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# Prognostic impact of *DNMT3A* mutation in acute myeloid leukemia with mutated *NPM1*

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**Key Points**

- Patients with *DNMT3A*$^{mut}$ have worse *NPM1* MRD clearance, which can be counteracted by preemptive allogeneic transplantation.
- *DNMT3A*$^{mut}$ does not modify the prognostic value of the *FLT3*-ITD allelic ratio in AML-*NPM1*.

The negative prognostic impact of internal tandem duplication of *FLT3* (*FLT3*-ITD) in patients with acute myeloid leukemia with mutated *NPM1* (AML-*NPM1*) is restricted to those with a higher *FLT3*-ITD allelic ratio (*FLT3*$^{high}$; ≥0.5) and considered negligible in those with a wild-type (*FLT3*$^{WT}$)/low ITD ratio (*FLT3*$^{low}$). Because the comutation of *DNMT3A* (*DNMT3A*$^{mut}$) has been suggested to negatively influence prognosis in AML-*NPM1*, we analyzed the impact of *DNMT3A*$^{mut}$ in *FLT3*-ITD subsets (absent, low, and high ratios). A total of 164 patients diagnosed with AML-*NPM1* included in 2 consecutive CETLAM protocols and with *DNMT3A* and *FLT3* status available were studied. Overall, *DNMT3A*$^{mut}$ status did not have a prognostic impact, with comparable overall survival ($P = .2$). Prognostic stratification established by *FLT3*-ITD (*FLT3*$^{WT}$ = *FLT3*$^{low}$ > *FLT3*$^{high}$) was independent of *DNMT3A*$^{mut}$ status. Measurable residual disease (MRD) based on *NPM1* quantitative polymerase chain reaction was available for 94 patients. *DNMT3A*$^{mut}$ was associated with a higher number of mutated *NPM1* transcripts after induction ($P = .012$) and first consolidation (C1; $P < .001$). All *DNMT3A*$^{mut}$ patients were MRD$^{+}$ after C1 ($P < .001$) and exhibited significant MRD persistence after C2 and C3 (MRD$^{+}$ vs MRD$^{-}$; $P = .027$ and $P = .001$, respectively). Finally, *DNMT3A*$^{mut}$ patients exhibited a trend toward greater risk of molecular relapse ($P = .054$). In conclusion, *DNMT3A*$^{mut}$ did not modify the overall prognosis exerted by *FLT3*-ITD in AML-*NPM1* despite delayed MRD clearance, possibly because of MRD-driven preemptive intervention.

## Introduction

In recent years, the role of molecular genetics has proven to be essential in deciphering the heterogeneity of acute myeloid leukemia (AML)[1,2] and defining genetic markers of prognostic significance that can guide risk-adapted treatment.[3]

AML with mutations in the nucleophosmin 1 gene (AML-*NPM1*) forms a specific category in the latest World Health Organization classification because of its singular characteristics.[4] The cooccurrence of

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mutated *NPM1* (*NPM1*$^{mut}$) and the internal tandem duplication of *FLT3* (*FLT3*-ITD) in de novo AML with intermediate-risk cytogenetics results in a different prognostic impact depending on the *FLT3* allelic burden.[5-7] Previous studies have shown that patients with *NPM1*$^{mut}$ and an *FLT3*-ITD low ratio (*FLT3*$^{low}$; *FLT3*-ITD/FLT3$^{WT}$ ratio of $<0.5$) had overall survival (OS) and risk of relapse (RR) similar to those of patients with *NPM1*$^{mut}$ and wild-type (WT) *FLT3* (*FLT3*$^{WT}$).[8,9] Since 2012, these findings have been included in our latest protocol (CETLAM [Spanish Cooperative Group for the Diagnosis and Treatment of Acute Myeloid Leukemia and Myelodysplastic Syndromes] AML-12), and patients with *FLT3*$^{low}$-*NPM1*$^{mut}$ AML are not considered for allogenic hematopoietic stem cell transplantation (alloHSCT) in first complete remission (CR1). However, a molecular-based measurable residual disease (MRD) monitoring protocol is strictly followed to allow early-intervention strategies.

The DNA (cytosine-5)-methyltransferase 3A (*DNMT3A*) gene is located on the short arm of chromosome 2 and encodes for a DNA methyltransferase that methylates unmodified DNA cytosine residues modulating the expression of several genes.[10,11] Almost all *DNMT3A* mutations are heterozygous, and more than two-thirds cluster at the methyltransferase domain in codon R882, causing loss of methylation activity by disturbing *DNMT3A* tetramerization.[12-16] However, although a precise methylation pattern alteration resulting from mutations in *DNMT3A* has not yet been established,[17-21] a new mechanism of leukemogenesis characterized by the upregulation of the hepatic leukemia factor (a specific leukemic transcription factor) has been shown to be related to the cooccurrence of *DNMT3A*, *NPM1*, and *FLT3* mutations.[22]

