HHS Public Access

Author manuscript

Leukemia. Author manuscript; available in PMC 2021 August 30.

Published in final edited form as:

Leukemia. 2018 February ; 32(2): 550–553. doi:10.1038/leu.2017.295.

Distinct clinical and biological implications of various *DNMT3A* mutations in myeloid neoplasms

SK Balasubramanian[1], **M Aly**[1], **Y Nagata**[1], **T Bat**[1], **BP Przychodzen**[1], **CM Hirsch**[1], **V Adema**[1], **V Visconte**[1], **T Kuzmanovic**[1], **T Radivoyevitch**[1], **A Nazha**[2], **S Mukherjee**[2], **MA Sekeres**[2,3], **JP Maciejewski**[1]

[1]Department of Translational Hematology and Oncology Research, Cleveland Clinic, Cleveland, OH, USA

[2]Department of Hematology and Medical Oncology, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, USA

[3]Department of Hematology and Medical Oncology, Leukemia Program, Cleveland Clinic, Cleveland, OH, USA

While *DNMT3A* mutations (*DNMT3A*^{MT}) mostly represent early event in the clonal hierarchy, a broad analysis of the clonal architecture of *DNMT3A*-associated neoplasia has not been conducted across disease sub-entities and/or mutation types. Moreover, the prognostic impact of *DNMT3A*^{MT} has been studied mostly in AML,[1,2] and only a few studies compared the clinical impact of various types of *DNMT3A*^{MT}, their position within the clonal hierarchy (primary vs secondary), and their configuration (canonical vs non-canonical *DNMT3A*^{MT}).[3]

Heterozygous *DNMT3A*^{MT} are most commonly found in noncore binding factor (NCBF) AML, but also occur in MDS and related neoplasms.[1,4–6] Subclonal *DNMT3A*^{MT} were found in otherwise asymptomatic older individuals and linked to increased risk of subsequent leukemia.[7,8] While most common *DNMT3A*^{MT} affect the canonical site (R882), nonsense variants have also been reported, but 2p deletions or microdeletions affecting the *DNMT3A* locus are rare.[9] The canonical R882 profoundly reduces methyltransferase activity, suggesting a dominant-negative effect,[10] whereas isolated nonsense mutations produce haploinsufficiency. The lack of somatic uniparental disomy, hemizygous deletions, or biallelic mutations suggests that such lesions would be non-competitive/lethal to the clone.

maciejj@ccf.org .

AUTHOR CONTRIBUTIONS

SKB designed the research, analyzed the data, and wrote the paper, MA, TB, YN, VA, VV, AN, SM, HEC, MAS helped with interpretation of the results and edited the manuscript, KT collected the clinical data and CH, BPP performed research procedures, TR helped with statistics, JPM designed the study and wrote the manuscript.

Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)

CONFLICT OF INTEREST

The authors declare no conflict of interest.

We investigated the role of mutation topography, clonal hierarchical position and clinical features in MDS, overlap neoplasms (MPN and MDS/MPNs), and primary and secondary AML to elucidate the clinical characteristics of *DNMT3A*^{MT} cases with respect to molecular biology and clinical phenotypes.

Mutational status was analyzed in 422 MDS, 240 MDS/MPN, 163 sAML and 234 pAML cases (n = 1059) followed for 1–311 mo. (median 53 mo.; Supplementary Table S1, Supplementary Figure S1A). Among them, 155 samples were subjected to whole-exome sequencing and 904 tested for mutations in 62 genes using targeted deep NGS (Supplementary Tables S2 and S3). Somatic *DNMT3A*^{MT} were identified in 212 (21%) patients (Table 1, Figure 1a). Alterations predicted to have functional consequences based on ≥ 5/9 databases are shown in Supplementary Table S4. Of the 212 mutations, 150 were missense (78 canonical R882, 72 non-canonical) and 62 truncations/frame shifts (Figure 1a) that were all heterozygous; 17/1059 patients had germline variants of unknown significance (Supplementary Table S5).

