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TP53 Mutations are Infrequent in Newly-Diagnosed Chronic Lymphocytic Leukemia

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Running title: TP53 mutations in newly-diagnosed CLL.

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Abstract

TP53 mutations in the absence of 17p-deletion correlate with rapid disease progression and poor survival in chronic lymphocytic leukemia (CLL). Herein, we determined the *TP53* mutation frequency in 268 newly-diagnosed CLL patients from a population-based material. Overall, we detected *TP53* mutations in 3.7% CLL patients, where 7/10 cases showed a concomitant 17p-deletion, confirming the high prevalence of *TP53* mutation in 17p-deleted patients. However, only 3 (1.1%) patients from our newly-diagnosed CLL cohort carried *TP53* mutations without 17p-deletion, a frequency that is much lower than previous reports on referral cohorts (3-6%). Our findings imply that *TP53* mutations are rare at CLL onset and instead arise during disease progression.

Introduction

Among chronic lymphocytic leukemia (CLL) patients, a deletion of chromosome 17p13, which harbors the tumor suppressor gene *TP53*, has been repeatedly shown to be associated with the worst prognosis due to advanced disease at diagnosis, early need for treatment, and resistance to chemotherapy [1]. The 17p-deletion is detected in 5–7% of CLL patients in early stage disease, and is more common among patients carrying other poor-prognostic factors, such as unmutated immunoglobulin heavy variable (IGHV) genes, high ZAP70, and CD38 expression [1]. Identification of patients with 17p-deletions is thus of clinical relevance and can support the allocation of these CLL patients to alternative treatment regimes, such as those including alemtuzumab and prednisolone.

More than 70% of CLL patients with 17p-deletion harbor *TP53* mutations on the remaining allele [2]. However, recent studies have reported that 3–5% of CLL patients carry *TP53* mutations without 17p-deletion [2-5] and an even higher incidence was observed in patients (up to 18%) with fludarabine-refractory CLL [6]. These studies have revealed a strong correlation between *TP53* mutations, in the absence of 17p deletion, with rapid disease progression, chemorefractoriness and complex karyotype [3,4], and also demonstrated *TP53* mutation status as an independent predictor of poor survival in CLL [2].

In the present study, we aimed to analyze for the presence of *TP53* mutations in 268 newly-diagnosed CLL patients from a population-based cohort of Scandinavian patients, as compared to previous studies on cohorts from referral centers.

Materials and methods

Patients

A total of 268 patients below 75 years of age from the Swedish part of a population-based case-control study called SCALE (Scandinavian Lymphoma Etiology) were included in the present study. All samples displayed the typical CLL immunophenotype (CD5+/CD19+/CD23+) and met the recently revised diagnostic criteria presented by the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) [7]. Survival data was available on all patients, with a median follow-up time of 90 months (quartile range, 3-109 months) for surviving patients, while data on time to treatment was available for 233 patients. Informed consent was obtained according to the Declaration of Helsinki and the study was approved by the local ethical review committee.

Assessment of IGHV gene mutational status

The IGHV gene mutational status was assessed by PCR amplification with IGHV subgroup specific primers, followed by sequence analysis. Sequences were submitted to IMGT/V-QUEST to determine the IGHV germline identity. The IGHV gene was considered mutated when the identity to the corresponding germline gene was less than 98%.

Analysis of recurrent genomic aberrations

All 268 CLL samples were analyzed using high-resolution Affymetrix 250K SNP-arrays from which data on known recurrent genomic aberrations (e.g. 11q-, +12, 13q- and 17p-) were extracted [8].

***TP53* mutation analysis**

The PCR products of exons 4 to 8 of the *TP53* gene were purified and sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and an ABI 3730 automatic sequencer, as previously described [9]. ContigExpress (Vector NTI Advance version 10.3.1, Invitrogen), BioEdit Sequence Alignment Editor Version 7.0.5.3 and the GenBank data library (release 174.0) were used to analyze and align sequences. Mutations were validated using the IARC *TP53* Mutation Database.

