Impact of genetic alterations on central nervous system progression of primary vitreoretinal lymphoma

Kota Yoshifuji,[1*] Daichi Sadato,[2*] Takashi Toya,[3] Yotaro Motomura,[1] Chizuko Hirama,[2] Hiroshi Takase,[4] Kouhei Yamamoto,[5] Yuka Harada,[6] Takehiko Mori[1] and Toshikage Nagao[1]

[1]Department of Hematology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU); [2]Clinical Research Support Center, Tokyo Metropolitan Komagome Hospital; [3]Hematology Division, Tokyo Metropolitan Komagome Hospital; [4]Department of Ophthalmology and Visual Science, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU); [5]Department of Pathology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University and [6]Clinical Laboratory, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

**KYo and DS contributed equally as first authors.*

Correspondence: K. Yoshifuji
yoshhema@tmd.ac.jp

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Supplementary data

Supplemental Method

Treatment for the patients

All patients underwent initial treatment with weekly intravitreal MTX injections (400 μg/100 μL) in the affected eyes until the lesions resolved. Thereafter, systemic HD-MTX (3.5 g/m2 every other week for a total of five cycles) was administered to 20/36 patients, and the remainder were carefully observed without any additional chemotherapy, according to the decision of the physician. If the treatment was not tolerated, it was discontinued at the discretion of the physician.

Flow cytometry analysis

The infiltrating cells were isolated from the vitreous humor and obtained for flow cytometry. The surface expression of B-cell markers (CD19 and CD20), T-cell markers (CD3, CD4, CD5, and CD8), and κ and λ light chains were examined. Using the criteria suggested by Levy et al.[1], we defined a monoclonal κ population as one where the κ/λ ratio was 3:1 or greater, and monoclonal λ population as one that had a λ/κ ratio in excess of 2:1.

PCR analysis of IGH rearrangement

PCR analysis of IGH rearrangement was outsourced to LSI Medience Corporation (Tokyo, Japan).

Cytokine measurement

The IL-6 and IL-10 concentrations in a vitreous humor were measured at our laboratory and SRL Corporation (Tokyo, Japan). In total, 50 μL of vitreous supernatant from each patient was used for ELISA according to the given manufacturer instructions (Invitrogen, Camarillo, CA, USA).

Amplicon-based targeted sequencing

The custom gene panel of 107 genes frequently mutated in lymphoma, and PVRL was designed using Illumina Design Studio. Covered bases were 406,093 bp, and there were 3,044 (5–157 amplicons/gene)

designed panel amplicons. This custom gene panel was designed to cover all exons of each gene on genomic DNA. As a template, 10 ng DNA amplified the target genes. Libraries were synthesized using AmpliSeq Library Plus for Illumina (Illumina, San Diego, CA, USA). The libraries were analyzed using MiSeq Reagent Kit v2 (500 cycles) with MiSeq (Illumina) platform following the provided manufacturer instructions.

Whole exome sequencing

Genomic DNA capture, enrichment, and elution were performed using Agilent SureSelect Human V6 (Agilent Technologies, Santa Clara, CA) following protocols by the manufacturer. In total, 600 ng of each genomic DNA sample was used as bait. After ligation on adaptor oligonucleotides, tail repairing, and purification, libraries were quantified by qPCR to obtain an adequate DNA template for sequencing. Synthesized libraries were sequenced on the NovaSeq 6000 (Illumina) as 150 bp pair-ended reads. Sequencing was performed by Rhelixa (Tokyo, Japan).

Gene variant discovery

Fastq files from next-generation sequencing were cleaned with Trimmomatic,[2] and the results were aligned to the human reference genome, hg19, using Burrows–Wheeler Alignment (BWA)[3]. Qualimap[4] was used to analyze coverages of mapped reads. Gene variants were detected using HaplotypeCaller included in the GATK tool[5]. Gene variants obtained from HaplotypeCaller were filtered with the parameters of quality/depth, mapping quality, and strand bias to exclude false-positive variants.[6] Variants were annotated with information from the Refseq, 1000G, and Exac databases in Illumina VariantStudio 3.0 software (Illumina). Variants with a prevalence of >1% in each regional population were excluded. COSMIC and CLINVAR databases and previous genomics research papers (Table S1) were referred to judge whether the variants were pathogenic or not.

Detection of copy number alteration

Copy number alteration for each PVRL sample were analyzed using CNVkit[7] with bam files generated by the mapping process of gene variant discovery. Consequently, the normalized coverage values of PVRL data were compared to that of uveitis cases as controls and gene copy numbers were obtained. During the

calculation process, the number of amplicons and the $\log_2$ value in control data (−5 or less) and spread of read depth (1 or more) were applied as a filter, resulting in copy number of read depth (20 or more) with low spread read depth gene regions. The $\log_2$ copy number of >0.25 was decided as gain and the $\log_2$ copy number less than −0.25 was considered as loss. CNA of *HIST1H1B*, *HIST1H1C*, *HIST1H1E*, *HIST1H4H,* and *SOCS1* were excluded from the analysis because the copy number variation between the samples was too large. In the annotation process, copy number gain of oncogene and loss of tumor suppressor gene were defined as pathogenic and incorporated into analysis. Genes with the gain of function mutations had oncogenic function were considered as oncogenes, and genes with the loss of function mutations contributed to tumorigenic pathway were considered as tumor suppressor genes (Table S1).

Table S1. Target genes included in the sequencing panel of 107 genes and the reference used for gene annotation

Gene	Mutation effect	Characteristics of mutations	Reference
ACTB	N.I.	Missense in N-terminal	Lohr et al[8], Wang et al[9]
APC	Loss of function	-	Zhang et al[10], Schmitz et al[11]
ARID1A	Loss of function	-	Zhang et al[10], Schmitz et al[11]
ARID2	Loss of function	-	Wang et al[9]
AXIN1	N.I.	-	Wang et al[9]
ATM	Loss of function	-	Schmitz et al[11]
B2M	Loss of function	-	Challa-Malladi et al[12], Schmitz et al[11]
BCL10	N.I.	-	Morin et al[13], Schmitz et al[11]
BCL2	Gain of function	-	Morin et al[13], Wang et al[9]
BCL6	N.I.	-	Morin et al[13], Schmitz et al[11]
BCL7A	Loss of function	Missense in N-terminal	Schmitz et al[11], Baliñas-Gavira et al[14]
BRCA1	Loss of function	-	Wang et al[9]
BRAF	Gain of function	Missense in hotspot (e.g. V600)	Schmitz et al[11]
BTG1	Loss of function	Missense in N-terminal	Lee et al[15], Bonzheim et al[16], Mlynarczyk et al[17]
BTG2	Loss of function	Missense in N-terminal	Lee et al[15], Bonzheim et al,[16] Wang et al[9], Mlynarczyk et al[17]
CACNA1C	N.I.	-	Lee et al[15]
BTK	Loss of function	-	Lohr et al[8], Schmitz et al[11], Hu et al[18]
CCND3	Loss of function	Missense in C-terminal hotspot	Morin et al[13], Schmitz et al[19], Schmitz et al[11]
CD274	Gain of function	-	Kataoka et al[20], Schmitz et al[11]
CD58	Loss of function	-	Challa-Malladi et al[12], Schmitz et al[11]
CD70	Loss of function	-	Schmitz et al[11]
CD79A	Gain of function	Missense in immunoreceptor tyrosine-based activation motif	Davis et al[21], Schmitz et al[11]
CD79B	Gain of function	Missense in immunoreceptor tyrosine-based activation motif (e.g. Y196)	Davis et al[21], Bonzheim et al[16], Wang et al[9]
CDKN2A	Loss of function	-	Nayyar et al[22], Wang et al[9]
CDKN2B	Loss of function	-	Nayyar et al[22], Wang et al[21]
CIITA	Loss of function	-	Mottok et al[23], Wang et al[9]
CREBBP	Loss of function	-	Bonzheim et al[16], Wang et al[9]
CSMD1	Loss of function	-	Escudero-Esparza et al[24], Lee et al[15]
CXCR4	Gain of function	Nonsense in C-terminal hotspot (e.g. S342*)	Treon et al[25], Lee et al[15]
DTX1	Loss of function	-	de Miranda et al[26], Lee et al[15]
DUSP2	N.I.	-	Lee et al[15], Wang et al[9]
EHD1	N.I.	-	Lee et al[15]
EP300	Loss of function	-	Schmitz et al[11]
ERBB4	Gain of function	-	Wang et al[9]
ETS1	Loss of function	-	Morin et al[13], Bonetti et al[27], Wang et al[9]
ETV6	Loss of function	-	Bonzheim et al[16], Wang et al[9]
EZH2	Gain of function	-	Zhang et al[10], Schmitz et al[11]
FAS	Loss of function	-	Grønbaek et al[28], Schmitz et al[11]
FAT1	Loss of function	-	Laginestra et al[29], Wang et al[9]
FAT4	Loss of function	-	Cai et al[30], Lee et al[15]
FBXW7	Loss of function	-	Wang et al[9]

