LPL is the strongest prognostic factor in a comparative analysis of RNA-based markers in early chronic lymphocytic leukemia

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Online Supplementary Table S1. Taqman MGB® probes used for RQ-PCR analysis.

Gene name	TaqMan® Gene Expression Assay ID
LPL	Hs00173425_m1
ZAP70	Hs00896347_m1
TCL1A	Hs00172040_m1
CLLU1	Hs02576184_m1
MCL1	Hs01050896_m1
β2M	Hs99999907_m1

RQ-PCR was performed according to the manufacturer's recommendation: 50°C for 2 min followed by 95°C for 10 min and 45 cycles of 95°C for 15 s and 60°C for 1 min. All samples were run in triplicates and the mean values were used for calculating relative expression. Samples in which one replicate deviated by more than 0.5 in Ct value from any other replicate were rerun. Additionally, 12 randomly chosen samples were rerun to cross-check relative expression where no significant differences were noted.

Online Supplementary Table S2. Multivariate Cox's regression analysis of RNA-based markers within Binet A subgroup.

Variable	Overall survival (n=185)			Time to treatment (n=170)		
	HR	95% CI	*P*	HR	95% CI	*P*
LPL	7.90	3.92 - 15.92	< 0.0001	4.09	2.33 - 7.18	< 0.0001
ZAP70	1.36	0.78 - 2.37	0.27	1.47	0.88 - 2.46	0.15
TCL1A	1.10	0.62 - 1.95	0.75	1.57	0.92 - 2.66	0.10
CLLU1	0.91	0.50 - 1.65	0.76	1.12	0.66 - 1.91	0.68
MCL1	1.21	0.69 - 2.11	0.51	0.91	0.55 - 1.53	0.73

The threshold values used in the analysis are determined based on ROC curve analysis. HR: Hazard ratio, CI: Confidence interval.

Online Supplementary Table S3. Multivariate Cox's regression analysis of *LPL* and established markers excluding IGHV mutational status.

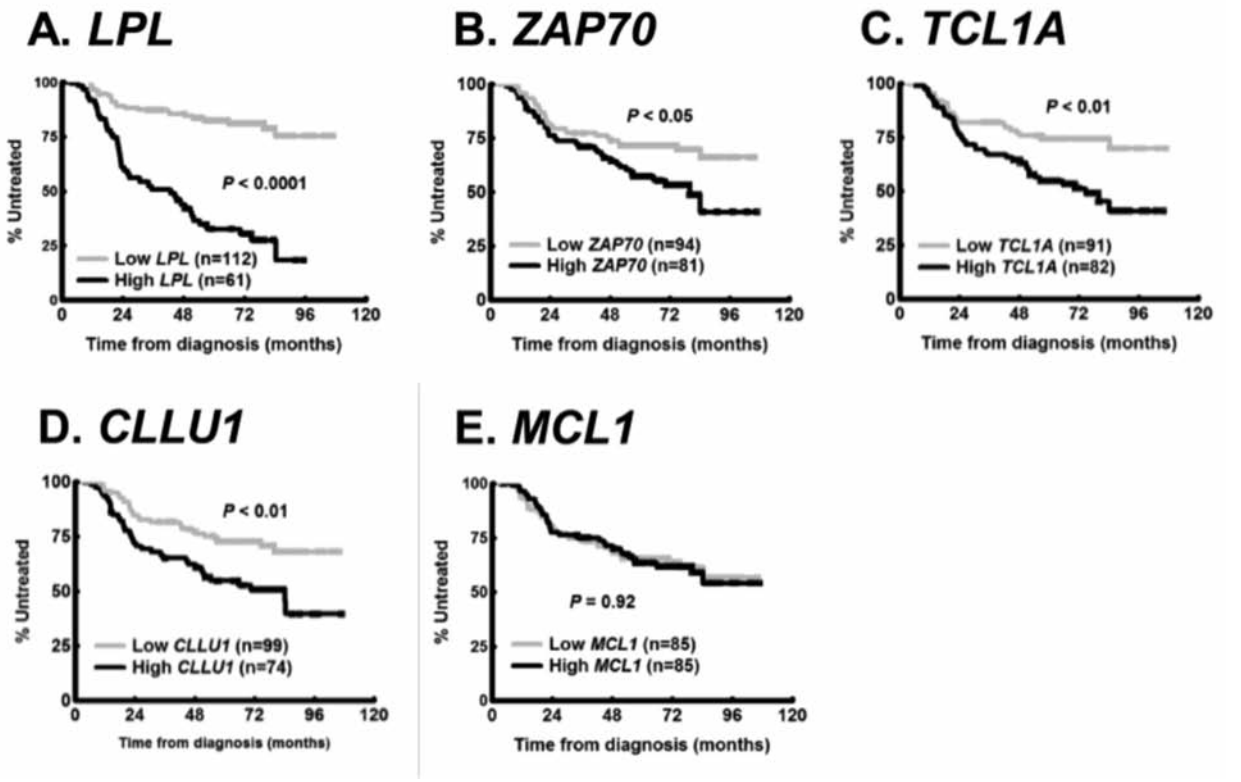
Variable	Time to treatment (n=215) Analysis excluding IGHV status		
	HR	CI	P
Age at diagnosis	0.94	0.64 - 1.37	0.74
Gender	1.15	0.76 - 1.72	0.51
Trisomy 12	1.44	0.77 - 2.68	0.25
del(11q)	1.65	0.95 - 2.85	0.07
del(17p)	2.46	1.10 - 5.53	0.03
CD38	2.09	1.33 - 3.30	0.001
LPL	2.59	1.61 - 4.16	< 0.0001

