Clinical Cancer Research

Survival of Del17p CLL Depends on Genomic Complexity and Somatic Mutation

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Abstract

Purpose: Chronic lymphocytic leukemia (CLL) with 17p deletion typically progresses quickly and is refractory to most conventional therapies. However, some del(17p) patients do not progress for years, suggesting that del(17p) is not the only driving event in CLL progression. We hypothesize that other concomitant genetic abnormalities underlie the clinical heterogeneity of del(17p) CLL.

Experimental Design: We profiled the somatic mutations and copy number alterations (CNA) in a large group of del(17p) CLLs as well as wild-type CLL and analyzed the genetic basis of their clinical heterogeneity.

Results: We found that increased somatic mutation number associates with poor overall survival independent of 17p deletion ($P = 0.003$). *TP53* mutation was present in 81% of del(17p) CLL, mostly clonal (82%), and clonal mutations with del(17p) exhibit shorter overall survival than subclonal mutations with del(17p) ($P = 0.019$). Del(17p) CLL has a unique driver mutation profile, including *NOTCH1* (15%), *RPS15* (12%), *DDX3X* (8%), and *GPS2* (6%). We found that about half of del(17p) CLL cases have recurrent deletions at 3p, 4p, or 9p and that any of these deletions significantly predicts shorter overall survival. In addition, the number of CNAs, but not somatic mutations, predicts shorter time to treatment among patients untreated at sampling. Indolent del(17p) CLLs were characterized by absent or subclonal *TP53* mutation and few CNAs, with no difference in somatic mutation number.

Conclusions: We conclude that del(17p) has a unique genomic profile and that clonal *TP53* mutations, 3p, 4p, or 9p deletions, and genomic complexity are associated with shorter overall survival. *Clin Cancer Res; 23(3); 735–45. ©2016 AACR.*

Introduction

Chronic lymphocytic leukemia (CLL) is a common leukemia with a heterogeneous disease course that varies based on the degree of somatic hypermutation in the immunoglobulin heavy chain variable region (*IGHV*) and by chromosome abnormalities (1–3). Metaphase analysis of CLL chromosomes revealed that complex karyotype, defined as three or more gross chromosomal abnormalities, is predictive of worse disease outcome (4, 5). In 2000, Döhner and colleagues used FISH to detect recurrent chromosomal abnormalities and established a hierarchical prognostic model for overall survival (OS; ref. 3), in which 17p deletion is associated with the worst disease outcome. Many studies have since confirmed that del(17p) CLL responds poorly to conventional chemoimmunotherapies and has a median OS of less than 3 years, at least prior to the newly approved targeted therapies (6, 7).

Del(17p) is found in 5% to 10% of patients at diagnosis but in up to 40% of patients relapsing after fludarabine-based therapies (8, 9). Del(17p) causes loss of one allele of the tumor suppressor *TP53*, which plays an important role in DNA repair, cell-cycle arrest, and apoptosis in response to genotoxic insults (10). Somatic mutations in *TP53* occur in the other allele of *TP53* in about 80% of del(17p) CLL, resulting in biallelic inactivation (11–14). About 4% to 30% of *TP53* mutations are monoallelic without coexisting del(17p) (11, 13, 15, 16). Similar to 17p deletion, *TP53* mutations alone, even when present as very small subclones (17, 18), have been associated with worse treatment response, shorter failure-free survival (FFS), and shorter OS (11–14). Notably, however, del(17p) CLL exhibits considerable heterogeneity. A subset of CLL patients with del(17p) has stable disease without a need for treatment for more than 5 years (19, 20). Delgado and colleagues showed that genomic complexity measured by SNP array and *IGHV* mutational status were the main predictors of OS in CLL patients with *TP53* disruption, but their analysis was limited by an absence of somatic mutation data (21).

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

Chronic lymphocytic leukemia (CLL) with 17p deletion represents the most challenging disease group, with poor overall survival, yet significant heterogeneity in survival. We hypothesized that the concurrent genomic landscape of del(17p) CLL would predict this heterogeneity in outcome and therefore undertook to characterize the somatic mutation and copy number landscape in this subgroup of CLL, which has been underrepresented in all prior sequencing studies. Our results demonstrate that subclonal rather than clonal mutation of the second *TP53* allele and lack of genomic complexity of any type are characteristic of patients with longer overall survival, who may not require therapy for an extended period. These results have direct implications for clinical prognostication.

Recent work by our group and others has used genomic profiling to characterize the landscape of genomic alterations in CLL, including recurrent copy number alterations (CNA; ref. 22) and somatic mutations in CLL driver genes (23–26). Surprisingly, none of these studies included significant numbers of CLLs with 17p deletion, for example, only 17 in the recent study of 500 CLLs from Puente and colleagues (24). We undertook this study to systematically characterize the coexisting genetic aberrations in the largest cohort of del(17p) CLL investigated to date using whole-exome sequencing (WES) and array analysis. Our results show that del(17p) CLL has distinct genetic profiles associated with different disease outcomes.

Materials and Methods

Patient samples

Matched peripheral blood (tumor) and saliva (germline) samples were collected from patients who consented to an IRB-approved tissue banking protocol. B-cell enrichment was performed using Easy Sep Human B cell Enrichment Kit (STEMCELL Technologies Inc.) for samples with WBC count lower than 25,000 μL or absolute lymphocyte count <20,000 μL. For samples with lymphocyte count >25,000 μL, peripheral blood mononuclear cells were separated using conventional Ficoll–Hypaque separation according to the manufacturer's instructions (STEMCELL Technologies Inc.). Tumor and saliva DNA were extracted using QIAamp Blood DNA (Qiagen Inc, Valencia, CA) and Oragene DNA (Oragene) kits respectively, according to the manufacturer's directions.

FISH cytogenetics were assessed by the clinical cytogenetics laboratory at the Brigham & Women's Hospital (Boston, MA). The cutoff for 17p deletion was 10% cells positive; 13q deletion, 2.5%; trisomy 12, 2%; and 11q deletion, 5%. *IGHV* status was assessed by the CLL Research Consortium tissue bank or by LabCorp; unmutated *IGHV* requires ≥98% homology to the closest germline match to an *IGHV* gene. CNA data, if available, were used to determine the 17p deletion status for patients without FISH cytogenetics [$n = 14$, all were 17p wild type (WT)].

