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Landscape of biallelic *DNMT3A* mutant myeloid neoplasms

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Abstract

DNA methyltransferase 3 A mutations (*DNMT3A*^{MT}) are frequent in myeloid neoplasia (MN) and mostly heterozygous. However, cases with multiple *DNMT3A*^{MT} can be also encountered but their clinical and genetic landscape remains unexplored. We retrospectively analyzed 533 cases with *DNMT3A*^{MT} identified out of 5,603 consecutive MNs, of whom 8.4% had multiple *DNMT3A*^{MT} hits. They were most frequent in acute myeloid leukemia (AML) with R882 variant accounting for 13.3% of the multi-hits. Multiple *DNMT3A*^{MT} more likely coincided with *IDH2* ($P=0.005$) and *ETV6* ($P=0.044$) mutations compared to patients with single *DNMT3A*^{MT}. When the sum of variant allele frequencies (VAFs) for multiple *DNMT3A*^{MT} exceeded 60%, we found a significant positive clonal burden correlation of the two *DNMT3A* variants ($P<0.0001$) suggesting that they occurred in biallelic configuration. AML patients with biallelic *DNMT3A* inactivation ($n=52$) presented with older age ($P=0.029$), higher leukocytes ($P<0.0001$) and peripheral blast counts ($P=0.0001$) and significantly poorer survival rate (5.6% vs. 47.6% at 2 years; $P=0.002$) than monoallelic *DNMT3A*^{MT}. Multivariate analysis identified biallelic *DNMT3A*^{MT} (HR 2.65; $P=0.001$), male gender (HR 2.05; $P=0.014$) and adverse genetic alteration according to the European LeukemiaNet 2022 classification (HR 1.84; $P=0.028$) as independent adverse factors for survival, whereas intensive chemotherapy (HR 0.47; $P=0.011$) favorably influenced outcomes. Longitudinal molecular analysis of 12 cases with biallelic *DNMT3A*^{MT} demonstrated that such clones persisted or expanded in 9 relapsed or transformed cases (75%) suggesting the early origin of biallelic hits with strong leukemogenic potential. Our study describes the likelihood that biallelic *DNMT3A*^{MT}, while rare, are indeed compatible with clonal expansion and thus questions the applicability of synthetic lethality strategies.

Keywords *DNMT3A* mutation, Acute myeloid leukemia, Myelodysplastic syndrome, Myeloproliferative neoplasms

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To the editor

DNA methyltransferase 3 A mutations (*DNMT3A*^{MT}) are one of the most common aberrations in *de novo* acute myeloid leukemia (AML; 20–25%) [1–3], myelodysplastic syndromes (MDS; about 10%) [4] and clonal hematopoiesis of indeterminate potential [5, 6] and are mostly heterozygous [7]. Decreased DNMT3A function results in genomic hypermethylation with a significant enrichment of promoters of the transcription start sites of genes involved in differentiation [2, 7–9]. Multiple *DNMT3A*^{MT} seem to be associated with shorter event-free survival and overall survival (OS) in AML [10] but little is known about the landscape of biallelic *DNMT3A*^{MT}.

We analyzed 5,603 consecutive cases with myeloid neoplasia (MN) followed from 2002 to 2023 at The Cleveland Clinic for the presence of *DNMT3A*^{MT} using NGS to determine the genetic and clinical landscape of biallelic *DNMT3A*^{MT}. Clinical features of total patients subjected to NGS are summarized in Table S1.

DNMT3A^{MT} were identified in 533 patients (9.5%), of whom 488 carried single and 45 carried multiple hits (2 hits in 44 cases and 3 hits in 1 case; Fig. 1A; Supplementary Tables S2-S4). When compared with single-hit, multiple *DNMT3A* hits were not found in myeloproliferative neoplasm (MPN), but were encountered more often in AML than in MDS and MDS/MPN overlap (62.2% vs. 33.3% vs. 2.2%; $P=0.027$). However, no significant differences in the percentage of blasts or routine blood parameters were found according to the number of *DNMT3A* hits (Supplementary Table S5). Patients harboring multiple *DNMT3A*^{MT} carried more likely *IDH2* (24.4% vs. 9.6%; OR 3.03; $P=0.005$) and *ETV6* (6.7% vs. 1.4%; OR 4.91; $P=0.044$) mutations compared to those with single *DNMT3A*^{MT} (Fig. 1B-D).