*DNMT3A* is considered a founder mutation in AML.[23,24] It has been associated with age-related clonal hematopoiesis, with increasing frequency in healthy elderly individuals,[25] although a recent study found a correlation of *DNMT3A* mutations with younger age in *NPM1*$^{mut}$ AML.[26] Patients with AML and mutated *DNMT3A* (*DNMT3A*$^{mut}$) are frequently older and present with higher white blood cell (WBC) counts and higher platelet counts compared with WT *DNMT3A* (*DNMT3A*$^{WT}$).[13,17,27] *DNMT3A* is the third most frequently mutated gene in AML patients included in intensive chemotherapy trials. It is predominantly observed in AML-*NPM1* (73%) and less frequently in patients with mutations in chromatin remodeling genes or genes involved in spliceosome function. Interestingly, a recurrent association of *NPM1*$^{mut}$/*DNMT3A*$^{mut}$/*FLT3*-ITD has been observed in 6% of AML cases.[28,29] The prognostic significance of mutations in *DNMT3A* has been controversial; some studies have found no significant influence on survival outcomes,[17] whereas others have suggested that the cooccurrence of *NPM1*$^{mut}$/*DNMT3A*$^{mut}$/*FLT3*-ITD in AML patients is associated with adverse outcomes.[7,13,30-33]

In 2013, the German AML Study Group described the clinical impact of *DNMT3A*$^{mut}$ in younger adults with AML.[34] In a univariable exploratory subset analysis, the group showed a significant prognostic impact of *DNMT3A*$^{mut}$ in unfavorable European LeukemiaNet (ELN) AML, whereas no impact was observed in favorable ELN AML. In 2016, the proposed AML gene classification by Papaemmanuil et al[28] showed a deleterious effect of *DNMT3A*$^{mut}$ when specifically associated with *FLT3*-ITD independently of its allelic ratio.

We analyzed whether this triple-gene alteration led to an unfavorable prognosis in AML-*NPM1* patients, with particular attention to those harboring *FLT3*$^{low}$.

# Methods

## Patients and samples

Patients with de novo AML and intermediate-risk cytogenetics according to the Medical Research Council,[35] *NPM1*$^{mut}$, and available bone marrow sample from diagnosis were selected. All patients were diagnosed between 2003 and 2017 and were included in the CETLAM intensive treatment protocols AML-03 and AML-12 (registered at www.clinicaltrials.gov as #NCT01723657 and #NCT04687098) provided they met the criteria for eligibility. The present study was reviewed and approved by the ethics committee of the Hospital de la Santa Creu i Sant Pau (Comitè ètic d'Investigació Clínica). Informed consent for both bone marrow analysis and treatment was obtained in all cases according to the Declaration of Helsinki.

## Molecular studies

Diagnostic bone marrow samples from all patients were analyzed for *DNMT3A*$^{mut}$ as previously described.[17] The mutational status of the *FLT3* gene was also established. In mutated cases, the allelic ratio was calculated by dividing the area under the curve of the *FLT3*-ITD peak and the area under the curve of the *FLT3*$^{WT}$ peak. Patients were stratified into 2 groups: those with a high ratio (*FLT3*$^{high}$) if the ratio of *FLT3*-ITD/*FLT3*$^{WT}$ was $\geq 0.5$ and those with *FLT3*$^{low}$ if the ratio of *FLT3*-ITD/*FLT3*$^{WT}$ was $<0.5$.

Monitoring of *NPM1* MRD was performed on bone marrow samples by quantitative reverse transcription polymerase chain reaction (sensitivity $10^{-4}$ to $10^{-6}$) as previously described.[36] After each treatment cycle, absolute transcript reduction was estimated, and its logarithm (log10) reduction from diagnosis was also explored. Based on the latest ELN MRD working party recommendations,[37] MRD positivity was considered when *NPM1* transcripts were amplified in at least 2 of 3 replicates with cycle threshol values of $\leq 40$ at a cycling threshold of 0.1. Molecular relapse was confirmed if the MRD level (in a patient previously MRD$^-$) increased $\geq 1$ log10 between 2 consecutive positive samples, and molecular progression was confirmed if copy numbers increased $\geq 1$ log10 between 2 positive samples in a patient with MRD$^+$.

## Statistical analysis

Analysis of the relationship between categorical variables was performed using the $\chi^2$ or Fisher's exact test. Differences between groups for continuous variables were established through the independent samples *t* test or Mann-Whitney U test. All tests were 2 sided and considered significant where $P < .05$. OS was calculated from diagnosis to death, whereas leukemia-free survival (LFS) was calculated from CR to death or relapse; both functions were estimated with the Kaplan-Meier method. Unless specified otherwise, all survival results reported reflect 5-year estimates. A log-rank test was run to determine differences in the survival distribution, with a significance threshold of $P \leq .05$. RR was estimated using the cumulative incidence method, defining relapse as the main event and death without relapse as the competitive event. Molecular LFS (molLFS) was estimated from CR to molecular failure, overt hematological relapse, or death. All statistical analyses were performed with SPSS software (version 26; IBM, Armonk, NY) and R statistics (version 3.6.1; R Foundation for Statistical Computing, Vienna, Austria).

