

SUPPLEMENTAL MATERIAL

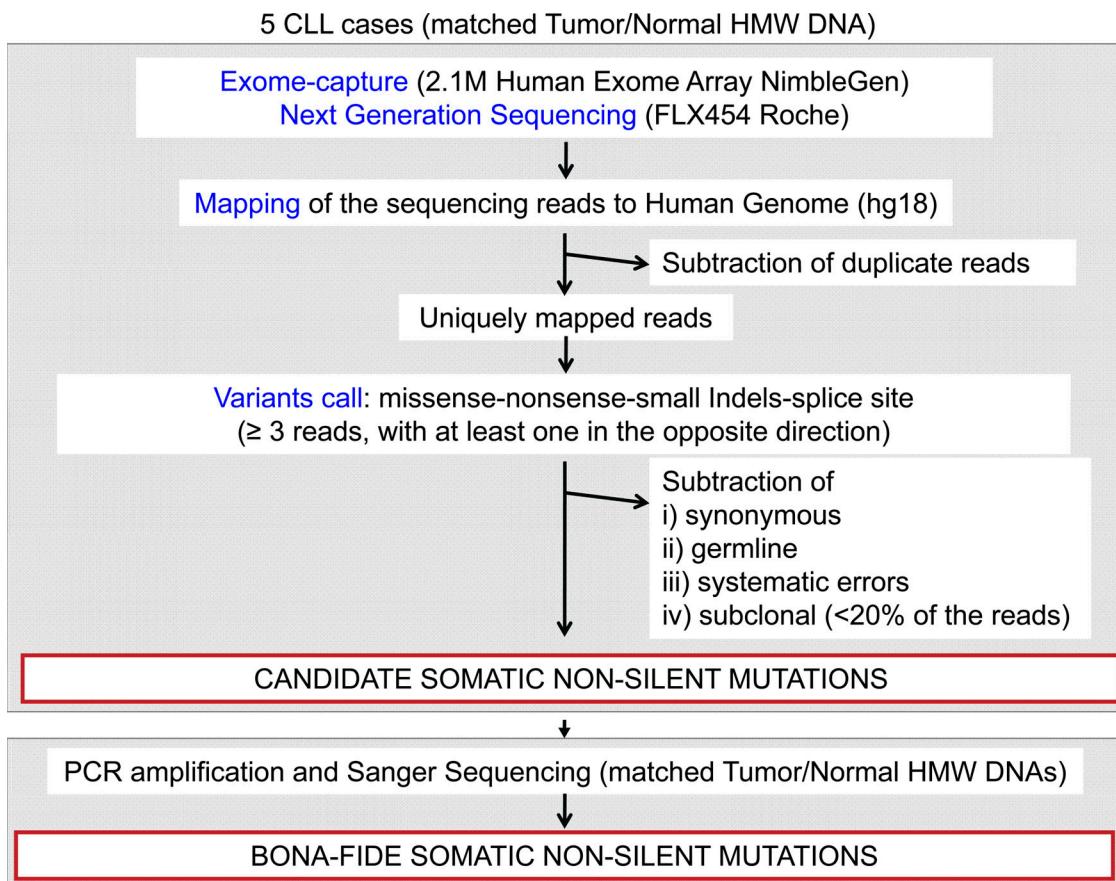
Fabbri et al., <http://www.jem.org/cgi/content/full/jem.20110921/DC1>

Figure S1. WES approach. Computational pipeline used for the analysis of 454 WES data and the validation of candidate sequence variants.

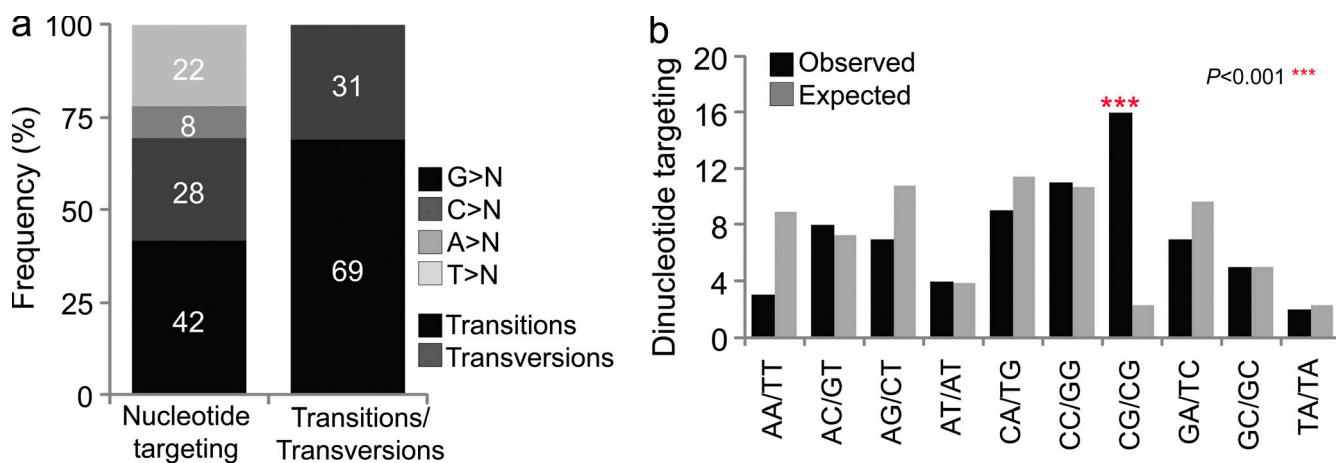


Figure S2. Mutation spectrum in CLL. (a) Nucleotide substitution pattern of nonsynonymous mutations identified in the exomes of the 5 CLL discovery cases. (b) Number of mutations observed at specific dinucleotides (black bars). Gray bars indicate the expected number of events calculated based on the sequence composition of the target exomic region (see Materials and methods for details).

