



## The 65th ASH Annual Meeting Abstracts

## 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  $\leq 10\%$  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  $\leq 1\%$ , 14 FP of VAF  $> 1$  and  $\leq 2\%$ , 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<sup>st</sup> 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).

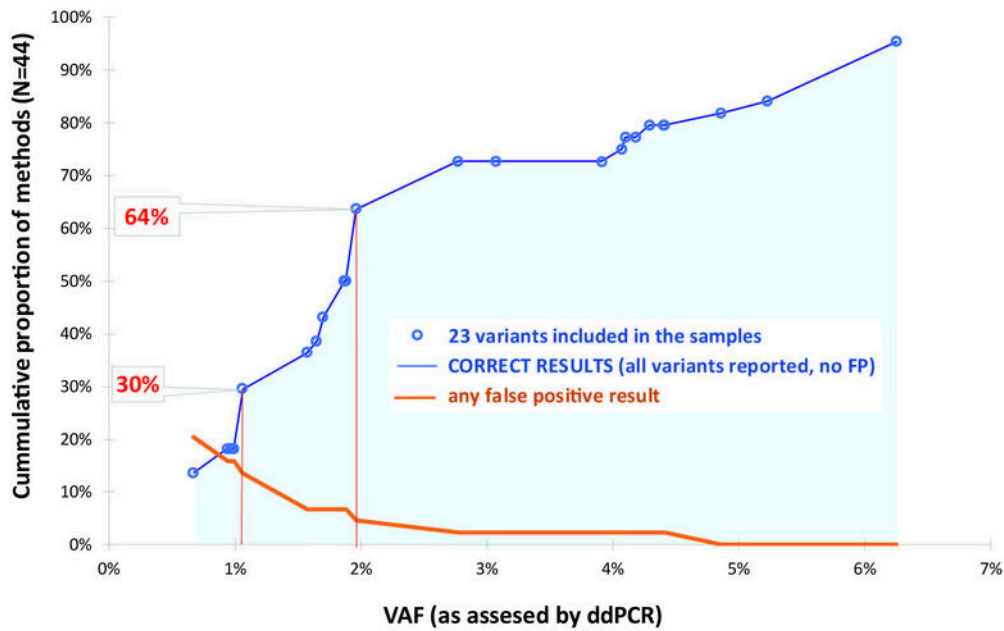
OS of patients with low-VAF variants was significantly shorter compared to the wt- *TP53* group in patients never treated with targeted treatment ( $P=0.033$ ; median OS wt- *TP53* 6.6 y, low-VAF 3.2 y and high-VAF 2.1 y). Targeted therapy in 2<sup>nd</sup> or later therapy lines diminished the difference 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  $\geq 1.1\%$  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.

