave the final approval for the paper.

DISCLOSURES

Authors declare no relevant conflict of interest.

[1–3] Relapse continues to be a major cause of failure to achieve long term disease-free survival using available treatment strategies.[4] Recently, several groups have identified a number of recurring mutations in patients with *de novo* AML.[5–7] The suitability of these mutations as a marker of minimal residual disease (MRD) is being studied.[7, 8] Molecular markers that are reliably stable during the disease course and clonal evolution are sought after as markers for MRD detection. Mutations in Nucleophosmin-1 (*NPM1*) gene have been described in about 35% of adult patients with de novo AML and 45–60% of patients with a normal karyotype (NK).[9] Mutations in exon 12 are considered to be the most frequent mutations in AML (>50%) patients with normal karyotype (NK).[9] Since *NPM1* mutation is considered as a founder mutation in AML leukemogenesis, its prognostic impact has been evaluated by a number of groups.[9–15] Furthermore, the most recent WHO classification of myeloid neoplasms considers *NPM1* mutated AML as a separate entity.[16–18] Among patients with NK AML, the presence of *NPM1* mutations predicts for a higher likelihood of achieving complete remission (CR), lower relapse rates and better overall outcomes. [10] [19–22] NPM1 is a phosphoprotein encoded by a gene on chromosome 5. It is thought to play a role in various intracellular processes [23] such as ribosomal assembly, shuttling or transport,[24, 25] DNA repair, stress responses and protection against P53 induced apoptosis.[26, 27] The cytoplasmic localization of the mutant *NPM1* is considered to be the key event in inducing intracellular signaling pathways, although this localization is not always concordant with the presence of the mutations.[28] *NPM1* mutations have also been shown to induce CD4 and CD8 T cell responses and are being explored as an immunotherapeutic target.[29] Cup like nuclei are recognized as a common feature of the AML blasts from patients with AML and *NPM1* and/or *FLT3* mutations.[14]

Different techniques have been used to analyze *NPM1* mutations including RNA or DNA based real time quantitative polymerase chain reaction (RQ-PCR), [8] imaging flow cytometry, [30] and next generation sequencing.[31] Few prior studies have examined the role of *NPM1* mutations as markers for MRD assessment. [8, 32, 33] In these reports, the investigators examined the presence of *NPM1* mutations in paired samples at the diagnosis, and at the time of relapse. [8, 32–34] Kronke et al reported that among 245 patients with AML aged < 60 years, early detection of relapse was possible in patients with >200 *NPM1* mutation/10^4 ABL copies (n=36) as assessed by real time PCR.[8] However, 9% of the relapsed samples did not contain a mutant *NPM1* clone in this study. In another study of paired samples (at diagnosis and at relapse) from 84 patients with *NPM1* mutated AML, *NPM1* was found to be expressed at high levels (2 log range) at the time of relapse and was a stable MRD marker.[32] Two other studies have suggested that *NPM1* mutations may not reliably recur at the time of relapse.[19, 20]

We have conducted this study to assess the prognostic significance of *NPM1* mutations in patients with AML at the diagnosis, at CR and at the time of relapse in our single institute database.

PATIENTS AND METHODS

Population studied

We conducted a retrospective analysis of patients (n=360, NK; n=137) with newly diagnosed AML who underwent testing for *NPM1* status and who were treated at our institution between 2008 and 2012 (patients with acute promyelocytic leukemia were excluded). *NPM1* mutations were detected in 60 (16.6%) patients and were undetectable in the other 300. All patients were treated on frontline induction protocols and had bone marrow biopsy and/or aspiration, cytogenetic, and molecular studies at the time of diagnosis. Cytogenetic and molecular studies at complete remission (CR) and relapse were performed at the discretion of the treating physician. All patients signed an informed consent for participation and the trials were conducted in accordance with the Declaration of Helsinki. All studies have been approved by the Institutional Review Board of the University of Texas - MD Anderson Cancer Center. CR and relapse were defined as described previously.[35] The available bone marrow samples at diagnosis, CR and first relapse were reviewed for the presence of *NPM1* mutated clones (Figure 1A–B). A subset of *NPM1* mutated patients were *FLT3*-ITD positive n=24 (41%) and were also analyzed for time to event variables.

Detection of *NPM1* Mutations

NPM1 mutation status was determined from DNA from unsorted bone marrow (BM) aspirate samples by a PCR-based method at baseline, remission, and relapse samples, when available. Genomic DNA from bone marrow samples was isolated using the Autopure extractor (QIAGEN/Gentra, Valencia, CA). Mutations in exon 12 of *NPM1* were assessed by a DNA-based semi-quantitative polymerase chain reaction capillary electrophoresis (PCR-CE) assay with analytical sensitivity of approximately 2.5%. *FLT3*-ITD mutations were also assessed using the same method.