Only samples that provided an evaluable karyotype by whichever method they were assessed were considered to have available data. For stimulated karyotyping since 2011, 0.8 to 1.0 mL of either bone marrow or peripheral blood was added to each of the two 5.0 mL MarrowMAX media (Life Technologies) replicate cultures and stimulated with a B-cell cocktail consisting of 100 μL CpG-ODN-GNKG685 (10 μg/mL, Sigma), 50 μL pokeweed mitogen (PWM) from *Phytolacca americana* (10 μg/mL, Sigma), and 100 μL phorbol 12-myristate 13-acetate (40 ng/mL, Sigma). Prior to 2011, the cells were stimulated only with PWM. In all cases, the cells were cultured at 37°C with 5% CO_2 for 3 days and harvested/banded according to standard procedures. GTG-banded metaphase chromosomes were captured and processed using CytoVision Ultra Workstations (Leica Microsystems, version 4.5.2). Twenty metaphases from across both of the two cultures were analyzed.

SNP arrays

Matched tumor and normal DNA of 40 CLL patients with 17p deletion were run on the Affymetrix CytoScan HD array according to the manufacturer's protocol. CNAs were inferred using the Affymetrix Chromosome Analysis Suite [version 2.0.1.2 (r5919), genome build hg19]. Tumor and germline DNA of 160 CLL patients, including 15 del(17p) and 145 WT, were run on Affymetrix SNP6.0 arrays, and CNAs were inferred using Nexus Copy Number 7.0 (Biodiscovery, significance threshold set at 1.0E−10). All CNAs and LOHs were manually reviewed to ensure call quality and to remove small germline copy number variants. Neighboring segments with similar copy number ratios were joined as single segments. The final set of copy number segments was checked for probe densities on both the SNP6.0 platform and the Cytoscan HD platform, and all segments had at least 60 probes on both platforms. The 160 SNP6.0 arrays have been described previously (22). Sex chromosomes and immunoglobulin switch regions were excluded in the copy number analysis. A cutoff of 25 probes and 200 kb for deletion CNAs or 400 kb for gain CNAs was used. A size cutoff of 10 MB genome-wide and 3 MB within genic regions was used to detect LOH events. Chromothripsis events were identified using the criteria defined by Korbel and Campbell (27). A region was considered to have undergone chromothripsis if it satisfied at least two criteria: the first being the presence of oscillating patterns of retention of heterozygosity alternating with areas of LOH, and second, alternating patterns of copy number states with clustering of at least 5 to 10 breakpoints within a 50-Kb region.

WES

Purified genomic DNAs of 176 CLL tumor–germline pairs were submitted to the Genomics Platform at the Broad Institute (Cambridge, MA) for WES. Mean target coverage of all samples is 80×. A total of 134 of the 176 whole exomes were included in a previous study (25). PicoGreen-based dsDNA quantitation (Life Technologies) was performed to ensure sample purity, and DNA fingerprinting was used to confirm the match between the tumor and its cognate normal, prior to WES library preparation. Libraries were sequenced on either an Illumina HiSeq2000 or GA-IIX, and aligned reads were generated by the Picard pipeline (28). Somatic mutations and indels were identified using MuTect (29) and indelocator (https://www.broadinstitute.org/cancer/cga/indelocator), respectively, integrated in the Call Somatic Mutations for Capture workflow and the Call Indels for Capture workflows in the Broad Firehose pipelines. Somatic mutations with less than 8 sequencing reads of the alternative alleles were filtered out in the analysis as

described previously (30). ABSOLUTE (31) was used to assess cancer cell fraction (CCF) of somatic mutations. Somatic mutations were considered clonal when the CCF was 85% or greater as described previously (26).

Sanger sequencing of the *TP53* gene

Exons 4–9 of *TP53* were sequenced according to the protocol described in the IARC *TP53* database (p53.iarc.fr). Primers 326/327 were used to amplify and sequence exon 4, primers 236/240 for exons 5 and 6, primers 237/238 for exon 7, and primers 314/315 for exons 8 and 9. Sequencing results were aligned to the *TP53* reference sequence and manually reviewed.

Statistical analysis

OS and FFS were estimated using the Kaplan–Meier method. FFS was measured from the time of sampling to first treatment after sampling or death, whichever occurred first. OS was measured from the time of sampling to death, to control for differences in the time of sampling in relation to diagnosis. Patients were censored at the date last known alive. Because genetic changes may evolve over time, all time to event endpoints were measured from time of sampling. For OS and FFS, univariable and multivariable Cox regression analysis was also performed. All Cox models were stratified by treatment status at sampling as previously treated and untreated patients represent two different patient populations with different prognoses. Prior to performing the regression analysis, the proportional hazards assumption for each variable was tested and interaction terms were examined. The linearity assumption for continuous variables was examined using restricted cubic spline estimates of the relationship between the continuous variable and log relative hazard (32). Natural log or log10 transformation was attempted to normalize mutation and CNA event parameters. However, due to the influence of some large values in the SNP parameters, all continuous variables from WES and SNP analyses were dichotomized for consistency of presentation, and optimal cut-off values were identified using the method of recursive partitioning for survival trees and the restricted cubic spline estimation method (33). These cut-off values are presented in tables and figures throughout the article. Furthermore, the C-statistic (34) was used to guide and compare the predictive ability of selected parameters from the Cox model. The Fisher exact test was used for comparison of binary variables and the Wilcoxon rank-sum test was used for comparison of continuous variables. The log-rank test was used to assess the difference in FFS or OS between groups. All statistical tests were two-sided at a significance level of 0.05, and multiplicity was not considered. All statistical analyses were performed using SAS 9.3 (SAS Institute Inc.) and R version 3.2.0 (The Comprehensive R Archive Network, www.cran.r-project.org). The heatmap was generated using GENE-E (http://www.broadinstitute.org/cancer/software/GENE-E).

Results

Patient characteristics

A total of 277 CLL patients were included in this study: 69 with del(17p) and 208 without (Table 1 and Supplementary Table S2). Of these 277 patients, 176 were profiled by WES for somatic mutations and 200 were profiled by SNP arrays for CNAs (Table 1). Ninety-nine patients were characterized by both WES and SNP arrays. As is well documented in the literature (3, 6, 11), del(17p) CLL exhibits different clinical characteristics with markedly fewer mutated *IGHV* (16% del

