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ARTICLE

Detection of clinically relevant variants in the *TP53* gene below 10% allelic frequency: A multicenter study by ERIC, the European Research Initiative on CLL

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Abstract

In chronic lymphocytic leukemia, the reliability of next-generation sequencing (NGS) to detect *TP53* variants ≤10% allelic frequency (low-VAF) is debated. We tested the ability to detect 23 such variants in 41 different laboratories using their NGS method of choice. The sensitivity was 85.6%, 94.5%, and 94.8% at 1%, 2%, and 3% VAF cut-off, respectively. While only one false positive (FP) result was reported at >2% VAF, it was more challenging to distinguish true variants <2% VAF from background noise (37 FPs reported by 9 laboratories). The impact of low-VAF variants on time-to-second-treatment (TTST) and overall survival (OS) was investigated in a series of 1092 patients. Among patients not treated with targeted agents, patients with low-VAF *TP53* variants had shorter TTST and OS versus wt-*TP53* patients, and the relative risk of second-line treatment or death increased continuously with increasing VAF. Targeted therapy in ≥2 line diminished the difference in OS between patients with low-VAF *TP53* variants and wt-*TP53* patients, while patients with high-VAF *TP53* variants had inferior OS compared to wild type-*TP53* cases. Altogether, NGS-based approaches are technically capable of detecting low-VAF variants. No strict threshold can be suggested from a technical standpoint, laboratories reporting *TP53* mutations should participate in a standardized validation set-up. Finally, whereas low-VAF variants affected outcomes in patients receiving chemoimmunotherapy, their impact on those treated with novel therapies remains undetermined. Our results pave the way for the harmonized and accurate *TP53* assessment, which is indispensable for elucidating the role of *TP53* mutations in targeted treatment.

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INTRODUCTION

Genetic alterations (mutations and/or deletions) in the *TP53* gene have been repeatedly associated with poor prognosis in chronic lymphocytic leukemia (CLL).[1,2] While patients with *TP53* aberrations usually relapse early after chemoimmunotherapy-based regimens, they strongly benefit from targeted treatment with kinase and BCL2 inhibitors.[3–6] As such, *TP53* aberrations should be assessed before each therapy line both by sequencing and interphase fluorescence in-situ hybridization (I-FISH).[7] Historically, *TP53* gene sequencing was performed with Sanger sequencing with a detection limit >10% variant allele frequency (VAF). The introduction of NGS allowed the detection of clones carrying mutations «10%, and this raised questions about the technical reliability and clinical utilization of detecting these very small clones.

Decreasing the VAF threshold for detecting variants increases the risk of an erroneous result. The wrong interpretation of the background noise may result in reporting a false positive result or missing the true variant present in the position where the background noise is high.[8] In addition, insufficient DNA input and sequencing coverage or poor sequencing quality may lead to omitting a low-VAF variant. Various approaches have been introduced to correctly identify true low-VAF variants, involving wet-lab modifications, for example, unique molecular identifiers (UMI), either single or duplex, and advanced error-correction methods embedded in the bioinformatics pipelines.[9–11] Nowadays, multiple commercially available gene panels with inbuilt bioinformatics analysis aim to detect variants of 5% VAF or even lower, and several single-gene kits detecting *TP53* variants down to 1% VAF are available.

In CLL, several groups demonstrated a strong selective advantage of small *TP53*-aberrant subclones under the selection pressure of chemoimmunotherapy, resulting in their clonal expansion in relapse and disease deterioration.[12–14] Studies employing sensitive, custom-based NGS methods with tailored bioinformatics tools showed the negative prognostic impact of *TP53* variants <10% VAF in patients treated with chemoimmunotherapy.[12,14–18] Collectively, these results and the wide implementation of NGS challenged the arbitrary Sanger-equivalent 10% VAF threshold for reporting *TP53* variants, which was previously recommended by the European Research Initiative on CLL (ERIC) in CLL in 2018.[19] This approach was recently re-evaluated and specific VAF cut-off for reporting is no longer recommended.[20]

We present here the results of a multicenter study organized by the *TP53* Network of ERIC addressing (i) the reliability and reproducibility of NGS-based methods used in diagnostic and research laboratories for the detection of low-VAF *TP53* variants and (ii) the impact of low-VAF *TP53* variants identified in these laboratories on patients' outcome.

METHODS

Samples, cell lines, and patients

Reference samples for interlaboratory comparison of NGS-based methods were prepared by diluting primary CLL samples ($N = 7$; including one sample containing two *TP53* variants), and *TP53*-mutated tumor cell lines ($N = 8$) (Supporting Information S1: Table S1) in DNA isolated from peripheral blood of the healthy donor. *TP53* variants were described using the reference MANE transcript NM_000546.6. The samples were prepared at University Hospital Brno, Czech Republic (Samples 2 and 4–7) and IRCCS Ospedale San Raffaele, Milan, Italy (Samples 1 and 3), and sent to 41 laboratories participating in the study.

To analyze the impact of low-VAF *TP53* mutations on time to second treatment (TTST) and overall survival (OS), data from 1210 patients with CLL were collected from 12 centers. The inclusion criteria were as follows: patients treated for CLL with *TP53* analysis performed before the first therapy was initiated. Clinical data were curated to reveal discrepancies in disease timeline and inconsistencies with inclusion criteria; cases with discordant data and patients untreated until the last follow-up were excluded from the study. The final dataset included 1092 cases from whom peripheral blood samples were collected between 1998 and 2021. Informed consents, approved by the ethical committees of the participating hospitals, were obtained in accordance with the Declaration of Helsinki. Diagnostic criteria, indications for treatment initiation, and response assessment followed the International Workshop on CLL (iwCLL) guidelines.[7]

Droplet digital polymerase chain reaction (ddPCR)

The variants present in reference samples were validated using ddPCR. ddPCR was performed from 60 ng DNA in triplicates using custom ddPCR assays specific to each *TP53* variant and the QX200 Droplet Digital PCR System (BioRad).

Next-generation sequencing (NGS) methods

NGS-based assays ($N = 44$) were used by the participating laboratories ($N = 41$; three laboratories participated with two methods) to analyze the reference samples (detailed in Supporting Information S1: Table S2). The methods used by laboratories participating in the second part of the study ($N = 12$) to identify *TP53* variants in patients with CLL are shown in Supporting Information S1: Table S3; eight centers used an identical method as for the reference samples, and the remaining four centers applied an extended panel for all or part of the samples.

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Curation of *TP53* variants

For all *TP53* variants identified in patient samples by individual centers in the second phase of the study, data from *in-vitro* functional studies[21–23] were recorded from The *TP53* Database (https://tp53.cancer.gov)[24] and The *TP53* Website (https://p53.fr/).[25] The presence of variants in the human population was collected from GnomAD.[26] Common population variants c.215C>G, c.639A>G, and c.108G>A were not considered. Variants with concordant results from *in-vitro* functional studies pointing to preserved activity and/or variants interpreted as likely benign or benign by the ClinGen *TP53* Variant Curation Expert Panel,[27] or described as a rare missense polymorphism present in human populations[28] were excluded from further analyses ($N = 9$; Supporting Infomaton S1: Table S4).

Statistical analysis

All statistical analyses were performed in R version 4.1.1[29] with additional packages. Statistical significance was defined as $p < 0.05$. Graphical visualizations were performed in R and GraphPad Prism. The correlations of ddPCR to median and individual NGS results were calculated using the Spearman correlation coefficient. The impact of assay design on method performance and correlation of the results to ddPCR were assessed by the Kruskal–Wallis test followed by post hoc tests (continuous values) or Fisher exact test (categorical values).

Survival analyses were performed by the Kaplan–Meier method and between-group comparisons were performed using the log-rank test; the association between variables and survival was estimated by Cox regression with Benjamini-Hochberg correction for multiple testing (survival R-package). TTST was measured from the start date of first-line treatment to the date of second-line treatment or death, whichever came first (event) or last follow-up (censoring). Overall survival (OS) was considered from the date of first-line treatment to the date of death from any cause (event) or last follow-up (censoring). To visualize changes in the relative risk of second-line treatment and death, spline curves were constructed in R.

TABLE 1 List of reference samples and pathogenic variants used in interlaboratory comparison.