Statistical analysis

Differences among variables were evaluated by the chi-square test and Mann- Whitney test for categorical and continuous variables, respectively. Survival curves were calculated for overall survival (OS; defines as time from diagnosis to death), event free survival (EFS; defined as time from diagnosis to time of treatment failure, relapse or death), complete remission duration (CRD; defined as time from documentation of CR to first relapse) according to the Kaplan-Meier method and compared by use of a 2 sided log rank test. All P values were two sided and values less than 0.05 were considered to be significant. Statistica (version 9) software was used for statistical analysis.

RESULTS

Patient characteristics

Data from 360 previously untreated patients with AML, who had available *NPM1* analysis on their BM at the time of initial diagnosis, was collected. The characteristics of the patients (both overall and those with NK) are shown in Tables 1 and 3. Median age was 60 years (range 19 – 83 years). Median WBC count at diagnosis was 5.2×10^9/L and was similar in *NPM1* mutated and wild-type patients. Patients with mutated *NPM1* exhibited significantly

higher BM blasts percentage as compared to patients with wild-type *NPM1* (Median 62% vs. 40% respectively; p<0.001) and significantly lower % of CD34+ cells (Median 0.7 vs. 72.5% respectively; p<0.001). *NPM1* mutations were present with similar frequency in men and women.

Cytogenetics was normal in 137 (38%) patients of whom 46 (33%) had mutated *NPM1* as compared to wild type *NPM1* in 91 (66%) patients. Patients with *NPM1* mutations had a lower proportion of other cytogenetic abnormalities as compared to patients with wild type *NPM1*. Overall, 60 of 360 (16.6%) patients including the previously mentioned 46 of the 137 (33%) NK patients had mutated *NPM1* at the baseline. *RAS* mutations were equally present among the two groups. 262 patients (72%) had de novo AML, and 98 (27%) secondary or therapy-related AML. Secondary leukemia was more common in the *NPM1* wild type (30%) than in the *NPM1* mutated (13%) category (p=0.008). *NPM1* mutated patients had higher proportions of *FLT3*-ITD *positive* cases as compared to *NPM1* wild type including in NK (n=24; 41% and n=22; 49% in NK group respectively).

Prognostic significance of *NPM1* mutations

Overall, median follow up of the patients was 61 weeks (Range 0–163) while in NK it was 70 weeks (3–163 weeks). Patients with mutated *NPM1* had a significantly longer complete remission duration (CRD) (P=0.03) and a higher proportion n=54 (90%) achieved complete remission as compared to *NPM1* wild type patients (P=0.002) (Table 2 and Figure 2A). Only 6 of 95 (6%) patients who did not achieve CR had mutated *NPM1*. Both event-free survival (EFS) and overall survival (OS) were significantly longer in patients with *NPM1* mutation (P <0.001 and 0.001 respectively) (Figure 2B–C). When analyzed by age, in patients < 60 years (n=175), OS, EFS and response rates were significantly superior in *NPM1* mutated subgroup (p=0.001, 0.007, 0.02 respectively). Among patients ≥ 60 years (n=185) EFS and response rates were also significantly higher in the *NPM1* mutated subgroup (p=0.008, 0.03 respectively) (Table 2).

Due to a significant association between *NPM1* mutated patients with *FLT3*-ITD positivity, we have further analyzed the EFS and OS among *NPM1+/FLT3*-ITD+, *NPM1+/FLT3*-ITD-, *NPM1−/FLT3*-ITD+, and *NPM1−/FLT3*-ITD- subgroups (Figure 3A–D). Patients who were *NPM1−/FLT3*-ITD+ had significantly inferior EFS and OS as compared to other categories including in patients with normal karyotype.

Analysis for detecting both *IDH1* and *IDH2* mutations were performed at baseline in 103 patients. Among the 300 patients with wild type *NPM1* at baseline 85 patients had testing for *IDH1* and *IDH2* mutations at baseline; 5 (6%) were *IDH1* mutated and 80 (94%) were *IDH1* wild type. 7 of 85 (8%) patients were *IDH2* mutated and 78 (92%) were *IDH2* wild type. On the other hand, among the 60 patients who were *NPM1* mutated at baseline, 18 patients had testing for *IDH1* and *IDH2* mutations. Four (22%) had *IDH1* mutation and fourteen (78%) were *IDH1* wild type. One (5%) patient was *IDH2* mutated and 17 (94%) were *IDH2* wild type.

Characteristics and outcome of patients with normal karyotype (NK)

A total of one hundred and thirty seven patients with NK (normal karyotype) were evaluated. Table 3 shows the main characteristics of patients with NK with or without mutated *NPM1*. Median age was 61 years (range 20 – 81 years). Median WBC count at diagnosis was significantly higher in *NPM1* mutated patients as compared to *NPM1* wild type (Median WBC 6.3 vs 2.4 K/μL; P=0.04 respectively). Patients with *NPM1* mutation exhibited significantly higher bone marrow (BM) blasts percentage as compared with *NPM1* wild type cases (Median 59% vs. 36% respectively; p<0.001). Higher proportions of *NPM1* mutated patients presented with acute monocytic (AMoL) and acute myelomonocytic leukemia (AMML) as compared to *NPM1* wild type patients (P<0.001). Proportions of secondary leukemia was higher in *NPM1* negative as compared to *NPM1* mutated patients (P=0.02). As expected, *FLT3*-ITD mutations were higher in *NPM1* mutated as compared to *NPM1* wild type (P=0.004). [2, 11]

