UGT2B17 expression: a novel prognostic marker within IGHV-mutated chronic lymphocytic leukemia?

Sujata Bhoi,[1] Panagiotis Baliakas,[1] Diego Cortese,[1] Mattias Mattsson,[1,2] Marie Engvall,[1] Karin E. Smedby,[2] Gunnar Juliusson,[4] Lesley-Ann Sutton,[1] and Larry Mansouri[1]

[1]Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University; [2]Department of Medical Sciences, Uppsala University; [3]Department of Medicine Solna, Clinical Epidemiology Unit, Karolinska Institutet, Stockholm; and [4]Department of Laboratory Medicine, Stem Cell Center, Hematology and Transplantation, Lund University, Sweden

Correspondence: larry.mansouri@igp.uu.se
doi:10.3324/haematol.2015.136440

UGT2B17 expression in chronic lymphocytic leukemia: a novel prognostic marker within IGHV mutated CLL?

Supplemental Methods

Patients

Two-hundred-and-fifty-three CLL patients from the Swedish part of the Scandinavian population-based case control study, known as Scandinavian Lymphoma Etiology (SCALE) were included in this study.[1] All samples were taken before the administration of any treatment. Median time between diagnosis and sample collection was 3 months, while median age at diagnosis was 64 years (range, 30-75). Follow-up samples (n=91) were collected with a median time of 6.7 years from diagnosis (range, 5.0-8.1 years). All cases were diagnosed and classified according to recently revised criteria displaying typical CLL immunophenotype and >70% tumour cells.[2] Informed consent was obtained according to the Helsinki declaration and the study was approved by the local ethics review committee.

UGT2B17 expression analysis

RNA was extracted from peripheral blood mononuclear cells (PBMCs) using the RNA easy Mini kit (Qiagen, Hilden, Germany) and RNA quality was assessed using the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and cDNA was synthesized from 400 ng of total RNA using the M-MLV reverse transcriptase Kit (Invitrogen, Carlsbad, CA) and random hexamers (Thermo Scientific, Pittsburgh, PA). The expression level of *UGT2B17* was quantified by real-time quantitative polymerase chain reaction (RQ-PCR) analysis using SYBR Green master mix (Thermo Scientific) and using primers for UGT2B17 that have previously been described. Reactions were run on a Stratagene MX 3005p instrument (Agilent Technologies, Santa Clara, CA) and the data obtained was normalised against beta-actin using the comparative delta delta Ct method.

Assessment of biological markers

IGHV subgroup-specific polymerase chain reaction (PCR) and sequence analysis was performed on genomic DNA as previously described,[3, 4] while standard procedure was also followed for immunophenotyping of CD38 expression. The expression of *LPL* was analyzed by RQ-PCR. High-resolution genomic screening was performed using Affymetrix 250K SNP-arrays to detect recurrent genomic aberrations [del(13q), trisomy 12, del(11q) and del(17p)] and mutational screening was performed for the following genes: *NOTCH1*: targeted analysis for del7544-45/p.P2514Rfs*4; *TP53*: exons 4-8; *SF3B1*: exons 14-16; all analystical methods have been described previously.[5]

Statistical analysis

Receiver operating characteristics (ROC) curve analysis using median survival for the studied cohort as the classification variable was used to determine the expression threshold value for *UGT2B17* expression (0.0317 normalized units) and patients were classified as low or high expressing cases. $\chi 2$ test was used to investigate the association between *UGT2B17* expression and other prognostic markers. Kaplan-Meier survival analysis was performed to construct survival curves for overall survival (OS) and time to first treatment (TTFT). OS was calculated from time of diagnosis until date of death or last follow-up. TTFT was calculated from date of diagnosis until the starting date of initial treatment. Log-rank test was used to evaluate differences between subgroups. Multivariate cox-regression analysis was utilized to compare the prognostic value of *UGT2B17* expression in relation to other prognostic markers. Differences in expression levels between diagnosis and follow up were assessed using the Paired t-test. Correlation between expression levels in diagnostic vs follow up samples was assessed by Spearman's rank correlation coefficient. All statistical analyses were carried out using Statistica Software (StatSoft, Tulsa, OK).