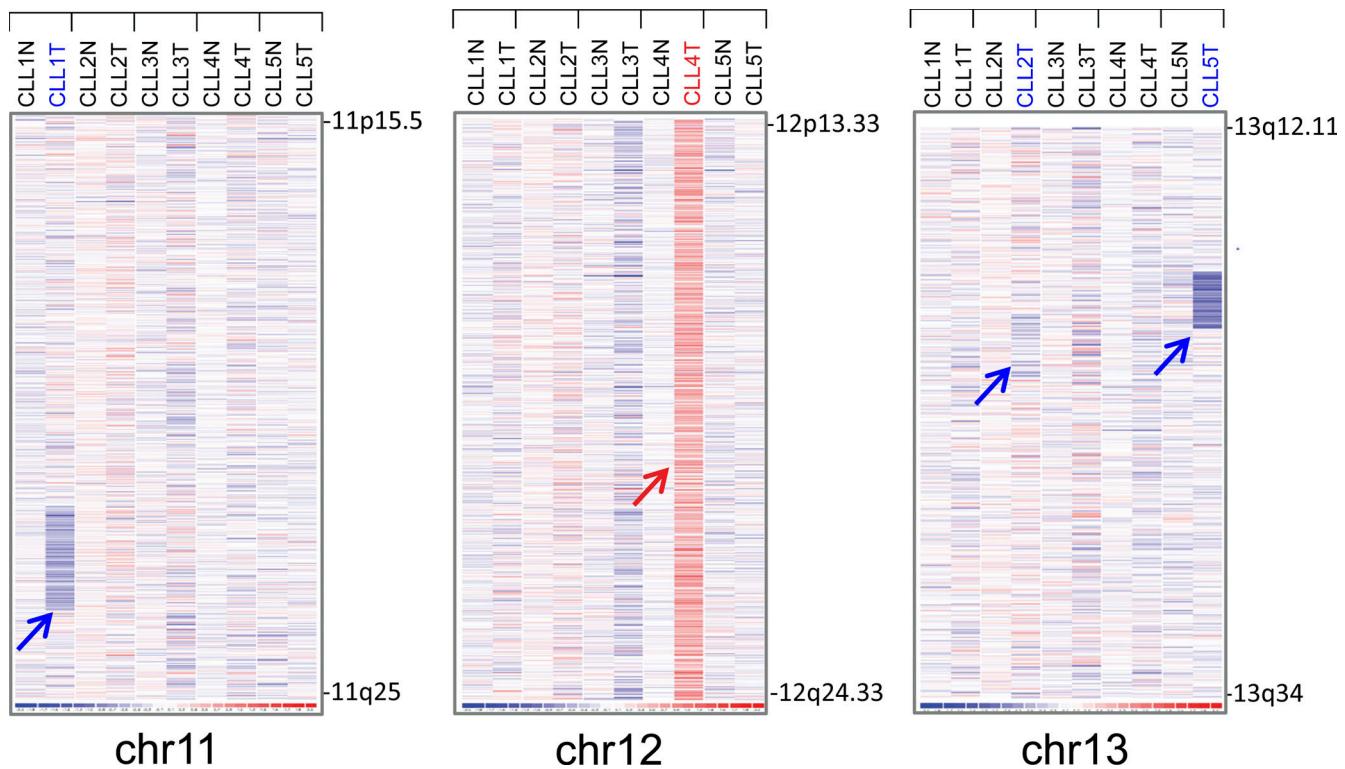


Figure S3. Genome-wide SNP array analysis identifies known CLL-associated CNAs. Heatmap showing median-smoothed \log_2 copy number ratio of three chromosomal regions that were found to carry genomic aberrations by SNP array analysis, in concordance with the results of FISH analysis. Arrows point to the aberrant interval. In the red-blue scale, white corresponds to a normal (diploid) copy number log ratio, blue is deletion, and red is gain. CNA, copy number aberrations.

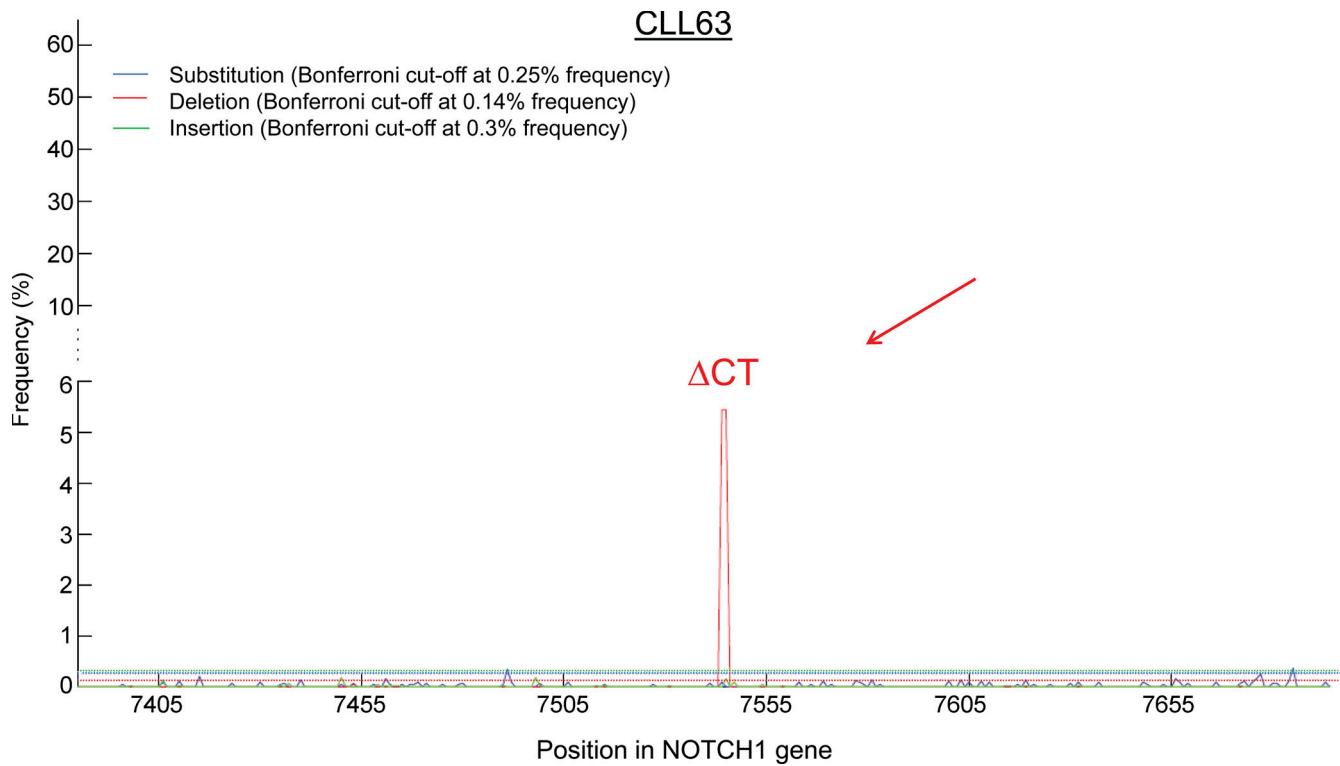


Figure S4. Detection of a subclonal *NOTCH1* mutation in the CLL phase of the CLL63/RS63 pair by ultradepth 454 sequencing. 30 mo elapsed between collection of the CLL sample (diagnosis of CLL) and collection of the RS sample (diagnosis of RS). The CT deletion at position 7544–7545 (available from EMBL/GenBank/DDBJ under accession no. NM_017617.2) was detected at 5.3% frequency, passing the cut-offs established by Bonferroni-corrected probabilities derived from fitting a negative binomial or Luria-Delbrück distribution to variant depths (see Materials and methods). The change in the y-axis scale is devised to compare the cut-off frequencies to the mutation frequencies.

