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ORAL ABSTRACTS

509.BONE MARROW FAILURE AND CANCER PREDISPOSITION SYNDROMES: CONGENITAL

Compound Heterozygosity Involving Other *Dead* (*DDX*) and *Deah* (*DHX*) Box Helicases Explains Seemingly Monoallelic Somatic and Germline *DDX41* Mutations

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DDX41 mutations typify a seemingly distinct group of AML/MDS that appear to differ in many features from wild type entities. Classical *DDX41* mutant myeloid neoplasia (MN) harbors biallelic mutations whereby, canonical germline (GL), mostly frameshifts (fs, chiefly at amino acid position 140) predispose to the acquisition of somatic canonical R525H and possible other somatic variants in the contralateral allele. Yet, in many instances, only GL or somatic mutations can be found. We stipulated that somatic *DDX41* is not present in mature leukocytes but would be detectable in hematopoietic stem cells (HSCs). Indeed, higher VAF is detected in HSCs *vs* neutrophils likely due to differentiation arrest. However, it is also possible that other somatic mutations (*e.g.*, in other RNAhelicases) and conversely in cases with R525H without GL *DDX41* might be present. In either case, upon our original discovery based on lower ATPase activity of R525H mutants [1] we stipulated that this mutation would be hypomorphic, an indirect evidence that has been, to date, not further substantiated. Here, we propose that R525H may indeed be functional *via* some unknown mechanisms, such as a GOF variant selected in the mode of somatic gene rescue (SGR) as a maladaptive response to relieve the GL LOF fs alteration.

To that end, we studied the molecular makeup of a cohort of 1,893 MN patients (including public datasets [2,3]) using NGS. In total, 101 patients with MN harbored *DDX41* lesions. Among these, 35 patients had somatic hits other than R525H, detected as a seemingly monoallelic configuration and 15 patients harbored biallelic hits involving D140fs (n=3), M1I/L (n=2), and other pathogenic GL variants (n=10). A total of 51 patients carried GL mutations without matching a second allele hit; including D140 as monoallelic mutation in 4 patients, fs/del/splice sites in 10 patients, and missense variants in 37 patients (among this, M1I/L accounted for 24%).

When we studied these monoallelic GL cases, we found a second somatic alteration in other *DDX/ DHX* helicases in carriers of seemingly monoallelic fs/splice sites. We found somatic hits in *DDX10* (n=7), *DDX25* (n=2), *DDX52* (n=1), *DHX16* (n=4) and *DHX34* (n=1) suggesting that neoplastic progression occurring in these cases was due to compound heterozygosity involving other helicases substituting for the absence of canonical biallelic configuration. Of note is that no hit in any other helicases was detected in cases with sole GL D140, leaving unexplained the somatic cause driving neoplastic progression in these cases. However, upon manual inspection, R525H was detected in 2 patients with D140 and one patient with M1I albeit at very low VAFs%. Ultra low input NGS of pre-sorted bone marrow cellular fractions showed higher clonal burden of somatic R525H in HSCs, revealing the truly biallelic nature of these cases. [4]

We then sought to explain the absence of GL in cases with R525H and other pathogenic lesions. For instance, we found one suspicious GL variant in *DDX20* in one patient with R525H and one in *DDX31* in one patient with non-R525 hit. These results suggest that monoallelic *DDX41* cases may have compound heterozygosity (somatic/GL *vs* GL/somatic) for *DDX41* and other *DDX* genes.

These compound heterozygous cases prompted us to screen other patients with wild type *DDX41* for the presence of *DDX/DHX* mutations. In total, we identified 38 monoallelic cases. Of those, 22 were GL with the most frequent being *DDX54*

(n=4), *DDX11*, and *DDX20* (n=3; for both), followed by others. We are currently exploring whether these cases might harbor cryptic R525H, which might have been not resolved in bulk sequencing.
In summary, we show that the canonical *DDX41* biallelic constellation is a more ubiquitous phenomenon in the disease evolution to MN and involves other DDX/DHX helicases. In fact, a proportion of seemingly monoallelic cases might be explained by compound heterozygosity of other RNA helicases. Moreover, most GL *DDX41* are cryptic biallelic or compound heterozygous, while cases with somatic canonical *DDX41* should be investigated for the presence of GL *DDX41* or other RNA helicases using ultra low input NGS. We propose that somatic *DDX41* hits correspond to maladaptive SGR of hypomorphic GL mutants in analogy but in reverse fashion compared to GL SAMD9/9L. Studies to be presented at ASH will further substantiate this possibility.

Disclosures Maciejewski: *Omeros:* Consultancy; *Regeneron:* Consultancy, Honoraria; *Alexion:* Membership on an entity's Board of Directors or advisory committees; *Novartis:* Honoraria, Speakers Bureau.

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