**Table 1. Patient characteristics according to *DNMT3A*$^{mut}$ status**

| | No. (%) | | |
|---|---|---|---|
| | *DNMT3A*$^{WT}$ (n = 85) | *DNMT3A*$^{mut}$ (n = 79) | *P* |
| **Female sex** | 51 (60) | 36 (46) | .08 |
| **Age, y** | | | .7 |
| Median | 53 | 53 | |
| Range | 18-71 | 25-72 | |
| **WBC, × 10$^9$/L** | | | <.001 |
| Median | 16 | 50 | |
| Range | 0.55-408 | 1.3-384 | |
| **BM blasts, %** | | | .7 |
| Median | 73 | 73 | |
| Range | 20-100 | 20-100 | |
| **Platelet count, × 10$^9$/L** | | | .4 |
| Median | 64 | 70 | |
| Range | 8-488 | 12-625 | |
| ***FLT3* mutational status** | | | .6 |
| WT | 46 (54) | 40 (50) | |
| *FLT3*$^{low}$ | 19 (22) | 15 (19) | |
| *FLT3*$^{high}$ | 19 (22) | 23 (30) | |
| **Treatment protocol** | | | |
| AML-03 | 26 (31) | 22 (28) | |
| AML-12 | 59 (69) | 57 (72) | |
| **Postinduction CR** | 75 (88) | 69 (87) | .7 |
| **No. of cycles to achieve CR (1 vs 2)** | 67 vs 8 | 61 vs 8 | .86 |
| **No. of patients undergoing alloHSCT in CR1** | 16 (19) | 22 (28) | .9 |

BM, bone marrow.

# Results

## Patient characteristics according to *DNMT3A*$^{mut}$ status

A total of 164 patients with AML-*NPM1* were included. Clinical and genetic characteristics at diagnosis are described in Table 1. Patients were included in protocols AML-03 (n = 48) and AML-12 (n = 116). *DNMT3A*$^{mut}$ was found in 79 patients (48%); in 62 cases (38%), mutations were in codon R882 or were insertions/deletions, whereas 17 (10%) were different missense mutations. Seventy-six patients (46%) harbored *FLT3*-ITD, 42 of whom had *FLT3*$^{high}$ (55%). According to *DNMT3A*$^{mut}$ status, patient characteristics were comparable, except for a higher WBC presentation among *DNMT3A*$^{mut}$ patients. Of note, the proportion of *FLT3*-ITD allelic burden subsets (ie, *FLT3*$^{WT}$, *FLT3*$^{low}$, and *FLT3*$^{high}$) was independent of *DNMT3A*$^{mut}$ (*P* = .6).

Eighty-eight percent of patients achieved CR after 1 or 2 cycles of induction therapy (n = 128 and 16, respectively); 6% (n = 10) had refractory disease, and 10 patients died during induction. As consolidation therapy, 58 patients received intensive treatment consisting of 2 to 3 high-dose cytarabine cycles. AlloHSCT was performed in 65 patients (CR1, n = 44; CR2, n = 15; with refractory disease, n = 4); 14 patients underwent autologous transplantation. The median follow-up time was 30 months.

## Prognostic impact of *DNMT3A*$^{mut}$

In the entire cohort of AML-*NPM1* patients, OS, LFS, and RR were 59% ± 4%, 60% ± 5%, and 27% ± 7%, respectively. *FLT3*-ITD allelic ratio confirmed its prognostic impact, with a similar outcome for patients with *FLT3*$^{WT}$ or *FLT3*$^{low}$ and a significantly worst prognosis for cases with *FLT3*$^{high}$. OS was 67% ± 6% vs 62% ± 9% vs 40% ± 8% (*P* = .002; supplemental Figure 1), respectively; RR was 18% ± 9% vs 27% ± 16% vs 41% ± 17% (*P* = .008), respectively; and LFS was 71% ± 5% vs 56% ± 9% vs 40% ± 9% (*P* = .002), respectively. In contrast, *DNMT3A*$^{mut}$ did not exert a significant effect on overall outcomes (Figure 1), with OS in *DNMT3A*$^{WT}$ vs *DNMT3A*$^{mut}$ patients of 62% ± 6% vs 56% ± 6% (*P* = .2), respectively; LFS of 65% ± 6% vs 54% ± 6% (*P* = .1), respectively; and RR of 22% ± 11% vs 31% ± 11% (*P* = .2). The outcomes of *DNMT3A* subsets among the entire AML-12 cohort are available in supplemental Figure 2.

Additionally, the individual effect of R882 *DNMT3A*$^{mut}$ was analyzed separately and did not show any prognostic impact (*P* = .4; supplemental Figure 3). Multivariate analysis performed for OS included age, protocol, WBC count, *DNMT3A*$^{mut}$ status, and *FLT3*-ITD subsets, the latter being the only statistically significant variable (data not shown).

The effect of the cooccurrence of *DNMT3A*$^{mut}$ and *FLT3*-ITD was analyzed separately. In the *DNMT3A*$^{mut}$ cohort, whereas OS between *FLT3*$^{WT}$ and *FLT3*$^{low}$ was similar, patients with *FLT3*$^{high}$ showed particularly worse outcomes (*FLT3*$^{WT}$ vs *FLT3*$^{low}$ OS, 68% ± 8% vs 56% ± 13%, respectively; *P* = .3; *FLT3*$^{WT/low}$ vs *FLT3*$^{high}$ OS, 65% ± 7% vs 28% ± 13%, respectively; *P* = .015). This was further validated in terms of LFS (*FLT3*$^{WT}$ vs *FLT3*$^{low}$, 68% ± 8% vs 50% ± 13%, respectively; *P* = .1; *FLT3*$^{WT/low}$ vs *FLT3*$^{high}$, 62% ± 7 vs 20% ± 16%, respectively; *P* = .014) and RR in the same groups (23% ± 15% vs 29% ± 23%, respectively; *P* = .4; 25% ± 13% vs 48% ± 20%, respectively; *P* = .017; Figure 2).