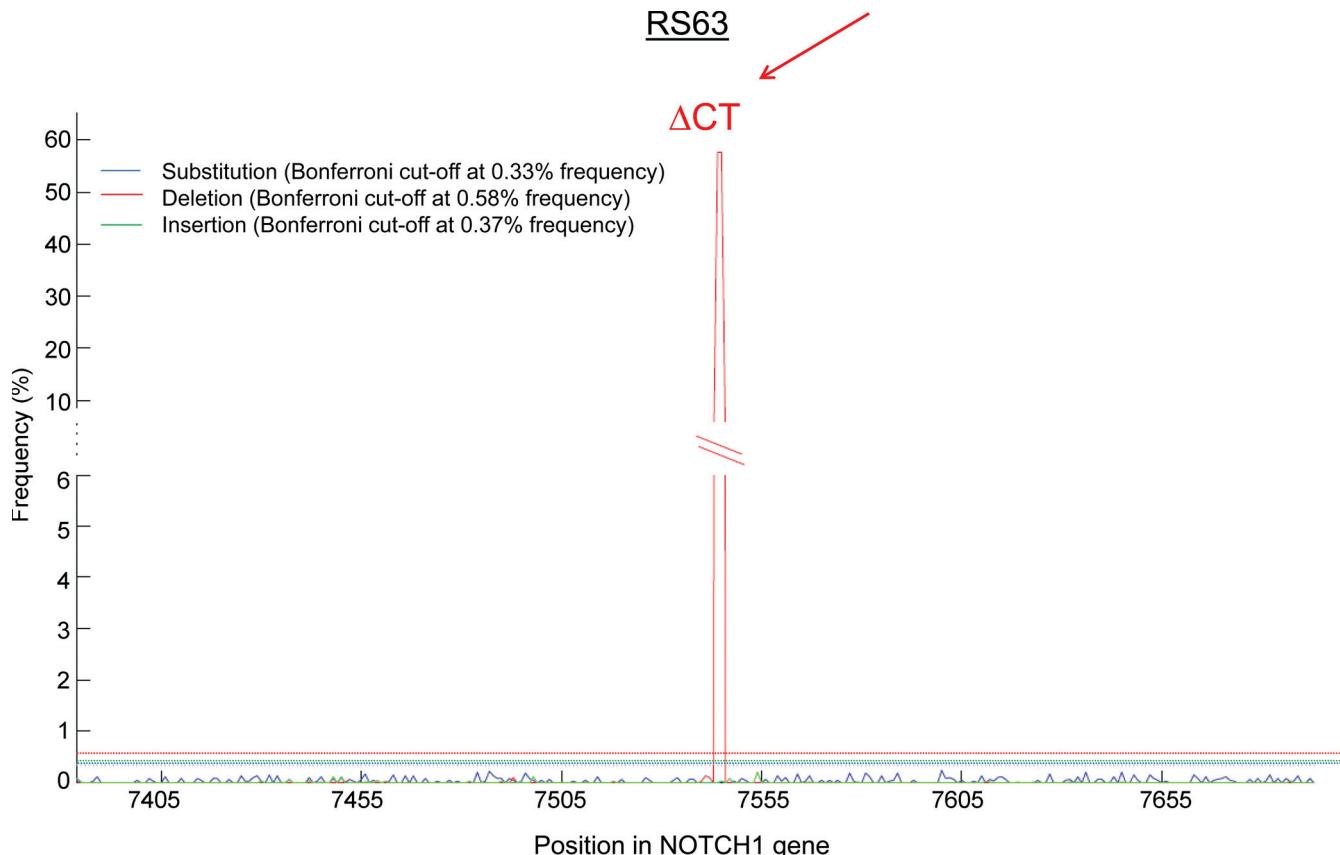


Figure S5. Detection of a *NOTCH1* mutation in the RS phase of the CLL63/RS63 pair through ultradeep 454 sequencing. 30 mo elapsed between collection of the CLL sample (diagnosis of CLL) and collection of the RS sample (diagnosis of RS). The CT deletion at position 7544–7545 (accession no. NM_017617.2) was detected at 58.6% frequency. The change in the y-axis scale is devised to compare the Bonferroni cut-off frequencies (see Materials and methods) to the mutation frequency.

SUPPLEMENTAL RESULTS

Identification and validation of WES candidate somatic mutations

The WES approach provided \sim 2.5 millions reads/case (average length 305 bp), with a mean depth of $10.1\times$ (range: 8.6 to 11.8 per case), and \sim 79% of the target sequence being covered by at least 5 reads (range: 74.6 to 86.3%; Table S2). Based on comparison with the heterozygous SNP call rate obtained in the same cases by analysis of Affymetrix SNP 6.0 array data, the sensitivity for the identification of heterozygous somatic mutations was estimated to be \sim 60%. This level of resolution allowed us to provide an initial estimate of the order of magnitude of the mutation load in this disease, and to identify genes that are recurrently mutated at high frequency (probability of detecting mutations in genes that are affected at \sim 30% prevalence = 63%).

Overall, 276 variants were predicted by the WES analysis to be specifically associated with the tumor sample in the 5 patients analyzed. Of these, 38 were confirmed to be somatic in origin by Sanger sequencing of the corresponding paired tumor and normal genomic DNA, whereas 170 were also found in the matched normal genomic DNA, thus representing germline polymorphisms that were not previously annotated and escaped detection during the high-throughput sequencing analysis, possibly because of the relatively low mean depth of coverage. The remaining 68 variants were absent in both tumor and normal genomic DNA when tested by Sanger sequencing.

