

Toll-like receptor signaling pathway in chronic lymphocytic leukemia: distinct gene expression profiles of potential pathogenic significance in specific subsets of patients

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Online Supplementary Table S1. Clinical and biological data of the patients.

Parameter	Number
Gender	
Male	113
Female	79
Binet stage at diagnosis	
A	149
B	12
C	4
CD38 expression (cut-off value 7%)	
Positive	57
Negative	120
Surface Ig expression	
MD-M	143
G	26
IGHV gene mutational status	
Mutated	124
Unmutated	67
Disease progression	
Progressive	50
Stable	113

Online Supplementary Table S2. Genes evaluated in the present study.

Approved Gene Symbol (HUGO)	Symbol on array	Gene Name	Other symbols
BTK	BTK	Brunton agammaglobulinemia tyrosine kinase	ATK, XLA, PSCTK1, AGMX1, IMD1
CASP8	CASP8	Caspase 8, apoptosis-related cysteine peptidase	MCH5, MACH, FLICE, Casp-8
CCL2	CCL2	Chemokine (C-C motif) ligand 2	MCP1, MCP-1, MCAF, SMC-CF, GDCF-2, HC11, MGC9434, SCYA2
CD14	CD14	CD14 molecule	
CD180	CD180	CD180 molecule	RP105, Ly78, LY64
CD80	CD80	CD80 molecule	CD28LG, CD28LG1
CD86	CD86	CD86 molecule	CD28LG2
CHUK	CHUK	Conserved helix-loop-helix ubiquitously expressed kinase	TCF16, IKK1, IKK-alpha, IkbkA, NFKBIKA, IKKA
CLEC4E	CLEC4E	C-type lectin domain family 4, member E	mincle, CLECSF9
CSF2	CSF2	Colony stimulating factor 2 (granulocyte-macrophage)	GM-CSF
CSF3	CSF3	Colony stimulating factor 3 (granulocyte)	MGC45931, GCSF, G-CSF, C17orf33
CXCL10	C6L10	Chemokine (C-X-C motif) ligand 10	IF110, IP-10, crg-2, mob-1, C7, gIP-10, INP10, SCYB10
ECSIT	ECSIT	ECSIT homolog (Drosophila)	SITPEC
EIF2AK2	EIF2AK2	Eukaryotic translation initiation factor 2-alpha kinase 2	PKR, EIF2AK1, PRKR
ELK1	ELK1	ELK1, member of ETS oncogene family	
FADD	FADD	Fas (TNFRSF6)-associated via death domain	MORT1, GIG3
FOS	FOS	V-fos FBJ murine osteosarcoma viral oncogene homolog	c-fos, AP-1
HMGB1	HMGB1	High-mobility group box 1	HMG3, SBP-1, DKFZp686A04236, HMG1
HRAS	HRAS	V-Ha-ras Harvey rat sarcoma viral oncogene homolog	HRAS1
HSPA1A	HSPA1A	Heat shock 70kDa protein 1A	HSP70-1, HSPA1
HSPD1	HSPD1	Heat shock 60kDa protein 1 (chaperonin)	GROEL, HSP60, SPG13
IFNA1	IFNA1	Interferon, alpha 1	IFNA@, IFL, IFN, IFN-ALPHA, IFNA13
IFNB1	IFNB1	Interferon, beta 1, fibroblast	IFB, IFF, IFNB
IFNG	IFNG	Interferon, gamma	
IKKBK	IKKBK	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	IKK2, NFKBIKB, IKK-beta, IKKB
IL10	IL10	Interleukin 10	CSIF, TGIF, IL10A, IL-10
IL12A	IL12A	Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)	CLMF, IL-12A, p35, NFSK, NKSF1
IL1A	IL1A	Interleukin 1, alpha	IL1F1, IL-1A, IL1
IL1B	IL1B	Interleukin 1, beta	IL1F2, IL-1B
IL2	IL2	Interleukin 2	
IL6	IL6	Interleukin 6 (interferon, beta 2)	IL-6, BSF2, HGF, HSF, IFNB2
IL8	IL8	Interleukin 8	SCYB8, LUCT, LECT, MDNCF, TSG-1, CXCL8, IL-8, NAP-1, 3-10C, MONAP, AMCF-1, LYNAP, NAF, b-ENAP, GCP-1, K60
IRAK1	IRAK1	Interleukin-1 receptor-associated kinase 1	IRAK, pelle
IRAK2	IRAK2	Interleukin-1 receptor-associated kinase 2	
IRF1	IRF1	Interferon regulatory factor 1	MAR
IRF3	IRF3	Interferon regulatory factor 3	
JUN	JUN	Jun oncogene	c-Jun, AP-1
LTA	LTA	Lymphotoxin alpha (TNF superfamily, member 1)	TNFB
LY86	LY86	Lymphocyte antigen 86	MD-1, dJ80N2.1
LY96	LY96	Lymphocyte antigen 96	MD-2
MAP2K3	MAP2K3	Mitogen-activated protein kinase kinase 3	MEK3, MKK3, MAPKK3, PRKMK3
