

Missense Mutations Located in Structural p53 DNA-Binding Motifs Are Associated With Extremely Poor Survival in Chronic Lymphocytic Leukemia

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ABSTRACT

Purpose

There is a distinct connection between *TP53* defects and poor prognosis in chronic lymphocytic leukemia (CLL). It remains unclear whether patients harboring *TP53* mutations represent a homogenous prognostic group.

Patients and Methods

We evaluated the survival of patients with CLL and p53 defects identified at our institution by p53 yeast functional assay and complementary interphase fluorescence in situ hybridization analysis detecting del(17p) from 2003 to 2010.

Results

A defect of the *TP53* gene was identified in 100 of 550 patients. p53 mutations were strongly associated with the deletion of 17p and the unmutated *IgVH* locus (both $P < .001$). Survival assessed from the time of abnormality detection was significantly reduced in patients with both missense ($P < .001$) and nonmissense p53 mutations ($P = .004$). In addition, patients harboring missense mutation located in p53 DNA-binding motifs (DBMs), structurally well-defined parts of the DNA-binding domain, manifested a clearly shorter median survival (12 months) compared with patients having missense mutations outside DBMs (41 months; $P = .002$) or nonmissense alterations (36 months; $P = .005$). The difference in survival was similar in the analysis limited to patients harboring mutation accompanied by del(17p) and was also confirmed in a subgroup harboring *TP53* defect at diagnosis. The patients with p53 DBMs mutation (at diagnosis) also manifested a short median time to first therapy (TTFT; 1 month).

Conclusion

The substantially worse survival and the short TTFT suggest a strong mutated p53 gain-of-function phenotype in patients with CLL with DBMs mutations. The impact of p53 DBMs mutations on prognosis and response to therapy should be analyzed in investigative clinical trials.

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) is characterized by a distinctively variable clinical course. Two major prognostic factors are mutational status of the immunoglobulin heavy-chain variable region (*IgVH*)^{1,2} and the presence of cytogenetic aberrations.³ A particularly poor prognosis is associated with the presence of del(17p).^{4,5} This deletion is in nearly all cases of progressive leukemia accompanied by *TP53* gene mutation.⁶ It has recently become clear that p53 mutation itself is responsible for an inferior prognosis in CLL, regardless of whether it is accompanied by del(17p).⁶⁻⁹ The proportion of patients with a sole *TP53* mutation [without del(17p)]

may vary from 3% to 4% among cohorts at diagnosis or before first therapy⁸⁻¹⁰ to 12% in fludarabine-refractory CLL.¹¹ Patients with the p53 defect respond poorly to therapy involving DNA-damaging agents (eg, fludarabine, chlorambucil) and have a short response duration to chemioimmunotherapy or alemtuzumab.¹² According to the revised National Cancer Institute (NCI) Working Group/International Workshop on Chronic Lymphocytic Leukemia guidelines for CLL,¹³ patients with del(17p) (p53 defect) should be offered investigative clinical protocols or should be appointed for allogeneic stem-cell transplantation. In this respect, it is critical to know whether all p53 mutations in CLL lead to a similar phenotype and prognosis.

Prognostic stratification based on the type of mutation and its position in p53 protein was demonstrated as valuable in several cancers. For example, mutations at the residues, which are closely involved in the p53 binding to DNA, have been associated with a particularly severe phenotype in, for example, breast tumors¹⁴ or diffuse large B-cell lymphoma.¹⁵

In collaboration with other groups, we recently reported that a majority of p53 mutations in CLL represent missense substitutions,¹⁶ which occur in the DNA-binding domain (DBD) of p53 protein.¹⁷ It is important to note that, in addition to a simple loss of protein function encoded from the affected allele, p53 missense mutations may result in a gain-of-function (GOF) phenotype reflecting a highly oncogenic activity of the altered protein.¹⁸ A pivotal mechanism of the mutated p53 GOF seems to be an interference with the p53-related proteins (ie, p63 and p73).¹⁹ Alternatively, or in parallel, some p53 mutants have been shown to upregulate genes that support cancer progression (eg, nuclear factor- κ B)²⁰ or aggravate effective therapy (eg, multidrug resistance 1 gene [*MDR1*]).²¹ *MDR1*, which codes for P-glycoprotein, is involved in a transport of certain drugs used in CLL therapy (eg, doxorubicin, vincristine).²²

With this report, we show that patients with CLL harboring *TP53* mutation constitute two readily distinct prognostic subgroups. Thus, missense substitutions located in structural p53 DNA-binding motifs (DBMs) can be identified with clearly reduced survival rates compared with other p53 mutations. Our study clinically demonstrates the mutated p53 GOF phenotype.