Table S1. Features of the 5 CLL discovery cases analyzed by Whole Exome Sequencing

Characteristic	CLL patient ID				
	CLL1	CLL2	CLL3	CLL4	CLL5
Age at diagnosis, years	67	71	46	69	63
Sex	F	F	M	F	M
Rai stage	0	IV	II	0	II
Lymphocytes, x10 ⁹ /Liter	38	124	36	16.7	68.5
Hb, g/dl	13.4	9.5	14.4	13.1	12
Platelets, x10 ⁹ /Liter	249	159	166	155	135
B2M, mg/Liter	2.7	6.3	2.2	3.3	3.2
LDH, U/Liter	551	500	340	480	372
Initial management	Wait and see	Treatment	Wait and see	Wait and see	Wait and see
Time to first treatment (months)	86	Therapy started at diagnosis	33	6	9
Transformation to DLBCL	yes	no	no	no	no
Survival (months)	166	55	107	216	108
Status	Death	Alive	Alive	Death	Alive
CD19+/CD5+ cells (%)	90	80	90	90	95
IGHV mutational status	Unmutated	Unmutated	Unmutated	Mutated	Mutated
V gene	1-69*01	3-30*04	1-69*01	4-34*01	3-23*01
D gene	3-3*01	5-24*01	3-16*02	3-22*01	5-5*01
J gene	6*02	6*02	6*02	3*02	4*02
IGHV homology (%)	100	100	100	94.38	92.34
Stereotyped HCDR3	Stereotyped	Not stereotyped	Not stereotyped	Not stereotyped	Not stereotyped
FISH ^a	del11q22-q23	del13q14 ^c	Normal	+12, 14q translocation	del13q14
ZAP-70 expression ^b	positive	negative	positive	negative	negative
CD38 expression ^b	negative	negative	positive	positive	negative
TP53 mutational status	Unmutated	Unmutated	Unmutated	Unmutated	Unmutated

^a based on FISH analysis for del13q, del11q, trisomy12, del17p, and 14q translocation. ^b Evaluated by FACS analysis. ZAP-70 expression was considered positive if the percentage of positive leukemic cells was above 20%, CD38 expression was considered positive if the percentage of positive leukemic cells was above 30%.

^c present in 18% of nuclei.

Table S2. Results of Titanium 454 sequencing and mapping after whole exome capture

Sample ID	Target Region Coverage (%)			Mean Depth ^b	N of mapped reads (%)	Unique reads in region (%)	N of variant reads (HC)		
	1X	≥5X	HCC ^a				Total	Known	Novel ^c
CLL1-T	95.8	74.6	67.2	8.6	2374973 (98.78)	1556726 (68.6)	9730	9163	567
CLL1-N	95.9	77.2	70	9.1	2490456 (99.06)	1679964 (70.4)	10162	9531	631
CLL2-T	96.5	86.3	81	11.8	2684394 (99.35)	2008402 (77.8)	12457	11675	782
CLL2-N	95.8	80.8	75.3	11.2	2643134 (99.18)	1870633 (73.9)	11392	10652	740
CLL3-T	95.7	76.5	69.6	9.1	2399542 (99.17)	1664769 (72.6)	10459	9744	715
CLL3-N	95.8	78.4	73.1	11.1	2531945 (99.30)	1849789 (76.2)	10925	10240	685
CLL4-T	95.7	75.8	68.8	9.1	2157095 (99.14)	1560948 (75.7)	10201	9603	598
CLL4-N	96.2	78.6	71	9	2389230 (99.10)	1633385 (71.7)	10956	10244	712
CLL5-T	96.4	82.2	74.9	10.3	2440574 (98.95)	1746590 (75.3)	12459	11556	903
CLL5-N	96.5	83.7	77.3	11.6	2695359 (99.27)	1969017 (76.3)	12367	11520	847

^a High Confidence Coverage, i.e. % of the target region where coverage depth was estimated to be sufficient for detecting both alleles of heterozygous variants. This value can be taken as an estimate of the mutation-detection sensitivity (see methods).

^b Mean number of sequence reads covering the target exome.

^c N of variant reads not reported in dbSNP database, before cross-comparison with paired normal.

Abbreviations: HC, high confidence.

Table S3. Validated somatic mutations identified by whole exome sequencing in the CLL discovery panel

