REGULAR ARTICLE

blood advances

Differential prognosis of single and multiple *TP53* abnormalities in high-count MBL and untreated CLL

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Key Points

- Similar mutation frequency, type, and location in *TP53* between 849 patients newly diagnosed with CLL and 381 patients with HCMBL.
- Having multiple *TP53* abnormalities increased the risk of progression to therapy and shortened the overall survival.

TP53 aberrations, including mutations and deletion of 17p13, are important adverse prognostic markers in chronic lymphocytic leukemia (CLL) but are less studied in high count monoclonal B-cell lymphocytosis (HCMBL), an asymptomatic pre-malignant stage of CLL. Here we estimated the prevalence and impact of *TP53* aberrations in 1,230 newly diagnosed treatment-naïve individuals (849 CLL, 381 HCMBL). We defined *TP53* state as: wild-type (no *TP53* mutations and normal 17p), single-hit (del(17p) or one *TP53* mutation), or multi-hit (*TP53* mutation and del(17p), *TP53* mutation and loss of heterozygosity, or multiple *TP53* mutations). Cox regression was used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for time to first treatment and overall survival by *TP53* state. We found 64 (7.5%) CLL patients and 17 (4.5%) HCMBL individuals had *TP53* mutations with variant allele fraction >10%. Del(17p) was present in 58 (6.8%) of CLL and 11 (2.9%) of HCMBL cases. Most individuals had wild-type (N=1,128, 91.7%) *TP53* state, followed by multi-hit (N=55, 4.5%) and then single-hit (N=47, 3.8%) *TP53* state. The risk of shorter time to therapy and death increased with the number of *TP53* abnormalities. Compared to wild-type patients, multi-hit patients had 3-fold and single-hit patients had 1.5-fold increased risk of requiring therapy. Multi-hit patients also had 2.9-fold increased risk of death compared to wild-type. These results remained stable after accounting for other known poor prognostic factors. Both *TP53* mutations and del(17p) may provide important prognostic information for HCMBL and CLL that would be missed if only one were measured.

Introduction

Chronic lymphocytic leukemia (CLL), a neoplasm of B-cell lymphocytes, is characterized by clinical and biologic heterogeneity. Distinct genetic profiles exist in CLL, including recurrent cytogenetic abnormalities and somatic mutations that influence disease aggressiveness.[1-3] One of the most well-known and

Submitted 28 September 2022; accepted 14 February 2023; prepublished online on *Blood Advances* First Edition 6 March 2023. https://doi.org/10.1182/bloodadvances.2022009040.

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Supplemental Table 1 provides details on all *TP53* mutations and 17p deletions observed in our participants.

Data are available on request from the corresponding authors.

The full-text version of this article contains a data supplement.

clinically important prognostic markers in CLL would be abnormalities in *TP53*. Somatic aberrations include *TP53* mutations and interstitial or complete deletions of the short arm of chromosome 17, del(17p). Prevalence of *TP53* mutations (median variant allele fraction [VAF], 0.6%)[4] ranges from 7% to 11% and that of del(17p) ranges from 4% to 8% in untreated CLL.[3,5-7] Of patients with a *TP53* mutation, ~62% to 76% also had a del(17p).[5,7,8] *TP53* abnormalities increase with disease progression and relapse after treatment.[6]

Patients with CLL and *TP53* abnormalities have a shorter progression-free survival, event-free survival, and overall survival (OS) when treated with chemoimmunotherapy and are less responsive to chemoimmunotherapy than patients with no *TP53* abnormalities.[6,7,9-12] However, the prognostic importance of *TP53* mutations with a low VAF and that of 17p deletions in few cells remain ambiguous. Prior studies have compared the OS of patients with wild-type vs mutated *TP53*; some studies[13-15] found shorter survival among patients carrying *TP53* mutations regardless of VAF, but another study[16] found shorter survival only in patients carrying *TP53* mutations with VAFs ≥12%. Currently, the *TP53* Network of the European Research Initiative on Chronic Lymphocytic Leukemia (ERIC) recommends evaluating *TP53* mutations with VAFs >10% because the clinical utility of low-VAF mutations remains unclear.[17] In a study of the effect of 17p deletion clone size identified via fluorescence in situ hybridization (FISH) analysis,[18] survival was most favorable among patients with less than 25% deleted nuclei (3-year survival rate, 92%), intermediate among patients with between 25% and 74% deleted nuclei (3-year survival rate, 67%), and least favorable among patients with 75% or more deleted nuclei (3-year survival rate, 40%; $P < .001$).

In addition, data from prior studies are inconclusive as to whether a single *TP53* abnormality vs multiple *TP53* abnormalities affects outcomes.[3,19,20] In the chemoimmunotherapy era, survival was significantly shorter among patients with a single *TP53* hit and further shortened among patients with multiple *TP53* hits.[20] More recent data indicate that patients with a single *TP53* hit treated with ibrutinib had better OS than patients with multiple *TP53* hits (5-year OS, 100% in single-hit vs 69% in multihit).[19] Although these studies have shown the impact of *TP53* aberrations in patients with CLL who have been treated, there are limited data on the prognostic impact of *TP53* mutations, in particular the role of single vs multiple *TP53* abnormalities in patients with newly diagnosed treatment-naive CLL.

Most CLL cases are preceded by a premalignant condition of circulating clonal B cells between 0.5 × 10^9/L and 4.9 × 10^9/L, called high-count monoclonal B-cell lymphocytosis (HCMBL) with a CLL-like immunophenotype.[21,22] *TP53* mutations (3.0%; 2/66) and del(17p)s (3.8%; 4/105) have been observed in HCMBL at a lower rate than in patients with CLL.[23] Prior work by our group demonstrated the ability to predict time to first treatment (TTFT) using the CLL International Prognostic Index and found *TP53* disruption, either via del(17p) FISH or *TP53* mutation, among 4.8% (20/415) of individuals with HCMBL.[24] However, in this study, *TP53* mutations were detected via Sanger sequencing, limiting the ability to detect low-VAF mutations.[24] Little is known about the impact of *TP53* abnormalities on prognosis among individuals with HCMBL.