PATIENTS AND METHODS

Patients

The analyzed cohort consisted of 550 patients with CLL monitored and/or treated at the Department of Internal Medicine–Hematology, University Hospital Brno (Brno, Czech Republic), between the years 2003 and 2010. CLL was diagnosed, and the patients were treated according to the 1996 NCI-sponsored CLL Working Group guidelines²³ or updated 2008 NCI Working Group/International Workshop on Chronic Lymphocytic Leukemia guidelines.¹³ All blood samples were processed with written informed consent, and the study was approved by the Ethical Commission of the University Hospital Brno.

Our patient cohort is biased toward more severe CLL as evidenced by 65% of patients harboring the unmutated *IgVH* locus and almost one third of patients (30%) having been treated before the first *TP53* investigation. This bias emerged from a local concentration of patients with inferior CLL at the University Hospital Brno; noncomplicated patients are monitored at regional hematologic centers elsewhere in the Czech Republic.

Analysis of *TP53* Mutations and Deletions

p53 mutations were identified by a yeast functional analysis (FASAY),²⁴ and cytogenetic deletions of the *TP53* (17p13.1) locus were detected by routine interphase fluorescence in situ hybridization analysis using a probe from Vysis–Abbott (Chicago, IL). We have previously described the experimental conditions for both methodologies.⁶ In addition to this earlier study, we performed a direct sequencing of genomic DNA (whole coding region, exons 2 to 11) in patients with del(17p) and wild-type p53 output by FASAY ($n = 6$). A mutation was identified in four patients (2-nt deletion in codon 209, $n = 2$; 1-nt insertion in codon 215, $n = 1$; and nonsense mutation in codon 317, $n = 1$). These mutations were not identified by FASAY, most likely because of the nonsense-mediated mRNA decay of corresponding molecules. In patients in whom the FASAY exceeded 50% of red colonies and we detected only one mutation and no del(17p), uniparental disomy (UPD) presence was considered.¹¹ These patients ($n = 5$) were analyzed using Affymetrix Cytogenetic

2.7M Array (Affymetrix, Santa Clara, CA) to confirm or exclude the UPD (Appendix Table A1, online only).

Classification of *TP53* Defects and Mutations

Monoallelic defects were classified as either sole mutation or sole deletion. Biallelic defects were classified as deletion accompanied by mutation of the other allele, two or more mutations, or a mutation accompanied by loss of heterozygosity through the UPD. Nonmissense mutations were defined as any mutation other than missense (eg, nonsense mutation, in-frame or frameshift deletion, insertion or mutation leading to aberrant splicing). Missense mutations involved in the direct contact of p53 with DNA were adopted from the database of the International Agency for Research on Cancer²⁵ (in our study, codons 239, 241, 248, 273, 275, 277, and 280). Missense mutations in the structural p53 DBMs are mutations localized in the L2 and L3 loops involved in interaction with DNA in the minor groove (codons 164 to 194 and 237 to 250, respectively) and mutations localized in the loop-sheet-helix motif involved in interaction with DNA in the major groove (codons 119 to 135 and 272 to 287).¹⁷ The DBMs are a part of the DBD (codons 102 to 292).¹⁷

Statistical Evaluation and Survival Analysis

The χ^2 test or Fisher's exact test were used to assess the association between *TP53* mutations and categorical variables. The unpaired t test was used to compare the age in individual groups. We previously reported⁶ that survival is reduced dramatically in patients with novel p53 defects. Therefore, this survival analysis was performed from the time of p53 mutation detection/investigation showing wild-type p53, unless stated differently. For survival evaluation, only patients with one discrete mutation were considered to assess an impact of particular mutation. Survival analysis and the time to first treatment (TTFT) analysis were done using the Kaplan–Meier survival estimator. Median survival, median TTFT, and differences between the curves were evaluated by the log-rank test using the GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). Hazard ratios were determined by the univariate Cox proportional hazards model using MedCalc (MedCalc Software, Mariakerke, Belgium).

RESULTS

p53 Defects in Patients With CLL

An abnormality of the *TP53* gene was identified in 100 of the 550 analyzed patients. Clinical and biologic characteristics of the patients are listed in Table 1. Types of p53 defects are listed in Table 2; and all mutations, detected in 96 patients, are listed in Appendix Table A1. A higher proportion of affected patients (18%) than is usually reported (10% to 15%) is in line with the unfavorable structure of our cohort (see Patients and Methods) and also reflects a repeated investigation in a proportion of patients ($n = 195$). This analysis disclosed 19 novel p53 defects, in all instances after previous therapy (97 of 195 patients were treated; median time to repeated investigation was 18 months). We previously discussed this negative impact.⁶

Missense substitutions accounted for 78% of mutations and were all located in the p53 DBD, specifically between amino acids 109 and 286. Thirteen of the 16 nonmissense mutations also directly affected the DBD, but the remaining three were located as far as in the C-terminal part of the protein. The p53 mutations were strongly associated with the unmutated *IgVH* locus and presence of del(17p) (both $P < .001$).

