Somatic mutations of cell-free circulating DNA detected by next-generation sequencing reflect the genetic changes in both germinal center B-cell-like and activated B-cell-like diffuse large B-cell lymphomas at the time of diagnosis

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Supplementary file 1. Lymphopanel and PGM data analysis

A. Lymphopanel set used for NGS experiments

Gene	Transcript Reference	Hotspot / Exons partially sequenced	Chromosomal location	Size sequenced (bp)
B2M	NM_004048	Hotspots exons 1 & 2, exon 3	15q21.1	360
BCL2	NM_000633	Hotspot exon 2	18q21.33	585
BRAF	NM_004333	Exon 15	7q34	119
CARD11	NM_032415	Coiled-coil domain exons 4-9	7p22.2	1121
CD58	NM_001779	Exons 1-6	1p13.1	753
CD79A	NM_001783	ITAM domain exons 4 & 5	19q13.2	183
CD79B	NM_000626	ITAM domain exons 5 & 6	17q23.3	141
CDKN2A	NM_000077+NM_058195+ NM_058197+NM_001195132	Exons 1, 2A, 2B, 3, 4 & 5	9p21.3	1,737
CDKN2B	NM_004936 + NM_0078487	Exons 1A, 1B & 2	9p21.3	1289
CIITA	NM_000246	Exons 1-19	16p13.13	3393
CREBBP	NM_004380	Exons 1-31	16p13.3	7323
EP300	NM_001429	Exons 1-31	22q13.2	7245
EZH2	NM_004456	SET domain, hotspots exon 16 & 18	7q36.1	177
FOXO1	NM_002015	Hotspots exon 1 & FH domain exon 2	13q14.11	780
GNA13	NM_006572	Exons 1-4	17q24.1	1134
ID3	NM_002167	Exons 1 & 2	1p36.12	360
IRF4/MUM1	NM_002460	Exons 2-9	6p25.3	1356
ITPKB	NM_002221	Exons 2-8	1q42.12	2830
KMT2D/MLL2	NM_003482	Exons 1-54	12q13.12	16614
MEF2B	NM_001145785	Exons 2-9	19p13.11	1107
MFHAS1	NM_004225	Exons 1-3	8p23.1	3159
MYC	NM_002467	Exons 1-3	8q24.21	1365
MYD88	NM_001172567	Exons 2-5	3p22.2	587
NOTCH1	NM_017617	PEST domain exon 34	9q34.3	1488
NOTCH2	NM_024408	Exons 26-28 & 34 (HD/PEST domains)	1p12-p11.2	2091
PIM1	NM_002648	Exons 1-6	6p21.2	942
PRDM1/BLIMP1	NM_001198	Exons 1-7	6q21	2478
SOCS1	NM_003745	Exon 2	16p13.13	636
STAT6	NM_001178078	Exons 9-14 (DNA binding domain hotspot)	12q13.3	795
TCF3	NM_001136139	B-HLH domain of E47 isoform exons 17 & 18	19p13.3	370
TNFAIP3	NM_006290	Exons 2-9	6q23.3	2373
TNFRSF14	NM_003820	Exons 1-8	1p36.32	852
TP53	NM_000546	Mutation hotspots exons 4-10	17p13.1	1004
XPO1	NM_003400	Exons 15-18	2p15	640

B. PGM Data analysis

Torrent Suite™ version 4.0 (Life Technologies) software was used to perform primary analysis, including signal processing, base calling, sequence alignment to the reference genome (hg19) and generation of Binary Alignment/Map (BAM) files. BAM files were used by Torrent Suite™'s Variant Caller to detect point mutations as well as short insertions and deletions using the "Somatic" and "Low Stringency" default parameters. VCF files generated by Variant Caller were annotated by ANNOVAR(1).