Sample ID	Gene ID	Mutation type	Chromosomal position ^a	Base change	Aminoacid change	read%	CovT	CovN	Affected domain	Polyphen-2 Prediction	Score	Expression ^b	COSMIC v52 (N of Mutated cases)
CLL1	ACTN1	missense	chr14:68422007	C>T	A425T	67%	7	6	spectrin-2	benign	0	A	1
CLL1	AP1G2	nonsense	chr14:23102859	G>A	R380*	30%	13	9	nd	altering	N/A	P	1
CLL1	BBS2	nonsense	chr16:55105893	G>T	Y106*	43%	7	15	nd	altering	N/A	P	1
CLL1	BIRC3	frameshift deletion	chr11:101706960	Δ 32bases	E368fs	30%	11	9	interdomain (BIR3 and CARD)	altering	N/A	P	4
CLL1	FRMPD1	missense	chr9:37736153	C>T	P1375L	75%	4	4	nd	benign	0.025	A	7
CLL1	FSCN3	nonsense	chr7:12025782	C>T	R340*	50%	8	7	actin-crosslinking domain	altering	N/A	A	1
CLL1	GOLM1	missense	chr9:87882296	G>A	R54C	42%	12	8	lumenal	altering	0.948	A	-
CLL2	ABCA7	missense	chr19:996094	G>A	G437S	40%	15	11	extracellular	altering	0.98	P (377.6)	3 (8) ^e
CLL2	ALPK3	missense	chr15:83202310	G>A	G1315S	33%	14	6	nd	altering	0.949	A (5.3)	7
CLL2	DNAH2	missense	chr17:7618928	T>C ^c	L1543P	20%	16	17	interdomain (TPR1-TPR2)	altering	0.999	NA	7
CLL2	GPRASP1	missense	chrX:101798114	A>T	I873F	23%	23	12	Glu-rich region	altering	0.569	P (1220.8)	2
CLL2	INHBC	missense	chr12:56115006	G>A	G24S	47%	16	10	propeptide (AA19-236)	benign	0.019	P (73.7)	-
CLL2	NOTCH1	frameshift deletion	chr9:138510742	Δ 28 bases ^d	P2415fs	-	-	-	PEST domain	altering	N/A	NA	628 (812) ^e
CLL2	SCGN	missense	chr6:25809474	G>A ^c	V255M	18%	18	11	calcium binding	benign	0.002	A	3
CLL2	WHSC1	missense	chr4:1932599	G>A	E109K	33%	13	27	SET domain	altering	0.982	P (290.5)	6
CLL2	YBX1	missense	chr14:2935456	G>A	A226T	43%	7	6	nd	benign	0	P (7900.4)	4
CLL2	ZNF300	missense	chr5:150255232	G>C	Q588E	50%	8	3	ZF N.12 (AA 577-599)	altering	0.984	A (15.3)	1
CLL3	EMX2	nonsense	chr10:119297581	C>T	Q203*	38%	14	11	homeobox DNA binding	altering	N/A	NA	-
CLL3	MPP2	missense	chr17:39312768	C>T	E447K	33%	9	14	guanylate kinase-like	altering	0.418	A (93.5)	-
CLL3	NOTCH1	frameshift deletion	chr9:138510462	Δ C ^c T ^d	P2515fs	-	-	-	PEST domain	altering	N/A	NA	628 (812) ^e
CLL3	OR4D11	missense	chr11:59028199	C>A	T192N	37%	10	20	extracellular	altering	0.924	NA	1
CLL3	SNX9	frameshift deletion	chr6:158250968	Δ ACTG	F290fs	44%	9	11	PX domain	altering	N/A	P (829.5)	2
CLL3	TGM7	missense	chr15:41366909	G>T	N242K	33%	9	25	nd	altering	0.978	A (9.5)	2
CLL4	ASPHD1	missense	chr16:29820259	C>T	R156C	33%	9	2	lumenal	altering	0.974	A (97.3)	-
CLL4	FAM123B	missense	chrX:63329239	T>A	E218V	54%	13	9	nd	altering	0.903	A (12.1)	110
CLL4	HIST1H2AM	missense	chr6:27968622	G>C	N95K	33%	12	14	kistone-fold	altering	0.983	P (180.8)	2
CLL4	MOG	missense	chr6:29735146	C>T	R54C	71%	7	9	Ig-like V-type, EC	altering	0.999	A (30.2)	1
CLL4	OR5AN1	missense	chr11:58888757	T>G	S84A	45%	11	12	extracellular	altering	0.508	NA	1
CLL4	PLEKHG5	missense	chr1:6453687	A>T	Y525N	33%	12	8	nd	altering	1	A (25.3)	1
CLL4	SLC1A1	missense	chr9:4575404	A>G	D474G	56%	9	10	extracellular	benign	0.012	A (64.4)	-
CLL5	ACTL6A	missense	chr3:180770679	C>T	P78L	57%	7	7	nd	altering	0.979	P	1
CLL5	ADCY8	missense	chr8:131862024	T>C	R1184G	75%	8	0	cytoplasmic	benign	0.174	NA	2
CLL5	GDF2	missense	chr10:48033768	G>A	T369M	60%	7	0	chain (AA320-429)	altering	0.999	A	2
CLL5	HEXIM1	missense	chr17:40582696	C>T	A119V	56%	12	0	nd	benign	0	P	1
CLL5	MYD88	missense	chr3:38157645	T>C	L265P	50%	9	10	TIR domain	altering	0.999	P	92 (93) ^e
CLL5	NHL2	missense	chrX:71275588	G>A	G489S	100%	15	0	nd	altering	0.906	A	-
CLL5	PUM2	missense	chr2:20317179	T>C	N983S	67%	12	11	Pumilio 4	benign	0	P	1
CLL5	SORCS1	missense	chr10:108327217	G>C	P1153R	44%	9	0	cytoplasmic	benign	0.001	P	1
CLL5	ST18	missense	chr8:53240291	T>C	T418A	50%	8	0	ZF N.2 (AA 409-439)	altering	0.988	NA	4
CLL5	ZNF644	missense	chr19:1176521	T>G	Y993S	55%	12	13	ZF N.6 (AA 963-987)	altering	0.995	P	2

^a Numbering according to NCBI Build 36.1 (hg18).^b Expression refers to the mutated case or to an independent panel of 16 CLL samples. A gene is considered expressed if the probe is called Present in the mutated case or if it has >90% of Present calls in the 16 CLL cases panel. The value reported in brackets corresponds to the signal value from the most highly expressed probe in the U133 Plus 2.0 platform.^c Subclonal mutation based on Sanger sequencing.^d Mutation identified through Sanger targeted re-sequencing of the mutational hotspots on NOTCH1 (HD-JME-PEST).^e When the number of mutations exceeds the number of mutated cases, its value is reported in brackets.

Abbreviations: read%, fraction of sequence reads showing the variant nucleotide; CovT, coverage tumor; CovN, coverage normal; nd, not determined; N/A, not available; NA, probe not informative or not present; A, Absent call; P, Present call; HD, heterodimerization domain; JME, juxtamembrane domain.

Table S4. Segments of tumor-acquired copy number changes identified in the CLL discovery panel

Sample ID	Chr	Cytoband	Start ^a	End ^a	Segment size(kb)	Copy number	N of genes in segment	First 10 genes in segment	N of miRNA in segment	First 10 miRNA in segment
CLL1	4	q21.1--q21.23	77636110	86139515	8503.405	1.43	44	SHROOM3 ANKRD56 SEPT11 CCNI CCNG2 CXCL13 CNOT6L MRPL1 FRAS1 ANXA3	1	hsa-mir-575
CLL1	4	q21.23	86587986	86867769	279.783	1.42	1	ARHGAP24	0	-
CLL1	6	p12.1	55703661	55723285	19.624	1.03	0	-		
CLL1	11	q21.1--q23.3	94306466	116378519	22072.053	1.4	124	CWC15 KDM4D KDM4DL SFRS2B ENDOD1 SESN3 FAM76B CEP57 MTMR2 MAML2	2	hsa-mir-34b hsa-mir-34c
CLL1	15	q26.1	89423285	89433097	9.812	1.06	0	-		
CLL2	10	q26.13	123263324	123337486	74.162	1.53	1	FGFR2	0	-
CLL2	13	q14.2--q21.2	47171346	60112584	12941.238	1.63	62	SUCLA2 NUDT15 MED4 ITM2B RB1 LPAR6 RCBTB2 CYSLTR2 FNDC3A MLNR	3	hsa-mir-16-1 hsa-mir-15a hsa-mir-1297
CLL3	14	q23.2	63771822	63773115	1.293	0.78	1	ESR2	0	-
CLL4	12	p13.33--q24.33	20691	132288250	132267.559	2.99	1085	IQSEC3 SLC6A12 SLC6A13 KDM5A CCDC77 B4GALNT3 NINJ2 WNK1 RAD52 ERC1	28	hsa-mir-3649 hsa-mir-200c hsa-mir-141 hsa-mir-1244-3 hsa-mir-613 hsa-mir-614 hsa-mir-3974 hsa-mir-920 hsa-mir-4302 hsa-mir-4698
CLL5	8	q24.3	140701232	141264442	563.210	1.06	2	KCNK9 TRAPPC9	0	-
CLL5	13	q14.11--q14.3	40457346	50287238	9829.892	1.06	69	ELF1 WBP4 KBTBD6 KBTBD7 MTRF1 NAA16 OR7E37P C13orf15 KIAA0564 DGKH	2	hsa-mir-16-1 hsa-mir-15a
CLL5	18	p11.32	2664799	2723565	58.766	0.96	1	SMCHD1		

^a Numbering according to NCBI Build 36.1 (hg18).