MAP2K4	MAP2K4	Mitogen-activated protein kinase kinase 4	MEK4, JNKK1, PRKMK4, MKK4, SERK1
MAP3K1	MAP3K1	Mitogen-activated protein kinase kinase kinase 1	MEKK, MAPKKK1, MEKK1
MAP3K7	MAP3K7	Mitogen-activated protein kinase kinase kinase 7	MEKK7, TAK1
MAP4K4	MAP4K4	Mitogen-activated protein kinase kinase kinase kinase 4	HGK, NIK, FLH21957
MAPK8	MAPK8	Mitogen-activated protein kinase 8	JNK, JNK1, SAPK1, PRKMB
MAPK8IP3	MAPK8IP3	Mitogen-activated protein kinase 8 interacting protein 3	KIAA1066, JSAP1, JIP3, syd
MYD88	MYD88	Myeloid differentiation primary response gene (88)	
NFKB1	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	KBF1, p105, NFKB-p50, p50, NF-kappaB
NFKB2	NFKB2	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)	LYT-10, p52
NFKBIA	NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	IKBA, MAD-3, IkappaBalpha, NFKBI
NFKBIL1	NFKBIL1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1	IKBL, NFKBIL
NFRKB	NFRKB	Nuclear factor related to kappaB binding protein	DKFZp547B2013, INO80G
NR2C2	NR2C2	Nuclear receptor subfamily 2, group C, member 2	TR2R1, hTAK1, TR4
PELI1	PELI1	Pellino homolog 1 (Drosophila)	
PPARA	PPARA	Peroxisome proliferator-activated receptor alpha	hPPAR, NR1C1, PPAR
PRKRA	PRKRA	Protein kinase, interferon-inducible double stranded RNA dependent activator	PACT, RAX, HSD14, DYT16
REL	REL	V-rel reticuloendotheliosis viral oncogene homolog (avian)	I-Rel, c-Rel
RELA	RELA	V-rel reticuloendotheliosis viral oncogene homolog A (avian)	NFKB3, p65
RIPK2	RIPK2	Receptor-interacting serine-threonine kinase 2	RICK, RIP2, CARDIAC, CARD3
SARM1	SARM1	Sterile alpha and TIR motif containing 1	SARM, SAMD2, KIAA0524
SIGIRR	SIGIRR	Single immunoglobulin and toll-interleukin 1 receptor (TIR) domain	TIR8
TAB1	MAP3K7IP1	Mitogen-activated protein kinase kinase kinase 7 interacting protein 1	MAP3K7IP1
TBK1	TBK1	TANK-binding kinase 1	NAK
TICAM1	TICAM1	Toll-like receptor adaptor molecule 1	TRIF, TICAM-1, MGC35334, PRVTIRB
TICAM2	TICAM2	Toll-like receptor adaptor molecule 2	TRAM, TICAM-2, TIRP
TIRAP	TIRAP	Toll-interleukin 1 receptor (TIR) domain containing adaptor protein	Mal, wyatt
TLR1	TLR1	Toll-like receptor 1	rsc786, KIAA0012, CD281
TLR10	TLR10	Toll-like receptor 10	CD290
TLR2	TLR2	Toll-like receptor 2	TIL4, CD282
TLR3	TLR3	Toll-like receptor 3	CD283
TLR4	TLR4	Toll-like receptor 4	hToll, CD284
TLR5	TLR5	Toll-like receptor 5	TIL3, SLEB1, FLJ10052, MGC126430, MGC126431
TLR6	TLR6	Toll-like receptor 6	CD286
TLR7	TLR7	Toll-like receptor 7	
TLR8	TLR8	Toll-like receptor 8	CD288
TLR9	TLR9	Toll-like receptor 9	CD289
TNF	TNF	Tumor necrosis factor (TNF superfamily, member 2)	TNFSF2, DIF, TNF-alpha, TNFA
TNFRSF1A	TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A	TNF-R, TNFAR, TNFR60, TNF-R-I, CD120a, TNF-R55, TNFR1
TOLLIP	TOLLIP	Toll interacting protein	IL-1RAcPIP
TRAF6	TRAF6	TNF receptor-associated factor 6	RNF85
UBE2N	UBE2N	Ubiquitin-conjugating enzyme E2N (UBC13 homolog, yeast)	UbcH-ben, UBC13, MGC8489
UBE2V1	UBE2V1	Ubiquitin-conjugating enzyme E2 variant 1	UEV-1, CROC-1, UEV1A, CROC1, UBE2V
Housekeeping genes			
B2M	B2M	Beta-2-microglobulin	-
HPRT1	HPRT1	Hypoxanthine phosphoribosyltransferase 1	HGPRT, HPRT
RPL13A	RPL13A	Ribosomal protein L13a	TSTA1
GAPDH	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	GAPD
ACTB	ACTB	Actin, beta	-