Data generated from tumor DNA samples were considered of sufficient quality when more than 90% of targeted bases were read at least 20 times with sequencing and mapping precisions of at least Q20. Only frameshift deletions and insertions, nonframeshift deletions and substitutions, splicing, nonsynonymous, stopgain or stoploss Single Nucleotide Variations (SNVs) were kept. Variants with a minimal Variant Allele Frequency (MAF) greater than 1% in the 1000 genome database were considered as polymorphisms and were discarded(2). A normal probability plot defined thresholds separating true positives [confirmed by Sanger sequencing, TVC (Torrent variant calling) quality score ≥ 22] from true negatives (discredited by Sanger sequencing, TVC score < 9.5) and highlighted a gray zone (9.5 ≤ TVC score < 22) in which variants must be confirmed by Sanger sequencing or pyrosequencing..

Further verification by Sanger sequencing was performed using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies) and an ABI PRISM 3130 analyzer (Life Technologies). Further verification by pyrosequencing was performed using the PyroMark PCR kit (Qiagen, France) with internal and sequencing primers designed using PyroMark software (Qiagen).

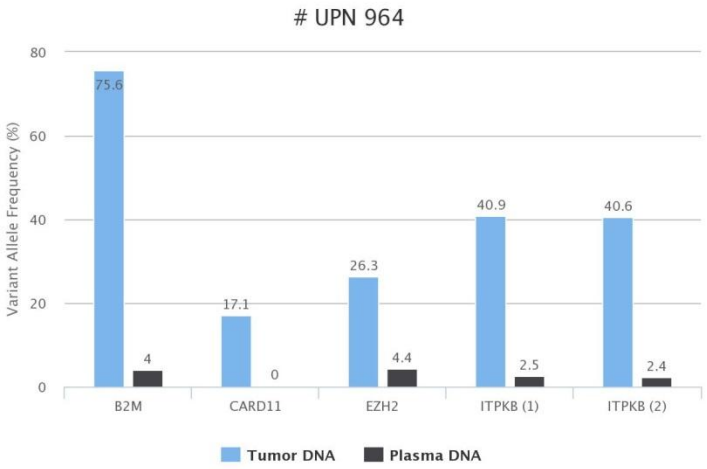
For circulating DNA variant calling, the parameter file was modified as follows: hotspot_min_variant_score: 3; hotspot_strand_bias: 1; downsample_to_coverage: 30 000. In addition, a dedicated hotspot VCF file generated from variants found in tumor DNA was used. This file instructs the Variant Caller to include these positions in its output files, including evidence for a variant and the filtering thresholds that disqualify a variant candidate.

References

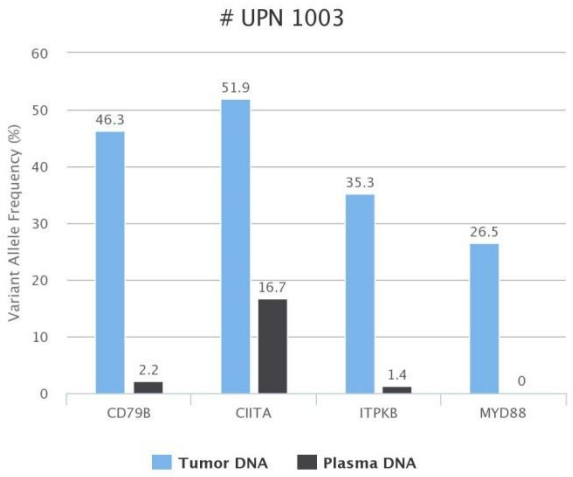
1. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic acids research. 2010 Sep;38(16):e164.
2. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. An integrated map of genetic variation from 1,092 human genomes. Nature. 2012 Nov 1;491(7422):56-65.