Among patients with NK, those with *NPM1* mutations had a higher proportions (89%) achieving CR, as compared to those with wild type *NPM1* (80%; P=NS) (Table 4). They also had a significantly longer CRD (P=0.004) (Figure 4A). Both EFS and OS were also significantly longer in patients with mutated *NPM1* (P =0.003 and 0.002 respectively) (Figure 4B–C). When analyzed by age, in patients < 60 years (n=60), OS, EFS and CRD were significantly superior in *NPM1* mutated subgroup (p=0.007, 0.007, 0.02 respectively), while among patients ≥ 60 years (n=77) none were significantly higher in the *NPM1* mutated subgroup except that CRD showed a trend of being longer in *NPM1* mutated as compared to *NPM1* wild type (104 weeks vs 60 weeks respectively) (Table 2).

NPM1 mutations at CR and at relapse

Among the 300 patients (including 91 with NK) with wild type *NPM1* at diagnosis, none acquired a mutated *NPM1* clone, either at CR or at the time of relapse suggesting that *NPM1* mutations are stable. Among the 60 patients who had *NPM1* mutation at the time of diagnosis, 39 patients (including 30 with NK) had available *NPM1* status at the time of CR and all (100%) were negative for the mutated clone (Figure 1A–B). Among these 39 patients who have achieved a *NPM1* negative status at CR, 10/39 (25%) patients overall [7/30 (23%) NK patients] relapsed. *NPM1* status was available for 8 patients overall including 6 with NK at the time of relapse. Among them, 7/8 (87%) overall [including 5/6 (83%) NK patients] had mutated *NPM1*, while 1/8 (12%) [1/6 (16%) NK patients] remained wild type for *NPM1*. This patient relapsed with extramedullary disease (leukemia cutis) without any BM involvement.

Seven patients relapsed with *NPM1* mutated leukemia including 3 with acquired *FLT3* mutation; 3 had wild type *FLT3* and 1 in whom *FLT3* mutational analysis was not done. Among these patients, the distribution of other mutations was *N-RAS*: 4/7 wild type and 3 not done, *IDH1:* 1/2 wild type and 1 mutated, *IDH2:* 2/2 wild type, *CEBPA:* 2/2 wild type and *KIT:* 2/2 wild type.

Among the 60 patients with mutated *NPM1* at baseline, 17 relapsed. Of these 17 patients, only 13 had mutated *NPM1* at the time of relapse. We compared the characteristics of these

17 patients with those *NPM1* mutated patients who did not relapse (n=37)(Table 5). Among these 17 patients, 8 achieved a second CR, 6 were non-responders (NR) and 3 were not evaluable for response. Their median survival was 33 weeks (range 1–131 weeks).

DISCUSSION

In this study, we have examined and confirmed the prognostic relevance of *NPM1* mutations in previously untreated patients with AML who were evaluated and treated at our institute. We have also examined (among patients with evaluable samples) the fate of *NPM1* mutations by analyzing BM samples at diagnosis, remission and at relapse. There are some shortcomings in our study including lack of serial samples in all the patients studied, use of a semi quantitative low sensitivity technique rather than a more sensitive quantitative method for detecting *NPM1* mutations, and lack of subtyping of *NPM1* mutations. Although, this type of analysis has been reported by other groups in the literature, we believe that this study further confirms that *NPM1* mutations are stable markers of the disease and, unlike *FLT3* mutations, do not fluctuate during the disease course, in particular, at relapse.

Monitoring minimal residual disease (MRD) by detection of mutations present in the leukemic clone is being increasingly utilized for the management of patients with leukemia.[1, 36] Various techniques and markers have been studied to detect MRD.[37] *NPM1* mutations are an ideal target for use in clinical practice as they are present in about 40–50% of patients with de novo AML.[11, 38] Other mutations such as *FLT3*-ITD have been evaluated but their applicability to common practice is controversial.[39] Here, we have shown that none of the 300 patients who were initially negative for mutated *NPM1* were positive at the time of CR or became positive at the time of relapse (100% of patients with evaluable samples retained their initial *NPM1* status). Furthermore, among the 39 evaluable patients with mutated *NPM1* at diagnosis, all became negative at the time of CR and the majority (>85%) regained the mutated *NPM1* clone at the time of relapse; one patient with only extramedullary relapse remained negative. Our findings are consistent with two other large studies which have evaluated paired samples of *NPM1* mutated patients at the time of CR and relapse.[8, 32] However, two other studies have reported that *NPM1* mutations do not always reappear at the time of relapse; although this occurred in only 2/17 and 2/27 relapsed patients in these studies. Furthermore, in one study, the lack of *NPM1* mutated clone at relapse was potentially attributable to therapy and clonal evolution.[19, 20]. In addition, we also found that all of our patients (irrespective of whether they were originally mutated or wild type for *NPM1*) were negative for the mutant clone at the time of CR, further supporting the usefulness of *NPM1* mutations as a marker for MRD.