Reference:

1. Smedby KE, Hjalgrim H, Melbye M, et al. Ultraviolet radiation exposure and risk of malignant lymphomas. J Natl Cancer Inst. 2005;97(3):199-209.
2. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 2008;111(12):5446-5456.
3. Agathangelidis A, Darzentas N, Hadzidimitriou A, et al. Stereotyped B-cell receptors in one-third of chronic lymphocytic leukemia: a molecular classification with implications for targeted therapies. Blood. 2012;119(19):4467-4475.
4. Murray F, Darzentas N, Hadzidimitriou A, et al. Stereotyped patterns of somatic hypermutation in subsets of patients with chronic lymphocytic leukemia: implications for the role of antigen selection in leukemogenesis. Blood. 2008;111(3):1524-1533.
5. Cortese D, Sutton LA, Cahill N, et al. On the way towards a 'CLL prognostic index': focus on TP53, BIRC3, SF3B1, NOTCH1 and MYD88 in a population-based cohort. Leukemia. 2014;28(3):710-713.

Supplemental Data

Supplemental Table 1A. Univariate analysis for overall survival.

Variable	N (events)	Median (months)	p-value
Binet stage	236		p<0.0001
A	183	165	
B/C	53	74	
IGHV	251		p<0.0001
Mutated	149	182	
Unmutated	102	77	
Unfavourable genetics	237		p<0.0001
Positive	33	57	
Negative	204	143	
del(11q)	245		p<0.01
Positive	30	91	
Negative	215	138	
LPL expression	226		p<0.0001
High expression	100	84	
Low expression	126	182	
UGT2B17 expression	253		
High expression	117	85	p<0.0001
Low expression	136	182	

The genomic lesions which have been associated with unfavorable clinical outcome [TP53 abnormalities (del(17p) and/or *TP53* mutations), *NOTCH1* and *SF3B1* mutations] were grouped together (unfavorable genetics) due to their low frequency in the present series which mostly consists of early stage cases.

Supplemental Table 1B. Univariate analysis for time to first treatment.

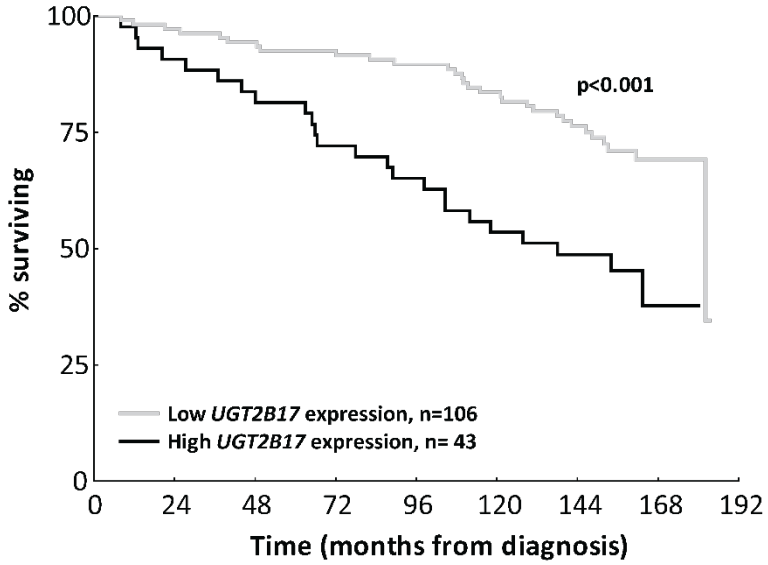
Variable	N (events)	Median (months)	p-value
Binet stage	214		p<0.0001
A	168	115	
B/C	46	2	
IGHV	215		p<0.0001
Mutated	135	183	
Unmutated	80	14	
Unfavourable genetics	202		p<0.001
Positive	30	3	
Negative	172	85	
del(11q)	209		p<0.0001
Positive	27	6	
Negative	182	84	
LPL expression	203		p<0.0001
High expression	90	18	
Low expression	113	183	
UGT2B17 expression	217		
High expression	98	20	p<0.0001
Low expression	119	183	

The genomic lesions which have been associated with unfavorable clinical outcome [TP53 abnormalities (del(17p) and/or *TP53* mutations), *NOTCH1* and *SF3B1* mutations] were grouped together (unfavorable genetics) due to their low frequency in the present series which mostly consists of early stage cases.