HR: Hazard ratio, CI: Confidence interval. The threshold values used in the analysis are as follows; age at diagnosis: median (63.9); recurrent genomic aberrations: HR is given in comparison to cases with no detected aberrations/del(13q); CD38: 7%; LPL*: threshold value based on ROC curve analysis. Binet stage is not included since most Binet stage B/C patients receive treatment at or shortly following diagnosis. HR: Hazard ratio, CI: Confidence interval.*

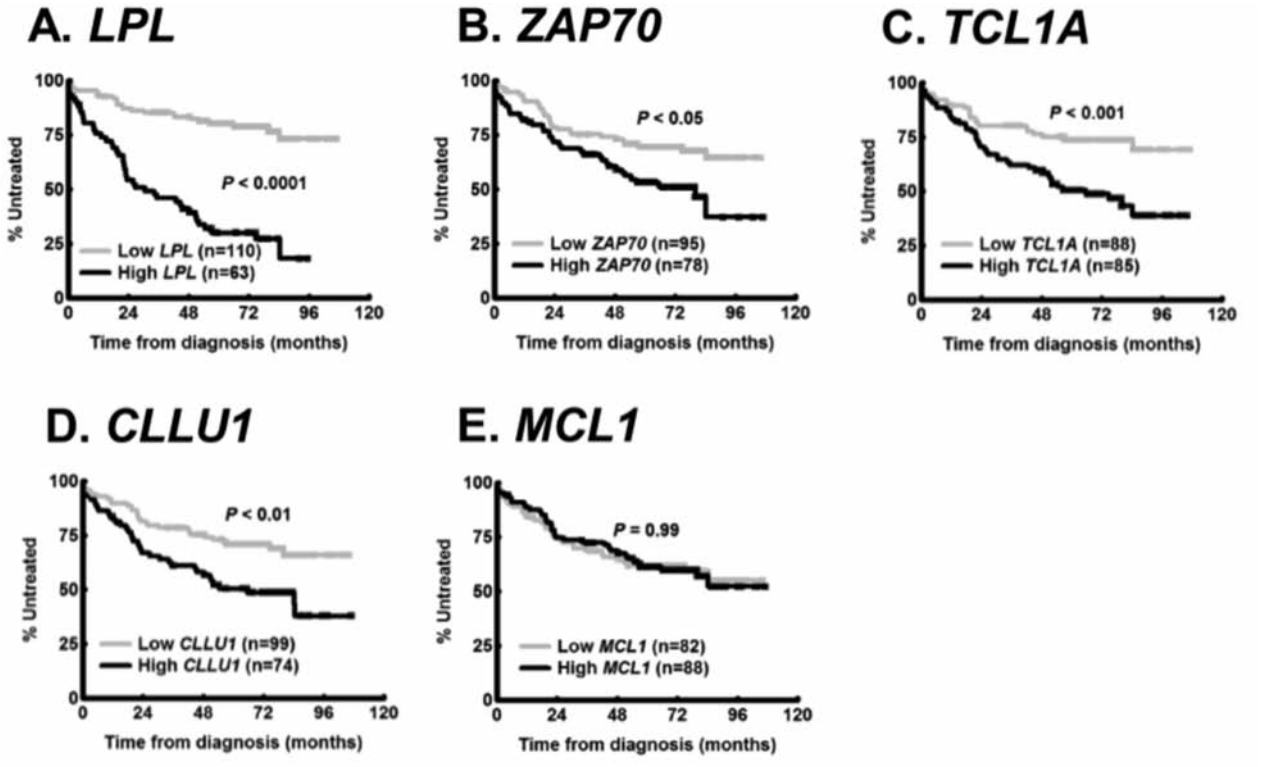
Online Supplementary Table S4. The transcriptional expression of RNA-based markers in relation to clinical and molecular characteristics.