The characteristics of our patients with mutated *NPM1* and its prognostic relevance are consistent with other published studies.[2, 9–11, 13] In our experience, presence of mutated *NPM1* predicted for significantly longer CRD, EFS, and OS in the overall population and in patients with normal karyotypes (NK) (Figures 2 and 4, respectively). Furthermore, we confirm the findings from other groups that NPM1+/FLT3 (ITD) patients have significantly inferiors EFS and OS as compared with other subgroups. Of note, however, is that in our study, the patients with mutated *NPM1* constituted only 33% (46/137) of those with NK which is lower than what has been reported in a number of other published reports (45–50%

NPM1 mutated in NK AML)[2, 9–11, 13] but consistent with several other studies,[20, 21, 40, 41] including a prior study from our center.[28] This difference could possibly be explained by a higher proportion of older patients in our cohort (185 older than 60 compared with 175 who were <60). This is consistent with the reports that the incidence of *NPM1* mutations decreases with advancing age [42] with some exceptions as in the study by Becket et al.[43]

In our experience patients with mutated *NPM1* have a favorable prognosis and mutated *NPM1* is a stable and reliable marker which does not fluctuate and can detect relapse in patients with AML harboring the mutation. *NPM1* status should be further evaluated for routine use as a potential MRD marker among the commonly available markers in management of patients with AML. Prospective studies with high sensitivity assay to detect *NPM1* mutations should be designed to better define the dynamics of *NPM1* mutations in predicting relapse, and to prospectively evaluate *NPM1* in conjunction with other molecular markers to develop a prognostic model and molecular MRD panel in patients with AML.

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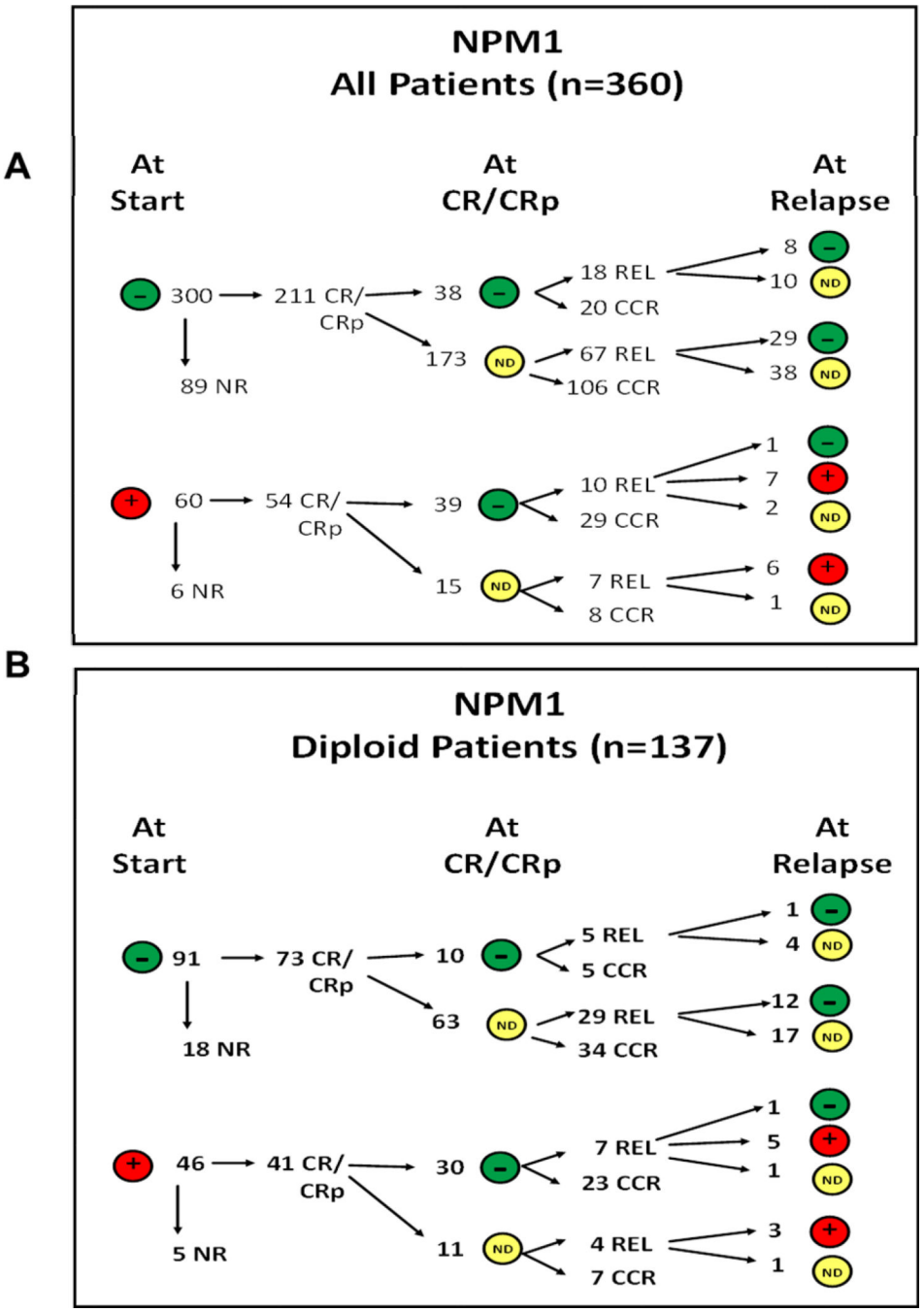

Figure 1.