Survival in Relation to p53 Mutations

Two previous studies^{26,27} have shown that survival of patients with CLL harboring *TP53* abnormalities is greatly influenced by the mutational status of the *IgVH* gene. Therefore, we first determined the impact of the *IgVH* status on the survival rate of p53-affected patients.

Table 1. Demographics and Clinical and Biologic Characteristics of Patients With CLL

Demographic or Characteristic	No <i>TP53</i> Mutation				<i>TP53</i> Mutation				<i>P</i> *
	Mutated <i>IgVH</i> (n = 160)		Unmutated <i>IgVH</i> (n = 241)		Mutated <i>IgVH</i> (n = 11)		Unmutated <i>IgVH</i> (n = 82)		
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	
Median age at diagnosis, years	59.6		61.7		55.2		59.0		.1889
Sex									
Male	108	67.5	161	66.8	3	27.3	56	68.3	.8919
Female	52	32.5	80	33.2	8	72.7	26	31.7	
Stage (at the time of <i>TP53</i> examination)	158		237		9		79		
Low risk, Rai 0	73	46.2	62	26.2	2	22.2	8	10.1	
Intermediate risk, Rai I/II	60	38.0	93	39.2	2	22.2	27	34.2	
High risk, Rai III/IV	25	15.8	82	34.6	5	55.6	44	55.7	< .001
Hierarchical cytogenetics (I-FISH)	159		240		10		81		
17p–	0	0	3	1.3	7	70.0	52	64.2	< .001
11q–	4	2.5	86	35.8	1	10.0	12	14.8	< .001
Trisomy 12	16	10.1	33	13.8	1	10.0	1	1.2	< .001
13q– sole	91	57.2	54	22.5	1	10.0	11	13.6	.0822
Normal	48	30.2	64	26.7	0	0	5	6.2	< .001

NOTE. Four patients with p53 defect and 52 patients with wild-type p53 had unknown status of *IgVH*.

Abbreviations: CLL, chronic lymphocytic leukemia; I-FISH, interphase fluorescence in situ hybridization.

*The statistical evaluation concerns a comparison of the following groups: no *TP53* mutation with the unmutated *IgVH* versus *TP53* mutation with the unmutated *IgVH*.

The data are presented in Figure 1. The patients who had p53 mutation but also the mutated *IgVH* gene (range of homology, 92.4% to 97.9%) had substantially better survival than p53-mutated patients with unmutated *IgVH* (homology \geq 98%; $P = .018$). Therefore, we omitted the small subgroup (n = 11) of p53-affected patients with mutated *IgVH* gene from the subsequent analysis because their survival data would be misleading. Thus, only the wild-type p53 patients harboring the unmutated *IgVH* gene were used as a control group in

all subsequent survival evaluations. In this respect, p53 mutations were clearly associated with a higher risk Rai stage and presence of del(17p). However, patients with p53 mutation less frequently exhibited del(11q), trisomy 12, and normal karyotype (Table 1).

Overall, p53 mutations were associated with an obviously reduced survival compared with wild-type p53 patients (median survival, 23 v 69 months, respectively; $P < .001$; data not shown). The analysis structured according to the type of p53 mutation showed that

Table 2. Summary of *TP53* Defects Identified in 550 Patients With CLL

Type of Defect	No. of Patients	Comment
Patients with defective p53	100	
p53 defect/mutated <i>IgVH</i>	11	Not analyzed further in this study*
p53 defect/ <i>IgVH</i> not analyzed	4	Not analyzed further in this study
p53 defect/unmutated <i>IgVH</i>	85†	
Monoallelic alteration	22	
Missense mutation	17	
Nonmissense mutation	2	
Del(17p)	3	
Biallelic alteration	62	
Del(17p)/missense mutation	33	
Del(17p)/nonmissense mutation	13	
Missense mutation and UPD	4	
\geq 2 mutations‡	12	Not analyzed further in this study§

Abbreviations: CLL, chronic lymphocytic leukemia; UPD, uniparental disomy.

*Figure 1 shows a substantially better survival ($P = .018$) of these patients compared with patients with p53 mutation and unmutated *IgVH*.

†One patient with unavailable fluorescence in situ hybridization result (not analyzed further).

‡Six patients also harbored del(17p).

§It is impossible to assign the patients to individual mutation categories.