Table 1. Patient characteristics and summary of CNAs and somatic mutations

	All	wt 17p	del(17p)	*P*
N	277 (100%)	208 (75%)	69 (25%)	
Male	163 (59%)	118 (57%)	45 (65%)	0.26
Age of onset	55 (32–86)	54 (32–78)	61 (38–86)	2.2e−05
Treated before sampling	74 (27%)	39 (19%)	35 (51%)	6.4e−07
IGHV mutated	135 (52%)	125 (64%)	10 (16%)	1.2e−11
Complex karyotype	42 (32%)	20 (21%)	22 (61%)	4.03e−05
FISH cytogenetics				
13q14 loss	170 (61%)	139 (67%)	31 (45%)	1.9E−4
11q loss	37 (13%)	29 (14%)	8 (12%)	0.55
Trisomy 12	36 (13%)	25 (12%)	11 (16%)	0.54
Profiled by WES[a]	176 (100%)	123 (70%)	53 (30%)	
Total mutations	19 (0–94)	18 (0–94)	21 (7–68)	0.0048
Nonsynonymous mutations	14 (0–70)	13 (0–70)	16 (5–54)	0.0055
Synonymous mutations	4 (0–24)	4 (0–24)	4 (1–14)	0.14
Subclonal mutations	9 (0–89)	9 (0–89)	8 (2–35)	0.9
Clonal mutations	9 (0–34)	7 (0–24)	12 (0–34)	5.8E−4
Profiled by SNP[a]	200 (100%)	145 (72%)	55 (28%)	
# of CNAs	1 (0–36)	1 (0–16)	7 (0–36)	1.5e−16
# of losses	1 (0–35)	1 (0–16)	6 (0–35)	5e−15
# of gains	0 (0–19)	0 (0–3)	1 (0–19)	3.3e−08
Lost Mb	3 (0–530)	1.2 (0–88)	97 (0–530)	2.9e−18
Gained Mb	0 (0–340)	0 (0–260)	5.9 (0–340)	9.9e−6
8p loss	21 (10%)	6 (4%)	15 (27%)	1.1e−05
3p loss	15 (8%)	0 (0%)	15 (27%)	8.1e−10
4p loss	14 (7%)	2 (1%)	12 (22%)	4.1e−06
9p loss	15 (8%)	2 (1%)	13 (24%)	1.1e−06
Loss in 3p, 4p, or 9p	31 (16%)	3 (2%)	28 (51%)	8.6e−16

[a]Median values and ranges are presented for the WES and SNP analysis.

Table 2. Cox model stratified by previous treatment status at sampling

	Univariable analysis		Multivariable analysis[a]	
	HR (95% CI)	P	HR (95% CI)	P
WES analysis ($N = 176$)				
Total no. of mutations: high vs. low	4.82 (2.08–11.2)	0.0002	3.55 (1.55–8.16)	0.003
Clonal mutations: high vs. low	6.62 (1.95–22.5)	0.002	7.39 (2.11–25.9)	0.003
Subclonal mutations: high vs. low	3.35 (1.34–8.38)	0.01	2.25 (0.87–5.82)	0.1
Nonsynonymous mutations: high vs. low	4.69 (1.75–12.6)	0.002	4 (1.45–11.1)	0.007
Synonymous mutations: high vs. low	2.79 (0.957–8.14)	0.06	2.45 (0.828–7.24)	0.1
SNP analysis ($N = 200$)				
Total no. of events: high vs. low	3.21 (1.68–6.16)	4e−04	2.28 (1.12–4.66)	0.02
No. of gain events: high vs. low	1.98 (1.01–3.88)	0.045	1.58 (0.80–3.12)	0.2
No. of loss events: high vs. low	3.63 (1.9–6.95)	1e−04	2.24 (1.06–4.76	0.04
Gain MB: high vs. low	2.4 (1.29–4.47)	0.006	1.86 (0.98–3.52)	0.06
Loss MB: high vs. low	5.26 (2.61–10.6)	4e−06	3.86 (1.74–8.55)	0.0009

NOTE: The optimal cut-off values for separating the high and low groups were identified using the method of recursive partitioning for survival trees. Cut-off values for total number of mutations, 21; clonal mutations, 8; subclonal mutations, 22, nonsynonymous mutations, 15; and synonymous mutations, 3. For CNAs, total number of events, 4; number of gain events, 2; number of loss events, 5; gain MB, 0.675 MB; loss MB, 25 MB.

Abbreviation: CI, confidence interval.

[a]Adjusted for *IGHV* mutational status and del(17p).

(17p) vs. 64% WT, $P < 0.0001$). A total of 73% of all patients were untreated at sampling, with 34% subsequently undergoing therapy, with a median follow-up time from sampling of 6.0 years among survivors (range, 3 days–8.8 years). As expected, a higher percentage of del(17p) patients had been treated prior to sampling (51% vs. 19%, $P < 0.0001$). Among the 35 del(17p) patients who were untreated at sampling, 24 (70%) patients subsequently underwent therapy at a median follow-up of 22 months.

Overview of somatic mutations and CNAs

We characterized the somatic mutation spectrum of 17p deleted CLL by WES on paired tumor and germline samples from 176 CLL patients, including 53 with 17p deletions, significantly more than prior studies (23–26). Comparing CLL with wild-type 17p, del(17p) CLL has more somatic mutations (median 21 vs. 18, $P = 0.0048$), nonsynonymous mutations (median 16 vs. 13, $P = 0.0055$), and clonal mutations (median 12 vs. 7, $P = 5.8E{-}4$; Table 1). Synonymous mutations and subclonal mutations are not significantly different between del(17p) and wild type. Furthermore, a Cox model stratified by previous treatment status at sampling revealed that increasing total mutations ($P = 0.003$), clonal mutations ($P = 0.003$), and nonsynonymous mutations ($P = 0.007$) are associated with shorter OS in both univariable and multivariable analysis controlling for del(17p) and *IGHV* mutational status, while synonymous mutations and subclonal mutations are not ($P > 0.05$; Table 2). The number of mutations stratified OS risk in both the previously treated and untreated groups (Fig. 1A) and stratified del(17p) into poor versus intermediate risk (Fig. 1B).

CNA data were obtained for 55 del(17p) CLL and 145 WT CLL, with a median of 1 CNA per patient in the entire cohort similar to our own and others' previous reports (22, 35, 36). 17p deletion has a minimally deleted region (MDR) of 34 kb, which, as expected, targets the *TP53* gene (Supplementary Table S1; Supplementary Fig. S1). Supplementary Figure S2 provides an overview of CNA events, showing fewer CNAs in patients who did not require therapy in the follow-up period. More CNAs were seen in del(17p) than WT (median 7 vs. 1, $P < 0.0001$, see Table 1). In addition, del(17p) CLL has a longer total length of deleted (97 Mb vs. 1.2 Mb, $P < 0.0001$) and gained DNA (5.9 Mb vs. 0 Mb, $P < 0.0001$). High total number of CNAs, as well as number of loss events and total length of loss, were all associated with shorter OS in the entire cohort in both univariable and multivariable analysis, stratified by treatment status at sampling and adjusted for 17p deletion and *IGHV* mutational status (Table 2; Fig. 1C; refs. 22, 36). The total number of CNA events further stratified OS risk in both del(17p) and WT CLL (Fig. 1D). In del(17p) CLL in our cohort, mutated *IGHV* was associated with fewer CNAs ($P < 0.01$), but not somatic mutations ($P > 0.1$), and was predictive of longer OS ($P < 0.01$).