Online Supplementary Table S3. Monoclonal antibodies used for the FACS analysis in the present study.

Description	Provider	Isotype Control	Quantity
TLR1 antibody [GD2.F4] (FITC) (ab59702)	AbCam, Cambridge, UK	IgG1	10µl
TLR2, Mouse Anti-Human, (Alexa Fluor® 488) Cat. No. MHTLR220	Invitrogen, Carlsbad, CA, USA	IgG2a	10µl
TLR4 antibody [HTA125] (Phycoerythrin) (ab11227)	AbCam, Cambridge, UK	IgG2a	10µl
TLR6 antibody [TLR6.127] (FITC) (ab72362)	AbCam, Cambridge, UK	IgG1	10µl
TLR7 antibody (Phycoerythrin) (ab72331)	AbCam, Cambridge, UK	IgG	2µl
Monoclonal Antibody to TLR8/CD288 (Clone 303F1.14) Alexa 488 Conjugated	IMGENEX, San Diego, CA	IgG2a	2µl
TLR9 antibody [5G5] (FITC) (ab58864)	AbCam, Cambridge, UK	IgG2a	10µl
FITC Mouse Anti-Human CD80 (557226)	BD Pharmingen™, Franklin Lakes, NJ USA	IgG1	10µl
PE Mouse Anti-Human CD86 (555658)	BD, Franklin Lakes, NJ USA	IgG1	10µl

Online Supplementary Table S4. IGHV gene repertoires of the “mutated” (<98% identity to germline), “unmutated” (≥98% identity to germline) and “truly unmutated” (100% identity to germline) subgroups.

Genes	Total		"Mutated" (<98%)		"Unmutated" (≥98%)		"Truly unmutated" (=100%)	
	n	%	n	%	n	%	n	%
IGHV1-18	5	2,62	3	2,42	2	2,99	1	1,92
IGHV1-2	11	5,76	4	3,23	7	10,45	6	11,54
IGHV1-24	1	0,52	0	0,00	1	1,49	0	0,00
IGHV1-3	7	3,66	2	1,61	5	7,46	5	9,62
IGHV1-46	2	1,05	1	0,81	1	1,49	1	1,92
IGHV1-58	1	0,52	0	0,00	1	1,49	1	1,92
IGHV1-69	14	7,33	0	0,00	14	20,90	11	21,15
IGHV1-8	3	1,57	2	1,61	1	1,49	1	1,92
IGHV2-26	1	0,52	1	0,81	0	0,00	0	0,00
IGHV2-5	2	1,05	1	0,81	1	1,49	1	1,92
IGHV3-11	3	1,57	1	0,81	2	2,99	2	3,85
IGHV3-13	1	0,52	1	0,81	0	0,00	0	0,00
IGHV3-15	3	1,57	3	2,42	0	0,00	0	0,00
IGHV3-20	2	1,05	2	1,61	0	0,00	0	0,00
IGHV3-21	3	1,57	2	1,61	1	1,49	0	0,00
IGHV3-23	12	6,28	8	6,45	4	5,97	3	5,77
IGHV3-30	11	5,76	8	6,45	3	4,48	3	5,77
IGHV3-30-3	2	1,05	1	0,81	1	1,49	1	1,92
IGHV3-33	6	3,14	3	2,42	3	4,48	3	5,77
IGHV3-43	1	0,52	0	0,00	1	1,49	1	1,92
IGHV3-48	8	4,19	7	5,65	1	1,49	1	1,92
IGHV3-49	3	1,57	3	2,42	0	0,00	0	0,00
IGHV3-53	2	1,05	2	1,61	0	0,00	0	0,00
IGHV3-64	1	0,52	0	0,00	1	1,49	0	0,00
IGHV3-66	1	0,52	1	0,81	0	0,00	0	0,00
IGHV3-7	15	7,85	13	10,48	2	2,99	2	3,85
IGHV3-73	1	0,52	1	0,81	0	0,00	0	0,00
IGHV3-74	2	1,05	2	1,61	0	0,00	0	0,00
IGHV3-9	2	1,05	1	0,81	1	1,49	1	1,92
IGHV4-30-4	2	1,05	1	0,81	1	1,49	1	1,92
IGHV4-31	4	2,09	4	3,23	0	0,00	0	0,00
IGHV4-34	28	14,66	28	22,58	0	0,00	0	0,00
IGHV4-39	8	4,19	1	0,81	7	10,45	5	9,62
IGHV4-4	6	3,14	6	4,84	0	0,00	0	0,00
IGHV4-59	2	1,05	2	1,61	0	0,00	0	0,00
IGHV4-61	3	1,57	2	1,61	1	1,49	0	0,00
IGHV4-b	1	0,52	1	0,81	0	0,00	0	0,00
IGHV5-51	5	2,62	3	2,42	2	2,99	1	1,92
IGHV5-a	3	1,57	0	0,00	3	4,48	1	1,92
IGHV6-1	3	1,57	3	2,42	0	0,00	0	0,00
Total	191	100,00	124	100,00	67	100,00	52	100,00