Sample	Variant cDNA (NM_000 546.6)	Variant protein (NP_000 537.3)	ddPCR (% VAF)
mTP53_1	c.524G>A	p.(Arg175His)	4.3
mTP53_1	c.743G>A	p.(Arg248Gln)	2.8
mTP53_1	c.853G>A	p.(Glu285Lys)	4.2
mTP53_1	c.166G>T	p.(Glu56Ter)	3.9
mTP53_1	c.580C>T	p.(Leu194Phe)	4.1
mTP53_2	None	None	NA
mTP53_3	c.524G>A	p.(Arg175His)	1.1
mTP53_3	c.743G>A	p.(Arg248Gln)	0.7
mTP53_3	c.853G>A	p.(Glu285Lys)	1.0
mTP53_3	c.166G>T	p.(Glu56Ter)	0.9
mTP53_3	c.580C>T	p.(Leu194Phe)	1.0
mTP53_4	c.949dup	p.(Gln317ProfsTer20)	5.2
mTP53_4	c.173del	p.(Pro58GlnfsTer65)	6.3
mTP53_5	c.559+1G>A	p.?	1.7
mTP53_6	c.949dup	p.(Gln317ProfsTer20)	1.6
mTP53_6	c.173del	p.(Pro58GlnfsTer65)	1.9
mTP53_7	c.626_627del	p.(Arg209LysfsTer6)	4.1
mTP53_7	c.741_742delinsTT	p.(Arg248Trp)	4.9
mTP53_7	c.817C>T	p.(Arg273Cys)	1.9
mTP53_7	c.685_689del	p.(Cys229HisfsTer9)	3.1
mTP53_7	c.949C>T	p.(Gln317Ter)	1.7
mTP53_7	c.337T>G	p.(Phe113Val)	2.0
mTP53_7	c.569C>T	p.(Pro190Leu)	4.4[a]
mTP53_7	c.672+1G>T	p.?	4.4[a]

[a]The two variants are located on the same allele. Only the VAF of variant c.569 C>T was validated by ddPCR.

RESULTS

Interlaboratory comparison of NGS methods

Six reference samples were prepared by mixing healthy donor DNA with DNA containing 16 pathogenic *TP53* variants isolated from primary CLL samples and tumor cell lines (Supporting Information S1: Table S1) to reach ~1%–10% VAF. One reference sample contained healthy donor DNA only. Seven variants were represented twice, albeit with different VAFs; altogether, the samples contained 23 pathogenic *TP53* variants of various types (missense, nonsense, splice, in-frame delins, frameshift deletion, and duplication), at different VAF, located within exons 4 to 9 and adjacent splice sites (Table 1). The allelic frequency of variants in individual samples was quantified by ddPCR using custom assays and ranged from 0.7% to 6.3% VAF. The results of ddPCR were used as reference values.

Samples were tested in 41 laboratories, all of which had previously passed the ERIC *TP53* Certification (http://www.ericll.org/certification-of-tp53-analysis/), an external quality assessment program organized to ensure the quality of *TP53* mutation detection and interpretation. Participating laboratories performed NGS analysis, each using a methodology of their choice. Three laboratories analyzed the samples independently with two methods; altogether, 44 sets of results were obtained (Supporting Information S1: Table S5). The applied methods included a broad portfolio of commercial as well as laboratory-developed protocols (Table 2 and Supporting Information S1: Table S2). At the time of the study, most laboratories adhered to the ERIC *TP53* Recommendations 2018 by reporting variants with >5%–10% VAF, and they had not validated the LOD for the identification of low-VAF variants in routine practice. Yet, the laboratories were asked to estimate their method's LOD; the estimated LOD values are shown in Figure 1A. Since validation was not required prior to the study, and not all laboratories provided the LOD estimation, the performance of methods was assessed at 1%, 2%, and 3% VAF thresholds.

In total, 823 variants in the coding region of the *TP53* gene were reported. The variants were classified as follows (Figure 1A): (i) true positive (TP) results ($N = 785$), that is, variants present in the reference samples and confirmed by ddPCR; (ii) false positive (FP) results ($N = 38$), that is, variants not present in the reference samples, reported by one laboratory each; (iii) not reported ($N = 224$), that is, variants present in the reference samples but not reported by the laboratory. For the purpose of the performance analysis, all variants not reported above a certain cut-off (1%, 2%, and 3% VAF) were considered false negative (FN).

For each variant, the median VAF was calculated from values reported by the participating laboratories (Supporting Information S1: Figure S1). Although the reported VAF varied as expected, the

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TABLE 2 Summary of methods used by laboratories participating in interlaboratory comparison.

Total	44
Library preparation principle	
Amplicon-based	36[a]
Capture-based	8
Library preparation	
in-house (primers/probes designed in the laboratory)	11[b]
Agilent/Multiplicom *(CLL MASTR Plus; SureMASTR TP53)*	3
Agilent *(HaloPlex; SureSelect)*	2
ArcherDX *(VariantPlex)*	2
GenDx	1
Illumina *(TruSight)*	4
Paragon Genomics *(CleanPlex)*	1
Qiagen *(QIAseq)*	2
Roche+NimbleGen *(KAPA HyperPrep/SeqCap EZ)*	2
Seqplexing	1
Sophia Genetics	4
Thermo Fisher or Illumina *(AmpliSeq™ technology)*	11
Sequencing platform	
Illumina	33
Ion Torrent	11
Unique molecular identifiers (UMI)	
No	35
Yes	9
Bioinformatic pipeline	
Commercial	26
In-house	14
Combination of both	4

[a]Single-prime extension $N = 4$.

[b]One method commercialized later (EasySeq-RC-PCR, Nimagen).

median values significantly correlated with the values assessed by ddPCR (Figure 1B; Spearman $R = 0.9849$, $p < 0.001$).

The cumulative reliability of NGS methods for detecting low-frequent *TP53* variants increased continuously with variant allele frequency

To assess the performance of NGS methods, the following parameters were calculated: (i) proportion of detected variants that are true positives (positive predictive value, PPV = TP/[TP+FP]; reaches 100% if no false positive results are reported), (ii) proportion of known variants that were detected by the test system (Sensitivity = TP/[TP+FN]; reaches 100% if all known variants are reported) (Table 3 and Supporting Information S1: Table S5). In total, laboratories reported 77.8% of all variants present in reference samples, reaching the sensitivity of 85.6%, 94.5%, and 94.8% at 1%, 2%, and 3% VAF cut-off, respectively. In total, 38 FP variants were reported by 10 laboratories; of them, 37 with VAF <2%. The variants that laboratories failed to report often occurred near the declared LOD and can be attributed to measurement variance. Among variants exceeding the LOD more prominently, the most frequent error represented a nucleotide insertion within a homopolymer sequence (c.949dup; Figure 1A). The cumulative capacity of methods to detect all variants with no FP results reached 30% and 64% for variants ≥1.1% and 2% VAF, respectively (Figure 1C).

Next, we explored which parameters in the assay design and bioinformatics analysis might affect method sensitivity, PPV, and correlation to ddPCR results (Supporting Information S1: Tables S2 and S5). The capacity to detect all variants with very low VAF (100% sensitivity; Fisher exact test; Supporting Information S1: Figure S2) was higher in assays including only the *TP53* gene (vs. gene panels; $p = 0.003$ for variants >1% VAF, Fisher exact test), laboratory-developed, that is, in-house and custom assays (vs. predesigned solutions; $p = 0.004$ and 0.03 for all and >1% variants, respectively), and in-house bioinformatics pipelines ($p = 0.006$ and 0.03 for all and >1% variants, respectively). We observed no difference between capture and amplicon-based assays. The Illumina sequencing platform demonstrated higher sensitivity values at 1%, 2%, and 3% VAF threshold compared to Ion Torrent ($p < 0.05$; Kruskal–Wallis test; Supporting Information S1: Figure S3). On the other hand, no false positive variants >1% VAF were reported by laboratories using the Ion Torrent platform. These results were likely related to the fact that most laboratories using Ion Torrent employed predesigned assays that are designed to be robust. In this context, "robust" refers to the assay's ability to maintain consistent performance despite minor variations in method parameters and its reduced sensitivity to outliers, albeit at the cost of a higher limit of detection (LOD).

Furthermore, we observed differences even among laboratories using the same type of NGS assay, likely due to using different sequencing approaches and bioinformatics pipelines. The results obtained by three laboratories after changing the parameters of the bioinformatics pipeline demonstrated that the less stringent setting allows for the identification of a higher number of true variants, however, it increases the risk of FP results (Supporting Information S1: Figure S4).

All participants received individual feedback comparing their findings to both the ddPCR results and the results of other participants (see Supporting Information Material for an example of an individual report) with the aim of helping them to improve the reliability of their methods and help set a more proper LOD. Based on the obtained feedback, laboratories changed the assay (ID17, 12, 18), modified the bioinformatics pipeline (ID23, 4, 27), identified a pipeline error that skipped essential noise-filtering (ID32), or set the correct LOD (ID13).

