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NOTCH1 and *SF3B1* mutations can be added to the hierarchical prognostic classification in chronic lymphocytic leukemia

Leukemia (2013) **27,** 512–514; doi:10.1038/leu.2012.307

During recent years, a variety of novel molecular markers have been proposed as prognostic factors in chronic lymphocytic leukemia (CLL), which has significantly improved the subdivision of the disease. One of the strongest markers is fluorescence *in situ* hybridization detection of certain genomic aberrations, that is, del(11q), trisomy 12, del(13q) and del(17p), which is now included in routine diagnostics to guide decisions about therapy.[1] More specifically, CLL patients with the 13q deletion (as a single aberration) have a more indolent disease course, whereas patients with 11q or, in particular, 17p deletions experience a more rapid disease progression with need of early treatment, and a generally poor overall survival.[1,2] Notably, CLL patients with 17p deletions and/or *TP53* mutations belong to the category of patients with the worst outcome in CLL, as they generally do not respond to the current state-of-the-art treatment with fludarabine, cyclophosphamide and rituximab.[1,3]

More recently, the advent of next-generation sequencing has revealed a number of novel genes to be frequently mutated in CLL, such as *NOTCH1*, *SF3B1*, *BIRC3* and *MYD88*.[4–10] In the pivotal studies, *NOTCH1* mutations were identified in up to 12% of patients, and the mutations were strongly associated with progressive disease, treatment resistance, increased risk

Table 1a. Overall survival and time to treatment in a population-based Scandinavian cohort of CLL patients according to NOTCH1/SF3B1 mutations and established prognostic markers

Variable	*Overall survival*			*Time to treatment*		
	N	*Median*	P-*value*	N	*Median*	P-*value*
Binet stage	310		<0.0001*	289		<0.0001*
A	238	N.R.		221	98.4	
B	56	81.6		53	2.6	
C	16	86.4		15	0.6	
IGHV *mutational status*	329		<0.0001	272		<0.0001
Mutated	219	N.R.		183	118.8	
Unmutated	110	76.8		89	10.2	
Chromosomal aberration	356		<0.0001*	293		<0.0001*
del(13q)	170	N.R.		142	109.2	
No aberrations	106	N.R.		82	NR	
Trisomy 12	31	93.6		25	22.8	
del(11q)	36	92.4		32	6.0	
del(17p)	13	21.6		12	1.0	
NOTCH1	359		<0.0001	296		0.001
Mutated	17	66.0		16	4.8	
Wild type	342	N.R.		280	58.8	
SF3B1	359		0.0001	296		0.004
Mutated	13	63.6		11	2.4	
Wild type	346	N.R.		285	56.4	

Abbreviations: CLL, chronic lymphocytic leukemia; SF3B1, splicing factor 3B subunit 1; N.R., not reached. **P*-value represents a combined *P*-value for the analysis and indicates that at least one group differs significantly from the rest. Median value is given in months.

Accepted article preview online 6 November 2012; advance online publication, 9 November 2012

Table 1b. Multivariate Cox regression analysis of *NOTCH1/SF3B1* mutations and recurrent chromosomal aberrations in CLL

Variable[a]	*Overall survival* (n = *356*)			*Time to treatment* (n = *293*)		
	HR	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*
Trisomy 12	1.84	1.11–3.03	0.017	2.63	1.68–4.13	<0.0001
del(11q)	1.89	1.16–3.07	0.011	3.37	2.21–5.15	<0.0001
del17p)/*TP53* mutation	2.66	1.48–4.80	0.001	2.45	1.35–4.45	0.003
NOTCH1 mutation	4.22	2.41–7.42	<0.0001	2.73	1.53–4.85	<0.001
SF3B1 mutation	3.47	1.86–6.48	<0.0001	3.18	1.65–6.14	<0.001

Abbreviations: CLL, chronic lymphocytic leukemia; SF3B1, splicing factor 3B subunit 1; HR, hazard ratio; CI, confidence interval. [a]Only recurrent genomic aberrations and *NOTCH1/SF3B1* mutations were included in the model. For recurrent genomic aberrations, HR is given in comparison with cases with no detected aberrations/del(13q).

