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

High *UGT2B17* mRNA expression has recently been correlated with poor prognosis in chronic lymphocytic leukemia (CLL).[1] In the present study, we investigated the expression of *UGT2B17* in a Scandinavian population-based CLL cohort (n=253) and can confirm that high expression of *UGT2B17* is associated with advanced clinical stage at diagnosis, unmutated IGHV genes (U-CLL) and poor clinical outcome. That said, we discovered a notable and novel finding based on the expression of *UGT2B17*, that of identifying patients with a poor prognosis within the IGHV-mutated group (M-CLL) (31/120, 26%), which previously could not be discriminated by any other established molecular marker, including recurrent genomic aberrations, novel mutations (*SF3B1, NOTCH1* and *TP53*) and CD38 expression. Interestingly, high *UGT2B17* expression arose as the strongest independent molecular prognostic marker of overall survival (OS) in multivariate analysis within M-CLL. The incorporation of *LPL* into our expression analysis enabled the further stratification of M-CLL, thus highlighting the potential use of RNA-based markers in the prognostic stratification of CLL, particularly for cases exhibiting an otherwise favorable clinicobiological profile.

During the last decades, a plethora of prognostic factors have been reported which can aid in the identification of CLL patients with a high risk of progression;[2-5] however, the usefulness of the majority of these parameters is generally restricted to U-CLL cases. Thus, amongst M-CLL cases, there is a lack of prognostic markers that can identify patients at greater risk of progression at diagnosis. For example, M-CLL patients generally lack poor-prognostic genomic aberrations and recurrent mutations e.g., *TP53, NOTCH1* or *SF3B1*, and CD38 is highly expressed in only a small proportion of M-CLL cases.[6-8]

It has recently been proposed that RNA-based markers could be used to predict clinical outcome, especially among cases with an otherwise favorable biological profile, namely early-stage, isolated del(13q) or M-CLL. One such example, which we and others have recently identified, is *LPL* expression, which retained its prognostic significance by multivariate analysis in the presence of other well-established markers, thereby highlighting not only the potential usefulness of RNA markers, but also the need for further investigation using this approach.[9,10] A potential candidate marker is *UGT2B17*, which has previously been associated with cancer and was recently reported to be overexpressed in high-risk CLL patients.[1] *UGT2B17*, a phase II metabolizing enzyme, is a member of the UGT2B super-family which conjugates various endogenous compounds, including steroid hormones as well as several pharmaceutical drugs. By catalyzing the transfer of glucuronic acid from uridine diphosphoglucuronic acid to a variety of substrates, *UGT2B17* detoxifies endogenous and exogenous steroid hormones and xenobiotics.[1,11,12]

In this study, we analyzed the expression of *UGT2B17* in 253 diagnosed CLL patients from a Scandinavian population-based CLL cohort (Table 1A, *Online Supplementary Methods*). One hundred and seventeen patients (46%) had high *UGT2B17* expression, as defined by ROC curve analysis and median survival (*Online Supplementary Methods*), while the remaining cases (n=136, 54%) exhibited either low (88/136, 65%) or no detectable expression (48/136, 35%) (Table 1A). A significant association was observed between high *UGT2B17* expression and U-CLL along with advanced clinical stage (Binet B/C) compared to cases with low expression (P<0.001 each, Table 1A).

Table 1A. Main clinicobiological characteristics of the population-based CLL cohort (n=253).

Variable	N 253	High *UGT2B17* expression (n=117)	Low *UGT2B17* expression (n=136)	*P*
Binet Stage	236			<0.001
A	183	73	110	
B	39	27	12	
C	14	11	3	
IGHV mutational status	251			<0.001
Mutated	149	45	104	
Unmutated	102	73	29	
Chromosomal aberration	245			
No aberration	72	25	47	0.020
del(13q)	112	50	62	ns
del(11q)	30	21	9	0.005
del(17p)	10	8	2	0.029
Trisomy 12	21	9	12	ns
Recurrent mutations*				
TP53 (n=251)	12	9	3	0.041
NOTCH1 (n=239)	14	10	4	0.053
SF3B1 (n=242)	11	6	5	ns
CD38 (20%)	253			<0.01
Low	193	80	113	
High	60	37	23	
LPL	226			<0.001
Low	126	37	89	
High	100	64	36	

**number of analyzed and mutated cases are indicated, ns: not significant.*

Table 1B. Multivariate Cox regression analysis of *UGT2B17* expression and established prognostic markers in CLL.