In patients without *DNMT3A*$^{mut}$, the allelic ratio of *FLT3*-ITD maintained its prognostic value, with similar outcomes between *FLT3*$^{WT}$ and *FLT3*$^{low}$ patients. Interestingly, in this group of patients, the deleterious effect of *FLT3*$^{high}$ was mildly modulated (*FLT3*$^{WT}$ vs *FLT3*$^{low}$ OS, 66% ± 8% vs 66% ± 11%, respectively; *P* = .5; *FLT3*$^{WT/low}$ vs *FLT3*$^{high}$ OS, 66% ± 7% vs 47% ± 12%, respectively; *P* = .028). Similar findings were seen in terms of LFS (*FLT3*$^{WT}$ vs *FLT3*$^{low}$, 74% ± 8% vs 61% ± 13%, respectively; *P* = .2; *FLT3*$^{WT/low}$ vs *FLT3*$^{high}$, 70% ± 7% vs 52% ± 13%, respectively; *P* = .083) and RR (14% ± 11% vs 26% ± 21%, respectively; *P* = .2; 17% ± 12% vs 33% ± 23%, respectively; *P* = .1; Figure 3).

The direct comparison of *DNMT3A*$^{mut}$ status according to each *FLT3* status (WT, low, and high) was not statistically different (supplemental Figure 4), although the general analysis of *DNMT3A* depending on *FLT3*$^{WT}$ or *FLT3*$^{mut}$ showed statistical differences. This might be due to the low number of patients analyzed when dividing into the 3 groups.

## MRD kinetics according to *DNMT3A*$^{mut}$ status

Patients included in the CETLAM AML-12 trial with AML-*NPM1* allocated to the favorable ELN category (*FLT3*$^{WT}$ or *FLT3*$^{low}$) were not intended to undergo alloHSCT in CR1 unless a molecular failure was identified. Therefore, patients were closely monitored for *NPM1* MRD at specific time points (postinduction, after each consolidation

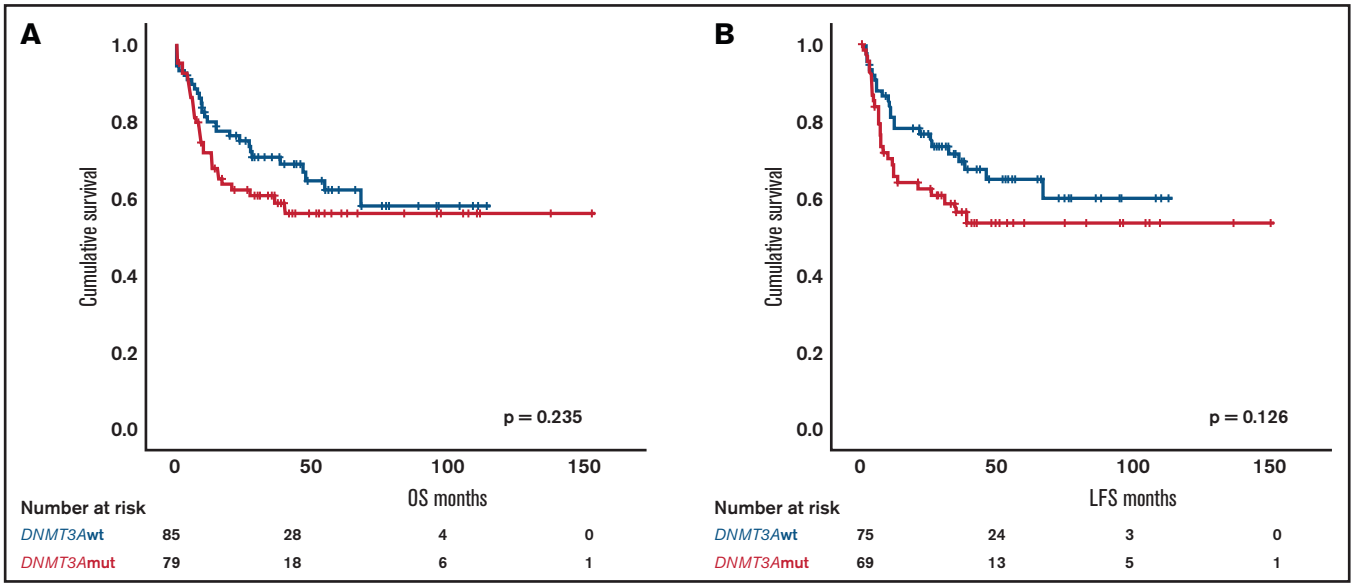


**Figure 1. Overall impact of *DNMT3A*$^{mut}$ on AML-*NPM1*.** OS (A) and LFS (B) of patients according to *DNMT3A*$^{mut}$ status.

cycle, and every 3 months for 3 years after treatment completion) to ensure rapid detection of molecular relapse and subsequent therapy initation.[38] Patients with *FLT3*$^{high}$ underwent alloHSCT after the first consolidation cycle (C1).

