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ORAL ABSTRACTS

642.CHRONIC LYMPHOCYTIC LEUKEMIA: CLINICAL AND EPIDEMIOLOGICAL

Laboratories Can Reliably Detect Clinically Relevant Variants in the *TP53* Gene below 10 % Allelic Frequency: A Multicenter Study of ERIC, the European Research Initiative on CLL

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The presence of mutations in the *TP53* gene is a powerful prognostic and predictive marker in chronic lymphocytic leukemia (CLL). Widespread use of NGS has enabled the detection of variants ≤10 % variant allelic frequency (low-VAF variants); however, the overall reliability and reproducibility of NGS techniques to identify such variants have been questioned repeatedly. Individual studies using sensitive, custom NGS-based assays have mostly demonstrated the shortened overall survival (OS) and event-free survival in patients with low-VAF *TP53* variants treated with chemoimmunotherapy (CIT) regimens with median survival ranging between that of TP53 variants >10 % VAF (high-VAF) and wild-type *TP53* (wt- *TP53*).

Within an ERIC multicenter study, we tested the ability of NGS methods used in diagnostic and research laboratories to detect low-VAF *TP53* variants and analyzed the impact of the identified low-VAF variants on patients' survival.

In the first phase of the study (Fig. 1), seven sample mixes containing 23 pathogenic *TP53* variants (range, 0.7-6.3% VAF) were analyzed in 41 ERIC centers using 44 NGS-based assays. All variants were validated with droplet digital PCR (ddPCR); obtained values were used as a reference for the assessment of each NGS method's performance. NGS results were categorized as true positive (TP), false positive (FP; not present in original samples and reported by one center each), and not reported/false negative (FN). In total, laboratories reported 77.8% of all variants (784 out of 1008), reaching a sensitivity [TP/ (TP + FN)] of 85.6%, 94.5%, and 94.8% at 1%, 2%, and 3% VAF cut-off, respectively. While the VAFs of individual variants reported by laboratories varied, median values strongly correlated with ddPCR (R^2=0.9841). Thirty-eight FP variants were reported by 10 laboratories, mainly <2% VAF (23 FP of VAF ≤1%, 14 FP of VAF >1 and ≤2%, 1 FP > 2%). Individual feedback was provided to improve the methods' performance and to help set an appropriate detection limit.

In the second phase of the study, 12 centers provided results of *TP53* NGS-based analysis of 1092 CLL clinical samples taken before first-line treatment (median time from sample to treatment 40 days). The impact of low-VAF variants (1-10% VAF; N=59) on time to second treatment (TTST; event: second treatment, death) and OS calculated from 1 st treatment initiation was compared to that of high-VAF variants (N=123) and wt- *TP53* using logrank test with Benjamini-Hochberg correction of p-values. TTST (Fig. 2) of the low-VAF group was significantly shorter compared to wt- *TP53* (P=0.013; median TTST wt- *TP53* 3.6 y, low-VAF 2.8 y, high-VAF 1 y) in patients not treated with targeted agents (N=999). If del(17p) status was considered, median TTST was the shortest in patients with a combination of del(17p) and either high (0.8 y) or low-VAF (1 y) *TP53* mutations, followed by high-VAF (1.5 y) and low-VAF (2.8 y) mutations in the absence of del(17p) (P<0.001, P=0.032, P<0.001, P=0.026, respectively, compared to wt- *TP53*/no del(17p) (3.6 y)). In patients receiving frontline targeted agents (N=73; enriched for *TP53* mutations), the results suggested shorter TTST for the high-VAF group only, but the difference was not significant (Fig. 2; P=0.06; median wt- *TP53* n.r., low-VAF 4.8 y and high-VAF 3.6 y).

OS of patients with low-VAF variants was significantly shorter compared to the wt- *TP53* group in patients never treated with targeted treatment (P=0.033; median OS wt- *TP53* 6.6 y, low-VAF 3.2 y and high-VAF 2.1 y). Targeted therapy in 2 nd or later therapy lines diminished the difference and only OS of the high-VAF group differed significantly from wt- *TP53* (P<0.001; median OS wt- *TP53* 10.6 y, low-VAF 8.6 y, and high-VAF 5.1 y).

Altogether, we show that the cumulative reliability (no FN and FP) of methods tested increased continuously with VAF (Fig. 1), reaching 30% and 64% for variants ≥1.1% and 2% VAF, respectively. The reliability was affected by the type of NGS method and bioinformatic pipeline settings. We conclude that no strict threshold can be suggested from a technical standpoint. However, our results emphasize a strong need to validate/verify the NGS method, describe its limits, and report only reliable results. From a clinical standpoint, while low-VAF variants impact clinical outcomes for patients receiving CIT in the frontline setting, their clinical impact for patients treated with novel therapies remains to be evaluated in larger cohorts.