(A-B). Schema describing the *NPM1* status at CR1 and first relapse. Panel A shows the numbers of patients overall and **panel B** shows number of patients (NK alone). ND indicates NPM1 mutation testing not done. CR/CCR/CRp indicates complete remission with without platelet recovery. One patient who remained negative at relapse had an extramedullary relapse.

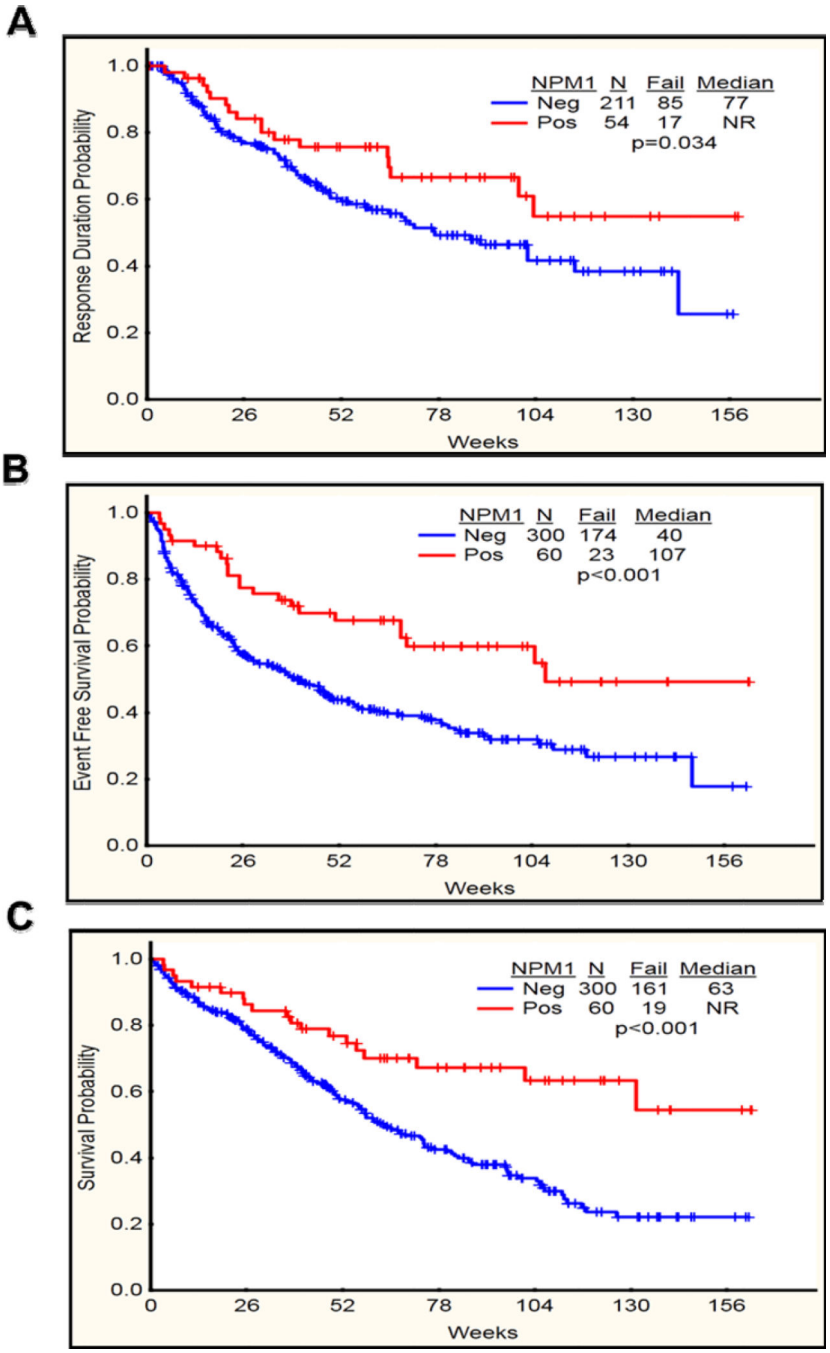

Figure 2.

(A-C). Complete remission duration (CRD), event free survival (EFS) and overall survival (OS) in *NPM1* mutated (Positive) vs *NPM1* wild type (Negative) among all the patients (n=360). A) CRD significantly longer in *NPM1* mutated as compared to *NPM1* wild type (P=0.034). **B)** EFS significantly longer in *NPM1* mutated as compared to *NPM1* wild type (P<0.001) **C)** OS significantly longer in *NPM1* mutated as compared to *NPM1* wild type (P<0.001).