We aim to (a) examine whether *TP53* aberrations occur less frequently in HCMBL cases than in CLL cases and to (b) evaluate the impact of single vs multiple aberrations compared with that of no *TP53* aberrations on TTFT and OS in HCMBL and CLL. We estimated the prevalence and impact of *TP53* abnormalities in a large cohort of individuals with HCMBL and those with newly diagnosed, treatment-naive CLL.

Methods

Participants

This research was approved by the Mayo Clinic institutional review board, and all participants provided written informed consent. *TP53* was characterized in individuals with CLL or HCMBL that was diagnosed between 2000 and 2019, in accordance with the Mayo Clinic CLL resource, using pretreatment peripheral blood mononuclear cells (PBMCs) collected on average within 12 days of initial diagnosis. CLL diagnosis was made based on the 2008 International Workshop CLL criteria[25] and updated to the 2018 International Workshop CLL criteria whenever possible.[22] Flow cytometry was performed for samples from all patients for diagnosis and identification of $CD19^+/CD5^+$ tumor cells. All HCMBLs had clonal B-cell counts between 0.5 × 10^9/L and <5 × 10^9/L.

DNA sequencing

DNA was extracted from either PBMCs with >80% $CD5^+/CD19^+$ clonal B cells or those enriched for $CD5^+/CD19^+$ clonal B cells. The sequencing of the entire coding regions and intron-exon junctions of *TP53* was part of a larger panel of 59 genes previously implicated in CLL.[26] The median coverage depth per sample across the 59 genes was 1799 (range, 65-3675) with >83% of the samples having a median coverage depth of >1000 per nucleotide, allowing for detection of mutations with VAFs as low as 1%. For *TP53* specifically, the median coverage was 1917 (range, 23-6004), with 83% of samples having a depth >1000, and 99% of samples having a depth of >100. Only mutations with at least 100 reads and 10 reads with the alternate allele were considered. Somatic mutations were called using MuTect2 in tumor-only mode. We included frameshift and in-frame deletions and insertions as well as nonsense, missense, and splicing mutations. Following the *TP53* ERIC-updated recommendations,[17] we evaluated *TP53* mutations with VAFs >10% in our primary analyses. In secondary analyses, we evaluated the role of *TP53* mutations with VAFs >1%.

Del(17p) status at time of sample collection or at diagnosis was available and extracted from the medical records for 82% (n = 1012) of the participants; individuals with >9.5% defective cells were considered to be $del(17p)^+$ using FISH. The number of defective nuclei detected via FISH was also deduced and used to evaluate the mean del(17p) abundance in our cohort of patients with CLL and HCMBL. FISH data were missing in 218 participants (18%), and we inferred del(17p) copy number variations from targeted DNA sequencing using PatternCNV.[27,28] First batch effects were adjusted by (a) quantifying the exon coverage of chromosomes without an established cytogenetic abnormality in CLL (ie, chromosomes 11, 12, 13, and 17 were excluded), (b) grouping patients into clusters with similar exon coverage patterns using principal component analysis and correlation matrixes, and then (c) rerunning PatternCNV on each cluster of patients to detect copy number variations on chromosomes 11, 12, 13, and 17. Del(17p)

was inferred when the log ratio of coverage decreased by −0.25 from the log2 normalized median coverage in the genomic region (hg19) from 17:7134079 to 7722415. Calls were 98.62% concordant between PatternCNV and FISH, when available.

TP53 state

Somatic mutations and del(17p) status were used to define *TP53* state for each patient based on the following criteria: (a) wild-type were patients with no *TP53* mutations and normal 17p; (b) single-hit were those with either 1 *TP53* heterozygous mutation (VAF <60%) or del(17p); and (c) multihit were those with *TP53* mutation and copy neutral loss of heterozygosity (cnLOH), multiple *TP53* mutations, or the combination of *TP53* mutations and del(17p) (supplemental Figure 1). A full list of *TP53* mutations and del(17p) status among patients with single-hit and multihit abnormalities is given in supplemental Table 1.

CLL and HCMBL outcomes

TTFT and OS were analyzed based on the *TP53* state. TTFT was defined as the time from sample collection to either first treatment, death, or last follow-up date, whichever occurred first. OS was defined as the time from sample collection to either death or last follow-up date, whichever occurred first. Median and 5-year TTFT and OS were estimated using the Kaplan-Meier method. We used Cox regression to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for TTFT and OS associations. To investigate the sensitivity of our findings, we stratified the Kaplan-Meier analyses and adjusted the Cox regressions based on the type of *TP53* and other known adverse prognostic factors, including age of >65 years, Rai stage I-IV, β 2-microglobulin (β2M) > 3.5 mg/L, or unmutated *IGHV*. For OS, we examined the effect of treatment by (a) evaluating OS based on the *TP53* state when OS is defined as time from treatment to either death or last follow-up date, (b) censoring OS at the time of treatment, and (c) subsetting of patients who received the most used inhibitors: B-cell lymphoma 2 or Bruton tyrosine kinase (all treatments administered are described in supplemental Table 2.)

Tumor characteristics based on the *TP53* state

Tumor mutational load (TML) was calculated as the number of mutated CLL driver genes (out of 59 total), as previously described.[26] We then compared TML and mutation status of individual genes based on single-hit and multihit *TP53* carriers.

Results

Patient characteristics

We characterized *TP53* in 1230 individuals: 849 with CLL and 381 with HCMBL (Table 1). Most participants were male (69.6%, CLL; 63.8%, HCMBL). The median age at diagnosis was 61 years for CLL and 67 years for HCMBL (Table 1). Most of the patients with CLL were diagnosed at Rai stage 0 (54.9%) and had a median β2M level of 2.4 mg/L, and 51.1% had unmutated *IGHV*. Chromosomal aberrations in the participants of the study included del(13q) (50.8%, CLL; 42%, HCMBL), tri(12) (13%, CLL; 12.6%, HCMBL), del(11q) (10.5%, CLL; 3.9%, HCMBL), and del(17p) (6.8%, CLL; 2.9%, HCMBL) (Table 1). Samples were collected within 2 years of diagnosis for most participants (85%). Median follow-up after sample collection was 6.3 years.