Using 99 samples that were profiled by both WES and SNP (heatmap, Fig. 1E), we tested whether the number of somatic mutations was correlated with the number of CNAs. Neither number of CNAs nor total length of gains or losses correlated with the number of somatic mutations in individual CLLs (Supplementary Fig. S3A–S3C), suggesting that mutations and CNAs result from different mechanisms in CLL. As the number of somatic mutations and CNAs are not correlated, we examined whether one is more predictive of survival than the other. The C-index from univariable Cox regression analysis stratified by treatment status at sampling was 0.765 for the total number of CNA events and 0.750 for the total number of mutations (Supplementary Table S3), suggesting that both are very predictive of OS as single variables. When we included both variables in a Cox model, the C-index was substantially increased to 0.850 (Supplementary Table S3; Supplementary Fig. S3D), indicating that both are independently significant in predicting OS as noted. It is notable that patients with a high number of both CNAs and mutations all died within 3 years (i.e., 0% 3 year OS) and 11 of 12 of these patients had del(17p).

TP53 disruption in CLL

As expected, somatic *TP53* mutation is the most abundant mutation in del(17p) CLL, seen in 43 patients (81%). In addition, *TP53* mutations were found in 6 (5%) patients without 17p deletion, two of whom have copy neutral LOH at 17p and are therefore considered to have biallelic loss (Supplementary Fig. S4A). We found that most *TP53* mutations in this del(17p) cohort are clonal (40/49, 82%, see Fig. 2A), similar to a previously reported rate of clonal *TP53* mutations in del(17p) patients (17). Del(17p) patients with subclonal *TP53* mutations had a longer OS than patients with clonal *TP53* mutations ($P =$

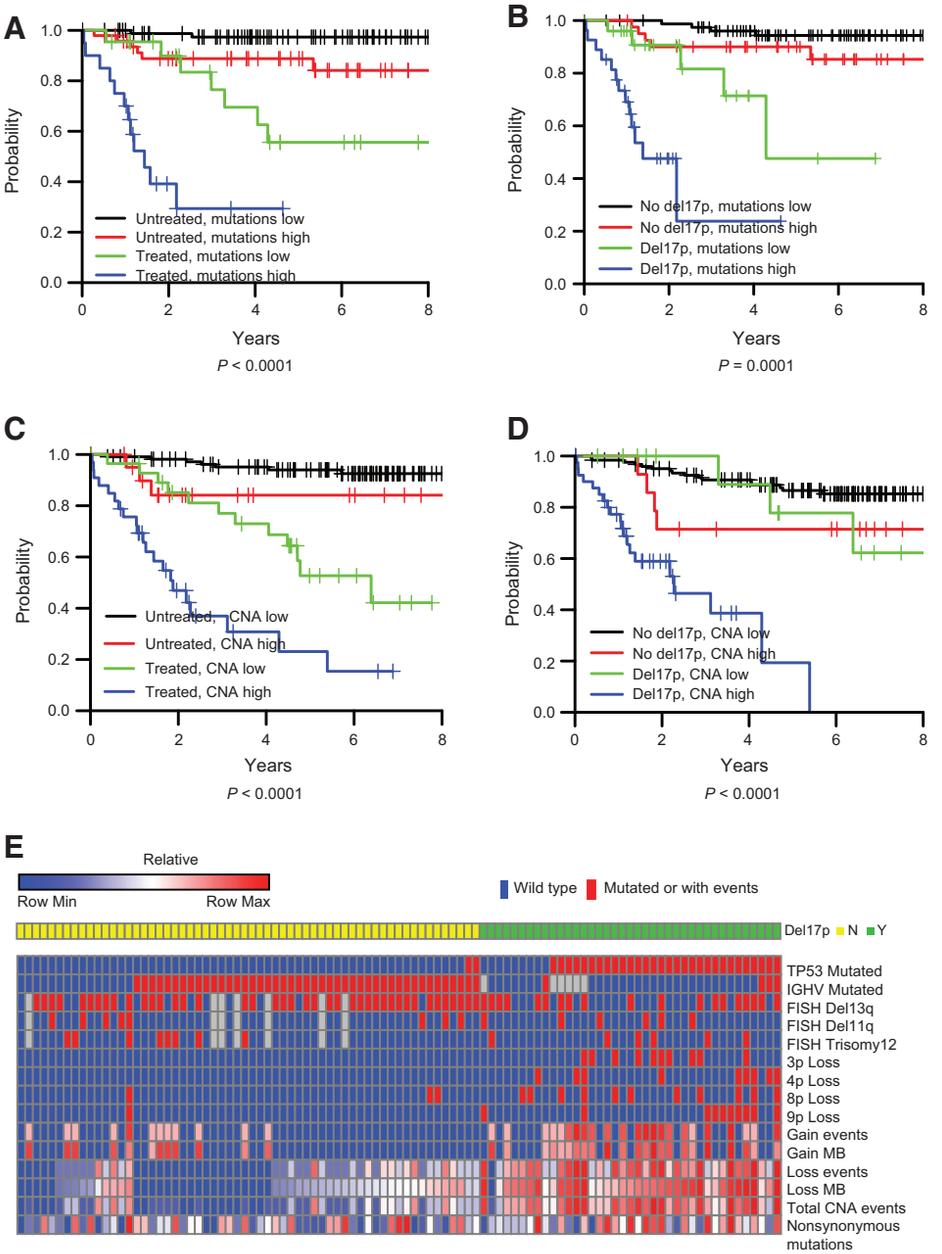

Figure 1.
OS and heatmap characteristics of patients. **A,** OS by total number of mutations and treatment status at sampling. **B,** OS by total number of mutations and del(17p) status. **C,** OS by total CNA events and treatment status at sampling. **D,** OS by total CNA events and del(17p) status. Mutations low, mutations < 21; mutations high, mutations ≥ 21; CNAs low, CNA < 4; CNAs high, CNA ≥ 4. The optimal cut-off values for total number of mutations and CNAs were identified using the method of recursive partitioning for survival trees. **E,** Heatmap characteristics of the 99 patients with both SNP and WES profiles. The first 9 rows are indicated by the presence (red) or absence (blue) of TP53 mutation, *IGHV* mutation, or abnormal cytogenetics by FISH. CNA events, weights, and nonsynonymous mutations represent percentiles of ranks across 99 observations; that is, the highest value in each row is denoted as 1 (100th percentile) and the lowest value is denoted as 0.01 (1st percentile). Gray indicates missing data.