Variable	Time to first treatment (n=189)			Overall survival (n=201)		
	HR	95% CI	*P*	HR	95% CI	*P*
Binet stage B/C	NA	NA	NA	1.84	1.19-2.83	0.005
U-CLL	2.63	1.48-4.65	<0.001	1.81	1.02-3.22	0.042
Unfavorable genetics	2.05	1.23-3.40	0.006	2.68	1.63-4.41	<0.001
del(11q)	1.37	0.80-2.34	0.259	0.91	0.53-1.57	0.740
High *LPL* expression	1.75	1.00-3.03	0.049	2.70	1.57-4.64	<0.001
High *UGT2B17* expression	1.62	1.07-2.46	0.023	1.68	1.08-2.61	0.021

Unfavorable genetics: [TP53 abnormalities (del(17p) and/or TP53 mutations), NOTCH1 and SF3B1 mutations], HR: Hazard ratio, CI: Confidence interval.

Of note, a considerable proportion of patients carrying mutated IGHV genes (45/149, 30%) or belonging to Binet stage A (73/183, 40%) displayed high *UGT2B17* expression (Table 1A). Thus, while confirming the results reported by Gruber *et al.*, i.e. that high expression of *UGT2B17* correlated to poor-risk CLL,[1] several differences between these two studies were also noted. More specifically, in our study, high expression of *UGT2B17* correlated significantly with del(11q) and del(17p) (P=0.005 and P=0.029, respectively) (Table 1A), whereas Gruber *et al.* reported a negative correlation with del(17p) and no association between *UGT2B17* expression and del(11q). In addition, we detected no correlation between *UGT2B17* expression and recurrently mutated genes such as *SF3B1* and *NOTCH1* (Table 1A).

Next, we assessed the prognostic significance of *UGT2B17* expression by studying time to first treatment (TTFT) and OS both within the entire cohort and also in specific subgroups of patients, most notably the group of M-CLL patients. Median follow-up for assessment of TTFT and OS was 4.3 and 10.1 years, respectively. Analysis of the entire cohort revealed that high expression of *UGT2B17* correlated with shorter TTFT and OS (P<0.001 each, Figure 1A,B). In multivariate analysis, *UGT2B17* expression, IGHV mutational status, *LPL* expression, and "unfavorable genetics" [*TP53* abnormalities (del(17p) and/or *TP53* mutations), *NOTCH1* and *SF3B1* mutations] were significant for TTFT (n=189), while *UGT2B17* expression, clinical stage, IGHV mutational status, *LPL* expression, and "unfavorable genetics" remained as independent prognostic factors for OS (n=201) (Table 1B, *Online Supplementary Table S1*). Genomic lesions, which have previously been associated with unfavorable clinical outcome (*TP53* abnormalities, *NOTCH1* and *SF3B1* mutations), were grouped together due to their low frequency in the present series, which predominantly comprised early stage cases.

A novel finding with potential prognostic impact relates to the observation that 45/149 (30%) of M-CLL cases exhibited high expression of *UGT2B17* and displayed poor clinical outcome (P<0.001, *Online Supplementary Figure S1*). Since the majority of these cases were negative for CD38 expression (134/149, 90%), carried only favorable genomic lesions (del(13q) or no recurrent aberrations (133/145, 92%) and did not display mutations in *TP53* (145/149, 97%), *NOTCH1* (139/142, 98%) or *SF3B1* (140/143, 98%), quantification of *UGT2B17* mRNA expression identified a subgroup of progressive M-CLL cases (31/120, 26%) for which, to date, no established prognostic marker has been successful in identifying (Figure 1C). Notably, within M-CLL, high *UGT2B17* expression remained as the strongest independent molecular prognostic marker for OS in multivariate analysis (*Online Supplementary Table S2*). Further evaluation of *UGT2B17* expression on clinical outcome in subgroups of CLL with favorable prognosis revealed high expression to be associated with both shorter TTFT and OS in Binet stage A cases (P<0.001 and P<0.001, respectively; *Online Supplementary Figure S2A,B*) as well as in cases with isolated del(13q) (P=0.012 and P<0.001, respectively; *Online Supplementary Figure S3 A,B*).