TP53 mutations

We found *TP53* mutations with VAFs >10% in 64 patients with CLL (7.5%) and 17 individuals with HCMBL (4.5%) (Table 2). Patients with *TP53* mutations were more likely to be older at diagnosis and have unmutated *IGHV* (Table 2). A total of 71 *TP53* mutations were identified in 64 patients with CLL (Figure 1A). In individuals with HCMBL, 19 *TP53* mutations were identified in 17 individuals (Figure 1B). For mutations with VAFs of >10%, the median *TP53* VAF was not significantly different between individuals with CLL and those with HCMBL (Figure 1C) (median VAF, 51% in CLL and 46% in HCMBL; Kruskal-Wallis $P > .68$). Most of the *TP53* mutations were missense, and we did not observe different patterns in the type or location of *TP53* mutations between HCMBL and CLL cases (Figure 1E).

Del(17p) was present in 58 patients with CLL (6.8%) and 11 individuals with HCMBL (2.9%) (Table 1; Figure 1A-B). Of those individuals with a FISH del(17p), the deletion occurred significantly more in leukemic cells in individuals with CLL (mean abundance, 58% of cells) compared with those in individuals with HCMBL (mean abundance, 28% of cells; Figure 1D; Kruskal-Wallis $P = .0069$). Most cases with del(17p) also had a *TP53* mutation, 41 CLL (70.7%) and 7 HCMBL (63.6%) cases (Table 2).

Single-hit vs multihit *TP53*

Most of the individuals in our HCMBL/CLL cohort (91.7%; n = 1128) had no *TP53* mutations with VAFs >10% and had normal 17p (wild-type *TP53*; supplemental Figure 1). The 47 individuals (3.8% of our cohort) with single-hit *TP53* mutations included 21 individuals with del(17p) but no *TP53* mutations, and 26 individuals with a single heterozygous *TP53* mutation and normal 17p. The 55 (4.5%) individuals with multihit *TP53* included 43 patients with both *TP53* mutation(s) and del(17p), 8 with normal 17p but multiple *TP53* mutations, and 4 with *TP53* mutation and cnLOH (supplemental Figure 1). Among those with an *TP53* aberration, CLL cases were more often multihit (56.8%; 46/81) than single-hit (43.2%; 35/81), and HCMBL cases were more often single-hit (57.1%; 12/21) than multihit (42.9%; 9/21), although these differences were not significant (Figure 1A-B). Because we did not observe significant differences between HCMBL and CLL, we evaluated *TP53* state based on the TTFT and OS in the entire cohort, with HCMBL and CLL combined.

TTFT

In the entire cohort of individuals with treatment data (n = 1120), 417 patients subsequently received treatment: 58% untreated at 5 years (95% CI, 54.6%-61.6%). Patients with any *TP53* abnormality had shorter TTFT than patients with wild-type *TP53* (39% vs 60% untreated, respectively, at 5 years; $P < .0001$). Dividing the patients based on the *TP53* state, we observed shorter TTFT among patients with multihit *TP53* (n = 53; 29 events; 27% untreated at 5 years) than patients with single-hit *TP53* (n = 42; 21 events; 52% untreated at 5 years; Figure 2A). In Cox regression, patients with multihit *TP53* (HR, 3.05; 95% CI, 2.08-4.46) and those with single-hit *TP53* (HR, 1.56; 95% CI, 1.00-2.42) had significantly shorter TTFTs compared with individuals with wild-type *TP53* (Figure 2B), with a trend toward increased risk with increased number of *TP53* abnormalities (likelihood ratio test, $P = 1.25 \times 10^{-6}$). These results did not change when restricted to individuals from whom a sample

Table 1. Characteristics of individuals with CLL and HCMBL

	CLL (n = 849)		MBL (n = 381)		Overall (n = 1230)	
Female, n, %	258	30.4	138	36.2	396	32.2
Male	591	69.6	243	63.8	834	67.8
Median age (IQR), y	61 (46-76)		67 (52-82)		63 (47-79)	
Rai stage 0, n, %	456	54.9				
Rai stage 1-4, n, %	374	45.1				
Median β2M (IQR), mg/L	2.4 (1.1-3.8)		2.1 (1.4-2.9)		2.3 (1.1-3.6)	
IGHV unmutated, n, %	406	51.1	71	24.6	477	44.0
IGHV mutated, n, %	389	48.9	218	75.4	607	56.0
FISH del(11q), n, %	89	10.5	15	3.9	104	8.5
FISH del(13q), n, %	431	50.8	160	42.0	591	48.0
FISH tri(12), n, %	110	13.0	48	12.6	158	12.8
FISH or CNV del(17p), n, %	58	6.8	11	2.9	69	5.6
No *TP53* mutations, n, %	748	88.1	348	91.3	1096	89.1
TP53 mutation(s) VAF >10%, n, %	64	7.5	17	4.5	81	6.6
TP53 state, wild-type, n, %	768	90.5	360	94.5	1128	91.7
TP53 state, single-hit, n, %	35	4.1	12	3.1	47	3.8
TP53 state, multihit, n, %	46	5.4	9	2.4	55	4.5
Single-hit *TP53* type, n	35		12		47	
No *TP53* mutation; del(17p), n, %	17	48.6	4	33.3	21	44.7
Single *TP53* mutation; normal 17p, n, %	18	51.4	8	66.7	26	55.3
Multihit *TP53* type, n	46		9		55	
TP53 LOH mutation, n, %	3	6.5	1	11.1	4	7.3
TP53 mutation(s); del(17p), n, %	37	80.4	6	66.7	43	78.2
Multiple *TP53* mutations, n, %	6	13.0	2	22.2	8	14.5
Median follow-up, y	5.95		7.27		6.27	

CNV, copy number variation.

was collected within 2 years of diagnosis (supplemental Figure 2A-B) nor when we removed 4 potentially benign mutations (p.Asn235Ser, p.Glu298Lys, p.Arg202Cys, and p.Gly360Val) during sensitivity analyses (supplemental Figure 13).