In 94 patients with available MRD data, we further investigated whether *DNMT3A*$^{mut}$ status influenced *NPM1* MRD. Although the probability of achieving hematological CR was not affected by *DNMT3A* (75 vs 69 patients in with *DNMT3A*$^{WT}$ and *DNMT3A*$^{mut}$, respectively; $P = .46$), *NPM1* MRD kinetics differed according to *DNMT3A*$^{mut}$ status. Patients with *DNMT3A*$^{mut}$ showed a higher number of absolute *NPM1*$^{mut}$ transcripts after induction ($P = .012$) and C1 ($P < .001$; Figure 4A). Similar findings were observed after C2 and C3 among patients not intended to undergo alloHSCT in CR1.

Therefore, we explored the relationship between *DNMT3A* subsets, posttreatment molecular MRD status (positive vs negative), and MRD response depth (log10 reduction; Figure 4B-G). After induction, all but 1 patient remained MRD$^{+}$. Although there were no statistical differences in the number of log10 reductions, a trend toward a more profound molecular response (≥4 log10) was observed in the *DNMT3A*$^{WT}$ group (*DNMT3A*$^{WT}$ vs *DNMT3A*$^{mut}$, 39% vs 15%, respectively; *P* not significant).

After C1, none of the *DNMT3A*$^{mut}$ patients achieved MRD$^{-}$ status, compared with 32% of *DNMT3A*$^{WT}$ patients ($P = .001$). Of note, patients without *DNMT3A*$^{mut}$ presented a deeper MRD reduction (≥4 log10 reduction in 77% of *DNMT3A*$^{WT}$ vs 46% of *DNMT3A*$^{mut}$ patients; $P = .033$). The relationship between *DNMT3A* and *NPM1* MRD was also sustained after C2 and C3 (Figure 4B-G; supplemental Figure 5). Additionally, when considering the triple-mutated group (*NPM1*, *FLT3*-ITD, and *DNMT3A*), all patients remained MRD$^{+}$ after induction, C1, and C2 regardless of *FLT3*-ITD allelic ratio.

Finally, the potential influence of *DNMT3A*$^{mut}$ status on molecular failure was explored. Among 85 cases included in the AML-12 protocol not initially considered for alloHSCT in CR1 (AML-*NPM1* with *FLT3*$^{WT}$ or *FLT3*$^{low}$, n = 63 and 22, respectively), the median molLFS was not reached at a mean follow-up of 30 months (supplemental Figure 6). When stratified by *DNMT3A*$^{mut}$ status, patients with the WT form exhibited a trend toward a long-term sustained molecular CR (molLFS, 63% ± 9% vs 50% ± 9% in *DNMT3A*$^{WT}$ (n = 42) vs *DNMT3A*$^{mut}$ (n = 35), respectively; $P = .054$; Figure 5).

Eleven patients in the favorable-risk group harbored *NPM1*$^{mut}$/*FLT3*$^{low}$/*DNMT3A*$^{mut}$; of these, only 5 experienced a molecular or hematological relapse and underwent alloHSCT. In total, 23 patients (27%) in this favorable subgroup underwent alloHSCT because of molecular or hematological relapse.

Overall, these findings suggest a deleterious effect of *DNMT3A*$^{mut}$ on *NPM1* MRD that should be validated in larger studies.

## Discussion

Several studies have been published attempting to elucidate the prognostic impact of *DNMT3A*$^{mut}$, but many have had contradictory results. This may be due to differences in the biological characteristics of the patients included (age, cytogenetics, availability of molecular studies), the treatment protocols, or other factors.[39] Of note, even those studies comparing the impact of *DNMT3A*$^{mut}$ status based on *NPM1*$^{mut}$ and *FLT3*$^{mut}$ status have shown contradictory results.[13,17,34] The aim of this study was not to analyze the impact of *DNMT3A*$^{mut}$ on AML outcomes, but rather to analyze its effect in the particular subset of patients with *NPM1*$^{mut}$ and *FLT3*-ITD, after the publication of a large study showing that *DNMT3A*$^{mut}$ have a deleterious effect on outcomes when cooccurring in this subgroup.[28] Our group described the effect of the *FLT3*-ITD ratio in 2012, and it was incorporated into the new treatment protocol. Consequently, patients with *NPM1*$^{mut}$ and *FLT3*$^{low}$ did not undergo alloHSCT in CR1. Therefore, we had a long follow-up in this group of patients treated following the ELN 2017 recommendations in which to analyze the possible effect of *DNMT3A*$^{mut}$.