Online Supplementary Table S5. Cases belonging to stereotyped subsets.

LAB IDENTIFIER	SUBSET	IGHV gene	IGHD gene	IGHJ gene	% identity	CDR3 LENGTH	CDR3 AA SEQ
P5588	1	IGHV1-2*02	IGHD5-5*01	IGHJ4*02	100,0	13	CARGGWGVVYFDYW
IT01-0298-H1	1	IGHV1-3*01	IGHD6-19*01	IGHJ4*02	100,0	13	CAREQLGPPYFDYW
IT01-0320-H1	1	IGHV1-3*01	IGHD6-19*01	IGHJ4*02	100,0	13	CAREQLAITHFDYW
N5760	1	IGHV1-3*01	IGHD6-19*01	IGHJ4*02	100,0	13	CAREQLVLHYPFDYW
P1173	1	IGHV5-a*01	IGHD6-19*01	IGHJ4*02	99,6	13	CAREQLVGIKNFDYW
P2355	1	IGHV1-2*02	IGHD6-19*01	IGHJ4*02	100,0	13	CARAQLVVTNFDYW
P3506	1	IGHV1-2*02	IGHD6-19*01	IGHJ4*02	99,7	14	CARAQLVLSVYFDYW
P3870	1	IGHV1-2*02	IGHD6-19*01	IGHJ4*02	100,0	13	CARGQLVQLNFDYW
P5092	1	IGHV5-a*03	IGHD6-19*01	IGHJ4*02	100,0	13	CAREQLVLEHFDYW
P3073	1	IGHV1-3*01	IGHD6-19*01	IGHJ4*02	100,0	13	CAREQLVRVNFYDW
P6090	2	IGHV3-21*02	ND	IGHJ6*02	96,2	9	CVTRNGMDVW
P326	2	IGHV3-21*02	ND	IGHJ6*02	98,6	9	CAIDRNGMDVW
P2920	4	IGHV4-34*01	IGHD3-10*01	IGHJ6*02	93,5	20	CARGYGDSPDKRYYYFGLDVW
P3020	4	IGHV4-34*01	IGHD3-10*01	IGHJ6*02	90,0	20	CARGYGTSDTRRYFYGMDVW
P6520	4	IGHV4-34*02	IGHD3-10*01	IGHJ6*02	94,4	20	CARGYEVPTTRRYYYGMELW
P103	4	IGHV4-34*01	IGHD3-10*01	IGHJ6*02	95,9	20	CARGYPDTPVRRYYGMDVW
P3916	4	IGHV4-34*01	IGHD3-10*01	IGHJ6*02	91,2	20	CARGYADSDVIRRYYYGMDVW
P1626	4	IGHV4-34*02	IGHD4-17*01	IGHJ6*02	94,8	18	CARSYGSTPTTRRYYYGMDVW
P1422	4	IGHV4-34*02	IGHD5-12*01	IGHJ6*02	91,9	20	CARGYADTPTFRYYGMDVW
P3551	4	IGHV4-34*01	IGHD5-12*01	IGHJ6*02	93,3	20	CARGWPEDAVTRRYYYGMEIW
P907	4	IGHV4-34*02	IGHD5-12*01	IGHJ6*02	93,2	20	CARGYGTSATTKRYYYGMDVW
IT01-0278-H1	4	IGHV4-34*01	IGHD2-21*02	IGHJ6*02	93,2	20	CARSYGDSPSVRRYYGLDVW
P2446	8	IGHV4-39*06	IGHD6-13*01	IGHJ5*02	99,7	19	CASLTGYSSSWYTPANWFDPW
P1050	8	IGHV4-39*01	IGHD6-13*01	IGHJ5*02	100,0	19	CAIYQGYSSSWFSQVNWFDPW
P1615	8	IGHV4-39*01	IGHD6-13*01	IGHJ5*02	100,0	19	CASRRGYSSSWFNVVAVFDPW
IT01-0252-H1	8	IGHV4-39*07	IGHD6-19*01	IGHJ5*02	100,0	19	CATRQSYSSGWYGGVNWFDPW
P781	16	IGHV4-34*04	IGHD1-26*01	IGHJ6*02	95,0	24	CAGRIFYCYGGNCCNANYYYYGMDVW
P1082	16	IGHV4-34*01	IGHD2-15*01	IGHJ6*02	96,3	24	CAGRIFYCSGAGCDSEGFYYYYGLDVW
N4932	33	IGHV4-39*01	IGHD3-22*01	IGHJ4*02	100,0	17	CARILWYYYYSSGNGGDYW
N4969	262	IGHV3-30-3*01	IGHD2-2*01	IGHJ6*02	100,0	29	CAGRIFYCSGAGCDSEGFYYYYGLDVW