Shorter TTFT was most pronounced among individuals with both del(17p) and a *TP53* mutation (supplemental Figure 3B), with a median TTFT of 1.8 years among those with del(17p) plus a single *TP53* mutation (n = 41; 21 treated) and a median of 0.1 year among those with del(17p) plus multiple *TP53* mutations (n = 5; 4 treated). In comparison, patients with wild-type *TP53* had a median TTFT of 7.9 years (n = 1025; 367 treated). In Cox regression, patients with both del(17p) plus *TP53* mutation(s) had a shorter TTFT compared with patients with wild-type *TP53* (HRs, 2.72 vs 9.02; supplemental Figure 3C). In sensitivity analyses, we analyzed these data among patients stratified based on other known prognostic factors, including (a) age, (b) Rai stage, (c) β2M, or (d) *IGHV* mutation status (supplemental Figures 4 and 5). Individuals with multihit *TP53* had significantly shorter TTFT regardless of age, Rai stage, or β2M risk group. Patients with multihit *TP53* had significantly shorter TTFT when *IGHV* was unmutated but not when *IGHV* was mutated (supplemental Figures 4D and 5D), as previously observed.[29] In multivariate Cox regression, multihit *TP53* remained a significant risk factor for shorter TTFT (HR, 2.00; 95% CI, 1.31-3.06; $P = .001$) after adjusting for age, Rai stage, β2M, and *IGHV* mutation status (Figure 3A). These data support that multiple hits to *TP53* reduces the time to treatment beyond other clinical factors.

OS

In the cohort of 1122 individuals (285 deaths) with survival data, 5-year survival was 88.4% (95% CI, 86.3%-90.5%). Patients with multihit *TP53* abnormalities (n = 49; 23 deaths) had shorter OS (5-year survival 62.3%; 95% CI, 48.6%-79.8%) compared with patients with wild-type *TP53* (n = 1032; 250 deaths; 5-year survival 90.5%; 95% CI, 88.5%-92.5%), and those with single-hit *TP53* abnormalities (n = 41; 12 deaths; 5-year survival 66.6%; 95% CI, 52%-85.3%; Figure 2C). In Cox regression, patients with multihit abnormalities had a 2.89-fold increased risk of death compared with those with wild-type *TP53* (95% CI, 1.88-4.43), and those with single-hit abnormalities had a 1.63-fold increased risk of death (95% CI, 0.91-2.91) compared with those with wild-type *TP53* (Figure 2D), with a significant trend in the HR as the number of *TP53* abnormalities increased (likelihood ratio test, $P = 5.42 \times 10^{-5}$). Results stayed consistent when restricted to individuals from whom a sample was collected within 2 years of diagnosis (supplemental Figure 2C-D).

Table 2. CLL and HCMBL by *TP53* mutation status

CLL	No TP53 mutation (n = 748)		VAF ≤10% (n = 37)		VAF >10% (n = 64)		Overall (n = 849)	
Female, n, %	223	29.8	13	5.0	22	8.5	258	30.4
Male, n, %	525	70.2	24	4.1	42	7.1	591	69.6
Median age (IQR), y	61 (46-76)		65 (55-75)		66.5 (50-83)		61 (46-76)	
Rai stage 0, n, %	405	55.4	20	55.6	31	49.2	456	54.9
Rai stage 1-4, n, %	326	44.6	16	44.4	32	50.8	374	45.1
Median β2M (IQR), mg/L	2.4 (1.1-3.7)		2.9 (1.1-4.8)		2.9 (1.1-4.6)		2.4 (1.1-3.8)	
IGHV unmutated, n, %	343	49.0	24	68.6	39	65.0	406	51.1
IGHV mutated, n, %	357	51.0	11	31.4	21	35.0	389	48.9
FISH del(11q), n, %	84	11.2	2	5.4	3	4.7	89	10.5
FISH del(13q), n, %	370	49.5	19	51.4	42	65.6	431	50.8
FISH tri(12), n, %	103	13.8	4	10.8	3	4.7	110	13.0
FISH/CNV del(17p), n, %	10	1.3	7	18.9	41	64.1	58	6.8
Median follow-up, y	6.21		5.49		4.54		5.95	
HCMBL	**No TP53 mutation (n = 348)**		**VAF ≤10% (n = 16)**		**VAF >10% (n = 17)**		**Overall (n = 381)**	
Female, n, %	130	37.4	1	6.3	7	41.2	138	36.2
Male, n, %	218	62.6	15	93.8	10	58.8	243	63.8
Median age (IQR), y	67 (53-81)		74 (63-85)		70 (57-83)		67 (52-82)	
Median β2M (IQR), mg/L	2.1 (1.4-2.8)		2.2 (1.5-2.9)		2.2 (0.6-3.9)		2.1 (1.4-2.8)	
IGHV unmutated, n, %	66	25.0	1	8.3	4	30.8	71	24.6
IGHV mutated, n, %	198	75.0	11	91.7	9	69.2	218	75.4
FISH del(11q), n, %	13	3.7	1	6.3	1	5.9	15	3.9
FISH del(13q), n, %	145	41.7	8	50.0	7	41.2	160	42.0
FISH tri(12), n, %	47	13.5	1	6.3	0	0.0	48	12.6
FISH/CNV del(17p), n, %	3	0.9	1	6.3	7	41.2	11	2.9
Median follow-up, y	7.18		7.65		6.2		7.27	

In our cohort, 417 individuals received treatment. To address possible OS variability based on treatment type, we censored at the time of treatment and still observed shorter OS among patients with multihit *TP53* compared with those with single-hit or wild-type *TP53*, but the effects were attenuated (supplemental Figure 6A). In addition, we stratified the data of 93 patients who received first-line B-cell lymphoma 2 or Bruton tyrosine kinase inhibitors and still observed significantly shorter OS among the patients with multihit *TP53* compared with those with wild-type *TP53* (supplemental Figure 6B).