Clinical impact of low-VAF *TP53* variants

In the second part of the study, we addressed the clinical relevance of low-VAF *TP53* variants identified independently of the method used. Twelve centers provided results of *TP53* analysis in patients with CLL followed at their clinics with the corresponding clinical and laboratory data. The curated cohort included 1092 CLL patients treated for CLL with *TP53* analysis performed before therapy initiation (Supporting Information S1: Figure S5 and Supporting Information S1: Table S6). The median time from sample collection to treatment was 40 days (interquartile range 3–198 days; 82% of samples taken <1 year before treatment initiation). Seventy-three patients receiving frontline therapy with kinase or BCL2 inhibitors were analyzed separately; this subgroup was enriched for *TP53* aberrations as targeted therapy was initially approved for patients with *TP53* gene disruption. The size of this subgroup and a short median follow-up of 3.9 years enabled us to analyze TTST only.

In total, 182/1092 (16.7%) patients carried one or more *TP53* mutations ≥1% VAF (237 mutations, 31 patients with ≥2 variants; Supporting Information S1: Table S7). In 59 patients, the allelic

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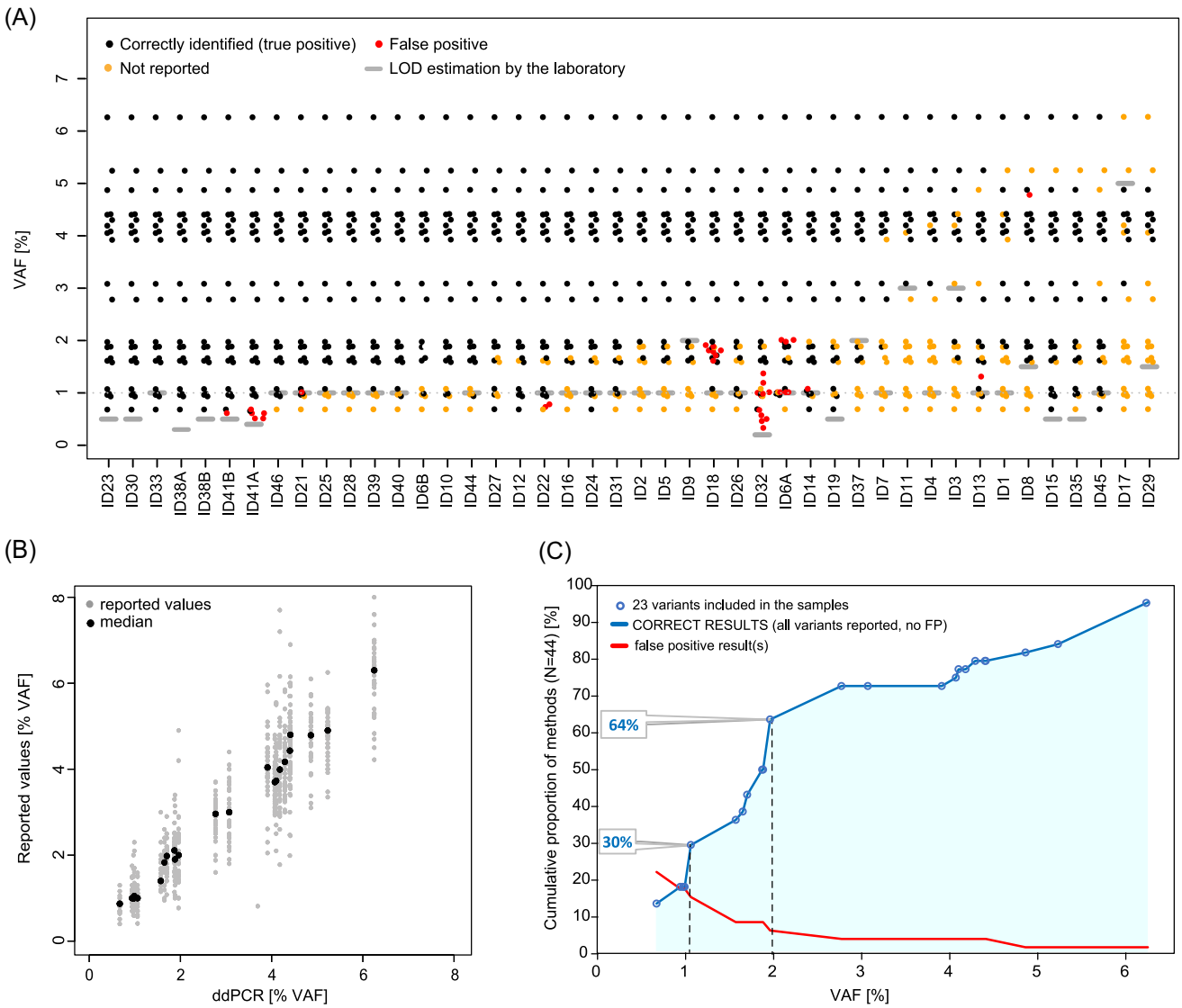

FIGURE 1 **Results of interlaboratory comparison of NGS methods. (A)** Results provided by 41 laboratories testing 44 methods. Black and orange dots represent variants present in reference samples with VAF values measured by ddPCR; the color distinguishes variants reported (black) and not reported (orange) by the laboratory. Methods (*X*-axis) are ordered by the lowest variant allele frequency (VAF) that was detected without missing any variant present in reference samples and without false positive results (red dots; VAF value reported by the laboratory). **(B)** Correlation of median VAF values reported by participants and VAF values obtained from droplet digital PCR (ddPCR) analysis for individual variants. Spearman correlation coefficient $R = 0.9849$. **(C)**. Overall performance of tested methods. Blue area shows the cumulative proportion of the methods (*Y*-axis) in relation to the lowest VAF (values measured by ddPCR; *X*-axis) that was reliably detected, that is, all variants present in reference samples were reported without false positivity. Red line shows the cumulative proportion of methods reporting false positive results (VAF value reported by the laboratory).

frequency of the variant with the highest VAF was ≤10%, and the sample was categorized as a "low-VAF *TP53* variant" (Table 4). As expected, we observed a significantly uneven distribution of main cytogenetic subgroups,[1] mainly concerning the high proportion of patients with del(17p) in the high-VAF *TP53* mutation subgroup (60.3%; $p < 0.001$ compared to both the wt-*TP53* and low-VAF *TP53* subgroups). The high-VAF subgroup was also enriched for patients with unmutated IGHV genes (82.5%; $p = 0.0001$ and $p = 0.0756$ compared to the wt-*TP53* and low-VAF *TP53* subgroups, respectively). In contrast, the proportion of patients with unmutated IGHV genes in the low-VAF subgroup did not significantly differ from that of the wt-*TP53* subgroup (69.6% vs. 65.3%, n.s.). Furthermore, this comparison confirmed that different types of frontline therapies had been used for patients with *TP53* mutations, especially in the high-VAF *TP53* mutation group, where the use of chemoimmunotherapy was limited. Targeted regimens were more frequently used in both the high-VAF and low-VAF *TP53* mutation groups. In addition, we observed that patients in the high-VAF subgroup were slightly but significantly older at diagnosis compared to others within this cohort.

The impact of low-VAF variants on TTST (Figure 2A–C) and OS (Figure 2D) was compared to that of the patients with high-VAF variants and wt-*TP53*. In patients not receiving targeted agents in the frontline (for details see Supporting Information S1: Table S6), the TTST (Figure 2A) in the low-VAF group was significantly shorter compared to wt-*TP53* and longer compared to the high-VAF group ($p = 0.013$ and 0.002, respectively; median TTST: wt-*TP53* 3.6 y, low-VAF 2.8 y, high-VAF 1 y). If del(17p) status was considered and groups were compared to wt-*TP53*/no del(17p) (median TTST 3.6 y),

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the shortest TTST was seen in patients with a combination of del(17p) and high-VAF *TP53* mutations (median TTST 0.8 y/$p < 0.001$ and 1 y/$p = 0.032$, respectively), followed by high-VAF and low-VAF mutations in the absence of del(17p) (median TTST 1.5 y/$p < 0.001$ and median TTST 2.8 y/$p = 0.026$, respectively) (Figure 2B). No TTST shortening was observed for patients with sole del(17p) (median TTST 3.9 y).