0.019, Fig. 2B). In contrast, *SF3B1* mutations ($n = 22$), the second most abundant mutation in our cohort (23), are mostly subclonal, and clonality of *SF3B1* is not associated with OS (Supplementary Fig. S4B and S4C).

Whether mutation of the second *TP53* allele impacts clinical outcome in del(17p) CLL remains an open question. Our cohort contains patients with biallelic disruption of *TP53* by somatic *TP53* mutation, with either 17p deletion ($n = 43$) or copy neutral LOH (cnLOH; $n = 2$), as well as monoallelic disruptions by either 17p deletion or *TP53* mutation ($n = 14$; Supplementary Fig. S4A). Interestingly, most biallelic disruptions have clonal *TP53* mutations (37 clonal vs. 8 subclonal). We tested whether there is a dose-dependent effect of *TP53* disruption on CNAs or somatic mutations in CLL and found more CNA events in biallelic disruption (median 9.5 in double null vs. 5 in single null, $P = 0.016$, Fig. 2C). No significant difference in the number of somatic mutations was seen based on mono- or biallelic *TP53* disruption (Fig. 2D), again suggesting that somatic mutations arise by a different mechanism than CNAs in CLL.

Mono- versus biallelic *TP53* disruption was also predictive of OS. With wild-type *TP53* having the best prognosis and biallelic *TP53* disruption having the worst prognosis, patients with monoallelic disruption of *TP53* by either del(17p) or somatic mutation showed intermediate OS ($P < 0.0001$, Fig. 2E). This dose-dependent pattern remained consistent when the analysis was restricted to previously untreated patients ($P < 0.0001$, Fig. 2F).

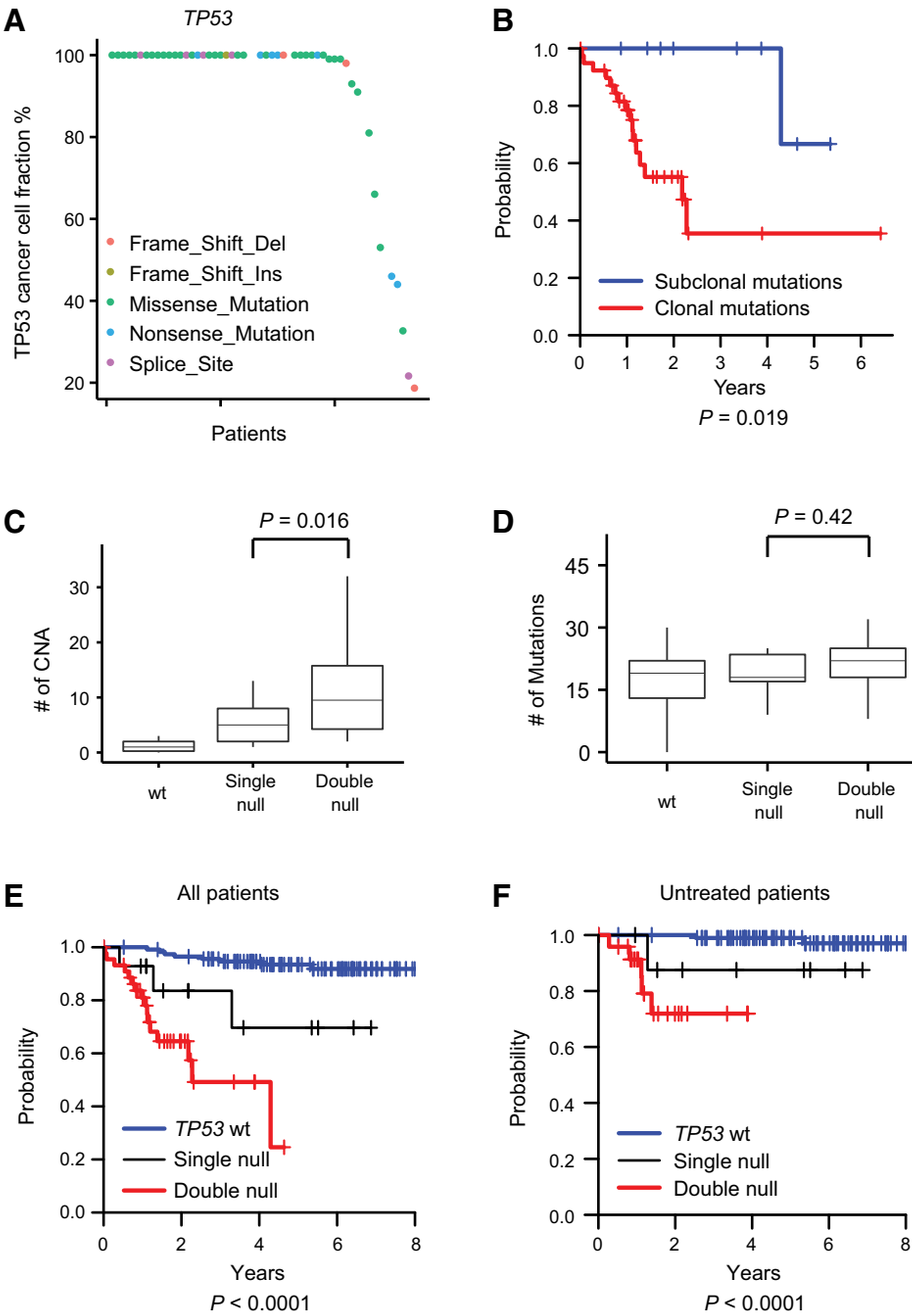

Figure 2.
Analysis of *TP53* disruptions. **A,** CCFs of the *TP53* mutations in each patient. **B,** Kaplan–Meier curves of OS by clonality status of *TP53* mutations. **C,** Relationship of CNAs and *TP53* disruptions. wt, no *TP53* mutation or deletion; single null, monoallelic *TP53* mutation or del(17p) deletion; double null, biallelic disruption. **D,** Relationship of somatic mutations and *TP53* disruptions. **E** and **F,** Dose-dependent impact of *TP53* disruption on overall survival in all patients (**E**) or previously untreated patients (**F**).