Next, we analyzed diverse *DNMT3A*^{MT} configurations based on the sum of VAFs (Supplementary Methods; Fig. 1E). *DNMT3A*^{MT} were classified into 5 groups, monoallelic ($n=265$), hemi/homozygous ($n=24$), biallelic ($n=28$), bi-clonal ($n=10$) or "undetermined" whether biallelic or bi-clonal ($n=7$) or whether monoallelic or hemi/homozygous ($n=199$). We identified a significant positive correlation between VAFs of two *DNMT3A* variants (Spearman $r^2=0.6060$; $P<0.0001$), suggesting that these hits occurred simultaneously. 52 MNs with biallelic or hemi/homozygous *DNMT3A*^{MT} were grouped as biallelic "*DNMT3A* impairments" which had inactivation of both parental copies.

Monoallelic hits were missense (73%) including R882 (35%), whereas biallelic hits had a constellation of missense/missense (39%), missense/frameshifts (25%), missense/nonsense (11%), missense/splice sites (11%), missense/in-frame ins/del (7%), frameshift/nonsense (3.5%) and frameshift/frameshift (3.5%; Fig. 1F). R882 accounted for only 13% of biallelic hits, indicating that biallelicity was confined also to non-R882.

Median age was higher in biallelic than monoallelic (73 vs. 69 years; $P=0.029$; Supplementary Table S4) and biallelic *DNMT3A* impairments were not found in MPN ($P<0.001$), but more often in AML (72%) than MDS (24%) and MDS/MPN overlap (6%). Patients with biallelic *DNMT3A* impairment presented higher WBC in both AML (median 16.06 vs. 2.99×10^3/μL, $P<0.0001$) and MDS (6.58 vs. 3.02×10^3/μL, $P=0.0032$) and higher ANC (1.66 vs. 0.63×10^3/μL, $P=0.0042$) and blasts (39% vs. 11%, $P=0.0001$) in AML (Supplementary Table S5).

With a median follow-up of 22 months (0.1–219.0), OS rates according to *DNMT3A* impairment were compared in patients with MDS ($n=111$) and newly diagnosed AML who received chemotherapy ($n=96$; Supplementary Table S6). Those with biallelic *DNMT3A* impairment showed significantly poorer 2-year OS than those with monoallelic *DNMT3A* mutant AML (5.6% [95%CI, 0.4–22.4%] vs. 47.6% [95%CI, 34.9–59.2%]; $P=0.002$; Fig. 1G). Although there was no statistically significant difference, the same trend was observed in MDS patients (45.5% [95%CI, 16.7–70.7%] vs. 60.6% [95%CI, 48.9–70.5%]; $P=0.086$; Fig. 1G) Both biallelic and hemi/homozygous *DNMT3A*^{MT} showed inferior OS compared to monoallelic *DNMT3A*^{MT} ($P=0.007$; $P=0.012$), while there was no significant difference between biallelic and hemi/homozygous in AML ($P=0.790$; Supplementary Fig. S1). Biallelic *DNMT3A* impairment (HR 2.65; $P=0.001$), male gender (HR 2.05; $P=0.014$) and adverse genetic alteration according to the European LeukemiaNet 2022 classification (HR1.84; $P=0.028$) were deemed as independent adverse factors, while 7+3 based intensive chemotherapy (HR 0.47, $P=0.011$) as favorable for OS in AML (multivariate analysis; Supplementary Table S7). In MDS, high IPSS risk (HR 2.63, $P=0.001$) was the only risk factor for survival, while biallelic *DNMT3A* impairment was not a significant risk factor (multivariate analysis; Supplementary Table S7).

Since the rank of *DNMT3A*^{MT} within the clonal hierarchy can be approximated using VAF for clinical purposes, we classified co-occurring *DNMT3A*^{MT} into ancestral, simultaneous, and secondary mutations (**Supplementary Methods**; Supplementary Table S8). Among 68 variants co-occurring with biallelic *DNMT3A*^{MT}, 54% were simultaneous to *DNMT3A*^{MT}, followed by secondary (37%) and ancestral hits (9%). Ancestral mutations frequently occurred in chromatin regulators (*BCORL1*, *ASXL1*, *EZH2*) with *EZH2* mutations identified as earlier events, whereas *CUX1*, *PTPN11* and *CEBPA* mutations were found only as subclonal^{MT} (Fig. 2A-C).

In 12 cases with biallelic *DNMT3A*^{MT}, the VAFs for both *DNMT3A* variants tracked together with longitudinal NGS results. (Fig. 2D-G; Supplementary Fig. S2).