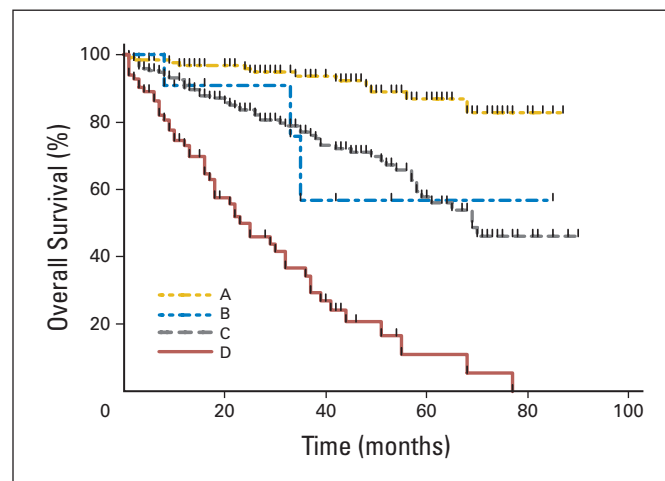


Fig 1. Survival of patients with p53 defect (mutation and/or 17p–) from the time of abnormality detection (or investigation showing wild-type p53) in relation to the *IgVH* mutational status: curve A, wild-type p53 and mutated *IgVH* (n = 131; median survival, not reached); curve B, p53 defect and mutated *IgVH* (n = 11; median survival, not reached); curve C, wild-type p53 and unmutated *IgVH* (n = 193; median survival, 69 months; 71 of 193 patients manifested a high-risk deletion of *ATM* [11q–]); and curve D, p53 defect and unmutated *IgVH* (n = 81; median survival, 23 months). Curve A v curve B, $P = .016$; curve A v curve C, $P < .001$; curve B v curve D, $P = .018$; curve C v curve D, $P < .001$.

both missense substitutions and nonmissense alterations substantially reduced the survival compared with wild-type p53 patients ($P < .001$ and $P = .004$, respectively), but mutations did not differ significantly from each other ($P = .17$; data not shown).

An interaction with the target DNA is crucial to the activity of the p53 protein. Therefore, we focused on the structurally well-defined DBMs of p53 (see Patients and Methods), which ensure a proper contact with the DNA. Figure 2A shows the survival analysis irrespective of the del(17p) presence. Missense mutations in DBMs ($n = 32$) led to a clearly shorter survival (12 months) compared with both remaining missense mutations ($n = 21$; 41 months; $P = .002$) and nonmissense alterations ($n = 15$; 36 months; $P = .005$). There was no difference in the survival of patients affected in the L2 or L3 loops compared with patients with mutation in the loop-sheet-helix motif (median survival, 13 v 10 months, respectively; data not shown). This suggests that patients with DBMs mutation form one uniform group in terms of survival. The analysis limited to patients with p53 mutation

and the accompanying del(17p) ($n = 50$) once again provided similar results (ie, a markedly reduced survival among patients with DBMs mutations; Fig 2B). In parallel, the analysis of a subgroup harboring a sole p53 mutation and intact remaining *TP53* allele (without 17p-) also noted a reduced survival rate for patients harboring DBMs mutation ($n = 8$; 9 months). Mutations outside DBMs, consisting of eight missense substitutions and two nonmissense alterations, resulted in a median survival time of 41 months (data not shown). However, this difference was not significant ($P = .36$), most likely because of the small number of patients studied.

We then specifically limited our subsequent analysis to mutations located at residues, which are in direct contact with DNA (see Patients and Methods). These patients ($n = 13$) also had a short median survival time (9 months; data not shown). This further supports the view that a modulation of p53-DNA interaction is critical in CLL.

A subset of p53 mutations in our study ($n = 28$) was already identified during diagnosis. Therefore, we verified the survival of this limited subgroup, divided again according to mutation presence in versus out of DBMs. In this analysis (Fig 3), the wild-type p53 subgroup showed a substantially longer survival (110 months) compared with the survival that had been observed from the time of p53 investigation (69 months). In contrast, patients with the p53 mutation in DBMs ($n = 12$) showed a short median survival of only 17 months ($P < .001$; hazard ratio compared with wild-type p53 patients, 20.8; 95% CI, 8.82 to 48.82), which was similar to the survival time measured from the time of abnormality detection (12 months). Mutations out of DBMs consisting of remaining missense substitutions ($n = 9$) and nonmissense alterations ($n = 7$) resulted in a median survival time of 51 months ($P < .001$; hazard ratio compared with wild-type p53 patients, 5.3; 95% CI, 2.41 to 11.69). Patients with mutations in versus out of the DBMs again differed significantly from each other in terms of survival ($P = .004$). The p53 mutations in DBMs compared with remaining p53 mutations were significantly associated with male sex ($P = .029$), whereas there was no correlation with age, Rai stage, or the hierarchical cytogenetics.³