Statistical analysis

Two-tailed P values were generated for two contingency tables, using Fisher's exact test, and for larger contingency tables, using Chi-square analysis. All tests were two-sided and the significance level was set to 0.05. Overall survival and time to treatment were estimated using the Kaplan-Meier method. Differences in median overall survival and time to treatment between groups were evaluated using the log-rank test. Overall survival was measured from the date of diagnosis to either the last follow-up date (defined as censored) or death. Time to treatment was evaluated by the time interval from the diagnostic date until date of initial treatment. Statistica 9.0 software (Statsoft, Tulsa, OK, USA) was employed for all calculations.

Results and Discussion

This study was based on a series of 268 CLL patients (176 men and 92 women) from a population-based Scandinavian CLL cohort on newly diagnosed patients with a median age at diagnosis of 64 years (range, 38-75 years). In total 173 of 259 (66.8%) patients harbored mutated IGHV genes and 192 of 248 (77.4%) had Binet stage A disease. The majority showed 13q-deletions (47%) or no recurrent aberration (29.5%), whereas 17p-deletion was detected in 10 (3.7%) cases (Table 3). Thus, our cohort was comprised of mainly 'low-risk' patients according to stage and mutation status, reflecting the population-based cohort, and fewer had 17p-deletion than in other studies [2-5].

Overall, we detected *TP53* mutations in 10 of 268 (3.7%) CLL patients, with 7 of these patients (2.6%) harboring 17p deletions on the remaining allele (Table 2). Among the three patients with only *TP53* mutations, all displayed mutated IGHV genes, two displayed 13q-deletions and one displayed trisomy 12 (Table 3). In addition, there were 3 patients with 17p deletions but no *TP53* mutation; all of them were diagnosed in Binet stage A, whereas most patients with *TP53* mutations were diagnosed in stage B (Table 3).

Hence, a lower frequency of *TP53* mutations in the absence of 17p-deletions (1.1%) was observed in our cohort compared to other studies in CLL, which have shown frequencies ranging from 3%–6% of cases [2-5] (Table 1). The lower overall incidence of *TP53* mutations in this study may reflect the inherent differences between this newly-diagnosed population-based CLL cohort, compared to patient materials collected via referral centers, as in most previous studies of *TP53* mutation in CLL [2-5]. These studies tend to have a selection bias towards more aggressive cohorts, whereas in contrast, our population-based cohort is comprised of more indolent cases, and therefore gives a more representative prevalence of *TP53* mutation in CLL at diagnosis.

The prognostic impact of 17p-deletion and *TP53* mutation, as well as *TP53* mutation in the absence of 17p-deletion has been documented in several recent studies [2-5]. We confirmed a significant decrease in the overall survival ($P<0.0001$; Figure 1A) and time to treatment ($P=0.01$; Figure 1B) in patients with *TP53* mutations and 17p deletions, compared to patients without any mutation or deletion. Due to the low number of patients with *TP53* mutation in the absence of 17p deletion in this material we could not perform a meaningful survival analysis. However, among the three patients with only *TP53* mutations, one was treated at 5 months and died 66 months after diagnosis, whereas the remaining two patients were alive after 118 and 121 months; one was treated after 24 months, whereas the other remained untreated. As mentioned, all of these patients showed mutated IGHV genes. Among

the three patients with 17p-deletions but without *TP53* mutations, one was treated almost immediately (5 days after diagnosis) and died 55 months later, and one patient received treatment 4 months and died 4 years after diagnosis. The final patient remained alive and untreated 9 years post-diagnosis. This particular patient had a mutated IGHV gene rearrangement.

Our findings support recent data by Best *et al.* [10], who have documented a subset of CLL patients with 17p-deletion and mutated IGHV genes having a stable disease for several years without requiring therapy. Thus, patients with 17p/*TP53* abnormalities do not necessarily result in an aggressive disease course and poor outcome in CLL.

In conclusion, we have confirmed the high prevalence of *TP53* mutations in 17p-deleted patients but observed a lower incidence of *TP53* mutations without 17p deletion in our population-based study compared to previous reports [2-5]. Our finding further supports that *TP53* mutations are gained during disease progression rather than at disease onset.

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Table 1. Comparison of the present study with earlier CLL studies.