DNMT3A^{MT} were most common in pAML (35%), followed by MDS or sAML (each 18%) and MDS/MPN (10%; $P < 0.000001$; Table 1, Supplementary Figure S1B). *DNMT3A*^{MT} were associated with older age compared to *DNMT3A*^{WT} (66 vs 60 y; $P = 0.0001$; Table 1, Figure 1a). R882 *DNMT3A*^{MT} coincided with pAML more than truncating or NCMS (non-canonical missense) variants (60 vs 29 vs 24%; $P = 0.004$; Figure 1a). R882 was, however, under-represented in MDS compared to non-R882 (22 vs 43%, $P = 0.03$; Table 1, Supplementary Figures S2A–D). Complex karyotypes were less frequent in *DNMT3A*^{MT} vs *DNMT3A*^{WT} cases (6 vs 14%; $P = .009$; Table 1).

Compared to *DNMT3A*^{WT}, *DNMT3A*^{MT} coincided with certain additional hits (Figure 1b), including those in cohesin genes, *NPM1, IDH1/2,* and *FLT3*. In contrast, PRC2 complex mutations (*SUZ12/EED/EZH2*) associated more with *DNMT3A*^{WT} than with *DNMT3A*^{MT}. Among *DNMT3A*^{MT} types, *NPM1* mutations were found more with R882 than with truncating and NCMS *DNMT3A*^{MT} (26 vs 8 vs 15%, $P = 0.001$). *APC* and *IDH1* were mutated more in truncating than in NCMS or R882 *DNMT3A*^{MT} (18 vs 3 vs 5%, $P = .01$ and 18 vs 1 vs 12%, $P = 0.006$). *ASXL1* (22 vs 13 vs 5%, $P = .02$), *PRPF8* (15 vs 5 vs 1%, $P = 0.007$), *RAD21* (14 vs 0 vs 1%, $P = 0.0006$) and *LUC7L2* (13% vs 0 vs 0, $P = 0.0001$) were frequently co-mutated with NCMS (Figure 1b).

Analyzing *DNMT3A*^{MT} in AML, pAML patients were more likely to harbor *NPM1, FLT3* and *IDH1* mutations than sAML (28 vs 0%, $P = 0.002$; 23 vs 3%, $P = 0.04$; 17 vs 3%, $P = 0.1$, respectively). Mutations in *ASXL1* and *SF3B1* more frequently coincided with MDS and sAML *vs.* pAML (15 vs 5%, $P = 0.05$; 12 vs 1%, $P = 0.008$; Supplementary Figure S3).

In a clonal hierarchy analysis to determine whether *DNTM3A*^{MT} were ancestral or secondary genetic events (Supplementary Table S6, Figure 1c, Supplementary Figure S4), the median VAF (variant allele frequency) for *DNMT3A*^{MT} cases was 35% (3–93%). Median VAFs among *DNMT3A*^{MT} types may differ at 41, 35 and 27% in R882, truncating, and NCMS *DNMT3A*^{MT}, respectively (Supplementary Figure S5). *DNMT3A*^{MT} was ancestral in 42% of cases. In these, the most common second hits were in *TET2, NPM1,*

FLT3, and *IDH1/2* (Supplementary Figure S4A). Approximately ½ (49%) of R882 variants were ancestral vs 1/3 of non-R882 variants (39% truncating, 33% NCMS, $P = 0.04$). The most common secondary hits in dominant R882 *DNTM3A*^{MT} cases were *TET2, FLT3* and *NPM1*, while in dominant truncating *DNMT3A*^{MT} cases, *BCOR* and *IDH1/2* variants were the most common. In dominant NCMS *DNMT3A*^{MT} cases, mutations in *NPM1, ASXL1* and *SF3B1* were the most common second hits (Figure 1c). *TET2* was a common ancestral hit associated with *DNMT3A*^{MT} (Supplementary Figure S4). Among the *DNMT3A*^{MT}cases (R882 and truncating mutations), *TET2* was the most frequent ancestral event (Figure 1c).