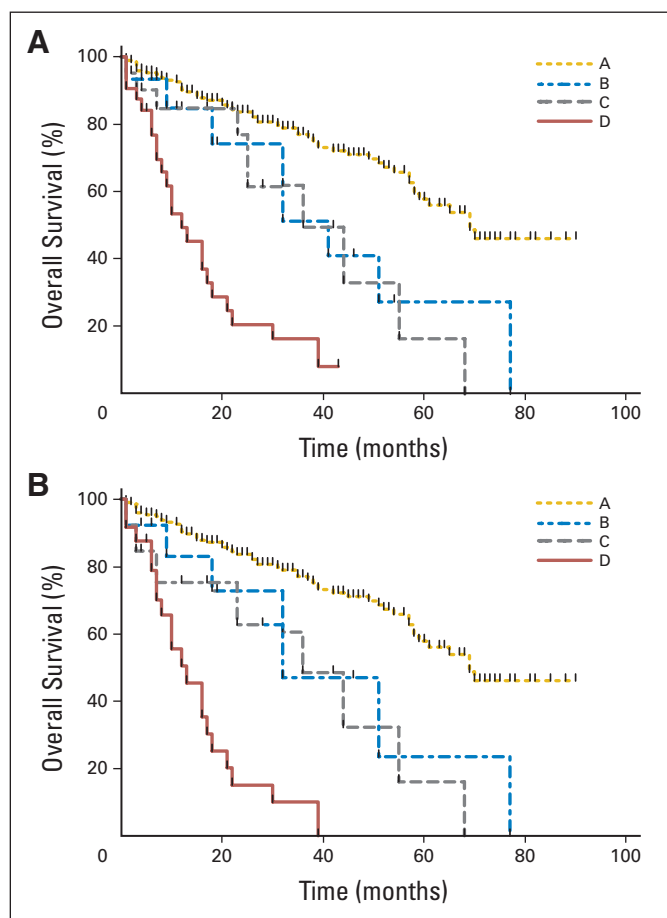


Fig 2. Survival of patients with p53 mutations in the DNA-binding motifs (DBMs) from the time of abnormality detection. (A) Analysis irrespective of del(17p) presence: curve A, wild-type p53 and unmutated *IgVH* (same as curve C in Fig 1); curve B, nonmissense p53 mutation ($n = 15$; median survival, 36 months); curve C, missense mutation outside DBMs ($n = 21$; median survival, 41 months); and curve D, missense mutation in DBMs ($n = 32$; median survival, 12 months). Curve D v curve C, $P = .002$; curve D v curve B, $P = .005$. (B) Only patients with accompanying del(17p): curve A, wild-type p53 and unmutated *IgVH* (same as curve C in Fig 1); curve B, nonmissense p53 mutation ($n = 13$; median survival, 36 months); curve C, missense mutation outside DBMs ($n = 13$; median survival, 32 months); and curve D, missense mutation in DBMs ($n = 24$; median survival, 13 months). Curve D v curve C, $P = .009$; curve D v curve B, $P = .002$.

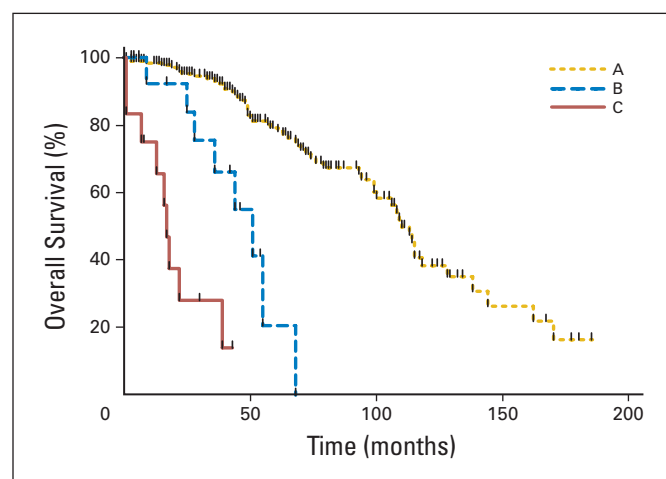


Fig 3. Survival of patients with p53 mutation in the DNA-binding motifs (DBMs) detected at diagnosis: curve A, wild-type p53 and unmutated *IgVH* ($n = 193$; median survival, 110 months); curve B, mutation outside DBMs ($n = 16$; median survival, 55 months); and curve C, missense mutation in DBMs ($n = 12$; median survival, 17 months). Curve A v curve B, $P < .001$; curve A v curve C, $P < .001$; curve B v curve C, $P = .004$.

TTFT

Patients with the p53 mutation identified at diagnosis and all wild-type p53 patients were also analyzed for TTFT. Median TTFT was only 1 month for patients with p53 DBMs mutation ($n = 12$) and 6 months for patients with p53 mutation outside DBMs ($n = 16$; $P = .042$). Both groups differed significantly from wild-type p53 patients with the unmutated *IgVH* ($n = 192$, including 70 patients harboring the high-risk 11q-), with a median TTFT of 19 months ($P < .001$ and $P = .024$, respectively). Within the 6-month period from diagnosis, significantly more patients with p53 DBMs mutations (11 of 12 patients) required therapy compared with the remaining p53-affected patients (eight of 16 patients; $P = .039$). This further confirms a more severe disease course associated with a p53 missense mutation in DBMs.