FLT3	Gain of function	Missense in thymidine kinase domain (e.g. D835)	Wang et al[9]
FLT4	Gain of function	Missense in thymidine kinase domain	Liu et al[31], Wang et al[9]
FOXO1	Loss of function	Missense in phosphoinositide 3-kinase/AKT phosphorylation sites	Trinh et al[32], Wang et al[9]
FRY	Loss of function	-	Lee et al[15], Mai et al[33]
GADD45B	N.I.	-	Wang et al[9]
GNA13	Loss of function	-	Muppidi et al[34], Schmitz et al[11]
GRHPR	Loss of function	-	Lee et al[15], Andrades et al[35]
HIST1H1B	Loss of function	-	Li et al[36], Lee et al[15]
HIST1H1C	Loss of function	-	Li et al[36], Lee et al[15]
HIST1H1E	Loss of function	-	Li et al[36], Lee et al[15]
HIST1H4H	Loss of function	-	Li et al[36], Lee et al[15]
IGLL5	Loss of function	-	Bonzheim et al[16], Lee et al[15]
IKZF3	Loss of function	-	Wang et al[9]
IRF4	Loss of function	Missense in DNA binding domain	Cherian et al[37], Lee et al[15], Bonzheim et al[16], Wang et al[9]
IRF8	Loss of function	Missense in DNA binding domain	Reddy et al[38], Lee et al[15]
ITPKB	Loss of function	-	Schmitz et al[11]
KLHL14	Loss of function	-	Choi et al[39], Lee et al[15]
KLHL6	Loss of function	-	Schmitz et al[11]
KMT2D	Loss of function	-	Lee et al[15], Wang et al[9]
LRP1B	Loss of function	-	Lee et al[15]
LRIG1	N.I.	-	Lee et al[15]
MCL1	N.I.	-	Wang et al[9]
MED12	N.I.	-	Wang et al[38]
MEF2B	N.I.	-	Pon et al[40], Wang et al[9]
MALT1	N.I.	-	Schmitz et al[11]
MPEG1	Loss of function	-	Schmitz et al[11], Lee et al[15]
MUC16	N.I.	-	Lee et al[15]
MTOR	Loss of function	-	Schmitz et al[11]
MYC	Gain of function	-	Wang et al[9]
MYD88	Gain of function	-	Lee et al[15], Bonzheim et al[16], Wang et al[9]
NFKB1	Loss of function	-	Wang et al[9]
NF1	Loss of function	-	Schmitz et al[11]
NFKBIA	Loss of function	-	Schmitz et al[11], Weniger et al[41]
NFKBIE	Loss of function	-	Schmitz et al[11], Weniger et al[41]
NFKBIZ	N.I.	-	Schmitz et al[11]
NOTCH1	Gain of function	-	Schmitz et al[11]
NOTCH2	Gain of function	-	Schmitz et al[11]
OSBPL10	N.I.	-	Dobashi et al[42], Lee et al[15]
OTOF	N.I.	-	Lee et al[15]
PCDH15	N.I.	-	Lee et al[15]
PAX5	Loss of function	-	Schmitz et al[11], Gu et al[43]
PIM1	Loss of function	-	Lee et al[15], Bonzheim et al[16], Wang et al[9]
PLCG2	N.I.	-	Wang et al[9]
PRDM1	Loss of function	-	Bonzheim et al[16], Wang et al[9]
RBMX	Loss of function	-	Schmitz et al[11], Zheng et al[44]
PTEN	Loss of function	-	Schmitz et al[11]
REL	N.I.	-	Schmitz et al[11]
RP1	N.I.	-	Lee et al[15]
RUNX1	Loss of function	-	Wang et al[9]
SETBP1	Gain of function	-	Wang et al[9]

RHOA	Gain of function	-	Schmitz et al[11]
SGK1	Gain of function	-	Schmitz et al[11]
SOCS1	Loss of function	-	Schmitz et al[11]
SPEN	Loss of function	-	Reddy et al[40], Schmitz et al[11]
STAT3	Gain of function	Missense in SH2 domain (e.g. Y640F, D661Y)	Koskela et al[45], Schmitz et al[11]
STAT6	Gain of function	Missense in DNA binding domain (e.g. D419)	Yildiz et al[46], Schmitz et al[11]
TBL1XR1	Loss of function	Missense in WD domain	Venturutti et al[47], Bonzheim et al[16], Wang et al[9]
TCF3	Gain of function	-	Schmitz et al[11]
TET2	Loss of function	-	Schmitz et al[11]
TMSB4X	N.I.	-	Lee et al[15]
TNFAIP3	Loss of function	-	Kato et al[48], Schmitz et al[11]
TNFRSF14	Loss of function	-	Schmitz et al[11], Wu et al[49]
TP53	Loss of function	-	Schmitz et al[11], Wang et al[9]
UBALD2	N.I.	-	Lee et al[15]
USH2A	N.I.	-	Lee et al[15]
ZFP36L1	Loss of function	-	Reddy et al[38], Lee et al[15]

N.I., Not identified. Details of references were listed in supplementary references.