DNMT3A^{MT} associated with worse overall survival (OS) than *DNMT3A*^{WT} (OS plots by univariate analyses) (Supplementary Figure S6A), failing to reach significance only in MDS/MPN and low-risk MDS, wherein *DNMT3A*^{MT} vs *DNMT3A*^{WT} median OS was 37 vs 102 mo. ($P = 0.12$) and 69 vs 101 mo. ($P = 0.54$), respectively (Supplementary Figures S6B, C); in high-risk MDS and pAML +sAML, median OS were 26 vs 75 mo. ($P < 0.0001$; Supplementary Figure S6D) and 16 vs 76 mo. ($P < 0.0001$) (Supplementary Figure S6E), respectively. There was no significant difference in OS in R882 vs NCMS or truncating *DNMT3A*^{MT} (26 vs 23 vs 27 months; $P = 0.72$; Supplementary Figure S6F). There was also no significant OS difference for patients with ancestral *vs.* secondary *DNMT3A*^{MT} (28 vs 17 mo., $P = 0.2$; Supplementary Figure S6G). Of note is that *DNMT3A*^{MT} with normal cytogenetics had better OS than those with abnormal/complex cytogenetics (32 vs 16 mo., $P = 0.001$; Supplementary Figure S6H). We also did a multivariate additive effects Cox model analysis which yielded hazard ratios (HR) relative to *DNMT3A*^{WT} low-risk MDS (Supplementary Table S7). These results show that assuming additive effects, the impact on survival of disease classification dwarfs the impact of different types of *DNMT3A*^{MT}, but given additional interaction model parameters and thus flexibility in data fitting, *DNMT3A*^{MT} type main effects differ and are strongest for R882 ($P = 0.03$).

DNMT3A mutation is considered an early lesion with leukemogenic potential.[11] By itself, *DNMT3A*^{MT} may be insufficient to cause neoplasia, as seen in asymptomatic older carriers[7,8] and in heterozygous murine models.[12] Our results demonstrate that clonal configuration and mutation type contribute to the heterogeneity of clinical outcomes and explain the difficulty in precisely assigning prognoses to a *DNMT3A*^{MT}. As in previous reports,[1,2,10] we identified these lesions in up to 35% of AML patients with canonical *DNMT3A*^{R882} being most common. The acuity of this disease suggests that R882 has the strongest leukemogenic potential. These findings are consistent with the dominant-negative role of *DNMT3A*^{R882} variant causing a degree of dysfunction beyond haploinsufficiency.[10] Indeed, R882 caused greater reductions in methyltransferase activity than other heterozygous hits.[13] In MDS/related disorders, *DNMT3A* truncations and NCMS variants predominate, implying that they associate with a more protracted disease. Likely, the non-canonical *DNMT3A* variants are less proleukemic, and require additional myeloid mutations.

The notion that *DNMT3A*^{R882} is more virulent is supported by the fewer secondary events. Also, as a secondary hit, *DNMT3A*^{R882} rapidly ‘sweeps’ the entire clonal population, thus requiring few other associated lesions. In contrast, non-R882 and truncating mutations may not suffice without other hits. The difference in associated mutations among the three

variants reinforces the notion that R882 *DNMT3A*^{MT} has a dominant-negative impact. It also suggests that each variant has distinct biological features.

The association of *DNMT3A*^{MT} with aggressive diseases is difficult to reconcile with the presence of subclonal founder lesions in asymptomatic, older adults.[7,8] This raises the question as to whether these asymptomatic carriers preferentially develop MDS, rather than pAML and/or are more likely to be affected by the less potent and non-canonical or truncation variants. Indeed, most of the *DNMT3A* clones reported in healthy, older individuals were not R882 (only 12–17% of healthy elderly *DNMT3A*^{MT} were R882).[7,8] Our results show that the spectrum of variants present in MDS is similar to that in asymptomatic carriers, consistent with the older age of onset of MDS. Consequently, additional hits may be needed to induce frank clonal neoplasia.