Next, to test robustness of our findings further, we limited our OS analyses to patients with well-known higher risk characteristics (supplemental Figures 7 and 8). In each of these high-risk groups, we observed significantly shorter OS among patients with multiple hits (supplemental Figure 7). After adjusting for these factors in multivariate analysis (Figure 3B), multihit *TP53* remained a significant risk factor for shorter OS (HR, 2.37; 95% CI, 1.52-3.70; $P < .001$). These data suggest that multihit *TP53* remains an important prognostic marker.

Tumor differences by *TP53* state

To investigate potential functional mechanisms underlying the differences in outcomes by *TP53* state, we explored global differences in CLL driver mutations (supplemental Figure 9). TML did not significantly differ based on the *TP53* state (Kruskal-Wallis, $P = .56$). Among the patients with single-hit *TP53*, *NOTCH1* and *SF3B1* were mostly commonly mutated, whereas among those with multihit *TP53*, *MGA* and *NOTCH1* were mostly commonly mutated (supplemental Figure 9B). However, these genes (and other driver genes) were not mutated at significantly different rates in the 2 groups (Fisher exact tests, all $P > .13$).

TP53 mutations with VAFs ≤10%

Given the depth of sequencing data, we were able to detect *TP53* mutations as low as VAFs >1%, which is well below the ERIC[17]-recommended VAF threshold of 10%. In our cohort, 37 patients with CLL (4.4%) and 16 with HCMBL (4.2%) had a *TP53* mutation with a VAF ≤10% (Table 2; supplemental Figure 10A-B). Eleven patients with CLL and 2 with HCMBL had both a mutation with a VAF >10% and a mutation with a VAF ≤10% (supplemental Figure 10D). Low-VAF mutations were split between Rai stages and were slightly more common among patients with *IGHV*-unmutated CLL (Table 2). Co-occurrence of del(17p) was much less frequent with mutations with VAFs ≤10% (12.1%, CLL; 9.1%, HCMBL) than mutations with VAFs >10% (70.7%, CLL; 63.6%, HCMBL; Table 2). The low-VAF mutations were most often missense mutations, and no distinct mutation patterns were observed between patients with CLL and those with HCMBL (supplemental Figure 10E).

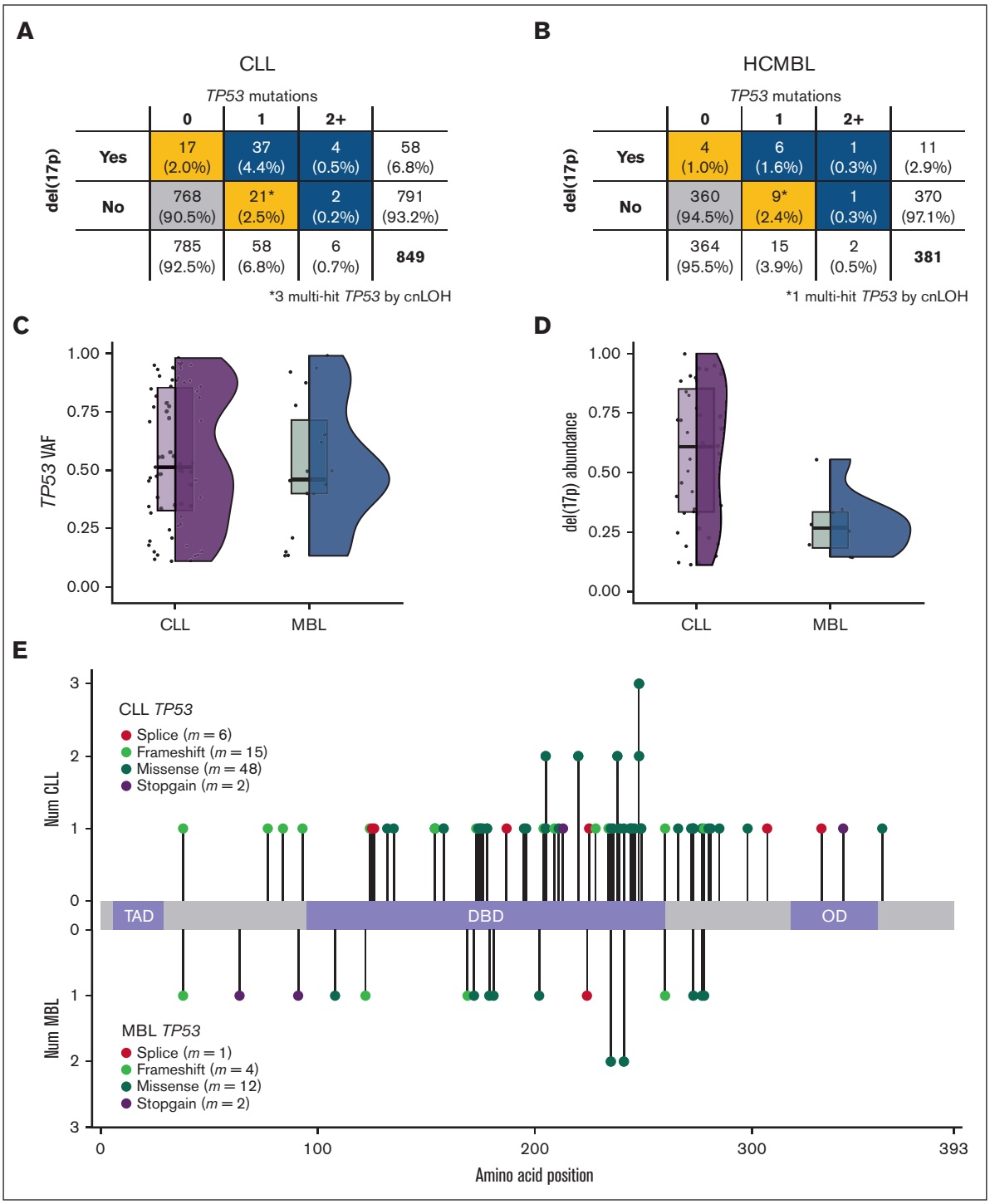

Figure 1. *TP53* mutations and del(17p) based on CLL and HCMBL. Note that only *TP53* mutations with VAF >10% were considered. Rate of mutations and del(17p) in CLL (A) and HCMBL (B) cases. Gold indicates single-hit (* denotes multihit based on cnLOH), and blue indicates multihit *TP53*. (C) VAF of *TP53* mutations. Box plot (quartiles and median shown) and violin plots show distribution. (D) Percent of cells with del(17p) by FISH. (E) Location, type, and counts of *TP53* mutations. Num, number.