Table S5. Features of the 48 CLL cases belonging to the screening panel

Case ID	Age at diagnosis, years	Sex	Rai stage	Lymphocytesx 10 ⁹ /L	Hb g/dL	Platelets x10 ⁹ /L	Initial management	Time to first treatment (months)	Transformation to DLBCL	Survival (months)	Status	CD38 ≥30%	ZAP70 ≥20%	V gene	D gene	J gene	IGHV homology (%)	IGHV M status	Stereotyped HCDR3	Trisomy 12	del17p13	del11q22-q23	del13q14	TP53 M
CLL9	60	M	0	18.9	17.0	219	Wait and see	Untreated	No	232	alive	Neg	Neg	2-5*01	6-13*01	4*02	95.53	M	No	Neg	Neg	Neg	Pos	UM
CLL10	64	M	I	37.9	13.5	193	Wait and see	Untreated	No	101	alive	Neg	Neg	4-30-4*01	1-7*01	6*03	90.90	M	No	Neg	Neg	Neg	Pos	UM
CLL11	64	F	0	12.2	13.1	166	Wait and see	Untreated	No	62	alive	Neg	Neg	4-61*01	5-24*01	5*02	92.85	M	No	Neg	Neg	Neg	Pos	UM
CLL12	67	M	0	15.2	15.4	257	Wait and see	Untreated	No	65	alive	Neg	Neg	3-33*01	6-13*01	4*02	92.27	M	No	Neg	Neg	Neg	Pos	UM
CLL13	71	M	0	16.6	15.5	126	Wait and see	Untreated	No	76	alive	Neg	Neg	4-61*02	3-10*01	4*02	93.78	M	No	Neg	Neg	Neg	Pos	UM
CLL14	73	F	I	23.1	14.9	164	Wait and see	27	No	76	alive	Neg	Neg	4-59*08	1-26*01	4*02	95.71	M	No	Neg	Neg	Neg	Neg	UM
CLL15	68	F	IV	170.5	6.9	101	Treatment	therapy started at diagnosis	No	49	alive	Neg	Neg	4-4*01	3-10*01	4*02	96.27	M	No	Neg	Neg	Neg	Pos	UM
CLL16	82	F	IV	12.5	13.2	92	Wait and see	25	No	30	death	Neg	Pos	4-39*01	1-1*01	4*02	95.53	M	No	Neg	Neg	Neg	Pos	UM
CLL17	68	F	0	19.7	13.4	179	Wait and see	Untreated	No	191	alive	Neg	Neg	4-39*01	6-19*01	6*02	96.56	M	No	Neg	Neg	Neg	Pos	UM
CLL18	64	M	I	16.0	16.2	168	Wait and see	Untreated	No	55	alive	Neg	Pos	4-34*01	2-2*01	4*02	93.89	M	No	Neg	Neg	Neg	Pos	UM
CLL19	74	F	0	16.9	12.3	371	Wait and see	Untreated	No	51	alive	Neg	Neg	4-34*01	6-6*01	4*02	94.81	M	No	Neg	Neg	Neg	Pos	UM
CLL20	59	M	0	15.3	14.3	242	Wait and see	110	No	123	alive	Neg	Neg	4-34*01	5-5*01	4*02	96.14	M	No	Neg	Neg	Neg	Pos	UM
CLL21	55	F	0	14.8	15.7	268	Wait and see	Untreated	No	124	alive	Neg	Neg	4-34*01	2-15*01	6*02	96.19	M	Yes	Neg	Neg	Neg	Neg	UM
CLL22	55	M	0	17.8	13.6	194	Wait and see	Untreated	No	300	alive	Neg	Neg	3-7*01	3-22*01	4*02	92.85	M	No	Neg	Neg	Neg	Pos	UM
CLL23	83	M	0	16.9	13.8	191	Wait and see	Untreated	No	57	death	Neg	Neg	3-74*01	2-2*01	4*02	93.40	M	No	Neg	Pos	Neg	Neg	M
CLL24	59	F	0	17.2	14.2	214	Wait and see	Untreated	No	53	alive	Neg	Neg	3-7*02	3-10*01	4*02	93.75	M	No	Neg	Neg	Neg	Pos	UM
CLL25	82	F	I	38.7	11.8	156	Wait and see	27	No	85	alive	Pos	Neg	3-48*03	na	5*02	93.75	M	No	Pos	Neg	Neg	Neg	UM
CLL26	73	F	0	16.8	12.6	238	Wait and see	129	No	159	alive	Neg	Neg	3-33*03	na	4*02	94.33	M	No	Neg	Neg	Neg	Neg	UM
CLL27	44	M	0	14.2	14.3	230	Wait and see	Untreated	No	88	alive	Neg	Neg	3-33*01	5-12*01	6*03	95.13	M	No	Neg	Neg	Neg	Pos	UM
CLL28	74	M	0	12.5	15.3	176	Wait and see	Untreated	No	84	alive	Neg	Pos	3-23*01	6-6*01	4*02	94.79	M	No	Neg	Neg	Neg	Neg	UM
CLL29	65	F	0	24.4	13.5	264	Wait and see	Untreated	No	100	alive	Neg	Neg	3-15*01	1-1*01	4*03	93.42	M	No	Neg	Neg	Neg	Pos	UM
CLL30	73	M	0	21.1	14.0	184	Wait and see	106	No	127	alive	Neg	Neg	3-23*01	5-24*01	4*02	93.69	M	No	Neg	Neg	Neg	Pos	UM
CLL31	81	F	I	13.7	12.8	161	Wait and see	12	No	111	alive	Neg	Neg	3-23*01	3-10*01	6*02	93.75	M	No	Pos	Neg	Neg	Pos	UM
CLL32	50	M	0	13.4	13.2	235	Wait and see	Untreated	No	114	alive	Pos	Pos	3-21*01	3-10*01	6*02	94.66	M	Yes	Neg	Neg	Neg	Neg	UM
CLL33	59	M	IV	15.6	7.1	45	Treatment	therapy started at diagnosis	No	77	alive	Neg	Neg	3-48*03	na	4*02	98.49	UM	No	Neg	Neg	Neg	Neg	M
CLL34	81	F	0	16.9	13.2	278	Wait and see	Untreated	No	80	alive	Neg	Neg	3-52*02	3-22*02	4*02	100.00	UM	No	Neg	Neg	Neg	Neg	UM
CLL35	77	M	IV	39.0	6.6	93	Treatment	therapy started at diagnosis	No	59	alive	Pos	Neg	4-39*01	5-24*01	4*02	100.00	UM	No	Neg	Neg	Neg	Neg	UM
CLL36	72	M	0	16.5	13.6	188	Wait and see	47	No	53	alive	Pos	Neg	5-51*01	3-3*01	4*02	100.00	UM	No	Neg	Neg	Neg	Pos	UM
CLL37	72	F	0	17.5	12.6	255	Wait and see	50	No	90	alive	Pos	Neg	1-69*02	5-12*01	4*02	100.00	UM	No	Pos	Neg	Neg	Pos	UM
CLL38	80	F	0	12.9	15.9	313	Wait and see	Untreated	No	38	alive	Pos	Neg	1-69*01	3-22*01	5*02	100.00	UM	Yes	Pos	Neg	Neg	Neg	UM
CLL39	67	M	I	21.5	13.5	126	Wait and see	29	No	73	alive	Pos	Neg	3-30-3*03	3-3*01	5*02	100.00	UM	No	Neg	Neg	Pos	Pos	UM
CLL40	71	F	0	81.0	12.0	191	Wait and see	8	No	86	alive	Pos	Neg	1-2*02	3-16*01	4*02	100.00	UM	No	Neg	Neg	Neg	Neg	UM
CLL41	79	M	I	49.4	12.3	109	Treatment	therapy started at diagnosis	No	18	death	Neg	Pos	4-30-4*01	3-22*01	5*02	100.00	UM	Yes	Neg	Pos	Neg	Pos	M
CLL42	48	M	0	13.8	14.8	170	Wait and see	40	No	63	alive	Neg	Pos	3-33*01	3-10*01	6*02	100.00	UM	No	Neg	Neg	Pos	Pos	UM
CLL43	62	F	I	16.9	12.0	120	Wait and see	Untreated	No	143	alive	Neg	Pos	3-20*01	2-2*03	6*03	99.60	UM	Yes	Pos	Neg	Neg	Neg	UM
CLL44	73	M	I	15.9	12.2	217	Wait and see	40	No	59	alive	Neg	Pos	1-69*01	3-16*01	6*02	99.24	UM	No	Neg	Neg	Neg	Pos	UM
CLL45	51	M	IV	23.0	14.2	82	Treatment	therapy started at diagnosis	No	147	death	Pos	Pos	5-51*01	3-22*01	4*02	100.00	UM	Yes	Neg	Neg	Pos	Pos	UM
CLL46	64	F	0	15.6	14.1	202	Wait and see	Untreated	No	96	alive	Pos	Pos	1-69*06	3-16*02	3*02	100.00	UM	Yes	Neg	Neg	Pos	Pos	UM
CLL47	46	M	I	24.4	14.3	391	Wait and see	6	No	69	alive	Pos	Pos	3-21*01	na	6*02	98.50	UM	Yes	Neg	Neg	Neg	Neg	UM
CLL48	91	M	I	70.8	11.5	132	Wait and see	Untreated	No	38	alive	Neg	Pos	5-51*01	5-18*01	6*02	100.00	UM	No	Pos	Neg	Neg	Neg	UM
CLL49	77	M	I	19.7	13.8	301	Wait and see	7	No	77	alive	Neg	Pos	3-48*01	3-10*01	4*02	100.00	UM	No	Pos	Neg	Neg	Neg	UM
CLL50	74	F	0	15.0	14.8	165	Wait and see	60	No	70	alive	Neg	Pos	3-30-3*01	3-3*01	3*02	100.00	UM	Yes	Pos	Neg	Neg	Neg	UM
CLL51	66	M	0	16.2	14.4	212	Wait and see	59	No	60	alive	Neg	Pos	1-69*01	3-10*01	6*02	100.00	UM	No	Neg	Neg	Neg	Neg	UM
CLL52	63	F	I	141.8	11.3	226	Treatment	therapy started at diagnosis	No	94	death	Neg	Pos	1-69*01	6-19*01	4*02	99.21	UM	No	Pos	Neg	Neg	Neg	UM
CLL53	58	F	I	20.5	13.1	183	Wait and see	14	No	88	alive	Pos	Pos	3-23*01	3-9*01	4*02	100.00	UM	No	Neg	Neg	Neg	Pos	UM
CLL54	62	M	I	19.5	16.0	255	Wait and see	5	No	46	alive	Pos	Pos	1-69*01	3-3*01	6*02	100.00	UM	No	Neg	Neg	Neg	Neg	UM
CLL55	65	M	I	13.7	13.5	188	Wait and see	13	No	81	alive	Pos	Pos	7-4-1*01	6-19*01	4*02	100.00	UM	Yes	Neg	Neg	Neg	Neg	UM
CLL56	62	M	IV	129.9	10.8	59	Treatment	therapy started at diagnosis	No	61	alive	Pos	Pos	3-30-3*01	3-3*02	6*02	100.00	UM	Yes	Neg	Neg	Neg	Neg	M