DISCUSSION

The adverse prognostic impact of a p53 defect mirrored by the presence of del(17p) is unquestionable in CLL.^{3-5,13} It is becoming clearer that a majority of affected patients harbor a mutation on the other *TP53* allele, and a subset of patients harbor a sole *TP53* mutation.⁶⁻¹¹ The p53 mutation has quite recently been shown to confer resistance to fludarabine-based therapeutic regimens in CLL.¹⁰ p53 status is and will continue to be one of the most carefully examined factors in CLL clinical trials investigating conventional or experimental therapy.²⁸ In this sense, it is worth knowing whether one can expect similar biologic behavior of different p53 mutations and, hence, a similar prognostic consequence with studied patients.

In our report, we show that both missense and nonmissense p53 mutations reduce the survival rate of patients with CLL. In addition, we show that patients harboring missense mutations in structural p53 DBMs constitute a readily distinctive prognostic subgroup with a prominently reduced survival rate and extremely short TTFT. Although all mutations in our study led to a basic loss of p53 transactivation activity, because they would not otherwise be detected by FASAY, the DBMs mutations clearly behave differently than remaining p53 alterations in CLL. The most probable explanation for this observation is the mutated p53 GOF effect.¹⁹ The GOF stems from a basic loss of p53 transactivation activity²⁹ and, therefore, should not be biased in the cohort screened by the FASAY, which is based on the detection of transactivation failure of mutants.

Although the mutated p53 GOF has not yet been tested directly in CLL cells, this effect can be anticipated. For example, two mutants detected in our study, R175H and R273H, have been recently shown to upregulate the mitogen-activated protein kinase kinase 3 (MAP2K3) through the involvement of the transcriptional cofactors NF-Y and NF- κ B,³⁰ and these proteins are known to support the survival of CLL cells on bone marrow stromal cells.³¹ Another described mechanism of the GOF effect predicts an interference with p53 homologues.¹⁹ It is important to study this potential interference in CLL cells because there are innovative studies focusing on activation of p73 in patients with a p53 defect.³²⁻³⁴ In this respect, it is advisable to compare a response of patients with absent p53 (eg, 17p- and frameshift mutation) versus patients with p53 missense mutation in DBMs and del(17p).

Our pivotal observation has been confirmed not only in sets of all identified mutations, but also in subgroups limited to patients with the accompanying del(17p). This analysis is crucial for the proof of clinically observed mutated p53 GOF because this aspect should be rigorously studied in the absence of wild-type p53.¹⁹ The setting is unique in our CLL study because the status of other alleles was not considered in reports concerning other tumors.^{14,15,35} Most importantly, we confirmed the negative prognostic role of DBMs mutations compared with other p53 alterations in patients investigated for p53 mutations at diagnosis. This confirms that the observation is not influenced by previous therapy and is not a result of bias imposed at the time of examination in samples analyzed during a disease course.

Remarkably, five of the six recently identified CLL-specific p53 mutation hot spots are located in the DBMs (codons 175, 179, 248, 273, and 281; the remaining hot-spot codon out of DBMs is 220).¹⁶ The survival of a subgroup of patients with CLL-specific p53 hot-spot mutations ($n = 11$) was only 10 months in our study (data not shown). Altogether, this indicates that DBMs mutations are preferentially selected in patients with CLL and supports the view that the alterations in p53 binding to DNA might be critical with CLL progression. Interestingly, our data are markedly similar to those obtained recently with diffuse large B-cell lymphoma, in which p53 DNA-binding mutations were the strongest predictor of poor survival.¹⁵ This observation indicates that the driving forces of p53 mutation selection might be similar in these closely related cancers.

A potential limitation with our study results from the unpredictable survival impact of diverse therapy (chemotherapy, chemoimmunotherapy, alemtuzumab, rituximab with glucocorticoids, and allogeneic stem-cell transplantation) given to patients with p53 defects. We cannot fully exclude that diverse therapy had an impact on some presented results. However, our data show how a strong variability in the p53 function is found in CLL cells, when differences between the mutant subgroups are clearly visible even in the heterogeneously treated cohort.

In summary, patients with p53 DBMs mutations seem to be the most critical subgroup of CLL. p53 is a transcription factor, in which subtle mutations lead to a markedly altered gene expression.³⁶ Hence, we propose that a genome-wide expression analysis might disclose whether there are any common coding genes or microRNAs³⁷ with altered expression in patients with p53 DBMs mutations. This analysis could indicate which processes might account for the hypothesized mutated p53 GOF anticipated in these patients. When prioritizing for allogeneic stem-cell transplantation,¹³ patients with p53 DBMs mutations should be considered primary candidates, because their long-term survival is otherwise improbable. In conclusion, the effect of p53 DBMs mutations on survival and therapeutic response should be analyzed in ongoing or planned clinical trials to confirm or exclude their more aggressive nature under particular clinical settings.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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Manuscript writing: All authors