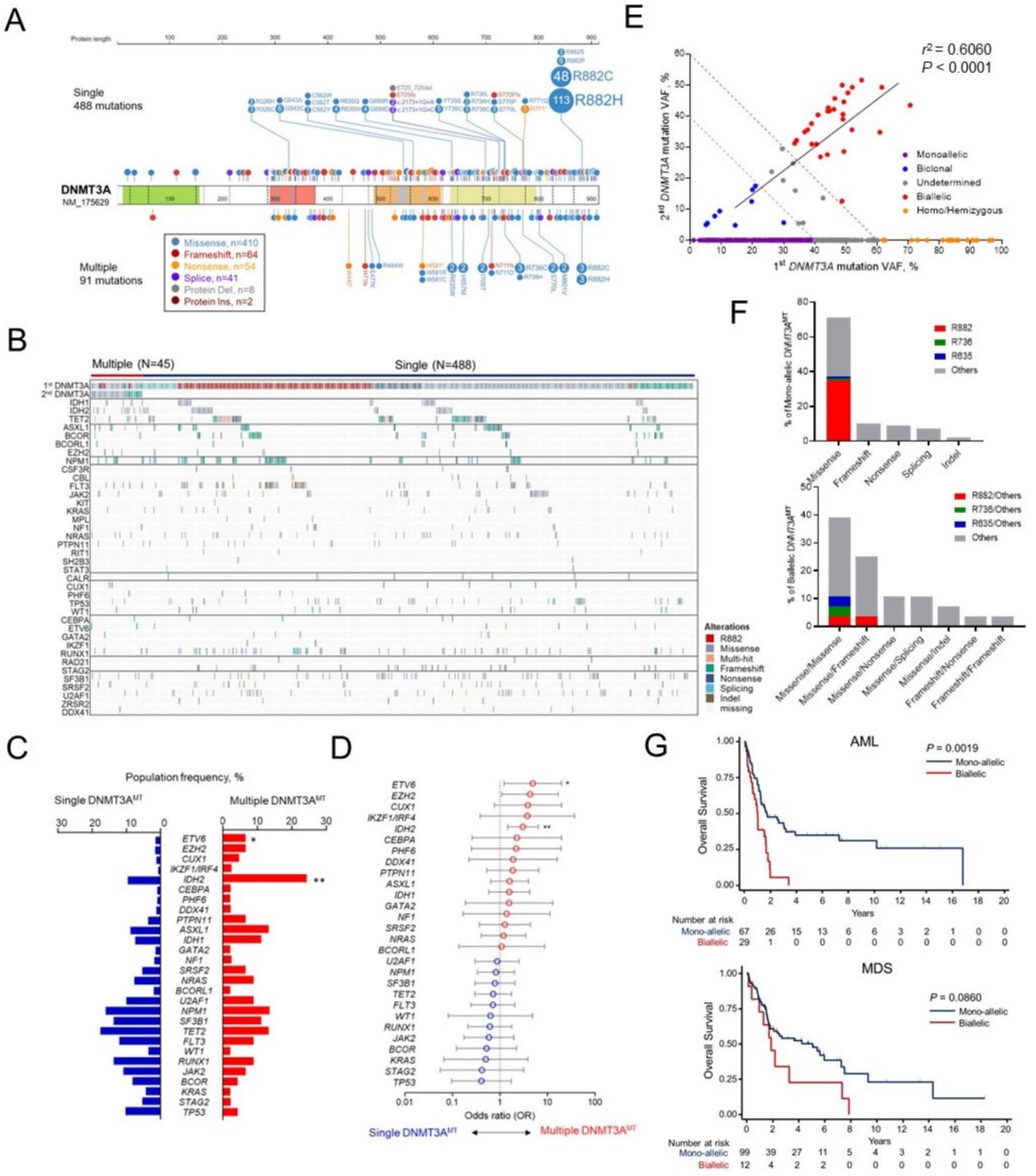
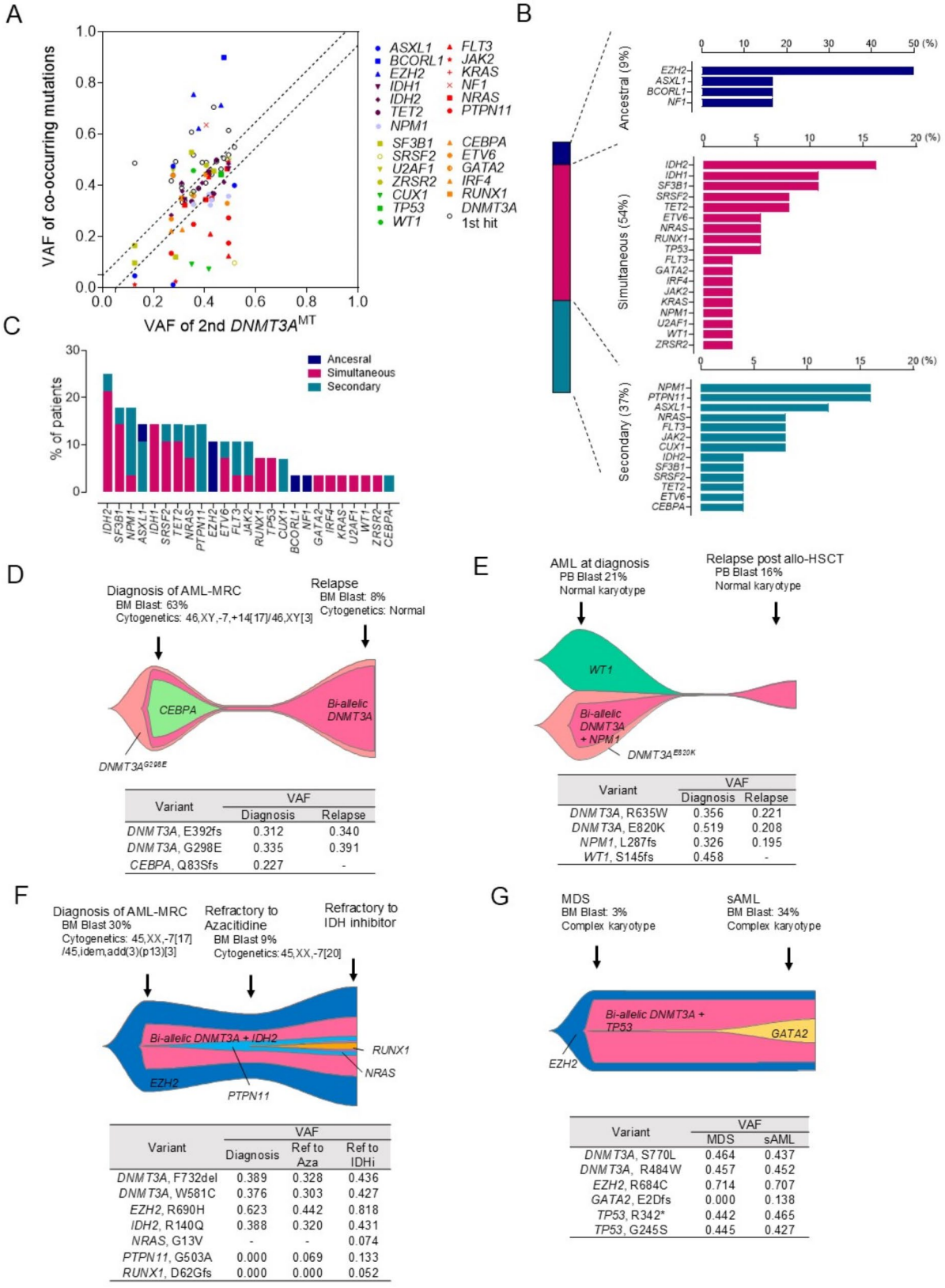