Table S2. Detected pathogenic genetic mutations

Case	Gene	Mutation type	cDNA change	AA change	VAF (%)	Read depth
1	*MYD88*	Missense	c.794T>C	p.Leu265Pro	29.36	453
1	*PIM1*	Frameshift	c.644_680delAGCCGGTGCAAGATCTCTTCGACTTCATCACGGAAAG	p.Glu215GlyfsTer138	20.99	567
1	*PIM1*	Nonsense	c.691C>T	p.Gln231Ter	40.71	565
1	*ETS1*	Nonsense	c.1323C>G	p.Tyr441Ter	27.11	439
1	*CD79B*	Missense	c.590A>G	p.Tyr197Cys	27.89	882
1	*BTG2*	Missense	c.133G>T	p.Ala45Ser	14.31	1,139
10	*TBL1XR1*	Missense	c.1108G>T	p.Asp370Tyr	55.50	582
10	*PIM1*	Splice	c.513+1G>C		57.03	619
10	*PRDM1*	Splice	c.291G>C	p.Glu97Asp	58.21	1,029
10	*PRDM1*	Splice	c.291+1G>A		58.41	1,029
10	*CDKN2A*	Missense	c.247C>T	p.His83Tyr	64.08	710
10	*ETV6*	Splice	c.33+1G>A		71.48	519
10	*CD79B*	Missense	c.590A>G	p.Tyr197Cys	78.99	2,385
10	*KLHL14*	Nonsense	c.289C>T	p.Gln97Ter	44.37	978
10	*IGLL5*	Nonsense	c.64C>T	p.Gln22Ter	42.40	500
10	*BTG2*	Missense	c.142G>A	p.Glu48Lys	43.54	2,522
10	*BTG2*	Missense	c.157C>T	p.His53Tyr	35.65	3,669
10	*BTG1*	Missense	c.498G>A	p.Met166Ile	39.23	1,300
10	*BTG1*	Missense	c.398G>A	p.Ser133Asn	44.41	1,504
10	*BTG1*	Missense	c.208A>G	p.Ile70Val	40.31	2,079
10	*BTG1*	Missense	c.129C>A	p.Ser43Arg	46.29	283
12	*MYD88*	Missense	c.794T>C	p.Leu265Pro	28.17	1,260
12	*PIM1*	Frameshift	c.149_156delGCAACGCC	p.Arg50HisfsTer13	56.27	670
12	*PIM1*	Frameshift	c.276delG	p.Met92IlefsTer93	60.48	625
12	*PIM1*	Nonsense	c.676G>T	p.Glu226Ter	48.71	1,944
12	*PIM1*	Nonsense	c.720_748delGCAGGTGCTGGAGGCCGTGCGGCACTGCC	p.Trp240Ter	24.73	1,326
12	*PRDM1*	Frameshift	c.500_522delCTCCCCGGGAGCAAAACCTGGCT	p.Ser167CysfsTer14	34.95	495
12	*ACTB*	Missense	c.143G>A	p.Gly48Asp	26.49	1,797
12	*ETV6*	Nonsense	c.19C>T	p.Gln7Ter	30.12	601
12	*ETV6*	Missense	c.1172A>G	p.Tyr391Cys	23.27	709
12	*BTG1*	Nonsense	c.103C>T	p.Arg35Ter	37.11	256

12	*KLHL14*	Frameshift	c.625_635delCTGGTGGAGGA	p.Leu209CysfsTer47	34.28	878
12	*KLHL14*	Nonsense	c.271C>T	p.Gln91Ter	29.34	634
12	*IGLL5*	Splice	c.206+2T>A		24.32	6,187
12	*BTG2*	Missense	c.83G>A	p.Gly28Asp	26.39	2,876
12	*BTG2*	Missense	c.185G>C	p.Gly62Ala	25.24	2,524
12	*BTG1*	Missense	c.304C>T	p.Leu102Phe	31.11	270
12	*BTG1*	Missense	c.116C>T	p.Thr39Ile	36.33	256
22	*MYD88*	Missense	c.794T>C	p.Leu265Pro	31.92	639
22	*TBL1XR1*	Missense	c.941T>A	p.Val314Asp	37.48	643
22	*HIST1H1B*	Missense	c.392C>G	p.Ala131Gly	73.16	395
22	*PIM1*	Nonsense	c.652C>T	p.Gln218Ter	48.17	546
22	*PRDM1*	Splice	c.291G>C	p.Glu97Asp	74.72	542
22	*CDKN2A*	Missense	c.197A>G	p.His66Arg	85.45	55
22	*PTEN*	Frameshift	c.149_153dupTTGAT	p.Asp52LeufsTer4	27.35	1,104
22	*MPEG1*	Nonsense	c.271C>T	p.Gln91Ter	33.27	505
22	*KMT2D*	Nonsense	c.6229C>T	p.Gln2077Ter	38.25	1,336
22	*CIITA*	Nonsense	c.657C>A	p.Cys219Ter	36.02	1,180
22	*CD79B*	Missense	c.590A>C	p.Tyr197Ser	34.73	976
26	*BTG2*	Nonsense	c.16G>T	p.Gly6Ter	47.99	2,761
26	*MYD88*	Missense	c.794T>C	p.Leu265Pro	39.86	1,041
26	*TBL1XR1*	Missense	c.1099T>C	p.Cys367Arg	38.34	866
26	*HIST1H1B*	Frameshift	c.230_257delAGAAGAATAACAGCCGCATTAAGCTGGG	p.Glu77AlafsTer6	18.61	1,752
26	*PIM1*	Nonsense	c.387C>G	p.Tyr129Ter	51.70	853
26	*PAX5*	Splice	c.41_46+13delGGACAGGTAGGACCGCGAT		35.36	1,151
26	*GRHPR*	Frameshift	c.129_130delGG	p.Glu44AlafsTer48	15.02	486
26	*GRHPR*	Frameshift	c.129_130delGG	p.Glu44AlafsTer48	15.02	486
26	*GRHPR*	Frameshift	c.129_130delGG	p.Glu44AlafsTer48	15.02	486
26	*MPEG1*	Nonsense	c.1201G>T	p.Glu401Ter	29.63	1,441
26	*MPEG1*	Frameshift	c.1195_1196delAA	p.Lys399ValfsTer10	36.38	1,443
26	*MPEG1*	Frameshift	c.920delG	p.Gly307AlafsTer21	29.60	1,108
26	*ETV6*	Splice	c.33+1G>C		57.42	404
26	*ETV6*	Nonsense	c.427C>T	p.Gln143Ter	54.02	1,405
26	*KMT2D*	Nonsense	c.14152G>T	p.Glu4718Ter	43.40	1,719
26	*CIITA*	Nonsense	c.1099C>T	p.Gln367Ter	30.15	617
26	*CIITA*	Frameshift	c.3052delG	p.Glu1018LysfsTer32	62.17	423

26	*BCL2*	Missense	c.351C>G	p.Ser117Arg	24.56	2,895
26	*BCL2*	Missense	c.20C>T	p.Thr7Ile	27.50	1,491
26	*GRHPR*	Frameshift	c.129_130delGG	p.Glu44AlafsTer48	15.02	486
26	*GRHPR*	Frameshift	c.129_130delGG	p.Glu44AlafsTer48	15.02	486
26	*BTG2*	Missense	c.96G>T	p.Glu32Asp	26.12	2,726
31	*MYD88*	Missense	c.794T>C	p.Leu265Pro	34.50	774
31	*TBL1XR1*	Missense	c.1051G>A	p.Glu351Lys	32.16	398
31	*TET2*	Frameshift	c.4745_4746delCT	p.Ser1582PhefsTer31	12.00	175
31	*PIM1*	Frameshift	c.201_214delGCACAGCCCCGGCT	p.His68ArgfsTer101	73.88	157
31	*PIM1*	Splice	c.513+1G>A		30.50	400
31	*PIM1*	Nonsense	c.652C>T	p.Gln218Ter	56.61	295
31	*PIM1*	Nonsense	c.691C>T	p.Gln231Ter	33.74	492
31	*PIM1*	Frameshift	c.711_724delCTTCTTCTGGCAGG	p.Phe238AlafsTer57	38.14	527
31	*PIM1*	Nonsense	c.1057G>T	p.Glu353Ter	24.67	608
31	*ETV6*	Splice	c.34-1G>A		29.54	799
31	*ETV6*	Splice	c.1254-2A>G		31.91	564
31	*KMT2D*	Nonsense	c.11911C>T	p.Gln3971Ter	33.17	612
31	*DTX1*	Nonsense	c.229C>T	p.Gln77Ter	32.22	239
31	*CD79B*	Missense	c.589T>C	p.Tyr197His	69.73	621
31	*BTG1*	Missense	c.347G>A	p.Gly116Glu	41.59	428
31	*BTG1*	Missense	c.145G>A	p.Ala49Thr	36.11	144
39	*MYD88*	Missense	c.794T>C	p.Leu265Pro	39.48	423
39	*KMT2D*	Nonsense	c.12844C>T	p.Arg4282Ter	34.29	35
49	*MYD88*	Missense	c.794T>C	p.Leu265Pro	46.60	515
49	*PIM1*	Nonsense	c.697G>T	p.Glu233Ter	73.22	956
49	*PIM1*	Frameshift	c.737_740delTGCG	p.Val246GlyfsTer118	73.17	954
49	*CD79B*	Missense	c.590A>C	p.Tyr197Ser	48.21	1,931
49	*KLHL14*	Nonsense	c.763C>T	p.Gln255Ter	47.42	1,242
49	*KLHL14*	Nonsense	c.735G>A	p.Trp245Ter	42.69	1,225
49	*BTG1*	Missense	c.123C>G	p.Ser41Arg	42.96	135
49	*BTG1*	Missense	c.108G>C	p.Gln36His	42.96	135
52	*PIM1*	Nonsense	c.927C>G	p.Tyr309Ter	33.15	374
52	*PRDM1*	Nonsense	c.232C>T	p.Gln78Ter	50.64	543
52	*ACTB*	Missense	c.217C>T	p.His73Tyr	68.18	396
52	*CSMD1*	Nonsense	c.9254G>A	p.Trp3085Ter	30.49	505
52	*ETV6*	Missense	c.1256T>G	p.Phe419Cys	33.62	687
52	*BCL7A*	Missense	c.91T>C	p.Trp31Arg	32.85	137