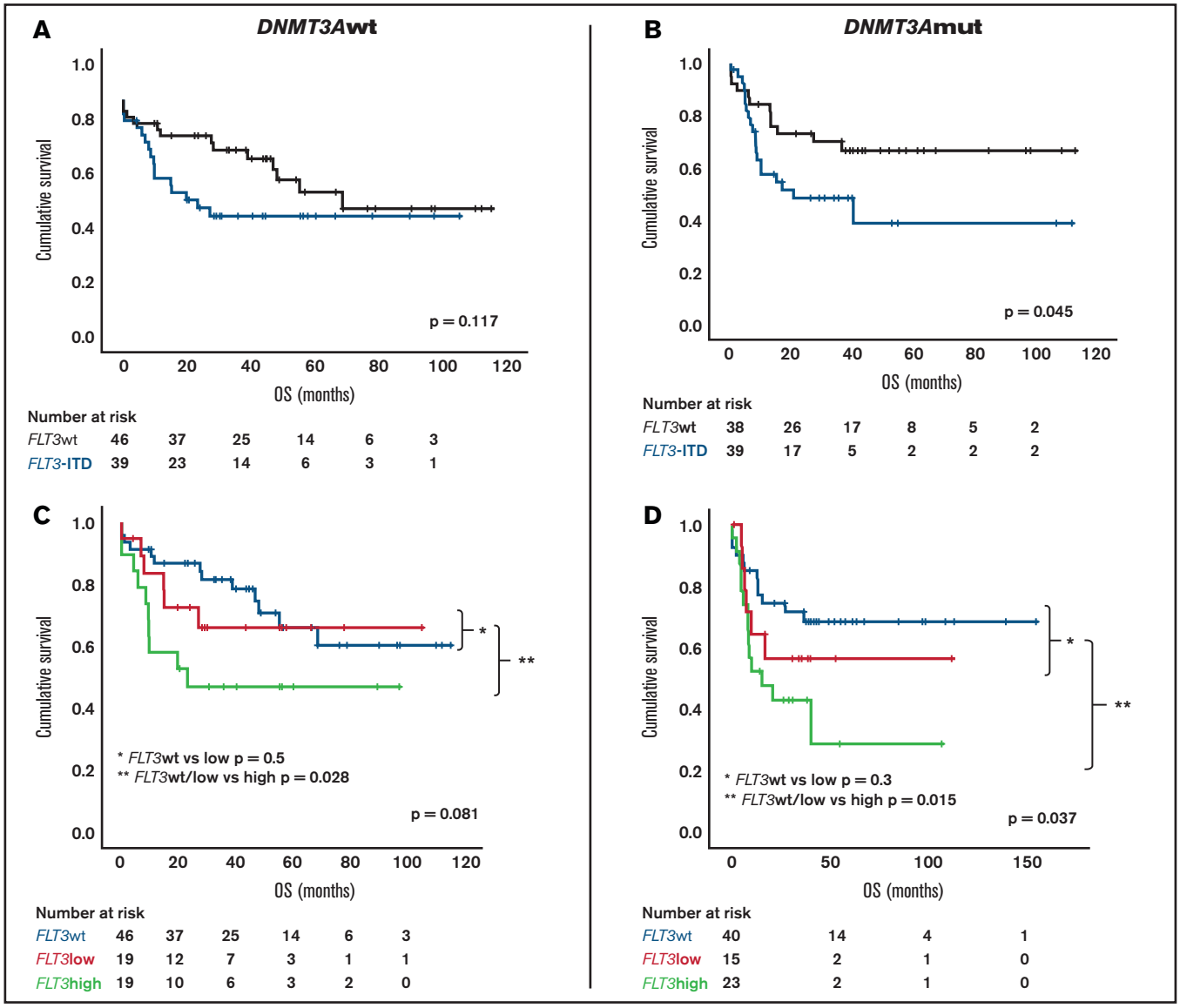


**Figure 2. *DNMT3A* influence over *FLT3*-ITD allelic ratio subgroups.** OS of *DNMT3A*$^{WT}$ (A,C) and *DNMT3A*$^{mut}$ (B,D) patients in different *FLT3* subsets.

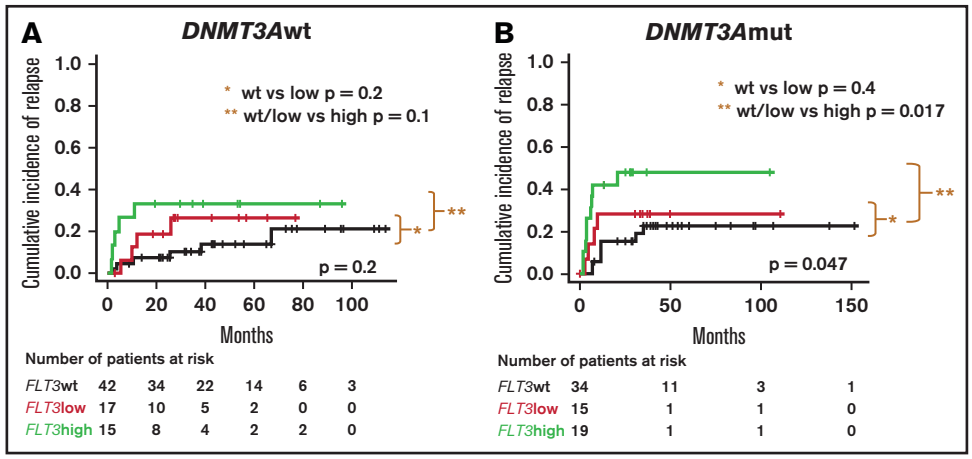


**Figure 3. Cumulative incidence of relapse in AML-*NPM1* patients based on *DNMT3A*$^{mut}$ status.** *DNMT3A*$^{WT}$ (A) and *DNMT3A*$^{mut}$ (B) patients according to *FLT3*-ITD subgroup.

