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Clinical Outcomes in Patients with Multi-Hit *TP53* Chronic Lymphocytic Leukemia Treated with Ibrutinib

Christian Brieghel¹, Kathrine Aarup¹, Mathias H. Torp², Michael A. Andersen¹, Christina W. Yde², Xin Tian³, Adrian Wiestner⁴, Inhye E. Ahn^{#4}, Carsten U. Niemann^{#1}

¹Department of Hematology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

²Center for Genomic Medicine, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

³Office of Biostatistics Research, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA

⁴Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA

These authors contributed equally to this work.

Abstract

Purpose: *TP53* aberration (*TP53* mutation and/or 17p deletion) is the most important predictive marker in chronic lymphocytic leukemia (CLL). While each *TP53* aberration is considered an equal prognosticator, the prognostic value of carrying isolated (single-hit) or multiple (multi-hit) *TP53* aberrations remains unclear, particularly in the context of targeted agents.

Experimental Design: We performed deep sequencing of *TP53* using baseline samples collected from 51 *TP53* aberrant patients treated with ibrutinib in a phase II study ([NCT01500733](#)).

Results: We identified *TP53* mutations in 43 patients (84%) and del(17p) in 47 (92%); 9 and 42 patients carried single-hit and multi-hit *TP53*, respectively. The multi-hit *TP53* subgroup was enriched with younger patients who had prior treatments and unmutated immunoglobulin heavy-chain variable region gene. We observed significantly shorter overall survival, progression-free survival (PFS), and time-to-progression (TTP) in patients with multi-hit *TP53* compared with those with single-hit *TP53*. Clinical outcomes were similar in patient subgroups stratified by 2 or >2 *TP53* aberrations. In multivariable analyses, multi-hit *TP53* CLL was independently associated with inferior PFS and TTP. In sensitivity analyses, excluding mutations below 1% VAF demonstrated similar outcome. Results were validated in an independent population-based cohort of 112 CLL patients treated with ibrutinib.

Corresponding author: Carsten Utoft Niemann, MD, PhD, Copenhagen University Hospital, Rigshospitalet, Department of Hematology, Blegdamsvej 9, Copenhagen 2100, Denmark, Phone: +45 35 45 78 30 | carsten.utoft.niemann@regionh.dk | Fax: NA.
AUTHORSHIP CONTRIBUTIONS

CB, AW, IEA, and CUN designed the study. IEA provided study materials or patients. CB, MHT, and CWY performed and interpreted sequence analyses. CB, KA, MAA, and IEA collected data. CB, XT, AW, IEA, and CUN performed and interpreted statistical analyses. CB, IEA, and CUN wrote the draft manuscript. All authors read and agreed to the final version of the manuscript.

Conclusions: In this study, single-hit *TP53* defines a distinct subgroup of patients with an excellent long-term response to single-agent ibrutinib, while multi-hit *TP53* is independently associated with shorter PFS. These results warrant further investigations on prognostication and management of multi-hit *TP53* CLL.

Keywords

Prognostication; targeted therapy; *TP53* mutation; deep sequencing; NGS

INTRODUCTION

Mutations in the *TP53* gene and deletion of the short arm of chromosome 17 [del(17p)] are associated with genomic instability and an adverse outcome in cancer (1, 2). In chronic lymphocytic leukemia (CLL), *TP53* aberrations [del(17p) and/or *TP53* mutation] predict unfavorable prognosis with shorter time to treatment initiation, higher likelihood of developing refractoriness to or early relapse after chemoimmunotherapy (CIT), and inferior overall survival (OS) (3–5). The prevalence of *TP53* aberrations differ by treatment settings; del(17p) affecting 4–8% of newly diagnosed CLL patients, and up to 40% at relapse (6–8). International guidelines in CLL recommend universal testing for *TP53* aberrations before any line of therapy using fluorescence *in situ* hybridization (FISH) to detect del(17p) and Sanger or next-generation sequencing (NGS) to detect *TP53* mutations down to 5–10% variant allele frequency (VAF) (9–11).

Although del(17p) and *TP53* mutations frequently co-occur, a monoallelic *TP53* aberration can be found in 30%–50% of *TP53* aberrant CLL patients, which has been associated with various poor outcome in patients treated with CIT (12–17). A recent study in myelodysplastic syndrome (MDS) demonstrated inferior outcome for patients with more than one *TP53* aberration (multi-hit) as compared to patients with only one *TP53* aberration (single-hit); the latter group demonstrated outcome similar to patients with *TP53* wild-type MDS (18). Similar data has been published for patients with acute myeloid leukemia (AML) receiving venetoclax-based therapies (19). Findings in CLL have been mixed. In a cohort of patients with newly diagnosed CLL, patients with concurrent del(17p) and *TP53* mutation had worse survival than those with a single *TP53* aberration only, suggesting the gene-dosage effect of *TP53* (15). Likewise, previous studies similarly support that monoallelic *TP53* do not impair the p53 protein completely in the era of chemotherapy (13, 20–22). Others, however, showed that an isolated *TP53* mutation was linked to negative outcomes comparable to those with concurrent aberrations in newly diagnosed patients and upon chemotherapy (14, 23). Further, there is an ongoing debate for the clinically meaningful allelic burden of *TP53* mutation. *TP53* mutations detected at low VAF (Sanger negative) are associated with poor outcomes once CLL-directed CIT begins (17, 24). For del(17p) by FISH, patients with a higher proportion of cells affected (<25% vs. 25%–75% vs. >75%) have shorter time to first treatment (25).