52	*CREBBP*	Missense	c.4463C>T	p.Pro1488Leu	48.61	1,473
52	*CD79B*	Missense	c.590A>G	p.Tyr197Cys	58.42	1,152
52	*MYD88*	Missense	c.794T>C	p.Leu265Pro	63.94	391
52	*BTG2*	Missense	c.273G>C	p.Gln91His	42.53	783
56	*PIM1*	Nonsense	c.382C>T	p.Gln128Ter	55.11	303
56	*ACTB*	Missense	c.137G>C	p.Gly46Ala	33.64	431
56	*CD79B*	Missense	c.590A>C	p.Tyr197Ser	34.32	1,110
56	*MYD88*	Missense	c.794T>C	p.Leu265Pro	43.04	381
56	*BTG1*	Nonsense	c.168G>A	p.Trp56Ter	57.03	626
56	*BTG1*	Missense	c.400A>T	p.Thr134Ser	50.12	431
56	*BTG1*	Missense	c.160C>T	p.His54Tyr	57.03	626
56	*BTG1*	Missense	c.8C>T	p.Pro3Leu	26.63	612
61	*PIM1*	Nonsense	c.387C>A	p.Tyr129Ter	48.55	1,584
61	*PIM1*	Splice	c.513+1G>A		38.99	418
61	*KMT2D*	Splice	c.10441-2A>G		66.88	2,962
61	*IKZF3*	Splice	c.826+1G>T		52.79	1,847
61	*BTG2*	Frameshift	c.100_124delAGGCTTAAGGTCTTCAGCGGGGCGC	p.Arg34SerfsTer59	41.52	2,271
61	*MYD88*	Missense	c.794T>C	p.Leu265Pro	94.93	592
61	*BTG1*	Missense	c.14A>T	p.Tyr5Phe	20.82	1,047
82	*SOCS1*	Frameshift	c.312_330delCGACAGCCGCCAGCGGAAC	p.Asp105AlafsTer7	23.05	564
87	*LRP1B*	Splice	c.1971-2A>T		47.28	1,303
87	*TBL1XR1*	Missense	c.920A>G	p.His307Arg	50.79	1,262
87	*BCL7A*	Splice	c.92+1G>A		41.35	237
87	*CREBBP*	Nonsense	c.5701C>T	p.Gln1901Ter	50.00	92
87	*NF1*	Nonsense	c.669G>A	p.Trp223Ter	45.19	135
87	*BTG2*	Missense	c.52G>A	p.Gly18Ser	53.98	2,321
87	*BTG2*	Missense	c.83G>A	p.Gly28Asp	53.83	2,326
87	*BTG2*	Missense	c.133G>T	p.Ala45Ser	53.64	2,321
87	*BTG2*	Missense	c.136C>T	p.Leu46Phe	40.55	2,328
97	*PIM1*	Nonsense	c.652C>T	p.Gln218Ter	80.38	581
97	*PIM1*	Nonsense	c.720G>A	p.Trp240Ter	41.68	715
97	*PIM1*	Nonsense	c.908G>A	p.Trp303Ter	41.99	443
97	*PRDM1*	Splice	c.291G>C	p.Glu97Asp	51.92	728
97	*GRHPR*	Splice	c.214+1G>A		41.75	103
97	*GRHPR*	Splice	c.287+1G>A		36.71	779
97	*KMT2D*	Frameshift	c.15891_15895dupGGTGC	p.His5299ArgfsTer8	37.80	463

97	*CD79B*	Missense	c.590A>G	p.Tyr197Cys	33.35	1,475
97	*BTG2*	Splice	c.142+1G>C		30.43	1,620
97	*MYD88*	Missense	c.794T>C	p.Leu265Pro	33.06	605
97	*MPEG1*	Nonsense	c.556C>T	p.Gln186Ter	29.55	714
114	*FBXW7*	Missense	c.1513C>T	p.Arg505Cys	47.07	2,422
114	*IRF4*	Missense	c.208C>G	p.Leu70Val	26.67	30
114	*PIM1*	Frameshift	c.245_249delGTCCC	p.Arg82LeufsTer90	60.07	263
114	*PIM1*	Frameshift	c.674_702delCGGAAAGGGGAGCCCTGCAAGAGGAGCTG	p.Thr225SerfsTer65	84.02	795
114	*CSMD1*	Splice	c.9814+1G>A		44.04	965
114	*ETV6*	Splice	c.12_33+24delTCCTGCTCAGTGTAGCATTAAGGTAAAAATCTTCTCCCCTCCTTCT		50.84	356
114	*BCL7A*	Missense	c.70G>A	p.Ala24Thr	46.23	106
114	*KLHL14*	Nonsense	c.562C>T	p.Gln188Ter	33.91	929
114	*KLHL14*	Nonsense	c.550C>T	p.Gln184Ter	42.80	736
114	*MYD88*	Missense	c.794T>C	p.Leu265Pro	45.77	627
114	*MEF2B*	Frameshift	c.396_399dupTGCA	p.Ala134CysfsTer21	46.81	94
114	*BTG2*	Missense	c.83G>A	p.Gly28Asp	32.40	1,923
114	*BTG2*	Missense	c.92G>A	p.Ser31Asn	32.40	1,923
126	*FAT4*	Nonsense	c.3754G>T	p.Gly1252Ter	36.60	806
126	*FRY*	Frameshift	c.2667delT	p.Leu890TrpfsTer30	20.41	49
132	*ITPKB*	Nonsense	c.691A>T	p.Lys231Ter	32.56	1,170
132	*MYD88*	Missense	c.794T>C	p.Leu265Pro	54.79	1,608
132	*TBL1XR1*	Missense	c.1184A>T	p.Tyr395Phe	44.29	736
132	*ACTB*	Missense	c.193C>T	p.Leu65Phe	28.15	959
132	*GRHPR*	Splice	c.214+1G>A		43.33	90
132	*ETV6*	Splice	c.33+1G>A		54.30	151
132	*IRF8*	Missense	c.197A>G	p.Lys66Arg	38.90	365
132	*BTG2*	Missense	c.83G>A	p.Gly28Asp	29.77	2,267
136	*MYD88*	Missense	c.794T>C	p.Leu265Pro	64.93	211
136	*HIST1H1E*	Missense	c.308G>A	p.Gly103Asp	22.31	130
136	*IGLL5*	Frameshift	c.32_41delAGACCCCTGA	p.Glu11GlyfsTer95	33.33	75
136	*RBMX*	Frameshift	c.1dupA	p.Met1?	36.17	47
136	*KMT2D*	Nonsense	c.2635G>T	p.Glu879Ter	38.58	127
136	*BCL7A*	Nonsense	c.92G>A	p.Trp31Ter	28.57	77
137	*CD58*	Nonsense	c.471C>G	p.Tyr157Ter	35.48	1,581
137	*CD58*	Nonsense	c.454C>T	p.Arg152Ter	38.45	1,597