Variable	Relative transcriptional expression level														
	LPL			*ZAP70*			*CLLU1*			*TCL1A*			*MCL1*		
	Low N (%)	High N (%)	P	Low N (%)	High N (%)	P	Low N (%)	High N (%)	P	Low N (%)	High N (%)	P	Low N (%)	High N (%)	P
Age at diagnosis			NS			NS			NS			NS			NS
< 63.9 years	78 (62)	48 (38)		72 (57)	54 (43)		72 (57)	54 (43)		56 (44)	70 (56)		66 (54)	57 (46)	
≥ 63.9 years	67 (53)	59 (47)		64 (51)	62 (49)		64 (51)	62 (49)		71 (56)	55 (44)		60 (48)	65 (52)	
Gender			NS			NS			NS			NS			NS
F	56 (61)	36 (39)		48 (52)	44 (48)		55 (60)	37 (40)		47 (51)	45 (49)		43 (48)	47 (52)	
M	89 (56)	71 (44)		88 (55)	72 (45)		81 (51)	79 (49)		80 (50)	80 (50)		83 (53)	75 (47)	
Binet Stage			<0.001			NS			0.038			NS			NS
A	119 (63)	69 (37)		103 (55)	85 (45)		107 (57)	81 (43)		93 (49)	95 (51)		91 (49)	94 (51)	
B	11 (28)	28 (72)		16 (41)	23 (59)		14 (36)	25 (64)		17 (44)	22 (56)		22 (56)	17 (44)	
C	7 (58)	5 (42)		6 (50)	6 (50)		8 (67)	4 (33)		9 (75)	3 (25)		5 (44)	7 (56)	
IGHV mutational status			<0.0001			<0.0001			<0.0001			0.016			NS
Mutated	126 (80)	32 (20)		105 (66)	53 (34)		102 (65)	56 (35)		88 (56)	70 (44)		83 (54)	71 (46)	
Unmutated	12 (14)	74 (86)		28 (33)	54(67)		28 (33)	58 (67)		34 (40)	52 (60)		39 (45)	47 (55)	
Genomic aberrations			<0.0001			NS			0.0067			NS			NS
del(13q)	84 (74)	29 (26)		71 (63)	42 (37)		70 (62)	43 (38)		62 (55)	51 (45)		58 (48)	53 (52)	
No aberration	46 (63)	27 (37)		37 (51)	36 (49)		42 (58)	31 (42)		41 (56)	32 (44)		39 (55)	32 (45)	
Trisomy 12	5 (25)	15 (75)		9 (45)	11 (55)		7 (35)	13 (65)		6 (30)	14 (70)		8 (40)	12 (60)	
del(11q)	3 (11)	25 (89)		11 (39)	17 (61)		8 (29)	20 (71)		10 (36)	18 (64)		14 (50)	14 (50)	
del(17p)	2 (20)	8 (80)		3 (30)	7 (70)		4 (40)	6 (60)		5 (50)	5 (50)		6 (60)	4 (40)	
CD38			<0.0001			<0.0001			<0.001			NS			0.014
< 7%	122 (72)	47 (28)		107 (63)	62 (37)		105 (62)	64 (38)		91 (54)	78 (46)		93 (56)	72 (44)	
≥ 7%	23 (28)	60 (72)		29 (35)	54 (65)		31 (37)	52 (63)		36 (43)	47 (57)		33 (40)	50 (60)	

The threshold values for RNA-based markers were determined based on ROC curve analysis. P *values shown in this table were derived from the* χ^2 *test. NS, not significant.*



Online Supplementary Figure S1. Time to treatment of patients when excluding cases treated within six months after diagnosis. Kaplan-Meier analysis of time to treatment of CLL cases according to the expression status of (A) *LPL*, (B) *ZAP70*, (C) *TCL1A*, (D) *CLLU1* and (E) *MCL1*.



Online Supplementary Figure S2. Time to treatment of patients within Binet A subgroup. Kaplan-Meier analysis of time to treatment of CLL cases according to the expression status of (A) *LPL*, (B) *ZAP70*, (C) *TCL1A*, (D) *CLLU1* and (E) *MCL1*.