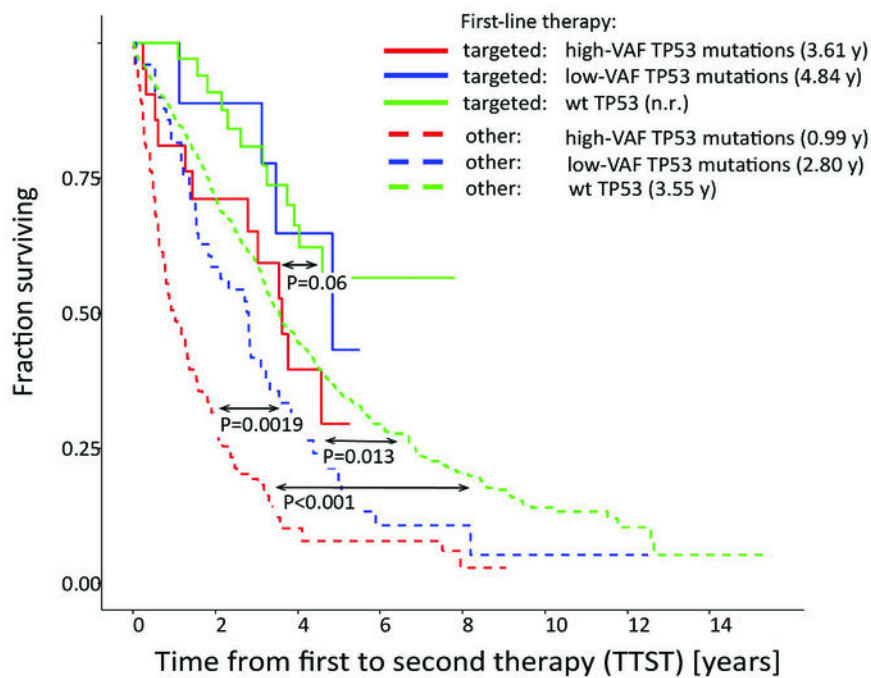
**Disclosures Brieghel:** Octapharma: Other: Travel grant. **Andres:** AstraZeneca, Novartis, Roche, Janssen-Cilag: Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Other: travel support. **Bellosillo:** ThermoFisher: Research Funding, Speakers Bureau. **Stilgenbauer:** Amgen: Consultancy, Honoraria, Other: travel support, Research Funding; **Abbvie:** Consultancy, Honoraria, Other: travel support, Research Funding; **Janssen:** Consultancy, Honoraria, Other: travel support, Research Funding; **Roche:** Consultancy, Honoraria, Other: travel support, Research Funding; **GSK:** Consultancy, Honoraria, Other: travel support, Research Funding; **Gilead:** Consultancy, Honoraria, Other: travel support, Research Funding; **Celgene:** Consultancy, Honoraria, Other: travel support, Research Funding; **AstraZeneca:** Consultancy, Honoraria, Other: travel support, Research Funding; **Novartis:** Consultancy, Honoraria, Other: travel support, Research Funding; **Sunesis:** Consultancy, Honoraria, Other: travel support, Research Funding. **Tausch:** Janssen-Cilag: Consultancy, Honoraria, Other: travel support, Speakers Bureau; **AstraZeneca:** Consultancy, Honoraria, Other: travel support, Speakers Bureau; **BeiGene:** Consultancy, Other: Travel support, Speakers Bureau; **Roche:** Consultancy, Honoraria, Research Funding, Speakers Bureau; **Abbvie:** Consultancy, Honoraria, Other: Travel support, Research Funding, Speakers Bureau. **Czekalska:** AstraZeneca: Honoraria. **Chatzidimitriou:** Novartis: Other; **Janssen:** Other. **Walewska:** AbbVie, AstraZeneca, Janssen, Beigene: Other: meeting attendancies. **da Silva:** AstraZeneca: Research Funding; **Janssen Cilag:** Consultancy, Research Funding; **Abbvie:** Consultancy, Research Funding; **Roche:** Consultancy, Research Funding; **Takeda:** Consultancy, Research Funding. **Rossi:** AbbVie, AstraZeneca, Gilead, BeiGene, BMS, Janssen, Lilly, Kyte: Honoraria, Research Funding. **Baliakas:** Gilead: Honoraria. **Kahre:** AstraZeneca Estonia: Honoraria. **Alcoceba:** Janssen, AstraZeneca: Honoraria, Other: Travel expenses. **Scarfo:** Octapharma: Speakers Bureau; **Lilly:** Consultancy; **Janssen:** Consultancy; **BeiGene:** Consultancy; **AstraZeneca:** Consultancy; **AbbVie:** Consultancy. **Costa:** Roche: Consultancy, Honoraria; **BMS:** Consultancy, Honoraria; **Astrazeneca:** Consultancy, Honoraria; **Genmab:** Consultancy, Honoraria; **Abbvie:** Consultancy, Honoraria; **Janssen:** Consultancy, Honoraria. **Davi:** Janssen, AstraZeneca:

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**Figure 1: Performance of NGS methods**



**Figure 2: Time to second therapy**



**Figure 1**

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