Driver genes in del(17p) CLL

To identify driver genes in del(17p) CLL, we ran the MutSigCV algorithm (30) on the 53 del(17p) CLL in our WES cohort. In addition to *TP53*, several other genes are significantly mutated, including *NOTCH1* (8 mutations, 15% of the cohort), *RPS15* (6, 12%), *DDX3X* (4, 8%), and *GPS2* (3, 6%; Fig. 3A). In contrast, MutSigCV identified *SF3B1* (18, 17%), *MYD88* (11, 10%), and *IGLL5* (4, 3%) as significantly mutated genes in 17p wild-type CLL (Fig. 3B). We therefore compared the mutation frequencies of known CLL driver genes in 17p versus WT CLL and found that mutations in *ATM* and *MYD88* occurred exclusively in 17p WT CLL, while mutations in *DDX3X*, *NOTCH1*, *RPS15*, and *TP53* occurred with higher frequency in del(17p) CLL ($P < 0.05$; Fig. 3C). Other mutations like *SF3B1* and *IGLL5* distributed without significant difference.

NOTCH1 and *DDX3X* have been previously implicated in CLL, and *NOTCH1* mutation has been correlated with poor outcome and risk of Richter transformation (24, 37, 38). The *RPS15* gene has recently been identified as a new CLL driver gene with a mutation frequency of 4.3% in 538 CLL cases (26) and 19.5% in relapsing CLL (39). In our cohort enriched for del (17p) CLL, we identify that the *RPS15* gene is more frequently

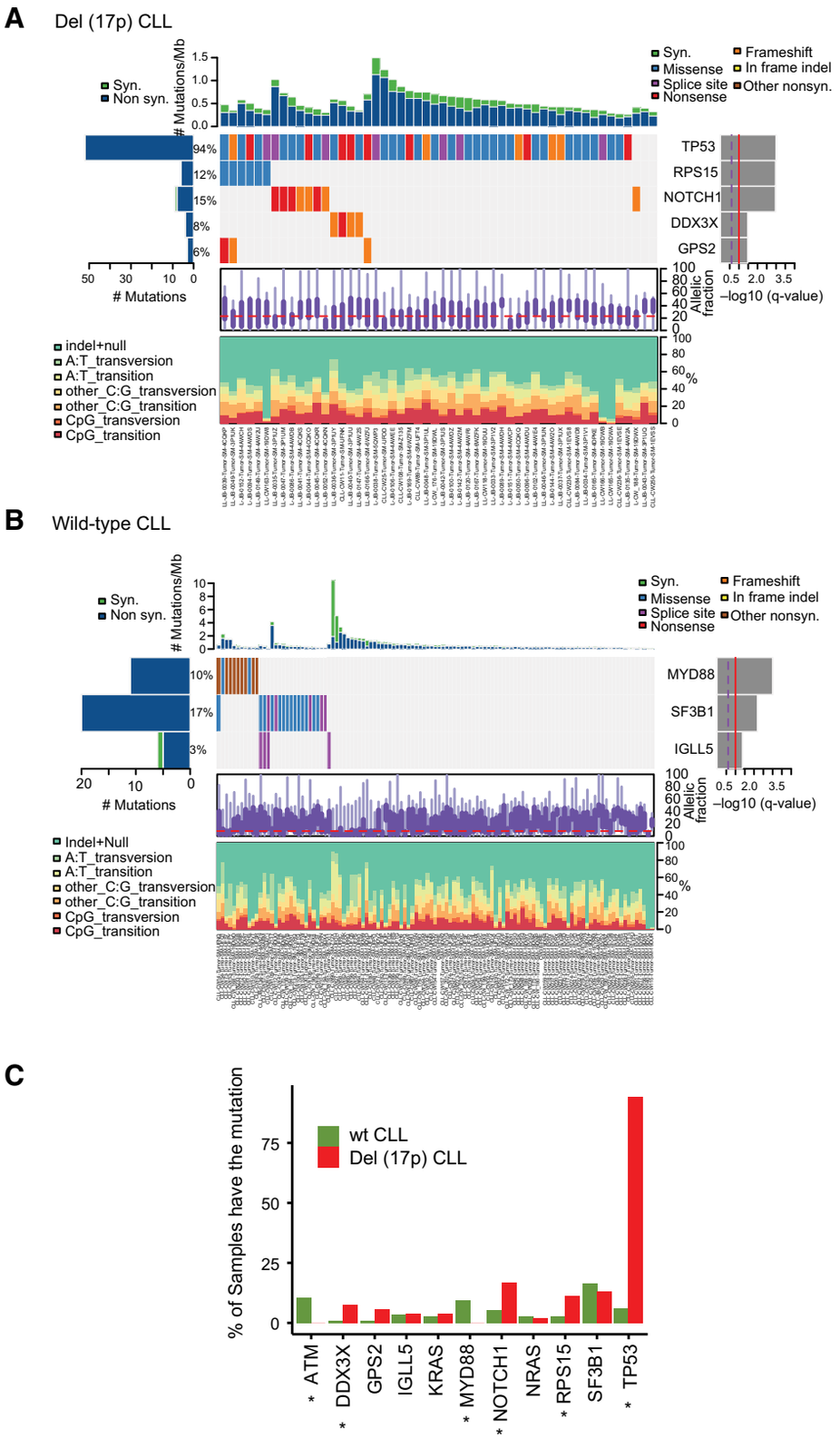

Figure 3.
Driver genes in del(17p) CLL. **A,** coMut plot of del(17p) CLL. **B,** coMut plot of wild-type CLL. Syn, synonymous; nonsyn, nonsynonymous. **C,** Mutation frequencies of known CLL driver genes in del(17p) and wt 17p CLL. *, $P < 0.05$.

mutated in del(17p) CLL than in WT (12% vs. 2.5%, $P < 0.05$). We confirmed all *RPS15* mutations by Sanger sequencing (Supplementary Fig. S5A). In the two samples with RNA-seq data available, we also confirmed that the mutations are expressed in the tumor mRNA (Supplementary Fig. S5B). *RPS15* encodes the ribosomal protein S15. Interestingly, all of the *RPS15* mutations occur in the far carboxyl end of the protein, ranging from amino acid 131 to 145 (Supplementary Fig. S5C; Supplementary Table S4), suggesting that mutation of this domain may be important in CLL development. Presence of an *RPS15* mutation was not associated with OS however (Supplementary Fig. S5D).

Recurrent deletions and chromothripsis in del(17p) CLL

To identify driver CNA events enriched in del(17p) CLL, we performed GISTIC analysis (40) and found recurrent chromosomal deletions at 3p, 4p, 8p, and 9p in del(17p) CLL (Supplementary Fig. S6). We have previously reported that 8p deletion is associated with del(17p) and worse prognosis (Supplementary Fig. S7; ref. 22). Here, we also note that over half of the del(17p) CLLs have at least one of 3p, 4p, or 9p deletions, yet WT CLL rarely shows these (Table 1; Fig. 1E). Each of these deletions is significantly associated with shorter OS in the entire cohort ($P < 0.0001$) and any one of these deletions stratifies OS in del(17p) CLL (Supplementary Fig. S8). MDRs of 3p, 4p, 8p, and 9p and the genes affected are included in Supplementary Table S1 and Supplementary Figs. S9–S12.