Using targeted gene panels, we reconstructed the clonal architecture of individual cases within disease subtypes. *DNMT3A*^{MT} were more often ancestral in pAML, whereas they can also be secondary subclonal in MDS and post-MDS (secondary) AML. In general, ancestral *DNMT3A*^{MT} or *TET2*^{MT} in MDS imply that such cases may have originated from clonal hematopoiesis of indeterminate potential (CHIP) of older adults. In contrast, other ancestral hits not observed in CHIP or subclonal *DNMT3A*^{MT} may point toward other etiologies. Nevertheless, truncations and NCMS *DNMT3A* lesions in MDS, which could be derived from CHIP, more likely represent secondary lesions. R882 is mostly a founder lesion, irrespective of histology, and is often followed by phenotype-determining mutations in *NPM1, FLT3,* or *IDH1,* which is consistent with murine models.[14]

The rank of the *DNMT3A*^{MT} in clonal hierarchy may have therapeutic implications. Ancestral *DNMT3A*^{MT} may be more likely to persist in relapses after treatments with *IDH1/2* or *FLT3* inhibitors. In contrast, secondary *DNMT3A* hits following *IDH1/2* or other targetable defects may be easier to eradicate. *DNMT3A*^{MT} confers a poor prognosis across different myeloid neoplasms. In terms of their position in the clonal hierarchy, no difference in outcomes between ancestral and secondary *DNMT3A* hits were observed which is consistent with *DNMT3A*^{MT} being clone sweeping events.[15]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This work was supported by the EPE1509JM - Edward P Evans Foundation, R01HL123904 – NHLBI, R01HL118281 – NHLBI, R01HL128425 – NHLBI.

REFERENCES

1. Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DEet al.DNMT3A mutations in acute myeloid leukemia. N Engl J Med2010; 363: 2424–2433. [PubMed: 21067377]
2. Ribeiro AFT, Pratcorona M, Erpelinck-Verschueren C, Rockova V, Sanders M, Abbas Set al.Mutant DNMT3A: a marker of poor prognosis in acute myeloid leukemia. Blood2012; 119: 5824–5831. [PubMed: 22490330]

3. Roller A, Grossmann V, Bacher U, Poetzinger F, Weissmann S, Nadarajah Net al.Landmark analysis of DNMT3A mutations in hematological malignancies. Leukemia2013; 27: 1573. [PubMed: 23519389]
4. Shih AH, Abdel-Wahab O, Patel JP, Levine RL. The role of mutations in epigenetic regulators in myeloid malignancies. Nat Rev Cancer2012; 12: 599–612. [PubMed: 22898539]
5. Walter MJ, Ding L, Shen D, Shao J, Grillot M, McLellan Met al.Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. Leukemia2011; 25: 1153–1158. [PubMed: 21415852]
6. Abdel-Wahab O, Pardanani A, Rampal R, Lasho T, Levine R, Tefferi A. DNMT3A mutational analysis in primary myelofibrosis, chronic myelomonocytic leukemia and advanced phases of myeloproliferative neoplasms. Leukemia2011; 25: 1219. [PubMed: 21519343]
7. Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SFet al.Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med2014; 371: 2477–2487. [PubMed: 25426838]
8. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BGet al.Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med2014; 371: 2488–2498. [PubMed: 25426837]
9. Yang L, Rau R, Goodell MA. DNMT3A in haematological malignancies. Nat Rev Cancer2015; 15: 152–165. [PubMed: 25693834]
10. Russler-Germain DA, Spencer DH, Young MA, Lamprecht TL, Miller CA, Fulton Ret al.The R882H DNMT3A mutation associated with AML dominantly inhibits wild-type *DNMT3A* by blocking its ability to form active tetramers. Cancer Cell2014; 25: 442–454. [PubMed: 24656771]
11. Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MCet al.Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med2014; 20: 1472–1478. [PubMed: 25326804]
12. Challen GA, Sun D, Jeong M, Luo M, Jelinek J, Berg JSet al.DNMT3A is essential for hematopoietic stem cell differentiation. Nat Genet2012; 44: 23–31.
13. Holz-Schietinger C, Matje DM, Reich NO. Mutations in DNA methyltransferase (DNMT3A) observed in acute myeloid leukemia patients disrupt processive methylation. J Biol Chem2012; 287: 30941–30951. [PubMed: 22722925]
14. Kim SJ, Zhao H, Hardikar S, Singh AK, Goodell MA, Chen T. A DNMT3A mutation common in AML exhibits dominant-negative effects in murine ES cells. Blood2013; 122: 4086–4089. [PubMed: 24167195]
15. Makishima H, Yoshizato T, Yoshida K, Sekeres MA, Radivoyevitch T, Suzuki Het al.Dynamics of clonal evolution in myelodysplastic syndromes. Nat Genet2016; 49: 204–212. [PubMed: 27992414]