Figure S1

Relevant reads sequenced
Tumor: 885
Plasma: 21,078



Relevant reads sequenced
Tumor: 582
Plasma: 18,827



Relevant reads sequenced
Tumor: 553
Plasma: 4,685

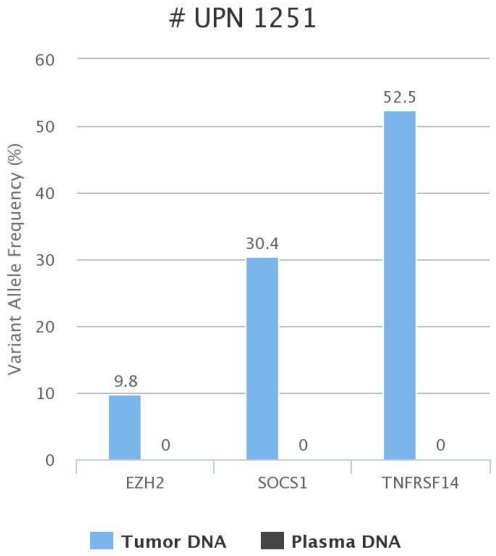
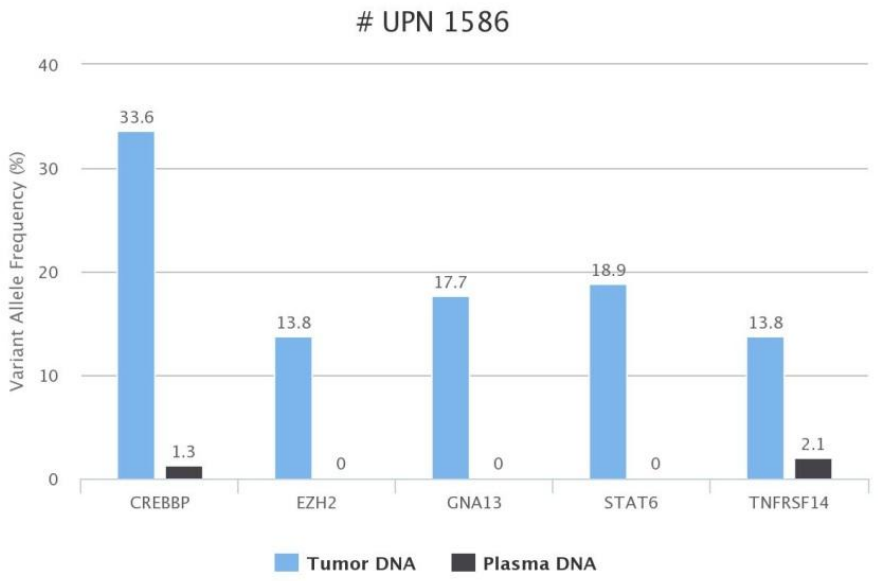