Previous studies have associated complex karyotype (CKT) with poor outcome (4, 5, 41, 42). We had karyotype information on 94 of the 200 patients profiled by SNP array. In this subgroup, and consistent with previous studies (4, 5, 41, 42), CLL patients with CKT had shorter OS than patients without CKT (Fig. 4A, $P = 0.0016$). A total of 63% of del(17p) CLL had CKT as compared with only 22% of WT ($P = 0.0002$). Patients with both del(17p) and CKT together had worse OS, suggesting possibly additive risks (Fig. 4B). As expected, we found that more CNA events measured by SNP array is associated with CKT (Supplementary Fig. S13). OS was similar among patients with either a high CNA count, CKT, or both, indicating that high CNA number and CKT reflect the same phenomenon and are not additive risk factors (Fig. 4C).

Chromothripsis has been previously reported in CLL, with a suggestion of association with del(17p) (43, 44). We found 3 cases of chromothripsis, affecting chromosomes 8, 11q, and 15q. 17p deletions were found in two cases, together with clonal *TP53* mutations, and the third case had cnLOH in 17p (Supplementary Fig. S14). We sequenced the *TP53* gene in the latter tumor sample with cnLOH and found a clonal missense mutation p.179H>R in exon 5 of *TP53* (Supplementary Fig. S15). Thus, all three cases had biallelic inactivation of *TP53*. The median number of CNA events was markedly higher in these 3 patients than in del(17p) patients overall (24 vs. 11). All 3 patients had a 3p deletion, and one had deletions in all of 3p, 4p, 8p, and 9p. The latter patient also harbored a potential chromoanasynthesis event adjacent to the chromothripsis region (Supplementary Fig. S14B). Chromoanasynthesis is characterized by stretches of euploidy, alternating with segments of higher ploidy, and is not accompanied by LOH events (45). All 3 patients had aggressive disease, with two treated before sampling and the third treated shortly after (6 days).

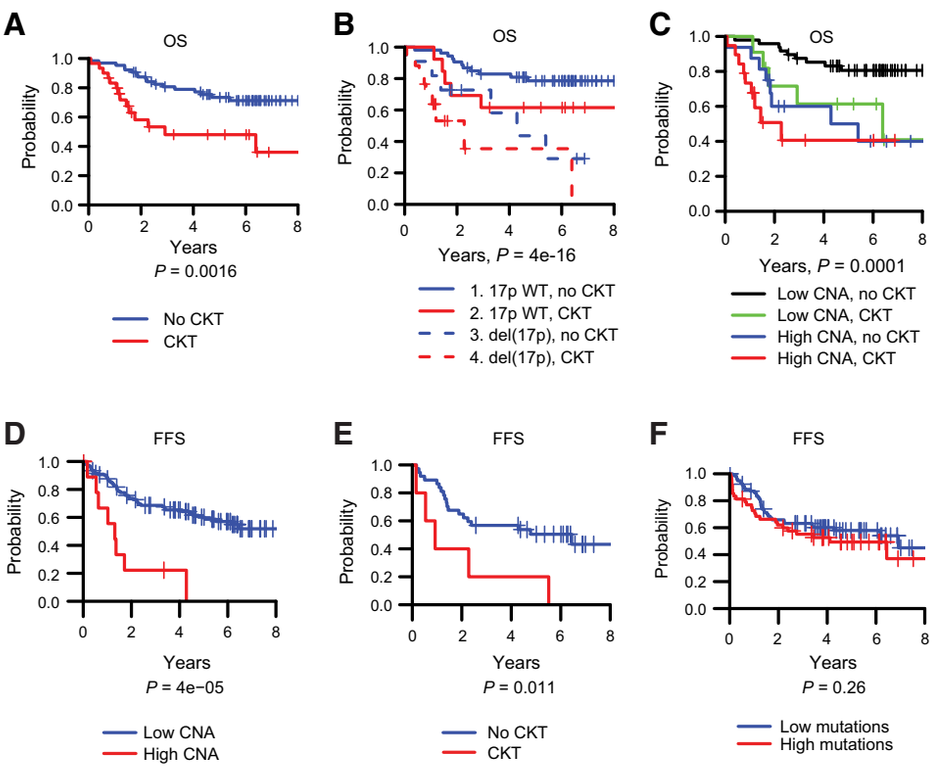

Figure 4.
OS and FFS. **A,** OS by CKT versus no CKT ($n = 94$). **B,** OS by del(17p) and CKT status. **C,** OS by CKT versus CNA numbers. Cutoff for high versus low CNAs is 4. **D–F,** FFS for previously untreated patients ($n = 122$). **D,** FFS by number of CNA events. The cutoff for high versus low is 4 CNA events. **E,** FFS by CKT status. **F,** FFS by number of total mutations, with cutoff for low versus high mutations 21.

CNAs, but not mutations, predict the need for treatment

A central question about the clinical heterogeneity of CLL, and 17p deleted CLL in particular, is what underlies the variable progression to treatment among treatment-naïve patients. For example, among 23 del(17p) patients treatment naïve at sampling in our SNP cohort, 7 (30%) have not yet required treatment, with a median follow-up of 57 months, while 16 (70%) underwent therapy soon after sampling. A visual inspection of the CNAs in the different treatment groups reveals that the never treated groups are largely devoid of large chromosomal deletions or gains with the exception of trisomy 12, as we have previously reported (Supplementary Fig. S2; ref. 22). This finding holds true in del(17p) CLL as well (Supplementary Fig. S2). The never treated del(17p) CLLs ($n = 7$) have a median of 1 CNA per sample, while the del(17p) CLLs treated after sampling have a median of 9 CNAs per sample, similar to the median in those treated prior to sampling (median = 8). *IGHV* mutational status has also been suggested to impact the outcome of del(17p) patients (20, 21), and mutated *IGHV* is overrepresented in our indolent del(17p) patients (57% mutated (4/7) versus 10% in all other del(17p) patients). We further analyzed the association between CNA events and the time from sampling to next treatment or death (FFS). In the entire cohort of previously untreated patients at sampling, excluding 16 patients who were treated within 30 days of sampling ($n = 122$), increasing CNA events are associated with shorter FFS (Fig. 4D). This result remains true in a multivariable model adjusting for del(17p) and *IGHV* mutational status ($P = 0.0002$). Complex karyotype, but not total somatic mutations, is also predictive of shorter FFS (Fig. 4E and F).