In patients receiving frontline targeted agents ($n = 73$), we did not observe any significant difference between the patient subgroups after the Benjamini-Hochberg correction, although a trend toward shorter TTST was observed for the high-VAF group (Figure 2C; $p = 0.06$; median wt-*TP53* not reached, low-VAF 4.8 y and high-VAF 3.6 y). The TTST analysis with the stratification based on the combinations of del(17p) and *TP53* mutation, and the type of regimen (detailed in Supporting Information S1: Table S6), was not possible due to the low numbers of patients in individual subgroups.

For OS, we analyzed separately patients receiving targeted treatment in the first or later relapses since it has been shown that targeted treatment also improves OS in patients with relapsed/refractory (R/R) disease.[3,6] Initially, these therapies were preferably used in patients with del(17p) and/or *TP53* mutations, including those with a low-VAF *TP53* clone before first-line therapy who experienced an expansion of the clone upon relapse. For patients who had not received targeted treatment until the last follow-up, OS was significantly shorter in both patients with low- and high-VAF variants compared to the wt-*TP53* group ($p = 0.033$ and $p < 0.0001$, respectively; median OS: wt-*TP53* 6.6 y, low-VAF 3.2 y and high-VAF 2.1 y). Among those receiving targeted therapy in 2nd or later therapy lines, only the OS of the high-VAF group differed significantly from wt-*TP53* ($p < 0.001$) while patients with wt-*TP53* and low-VAF *TP53* mutations detected at frontline therapy initiation demonstrated similar OS (median OS wt-*TP53* 10.6 y, low-VAF 8.6 y, and high-VAF 5.1 y) (Figure 2D).

TABLE 3 The performance of methods used by laboratories participating in interlaboratory comparison.

	Total	>1% VAF	>2% VAF	>3% VAF
Evaluated	1009[a]	833	528	484
True positive	785	713	499	459
False positive	38	15	1	1
PPV (TP/[TP+FP])	95.4%	97.9%	99.8%	99.8%
Any false positive (methods, *N*)	10	6	1	1
Any false positive (methods, %)	22.7%	13.6%	2.3%	2.3%
Not reported (false negative)	224	120	29	25
Sensitivity (TP/[TP+FN])	77.8%	85.6%	94.5%	94.8%
Any not reported (methods, *N*)	37	13	12	12
Any not reported (methods, %)	84.1%	29.5%	27.3%	27.3%

Note: For the purpose of the performance analysis, all variants not reported above the cut-off (1%, 2%, and 3% VAF) were considered false negative (FN).

[a]Three samples with one variant each were excluded from analysis due to technical issues.

TABLE 4 The summary of CLL cases included in the survival analyses.

	Total		*TP53* wt		High-burden *TP53* mutation (≥10% VAF)		Low-burden *TP53* mutation (1%–10% VAF)		
	Cases (No.)	% (range)	Cases (No.)	% (range)	Cases (No.)	% (range)	Cases (No.)	% (range)	*p*-Value[a]
No. of patients	**1092**	*100.0*	**910**		**123**		**59**		
Sex									n.s.
Female	395	*36.2*	324	*35.6*	46	*37.4*	25	*42.4*	
Male	697	*63.8*	586	*64.4*	77	*62.6*	34	*57.6*	
Age at diagnosis, median y (range)	64	*(28.6–94.0)*	63.9	*(28.6–94.0)*	67.3	*(29.4–89.8)*	64.88	*(41.1–91.0)*	0.004
IGHV status									0.0004
Mutated	344	*32.5*	306	*34.7*	21	*17.5*	17	*30.4*	
Unmutated	714	*67.5*	576	*65.3*	99	*82.5*	39	*69.6*	
FISH hierarchical									
Del(17p)	98	*9.7*	23	*2.7*	70	*60.3*	5	*9.1*	<0.0001
Del(11q)	213	*21.1*	193	*23.0*	10	*8.6*	10	*18.2*	0.0011
Trisomy 12	156	*15.4*	142	*16.9*	7	*6.0*	7	*12.7*	0.0063
Del(13q)	297	*29.4*	256	*30.5*	20	*17.2*	21	*38.2*	0.0053
Normal	247	*24.4*	226	*26.9*	9	*7.8*	12	*21.8*	<0.0001
First-line treatment									
TTFT from dg (median, months)	22	*(0–341.0)*	24.0	*(0–292.7)*	13.8	*(0–252.3)*	21.2	*(0–341.0)*	
Targeted treatment (BcR and BCL2 inhibitors)	73	*6.7*	42	*4.6*	22	*17.9*	9	*15.3*	<0.0001
FCR, FC-Ofa	467	*42.8*	417	*45.8*	27	*22.0*	23	*39.0*	<0.0001
BR	180	*16.5*	162	*17.8*	8	*6.5*	10	*16.9*	0.0032
Other than above	372	*34.1*	289	*31.8*	66	*53.7*	17	*28.8*	<0.0001

Abbreviations: B, bendamustine; C, cyclophosphamide; F, fludarabine; n.s., not significant; Ofa, ofatumumab; R, rituximab.

[a]Fisher exact test (categorical values) or Kruskal–Wallis test (age).

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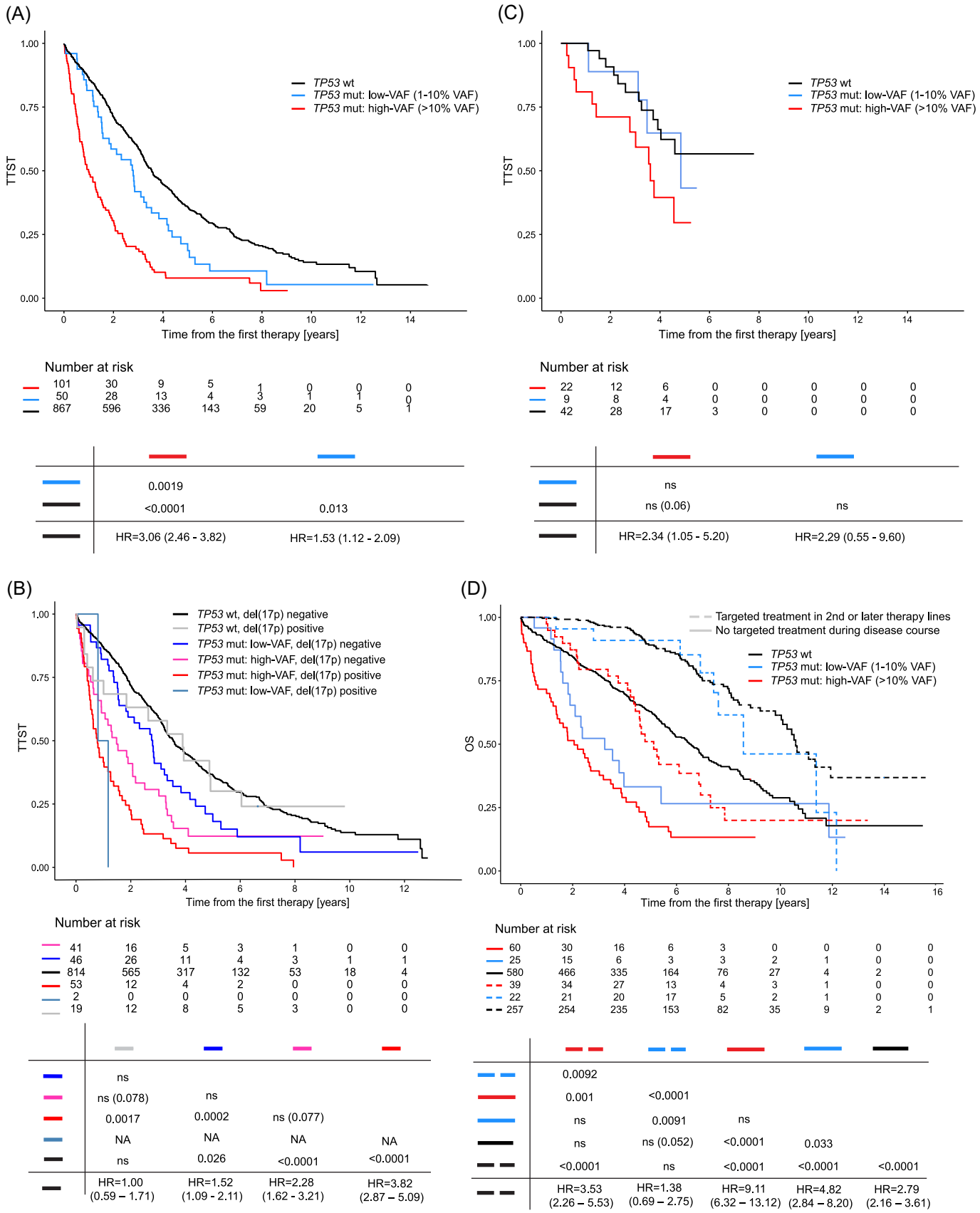

FIGURE 2 (See caption on next page).