Fig. 1 Landscape of single and multiple *DNMT3A* mutations. (**A**) Lollipop plot of somatic *DNMT3A* mutations. Variants identified as single and multiple hits are plotted at the top and bottom. (**B**) Oncoplot of pathogenic mutations identified in patients with single or multiple *DNMT3A* mutations. (**C**) Comparison of frequencies of recurrent somatic mutations in patients with single vs. multiple *DNMT3A* mutations. (**D**) Odds ratios and 95% confidential intervals of genes more likely to have single (blue) or multiple (red) *DNMT3A* mutations. * $P < 0.05$, ** $P < 0.01$, where P is based on Fisher's exact test. (**E**) Scatter plot of the variant allele frequencies (VAFs) of patients with *DNMT3A*^{MT}. The VAF of first hit *DNMT3A*^{MT} was plotted on the x-axis and that of the second –hit *DNMT3A*^{MT} on the y-axis. Patients were categorized into 5 groups by the sum of VAFs. (**F**) Percentage of different types of monoallelic (upper) and biallelic (lower) *DNMT3A*^{MT}. (**G**) Overall survival (OS) comparison between monoallelic (blue) vs. biallelic (red) *DNMT3A* mutants in *DNMT3A*^{MT} MDS and AML patients. Kaplan-Meier Curves were compared by Log-Rank test



Variant	VAF	
	Diagnosis	Relapse
DNMT3A, E392fs	0.312	0.340
DNMT3A, G298E	0.335	0.391
CEBPA, Q83Sfs	0.227	-