Discussion

Our somatic mutation analysis of 53 del(17p) CLLs is the largest cohort of del(17p) CLL to undergo WES to date (23–26, 46) and allowed us to identify increased somatic nonsynonymous mutations and a novel driver gene profile in del(17p) CLL. Clonal mutations are also increased, but this may be related to the older age of del(17p) patients in this cohort (median 65 vs. 60). In addition, we have found that total number of mutations, particularly nonsynonymous mutations, is associated with shorter OS independent of 17p deletion status but has a larger impact on OS in del(17p) CLL than WT. No association was observed between the number of somatic mutations and the number of CNAs, suggesting that the mechanisms by which they arise may be distinct. Somatic mutations are point mutations and small insertions or deletions, which reflect failed base or nucleotide excision repair or aberrant translesion synthesis. CNAs involve losses or gains of long stretches of DNA, which are likely a result of failed repair of double-strand DNA breaks. Our results suggest a greater impact of *TP53* inactivation on CNAs than somatic mutations.

Prior work has demonstrated that somatic point mutations of *TP53*, even in small subclones, associate with poor prognosis in CLL (17, 18). These studies have focused on all CLLs with and without del(17p) and have defined clonal mutations as variant allele frequency (VAF) greater than 12%, which translates to CCF greater than 12% to 24%, depending on the 17p deletion status. Therefore, these studies suggested that very small fractions of *TP53* mutation carry similar risk to *TP53* mutations with higher VAF >12% in all CLL cases but did not address the impact of clonality of somatic *TP53* mutations in the context of del(17p). In fact, Rossi and colleagues in their work showed that subclonal *TP53* mutations with 17p deletion or another clonal *TP53* mutation have shorter OS than subclonal or clonal *TP53* mutations alone, suggesting that *TP53* mutations in the context of 17p deletion represent a worse disease group, consistent with our results (17). As we present here, most *TP53* mutations are present in most cells in del(17p) patients (CCF > 0.85), and only 2 patients in our cohort have a VAF less than 0.24. It is clear from our analysis that, in the context of del(17p), *TP53* mutations are associated with different clinical outcome when the mutations are in most cells (CCF > 0.85) versus fewer cells (CCF $\leq$ 0.85). Our result showing mostly clonal *TP53* mutations with del(17p) likely reflects selection pressure favoring more clonal mutations in the context of del(17p).

We demonstrate a novel and distinct pattern of driver genes among the increased somatic nonsynonymous mutations in del(17p) CLL. The association of *NOTCH1* mutation with del(17p) has been previously reported (47). While we were preparing this article, Ljungström and colleagues reported recurrent *RPS15* mutations in relapsing CLL and the association with del(17p) (39). Here, we also identify *RPS15* mutations enriched in del(17p) CLL, with 12% frequency compared with less than 3% in WT CLL. We found *RPS15* mutations do not carry additional survival risk in del(17p) CLL, which is in agreement with Ljungström and colleagues' result (39). The *RPS15* mutations identified in our del(17p) enriched cohort cluster in a 15 amino acid region in the carboxyl terminus, suggesting potential gain of function activity. Ljungström and colleagues showed that *RPS15* mutations interact with *MDM2* and *MDMX* to cause reduced stability of *TP53*. It is paradoxical to find most *RPS15* mutations in del(17p) patients if the *RPS15* mutants have direct functional interaction with wild-type *TP53*, which is most often absent from del(17p) patients. In fact, we identified a case of *RPS15* mutation with biallelic disruption of *TP53* without any *TP53* protein expression by Western blot analysis. We therefore postulate that *RPS15* mutations may also have a functional consequence on other targets, possibly ribosomal, that cooperate less directly with the *TP53* pathway.

In addition to our mutational analysis, and consistent with our own and others' prior work (20–22, 48), we clearly show that increasing CNAs of any type associate with worse OS, in all CLL and in del(17p) CLL, and that biallelic *TP53* inactivation is associated with increased CNAs compared with monoallelic inactivation, which may be mechanistically related to loss of *TP53* function. We identify a novel association between del(17p) CLL and three high-risk CNAs affecting 3p, 4p, and 9p, the targets of which are as yet unknown but worthy of investigation. We further show that increasing CNAs correlate with complex karyotype, and either one has similar negative impact on OS, even in 17p deleted CLL. Prior studies have had limited ability to separate the effect of complex karyotype from 17p deletion, probably because they so commonly occur together. Our data are particularly interesting in light of recent reports that early relapse on ibrutinib can be predicted by 17p deletion, or by complex karyotype, but with numbers too small to easily segregate their separate effects (7, 49). Further follow-up of the RESONATE (50) study, which has both FISH and karyotype data on a large cohort of patients treated with ibrutinib, may also help elucidate this question.

Finally, our results shed light on why some del(17p)patients may remain untreated for extended periods. Del(17p) patients not treated during the 5-year follow-up of this study mostly have subclonal (4 subclonal and 1 clonal out of 7 cases) or no *TP53* mutation (2/7 cases). Correspondingly, these patients have very few CNAs, consistent with the observation that increasing CNAs occur in the context of greater *TP53* inactivation. Mutated *IGHV* also appeared to be enriched in this group, while the number of somatic mutations was similar to the rest of the cohort. Thus, the primary factor seems to be the very stable genomes of these patients, with few CNAs in the context of and possibly the result of only monoallelic or subclonal inactivation of *TP53*. This information can be clinically useful in our assessment of del (17p) CLL patients.

Strengths of our study include assembling the largest cohort of del(17p) patients analyzed by WES and SNP arrays to date, with comparison to a large control cohort. With this large cohort, we were able to perform multivariable analysis for overall survival. Weaknesses include the heterogeneity of the patient population and genomic sampling times, with not all patients undergoing all evaluations. We presented the analysis by optimal cut-off values for CNA events and number of mutations. The primary goal of exploring threshold values in the figures is to graphically illustrate our findings with interpretable OS curves, by low versus high CNAs or mutations. These cut-off values, however, would need to be validated in external independent studies, particularly if a different platform or analytic pipeline was used. That being said, the log-transformed continuous variable results are also highly significant and thus robust whether dichotomized or not and will be generalizable to del(17p) patients in various stages of disease.

In summary, we present the largest comprehensive genomic analysis of del(17p) CLL to date, showing that clonal and biallelic *TP53* mutation as well as increasing CNAs and increasing somatic mutations are associated with worse OS, although CNAs are a more significant predictor in all CLLs regardless of del(17p). We identify a unique driver gene profile in del(17p) CLL, including *NOTCH1* and *RPS15*, underscoring its unique pathogenesis, which will ultimately need to be understood to lead to effective therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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