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FIGURE 2 **Survival in patients analyzed in 12 participating centers stratified by *TP53* mutation status. (A, B)** Time to second treatment (TTST) in patients not receiving targeted treatment frontline stratified by variant allele frequency (VAF) **(A)** and by VAF and del(17p) presence **(B)**. **(C)** TTST in patients treated with frontline targeted agents stratified by VAF. Event = 2nd treatment or death, censored = untreated and alive at last follow-up. **(D)** Overall survival (OS) from therapy initiation. Low-VAF = 1%–10%; high-VAF = >10% VAF. Targeted treatment = BcR or BCL-2 inhibitor; for details on treatment see Supporting Information S1: Table 6. Group comparison–LogRank test with Benjamini-Hochberg correction of *p*-values. HR, hazard ratio: numbers in brackets show a 95% confidence interval.

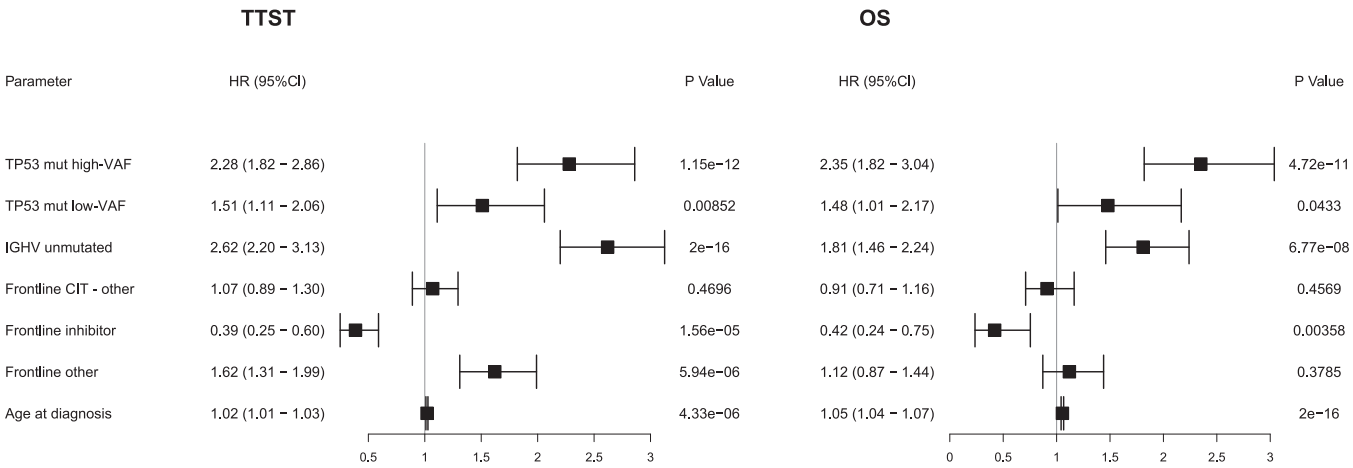

FIGURE 3 **Multivariate Cox model for TTST and OS**. Wild-type *TP53*, mutated IGHV, and FCR-like therapy were used as reference; 1058 patients with data available for all parameters were included. Frontline CIT–other, chemoimmunotherapy other than FCR-like; Frontline other–other therapies. CI, confidence interval; HR, hazard ratio.

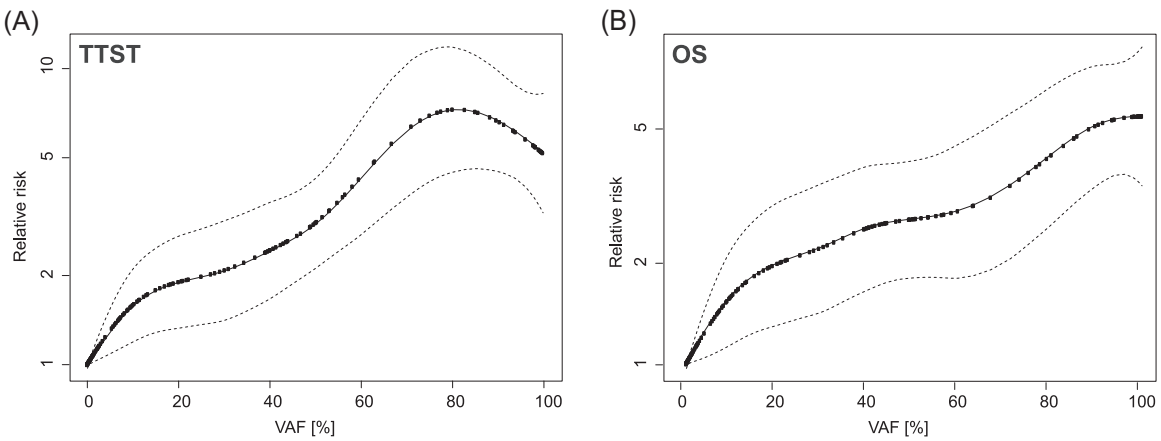

FIGURE 4 **Relative risk for TTST (A) and OS (B) in relation to *TP53* mutation VAF in patients not receiving targeted treatment frontline**. The increasing relative risks of the event (TTST: 2nd treatment or death, OS: death) were obtained from the Cox model with penalized spline term for continuous predictor "VAF highest" centered at 0, that is, *TP53* wildtype. VAF highest – % VAF of a single variant in the sample or the highest VAF if multiple variants were present.

Given that unmutated IGHV somatic hypermutation status is a known marker of poor prognosis and that we observed imbalanced proportions of IGHV status between cohorts with high-VAF and low-VAF *TP53* mutations, we next limited our analyses to 714 patients with unmutated IGHV genes. As shown in Supporting Information S1: Figure S6, the results were consistent with those of the whole cohort, though the difference in TTST between patients with wt-*TP53* and those with low-VAF mutations was less pronounced. Additionally, we analyzed the impact of IGHV status and therapy type across the entire cohort using a multivariate Cox model (Figure 3). Within our cohort, *TP53* mutation status, IGHV status, age, and type of frontline treatment were identified as independent prognostic factors for TTST and OS.

Since 517 patients were included in a previously published single-center cohort[14] (follow-up updated for this study), we assessed whether the conclusions stayed the same if this cohort was removed from the analysis (Supporting Information S1: Figure S7A–C). Although shifts toward lower statistical significance were observed since the lower number of patients in subgroups reduced statistical power, the overall pattern corresponded with the findings obtained from the whole cohort.

Finally, we demonstrated that the relative risk of second-line treatment or death increased continuously with increasing VAF in patients not receiving frontline targeted agents, indicating that no clinically significant VAF threshold for low-VAF variants can be applied (Figure 4).

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DISCUSSION

The present study was initiated after ERIC issued recommendations on the analysis of *TP53* variants in CLL in 2018[19] and aimed to address two main objections against decreasing the threshold for reporting *TP53* variants below 10% VAF. The first concerned the insufficient reliability of NGS methods when aiming for detection of variants <10% VAF, potentially leading to a high rate of incorrect results; the second concerned the lack of evidence about the clinical impact of low-VAF variants.

In the first part of the study, we documented that the majority of NGS-based approaches can reliably detect *TP53* variants below 5% VAF. Although background noise complicates variant detection at this level, most laboratories identified variants correctly while avoiding FP results. Since the information about the limits of a given method is an inseparable part of the reporting process, the participating laboratories were asked to provide the LOD, the parameter describing the lowest VAF value significantly exceeding background noise. Some laboratories had not established the LOD for the detection of low-VAF variants in routine practice, as they had reported variants >5%–10% VAF in line with ERIC *TP53* Recommendations valid at the time of the study,[19] and they used the current study to explore the potentialities of their methods. Others had implemented tools for assessing variant-specific LODs. For these reasons, we evaluated the overall performance of methods used at different VAF thresholds. All 13 variants present in the tested samples with VAF 2% and higher were reliably detected (without FP calls) by 64% of methods, and 30% of methods even successfully called all 19 variants ranging from 1.1% to 6.3% VAF. Thus, the data presented here show that NGS-based approaches used in molecular hematology laboratories are technically capable of detecting low-VAF variants. On the other hand, the fact that six laboratories reported FP variants >1% VAF emphasizes the need for thorough validation (laboratory-developed tests and ready-to-use commercial assays outside the recommended range of use) or verification (ready-to-use commercial methods) of all steps of the NGS-based approach, including the bioinformatics pipeline.[30–33] This process aims to set parameters of the method so that FP results are avoided while the number of false negative results is kept as low as possible.