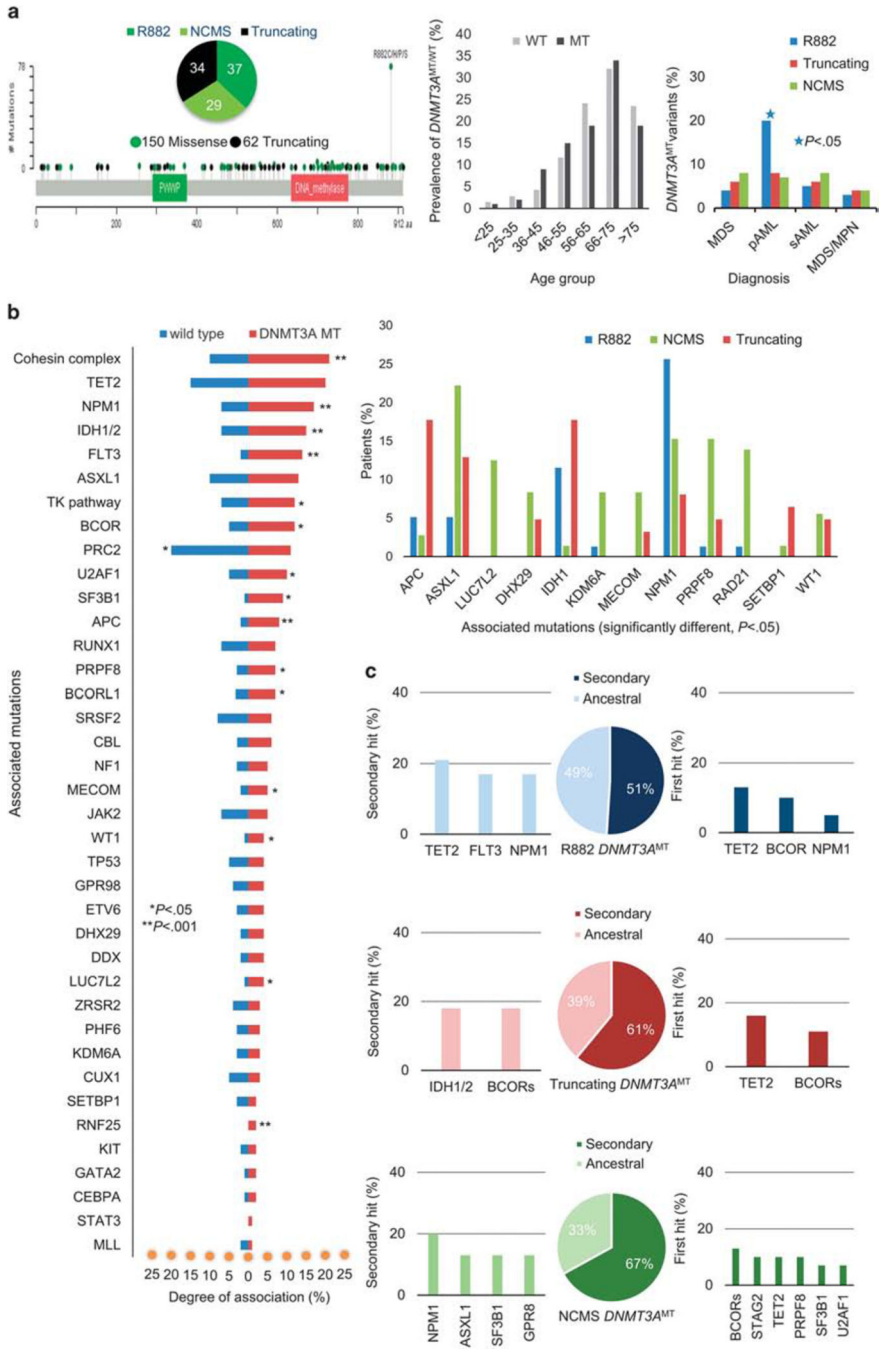

Figure 1.