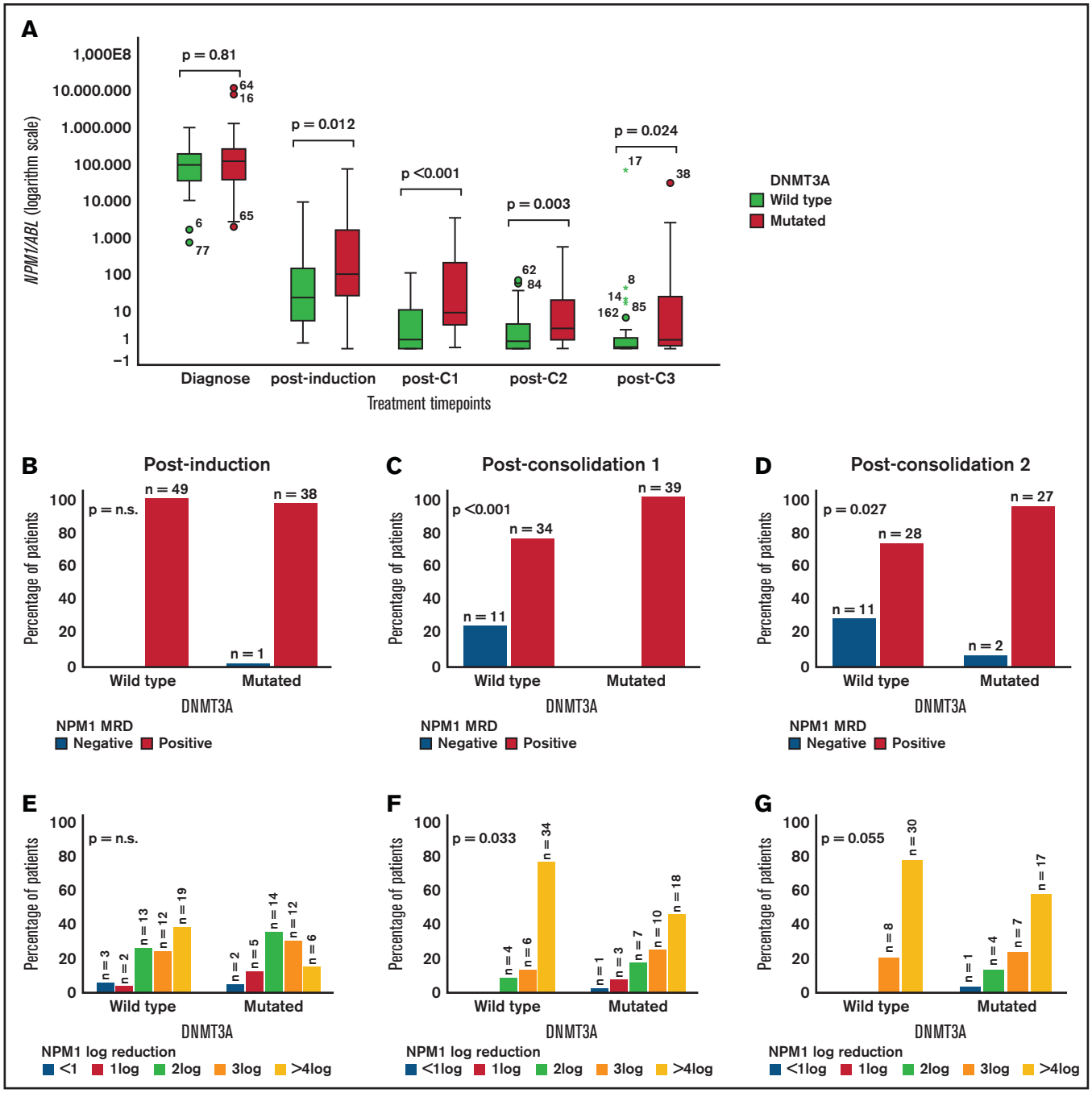


**Figure 4. *NPM1* MRD distribution at relevant clinical time points according to *DNMT3A*$^{mut}$ status.** (A) *NPM1* absolute transcript distribution in logarithmic scale. (B-G) MRD response, with MRD$^{+}$ and MRD$^{-}$ rates (B-D) and corresponding equivalent log10 reductions (E-G) at postinduction (B,E), post-C1 (C,F), and post-C2 (D,G).

In our study, survival analysis showed that *DNMT3A*$^{mut}$ did not have an impact in this particular group and that patients with *NPM1*$^{mut}$ and *FLT3*$^{low}$ had similar outcomes to patients with *NPM1*$^{mut}$ and *FLT3*$^{WT}$ regardless of *DNMT3A*$^{mut}$ status. However, because an effect of *DNMT3A*$^{mut}$ on *NPM1* MRD clearance was demonstrated, we investigated the influence of an early intervention planned in the treatment protocol when molecular relapse was detected. In the last few years, several publications analyzing the prognostic value of MRD follow-up based on *NPM1* transcript levels have been published. Although there is no consensus regarding the cutoff level or evaluation time points, all of them support the prognostic impact of MRD$^{+}$ persistence, with a higher incidence of relapse and shorter OS.[38,40-43] The largest study performed[44] evaluated the impact of MRD$^{+}$ in peripheral blood after the second chemotherapy cycle; it found the same impact on prognosis as previously reported, but the authors also reported that MRD persistence was the only independent prognostic factor for death in multivariate analysis. The ELN recommendations[37] also state that in AML-*NPM1*, rising MRD levels or the failure to achieve MRD$^{-}$ CR is associated with disease relapse and consequently advise that a change in therapy should be

