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POSTER ABSTRACTS

613.ACUTE MYELOID LEUKEMIAS: CLINICAL AND EPIDEMIOLOGICAL

Biallelic Landscape of *DNMT3A* Mutant Myeloid Neoplasia

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The structure of DNMT3A comprises an N-terminal regulatory part promoting nuclear localization and targeting chromatin and a C-terminal domain, mainly involved in DNA binding and methylation catalysis. [1] *DNMT3A* mutations in myeloid neoplasia (MN) and clonal hematopoiesis are mostly heterozygous. Homozygous null *Dnmt3a*mice are born runted and *Dnmt3a* $^{-/-}$ cells express a hypomethylation phenotype. [2] An invariant detection of heterozygous mutations without homozygous, hemizygous, or biallelic configurations suggests absence of acquisition of more malignant phenotypes by progressive inactivation of DNMT3A. Moreover, residual DNMT3A function is essential for cell survival and potentially suggests that synthetic lethality might be a viable therapeutic strategy whereby cases with two intact alleles would be more resistant allowing a therapeutic window. Consequently, identification of biallelic *DNMT3A* mutant cases may be extremely instructive for the development of therapeutics targeting DNMT3A.

We analyzed targeted NGS sequencing results of 4,895 cases with MN enrolled at our institution from 2002 to 2021 and retrospectively reviewed the clinical characteristics of all patients with deleterious *DNMT3A* mutations. *DNMT3A* mutations were identified in 461 patients, of whom 39 had double and 1 triple hits. Most of the mutants had AML (n=236) including biphenotypic AML (n=5), followed by MDS (n=142), MDS/MPN overlap (n=46) and MPN (n=37). In addition, mutations were identified in 9% of CHIP/CCUS (n=117) and 4% of aplastic anemia (n=68). Multiple *DNMT3A* hits were more often encountered in AML than MDS and MDS/MPN overlap (62.5% *vs.* 32.5% *vs.* 2.5%; $P = .045$). Single hits were represented by missense (73%) within the hotspot R882 (35%), frameshifts (10%), nonsense (9%), splice sites (6%) and in-frame ins/del (2%). Double hits configuration had a constellation of missense/missense (40%), missense/frameshifts (25%), missense/nonsense (13%), and missense/splice sites (10%) (**Fig1**). R882 accounted for 10% of the multi hit carriers. Of note is that biallelic involvement was not confined to non-R882. Patients harboring multi hits carriedmore likely *IDH2* (27.5% *vs.* 10.5%; OR 2.73; $P = .011$) and *ETV6* (7.5% *vs.* 1.7%; OR 4.32 $P = .043$) mutations compared to patients with single hits *DNMT3A* by multivariate logistic regression analysis.

Since deletion or UPD of *DNMT3A* locus has not been found, one can assume that all cases with one hit harbor monoallelic rather than biallelic subclones. For carriers of multiple mutations, one can stipulate that when the sum of VAFs exceeds the theoretical 60%, the presence of a biallelic subclone might be suspected. Indeed, 24 cases fulfilled this criterion. However, in 40% of patients with double hits, arithmetic VAF did not allow for unequivocal resolution of the mutational configuration, with either smaller biallelic subclones *vs.* subclonal mosaicism being possible.

Next, we compared clinical features and outcomes of patients with mono *vs.* biallelic cases. In MDS, compared with single hits *DNMT3A*, biallelic cases showed significantly higher WBC (median 7.49 *vs.* 3.15 k/μL; $P = .0052$), ANC (4.88 *vs.* 1.52 k/μL; $P = .0056$), and platelet count (264 *vs.* 83 k/μL; $P = .0036$). In high-risk MDS and AML, with a median follow-up of 12.5 months (0.2-201), patients with biallelic *DNMT3A*showed significantly poorer 1-year OS rate than patients with single hits (42.8% *vs.* 65.8%; $P = .0095$). Longitudinal molecular analysis of 8 cases (2 MDS at onset and upon progression to sAML; 4 AML at diagnosis and following relapse after conventional intensive chemotherapy-induced remission; 1 AML at diagnosis and CR post SCT; 1 AML and donor-derived AML post SCT) demonstrated an early origin of biallelic hits with leukemogenic potential. Indeed, biallelic clones persisted or expanded in 6 transformed cases.