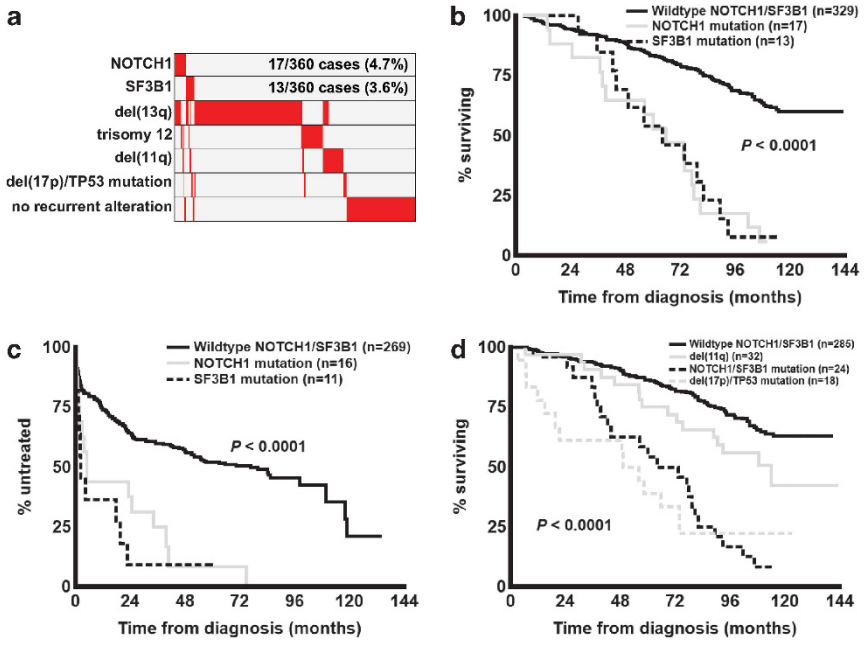

Figure 1. Relationship between *NOTCH1* and *SF3B1* mutations and other genomic aberrations (**a**). Prognostic impact of *NOTCH1* and *SF3B1* mutations on overall survival and time to treatment (**b** and **c**) and in relation to 11q and 17p deletions (**d**). In the latter analysis, six CLL cases with concurrent del(17p) and *NOTCH1* mutation were included in the 'del(17p)/*TP53*-mutated' subgroup, whereas the three cases that carried del(11q) and *SF3B1* mutations were grouped as '*SF3B1*-mutated'.

for Richter transformation and poor overall survival.[4,5,10] Furthermore, it has also been observed that trisomy 12 patients have a high frequency of *NOTCH1* mutations (up to 50%), and that CLL patients with concurrent trisomy 12 and *NOTCH1* alterations display a particularly poor prognosis.[11–13] Interestingly, the majority of cases with the *NOTCH1* lesions show a recurrent dinucleotide hotspot deletion within the *NOTCH1* PEST domain within exon 34. From *in vitro* studies, it appears that the mutated protein leads to upregulation of the NOTCH1 signaling pathway, resulting in an increased CLL cell survival and resistance to apoptosis.[5]

Using exome sequencing, a high frequency of mutations within the splicing factor 3B subunit 1 (*SF3B1*) gene was also reported in CLL, observed in up to 15% of patients in the initial studies.[6–8] These *SF3B1* mutations may potentially lead to a defective spliceosome complex and impaired splicing activity, although the precise role of such mutations in CLL pathogenesis remains to be further elucidated.[7] Similar to *TP53* and *NOTCH1* mutations, *SF3B1* mutations appear to be an independent prognostic marker, associated with very rapid disease progression and inferior survival.[6,8]

Most of the recent studies on *NOTCH1* and *SF3B1* mutations have been carried out on selected patient groups, which may influence the frequencies reported for these mutations as we recently observed for *TP53* mutations.[14] We here aimed to assess the mutation status of *NOTCH1* and *SF3B1*, by sequence analysis of exons with known hotspot regions (*NOTCH1*, exon 34; *SF3B1*, exon 14–16), in a well-characterized population-based CLL material from Scandinavia. As this material has already been analyzed for many of the established and novel prognostic markers (Table 1a), we also aimed to investigate the potential additive value of *NOTCH1* and *SF3B1* mutations in multivariable analysis.