Final approval of manuscript: All authors

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Appendix

Table A1. All TP53 Mutations Detected in 550 Patients With CLL

Patient No.	Codon	WT Codon	Mut Codon	WT aa	Mut aa	Mutation Type	Missense Mutation in DBMs (1 p53 mut/unmut <i>IgVH</i>)	17p– by FISH	<i>IgVH</i> Status
P1	109	TTC	TCC	Phe	Ser	Missense		Yes	Mut
P2	110	CGT	CCT	Arg	Pro	Missense	No	Yes	Unmut
P3	113	del 3 nt		Phe		Deletion in frame		No	Unmut
P4	120	AAG	ATG	Lys	Met	Missense		Yes	Unmut
P5	126	del 36 nt		Tyr		Deletion in frame		Yes	Unmut
P6	132	AAG	AGG	Lys	Arg	Missense	Yes	Yes	Unmut
P7	132	AAG	AAC	Lys	Asn	Missense		Yes	ND
P8	132	AAG	AGG	Lys	Arg	Missense		No	unmut
P9	134	TTT	ATT	Phe	Ile	Missense		No	Unmut
P10	134	TTT	TTG	Phe	Leu	Missense	Yes	No; UPD	Unmut
P11	135	TGC	GGC	Cys	Gly	Missense	Yes	Yes	Unmut
P12	138	GCC	CCC	Ala	Pro	Missense	No	No	Unmut
P13	143	GTG	ATG	Val	Met	Missense	No	No	Unmut
P14	155	ACC	ATC	Thr	Ile	Missense		No	Unmut
P15	157	GTC	TTC	Val	Phe	Missense		Yes	Unmut
P16	157	GTC	GGC	Val	Gly	Missense	No	Yes	ND
P9-M2	172	del 39 nt		Val		Deletion in frame			
P17	173	GTG	ATG	Val	Met	Missense	Yes	Yes	Unmut
P18	175	CGC	CAC	Arg	His	Missense	Yes	ND	Unmut
P19	175	CGC	CAC	Arg	His	Missense		No	Unmut
P20	175	CGC	CAC	Arg	His	Missense	Yes	Yes	Unmut
P21	176	TGC	TGG	Cys	Trp	Missense		No	Mut
P22	176	TGC	GGC	Cys	Gly	Missense		Yes	Mut
P23	178	CAC	CCC	His	Pro	Missense	Yes	Yes	Unmut
P24	179	CAT	CGT	His	Arg	Missense	Yes	Yes	Unmut
P25	181	CGC	TGC	Arg	Cys	Missense	Yes	Yes	Unmut
P26	184	ins 2 nt		Asp	Stop 247	Insertion frameshift		No; UPD	Unmut
P27	194	CTT	CGT	Leu	Arg	Missense	Yes	No	Unmut
P8-M2	194	del 14 nt		Leu	Stop 203	Deletion frameshift			
P28	195	ATC	ACC	Ile	Thr	Missense		Yes	Mut
P29	196	CGA	TGA	Arg	Stop	Nonsense		Yes	Mut
P30	196	CGA	GGA	Arg	Gly	Missense	No	No	Unmut
P31	205	TAT	CAT	Tyr	His	Missense	No	Yes	Unmut
P32	205	TAT	TGT	Tyr	Cys	Missense	No	Yes	Unmut
P33	209	del 2 nt		Arg	Stop 214	Deletion frameshift		Yes	Mut
P4-M2	209	del 2 nt		Arg	Stop 214	Deletion frameshift			
P34	211	ACT	ATT	Thr	Ile	Missense	No	Yes	Unmut
P35	213	CGA	TGA	Arg	Stop	Nonsense		Yes	Unmut
P36	215	AGT	AGA	Ser	Arg	Missense	No	Yes	Unmut
P37	215	ins 1 nt		Ser	Stop 221	Insertion frameshift	No	Yes	Unmut
P38	216	GTG	ATG	Val	Met	Missense	No	No	Unmut
P39	216	GTG	ATG	Val	Met	Missense	No	Yes	Unmut
P40	220	TAT	TCT	Tyr	Ser	Missense	No	Yes	Unmut
P41	220	TAT	TGT	Tyr	Cys	Missense		No	Mut
P42	220	TAT	TGT	Tyr	Cys	Missense	No	Yes	Unmut
P43	220	TAT	TGT	Tyr	Cys	Missense	No	Yes	Unmut
P44	226	del 2 nt		Gly	Stop 227	Deletion frameshift		Yes	Unmut
P45	234	TAC	AAC	Tyr	Asn	Missense	No	No	Unmut
P46	234	TAC	TGC	Tyr	Cys	Missense	No	No	Unmut
P47	234	TAC	TGC	Tyr	Cys	Missense	No	Yes	Unmut
P48	234	TAC	TGC	Tyr	Cys	Missense	No	No	Unmut
P49	236	TAC	TGC	Tyr	Cys	Missense	No	Yes	Unmut

(continued on following page)

Table A1. All *TP53* Mutations Detected in 550 Patients With CLL (continued)