137	*ITPKB*	Nonsense	c.622C>T	p.Gln208Ter	30.72	345
137	*MYD88*	Missense	c.794T>C	p.Leu265Pro	36.47	987
137	*TBL1XR1*	Missense	c.1200T>A	p.Ser400Arg	37.32	142
137	*TBL1XR1*	Missense	c.1124T>A	p.Ile375Lys	40.46	131
137	*TBL1XR1*	Missense	c.1123A>G	p.Ile375Val	40.46	131
137	*PIM1*	Nonsense	c.481G>T	p.Glu161Ter	38.11	677
137	*CDKN2A*	Nonsense	c.330G>A	p.Trp110Ter	36.89	862
137	*BCL7A*	Missense	c.86G>A	p.Arg29His	31.16	276
137	*CIITA*	Frameshift	c.3021delC	p.Ser1008GlnfsTer7	39.45	512
137	*CD79B*	Missense	c.589T>G	p.Tyr197Asp	34.08	1,247
137	*GNA13*	Nonsense	c.79C>T	p.Gln27Ter	27.96	651
139	*MYD88*	Missense	c.794T>C	p.Leu265Pro	54.98	1,346
139	*PIM1*	Nonsense	c.361G>T	p.Glu121Ter	39.10	693
139	*PRDM1*	Frameshift	c.485_486delTG	p.Val162GlufsTer26	81.73	197
139	*ETV6*	Splice	c.33+1G>C		83.65	159
139	*CREBBP*	Missense	c.4472A>C	p.Gln1491Pro	43.25	1,519
139	*TP53*	Missense	c.761T>A	p.Ile254Asn	82.95	733
139	*CD79B*	Missense	c.589T>G	p.Tyr197Asp	45.48	1,172
139	*IGLL5*	Frameshift	c.93_94delGG	p.Ala32HisfsTer59	91.19	590
139	*BTG1*	Missense	c.116C>T	p.Thr39Ile	46.44	239
144	*MYD88*	Missense	c.794T>C	p.Leu265Pro	34.62	1,352
144	*PRDM1*	Splice	c.291G>C	p.Glu97Asp	53.34	718
144	*CD79B*	Missense	c.590A>G	p.Tyr197Cys	32.83	1,185
144	*KLHL14*	Nonsense	c.289C>T	p.Gln97Ter	38.02	313
144	*BTG1*	Missense	c.17C>T	p.Thr6Ile	33.89	773
147	*GRHPR*	Splice	c.287+1G>A		22.31	1,013
147	*ETV6*	Splice	c.33+1delG		20.29	138
147	*IGLL5*	Frameshift	c.212delT	p.Leu71ArgfsTer38	31.77	1,432
147	*MYD88*	Missense	c.794T>C	p.Leu265Pro	31.90	1,279
147	*MPEG1*	Nonsense	c.2131C>T	p.Gln711Ter	17.74	248
173	*CSMD1*	Nonsense	c.585G>A	p.Trp195Ter	42.10	38
174	*MYD88*	Missense	c.794T>C	p.Leu265Pro	21.72	1,625
174	*TBL1XR1*	Missense	c.1100G>C	p.Cys367Ser	23.48	1,001
174	*PRDM1*	Splice	c.291G>A	c.291G>A(p.=)	27.79	662
174	*PRDM1*	Splice	c.291+1G>A		27.79	662
174	*CDKN2A*	Nonsense	c.329G>A	p.Trp110Ter	49.78	1,137
174	*KMT2D*	Nonsense	c.8050C>T	p.Gln2684Ter	25.77	2,716

174	*ZFP36L1*	Nonsense	c.567C>A	p.Cys189Ter	27.47	1,791
174	*KLHL14*	Nonsense	c.562C>T	p.Gln188Ter	35.16	2,076
179	*NOTCH2*	Nonsense	c.7090C>T	p.Gln2364Ter	55.59	1,423
179	*BTG2*	Frameshift	c.115_116insACTTAAGGTCTTCA	p.Ser39AsnfsTer67	29.44	1,155
179	*BTG2*	Frameshift	c.115_116insATTTAAGGTCTTCA	p.Ser39AsnfsTer67	20.61	1,155
179	*CD79B*	Missense	c.590A>C	p.Tyr197Ser	94.96	873
179	*GNA13*	Nonsense	c.111C>A	p.Cys37Ter	42.96	568
179	*BTG2*	Missense	c.83G>A	p.Gly28Asp	40.72	1,159
179	*BTG2*	Missense	c.121G>C	p.Ala41Pro	40.40	1,161
180	*MYD88*	Missense	c.794T>C	p.Leu265Pro	42.18	211
180	*IRF4*	Nonsense	c.178C>T	p.Gln60Ter	34.41	186
180	*IRF4*	Missense	c.208C>G	p.Leu70Val	94.69	113
180	*CD79B*	Missense	c.589T>G	p.Tyr197Asp	59.78	184
180	*KMT2D*	Nonsense	c.12253C>T	p.Gln4085Ter	42.86	252
180	*PIM1*	Splice	c.355+1G>C		96.80	437
180	*ETV6*	Splice	c.33+1G>C		54.95	202
180	*ETV6*	Splice	c.33+1delG		37.62	202
182	*GNA13*	Splice	c.283+1G>A		25.63	355
184	*MYD88*	Missense	c.794T>C	p.Leu265Pro	38.05	1,201
184	*TBL1XR1*	Missense	c.971C>T	p.Ser324Phe	78.37	1,946
184	*PIM1*	Splice	c.355+1G>A		39.38	678
184	*PIM1*	Nonsense	c.432C>A	p.Tyr144Ter	66.80	253
184	*PIM1*	Frameshift	c.704_711delCCCGCAGC	p.Ala235ValfsTer62	23.55	1,622
184	*GRHPR*	Splice	c.215-9_217delGCACAACAGGGG		44.26	1,803
184	*GRHPR*	Frameshift	c.220_221delAA	p.Asn74SerfsTer18	44.36	1,799
184	*MPEG1*	Nonsense	c.1687C>T	p.Gln563Ter	39.23	989
184	*ETV6*	Splice	c.31_33+8delAAGGTAAAAAT		80.45	133
184	*ZFP36L1*	Frameshift	c.750delG	p.Glu250AspfsTer52	29.03	31
184	*IGLL5*	Frameshift	c.158_179delGAGCCTCAGTTGGAAGCAGCCG	p.Gly53AspfsTer49	62.22	532
184	*BTG2*	Missense	c.20C>T	p.Thr7Ile	59.84	1,367
184	*BTG2*	Missense	c.277C>T	p.His93Tyr	37.53	1,327
184	*BTG1*	Missense	c.197G>A	p.Gly66Asp	43.70	540
184	*BTG1*	Missense	c.108G>C	p.Gln36His	39.51	205
184	*BTG1*	Missense	c.91C>T	p.Leu31Phe	40.10	207