Despite significant advances in targeted therapy and long-term outcomes in CLL, patients with *TP53* aberration have a shorter duration of response to ibrutinib compared to those without *TP53* aberrations (26–29). In patients treated with the BTK inhibitor ibrutinib, both

clonal selection due to previous therapy and genomic instability in *TP53* aberrated clones have been suggested as predisposing factors for the acquisition of mutations leading to treatment resistance (30, 31).

Traditionally, *TP53* aberrations are considered as a dichotomized prognostic marker (aberrated or not). Even so, advances in novel treatment and sequencing call for better delineation of differences in *TP53* aberrations. We thus applied sensitive NGS with a limit of detection (LOD) at 0.2% to investigate clinical outcomes in 51 patients treated with ibrutinib carrying either a single or multiple *TP53* hits. Whether single-hit or multi-hit *TP53* impacts clinical outcome on novel therapies as recently demonstrated in MDS and AML, remains unknown in CLL (18, 19).

MATERIALS AND METHODS

Patients with *TP53* aberrant CLL in a phase II study of single-agent ibrutinib were included ([clinicaltrials.gov: NCT01500733](https://clinicaltrials.gov/ct2/show/study/NCT01500733)) (32). *TP53* aberrations were defined by detection of 1) del(17p) based on FISH, 2) *TP53* mutations based on targeted NGS, or 3) tissue expression of p53 based on immunohistochemistry (26). Immunohistochemistry was only performed in two patients as supplemental evidence of *TP53* alteration. FISH cutoff for del(17p) was 8% or more interphase cells counted according to the local laboratory cutoff. All patients received ibrutinib 420 mg/day until progressive disease (PD) or intolerable side effects occurred. The study was approved by the Institutional Review Board and conducted according to the Declaration of Helsinki. Written informed consent was obtained from all patient.

We analyzed baseline samples collected prior to the initiation of ibrutinib for mutations in the *TP53* gene (exons 2–10 +2 base-pair intronic overlap) by deep NGS using methods previously described (22). In brief, the target region was amplified by PCR using 100 ng genomic DNA extracted from peripheral blood mononuclear cells. Library preparation was performed according to the manufacturer's protocol (Roche Nimblegen, Madison, WI, USA) and sequenced as paired-end on a NextSeq and MiSeq (2×150 and 2×125 base PE, respectively; Illumina, San Diego, CA, USA). To obtain comparable sequencing depth among samples, NextSeq FASTQ files were subsampled to 20%. We used a bioinformatics pipeline developed in CLC Biomedical Genomics Workbench 3.0 (Qiagen, Hilden, Germany) mapping to the hg19 reference genome. Variants were cross-referenced in the International Agency for Research on Cancer *TP53* database (<http://p53.iarc.fr>) excluding synonymous mutations and validated single nucleotide polymorphisms. The median coverage was 43,744x (95% above 11,000x), and the LOD of the assay was 0.2% VAF as previously described (22).

For external validation, we included a population-based cohort of 205 CLL patients treated with ibrutinib (33). Data were collected retrospectively from the Danish CLL registry and patient records (6). *TP53* mutations detected by either NGS or Sanger in this cohort had a LOD of 5%–20% (6).

Single-hit and multi-hit *TP53* were defined as having only one *TP53* aberration [del(17p) or a single *TP53* mutation] and more than one *TP53* aberrations [del(17p) and *TP53* mutation(s); or multiple *TP53* mutations], respectively. Primary outcomes were OS and progression-free survival (PFS) from the initiation of ibrutinib therapy. Death was censored for time-to-progression (TTP), whereas it was considered a competing risk for the cumulative incidence of relapse (CIR). A pairwise log-rank test was applied for OS, PFS, and TTP; Gray's test was applied for CIR. The study was adequately powered to detect a 30% survival difference using a two-sided significance level of 0.05. Discrimination capabilities were assessed by Harrell's C-index. We included all baseline characteristics in multivariable Cox regression analysis applying Firth's penalized likelihood in cases of zero recorded events (34). Statistical analyses were performed with R version 4.0.3 using *survival*, *survminer*, *coxphf*, and *Publish* (35).

RESULTS

Patient characteristics

Baseline characteristics of 51 CLL patients with *TP53* aberration treated with ibrutinib are summarized in Table 1. Efficacy and safety data of the study were previously reported (26). Due to eligibility criteria of the study, most patients had high-risk features including advanced Rai stage, relapsed/refractory (R/R) disease, elevated β 2-microglobulin (B2M), immunoglobulin heavy-chain variable region gene (IGHV) unmutated status (U-CLL), and del(17p).