Considering all *TP53* mutations (VAF > 1%), 1089 individuals had wild-type *TP53*, 74 individuals had a single-hit *TP53* [54 single heterozygous mutation, 20 del(17p)], and 67 individuals had multihit *TP53* [14 multiple mutations, 4 mutations and cnLOH, 37 single mutation and del(17p), and 12 multiple mutations and del(17p)]. TTFT remained shorter in patients with multihit *TP53* than those with single-hit or wild-type *TP53* (supplemental Figure 11A-B). Individuals with both a mutation and del(17p) had the shortest TTFTs (supplemental Figure 11C-E). These trends were the same for OS (supplemental Figure 12).

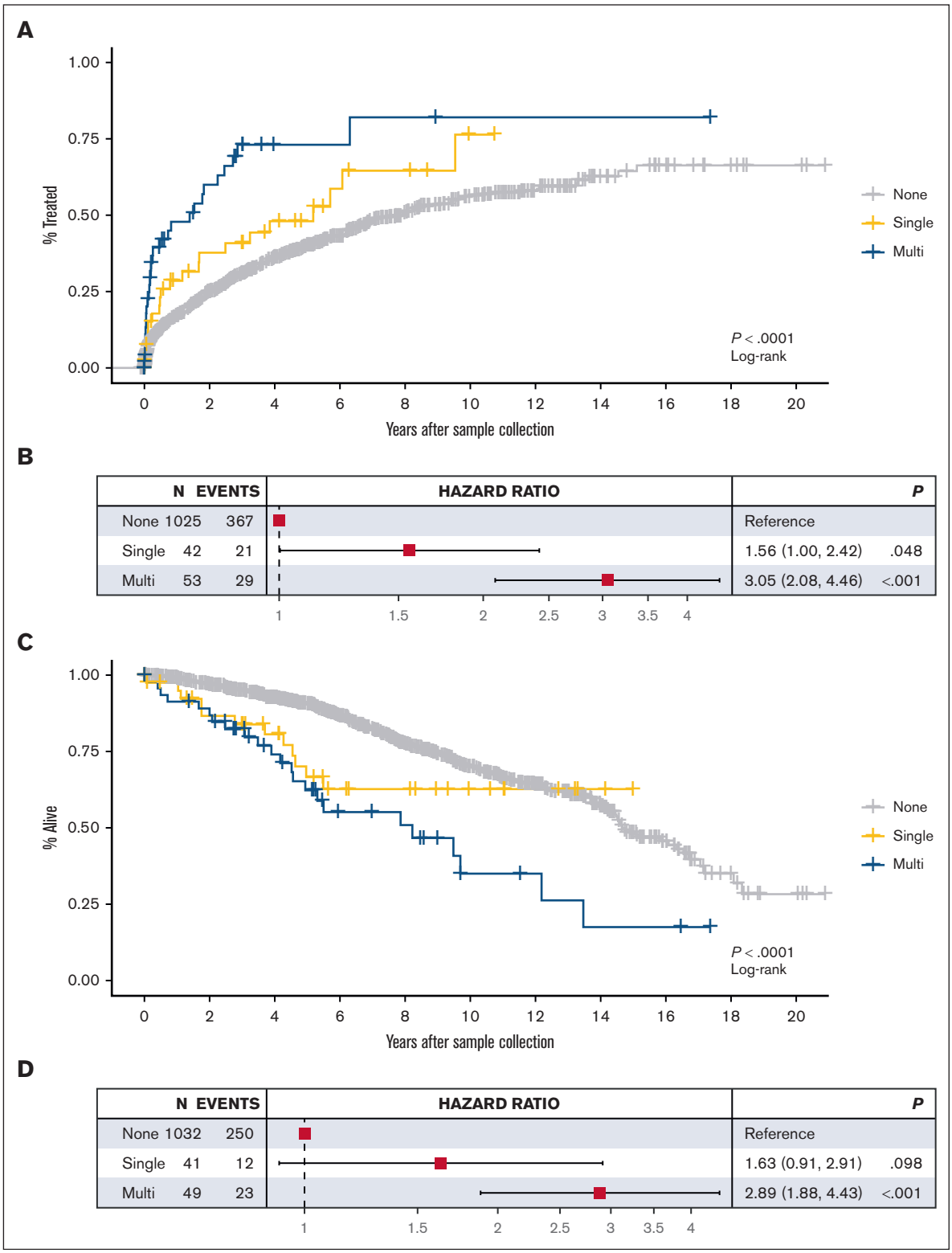

Figure 2. Kaplan-Meier curves and Cox regression forest plots. Based on *TP53* normal, single-hit, and multihit state for TTFT (A-B) and OS (C-D). Note that only *TP53* mutations with VAF >10% were considered.