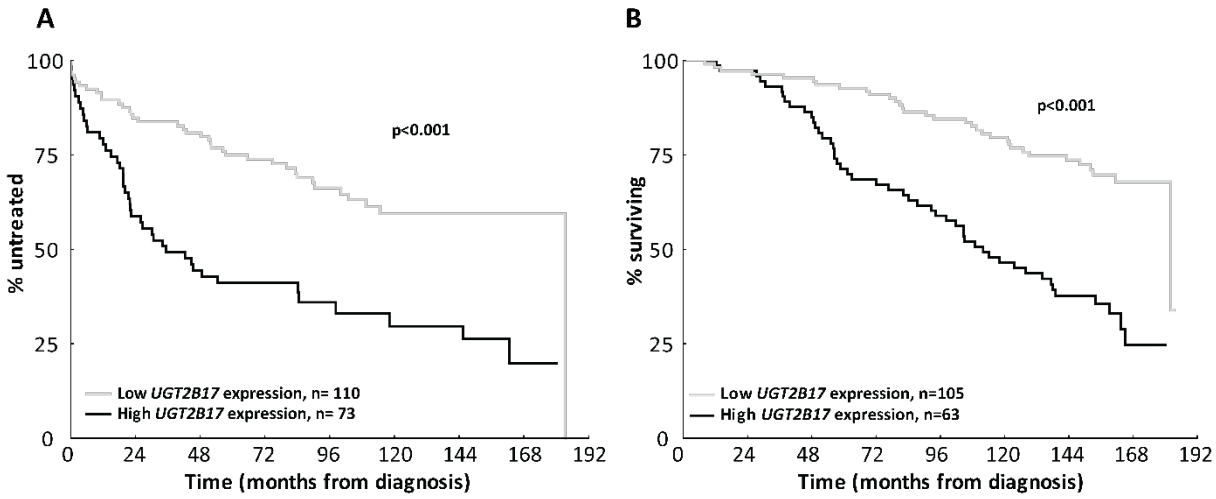
Supplemental Table 2. Multivariate Cox' regression analysis of *UGT2B17* expression and established prognostic markers in M-CLL.

Variable	Overall survival (n=131)		
	HR	**95% CI**	**p-value**
Binet stage B/C	3.56	1.76-7.20	<0.001
High *LPL* expression	2.57	1.34-4.93	0.004
High *UGT2B17* expression	2.66	1.45-4.89	0.001
del(11q)	1.47	0.40-5.41	0.55
Unfavourable genetics	2.84	1.12-7.18	0.02

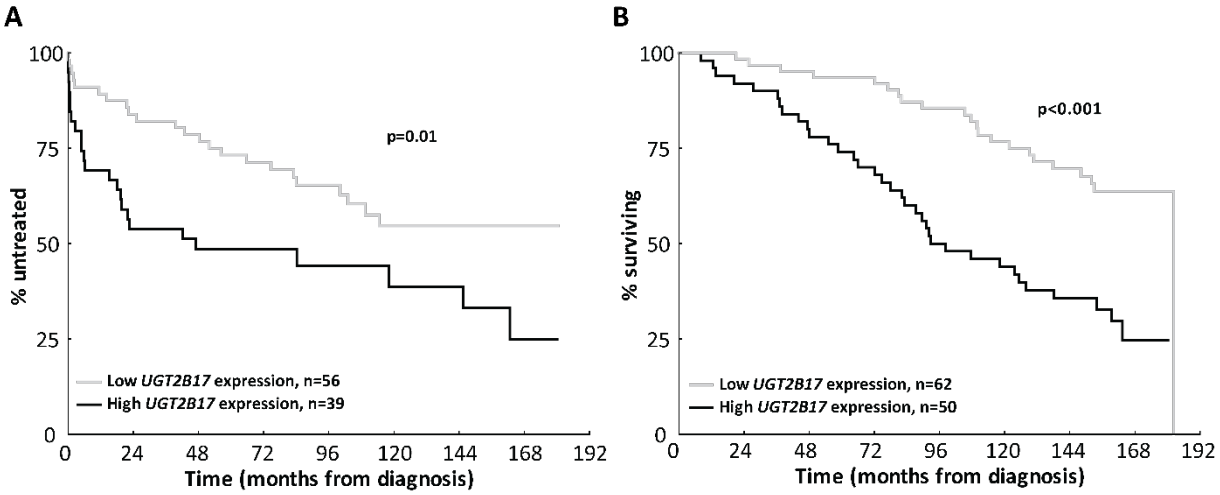
The genomic lesions which have been associated with unfavorable clinical outcome [TP53 abnormalities (del(17p) and/or *TP53* mutations), *NOTCH1* and *SF3B1* mutations] were grouped together (unfavorable genetics) due to their low frequency in the present series which mostly consists of early stage cases. HR: Hazard ratio, CI: Confidence interval.



Supplemental Figure 1. *UGT2B17* expression in M-CLL. Impact of high *UGT2B17* expression on overall survival (OS).



Supplemental Figure 2. *UGT2B17* expression in Binet stage A. Impact of high *UGT2B17* expression on (A) Time-to-first-treatment (TTFT); (B) Overall survival (OS).



Supplementary Figure 3. *UGT2B17* expression in cases with isolated del (13q). Impact of high *UGT2B17* expression on (A) Time-to-first-treatment (TTFT); (B) Overall survival (OS).