Patients characteristics	**Present study**	**Zenz *et al.* (2008)**	**Dicker *et al.* (2009)**	**Rossi *et al.* (2009)**	**Malcikova *et al.* (2009)**
Binet stage A (%)	192/248 (77.4)	59/123 (48.0)	107/142 (75.4)	231/308 (75.0)	[a]NA
Mutated IGHV gene (%)	173/259 (66.8)	40/113 (35.4)	99/174 (56.9)	188/303 (62.0)	135/355 (38.0)
TP53 mutation (%)	10/268 (3.7)	18/126 (14.3)	26/193 (13.5)	31/308 (10.0)	67/400 (16.8)
TP53 mutation and 17p deletion (%)	7/268 (2.6)	13/126 (10.3)	17/193 (8.8)	18/297 (6.1)	42/400 (10.5)
17p deletion without *TP53* mutation (%)	3/268 (1.1)	3/126 (2.4)	1/193 (0.5)	16/297 (5.4)	3/400 (0.8)
TP53 mutation without 17p deletion (%)	3/268 (1.1)	5/126 (4.1)	9/193 (4.7)	10/297 (3.4)	25/400 (6.3)

[a]Only Rai stage reported.

Table 2. *TP53* mutation profile in CLL.

Patient	**17p deletion status**	**Exon**	**Codon position**	**Nucleotide change**	**Amino acid change**	**Mutation type**
1	del(17p)	6	193	CAT - CTT	His-Leu	Missense
2	del(17p)	4	100	CAG - TAG	Gln-stop codon	Nonsense
3	del(17p)	7	250	CCC - CTC	Pro-Leu	Missense
4	del(17p)	5	165	CAG - TAG	Gln-stop codon	Nonsense
5	del(17p)	6	208	GAC - GTC	Asp-Val	Missense
6	del(17p)	7	249	AGG - ATG	Arg-Met	Missense
7	del(17p)	6	193	CAT - CGT	His-Arg	Missense
8	No del	5	175	CGC - CAC	Arg-His	Missense
9	No del	5	181	CGC - CAC	Arg-His	Missense
10	No del	6	193	CAT - CGT	His-Arg	Missense

Table 3. Characteristics of CLL patients according to *TP53* mutation status and/or 17p deletion.

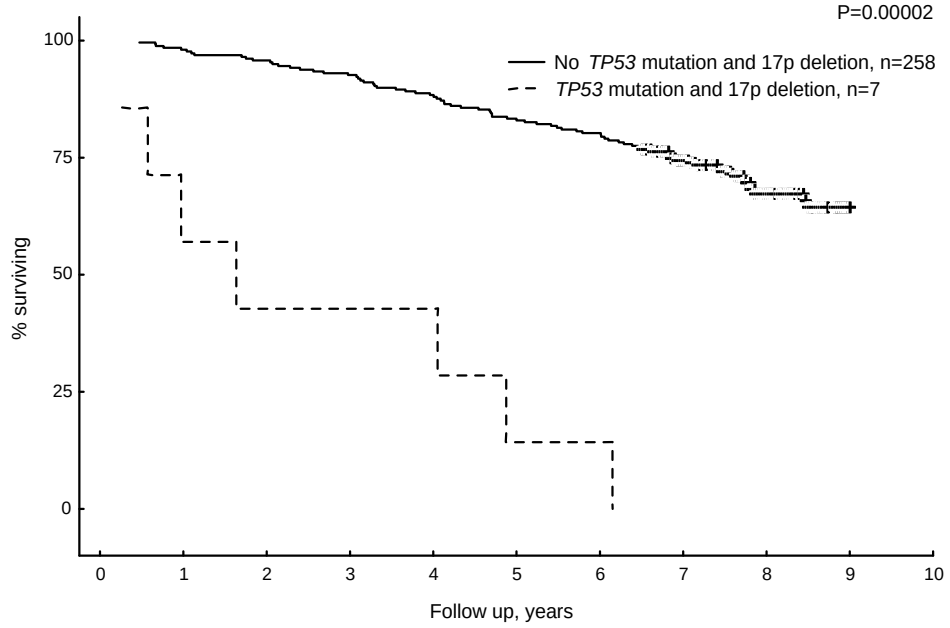
´Patients characteristics	All	No *TP53* mutation and/or 17p deletion	17p deletion only	*TP53* mutation with 17p deletion	*TP53* mutation only
Number of patients (%)	268	255 (95.1)	3	7	3
Median age at diagnosis, years (range)	64 (38-75)	64 (38-75)	58 (51-71)	65 (57-75)	62 (54-64)
Male/female (%)	176/92 (65.6/34.4)	169/86 (66.3/33.7)	3/0	2/5	2/1
Binet stage (%)					
A	192/248 (77.4)	186/235 (79.2)	3	2	1
B	43 (17.3)	36 (15.3)	0	5	2
C	13 (5.2)	13 (5.5)	0	0	0
IGHV mutation status (%)					
Mutated IGHV genes	173 (66.8)	167 (68.2)	1	1	3
Unmutated IGHV genes	86 (33.2)	78 (31.7)	2	6	0
Genomic aberrations (%)					
No aberration	79 (29.5)	79 (31.0)	0	0	0
13q deletion	126 (47.0)	124 (48.6)	0	0	2
11q deletion	30 (11.2)	30 (11.8)	0	0	0
17p deletion	10 (3.7)	0 (0)	3	7	0
Trisomy 12	23 (8.6)	22 (8.6)	0	0	1