TP53 mutations identified

At baseline, we identified 220 *TP53* mutations in 43 (84%) patients with a median VAF of 0.6% [interquartile range (IQR), 0.3%–1.6%]. The vast majority of the mutations were low burden with VAFs below 10% (177 mutations or 80%) including 146 minor *TP53* mutations (VAF < 1%). The remaining 43 *TP53* mutations had high burden VAFs (> 10%; Fig. 1A). At the individual patient level, 39 (76%) patients had at least one high burden mutation and four (8%) patients carried only low burden mutations. Mutations are characterized in detail in Fig. 1B and Supplementary Table S1 (16).

Among 51 patients analyzed, 47 (92%) patients carried del(17p), and 39 (76%) had concurrent del(17p) and *TP53* mutations (Fig. 1C). The median number of mutations per patient was one (IQR, 1–3 mutations), and mutations were enriched among patients with R/R disease as compared to treatment-naïve patients [median 1 (IQR, 1–2) vs. 3 (IQR, 1–13) mutations, respectively. $P = 0.006$; Wilcoxon signed-rank test]. Nine patients had single-hit *TP53* and 42 had multi-hit *TP53* including 19 patients with only 2 *TP53* aberrations. Among the 42 patients with multi-hit *TP53*, three carried *TP53* mutations only, 39 patients had del(17p) with one (n=19) or more (n=20) concomitant *TP53* mutations (Fig. 1D). All four patients carrying only *TP53* mutations had at least one high burden mutation (VAF > 10%); one patient carried only a single *TP53*:c.847C>T encoding functional p53. Patients with multi-hit *TP53* were younger and more frequently U-CLL compared to patients with single-hit *TP53* (Table 1).

Correlation of the number and allele burden of *TP53* aberrations with outcomes on ibrutinib

With a median follow-up time of 6.3 years (IQR, 6.1–7.2 years), 16 patients had died and 23 had PD; four patients died of causes unrelated to disease progression (26). OS and PFS were significantly longer for patients with single-hit *TP53* compared to those with multi-hit *TP53* ($P = 0.042$; Fig. 2A–B). For patients with multi-hit *TP53*, the median OS was not reached, while median PFS was 4.6 years (95% CI, 3.7 years to not reached). For patients with single-hit *TP53*, 5-year OS and PFS were 100% compared with 69% and 43%, respectively, for patients with multi-hit *TP53*. We observed no difference in OS and PFS in multi-hit patients with 2 and >2 *TP53* aberrations ($P = 0.32$; Fig. 2C–D) or between patients with *TP53* mutations only and those with del(17p) regardless of *TP53* mutational status (Supplementary Fig. S1). TTP and CIR were significantly superior in patients with single-hit *TP53* compared to patients with multi-hit *TP53*, whereas similar TTP and CIR were demonstrated for multi-hit *TP53* patients carrying 2 and >2 *TP53* aberrations (Supplementary Fig. S2).

To determine the optimal VAF cutoff that allows the selection of a clinically relevant burden of *TP53* mutations, we tested cutoffs ranging from 0.2%–10% VAF (Supplementary Fig. S3). The discrimination capability for OS increased with higher VAF cutoff as more patients were considered to have single-hit *TP53*, whereas the discrimination capability for PFS and TTP decreased with increasing VAF cutoff. By using a VAF cutoff of 1%, we observed similar outcome and discrimination capabilities as compared to using a VAF cutoff of 0.2%, while a VAF cutoff of 2% resulted in a less favorable PFS and TTP for patients with single-hit *TP53* aberration as well as lower discrimination capabilities (Supplementary Fig. S3).

Without any events among patients with single-hit *TP53*, we used Cox regression with Firth's penalized likelihood (34). Multivariable analyses confirmed that multi-hit *TP53* was independently associated with adverse PFS and TTP, while the analysis was unable to demonstrate any independent prognostic markers for OS (Fig. 3 and Supplementary Fig. S4). Due to the observed enrichment of multi-hit *TP53* in younger patients with R/R disease and U-CLL (Table 1), we performed subgroup analyses by log-rank for these three baseline characteristics. In line with results from the multivariable analyses, patients with multi-hit *TP53* had a consistently inferior outcome in all analyses compared to those with single-hit *TP53* (Supplementary Fig. S5). Within the multi-hit *TP53* subset, only R/R CLL was an inferior prognostic marker leading to further stratification of the subset in all outcomes.

External validation of the number of *TP53* aberrations

We utilized an independent group of 205 CLL patients in a Danish nationwide cohort treated with ibrutinib outside clinical trials (33). We excluded the 92 patients without *TP53* aberrations and one patient receiving combination targeted therapy from further analyses. Among 112 patients analyzed, the median age was 73 years (IQR, 66–77) and 77 (69%) had R/R disease. FISH was performed in all 112 patients, while *TP53* was sequenced in 61 (54%) of patients using NGS and Sanger with a LOD of 5% and 20% VAF, respectively. As a result, 94 patients carried del(17p) with no or unknown *TP53* mutation including 43

patients who were tested for the mutation and had wild-type *TP53* (single-hit *TP53*); 18 carried both del(17p) and at least 1 *TP53* mutation (multi-hit *TP53*). Except for del(17p) enriched in patients with multi-hit *TP53*, all other baseline characteristics were similar in the two groups (Table S2).