Table S6. Clinical and biological characteristics of the consecutive series of newly diagnosed and previously untreated CLL^a

	Number	%
Clinical features at CLL diagnosis		
Age >70	54/120	45.0
Male	73/120	60.8
Rai stage		
0	78/120	65.0
I	25/120	20.8
II	6/120	5.0
III	1/120	0.8
IV	10/120	8.3
Lymphocyte count (x 10 ⁹ /L) ^b	18.8	(9.5-28.8)
Hb <11 g/dL	7/120	5.8
Platelets <100 x 10 ⁹ /L	8/120	6.7
LDH > ULN	13/120	10.8
Beta-2-microglobulin		
<2.5 mg/L	78/120	65.0
2.6-3.5 mg/L	28/120	23.3
>3.5 mg/L	14/120	11.7
IGHV homology ≥98%	39/120	32.5
CD38 ≥30%	36/120	30.0
ZAP70 ≥20%	37/120	30.8
del17p13 and/or TP53 mutation	13/117	11.1
del11q22-q23	8/112	7.1
+12	17/112	15.2
del13q14	61/112	54.5
Normal FISH	32/112	28.6

^a ULN, upper limit of normal; *IGHV*, immunoglobulin heavy chain variable region gene; FISH, fluorescence in situ hybridization.

^b Median and 25th-75th percentiles are reported.