Online Supplementary Table S6. Average Δ Ct, median Δ Ct and SD values for the 83 genes analyzed in the present study.

Expression category	Median Δ Ct value
HIGH	≤ 6.6
INTERMEDIATE	$>6.6 \leq 9.9$
LOW	$>9.9 \leq 13.2$
NEG	>13.2

	Gene Symbol HUGO	Average Δ Ct value	Median Δ Ct value	SD	Expression Level
1	BTK	6,97	6,98	1,19	INTERMEDIATE
2	CASP8	8,90	8,98	1,13	INTERMEDIATE
3	CCL2	15,03	15,24	1,38	NEG
4	CD14	14,06	14,20	1,48	NEG
5	CD180	4,82	4,80	1,24	HIGH
6	CD80	11,47	11,51	2,51	LOW
7	CD86	7,12	6,76	2,23	INTERMEDIATE
8	CHUK	6,41	6,36	1,15	HIGH
9	CLEC4E	15,33	15,55	1,24	NEG
10	CSF2	15,32	15,44	1,13	NEG
11	CSF3	15,44	15,56	1,01	NEG
12	CXCL10	14,72	14,89	1,57	NEG
13	ECSIT	6,67	6,65	0,74	INTERMEDIATE
14	EIF2AK2	6,09	6,22	1,34	HIGH
15	ELK1	8,13	8,12	1,18	INTERMEDIATE
16	FADD	7,86	7,85	0,96	INTERMEDIATE
17	FOS	7,61	7,49	2,92	INTERMEDIATE
18	HMGB1	6,03	5,97	0,98	HIGH
19	HRAS	6,34	6,30	0,91	HIGH
20	HSPA1A	4,81	5,01	1,67	HIGH
21	HSPD1	5,84	5,91	1,08	HIGH
22	IFNA1	15,24	15,44	1,35	NEG
23	IFNB1	14,51	14,68	1,38	NEG
24	IFNG	10,77	10,52	2,77	LOW
25	IKBKB	4,85	4,90	1,20	HIGH
26	IL10	13,76	14,14	2,16	NEG
27	IL12A	7,79	7,73	1,13	INTERMEDIATE
28	IL1A	14,28	14,43	1,40	NEG
29	IL1B	12,49	12,71	2,22	LOW
30	IL2	15,05	15,19	1,39	NEG
31	IL6	13,56	14,14	2,46	NEG
32	IL8	11,43	11,16	2,79	LOW
33	IRAK1	8,60	8,36	1,74	INTERMEDIATE
34	IRAK2	8,17	8,16	1,23	INTERMEDIATE
35	IRF1	6,06	6,10	1,35	HIGH
36	IRF3	4,52	4,39	1,07	HIGH
37	JUN	4,65	4,74	1,86	HIGH
38	LTA	7,69	7,66	1,26	INTERMEDIATE
39	LY86	4,02	3,87	1,22	HIGH
40	LY96	6,80	6,82	0,91	INTERMEDIATE
41	MAP2K3	5,95	5,93	0,96	HIGH
42	MAP2K4	6,72	6,70	0,98	INTERMEDIATE
43	MAP3K1	2,51	2,40	1,05	HIGH
44	MAP3K7	7,67	7,68	1,00	INTERMEDIATE
45	MAP4K4	7,66	7,10	2,09	INTERMEDIATE
46	MAPK8	6,30	6,26	1,07	HIGH
47	MAPK8IP3	4,98	4,91	1,13	HIGH
48	MYD88	5,28	5,36	1,10	HIGH
49	NFKB1	4,86	4,89	1,01	HIGH
50	NFKB2	8,48	8,39	0,95	INTERMEDIATE
51	NFKBIA	2,97	2,99	1,08	HIGH
52	NFKBIL1	8,91	9,55	2,37	INTERMEDIATE
53	NFRKB	7,16	7,09	0,97	INTERMEDIATE
54	NR2C2	5,47	5,49	0,87	HIGH
55	PELI1	3,17	3,04	1,20	HIGH
56	PPARA	7,06	7,05	1,00	INTERMEDIATE
57	PRKRA	6,12	6,04	0,82	HIGH
58	REL	4,24	4,22	1,00	HIGH
59	RELA	4,72	4,82	1,18	HIGH
60	RIPK2	6,59	6,58	0,89	HIGH
61	SARM1	8,10	7,97	1,06	INTERMEDIATE
62	SIGIRR	13,58	13,42	1,31	NEG
63	TAB1	6,91	6,78	1,08	INTERMEDIATE
64	TBK1	7,29	7,37	0,80	INTERMEDIATE
65	TICAM1	6,00	6,00	0,87	HIGH
66	TICAM2	10,36	10,24	1,15	LOW
67	TIRAP	10,66	10,56	1,09	LOW
68	TLR1	8,85	8,91	0,98	INTERMEDIATE
69	TLR10	7,33	7,11	1,27	INTERMEDIATE
70	TLR2	10,78	10,70	1,68	LOW
71	TLR3	15,04	15,35	1,50	NEG
72	TLR4	12,05	11,96	2,06	LOW
73	TLR5	14,82	15,11	1,61	NEG
74	TLR6	6,99	6,94	0,91	INTERMEDIATE
75	TLR7	6,09	6,01	0,92	HIGH
76	TLR8	12,27	12,73	2,43	LOW
77	TLR9	10,63	10,51	1,27	LOW
78	TNF	7,51	7,61	1,51	INTERMEDIATE
79	TNFRSF1A	14,41	14,59	1,29	NEG
80	TOLLIP	7,71	7,66	0,82	INTERMEDIATE
81	TRAF6	6,29	6,34	0,87	HIGH
82	UBE2N	4,20	4,21	0,95	HIGH
83	UBE2V1	11,78	11,55	1,40	LOW

Online Supplementary Table S7. Flow cytometry results from 30 CLL cases analyzed in the present study. The data reported indicate percentage of positive cells.