At a median follow-up of 2.3 years (IQR 1.5–3.3 years), patients with multi-hit *TP53* demonstrated inferior OS and PFS than those with single-hit *TP53* or no known aberration ($P = 0.002$; Fig. 4). Restricting the analyses to include only patients with known *TP53* mutational status based on Sanger or NGS, we observed longer, but insignificant OS and PFS in patients with single-hit *TP53* compared to multi-hit *TP53* ($P = 0.11$ and $P = 0.08$, respectively; Supplementary Fig. S6).

DISCUSSION

We here demonstrate excellent OS and PFS in CLL patients with single-hit *TP53* treated with ibrutinib monotherapy. Multi-hit *TP53* was an independent risk factor associated with adverse PFS, with results validated in a population-based external cohort of CLL patients treated with ibrutinib.

CLL patients have heterogeneous outcomes during treatment with targeted agents. Several studies, including subgroup analyses of individual studies and pooled analyses of multiple trials, have identified clinical variables prognostic for ibrutinib treated patients (28, 32). Predictors of unfavorable outcomes on ibrutinib include *TP53* aberration, complex karyotype, history of previous treatments, and elevated biochemical factors such as B2M and lactate dehydrogenase (36–38). However, previous prognostic studies in CLL patients treated with ibrutinib have several limitations including various treatment regimens used in the cohort (37), high rates of missing data for *TP53* mutation (37), and a relatively small number of patients with *TP53* aberration (28). Unlike previous studies, all patients in the present study were *TP53* aberrant, systematically analyzed by deep NGS and uniformly treated with single-agent ibrutinib.

While previous studies have demonstrated an additive effect of *TP53* mutation and del(17p) on clinical outcome following CIT (13, 15, 20), we here show for the first time an additive effect of *TP53* aberrations in patients treated with single-agent ibrutinib. By including all patients with *TP53* aberrant CLL from a prospective study, we sought to limit selection bias. Results were externally validated, although outcomes for single-hit *TP53* patients were not as impressive as for patients in the phase II study (2-year PFS of 75% vs. 100%). This is likely a result of an older patient population (median 73 years vs. 62 years) with a higher proportion of R/R status (69% vs. 33%) in the validation cohort as multi-hit *TP53* aberration and prior treatment demonstrated independent prognostic impact in multivariable analysis. Limiting the comparison of results in the two cohorts, we note the markedly shorter follow-up time with broader IQR in the validation cohort. Further, we could not perform a multivariate analysis of the validation cohort due to high rates of missing data in variables.

Interestingly, no differences in outcomes were detectable between multi-hit patients carrying 2 or >2 *TP53* aberrations. In agreement with our findings, a study recently reported poor

prognosis of multi-hit *TP53* for patients with MDS and AML, whereas patients harboring *TP53* wild-type and single-hit demonstrated favorable outcomes (18, 19). The study on MDS indicates that biallelic targeting of the *TP53* locus is present in multi-hit *TP53* cases including patients carrying multiple mutations (18). Although our study does not provide data on allele-specific aberrations, the fact that two or more *TP53* aberrations correlate with poor outcomes on ibrutinib is consistent with the *two-hit hypothesis* (12), in which tumor suppressor genes require biallelic disruption to cause a phenotypic change (i.e. loss of p53 function) (39). However, with up to 37 distinct mutations in one patient sample in the present study, we speculate that multiple *TP53* aberrations could also represent intratumoral heterogeneity and differences in the burden of *TP53* aberrations suggest interclonal selection (2, 30, 31, 40). Such early clonal shifts associated with *TP53* aberrations have previously been linked to an increased risk of progression on ibrutinib (41).

The number of patients with *TP53* aberrations in our study is similar to other studies reporting *TP53* aberrations in CLL (14, 15, 17, 20, 22, 24, 25, 28, 42). However, as the proportion patients with del(17p) was high (92%), only few patients had isolated *TP53* mutations, and the proportion of patients with single-hit was lower than expected (13–15, 17, 20, 24). This probably reflects that most *TP53* aberrant patients in the original phase II study were included based on del(17p). Moreover, in the population-based validation cohort, a different cutoff for *TP53* mutation VAF was used. With the LOD for *TP53* mutations in the population-based cohort being considerably higher (5–20%) compared to that applied to the phase II study (0.2% VAF), a different distribution of patients with single- and multi-hit *TP53* is seen. In addition, patients in population-based cohorts are routinely screened for del(17p) by FISH, while testing for *TP53* mutations is inconsistent, despite clear clinical recommendations (9–11), which may have led to selection bias in the validation cohort. Data for the validation cohort were collected retrospectively, and more *TP53* aberrations could have been identified if all pretreatment samples had been available for deep sequencing. The threshold for del(17p) by FISH is markedly higher (10% of 200 cells analyzed) than that of *TP53* mutations by deep NGS (0.2% VAF), thus no data on outcome for patients with minor del(17p) subclones by FISH are available. Multiple del(17p) and biallelic del(17p) are rare, thus FISH may still be considered sufficient for detection of del(17p) with a cutoff above 10% affected cells (20, 43). By deep NGS, we could identify numerous minor *TP53* mutations below 1% VAF (146 or 66%) enriched in patients previously treated with CIT, consistent with previous studies (22, 42). As DNA was extracted from peripheral blood mononuclear cells, we are unable to discern whether such minor mutations exist in CLL or non-CLL cells. Like our study, others have not found *TP53* mutations enriched in elderly patients with CLL (15).