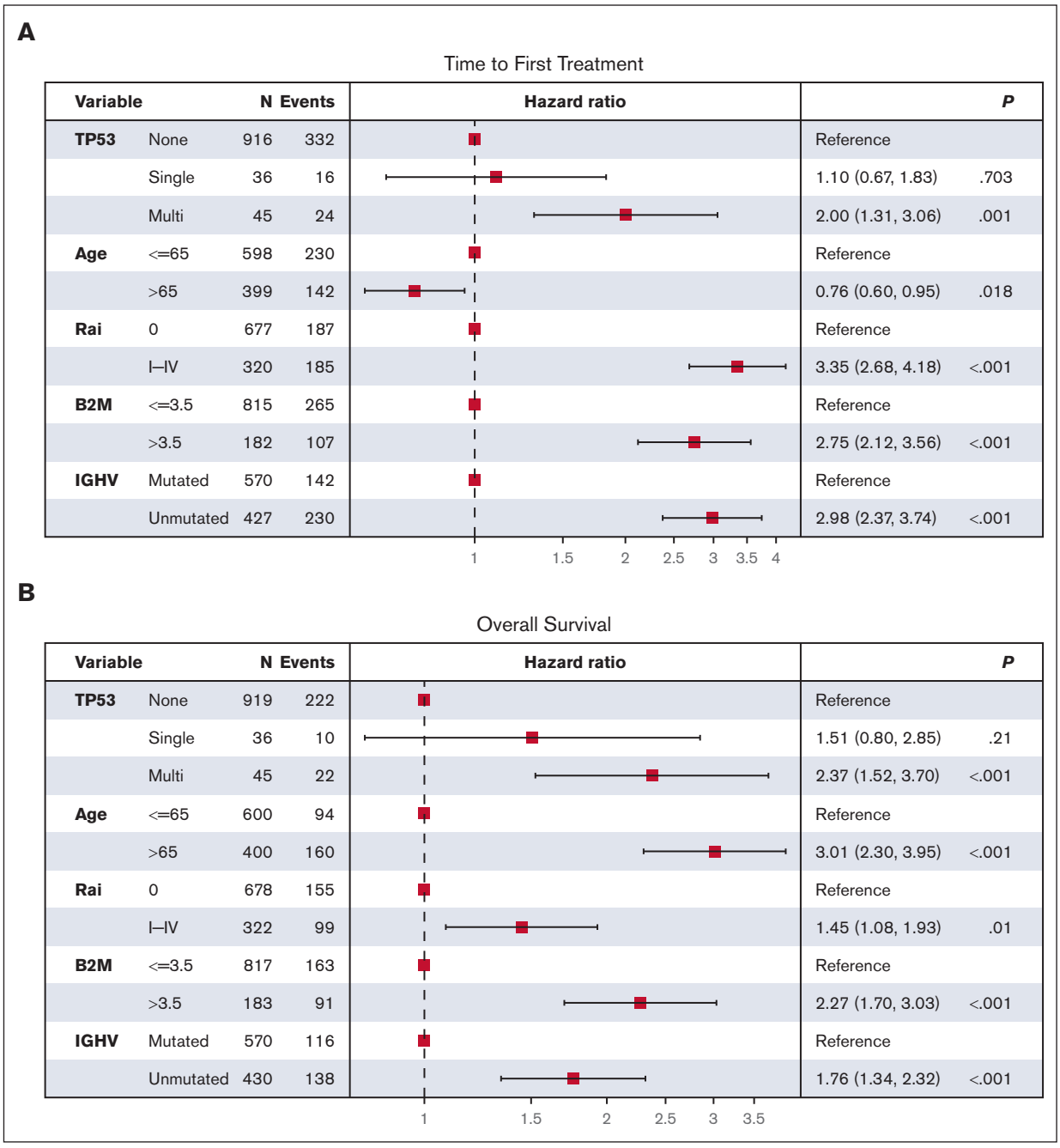

Figure 3. Multivariate Cox regression. (A) TTFT and (B) OS. Note that only *TP53* mutations with VAF >10% were considered.

Discussion

The results of our study of newly diagnosed, treatment-naive CLL and HCMBL, which is the largest to date, to our knowledge, show that (a) 1 in 15 patients have a *TP53* mutation (65% missense) at the time of initial diagnosis, with an average VAF of 50%; (b) 1 in 3 patients with a *TP53* aberration have a *TP53* mutation identified using sequencing studies in the absence of del(17p) via FISH testing; (c) compared with patients with no *TP53* abnormalities, those with multihit *TP53* have a threefold higher risk of needing first-line CLL therapy and increased risk of death; and (d) these results are true even after adjusting for other known prognostic factors, such as *IGHV* mutation status (for TTFT) and receipt of novel agent treatment (for OS). Collectively, these data have important implications in the counseling and management of patients with newly diagnosed CLL and HCMBL.

Overall, patients with HCMBL had lower VAF *TP53* mutations and fewer del(17p) leukemic cells than patients with CLL. Regardless of HCMBL/CLL status, multiple *TP53* abnormalities led to shorter TTFT and OS than single-hit *TP53* or wild-type *TP53*. As in prior studies, most of the *TP53* mutations were missense, and we did

not observe different patterns in the type or location of *TP53* mutations between HCMBL and CLL cases. Co-occurrence of *TP53* mutations and del(17p) had the poorest outcomes, even after accounting for other known prognostic factors. These findings remained consistent even when low-VAF mutations were included. Thus, an increasing number of *TP53* abnormalities may lead to worse prognosis for both patients with HCMBL and those with newly diagnosed CLL.

In CLL, many prior studies have focused on profiling *TP53* at the time of treatment iniatition.[6,8,16,19,20] Our study evaluated CLL at the time of diagnosis and found a similar prevalence of *TP53* aberrations as prior studies that evaluated patients with early-stage disease.[4,7,8,13-15,30] Together, these studies support evaluating *TP53* status at the time of CLL diagnosis to gain prognostic information.