CASE ID	TLR1	TLR2	TLR4	TLR6	TLR7	TLR8	TLR9	TLR10	CD80	CD86
P103	8,2	21,1	2,0	5,0	70,7	54,7	5,1	21,2	0,7	63,9
P1156	18,1	22,3	3,1	1,8	71,8	24,6	0,9	75,7	0,4	15,5
P1188	51,4	36,6	6,4	4,9	75,5	26,6	0,9	80,3	0,4	54,5
P1540	55,2	40,2	4,8	6,4	71,6	3,6	1,6	84,2	4,2	75,7
P1626	21,3	19,1	2,6	8,1	63,5	21,1	44,9	34,3	3,9	9,9
P1697	8,5	58,5	0,6	4,1	68,6	35,0	19,5	39,9	0,4	39,5
P2355	63,0	25,9	1,9	18,7	87,1	32,5	3,7	46,9	0,2	15,1
P3492	50,9	41,6	6,5	8,9	71,4	25,7	4,4	38,1	0,1	6,5
P3870	62,0	43,9	1,5	6,2	81,1	56,9	3,0	93,5	4,6	21,0
P427	19,9	28,5	2,9	4,1	65,6	45,7	3,0	48,1	2,7	22,4
P4383	41,2	37,7	3,7	5,7	60,9	20,3	2,5	76,8	0,5	18,1
P4557	37,3	12,1	2,4	7,1	54,6	25,2	1,8	69,4	0,6	44,9
P4699	85,4	43,9	2,8	10,1	93,7	30,7	0,9	97,7	2,0	51,1
P4712	21,6	31,7	2,4	6,2	62,8	35,4	4,0	42,7	3,1	18,0
P4994	28,5	21,2	0,6	6,0	59,6	92,4	2,3	18,3	1,0	39,1
P5017	27,7	34,6	2,7	4,4	66,7	23,9	1,1	53,1	0,1	13,5
P5092	24,8	45,0	2,1	4,7	64,6	33,8	3,9	53,3	0,2	15,8
P511	6,8	36,4	2,2	6,7	82,8	52,8	4,8	57,5	0,3	50,2
P5283	13,3	42,4	0,7	6,3	85,1	26,0	24,3	48,5	0,1	23,7
P571	67,3	56,9	3,0	13,8	92,0	45,8	1,9	82,1	0,1	23,9
P5949	36,0	37,2	4,0	4,6	86,0	34,9	2,2	75,3	0,1	19,1
P6124	37,2	32,3	3,1	5,6	83,6	57,0	1,2	54,5	0,1	34,7
P7317	5,6	17,2	3,0	4,9	77,7	16,1	3,1	19,8	0,5	13,7
P7395	56,0	34,6	6,3	5,8	80,5	50,0	6,9	70,7	0,1	5,3
P775	42,7	23,0	3,7	6,2	87,1	36,8	4,6	52,7	0,8	94,2
P781	29,7	32,0	2,3	5,7	92,5	67,7	3,9	46,0	2,6	52,2
P8762	46,0	30,8	1,8	3,4	75,3	63,3	2,1	70,6	0,2	27,5
P3551	39,6	42,0	2,1	3,5	70,9	62,3	2,7	33,6	2,8	45,3
P3916	12,7	15,3	2,2	7,0	71,2	45,6	2,4	26,2	2,8	42,0
P5610	44,3	36,4	2,8	6,0	86,8	98,6	4,5	44,5	2,1	60,5

Online Supplementary Table S8. Western blotting results from 59 CLL cases analyzed in the present study. Data reported indicate ratio between the OD of the sample and the OD of actin.

