



## Introduction

Deletion of the 17p13 locus, which harbors the tumour suppressor gene *TP53*, has long been known to define the group of chronic lymphocytic leukemia (CLL) patients with the poorest clinical outcome. Furthermore, over 70% of CLL patients with 17p-deletion harbor *TP53* mutations on the remaining allele<sup>1,2</sup>. However, recent studies report that 3–5% of CLL patients may carry *TP53* mutations without 17p-deletion<sup>3-6</sup> and that *TP53* mutation status can predict poor survival in CLL.

## Aim

Since most earlier studies reporting *TP53* mutation frequencies in CLL focused on cohorts collected via referral centres, we aim to investigate the frequency of *TP53* mutation within a Scandinavian population-based cohort, in order to gain a more representative prevalence of *TP53* mutation in CLL at diagnosis.

## Results 1

**Table 1. Patient characteristics of the current cohort**

Patient Characteristics		
	No.	%
Median age at diagnosis	64 years (range 38-75 yrs)	
<b>Gender</b>		
Male	176	66
Female	92	34
<b>Binet Stage</b>		
Stage A	192/248	78
Stage B	43/248	17
Stage C	13/248	5
<b>IGHV mutation status</b>		
IGHV mutated	173/259	67
IGHV unmutated	86/259	33
<b>Genomic aberrations</b>		
None	79/268	30
del(13q)	126/268	47
del(11q)	30/268	11
del(17p)	10/268	4
Trisomy 12	23/268	9

Sixty-seven percent of patients harbored mutated IGHV genes and 77% had Binet Stage A disease. The majority of patients (77%) showed 13q-deletions or no recurrent aberration. Del(17p) was detected at a low frequency in 10 (3.7%) cases. Thus, our cohort was comprised of mainly 'low-risk' patients according to stage and mutation status, reflecting the population-based cohort.

The *TP53* gene was analysed for the presence of mutations in 268 newly diagnosed CLL patients by direct sequencing of exons 4-8. The results are presented in Table 2.

**Table 2. Missense and nonsense mutations in TP53**

Pt	17p deletion status	Exon	Codon	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

- Ten cases carried missense or nonsense mutations in the *TP53* gene; 10/268 = **3.7%**
- Seven of the 10 cases carrying *TP53* mutations also carried del(17p)
- Only 3 cases carried a mutation in the absence of del(17p) 3/268 = **1.1%**

## References

1. Dohner H, et al. Blood 1995;85 (6):1580-9.
2. Krober A, et al. Blood 2002;100 (4):1410-6.
3. Dicker F, et al. Leukemia 2009;23 (1):117-2
4. Malcikova J et al, Blood 2009;114(26):5307-14
5. Rossi D, et al. Clin Cancer Res 2009;15 (3):995-1004.
6. Zenz T, et al. Blood 2008;112 (8):3322-9.