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Figure 1: Performance of NGS methods

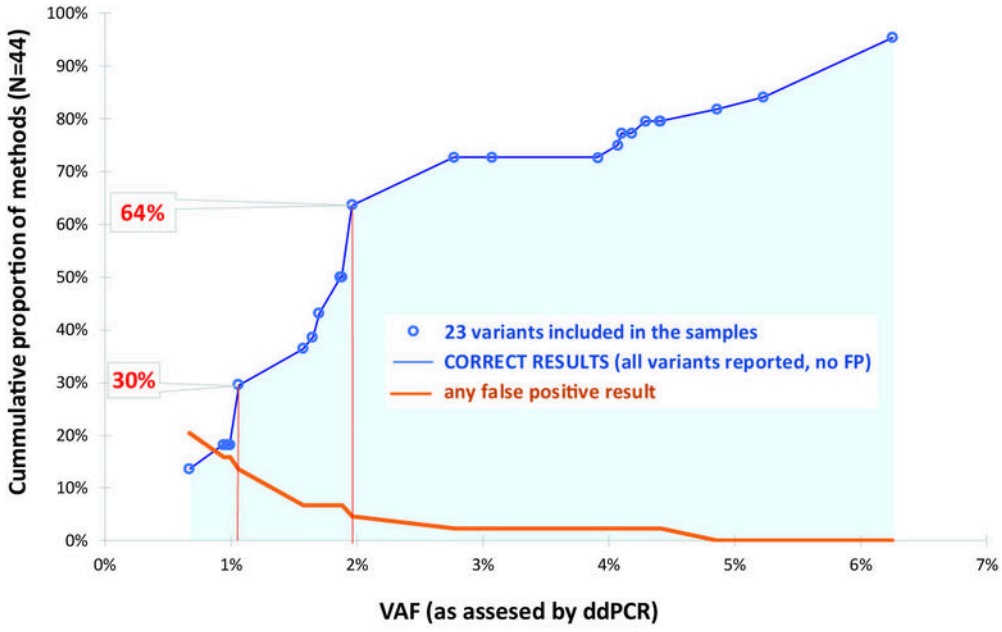

Figure 2: Time to second therapy

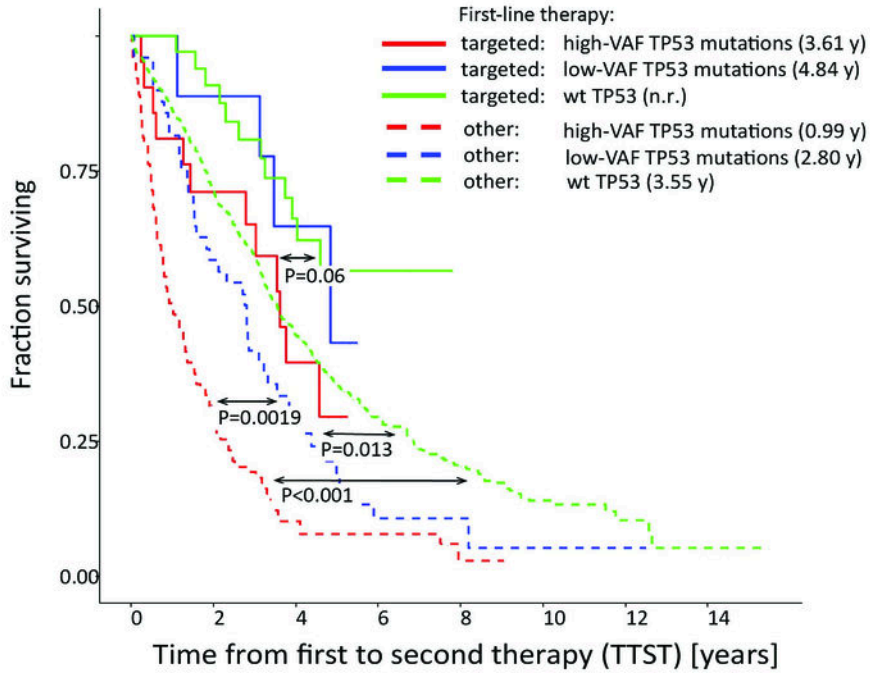

Figure 1

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