The validation and verification of an NGS assay aiming below the LOD of Sanger sequencing is challenging. Besides sequencing healthy controls, the procedure should involve as many variants as possible, as the background noise of NGS is position and variant-specific, and pathogenic variants can occur in virtually any position of the *TP53* gene.[20,24,25,34] No certified reference material containing multiple types of *TP53* variants is currently available, and the only orthogonal method with sufficient sensitivity is ddPCR. Although we proved the usability of this method here, it requires the preparation of a specific assay for each variant, thus rendering thorough validation expensive. The above-described challenges underscore the substantial value of participation in interlaboratory comparison programs after completing the validation/verification process. We showed here that the median VAF reported by laboratories participating in interlaboratory comparison can be used as a reliable reference since VAF values correlated with the results of ddPCR. More recently, ERIC advocates the inclusion of samples with *TP53* variants <10% VAF of various types in the external quality assessment programs organized in cooperation with GenQA/NEQAS-Li (ERIC *TP53* Certification, CLL *TP53* EQA of GenQA). While our study showed that some laboratory-developed assays targeting only the *TP53* gene on the Illumina platform could detect variants with very low VAF, these assays are typically designed for use in a single laboratory by experienced users and require thorough validation for routine diagnostics. Commercial assays, on the other hand, require a less extensive verification process, but because they are typically designed to be more robust and to minimize false positive results, they tend to have a higher LOD. Given that laboratories have different infrastructures available, we adhere to the current ERIC recommendation that the laboratories select their assay based on the available resources and equipment (including computational infrastructure), their specific focus (e.g., parallel analysis of other genes and diseases versus a stand-alone assay for *TP53*), and regional legal and reimbursement requirements.[20]

In the second part of the study, we tested the impact of low-VAF variants on patient survival. In patients treated with chemoimmunotherapy, we confirmed the previously published negative impact of low-VAF *TP53* mutations. The inferior survival of these patients is in line with the fact that *TP53*-aberrant clones unambiguously possess a strong selective advantage under the pressure of chemoimmunotherapy. This selective advantage manifested in significantly shortened OS for patients with low-VAF *TP53* subclones compared to wt-*TP53* patients in four of six studies.[12,14–18] The impact on progression-free survival after first-line treatment was less studied and appeared less prominent, probably due to the time required for the expansion of *TP53*-aberrant clones.[14,16,18] Overall, the pattern of Kaplan–Meier curves is similar among studies, including the data presented here, with the curve of low-VAF variants located between that of wild-type *TP53* and high-VAF mutations. Nevertheless, differences between the studies were apparent with the survival of the low-VAF group being reported as highly similar to that of the high-VAF group,[12,15] different both from the high-VAF and wt *TP53* groups,[14] and differing neither from the high-VAF nor from the wt *TP53* groups.[16] These differences may be attributed to different cohort compositions since some studies analyzed early-stage cohorts with a higher proportion of patients carrying mutated IGHV genes, while others studied patients with active disease where those with unmutated IGHV genes predominated. Further, treatment type and intensity differed among the studies, which potentially affected the result as the selection advantage of *TP53*-aberrant clones depends on the level of resistance to the specific drug, the rate of elimination of leukemic cells, and the growth advantage of the residual population. Less efficient drugs like chlorambucil exert weaker selective pressure than regimens efficiently eliminating wild-type cells, for example, FCR (fludarabine-cyclophosphamide-rituximab).[14,35] At the same time, the selective potential of kinase and BCL-2 inhibitors against *TP53*-aberrant CLL cells appears to be less impactful, with all scenarios of clonal development being observed (persistence, expansion, and disappearance),[14,36–42] although the frequent expansion of *TP53* mutated clones was documented in patients with progressive disease receiving BTKi in pretreated cohorts.[42,43] Accordingly, patients with *TP53* aberrations who respond poorly to chemo- and chemoimmunotherapy benefit from targeted treatment,[3–6] as also shown in our study (Figure 2). Still, the presence of a *TP53* defect remains a negative prognostic marker, at least in cohorts of patients with R/R CLL and in frontline fixed-duration venetoclax-obinutuzumab regimen.[44–46]

In the era of chemoimmunotherapy, some studies reported that patients carrying the combination of *TP53* mutation and 17p13 deletion had shorter median survival compared to patients with isolated *TP53* aberration,[47,48] which is what we also observed here for TTST. Today, this observation might be even more relevant for patients treated with targeted agents, as some studies have shown that only the combination of *TP53* mutation and 17p13 deletion negatively impacts outcome, while the targeted therapy may overcome the negative impact of sole *TP53* mutations.[49–51] Altogether, the impact of the type of alteration and clonal size in patients treated with targeted agents remains to be resolved. Unfortunately, an insufficient number of patients treated with frontline targeted treatment in the present study, regimen heterogeneity, and short follow-up did not

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allow us to reach firm conclusions on this issue. In particular, while our study demonstrates that the relative risk associated with increasing VAF of *TP53* mutations rises continuously in patients treated with frontline chemotherapy, it remains unclear whether this pattern also applies to patients treated with targeted therapies. Conducting similar analyses in large cohorts of patients treated solely with targeted therapies, stratified by continuous and fixed duration regiments, is crucial to address this gap in knowledge. Studies addressing this important question must build on reliable, validated methodology, which is what this publication aims at as part of ERIC harmonization activities.

We acknowledge that our study has limitations and biases inherent to a retrospective real-world multicentric study. First, collecting data from several centers and time periods resulted in a wide range of treatment regimens. Second, the type of sample processing differed among centers, with some using mononuclear cell separation while others employing separation of CD19 cells. When mononuclear cell fraction is used for *TP53* analysis in a sample with a low proportion of leukemic cells, some low-VAF variants may not be truly low-burden.[8] This, however, reflects routine practice in many diagnostic laboratories; not reporting such cases may result in omitting *TP53* aberrations present in the majority of cancer cells.

CONCLUSION

We conclude that no universal LOD cut-off for reporting *TP53* variants is currently justifiable either from a technical or clinical standpoint. We argue that when low-VAF variants are identified using a reliable, validated method, they should be included in the clinical report to allow the referring physician to decide about treatment.[20] This is a practical approach, especially considering that the treatment portfolio is dynamically evolving and the availability of novel drugs differs worldwide. While low-VAF variants impact clinical outcomes for patients receiving CIT, their clinical impact for patients treated with novel therapies remains to be evaluated in larger cohorts. Moreover, longer follow-up is needed to resolve whether targeted therapies might impact clonal dynamics of *TP53* aberrant clones during long-term treatment and after several lines of chemo-free therapy. The data from prospective clinical trials will hopefully resolve the question as to whether low-VAF *TP53* variants represent a risk for inferior outcomes in patients treated with targeted agents.

AUTHOR CONTRIBUTIONS

All authors contributed to the article, critically evaluated the content, and approved the submitted version. Sarka Pavlova, Jitka Malcikova, and Silvia Bonfiglio designed and coordinated the study, and performed variant curation. Sarka Pavlova and Jitka Malcikova wrote the manuscript, and Kostas Stamatopoulos, Paolo Ghia, and Richard Rosenquist critically revised the text. Sarka Pavlova, Silvia Bonfiglio, and Christian Brieghel prepared reference samples and/or performed ddPCR. Lenka Radova and Tereza Pikousova performed statistical analyses. Jack B. Cowland, Mette K. Andersen, Maria Karypidou, Bella Biderman, Michael Doubek, Gregory Lazarian, Inmaculada Rapado, Andrew Hindley, David Donaldson, Hero Nikdin, Anastasia Chatzidimitriou, Stamatia Laidou, Svetlana Smirnova, Eugene Nikitin, Amy Christian, Renata Walewska, David Oscier, Marta Sebastião, MGS, Tereza Pikousova, Terezia Kurucova, Lydia Scarfo, Francesca Gandini, Ettore Zapparoli, Adoración Blanco, Pau Abrisqueta, Paula Gameiro, Joaquin Martinez-Lopez, Bárbara Tazón-Vega, Fanny Baran-Marszak, Zadie Davis, Andrey Sudarikov, and Carsten U. Niemann performed NGS for reference and clinical samples and/or analyzed NGS data, and provided clinical data. Lenka Radova, Michael Doubek extracted clinical data. Matthijs Vynck, Naomi A. Porret, Martin Andres, Dina Rosenberg, Dvora Sahar, Carolina Martínez-Laperche, Ismael Buño, Julio B. Sánchez, José A. García-Marco, Alicia Serrano-Alcalá, Blanca Ferrer-Lores, Concepción Fernández-Rodriguez, Beatriz Bellosillo, Stephan Stilgenbauer, Eugen Tausch, Fiona Quinn, Emer Atkinson, Lisette van de Corput, Cafer Yildiz, Cristina Bilbao-Sieyro, Yanira Florido, Christian Thiede, Caroline Schuster, Anastazja Stoj, Sylwia Czekalska, Audrey Bidet, Charles Dussiau, Friedel Nollet, Giovanna Piras, Maria Monne, Ivan Sloma, Alexis Claudel, Laetitia Largeaud, Loïc Ysebaert, Peter J. M. Valk, Piero Galieni, Mario Angelini, Davide Rossi, Valeria Spina, Sónia Matos, Vânia Martins, Tomasz Stokłosa, Monika Pepek, Panagiotis Baliakas, Rafa Andreu, Irene Luna, Tiina Kahre, Ülle Murumets, Sophie Laird, Daniel Ward, Miguel Alcoceba, Ana Balanzategui, Ana E. Rodríguez-Vicente, Rocío Benito, Frédéric Davi, and Clotilde Bravetti performed NGS for reference samples and/or analyzed NGS data.