Figure S1

Relevant reads sequenced
Tumor: 1,263
Plasma: 21,661



Relevant reads sequenced
Tumor: 1,381
Plasma: 51,195

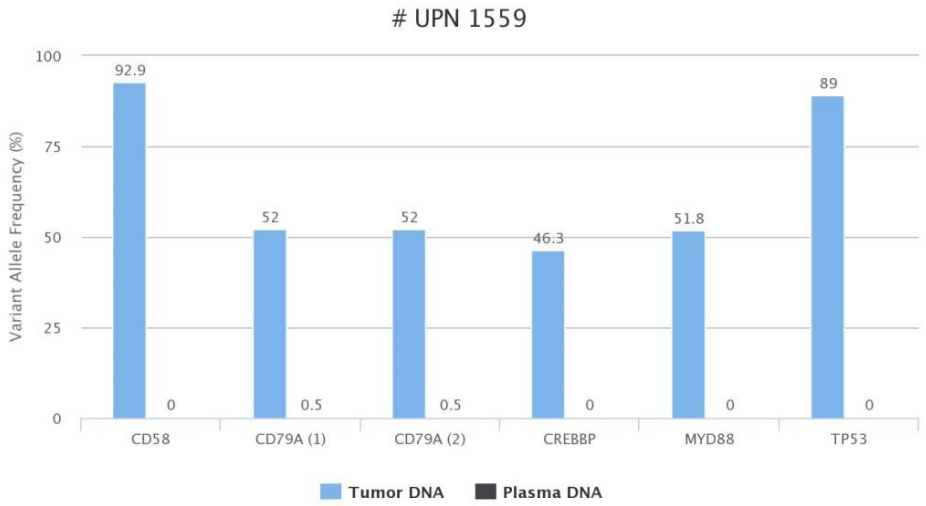


Figure S1

Relevant reads sequence
Tumor: 1,886
Plasma: 70,466

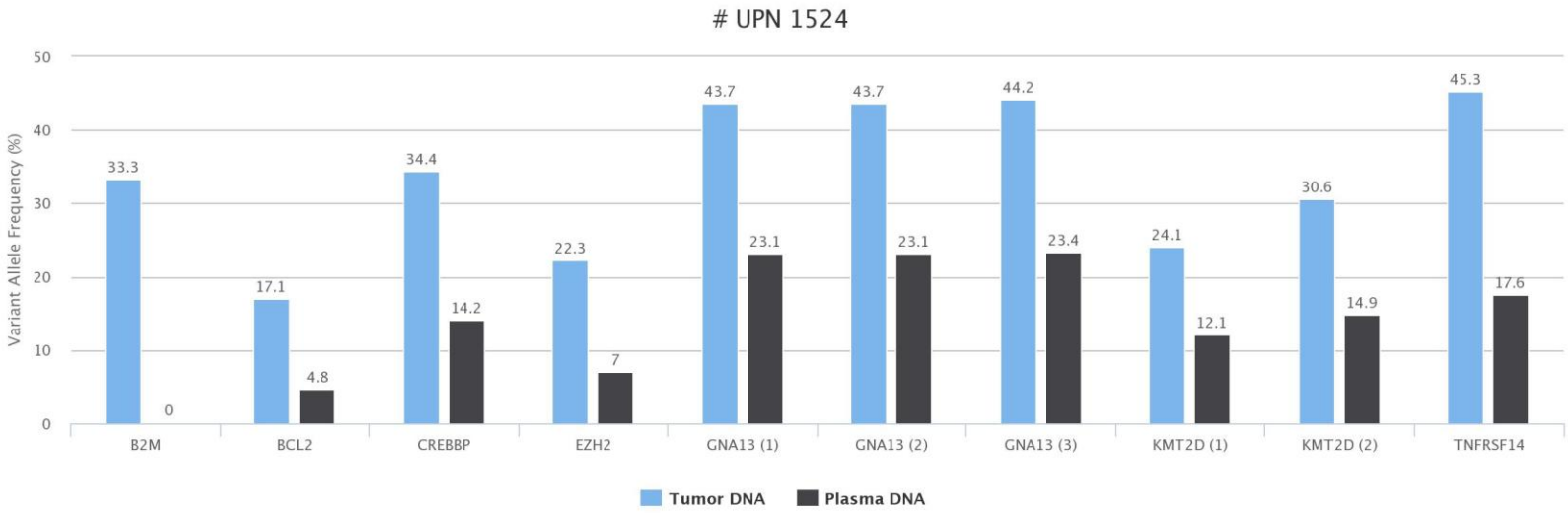
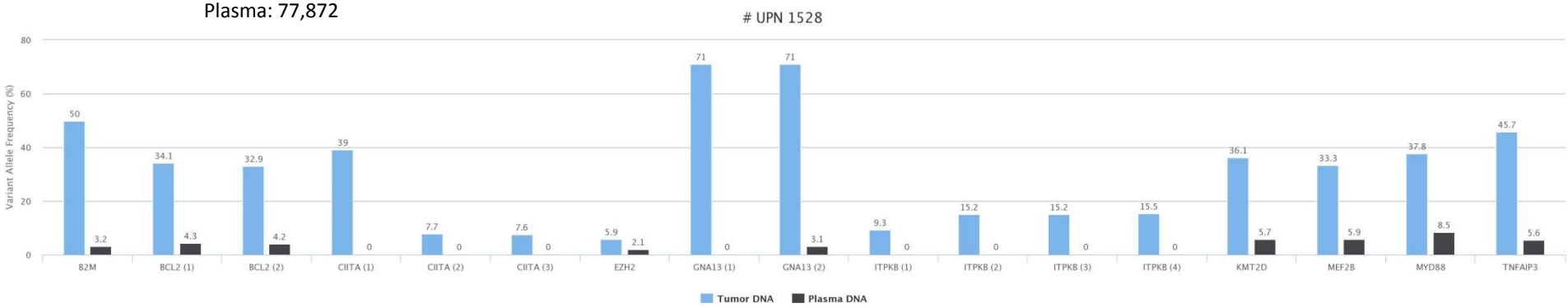