189	*MYD88*	Missense	c.794T>C	p.Leu265Pro	88.47	1,102
189	*CD79A*	Nonsense	c.553G>T	p.Glu185Ter	52.75	728
189	*IGLL5*	Splice	c.206+1G>T		35.00	3,177
197	*FBXW7*	Missense	c.1393C>T	p.Arg465Cys	24.71	603
197	*CD79B*	Missense	c.589T>A	p.Tyr197Asn	28.35	1,252
197	*EP300*	Splice	c.1529-2A>T		33.33	222
204	*HIST1H1C*	Frameshift	c.199_200delGC	p.Ala67CysfsTer5	40.15	259
204	*IRF8*	Missense	c.67T>C	p.Tyr23His	45.67	1,743
204	*CD79B*	Missense	c.590A>C	p.Tyr197Ser	38.41	2,114
204	*GNA13*	Frameshift	c.93delC	p.Lys32ArgfsTer14	39.48	1,145
204	*GNA13*	Frameshift	c.80_89delAGCAACGCAA	p.Gln27ArgfsTer16	39.60	1,149
204	*IGLL5*	Splice	c.206+17_206+18insTCAGGTAAGGGGCAAGAGATT		49.00	1,945
204	*MYD88*	Missense	c.794T>C	p.Leu265Pro	46.65	701
204	*BTG1*	Missense	c.206G>A	p.Cys69Tyr	27.07	1,330

Abbreviations: AA, amino acid; VAF, variant allele frequency

Table S3. Detected pathogenic copy number alterations

Case	Gene	CNA	Log_2	Case	Gene	CNA	Log_2	Case	Gene	CNA	Log_2
1	*CDKN2A*	Loss	−0.91	**49**	*BCL2*	Gain	0.78	**139**	*FLT3*	Gain	0.45
1	*CDKN2B*	Loss	−0.64	**49**	*CD58*	Loss	−0.28	**139**	*PRDM1*	Loss	−0.75
1	*CSMD1*	Loss	−0.51	**49**	*CDKN2A*	Loss	−3.17	**139**	*SGK1*	Loss	−0.89
1	*IGLL5*	Loss	−0.45	**49**	*CDKN2B*	Loss	−4.32	**139**	*TNFAIP3*	Loss	−0.94
1	*MYC*	Gain	0.53	**49**	*CIITA*	Loss	−0.74	**139**	*TP53*	Loss	−0.82
1	*PRDM1*	Loss	−0.37	**49**	*ETV6*	Loss	−0.95	**144**	*CDKN2A*	Loss	−1.12
1	*SGK1*	Loss	−0.47	**49**	*IGLL5*	Loss	−3.46	**144**	*CDKN2B*	Loss	−1.68
1	*TNFAIP3*	Loss	−0.52	**49**	*MALT1*	Gain	0.78	**144**	*PRDM1*	Loss	−0.53
10	*BCL7A*	Loss	−0.45	**52**	*CDKN2A*	Loss	−1.28	**144**	*SGK1*	Loss	−0.36
10	*CDKN2A*	Loss	−0.68	**52**	*CDKN2B*	Loss	−2.51	**144**	*STAT6*	Gain	0.80
10	*CDKN2B*	Loss	−1.29	**52**	*MEF2B*	Loss	−0.61	**144**	*TNFAIP3*	Loss	−0.55
10	*ETV6*	Loss	−0.54	**56**	*CDKN2A*	Loss	−2.61	**179**	*STAT6*	Gain	0.83
10	*KMT2D*	Loss	−0.65	**56**	*CDKN2B*	Loss	−3.49	**179**	*CDKN2A*	Loss	−3.74
10	*PRDM1*	Loss	−1.30	**56**	*PRDM1*	Loss	−2.36	**179**	*CDKN2B*	Loss	−3.79
12	*CDKN2A*	Loss	−0.78	**61**	*BCL7A*	Loss	−0.56	**180**	*BCL2*	Gain	0.87
12	*CDKN2B*	Loss	−0.84	**61**	*CDKN2A*	Loss	−3.11	**180**	*BCL7A*	Loss	−0.69
12	*IGLL5*	Loss	−0.49	**61**	*CDKN2B*	Loss	−2.27	**180**	*CD274*	Gain	0.74
12	*PIM1*	Loss	−0.55	**61**	*IGLL5*	Loss	−1.43	**180**	*CDKN2A*	Loss	−3.09
12	*PRDM1*	Loss	−0.36	**61**	*PRDM1*	Loss	−0.92	**180**	*CDKN2B*	Loss	−1.34
12	*SGK1*	Loss	−0.33	**61**	*SGK1*	Loss	−0.70	**180**	*MALT1*	Gain	0.55
12	*TNFAIP3*	Loss	−0.37	**61**	*TNFAIP3*	Loss	−0.90	**180**	*SETBP1*	Gain	0.69
22	*CDKN2A*	Loss	−2.48	**87**	*CDKN2A*	Loss	−4.01	**184**	*ARID2*	Loss	−0.75
22	*CDKN2B*	Loss	−2.33	**87**	*CDKN2B*	Loss	−4.51	**184**	*CDKN2A*	Loss	−2.52
22	*CSMD1*	Loss	−0.76	**97**	*CDKN2A*	Loss	−3.14	**184**	*CDKN2B*	Loss	−2.35
22	*ETV6*	Loss	−0.68	**97**	*CDKN2B*	Loss	−1.54	**184**	*CSMD1*	Loss	−0.61
22	*IGLL5*	Loss	−2.77	**97**	*ETV6*	Loss	−1.54	**184**	*ETV6*	Loss	−0.51
22	*MEF2B*	Loss	−0.52	**97**	*NFKBIZ*	Gain	0.47	**184**	*PRDM1*	Loss	−0.82
22	*PRDM1*	Loss	−0.81	**114**	*CDKN2A*	Loss	−1.05	**184**	*SGK1*	Loss	−1.10
26	*CDKN2A*	Loss	−0.32	**114**	*CDKN2B*	Loss	−1.66	**184**	*TNFAIP3*	Loss	−0.81
26	*ETV6*	Loss	−0.42	**114**	*MPEG1*	Loss	−0.78	**189**	*MCL1*	Gain	0.81
26	*IGLL5*	Loss	−1.69	**132**	*CDKN2A*	Loss	−1.90	**189**	*BRAF*	Gain	0.38
26	*MALT1*	Gain	0.60	**132**	*CDKN2B*	Loss	−1.75	**189**	*STAT6*	Gain	0.38

26	*SETBP1*	Gain	0.56	**132**	*NFKBIZ*	Gain	0.50	**189**	*PRDM1*	Loss	−0.75
31	*BCL2*	Gain	0.48	**132**	*RHOA*	Gain	0.81	**189**	*SGK1*	Loss	−0.71
31	*CDKN2A*	Loss	−1.46	**132**	*SGK1*	Loss	−0.71	**189**	*TNFAIP3*	Loss	−0.72
31	*CDKN2B*	Loss	−1.67	**132**	*TNFAIP3*	Loss	−0.91	**197**	*STAT6*	Gain	0.63
31	*MALT1*	Gain	0.51	**136**	*CDKN2A*	Loss	−0.83	**197**	*CDKN2A*	Loss	−1.04
31	*PRDM1*	Loss	−0.56	**136**	*CDKN2B*	Loss	−0.85	**197**	*IGLL5*	Loss	−1.11
31	*SETBP1*	Gain	0.53	**136**	*PRDM1*	Loss	−0.50	**204**	*CDKN2A*	Loss	−4.93
31	*TMSB4X*	Gain	0.96	**136**	*SGK1*	Loss	−0.79	**204**	*CDKN2B*	Loss	−2.11
39	*CD274*	Gain	0.68	**136**	*TNFAIP3*	Loss	−0.38	**204**	*CREBBP*	Loss	−0.80
39	*CDKN2A*	Loss	−3.86	**137**	*SGK1*	Loss	−0.79	**204**	*IGLL5*	Loss	−1.19
39	*CDKN2B*	Loss	−4.63	**139**	*CDKN2A*	Loss	−2.85	**204**	*PRDM1*	Loss	−0.90
39	*ETV6*	Loss	−2.81	**139**	*CDKN2B*	Loss	−3.62	**204**	*SGK1*	Loss	−0.93
39	*IGLL5*	Loss	−0.86	**139**	*ETV6*	Loss	−0.57	**204**	*TNFAIP3*	Loss	−1.11