CASE ID	TLR1	TLR2	TLR8	TLR9
N1713	0,193	0,313	0,000	0,000
N1777	0,192	0,132	0,000	0,000
N1887	0,074	0,185	0,108	0,000
N2687	0,422	0,310	0,081	0,000
N3517	0,215	0,128	0,148	0,131
N3527	0,065	0,156	0,097	0,000
N4617	0,168	0,170	0,000	0,000
N4691	0,156	0,062	0,138	0,113
N4707	0,060	0,114	0,137	0,000
N4969	0,206	0,254	0,118	0,000
N6015	0,275	0,179	0,000	0,062
N6084	0,187	0,240	0,119	0,123
P103	0,154	0,154	0,104	0,000
P1050	0,118	0,105	0,000	0,060
P1060	0,090	0,162	0,083	0,070
P1097	0,228	0,155	0,101	0,000
P1289	0,115	0,143	0,189	0,000
P1615	0,065	0,120	0,093	0,000
P1894	0,087	0,198	0,071	0,040
P2329	0,178	0,293	0,000	0,000
P2528	0,078	0,098	0,170	0,000
P2685	0,110	0,110	0,117	0,000
P2740	0,175	0,090	0,091	0,070
P280	0,090	0,080	0,167	0,000
P3020	0,084	0,122	0,000	0,000
P3021	0,092	0,430	0,112	0,000
P3073	0,120	0,070	0,082	0,000
P317	0,096	0,189	0,162	0,031
P325	0,101	0,079	0,198	0,039
P326	0,063	0,090	0,142	0,000
P3492	0,112	0,050	0,000	0,000
P3716	0,130	0,065	0,101	0,107
P3870	0,076	0,090	0,078	0,000
P3966	0,080	0,173	0,125	0,000
P4086	0,214	0,264	0,136	0,084
P427	0,087	0,142	0,089	0,000
P4383	0,080	0,096	0,080	0,000
P4438	0,088	0,149	0,000	0,020
P450	0,059	0,103	0,138	0,037
P4994	0,088	0,146	0,203	0,000
P5092	0,218	0,268	0,103	0,051
P5231	0,155	0,213	0,122	0,000
P5359	0,190	0,100	0,000	0,000
P571	0,120	0,082	0,000	0,000
P585	0,110	0,115	0,101	0,035
P5949	0,130	0,184	0,000	0,050
P5975	0,205	0,264	0,101	0,000
P6079	0,159	0,166	0,130	0,000
P6090	0,160	0,730	0,125	0,000
P611	0,120	0,351	0,000	0,000
P6124	0,115	0,202	0,067	0,000
P6237	0,099	0,110	0,167	0,000
P6832	0,145	0,384	0,000	0,000
P7057	0,060	0,150	0,000	0,000
P711	0,143	0,620	0,062	0,034
P7395	0,154	0,108	0,084	0,000
P8071	0,160	0,223	0,093	0,000
N6450	0,285	0,195	0,000	0,000
N6490	0,234	0,186	0,196	0,000