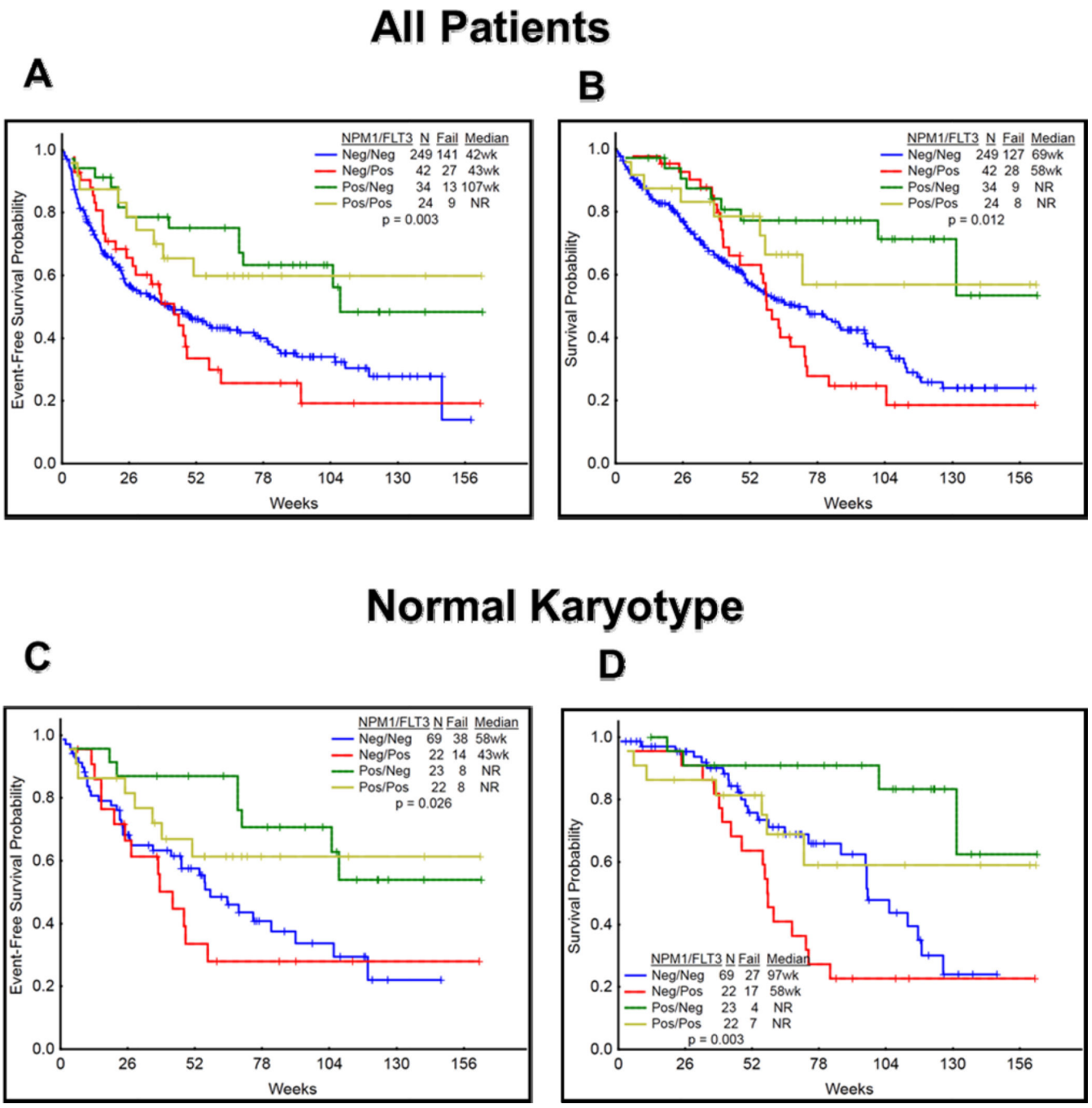

Figure 3.

(A-D). Event free survival (EFS) and overall survival (OS) among *NPM1+/FLT3+*, *NPM1+/FLT3−*, *NPM1−/FLT3+*, and *NPM1−/FLT3−* subgroups is shown (A–D). A) EFS was significantly inferior in *NPM1−/FLT3+* mutated as compared to other subgroups (P=0.003) B) OS significantly inferior in *NPM1−/FLT3+* mutated as compared to other subgroups (P=0.012). Similar results were seen in patients with normal karyotype (EFS: P=0.02; OS: P=0.003) in various subgroups. *NPM1+/FLT3−* have significantly better outcomes.