Current clinical practice usually does not consider single vs multihit *TP53* abnormalities; however, our study supports that collectively the number of *TP53* abnormalities lead to different outcomes. Individuals with multiple *TP53* abnormalities had shorter TTFTs and OSs than those with a single-hit *TP53*, and individuals with both a *TP53* mutation(s) and del(17p) had the poorest outcomes. These findings are consistent with prior studies among patients with CLL who were symptomatic.[4,6,13,20] Thus, we found that having 1 *TP53* mutation only increases risk of poor outcomes when del(17p) is also present. Some prior studies also observed this finding in that *TP53* mutations in the absence of del(17p) were not prognostic for TTFT[4] or OS.[6,15] However, other studies reported significantly shorter OS[7,8,13,14,30] in patients with only *TP53* mutations. These discrepancies may be because of underlying differences in patient populations (considering that we enrolled only newly diagnosed untreated CLL), type of treatment, or *TP53* sequencing and mutation detection strategies. A large, retrospective registry study (informCLL) reported that among 840 patients with CLL treated largely at community centers, only a third underwent testing for FISH, and only 1 in 10 patients underwent testing for *TP53* mutation before treatment.[31] Given that our study shows that patients with multihit *TP53* abnormalities experience poor outcomes, additional outreach efforts to community physicians need to occur for optimal outcomes of patients with CLL.

Although limited by sample size, we observed shorter OS in individuals with multihit *TP53* when restricted to the 93 patients who were treated with novel therapy in the front-line setting, patients with multihit *TP53* had 16-fold increased risk of death ($P = .003$) whereas those with single-hit *TP53* had a sixfold increased risk of death ($P = .15$) compared with patients with wild-type *TP53*. These results are in line with recent work showing that the concomitant presence of *TP53* mutations and del(17p) is an independent negative prognostic factor for OS among patients with CLL on ibrutinib treatment.[32] Screening for multihit *TP53* among patients receiving novel therapy may be an important prognostic factor for OS in CLL and may have prognostic and counseling impact on HCMBL.

To our knowledge, this is currently the largest study of *TP53* of HCMBL, and we found that *TP53* may be important for evaluating HCMBL clinical course. In our cohort, 9.4% of patients with HCMBL had a *TP53* abnormality, with 3.1% of these abnormalities being multihit (including all VAF mutations). *TP53* mutations and del(17p) were less common, and del(17p) abundance was significantly lower in HCMBL than in CLL; these observations were expected, given that HCMBL is a precursor disease of CLL. Patients with HCMBL with multihit *TP53* did, however, have an elevated risk of shorter TTFT and OS; multihit *TP53* may already play a role in HCMBL clinical course and might be a prognostic factor in progression.

Following the ERIC *TP53* mutation calling standards in CLL,[17] our primary analyses defined *TP53* mutations with VAFs >10%. However, we had the depth of sequencing to evaluate mutations with VAFs >1%. When all mutations with VAFs >1% were considered, we still observed shorter TTFT and OS in patients with multihit *TP53*. We also observed that patients with a single *TP53* hit also had significantly worse outcomes than those with wild-type *TP53*, suggesting that any *TP53* abnormality (single or multiple and mutation or deletion) is a significant prognostic factor. However, prior studies have found mixed results regarding the prognostic value of low-VAF *TP53* mutation. Some prior studies observed significantly shorter OS in patients with low-VAF *TP53* mutation, even when del(17p) status was included as a covariate.[13,14,30] Conversely, other studies did not find prognostic value of low-VAF *TP53* mutation in the absence of del(17p) within the context of treatment.[15,16,33] Our study, which includes highly robust clinical information on our patient cohorts, supports the importance of screening small subclones with *TP53* mutations at the time of diagnosis.

Our study is strengthened by deep targeted sequencing of *TP53* in a large cohort of individuals with CLL and HCMBL from a single institution in which the clinical courses are robustly annotated. We followed a systematic approach to call and screen *TP53* mutations to address the limitation of paired germ line sequencing not being available. Our findings are limited to the observational and retrospective nature of the study. Because of the relatively low frequency of *TP53* abnormalities in CLL/HCMBL, stratification of results is also limited by small sample sizes; replication in an independent data set would strengthen our findings. However, our results are largely in line with prior studies of mostly symptomatic CLL and add evidence of the importance of screening for *TP53* abnormalities in addition to measuring del(17p) at diagnosis in individuals with HCMBL and CLL.

In summary, we characterized *TP53* in 849 individuals with CLL and 381 with HCMBL and observed mutations or 17p deletions in 141 individuals with similar mutation frequencies, types, and locations between CLL and HCMBL. Multiple *TP53* hits reduced TTFT and OS, even after accounting for other clinical prognostic factors, primarily driven by individuals with both *TP53* mutations and del(17p). Many recent studies have identified other markers of high-risk disease (eg, complex karyotype,[34] subset #2[35]). Similarly, patients with multiple *TP53* aberrations may constitute an ultrahigh-risk group of patients with CLL. With many novel drugs being tested in early intervention trials, the accurate identification of high-risk patients may allow for an improved risk stratification.

Acknowledgments

The authors thank the individuals who participated in our studies for their time and contribution.

This study was supported by awards from the National Cancer Institute, National Institutes of Health (K00CA234943) (R.G.), (R01CA235026 and R01CA254951) (S.L.S. and E.B.), (R21CA256648) (S.L.S. and G.K.), and (P50CA097274). The

content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Authorship

Contribution: S.L.S. and E.B. conceived and designed the study; S.L.S., E.B., S.A.P., and J.R.C. acquired the data; R.G., J.E.W.-N., C.E.M., and D.R.O. analyzed the data; R.G., J.E.W.-N., S.L.S., and E.B. drafted the manuscript; and all authors interpreted the data and reviewed the manuscript.

Conflict-of-interest disclosure: N.E.K. serves on the advisory board for AbbVie, AstraZeneca, Beigene, Behring, Boehringer Ingelheim Pharmaceuticals Inc, Cytomx Therapy, Dava Oncology, Janssen, Juno Therapeutics, Oncotracker, Pharmacyclics, and Targeted Oncology; serves on the data safety monitoring committee for Agios Pharmaceuticals, AstraZeneca, Bristol Myers Squibb, Celgene, CytomX Therapeutics, Dren Bio, Janssen, MorphoSys, and Rigel; and has received research funding from AbbVie, Acerta Pharma, Bristol Myers Squibb, Celgene, Genentech, MEI Pharma, Pharmacyclics, Sunesis, TG Therapeutics, and Tolero Pharmaceuticals. The remaining authors declare no competing financial interests.

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