FIGURE LEGENDS

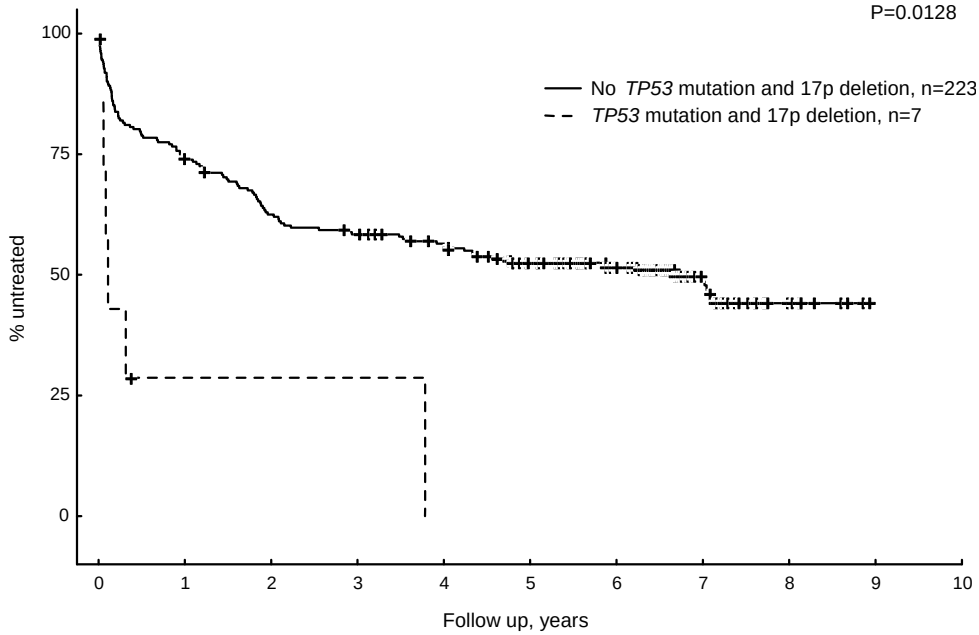
Figure 1. *TP53* mutations in relation to survival and time to treatment in CLL. Kaplan-Meier curves of (A) overall survival in 268 CLL patients and (B) time to treatment in 233 CLL patients. The presence of *TP53* mutations and 17p deletions was associated with poor survival (n=7; P=0.00002) and shorter time to treatment (n=7; P=0.0128). The cases with *TP53* mutation in the absence of 17p-deletion (n=3) and cases with 17p-deletion without *TP53* mutation (n=3) were not included.

Figure 1.

A


P=0.00002
No *TP53* mutation and 17p deletion, n=258
TP53 mutation and 17p deletion, n=7
% surviving
100
75
50
25
0
0 1 2 3 4 5 6 7 8 9 10
Follow up, years

B


P=0.0128
No *TP53* mutation and 17p deletion, n=223
TP53 mutation and 17p deletion, n=7
% untreated
100
75
50
25
0
0 1 2 3 4 5 6 7 8 9 10
Follow up, years