Characteristics of *DNMT3A*^{MT} in our cohort of patients with myeloid neoplasms. **(a)** Illustration of position of *DNMT3A*^{MT} in chromosome 2 in our cohort of patients with myeloid neoplasia (left). Prevalence of *DNMT3A*^{MT} in different age groups in comparison to *DNMT3A*^{WT} (center). Maximum prevalence of *DNMT3A*^{MT} between age group 66–75 years. Distribution of different *DNTMT3A*^{MT} variants among different myeloid malignancies in the whole cohort (right). Canonical R882 variant is strongly associated with pAML than truncating or NCMS *DNMT3A*^{MT} (*$P < 0.05$). **(b)** 'Tornado chart'

(left) demonstrating associated mutations in *DNMT3A*^{MT} and in wild-type patients with different myeloid neoplasia. Bar graph (right) shows significantly different other associated myeloid mutations in R882, NCMS and truncating *DNMT3A*^{MT} variants. There is a higher association of *NPM1* in R882 variant, *APC, IDH1* and *SETBP1* in truncating variant and *ASXL1, PRPF8* and *RAD21* in NCMS *DNMT3A*^{MT}. **(c)** Illustrates clonal architecture in R882, truncating and NCMS variants of *DNMT3A*^{MT}myeloid neoplasms.

Table 1.

Patient characteristics among different variants in *DNMT3A* mutation

	Total Cohort	*DNMT3A* ^{WT}	*DNMT3A* ^{MT}	R882	Truncating	NCMS
n	1059	847	212	78	62	72
Age (median)	65	60	66 *	67	68	67
Gender % (M)	58	58	56	56	55	60
Diagnosis						
MDS	422	347 (82%)	75 (18%)	17 (4%)	25 (6%)	33 (8%)
pAML	234	152 (65%)	82 (35%) **	47 (20%) ***	18 (8%)	17 (7%)
sAML	163	133 (82%)	30 (18%)	8 (5%)	9 (6%)	13 (8%)
MDS/MPN	240	215 (90%)	25 (10%)	6 (3%)	10 (4%)	9 (4%)
Normal karyotype		434 (51%)	85 (40%)	32 (41%)	20 (32%)	33 (45%)
Complex karyotype		116 (14%) ****	14 (6%)	5 (6%)	4 (6%)	5 (7%)
CBC (mean)						
WBC × 10^9/l		4	5	4	5.6	6.4
ANC × 10^9/l		2	2	2	2.3	2.7
Hb (g/dl)		10	9	10	9.6	9.6
Platelet count (k/μl)		81	44	36	63	33
PB Blast %		3	12	23 *****	10	6
BM blast %		10	18	30 *****	18	10

Abbreviations: ANC, absolute neutrophil count; BM, bone marrow; CBC, complete blood count; Hb, hemoglobin; MDS/MPN, Myelodysplastic syndrome/Myeloproliferative neoplasm; NCMS, non-canonical missense mutation in *DNMT3A* gene; pAML, primary AML; PB, peripheral blood; sAML, secondary AML; WBC, white blood cells.

* $P < 0.001$ between *DNMT3A*^{MT} vs wild type;

** $P < 0.001$ comparing prevalence of *DNMT3A*^{MT} among MDS, pAML, sAML and MDS/MPN;

*** $P < 0.001$ between R882 vs Truncating vs other non-canonical missense *DNMT3A*^{MT};

**** $P < 0.05$ between *DNMT3A*^{MT} vs wild type;

***** $P = 0.05$ between R882 *DNMT3A*^{MT} vs *DNMT3A*^{WT}.