## Summary:

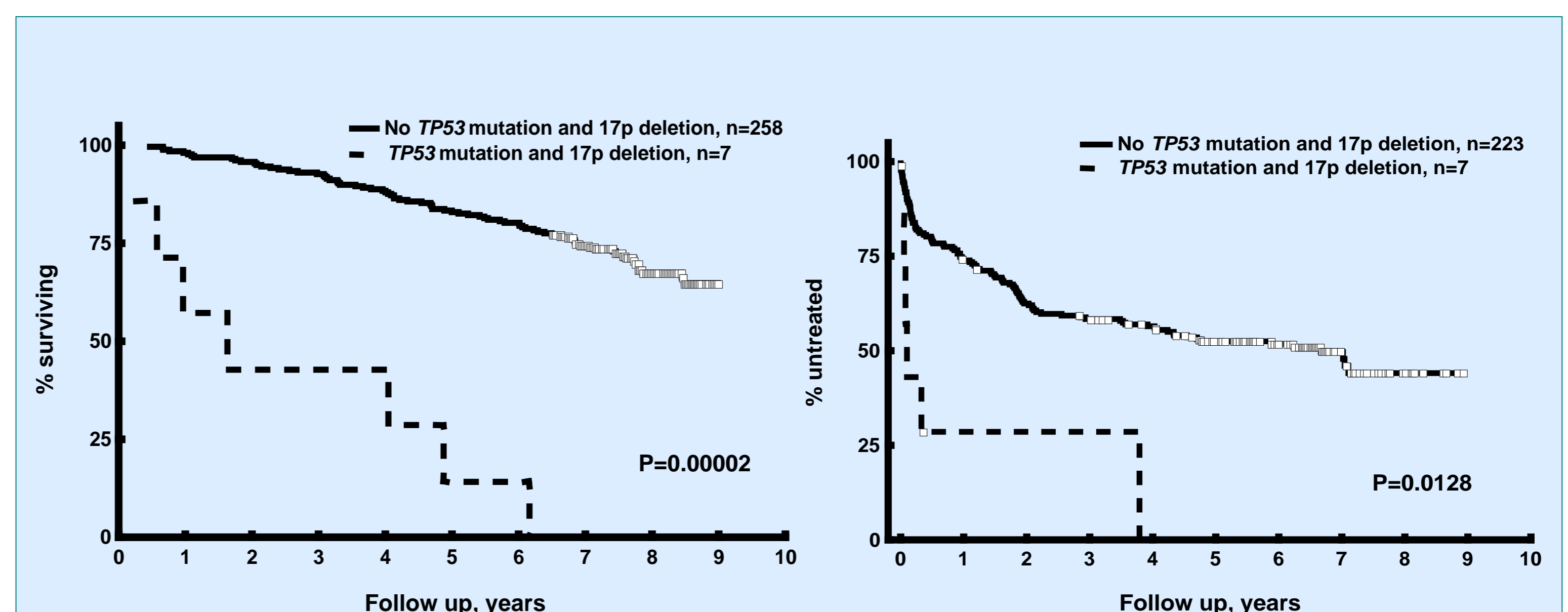
- We observed a high prevalence (70%) of *TP53* mutations in 17p-deleted patients
- However, the incidence of *TP53* mutations in the absence of 17p deletion was notably lower in our population-based study (1.1%) compared to studies based on hospital collected cohorts.
- Our finding implies that *TP53* mutations are gained during disease progression rather than at disease onset.

## Results 2

**Table 3. Patients with TP53 mutation with an intact 17p**

Patient	Binet	FISH code	Mutation Status	Still alive (Feb 2010)	Survival (Years)	Treatment initiated?
Pt1	B	del(13q)	Mutated	No	5,5	5 months
Pt2	B	Trisomy 12	Mutated	Yes	9	24 months
Pt3	B	del(13q)	Mutated	Yes	10	Not treated

Of the three patients carrying *TP53* mutations only, 2 were still alive more than 9 years after diagnosis and one remained untreated. Notably all 3 patients carried IGHV mutated genes.



We observed a significant decrease in the **overall survival and time to treatment** of patients with *TP53* mutations and 17p deletion (p=0.00002 and p=0.0128 respectively).

## Patient cohort

All samples were sourced from the Scandinavian Lymphoma Etiology (SCALE) project, a population-based case-control study of newly diagnosed lymphoma patients and controls. Samples were obtained from 650 CLL patients located throughout Denmark and Sweden. Comprehensive clinical data was also collected for all patients. Subsequently, 370 cases were run on Affymetrix (250K), SNP array gaining information on recurrent genomic aberrations.

## Methods

- The *TP53* exons 4-8 were amplified in 268 patients. Three different primer sets were used to cover all 5 exons. All amplicons were then direct sequenced.
- Vector NTI Advance (version 10.3.1), BioEdit Sequence Alignment Editor (Version 7.0.5.3) the GenBank data library were used to analyse and align sequences.
- All mutations identified were reconfirmed by sequencing of a second PCR product.
- Overall survival and time to treatment were estimated by the Kaplan-Meier method using Statistica 9.0 software.