CONFLICT OF INTEREST STATEMENT

Bárbara Tazón-Vega: Honoraria: Bristol Meyer Squibb. Beatriz Bellosillo: Advisory board honoraria, research support, travel support, speaker fees: Astra-Zeneca, BMS, Janssen, Merck-Serono, Novartis, Pfizer, Hoffman-La Roche, ThermoFisher. Christian Brieghel: Travel grant: Octapharma. Carsten U. Niemann: Research funding and/or consultancy fees: Abbvie, AstraZeneca, Beigene, Janssen, Genmab, Lilly, MSD, CSL Behring, Takeda, Octapharma. Davide Rossi: Honoraria: AbbVie, AstraZeneca, BeiGene, BMS, Janssen, Lilly. Research grants: AbbVie, AstraZeneca, Janssen. Travel grants: AstraZeneca, Janssen. Eugen Tausch: Honoraria and research support: Abbvie, AstraZeneca, BeiGene, Janssen, Hoffmann-La Roche; Research support from Abbvie, Roche, Gilead. Frédéric Davi: Honoraria: Janssen, AstraZeneca. Kostas Stamatopoulos: Research funding, honoraria and/or consultancy fees: Abbvie, AstraZeneca, Janssen, Lilly, Roche. Lydia Scarfo: Consultancy: AbbVie, AstraZeneca, BeiGene, Janssen, Lilly; Speaker Bureau: Octapharma. Miguel Alcoceba: Honoraria and travel grants: Janssen, AstraZeneca. Martin Andres: Consultancy, Honoraria, and travel support: AstraZeneca, Novartis, Roche, Janssen-Cilag. Maria Gomes da Silva: Consultancy and Research Funding: Janssen Cilag, AstaZeneca, Abbvie, Roche, Takeda. Pau Abrisqueta: Consultancy and Honoraria: Janssen, Abbvie, Roche, BMS, AstraZeneca, Genmab. Panagiotis Baliakas: Honoraria: Abbvie, Gilead, Janssen. Research funding: Gilead. Paolo Ghia: Honoraria: AbbVie, Astrazeneca, BeiGene, BMS, Galapagos, Janssen, Lilly/Loxo Oncology, MSD, Roche. Research funding: AbbVie, AstraZeneca, BMS, Janssen. Richard Rosenquist: Honoraria: AbbVie, AstraZeneca, Janssen, Illumina, Roche. Renata Walewska: Travel support: AbbVie, AstraZeneca, Janssen, Beigene. Sylwia Czekalska: Honoraria: AstraZeneca. Funding: Janssen, AstraZeneca. Stephan Stilgenbauer: Advisory board honoraria, research support, travel support, speaker fees: AbbVie, Acerta, Amgen, AstraZeneca, BeiGene, BMS, Celgene, Gilead, GSK, Hoffmann-La Roche, Infinity, Janssen, Lilly, Novartis, Sunesis, Verastem. Tiina Kahre: Honoraria: AstraZeneca. Tomasz Stokłosa: Honoraria and Research Funding: Janssen, AstraZeneca. The remaining authors have no competing interests to declare.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

REFERENCES

1. Döhner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med*. 2000; 343(26):1910-1916.
2. Hallek M, Fischer K, Fingerle-Rowson G, et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. *Lancet*. 2010;376(9747):1164-1174. doi:10.1016/S0140-6736(10)61381-5
3. Byrd JC, Hillmen P, O'Brien S, et al. Long-term follow-up of the RESONATE phase 3 trial of ibrutinib vs ofatumumab. *Blood*. 2019; 133(19):2031-2042. doi:10.1182/blood-2018-08-870238
4. Woyach JA, Ruppert AS, Heerema NA, et al. Ibrutinib regimens versus chemoimmunotherapy in older patients with untreated CLL. *N Engl J Med*. 2018;379(26):2517-2528. doi:10.1056/NEJMoa1812836
5. Sharman JP, Egyed M, Jurczak W, et al. Efficacy and safety in a 4-year follow-up of the ELEVATE-TN study comparing acalabrutinib with or without obinutuzumab versus obinutuzumab plus chlorambucil in treatment-naïve chronic lymphocytic leukemia. *Leukemia*. 2022;36(4):1171-1175. doi:10.1038/s41375-021-01485-x
6. Seymour JF, Kipps TJ, Eichhorst BF, et al. Enduring undetectable MRD and updated outcomes in relapsed/refractory CLL after fixed-duration venetoclax-rituximab. *Blood*. 2022;140(8):839-850. doi:10.1182/blood.2021015014
7. Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood*. 2018;131(25):2745-2760. doi:10.1182/blood-2017-09-806398
8. Pandzic T, Ladenvall C, Engvall M, et al. Five percent variant allele frequency is a reliable reporting threshold for *TP53* variants detected by next generation sequencing in chronic lymphocytic leukemia in the clinical setting. *Hemasphere*. 2022;6(8):e761. doi:10.1097/HS9.0000000000000761
9. Salk JJ, Schmitt MW, Loeb LA. Enhancing the accuracy of next-generation sequencing for detecting rare and subclonal mutations. *Nat Rev Genet*. 2018;19(5):269-285. doi:10.1038/nrg.2017.117
10. Spencer DH, Tyagi M, Vallania F, et al. Performance of common analysis methods for detecting low-frequency single nucleotide variants in targeted next-generation sequence data. *J Mol Diagn*. 2014;16(1): 75-88. doi:10.1016/j.jmoldx.2013.09.003
11. Kennedy SR, Schmitt MW, Fox EJ, et al. Detecting ultralow-frequency mutations by Duplex Sequencing. *Nat Protoc*. 2014;9(11): 2586-2606. doi:10.1038/nprot.2014.170
12. Rossi D, Khiabanian H, Spina V, et al. Clinical impact of small TP53 mutated subclones in chronic lymphocytic leukemia. *Blood*. 2014; 123(14):2139-2147. doi:10.1182/blood-2013-11-539726
13. Landau DA, Tausch E, Taylor-Weiner AN, et al. Mutations driving CLL and their evolution in progression and relapse. *Nature*. 2015;526(7574): 525-530. doi:10.1038/nature15395
14. Malcikova J, Pavlova S, Kunt Vonkova B, et al. Low-burden TP53 mutations in CLL: clinical impact and clonal evolution within the context of different treatment options. *Blood*. 2021;138(25): 2670-2685. doi:10.1182/blood.2020009530
15. Nadeu F, Delgado J, Royo C, et al. Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. *Blood*. 2016;127(17):2122-2130. doi:10.1182/blood-2015-07-659144
16. Blakemore SJ, Clifford R, Parker H, et al. Clinical significance of TP53, BIRC3, ATM and MAPK-ERK genes in chronic lymphocytic leukaemia: data from the randomised UK LRF CLL4 trial. *Leukemia*. 2020;34(7):1760-1774. doi:10.1038/s41375-020-0723-2
17. Brieghel C, Kinalis S, Yde CW, et al. Deep targeted sequencing of TP53 in chronic lymphocytic leukemia: clinical impact at diagnosis and at time of treatment. *Haematologica*. 2019;104(4):789-796. doi:10.3324/haematol.2018.195818