Abbreviations: CNA, copy number alteration

Table S4. Relationship between *ETV6* loss and clinical findings

Factors	*ETV6* loss		p-value
	Positive (n = 8)	Negative (n = 27)	
Sex, male/female	3/5	11/16	1
Age, median (range), years	71.5 (45–83)	69 (43–84)	0.70
Laterality, unilateral/bilateral	3/5	12/15	1
IL-10 level (pg/mL), median (range)	890 (17–5005)	1008 (10-130,125)	0.50
IL-10/IL-6 ratio	12.6 (0.29–98.1)	15.6 (0.46–1161.8)	0.26
Cytopathology positive (class ≥IIIb)	6/2	17/10	0.69
Detection of B-cell clonality (FCM analysis)	5/1	18/6	1
Positive for *IGH* rearrangement (PCR)	8/0	20/6	0.30
WBC (/μL), median (range)	5950 (4500–13,000)	6200 (3600–12,400)	0.84
ANC (/μL), median (range)	3735 (2547–10,946)	4018 (2051–11,284)	0.95
ALC (/μL), median (range)	1905 (1363–2539)	1488 (792–3834)	0.24
LDH (U/L), median (range)	220.5 (163–376)	199 (141–274)	0.38
sIL-2R (U/mL), median (range)	269.5 (208.4–4040)	321 (107–762)	0.40
CRP (mg/dL), median (range)	0.06 (0.02–0.48)	0.05 (0.02–0.54)	0.41

p-values < 0.05 were considered statistically significant.

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; CRP, C-reactive protein; FCM, flow cytometry; IL, interleukin; LDH, lactate dehydrogenase; PCR, polymerase chain reaction; sIL-2R, soluble interleukin-2 receptor, WBC, white blood cell

Table S5. Relationship between *PRDM1* alteration and clinical findings

Factors	*PRDM1* alteration		p-value
	Positive (n = 17)	Negative (n = 18)	
Sex, male/female	9/8	5/13	0.18
Age, median (range), years	72 (45–83)	69.5 (43–84)	0.47
Laterality, unilateral/bilateral	5/12	10/8	0.18
IL-10 level (pg/mL), median (range)	738 (137–130,125)	1192 (10–10,596)	0.64
IL-10/IL-6 ratio	17.4 (1.2–1161.8)	13.0 (0.29–190.6)	0.22
Cytopathology positive (class ≥IIIb)	12/5	11/7	0.73
B-cell clonality (FCM analysis)	11/4	12/3	1
Positive for *IGH* gene rearrangement (PCR)	12/4	16/2	0.39
WBC (/μL), median (range)	6000 (4100–13000)	6200 (3600–12,400)	0.87
ANC (/μL), median (range)	3870 (2378–10946)	3959 (2051–11,284)	0.88
ALC (/μL), median (range)	1488 (968–2539)	1632.5 (792–3834)	0.82
LDH (U/L), median (range)	199 (157–376)	212.5 (141–274)	0.88
sIL-2R (U/mL), median (range)	287.1 (125–4040)	341.5 (107–762)	0.31
CRP (mg/dL), median (range)	0.04 (0.02–0.48)	0.065 (0.02–0.54)	0.69

p-values < 0.05 were considered statistically significant.

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; CRP, C-reactive protein; FCM, flow cytometry; IL, interleukin; LDH, lactate dehydrogenase; PCR, polymerase chain reaction; sIL-2R, soluble interleukin-2 receptor; WBC, white blood cell

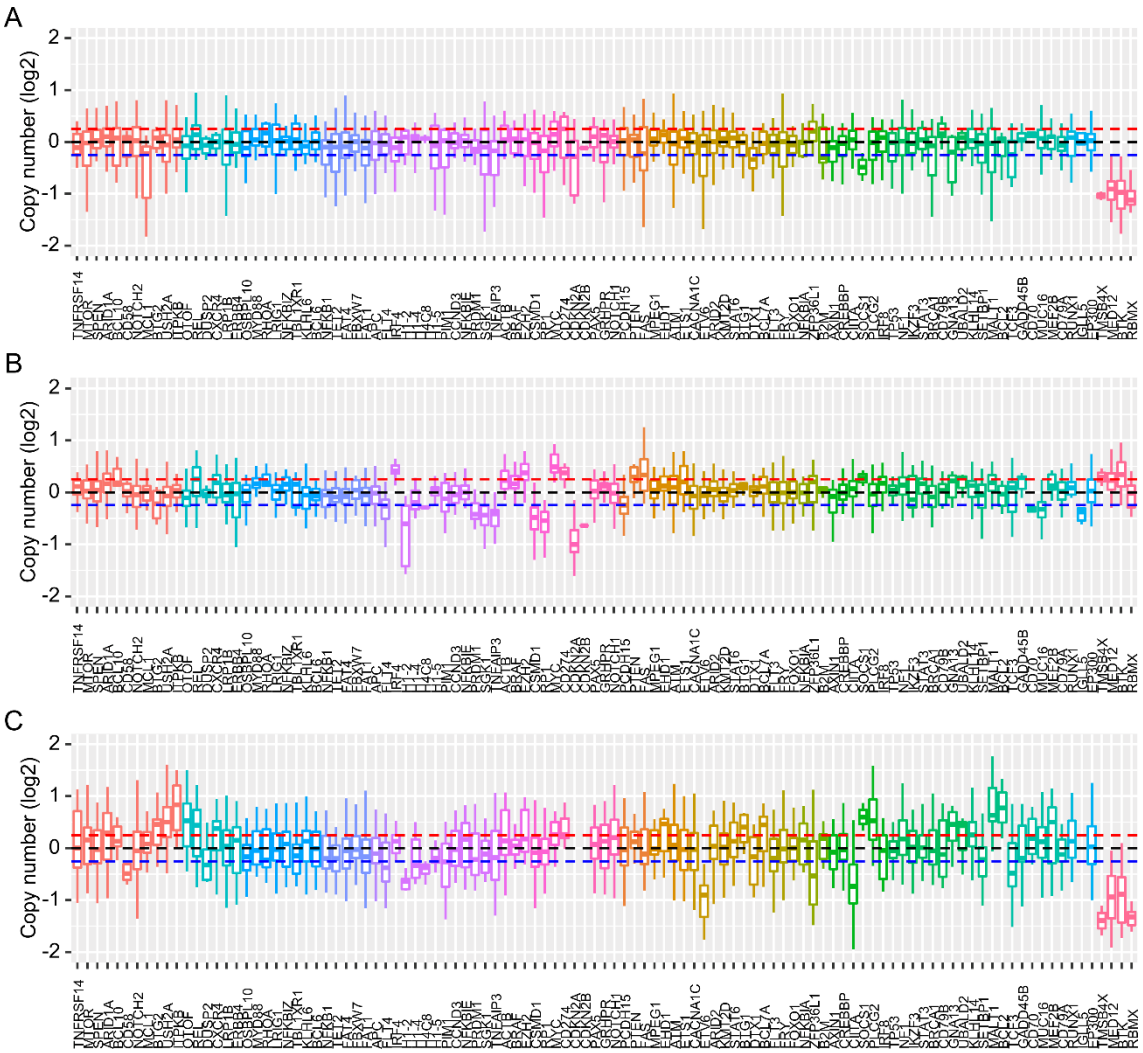
Table S6. Pathogenic gene alteration in primary vitreoretinal lymphoma patients with central nervous system progression