Our study describes the likelihood that biallelic configuration of *DNMT3A* mutations, while rare, is indeed compatible with clonal expansion and thus questions the applicability of a synthetic lethality strategy. We are currently confirming that, indeed, truly biallelic cases *vs.* mosaicism are permissive using DNASeq of single sorted progenitor cells. The results of this analysis will be available at the time of ASH meeting.

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Figure 1. Schematic representation of DNMT3A hits.

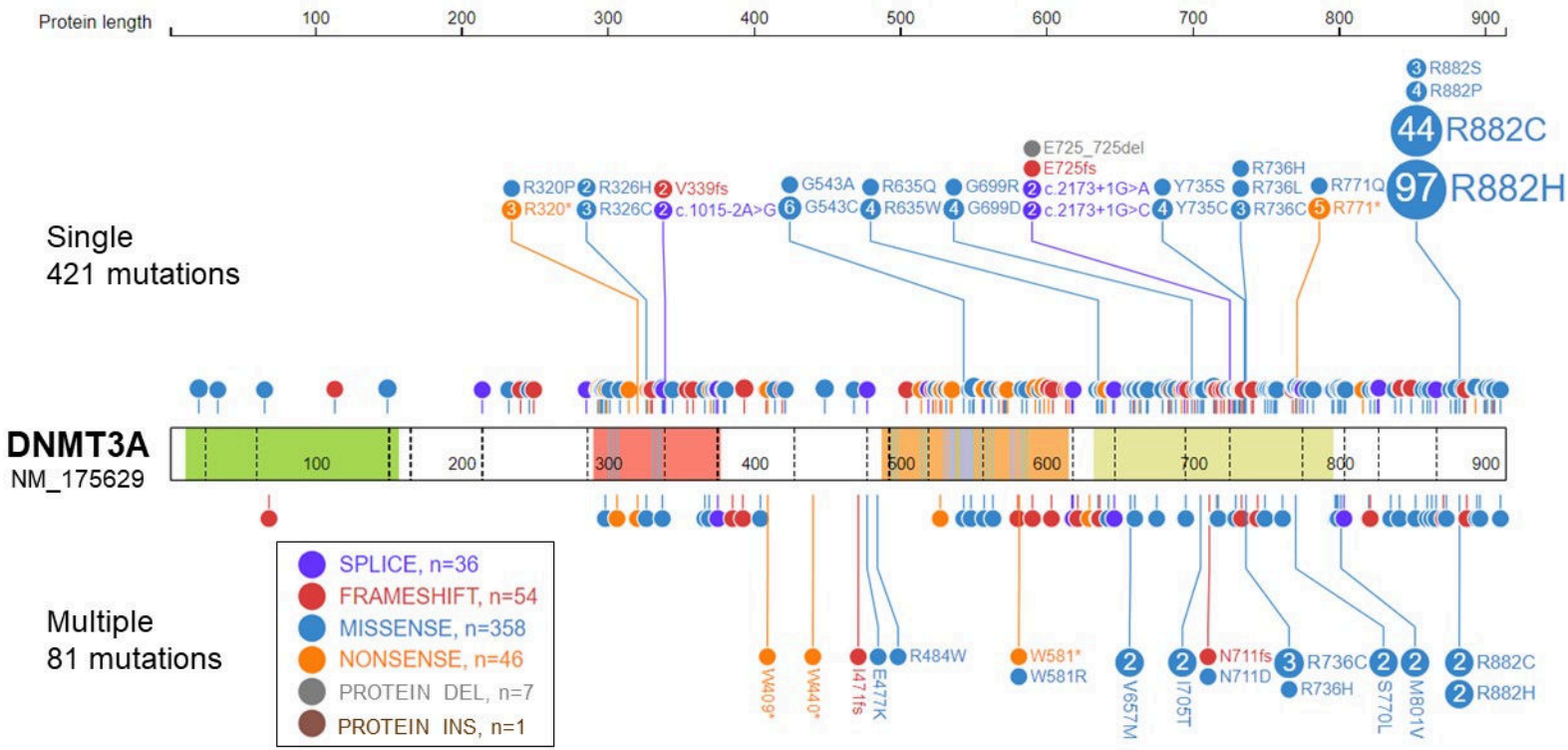

Figure 1