Variant	VAF	
	Diagnosis	Relapse
DNMT3A, R635W	0.356	0.221
DNMT3A, E820K	0.519	0.208
NPM1, L287fs	0.326	0.195
WT1, S145fs	0.458	-

Variant	VAF		
	Diagnosis	Ref to Aza	Ref to IDHi
DNMT3A, F732del	0.389	0.328	0.436
DNMT3A, W581C	0.376	0.303	0.427
EZH2, R690H	0.623	0.442	0.818
IDH2, R140Q	0.388	0.320	0.431
NRAS, G13V	-	-	0.074
PTPN11, G503A	0.000	0.069	0.133
RUNX1, D62Gfs	0.000	0.000	0.052

Variant	VAF	
	MDS	sAML
DNMT3A, S770L	0.464	0.437
DNMT3A, R484W	0.457	0.452
EZH2, R684C	0.714	0.707
GATA2, E2Dfs	0.000	0.138
TP53, R342*	0.442	0.465
TP53, G245S	0.445	0.427

Fig. 2 (See legend on next page.)

(See figure on previous page.)

Fig. 2 Clonal architecture of biallelic *DNMT3A*^{MT}cases. (**A**) Scatter plot shows variant allele frequencies (VAFs) of co-occurring mutations (y-axis) and 2nd hit *DNMT3A* mutations (x-axis) in biallelic *DNMT3A* mutant cases. (**B**) VAFs were used to categorize co-occurring mutations into ancestral, simultaneous, or secondary mutations compared with biallelic *DNMT3A* mutations. The bar graphs show the percentages of the corresponding genes. (**C**) The prevalence of patients in each mutational type of co-occurring mutations. (**D**-**G**) Fish plots of representative longitudinal clonal hierarchy of biallelic *DNMT3A*^{MT} cases are shown. Clonal architecture of AML at diagnosis and following relapse after conventional intensive chemotherapy-induced remission (**D**) and allo-HSCT (**E**). (**F**) AML-MRC at diagnosis and after refractoriness to the initial therapy. (**G**) MDS at onset and upon progression to secondary AML (sAML). The variant allele frequencies (VAFs) of each variant are shown at the bottom

Except for 1 case in CR post allo-HSCT and 1 donor-derived leukemia (Supplementary Fig. S2**A-B**), biallelic *DNMT3A*^{MT} clones persisted/ expanded in 9/12 cases (Fig. 2D-G; Supplementary Fig. S2C-G) or was newly acquired at relapse post allo-HSCT in 1 case (Supplementary Fig. S2H). Furthermore, mono-allelic *DNMT3A*^{MT} clones were lost and only biallelic *DNMT3A*^{MT} clones dominated at relapse in 2 cases (Fig. 2E and Supplementary Fig. S2C), in which only mono-allelic *DNMT3A*^{MT} clones persisted during CR (Supplementary Fig. S2C).

Our study describes the role of multiple *DNMT3A*^{MT} in AML development and persistence, highlighting the likelihood that biallelic *DNMT3A*^{MT}, while rare, are indeed compatible with clonal expansion and that the acquisition of a second *DNMT3A*^{MT} may confer a stronger leukemogenic drive accelerating clonal progression. Given the lack of sample availability, our study is limited to estimation of *DNMT3A* mutational configuration using VAF-method. Further studies using single-cell DNA sequencing are warranted.

Abbreviations

DNMT3A^{MT}	DNA methyltransferase 3 A mutations
AML	Acute myeloid leukemia
MDS	Myelodysplastic syndromes
MN	Myeloid neoplasia
NGS	Next-generation sequencing
VAFs	variant allele frequencies
OS	Overall survival
OR	Odds ratio
HR	Hazard ratio

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

N.K. supervised the study, collected, analyzed, interpreted clinical and molecular data and wrote the manuscript; Y.K., C.B-P., L.G., A.D., and C.G., V.V. collected, analyzed, interpreted clinical and molecular data and edited the manuscript. N.W., A.A., and M.W. collected clinical and molecular data. J.P.M and V.V. provided invaluable help to the manuscript preparation, generated, and conceived the study design, designed figures and tables, and wrote the manuscript. All authors participated in the critical review of the final paper and submission.

Data availability

All data to reproduce our study are presented in the manuscript and tables within supplemental material. Additional information can be requested, if needed, by e-mailing the corresponding author: visconv@ccf.org.

Declarations

Ethics approval and consent to participate

This study was performed according to the Helsinki Declaration, and patients gave their written informed consent prior to the inclusion in the study. The protocol and informed consent form were approved by the institutional review board of The Cleveland Clinic.

Consent for publication

All patients gave their written informed consent prior to inclusion in the study.

Competing interests

The authors declare no competing interests.

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