Current clinical guidelines for *TP53* mutational analysis in CLL recommend reporting of *TP53* mutations down to 5% VAF with a caveat that the significance of mutations below 10% VAF is unknown (10). To investigate a clinically meaningful VAF cutoff, we gradually excluded low burden *TP53* mutations for the current study. By excluding minor mutations below VAF of 1%, a similar predictive performance for OS, PFS and TTP was demonstrated, while excluding low burden mutations below 2% VAF resulted in an inferior performance to predict PFS and TTP. Collectively, VAF cutoff of 1% for *TP53* mutations was a clinically meaningful threshold for patients treated with ibrutinib, similar to findings

from previous studies on CIT (17, 22, 24, 42). Ongoing efforts to improve inter-laboratory reproducibility of high-sensitivity DNA assays for *TP53* are needed before a lower LOD can be implemented in clinical practice (44).

In summary, the number of *TP53* aberrations assessed by the combination of deep NGS at 1% LOD and FISH correlates with OS and is an independent prognostic factor for PFS and TTP in patients with CLL on single-agent ibrutinib. Patients with multi-hit *TP53* aberrations may thus be prioritized for clinical trials. Whether novel therapies including combination targeted therapy or chimeric antigen receptor T-cell therapy benefit this patient population remains unknown (45–49). Patients with single-hit *TP53*, who mainly had isolated del(17p), can achieve durable responses to ibrutinib monotherapy. An assessment of *TP53* aberrations by using both FISH and deep NGS should be performed in all CLL patients considered for treatment with ibrutinib.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Translational relevance

Testing for *TP53* aberrations is recommended prior to treatment of patients with chronic lymphocytic leukemia (CLL) as *TP53* confers resistance to chemoimmunotherapy. While *TP53* aberrant patients receive targeted treatments, *TP53* aberration remains a predictor of poor outcome. Deletion of chromosome 17p [del(17p)] and *TP53* mutations mostly cooccur, while each lesion may be found as the sole *TP53* aberration – especially when using deep next-generation sequencing. Although currently considered equal prognostic markers, patients treated with single-agent ibrutinib carrying only a single *TP53* hit [del(17p) or a *TP53* mutation] demonstrate excellent progression-free and overall survival on ibrutinib compared to those with multiple *TP53* hits. Thus, testing for both del(17p) by FISH and *TP53* mutations by deep next-generation sequencing should be performed to improve risk stratification of and facilitate clinical trials testing novel treatment approaches in multi-hit *TP53* CLL.

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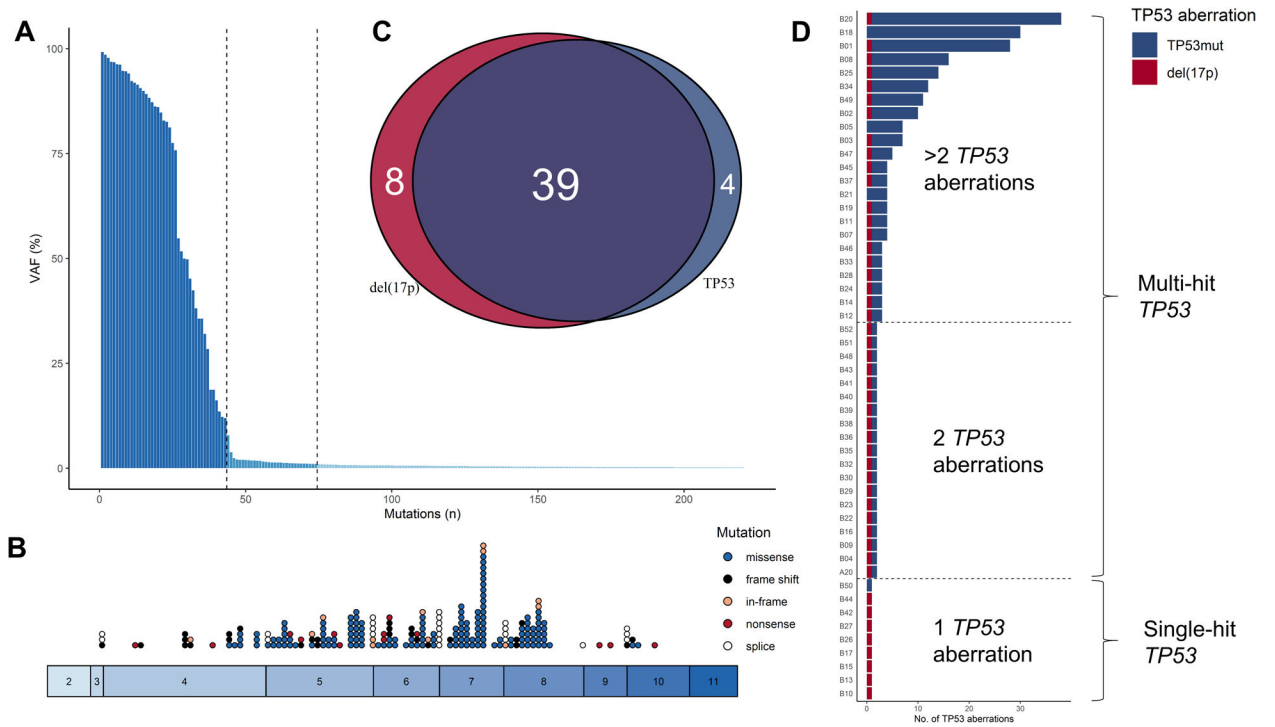