This study encompassed 360 CLL patients diagnosed in Sweden and Denmark during a definite time period (1999–2002), who were included in the Scandinavian population-based case–control study called SCALE (Scandinavian Lymphoma Etiology).[15] All CLL samples were diagnosed according to established criteria, showing a typical CLL immunophenotype. The mean age at

diagnosis was 64 years (range, 38–75 years) and, as expected, there was a male to female predominance of 2:1. Survival data were available for 359 patients, with a median follow-up time of 98 months, whereas data on time to treatment were available for 296 patients. Molecular and clinical data for all patients are summarized in Table 1a. Informed consent was collected according to the Helsinki Declaration, and ethical approval was granted by local ethical review committees.

PCR amplification of exon 34 of the *NOTCH1* gene was performed, followed by Sanger sequencing to detect the hotspot 2-bp deletion. Similarly, PCR amplification and sequencing of exons 14–16 of the *SF3B1* gene was carried out (protocols and primers available on demand). Overall survival was measured from the date of diagnosis until last follow-up or death. Time to treatment was evaluated from the diagnostic date until date of initial treatment. Survival curves were constructed with the Kaplan–Meyer method, and the log-rank test was used to determine differences between survival proportions. The Cox proportional hazards model was applied to assess the prognostic strength of each prognostic marker included. All statistical analyses were performed by using Statistica Software 10.0 (Stat Soft Inc., Tulsa, OK, USA).

Out of 360 investigated cases in this population-based cohort of newly diagnosed CLL, 17 patients displayed a *NOTCH1* mutation (4.7%), whereas 13 patients had an *SF3B1* mutation (3.6%). These mutations were mutually exclusive (Figure 1a). Notably, these are considerably lower frequencies than in previous reports,[5,7,8,10] which most probably reflects the unselected nature of this cohort. Hence, in parallel to our recent data on *TP53* mutations in early CLL,[14] the frequencies of these mutations are indeed lower than at later stages of the disease. In contrast to previous observations,[11–13] we did not find an association between trisomy 12 and *NOTCH1* mutations, as only 2 out of 31 trisomy 12 patients carried a *NOTCH1* mutation. That notwithstanding and in concordance with previous studies, presence of *NOTCH1* or *SF3B1* mutations was strongly associated with poor outcome, both in terms of shorter time to treatment and decreased overall survival (Figure 1b c). In addition, *NOTCH1* or *SF3B1* mutations had a similarly poor impact on prognosis as *TP53* aberrations (deletions/mutations; Figure 1d).

To test the additive value of *NOTCH1* and *SF3B1* mutations in this unselected cohort of CLL patients, we performed a multivariate analysis including genomic aberrations according to the hierarchical classification by Döhner *et al.*,[1] and *NOTCH1* and *SF3B1* mutations. As can be seen in Table 1b, 11q deletion, trisomy 12, *TP53* aberrations, *NOTCH1* and *SF3B1* mutations all remained as strong, independent prognostic markers in multivariate analysis for overall survival, as well as for time to treatment. In conclusion, the legitimacy of *NOTCH1* and *SF3B1* as poor prognostic markers has grown rapidly and we here propose to include *NOTCH1* and *SF3B1* mutations in the hierarchical classification of genetic aberrations, where these mutations will be added to the high-risk group of patients with *TP53* aberrations. Obviously, this proposal has to be tested in other larger, independent CLL cohorts as well as in prospective trials, before it can be transferred into routine clinical practice.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This research was supported by the Nordic Cancer Union, the Swedish Cancer Society, the Swedish Research Council and the Lion's Cancer Research Foundation, Uppsala.

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