Patient No.	Codon	WT Codon	Mut Codon	WT aa	Mut aa	Mutation Type	Missense Mutation in DBMs (1 p53 mut/unmut <i>IgVH</i>)	17p— by FISH	<i>IgVH</i> Status
P50	236	TAC	TGC	Tyr	Cys	Missense	No	Yes	Unmut
P51	236	TAC	GAC	Tyr	Asp	Missense	No	No	Unmut
P52	237	ATG	ATA	Met	Ile	Missense	Yes	No	Unmut
P53	239	AAC	GAC	Asn	Asp	Missense	Yes	No	Unmut
P54	241	TCC	TAC	Ser	Tyr	Missense	Yes	Yes	Unmut
P55	244	GGC	GAC	Gly	Asp	Missense	Yes	Yes	Unmut
P56	246	ATG	GTG	Met	Val	Missense	Yes	No	Unmut
P57	248	CGG	CAG	Arg	Gln	Missense	Yes	Yes	Unmut
P58	248	CGG	CAG	Arg	Gln	Missense	Yes	Yes	Unmut
P59	248	CGG	TGG	Arg	Trp	Missense	Yes	Yes	Unmut
P60	248	CGG	CAG	Arg	Gln	Missense	Yes	Yes	Unmut
P61	248	CGG	CAG	Arg	Gln	Missense	Yes	Yes	Unmut
P62	249	AGG	ACG	Arg	Thr	Missense	Yes	No	Unmut
P63	249	AGG	GGG	Arg	Gly	Missense	Yes	No; UPD	Unmut
P64	249	del 3 nt		Arg		Deletion in frame		No	Unmut
P65	249	AGG	GGG	Arg	Gly	Missense	Yes	Yes	Unmut
P66	252	del 3 nt		Leu		Deletion in frame		Yes	Unmut
P67	252	del 9 nt		Ile		Deletion in frame		Yes	Unmut
P68	255	ATC	TTC	Ile	Phe	Missense	No	No; UPD	Unmut
P15-M2	256	ACA	CCA	Thr	Pro	Missense			
P19-M2	266	GGA	GTA	Gly	Val	Missense			
P69	272	GTG	ATG	Val	Met	Missense	Yes	Yes	Unmut
P35-M2	273	CGT	CTT	Arg	Leu	Missense			
P70	273	CGT	CAT	Arg	His	Missense	Yes	Yes	Unmut
P71	273	CGT	CAT	Arg	His	Missense		No	Unmut
P72	275	TGT	TAT	Cys	Tyr	Missense	Yes	No	Unmut
P73	275	TGT	TAT	Cys	Tyr	Missense	Yes	No	Unmut
P74	275	TGT	TTT	Cys	Phe	Missense	Yes	Yes	Unmut
P29-M2	277	TGT	TTT	Cys	Phe	Missense			
P71-M2	277	TGT	TTT	Cys	Phe	Missense			
P75	277	TGT	TTT	Cys	Phe	Missense	Yes	No	Unmut
P76	277	TGT	TTT	Cys	Phe	Missense	Yes	Yes	Unmut
P14-M2	278	CCT	TCT	Pro	Ser	Missense			
P77	278	CCT	CGT	Pro	Arg	Missense	Yes	Yes	Unmut
P78	278	CCT	TCT	Pro	Ser	Missense	Yes	Yes	Unmut
P79	278	del 3 nt		Pro		Deletion in frame		Yes	Unmut
P80	280	AGA	GGA	Arg	Gly	Missense	Yes	No	Unmut
P26-M2	281	GAC	AAC	Asp	Asn	Missense			
P81	281	GAC	GAG	Asp	Glu	Missense		Yes	Mut
P82	282	CGG	CCG	Arg	Pro	Missense	Yes	Yes	Unmut
P83	286	GAA	GTA	Glu	Val	Missense		Yes	ND
P84	289	del 2 nt		Leu	Stop 304	Deletion frameshift		Yes	Unmut
P85	294	del 1 nt		Glu	Stop 344	Deletion frameshift		Yes	Unmut
P86	314	del 14 nt		Ser	Stop 331	Deletion frameshift		Yes	Unmut
P87	317	CAG	TAG	Gln	Stop	Nonsense		Yes	Unmut
P88	346	ins 1 nt		Glu	Stop 346	Insertion frameshift		Yes	Unmut
P89	ASHM							No	Mut
P90	ASHM							No	Mut
P91	ASHM							ND	Mut
P92	ASHM							Yes	Unmut
P93	Multiple							Yes	Unmut
P94	Multiple							Yes	Unmut
P95	Intron 5	G-A in splice site			Stop 190	Splice		Yes	Unmut
P96	Intron 5	del 22 nt			Stop 190	Splice		Yes	Unmut

Abbreviations: aa, amino acid; ASHM, aberrant somatic hypermutations in the *TP53* gene; CLL, chronic lymphocytic leukemia; DBM, DNA-binding motif; FISH, fluorescence in situ hybridization; mut, mutated; ND, not determined; unmut, unmutated; UPD, uniparental disomy; WT, wild type.