Figure 1. Characterization of *TP53* mutations. (A) Overall, 220 mutations were identified: 43 high burden [variant allele frequency (VAF) >10%] and 177 low burden mutations (VAF < 10%) including 146 minor mutations (VAF < 1%). (B) Mutations were mainly identified in hot spot exons 5–9. (C) In all 51 patients, 17p deletion [del(17p); red] and *TP53* mutations (blue) mainly cooccurred (purple). (D) Patients were grouped into single-hit and multi-hit *TP53* aberrations.

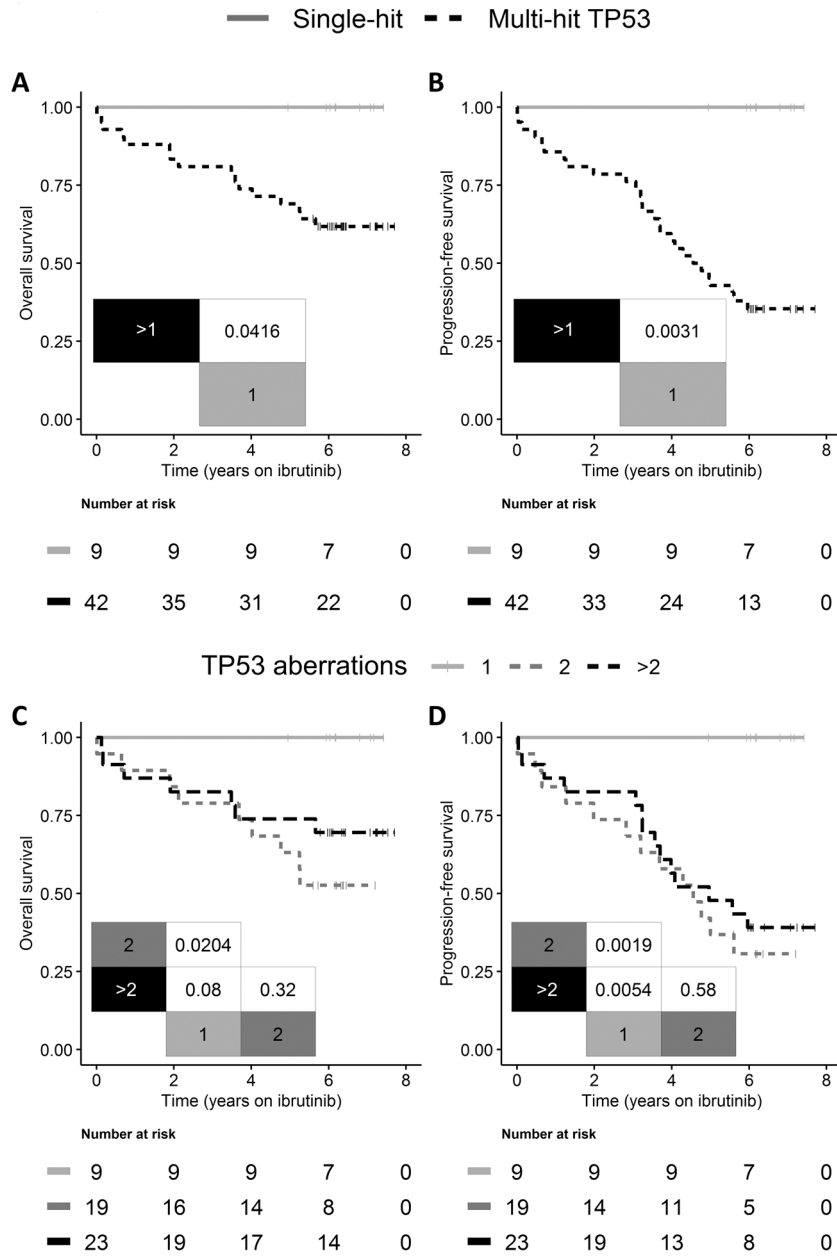


Figure 2. (A, C) Overall survival and (B, D) progression-free survival following initiation of single-agent ibrutinib stratified on (A-B) single-hit (gray) vs. multi-hit (black striped) *TP53* and in (C-D) patients carrying 1 (gray) vs. 2 (dark gray striped) vs. > 2 (black striped) *TP53* aberrations. Overall survival and progression-free survival were significantly shorter in patients with multi-hit *TP53*. However, stratifying multi-hit *TP53* patients further into carrying 2 and > 2 *TP53* aberrations demonstrated similar outcome.