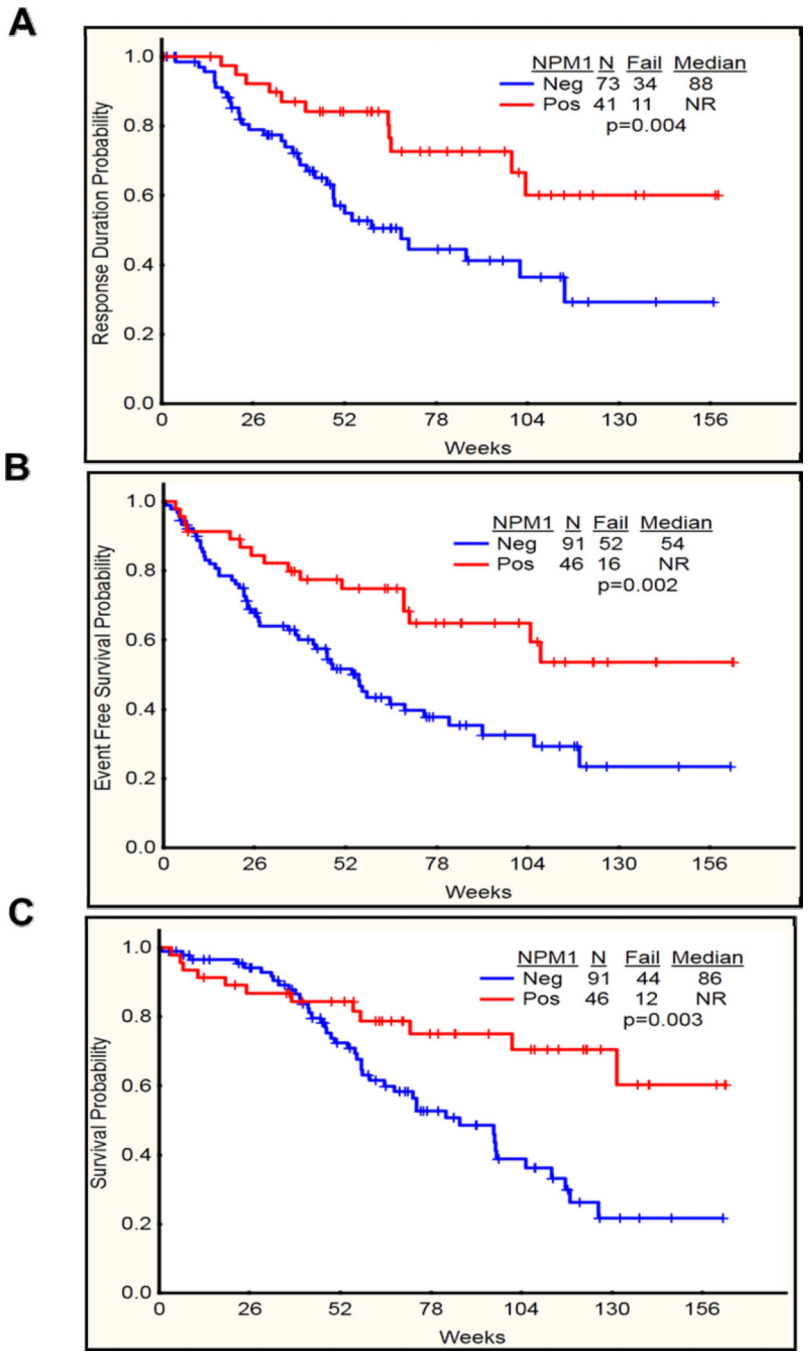

Figure 4.

(A-C). Complete remission duration (CRD), event free survival (EFS) and overall survival (OS) in *NPM1* mutated (Positive) vs *NPM1* wild type (Negative) among patients with AML with NK karyotype (DK) (n=137). A) CRD significantly longer in *NPM1* mutated as compared to *NPM1* wild type (P=0.004). **B)** EFS significantly longer in *NPM1* mutated as compared to *NPM1* wild type (P=0.002) **C)** OS significantly longer in *NPM1* mutated as compared to *NPM1* wild type (P=0.003).

Table 1

Patient characteristics (Overall population)

Parameter	*NPM1* WT	*NPM1* MUT	Total	P value
N	300	60	360	
Age	60 (19–83)	60 (23–80)	60 (19–83)	0.58
WBC (K/μL)	5.0 (0.1–191.0)	6.3 (0.1–228.5)	5.2 (0–228.5)	0.22
BM Blasts	40 (0–95)	62 (12–95)	42 (0–95)	**<0.001**
CD34+	72.5 (0–99.8)	0.7 (0–94.8)	54.9 (0–54.9)	**<0.001**
Female n (%)	137 (46)	29 (48)	166 (46)	
Male n (%)	163 (54)	31 (52)	194 (54)	0.7
Diagnosis:				
AML	209 (70)	33 (55)	242 (67)	
AMML	46 (15)	17 (28)	63 (18)	
AMoL	26 (9)	8 (13)	34 (9)	**0.07**
AEL	16 (5)	2 (3)	18 (5)	
Mega	3 (1)	0	3 (1)	
AML type:				
De novo	210 (58)	52 (87)	262 (73)	
Secondary	90 (42)	8 (13)	98 (27)	**0.008**
***FLT3* Status**				
***FLT3* WT**	249 (85)	34 (59)	283 (81)	
***FLT3* MUT**	43 (15)	24 (41)	67 (19)	**<0.001**
ND	8	2	10	
***RAS* Status**				
***RAS* WT**	236 (85)	43 (81)	279 (84)	
***RAS* MUT**	43 (15)	10 (19)	53 (16)	0.53
ND	21	7	28	
Karyotype:				
CBF [t(8;21) & inv(16)]	41 (15)	2 (3)	43 (12)	
Normal	91 (30)	46 (77)	137 (38)	
Miscellaneous	88 (29)	8 (13)	96 (27)	**<0.001**
−5/−7	73 (24)	1 (2)	74 (21)	
IM/ND	7 (2)	3 (5)	10 (3)	

