Title: Low Allele Fraction, High Stakes: Discerning Germline Mosaic *TP53* Variants from Clonal Hematopoiesis of Indeterminate Potential (CHIP)

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Background:

Clonal hematopoiesis of indeterminate potential (CHIP) occurs when acquired somatic variants cause hematopoietic cell expansion in the absence of an overt hematologic malignancy, and is most common at older ages. It can be characterized by the detection of variants with a low variant allele fraction (VAF) in epigenetic regulatory genes, including *DNMT3A*, *ASXL1*, and *TET2*. Likely pathogenic/pathogenic variants (PVs) in *TP53* are also frequently observed with low VAF and their detection during germline genetic testing presents a diagnostic challenge: these findings may represent germline mosaic Li-Fraumeni syndrome (LFS) or incidentally detected CHIP. This study aims to evaluate evidence from a genetic testing laboratory to inform the distinction between mosaic LFS and CHIP.

Methods:

We retrospectively reviewed de-identified clinical and genetic data from individuals who underwent multi-gene panel testing from November 2024-May 2025 and had a low VAF *TP53* PV (VAF <40%). Phenotype, confirmatory and cascade testing, and any co-occurring variants in *DNMT3A, ASXL1,* or *TET2* were evaluated with descriptive statistics.

Results:

A total of 208 individuals with a low VAF *TP53* PV were identified over 7 months. Preliminary data were available for 24 individuals. Of these, 2/24 (8.33%) had a co-occurring variant in a CHIP gene (*TET2* and *DNMT3A* splice-site variants at 47.2 and 54.8% VAF; *TP53* VAFs were ~13%). These individuals were aged 66 and 76 at testing, with personal histories of laryngeal squamous cell carcinoma (age 45) and uterine cancer (age 28), respectively. Another individual, aged 80 with a neuroendocrine tumor, had confirmatory testing on fibroblasts, which did not detect the *TP53* PV. Among the remaining 21 cases, (average age 72.8 years; range 31-90), there were 28 cancer diagnoses, including four diagnosed <age 46 (3 breast and 1 thyroid cancer). Cascade testing of four relatives from two families failed to clarify CHIP versus mosaicism.

Conclusions:

These data are consistent with literature showing that CHIP occurs at older ages, as the average age for our cohort with low VAF *TP53* PVs was >70 years. Additional data on co-

occurring CHIP variants, age at testing, and phenotype may help stratify whether a low VAF *TP53* PV result is more likely to be caused by CHIP or *TP53* mosaicism, which has clinical implications for patients and their families. We are in the process of more robustly analyzing a larger cohort to further elucidate these associations.