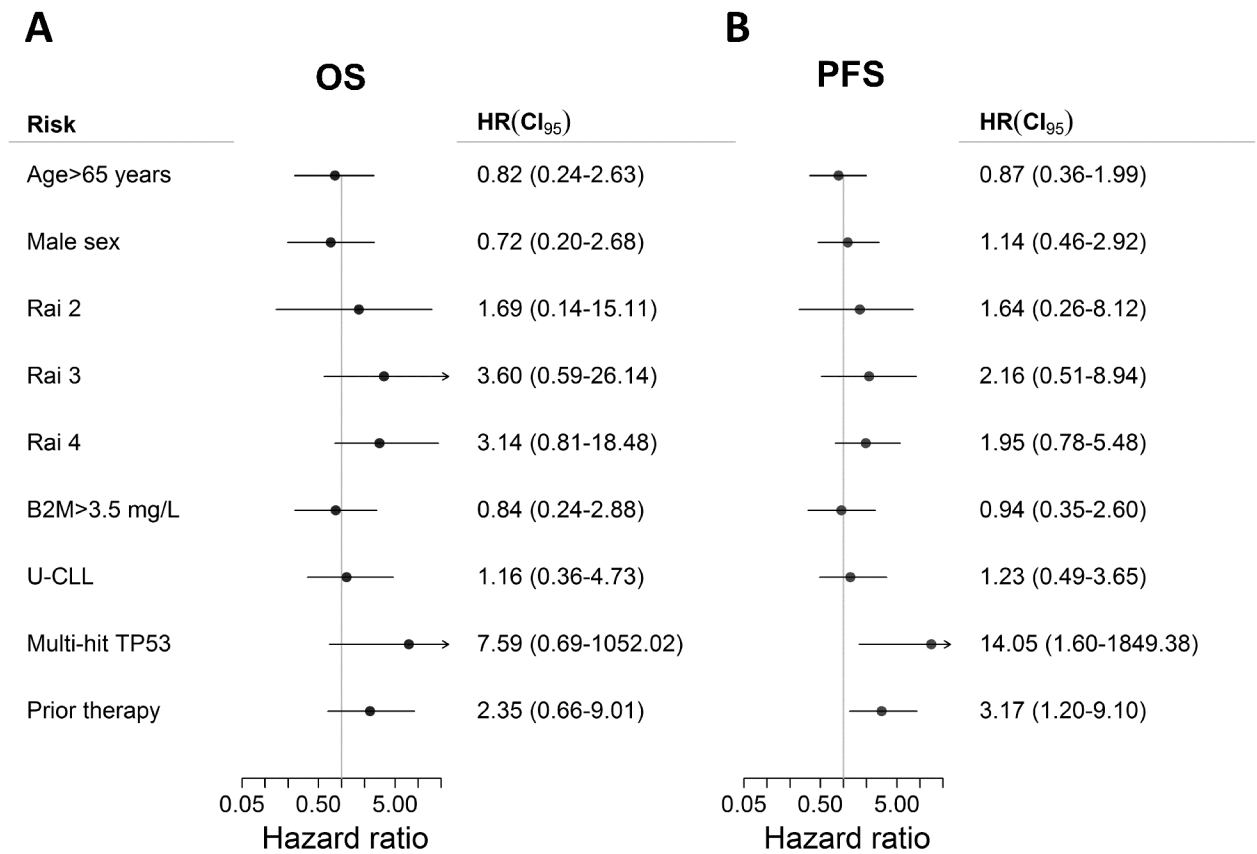


Figure 3. Multivariable analyses with Firth's penalized likelihood. **(A)** No baseline risk factors demonstrated impact on overall survival (OS), whereas **(B)** multi-hit *TP53* and prior therapy were independently associated with shorter progression-free survival (PFS). Abbreviations: B2M, β 2-microglobulin; U-CLL, unmutated immunoglobulin heavy-chain variable region gene (IGHV) status.