Figure S1

Relevant reads sequenced
Tumor: 3421
Plasma: 77,872



Relevant reads sequenced
Tumor: 636
Plasma: 386

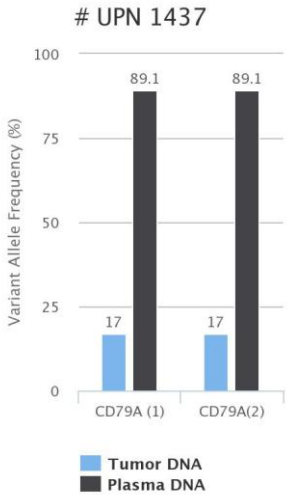
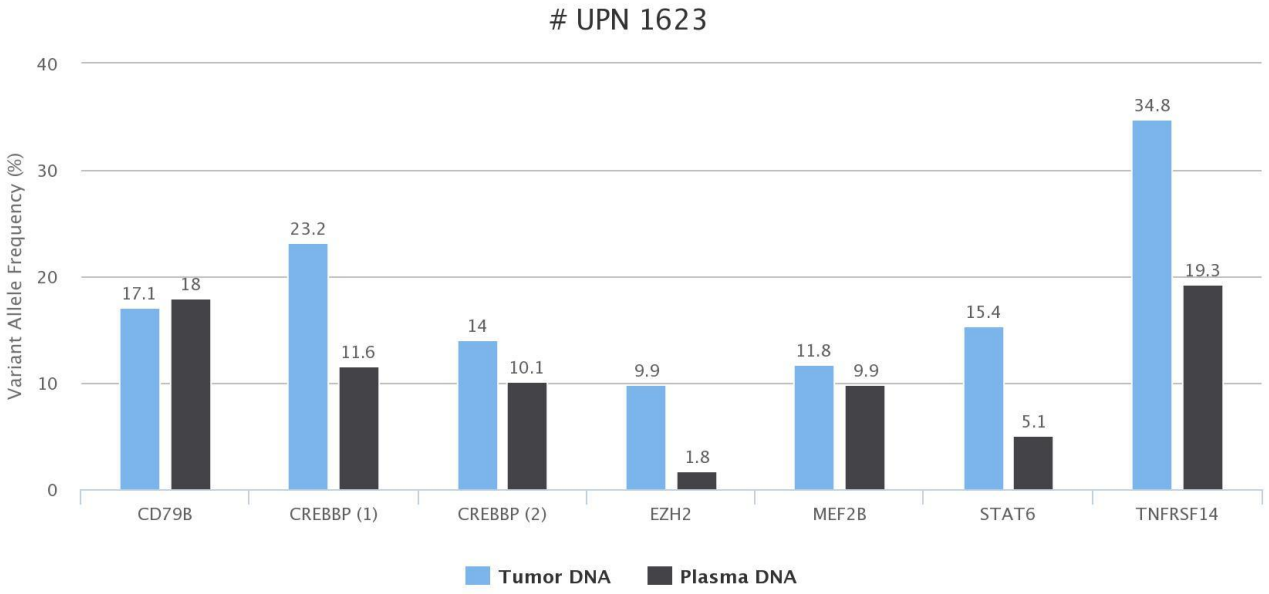