Taking a step further, we explored the co-expression of *UGT2B17* and *LPL* in 226 cases and found that 64/226 (28%) had high expression of both genes, 52/64 (81% of cases concerned U-CLL), while 36 and 37 cases displayed either high *LPL* or *UGT2B17* expression (15.9% and 16.4%, respectively, P<0.001, Table 1A). Interestingly, in contrast to cases with isolated *LPL* expression, the isolated *UGT2B17* high expressing subgroup was predominantly comprised of M-CLL (50% *vs.* 83%, P=0.003). Cases with high expression for both genes exhibited a median survival of 7.4 years, M-CLL cases with low expression for both genes displayed a median survival of 15.2 years, while cases displaying isolated expression of either gene had an intermediate survival rate (Figure 1D). Taking into consideration the indolent nature of the disease experienced by the majority of M-CLL patients, analysis of *UGT2B17* and *LPL* expression, which could distinguish those cases with the highest risk for progression and therefore shorter OS, is particularly appealing; especially if such markers can be assessed using standardized methodology such as RQ-PCR analysis.

Finally, in order to evaluate *UGT2B17* expression stability and reproducibility, we investigated follow-up samples from 91 cases (range: 5.0-8.1 years, median 6.7 years). *UGT2B17* expression remained stable over time (range at diagnosis: 0-3.9, average 0.29 normalized units *vs.* range at follow-up: 0-4.17, average 0.25 normalized units, P=0.54), a finding which also held true when including only those patients who had received treatment prior to second sampling (n=33, P=0.53). In the latter group, 31 out of 33 patients retained the same expression classification after treatment, while 2 of the patients changed from a low expression of *UGT2B17* to a high expression following treatment, thereby highlighting the potential role of *UGT2B17* as a suitable follow-up marker in CLL within clinical practice.

Data on the exact mechanism of the action of *UGT2B17* in CLL which could provide a rational explanation for the association with progressive disease, especially in M-CLL, is currently lacking. Gruber *et al.* have reported high expression of *UGT2B17* in cases with stable or progressive disease after fludarabine-based therapy administration, implying that *UGT2B17* expression could