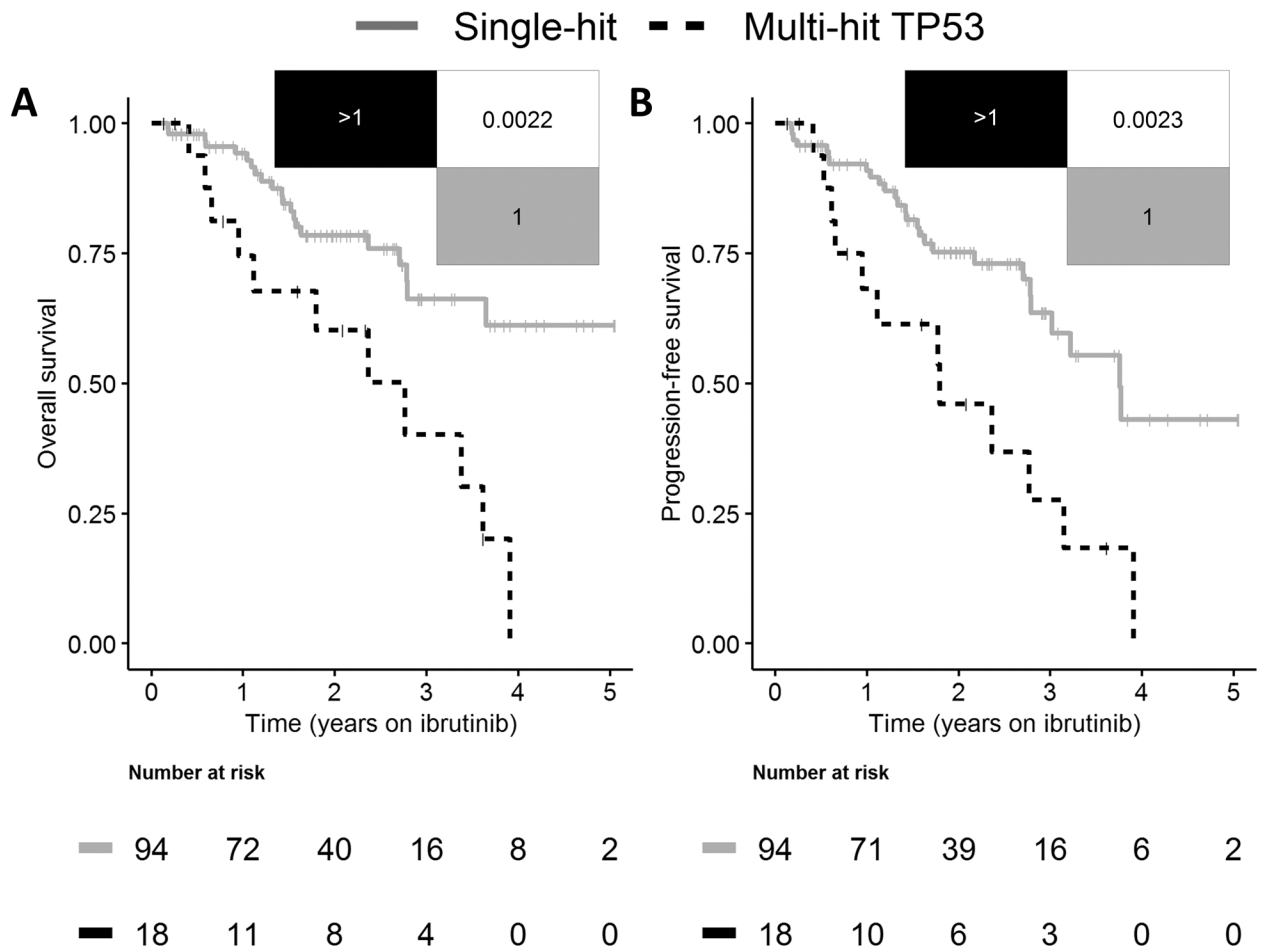


Figure 4. Validation of multi-hit *TP53* in 112 *TP53* aberrant patients with chronic lymphocytic leukemia following initiation of single-agent ibrutinib. (A) Overall survival and (B) progression-free survival in patients with single-hit (gray) and multi-hit (black striped) *TP53*. Overall survival and progression-free survival were significantly shorter in patients with multi-hit *TP53*.

Table 1.Baseline patient characteristics stratified on *TP53* aberrational status (single-hit vs. multi-hit *TP53*)

| Characteristic | Total (n = 51) | Single-hit <i>TP53</i> (n = 9) | Multi-hit <i>TP53</i> (n = 42) |
|--------------------------------|-----------------|-----------------------------------|-----------------------------------|
| | No. (%) | No. (%) | No. (%) |
| Age, years | | | |
| Median (IQR) | 62 (59–69) | 69 (66–76) | 62 (59–67.8) |
| Sex | | | |
| Female | 20 (39.2) | 4 (44.4) | 16 (38.1) |
| Male | 31 (60.8) | 5 (55.6) | 26 (61.9) |
| Rai stage | | | |
| 1 | 13 (25.5) | 1 (11.1) | 12 (28.6) |
| 2 | 5 (9.8) | 1 (11.1) | 4 (9.5) |
| 3 | 8 (15.7) | 2 (22.2) | 6 (14.3) |
| 4 | 25 (49.0) | 5 (55.6) | 20 (47.6) |
| B2M, mg/L | | | |
| Median (IQR) | 3.9 (2.9–5.8) | 3.3 (2.5–3.7) | 4.0 (3.0–6.1) |
| IGHV status | | | |
| M-CLL | 17 (33.3) | 7 (77.8) | 10 (23.8) |
| U-CLL | 34 (66.7) | 2 (22.2) | 32 (76.2) |
| del(17p) | | | |
| No | 4 (7.8) | 1 (11.1) | 3 (7.1) |
| Yes | 47 (92.2) | 8 (88.9) | 39 (92.9) |
| del(17p) positivity by FISH, % | | | |
| Median (IQR) | 56% (18.8–85.8) | 16.5% (14.5–53.8) | 62% (30.8–87.5) |
| Treatment status | | | |
| TN | 34 (66.7) | 8 (88.9) | 26 (61.9) |
| RR | 17 (33.3) | 1 (11.1) | 16 (38.1) |

Abbreviations: ab, aberration; B2M, β 2-microglobulin; IGHV, immunoglobulin heavy-chain variable region; M-CLL, mutated IGHV status; U-CLL, unmutated IGHV status; del(17p), deletion of chromosome 17p; TN, treatment-naïve; RR, relapsed-refractory, IQR, interquartile range.

* One patient had del(17p) detected by microarray.