Online Supplementary Table S9. Gene expression profiles in relation to BcR molecular features. Average ΔCt , $2^{-\Delta\Delta\text{Ct}}$ values, fold difference and t-test values in subgroups of CLL cases.

A. M VS UM						
GENE	M $\Delta\text{Ct AVERAGE}$	UM $\Delta\text{Ct AVERAGE}$	M vs UM $2^{-\Delta\Delta\text{Ct}}$	M vs UM FOLD DIFFERENCE	M vs UM t-TEST	
CD86	6,37	8,54	4,50	4,50	0,0000000002	
IFNG	10,30	11,67	2,58	2,58	0,0020	
CD80	11,02	12,31	2,44	2,44	0,0017	
TLR4	11,69	12,73	2,05	2,05	0,0019	
IL6	13,23	14,23	2,00	2,00	0,0047	
NFKBIL1	9,24	8,30	0,52	-2,02	0,0289	
TLR8	12,60	11,70	0,54	-2,07	0,0215	

B. #1 vs #4						
GENE	#1 $\Delta\text{Ct AVERAGE}$	#4 $\Delta\text{Ct AVERAGE}$	#1 vs #4 $2^{-\Delta\Delta\text{Ct}}$	#1 vs #4 FOLD DIFFERENCE	#1 vs #4 t-TEST	
CD86	8,13	5,46	0,16	-6,35	0,0016	
TLR4	13,78	11,82	0,26	-3,89	0,0254	
TLR7	5,39	6,42	2,05	2,05	0,0313	
NFKB1A	2,63	3,60	1,96	2,06	0,0244	

C. #1 vs #8						
GENE	#1 $\Delta\text{Ct AVERAGE}$	#8 $\Delta\text{Ct AVERAGE}$	#1 vs #8 $2^{-\Delta\Delta\text{Ct}}$	#1 vs #8 FOLD DIFFERENCE	#1 vs #8 t-TEST	
NFKB1A	2,63	4,58	0,26	-3,88	0,0078	
RIPK2	6,34	8,17	0,28	-3,55	0,0284	
MAP4K4	8,59	6,46	4,37	4,37	0,0314	
TLR4	13,78	9,42	20,56	20,56	0,0364	

D. #4 vs #8						
GENE	#4 $\Delta\text{Ct AVERAGE}$	#8 $\Delta\text{Ct AVERAGE}$	#4 vs #8 $2^{-\Delta\Delta\text{Ct}}$	#4 vs #8 FOLD DIFFERENCE	#4 vs #8 t-TEST	
LY96	6,76	5,74	2,03	2,03	0,0112	
RIPK2	6,70	8,17	0,36	-2,76	0,0385	
CD86	5,46	7,89	0,19	-5,40	0,0076	

E. #4 vs M						
GENE	#4 $\Delta\text{Ct AVERAGE}$	M $\Delta\text{Ct AVERAGE}$	#4 vs M $2^{-\Delta\Delta\text{Ct}}$	#4 vs M FOLD DIFFERENCE	#4 vs M t-TEST	
CD86	5,46	6,45	2,00	2,00	0,0015	
IL10	14,73	13,55	0,44	-2,26	0,0491	
IFNG	12,27	10,15	0,23	-4,37	0,0539	

F. #4 vs IGHV4-34						
GENE	#4 $\Delta\text{Ct AVERAGE}$	IGHV4-34 $\Delta\text{Ct AVERAGE}$	#4 vs IGHV4-34 $2^{-\Delta\Delta\text{Ct}}$	#4 vs IGHV4-34 FOLD DIFFERENCE	#4 vs IGHV4-34 t-TEST	
NFKB1A	3,60	2,60	0,50	-2,00	0,0078	
IL10	14,73	13,32	0,38	-2,66	0,0454	
CD86	5,46	6,41	1,94	2,04	0,0568	