	Vitreous humor					Brain				
Case	**Gene**	**Mutation type**	**cDNA change**	**AA change**	**VAF**	**Gene**	**Mutation type**	**cDNA change**	**AA change**	**VAF**
39	*MYD88*	Missense	c.794T>C	p.Leu265Pro	39.48	*MYD88*	Missense	c.794T>C	p.Leu265Pro	90.42
	KMT2D	Nonsense	c.12844C>T	p.Arg4282Ter	34.29	*KMT2D*	Nonsense	c.12844C>T	p.Arg4282Ter	70.33
	CD274	Gain				*CD274*	Gain			
	CDKN2A	Loss				*CDKN2A*	Loss			
	CDKN2B	Loss				*CDKN2B*	Loss			
	IGLL5	Loss				*IGLL5*	Loss			
	ETV6	Loss				-				
	-					*ACTB*	Gain			
	-					*CD58*	Frameshift	c.218delC	p.Ala73ValfsTer11	91.62
	-					*IRF4*	Missense	c.170C>T	p.Ala57Val	64.48
	-					*HIST1H1C*	Missense	c.347C>G	p.Ala116Gly	94.51
	-					*HIST1H4H*	Missense	c.28G>T	p.Gly10Cys	34.31
	-					*MYC*	Missense	c.63C>G	p.Ser21Arg	63.02
	-					*MYC*	Missense	c.106C>T	p.Pro36Ser	63.35
	-					*MYC*	Missense	c.650G>C	p.Ser217Thr	62.87
	-					*CD79B*	Missense	c.589T>C	p.Tyr197His	57.61
	-					*BRAF*	Gain			
	-					*STAT6*	Gain			
	-					*B2M*	Loss			
56	*ACTB*	Missense	c.137G>C	p.Gly46Ala	33.64	*ACTB*	Missense	c.137G>C	p.Gly46Ala	40.37
	CD79B	Missense	c.590A>C	p.Tyr197Ser	34.32	*CD79B*	Missense	c.590A>C	p.Tyr197Ser	39.56
	MYD88	Missense	c.794T>C	p.Leu265Pro	43.04	*MYD88*	Missense	c.794T>C	p.Leu265Pro	40.16
	BTG1	Missense	c.400A>T	p.Thr134Ser	50.12	*BTG1*	Missense	c.400A>T	p.Thr134Ser	39.05
	BTG1	Missense	c.160C>T	p.His54Tyr	57.03	*BTG1*	Missense	c.160C>T	p.His54Tyr	38.59
	BTG1	Nonsense	c.168G>A	p.Trp56Ter	57.03	*BTG1*	Nonsense	c.168G>A	p.Trp56Ter	38.59
	BTG1	Missense	c.8C>T	p.Pro3Leu	26.63	*BTG1*	Missense	c.8C>T	p.Pro3Leu	35.94
	PRDM1	Loss				*PRDM1*	Loss			
	CDKN2A	Loss				*CDKN2A*	Loss			
	CDKN2B	Loss				*CDKN2B*	Loss			
	PIM1	Nonsense	c.382C>T	p.Gln128Ter	55.11	-				

	KMT2D	Loss				-				
	BCL7A	Loss				-				
	-					*BTG1*	Missense	c.316G>A	p.Val106Ile	42.37
	-					*GNA13*	Nonsense	c.79C>T	p.Gln27Ter	41.29
82	*SOCS1*	Frameshift	c.312_330delCGACAGCCGCCAGCGGAAC	p.Asp105AlafsTer7	23.05	*SOCS1*	Frameshift	c.312_330delCGACAGCCGCCAGCGGAAC	p.Asp105AlafsTer7	49.62
	-					*MYD88*	Gain			
	-					*RHOA*	Gain			
	-					*TET2*	Loss			
	-					*FAT4*	Loss			
	-					*FBXW7*	Loss			
	-					*FAT1*	Loss			
	-					*CDKN2A*	Loss			
	-					*CDKN2B*	Loss			
	-					*IGLL5*	Loss			
	-					*MYD88*	Missense	c.794T>C	p.Leu265Pro	42.32
	-					*HIST1H1E*	Missense	c.536C>T	p.Ala179Val	24.25
	-					*PIM1*	Splice	c.356-1G>A		40.08
	-					*PRDM1*	Nonsense	c.1230C>A	p.Tyr410Ter	78.49
	-					*ACTB*	Missense	c.585G>C	p.Glu195Asp	31.11
	-					*CSMD1*	Splice	c.86-2A>G		26.09
	-					*BTG1*	Missense	c.136G>A	p.Glu46Lys	38.39
	-					*IGLL5*	Splice	c.206+1G>C		37.45
97	*PIM1*	Nonsense	c.720G>A	p.Trp240Ter	41.68	*PIM1*	Nonsense	c.720G>A	p.Trp240Ter	44.93
	PIM1	Nonsense	c.908G>A	pTrp303Ter	41.99	*PIM1*	Nonsense	c.908G>A	p.Trp303Ter	43.76
	GRHPR	Splice	c.287+1G>A		36.71	*GRHPR*	Splice	c.287+1G>A		47.28
	KMT2D	Frameshift	c.15891_15895dupGGTGC	p.His5299ArgfsTer8	37.80	*KMT2D*	Frameshift	c.15891_15895dupGGTGC	p.His5299ArgfsTer8	52.79
	CD79B	Missense	c.590A>G	p.Tyr197Cys	33.35	*CD79B*	Missense	c.590A>G	p.Tyr197Cys	37.82
	BTG2	Splice	c.142+1G>C		30.43	*BTG2*	Splice	c.142+1G>C		39.49
	MYD88	Missense	c.794T>C	p.Leu265Pro	33.06	*MYD88*	Missense	c.794T>C	p.Leu265Pro	47.34
	MPEG1	Nonsense	c.556C>T	p.Gln186Ter	29.55	*MPEG1*	Nonsense	c.556C>T	p.Gln186Ter	42.65
	PRDM1	Splice	c.291G>C	p.Glu97Asp	51.92	*PRDM1*	Missense	c.291G>C	p.Glu97Asp	78.68
	CDKN2A	Loss				*CDKN2A*	Loss			

	CDKN2B	Loss				*CDKN2B*	Loss			
	PIM1	Nonsense	c.652C>T	p.Gln218Ter	80.38	-				
	GRHPR	Splice	c.214+1G>A		41.75	-				
	ETV6	Loss				-				
	NFKBIZ	Gain				-				
	-					*PIM1*	Splice	c.356-9_357delCTTTCCTAGGC		29.60
	-					*MYC*	Missense	c.486G>T	p.Glu162Asp	48.26
	-					*STAT6*	Gain			
	-					*IGLL5*	Loss			

Abbreviations: AA, amino acid; CNS, central nervous system; VAF, variant allele frequency



Supplemental Figure 1. Representative copy number plot. Copy numbers of each amplicon were shown as boxplot organized by the gene level. Results of uveitis sample (control) and two PVRL cases (Case 1 and 49) are shown in (A) and (B) and (C), respectively. Black dotted line indicates copy number neutral value. Red and blue dotted lines are thresholds for gain and loss, respectively.

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