Table S7. NOTCH1 mutations in CLL, RS and de novo DLBCL

Case ID	Nucleotide change ^a	AA change ^a	Protein domain	Reported in T-ALL (COSMICv52)
CLL at diagnosis				
CLL2	Δ 28 bases (7247-7274)	P2415fs	PEST	Yes
CLL3	ΔCT (7544-7545) ^c	P2515fs	PEST	Yes
CLL34	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL40	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL52	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL55	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL56	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL38	+CCCC(7023-7024)	S2342fs	PEST	Yes ^f
CLL200	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL227	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL229	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL261	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL270	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL275	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL277	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL280	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL290	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL293	ΔCT (7544-7545)	P2515fs	PEST	Yes
Paired CLL/Richter				
RS-57	ΔCT (7544-7545) ^b	P2515fs	PEST	Yes
RS-62	ΔCT (7544-7545) ^d	P2515fs	PEST	Yes
RS-63	ΔCT (7544-7545) ^c	P2515fs	PEST	Yes
RS-64	ΔCT (7544-7545) ^c	P2515fs	PEST	Yes
RS-66	C7510T ^d	Q2504*	PEST	Yes
RS-72	ΔCT (7544-7545) ^c	P2515fs	PEST	Yes
RS-74	ΔCT (7544-7545) ^d	P2515fs	PEST	Yes
RS-78	ΔCT (7544-7545) ^e	P2515fs	PEST	Yes
RS-81	dupl.50bp (7295-7344) ^b	R2434fs	PEST	No
RS-85	C7321T ^b	Q2441*	PEST	Yes
RS-86-A	ΔCT (7544-7545) ^d	P2515fs	PEST	Yes
RS-86-B	G5164A ^b	V1722M	HD	Yes
RS-88	ΔCT (7544-7545) ^c	P2515fs	PEST	Yes
RS-91	ΔCT (7544-7545) ^d	P2515fs	PEST	Yes
RS-92	ΔCT (7544-7545) ^d	P2515fs	PEST	Yes
RS-98	ΔCT (7544-7545) ^e	P2515fs	PEST	Yes
RS-100	ΔCT (7544-7545) ^e	P2515fs	PEST	Yes
RS-103	ΔCT (7544-7545) ^c	P2515fs	PEST	Yes
RS-114	ΔCT (7544-7545) ^b	P2515fs	PEST	Yes
Chemorefractory CLL				
CLL-121-F-R	ΔC7446	F2482fs	PEST	Yes
CLL-123-A-R	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL-126-F-R	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL-129-A-R	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL-130-F-R	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL-137-F-R	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL-141-F-R	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL-146-F-R	ΔC7392	A2464fs	PEST	Yes ^f
CLL-152-F-R	ΔCT (7544-7545)	P2515fs	PEST	Yes
CLL-158-F-R	ΔCT (7544-7545)	P2515fs	PEST	Yes
de novo DLBCL				
2132	ΔCT (7544-7545)	P2515fs	PEST	Yes
2100	C7246T	Q2415*	PEST	No

^a Numbering according to GenBank accession No. NM_017617.2 (mRNA) and NP_060087.2 (protein) respectively.

^b Mutation not detectable at CLL diagnosis within the sensitivity threshold of Sanger sequencing.

^c Mutation represented at a subclonal level at CLL diagnosis according to Sanger sequencing.

^d Mutation represented at a clonal level at CLL diagnosis according to Sanger sequencing.

^e paired CLL phase not available.

^f Different nucleotide change causing frameshift at the same aminoacid position of the NOTCH1 protein.

Abbreviations: F-R, fludarabine-refractoriness; A-R, alkylator-refractoriness.

Table S8. Clinical and biological characteristics of the RS cohort^a

	Number	%
Clinical features at RS diagnosis		
Age >60 years	40/58	69.0
Male	37/58	63.8
ECOG PS >1	27/58	46.5
Ann Arbor stage III-IV	55/58	94.8
Rai stage		
0	0/58	0
I	21/58	36.2
II	7/58	12.1
III	7/58	12.1
IV	23/58	39.7
B symptoms	26/58	44.8
Extranodal sites >1	20/58	34.4
Tumor size >5 cm	32/58	55.1
Platelets <100 × 10 ⁹ /L	24/58	41.3
LDH >1.5 ULN	34/58	58.6
Prior CLL therapies >1	22/58	37.9
IPI >2	36/58	62.0
RS score >1	35/58	60.3
Pathologic features at RS diagnosis		
Non-GC phenotype	55/58	94.8
EBV infection	3/58	5.1
Genetic features		
<i>TP53</i> disruption	30/54	55.5
<i>MYC</i> aberrations	11/51	21.5
<i>IGHV</i> homology ≥98%	37/56	66.1
Stereotyped VH CDR3	22/56	39.3

^a ULN, upper limit of normal; IPI, international prognostic index; GC, germinal center; *IGHV*, immunoglobulin heavy chain variable region gene; VH CDR3, immunoglobulin heavy chain complementarity-determining region 3.

Table S9. Clinical and biological characteristics of the chemorefractory CLL cohort^a

	Number	%
Clinical features at diagnosis		
Age >70	33/48	68.8
Male	31/48	64.6
Rai stage		
0	0/48	0
I	8/48	16.7
II	8/48	16.7
III	3/48	6.3
IV	29/48	60.4
Lymphocyte count ($\times 10^9/L$) ^b	43.0	(14.4-99.1)
Hb <11 g/dL	23/48	47.9
Platelets <100 $\times 10^9/L$	29/48	60.4
LDH > ULN	24/48	50.0
Beta-2-microglobulin		
<2.5 mg/L	6/48	12.5
2.6-3.5 mg/L	13/48	27.1
>3.5 mg/L	29/48	60.4
Number of prior therapies		
1	20/48	41.7
2	16/48	33.3
>2	12/48	25.0
Treatment regimen at refractoriness		
FCR	12/48	25.0
FA	5/48	10.4
FR	2/48	4.2
FC	4/48	8.3
F	12/48	25.0
R-COP	13/48	27.1
<i>IGHV</i> homology $\geq 98\%$	30/48	62.5
CD38 $\geq 30\%$	24/48	50.0
ZAP70 $\geq 20\%$	24/48	50.0
del17p13 and/or <i>TP53</i> mutation	19/48	39.6
del11q22-q23	13/48	27.1
+12	16/48	33.3
del13q14	24/48	50.0
Normal FISH	5/48	10.4

^a ULN, upper limit of normal; FCR, fludarabine, cyclophosphamide, rituximab; FA, fludarabine, alemtuzumab; FR, fludarabine, rituximab; FC, fludarabine, cyclophosphamide; F, fludarabine; R-COP, rituximab, cyclophosphamide, vincristine, prednisone; *IGHV*, immunoglobulin heavy chain variable region gene; FISH, fluorescence in situ hybridization.

^b Median and 25th-75th percentiles are reported.