Figure S1

Relevant reads sequenced
Tumor: 3,724
Plasma: 36,037



Relevant reads sequenced
Tumor: 1,811
Plasma: 58,131

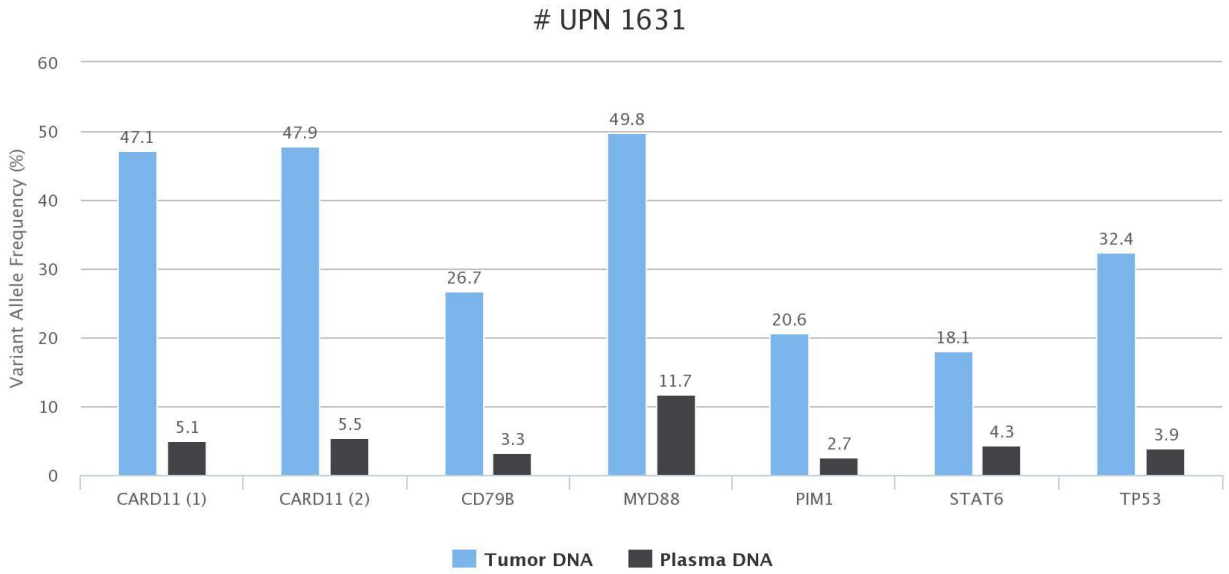
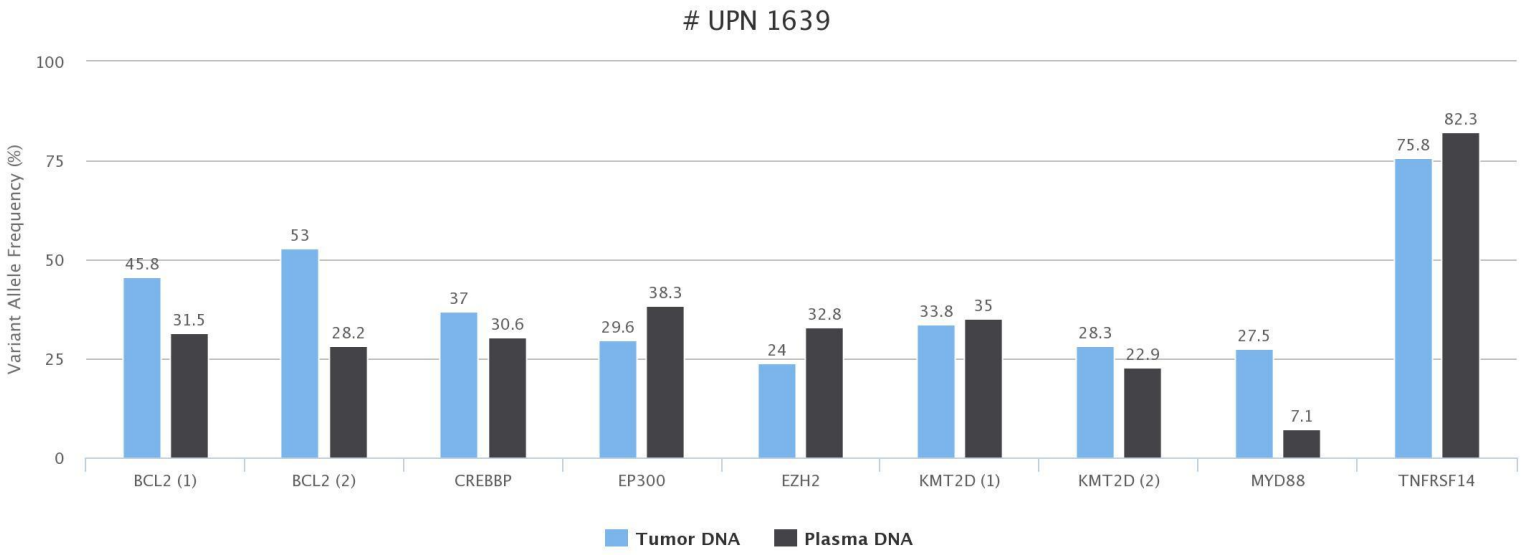
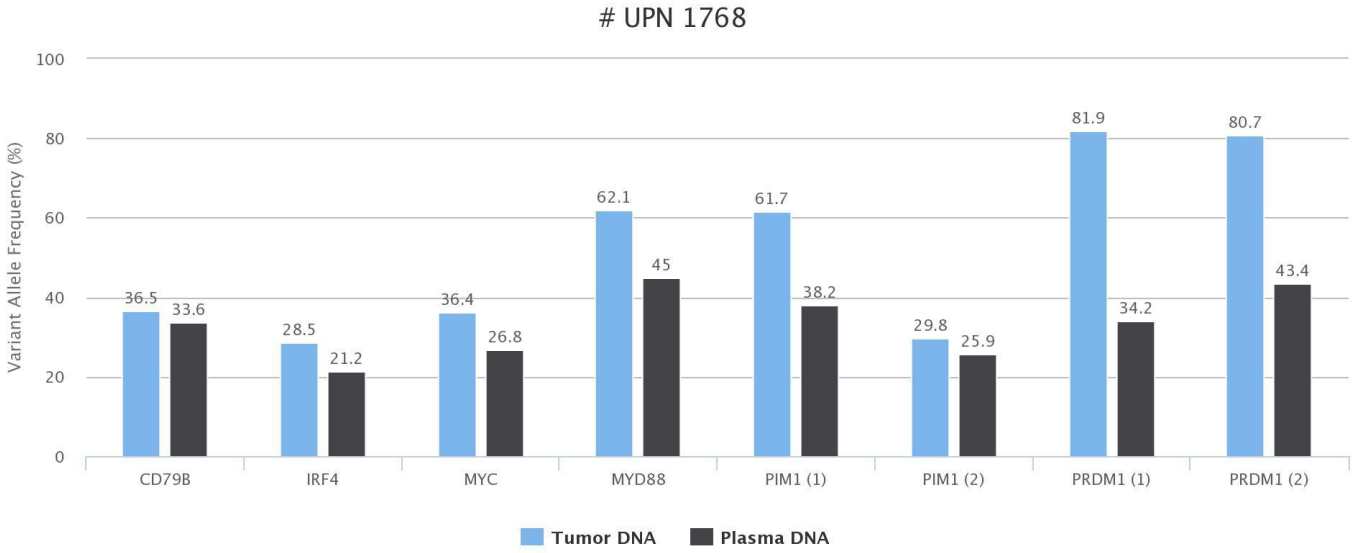