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18. Bomben R, Rossi FM, Vit F, et al. TP53 mutations with low variant allele frequency predict short survival in chronic lymphocytic leukemia. *Clin Cancer Res*. 2021;27(20):5566-5575. doi:10.1158/1078-0432.CCR-21-0701
19. Malcikova J, Tausch E, Rossi D, et al. ERIC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia-update on methodological approaches and results interpretation. *Leukemia*. 2018;32(5):1070-1080. doi:10.1038/s41375-017-0007-7
20. Malcikova J, Pavlova S, Baliakas P, et al. ERIC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia-2024 update. *Leukemia*. 2024;38(7):1455-1468. doi:10.1038/s41375-024-02267-x
21. Kato S, Han SY, Liu W, et al. Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. *Proc Natl Acad Sci USA*. 2003;100(14):8424-8429. doi:10.1073/pnas.1431692100
22. Giacomelli AO, Yang X, Lintner RE, et al. Mutational processes shape the landscape of TP53 mutations in human cancer. *Nat Genet*. 2018;50(10):1381-1387. doi:10.1038/s41588-018-0204-y
23. Kotler E, Shani O, Goldfeld G, et al. A systematic p53 mutation library links differential functional impact to cancer mutation pattern and evolutionary conservation. *Mol Cell*. 2018;71(1):178-190.e8. doi:10.1016/j.molcel.2018.06.012
24. de Andrade KC, Lee EE, Tookmanian EM, et al. The TP53 Database: transition from the International Agency for Research on Cancer to the US National Cancer Institute. *Cell Death Differ*. 2022;29(5):1071-1073. doi:10.1038/s41418-022-00976-3
25. Leroy B, Anderson M, Soussi T. TP53 mutations in human cancer: database reassessment and prospects for the next decade. *Hum Mutat*. 2014;35(6):672-688. doi:10.1002/humu.22552
26. Karczewski KJ, Francioli LC, Tiao G, et al The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581(7809):434-443. doi:10.1038/s41586-020-2308-7
27. Fortuno C, Lee K, Olivier M, et al. Specifications of the ACMG/AMP variant interpretation guidelines for germline TP53 variants. *Hum Mutat*. 2021;42(3):223-236. doi:10.1002/humu.24152
28. Doffe F, Carbonnier V, Tissier M, et al. Identification and functional characterization of new missense SNPs in the coding region of the TP53 gene. *Cell Death Differ*. 2021;28(5):1477-1492. doi:10.1038/s41418-020-00672-0
29. R Foundation for Statistical Computing. *A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing; 2021. http://www.R-project.org
30. ISO. International Standard ISO 15189: Medical laboratories—Requirements for quality and competence. In: Standardization tIOf, editor. Fourth edition; 2022.
31. Jennings LJ, Arcila ME, Corless C, et al. Guidelines for validation of next-generation sequencing–based oncology panels. *J Mol Diagn*. 2017;19(3):341-365. doi:10.1016/j.jmoldx.2017.01.011
32. Roy S, Coldren C, Karunamurthy A, et al. Standards and guidelines for validating next-generation sequencing bioinformatics pipelines. *J Mol Diagn*. 2018;20(1):4-27. doi:10.1016/j.jmoldx.2017.11.003
33. Mattocks CJ, Morris MA, Matthijs G, et al. A standardized framework for the validation and verification of clinical molecular genetic tests. *Eur J Hum Genet*. 2010;18(12):1276-1288. doi:10.1038/ejhg.2010.101
34. Donehower LA, Soussi T, Korkut A, et al. Integrated analysis of TP53 gene and pathway alterations in The Cancer Genome Atlas. *Cell Rep*. 2019;28(5):1370-1384. doi:10.1016/j.celrep.2019.07.001
35. Edelmann J, Tausch E, Landau DA, et al. Frequent evolution of copy number alterations in CLL following first-line treatment with FC(R) is enriched with TP53 alterations: results from the CLL8 trial. *Leukemia*. 2017;31(3):734-738. doi:10.1038/leu.2016.317
36. Bonfiglio S, Sutton LA, Ljungström V, et al. BTK and PLCG2 remain unmutated in one-third of patients with CLL relapsing on ibrutinib. *Blood Adv*. 2023;7(12):2794-2806. doi:10.1182/bloodadvances.2022008821
37. Landau DA, Sun C, Rosebrock D, et al. The evolutionary landscape of chronic lymphocytic leukemia treated with ibrutinib targeted therapy. *Nat Commun*. 2017;8(1):2185. doi:10.1038/s41467-017-02329-y
38. Cafforio L, Raponi S, Cappelli LV, et al. Treatment with ibrutinib does not induce a *TP53* clonal evolution in chronic lymphocytic leukemia. *Haematologica*. 2022;107(1):334-337. doi:10.3324/haematol.2020.263715
39. Gángó A, Alpár D, Galik B, et al. Dissection of subclonal evolution by temporal mutation profiling in chronic lymphocytic leukemia patients treated with ibrutinib. *Int J Cancer*. 2020;146(1):85-93. doi:10.1002/ijc.32502
40. Jain N, Croner LJ, Allan JN, et al. Absence of BTK, BCL2, and PLCG2 mutations in chronic lymphocytic leukemia relapsing after first-line treatment with fixed-duration ibrutinib plus venetoclax. *Clin Cancer Res*. 2024;30(3):498-505. doi:10.1158/1078-0432.CCR-22-3934
41. Naeem A, Utro F, Wang Q, et al. Pirtobrutinib targets BTK C481S in ibrutinib-resistant CLL but second-site BTK mutations lead to resistance. *Blood Adv*. 2023;7(9):1929-1943. doi:10.1182/bloodadvances.2022008447
42. Brown J, Mashima K, Fernandes S, et al. Mutations detected in real world clinical sequencing during BTK inhibitor treatment in CLL. *Res Sq*. 2024. doi:10.21203/rs.3.rs-3837426/v1
43. Woyach JA, Jones D, Jurczak W, et al. Mutational profile in previously treated patients with chronic lymphocytic leukemia progression on acalabrutinib or ibrutinib. *Blood*. 2024;144(10):1061-1068. doi:10.1182/blood.2023023659
44. Byrd JC, Furman RR, Coutre SE, et al. Ibrutinib treatment for first-line and relapsed/refractory chronic lymphocytic leukemia: final analysis of the pivotal phase Ib/II PCYC-1102 study. *Clin Cancer Res*. 2020;26(15):3918-3927. doi:10.1158/1078-0432.CCR-19-2856
45. Kater AP, Wu JQ, Kipps T, et al. Venetoclax plus rituximab in relapsed chronic lymphocytic leukemia: 4-year results and evaluation of impact of genomic complexity and gene mutations from the MURANO phase III study. *J Clin Oncol*. 2020;38(34):4042-4054. doi:10.1200/JCO.20.00948
46. Tausch E, Schneider C, Robrecht S, et al. Prognostic and predictive impact of genetic markers in patients with CLL treated with obinutuzumab and venetoclax. *Blood*. 2020;135(26):2402-2412. doi:10.1182/blood.2019004492
47. Gonzalez D, Martinez P, Wade R, et al. Mutational status of the TP53 gene as a predictor of response and survival in patients with chronic lymphocytic leukemia: results from the LRF CLL4 trial. *J Clin Oncol*. 2011;29(16):2223-2229. doi:10.1200/JCO.2010.32.0838
48. Stengel A, Kern W, Haferlach T, Meggendorfer M, Fasan A, Haferlach C. The impact of TP53 mutations and TP53 deletions on survival varies between AML, ALL, MDS and CLL: an analysis of 3307 cases. *Leukemia*. 2017;31(3):705-711. doi:10.1038/leu.2016.263
49. Huber H, Tausch E, Schneider C, et al. Final analysis of the CLL2-GIVe trial: obinutuzumab, ibrutinib, and venetoclax for untreated CLL with del(17p)/TP53mut. *Blood*. 2023;142(11):961-972. doi:10.1182/blood.2023020013
50. Brieghel C, Aarup K, Torp MH, et al. Clinical outcomes in patients with multi-Hit *TP53* chronic lymphocytic leukemia treated with ibrutinib. *Clin Cancer Res*. 2021;27(16):4531-4538. doi:10.1158/1078-0432.CCR-20-4890
51. Bomben R, Rossi FM, Vit F, et al. Clinical impact of TP53 disruption in chronic lymphocytic leukemia patients treated with ibrutinib: a campus CLL study. *Leukemia*. 2023;37(4):914-918. doi:10.1038/s41375-023-01845-9

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