Legends – WT: wild type; MUT: mutant; WBC: white blood count; BM: bone marrow; ND: not done; IM: insufficient metaphases

Table 2

Prognostic significance of *NPM1* mutations (Overall population)

Parameter	NPM1 WT	NPM1 MUT	Total	P value
CR/CRp	211 (70)	54 (90)	265 (74)	**0.002**
NR	89 (20)	6 (10)	95 (26)	
CRD (wk)	77	NR		**0.034**
OS (wk) (N=360)	63	NR		**<0.001**
OS (wk) (<60 yrs) (N=175)	82	NR		**<0.001**
OS (wk) (≥60 yrs) (N=185)	49	73		**0.064**
EFS (wk)	40	107		**0.001**
EFS (wk) (<60 yrs)	69	NR		**0.007**
EFS (wk) (≥60 yrs)	27	105		**0.008**

Legends – WT: wild type; MUT: mutant; CR: complete response; CRp: complete response without platelet recovery; NR: no response; CRD: CR duration; OS: overall survival; EFS: event-free survival; wk: week

Table 3

Characteristics of patients with *NPM1* mutations and normal karyotype

Diploid	*NPM1* WT	*NPM1* MUT	Total	P value
N	91	46	137	
Age	61 (20–81)	59 (23–80)	61 (20–81)	0.11
WBC (K/μL)	2.4 (0.5–132.3)	6.3 (1.1–100.2)	3.8 (0.5–132.3)	**0.04**
BM Blasts	36 (0–93)	59 (12–95)	44 (0–95)	**<0.001**
CD34+	79 (0–99)	1(0–64)	31 (0–100)	**<0.001**
Female n (%)	37 (41)	25 (54)	62 (45)	
Male n (%)	54 (59)	21 (46)	75 (55)	0.13
Diagnosis:				
AML	71 (78)	24 (52)	95 (69)	
AMML	8 (9)	14 (30)	22 (16)	
AMoL	3 (3)	7 (15)	10 (7)	**<0.001**
AEL	7 (8)	1 (2)	8 (6)	
Mega	2 (2)	0	2 (1)	
AML Type:				
De novo	69 (76)	42 (91)	111 (81)	
Secondary	22 (24)	4 (9)	26 (19)	**0.029**
***FLT3* Status:**				
***FLT3* WT**	69 (76)	23 (51)	92 (68)	
***FLT3* MUT**	22 (24)	22 (49)	44 (32)	**0.004**
ND		1	1	
***RAS* Status:**				
***RAS* WT**	77 (89)	34 (81)	111 (86)	
***RAS* MUT**	10 (11)	8 (19)	18 14)	0.29
ND	4	4	8	

Legends – WT: wild type; MUT: mutant; WBC: white blood count; BM: bone marrow; ND: not done

Table 4

Prognostic significance of *NPM1* mutations (normal karyotype)

Parameter	*NPM1* WT	*NPM1* MUT	Total	P value
CR/CRp	73 (80)	41 (89)	114 (83)	
NR	18 (20)	5 (11)	23 (17)	0.19
CRD (wk)	68	NR		**0.004**
OS (wk) (n=137)	86	NR		**0.003**
OS (wk) (<60 yrs) (n=60)	113	NR		**0.007**
OS (wk) (≥60 yrs) (n=77)	74	101		0.3
EFS (wk)	54	NR		**0.002**
EFS (wk) (<60 yrs)	58	NR		**0.007**
EFS (wk) (≥60 yrs)	54	105		0.13

Legends – WT: wild type; MUT: mutant; CR: complete response; CRp: complete response without platelet recovery; NR: no response; CRD: CR duration; OS: overall survival; EFS: event-free survival; wk: week

Table 5

Characteristics of *NPM1* mutated patients (relapsed vs. those remaining in remission)

	Remission **N=37**	**Relapsed** **N=17**	***P value***
Diploid (n)	30	11	
Other (n)	7	6	0.19
***FLT3*+**	15	6	
***FLT3*−**	21	10	0.78
***RAS*+**	8	2	
***RAS*−**	25	12	0.45
P.S.			
0–1	33	16	
2–3	4	1	0.56
Age (Yrs)	57 (27–75)	60 (23–77)	0.54
WBC (K/μL)	6.2 (1.1–186.5)	8.5 (1.6–228.5)	0.32