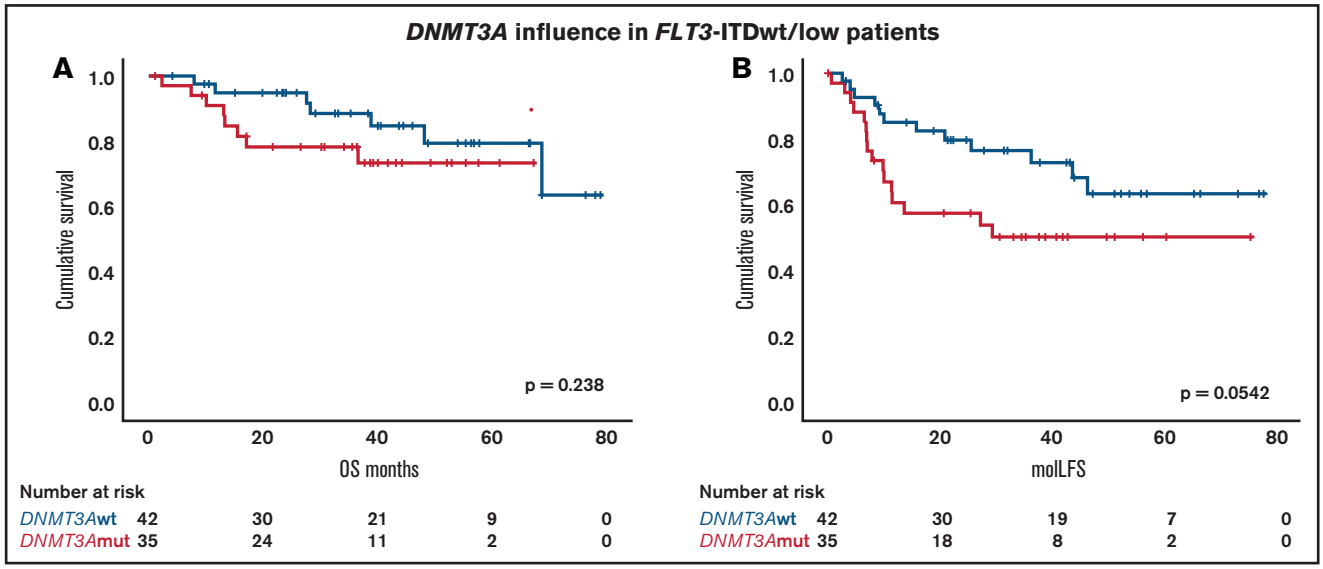


**Figure 5. *DNMT3A* influence in the favorable *FLT3*-ITD subgroup.** Distribution of patients with *FLT3*$^{WT}$ or *FLT3*$^{low}$ according to OS (A) and molLFS (B).

considered. Following the same reasoning, it was recently published by our group that an MRD ratio (*NPM1*$^{mut}$/ABL1X100) of ≥0.05 (in bone marrow) after the C1 was associated with significantly lower molLFS and that an early intervention resulted in a favorable outcomes.[38] Consequently, using the MRD level to guide postremission therapy can be considered a good strategy.

Interestingly, in the present study, a trend toward worse molLFS was observed in patients with *DNMT3A*$^{mut}$, but without an impact on OS. When only patients in the favorable ELN 2017 risk group were considered, we found that 27% of patients met either cytological or molecular relapse criteria. Of those, 70% underwent alloHSCT in CR1 (in molecular relapse) or CR2. As a result, the effect of this strategy might counteract the negative effect on OS seen in the *DNMT3A*$^{mut}$ subgroup. This intervention might be the most important difference between the treatment protocols for our patients and those included in the Papaemmanuil et al[28] study, which considered alloHSCT only in patients at high cytogenetic risk, whereas intermediate-risk patients underwent alloHSCT only when a sibling donor was available.[45-47]

Considering these findings, close MRD monitoring in *DNMT3A*$^{mut}$ AML-*NPM1* patients, along with early intervention strategies when a molecular relapse is detected, could be an appropriate approach, with a possible impact on OS.

Patients with *DNMT3A*$^{mut}$ and *FLT3*$^{high}$ had poorer outcomes than patients in the favorable ELN group (ie, *FLT3*$^{WT}$ or *FLT3*$^{low}$). Nonetheless, *DNMT3A*$^{mut}$ status did not seem to affect patients with FLT3$^{high}$, although a deleterious effect of this triple-mutation status (*NPM1*$^{mut}$/*FLT3*$^{high}$/*DNMT3A*$^{mut}$ vs *NPM1*$^{mut}$/*FLT3*$^{high}$/*DNMT3A*$^{WT}$) cannot be definitively excluded because of the small size of the subgroups analyzed. These findings may show a dosage effect on the interaction between *FLT3* and *DNMT3A*$^{mut}$ in AML-*NPM1*, highlighting the relevance of considering not only the presence of every single mutation but also the interaction among them.

In conclusion, patients with *NPM1*-AML with *FLT3*$^{low}$ and *DNMT3A*$^{mut}$ can be classified as favorable risk, but closer MRD follow-up is recommended to detect a molecular relapse and proceed to a therapeutic intervention.

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## Authorship

Contribution: G.O. and A.A. performed research. M.P. and G.O. designed research, analyzed data, and wrote the paper; J.S., J.F.N., and J.E. supervised research and wrote the paper; M.A., S.V., R.C., M.T., A.S., L.E., O.S., A.G., and J.B. collected and provided the clinical data; A.B., A.G., and M.H. analyzed data; and all authors reviewed the final manuscript.

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