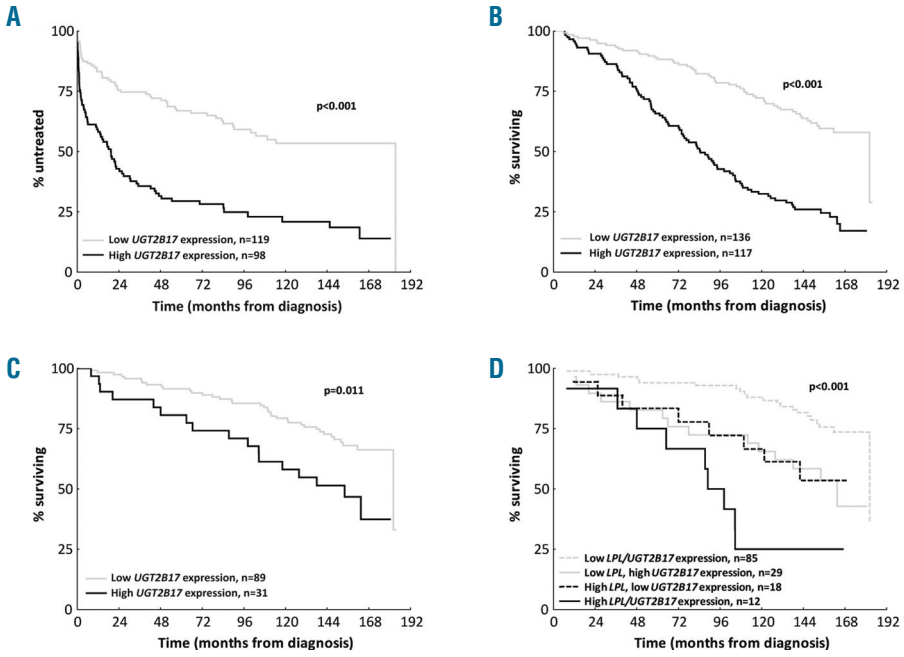

Figure 1. *UGT2B17* expression in CLL. Impact of high *UGT2B17* expression on (A) Time to first treatment (TTFT); (B) Overall survival (OS). (C) OS for M-CLL cases negative for poor prognostic recurrent genomic aberrations (del(17p), del(11q) and trisomy 12), novel mutations (*SF3B1*, *NOTCH1* and *TP53*) and CD38 expression. (D) *UGT2B17* and *LPL* expression subdivides OS in M-CLL for OS.

be linked to drug metabolism, either within the cancer cell population or in other sites where degradation of the drug occurs. Suboptimal treatment responses could possibly explain the impact of *UGT2B17* expression on OS.

In conclusion, we not only confirm that *UGT2B17* expression is associated with advanced clinical stage at diagnosis, U-CLL and a poor clinical outcome, but also note for the first time its prognostic role among M-CLL patients, whereby *UGT2B17* expression was able to identify poor risk cases which previously could not be discriminated using established markers, and within which it represented the most significant molecular factor affecting OS. Incorporation of *LPL* expression into our analysis enabled the further stratification of M-CLL, thus providing a rationale for the use of RNA-based markers in the prognostic stratification of CLL. In order to adopt such an approach into everyday clinical practice, the investigation of RNA markers in a large series of patients is imperative, while elucidation of the biological background and the exact mechanisms of action may provide novel therapeutic targets.

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References

1. Gruber M, Bellemare J, Hoermann G, et al. Overexpression of uridine diphospho glucuronosyltransferase 2B17 in high-risk chronic lymphocytic leukemia. Blood. 2013;121(7):1175-1183.
2. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999;94(6):1840-1847.
3. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999;94(6):1848-1854.
4. Mansouri L, Cahill N, Gunnarsson R, et al. NOTCH1 and SF3B1 mutations can be added to the hierarchical prognostic classification in chronic lymphocytic leukemia. Leukemia. 2013;27(2):512-514.
5. Rossi D, Rasi S, Spina V, et al. Integrated mutational and cytogenetic analysis identifies new prognostic subgroups in chronic lymphocytic leukemia. Blood. 2013;121(8):1403-1412.
6. Thunberg U, Johnson A, Roos G, et al. CD38 expression is a poor predictor for VH gene mutational status and prognosis in chronic lymphocytic leukemia. Blood. 2001;97(6):1892-1894.
7. Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000;343(26):1910-1916.
8. Baliakas P, Hadzidimitriou A, Sutton LA, et al. Recurrent mutations refine prognosis in chronic lymphocytic leukemia. Leukemia. 2015;29(2):329-336.
9. Kaderi MA, Kanduri M, Buhl AM, et al. LPL is the strongest prognostic factor in a comparative analysis of RNA-based markers in early chronic lymphocytic leukemia. Haematologica. 2011;96(8):1153-1160.
10. Sevov M, Rosenquist R, Mansouri L. RNA-based markers as prognostic factors in chronic lymphocytic leukemia. Expert Rev Hematol. 2012;5(1):69-79.
11. Hirata H, Hinoda Y, Zaman MS, et al. Function of UDP-glucuronosyltransferase 2B17 (UGT2B17) is involved in endometrial cancer. Carcinogenesis. 2010;31(9):1620-1626.
12. Angstadt AY, Berg A, Zhu J, et al. The effect of copy number variation in the phase II detoxification genes UGT2B17 and UGT2B28 on colorectal cancer risk. Cancer. 2013;119(13):2477-2485.