Figure S1

Relevant reads sequenced
Tumor: 2,041
Plasma: 85,369



Relevant reads sequenced
Tumor: 1,852
Plasma: 50,862



Supplementary table 1

UPN	Sex	Age	Stage	IPI	LDH (x UNL)	Bone marrow involvement	Phenotype	Gene	SNV	Tumor DNA			circulating DNA					Duration of Tumor/plasma storage (y) before sequencing
										VAF (%)	read number	mean VAF (%)	VAF (%)	read number	mean VAF (%)	Allele Call	concentration (ng/µl)	
964	F	17	IV	3	5	0	GCB	*ITPKB*	NM_002221:exon2:c.C731T-(p.T244I)	40.9	79/193	40.1	2.5	158/6313	2.3	Heterozygous	2.02	14
								ITPKB	NM_002221:exon2:c.726_728del-(p.242_243del)	40.6	78/192		2.4	152/6260		Heterozygous		
								CARD11	NM_032415:exon7:c.G1010A-(p.R337Q)	17.1	13/76		0.0	0/54		Absent		
								EZH2	NM_004456:exon16:c.A1937T-(p.Y646F)	26.3	76/289		2.8	126/4435		Heterozygous		
								B2M	NM_004048:exon1:c.T2A-(p.M1K)	75.6	102/135		4.0	130/4016		Heterozygous		
1003	F	75	III	3	2.5	0	ABC	*ITPKB*	NM_002221:exon2:c.541delC-(p.R181fs)	35.3	36/102	40.0	1.4	22/1540	5.1	Heterozygous	1.94	13
								MYD88	NM_002468:exon4:c.T695C-(p.M232T)	26.5	67/253		0.0	0/21		Absent		
								CIITA	NM_000246:exon13:c.C2819A-(p.T940K)	51.9	14/27		16.7	1/6		Heterozygous		
								CD79B	NM_000626:exon6:c.596delT-(p.L199fs)	46.3	93/201		2.1	369/17260		Heterozygous		
1251	M	42	IE	0	0.8	0	GCB	*TNFRSF14*	NM_003820:exon1:c.G3A-(p.M1I)	52.5	74/141	30.9	0.0	0/1113	0.0	Absent	0.46	10
								EZH2	NM_004456:exon16:c.A1937T-(p.Y646F)	9.8	38/389		0.0	0/2519		Absent		
								SOCS1	NM_003745:exon2:c.C174A-(p.F58L)	30.4	7/23		0.0	0/1053		Absent		
1437	M	76	III	4	5.85	0	ABC	*CD79A*	NM_001783:c.568-2A>G	17.0	53/318	17.0	89.1	344/386	89.1	Heterozygous	11.2	8
								CD79A	NM_001783:exon5:c573_617del-(p.191_206del)	17.0	53/318		89.1	344/386		Heterozygous		
1524	F	45	III	2	1.52	0	GCB	*TNFRSF14*	NM_003820:exon3:c.T269C-(p.L90P)	45.3	77/170	33.9	17.6	539/3064	14.0	Heterozygous	1.48	7
								EZH2	NM_004456:exon16:c.T1936A-(p.Y646N)	22.3	106/475		7.0	818/11751		Heterozygous		
								KMT2D	NM_003482:exon40:c.G13671C-(p.Q4557H)	24.1	64/266		12.1	1269/10509		Heterozygous		
								KMT2D	NM_003482:exon34:c.C8401T-(p.R2801X)	30.6	33/108		14.9	1015/6818		Heterozygous		
								B2M	NM_004048:exon1:c.T35C-(p.L12P)	33.3	44/132		0.0	0/4555		Absent		
								CREBBP	NM_004380:c.4394+2A>T	34.4	45/131		14.2	47/331		Heterozygous		
								GNA13	NM_006572:exon3:c.T533A-(p.L178Q)	43.7	52/119		23.1	1909/8254		Heterozygous		
								GNA13	NM_006572:exon3:c.T530C-(p.F177S)	43.7	52/120		23.1	1920/8314		Heterozygous		
								GNA13	NM_006572:exon3:c.T526A-(p.Y176N)	44.2	53/120		23.4	1946/8312		Heterozygous		
								BCL2	NM_000633:exon2:c.G17C-(p.R6T)	17.1	42/245		4.8	409/8558		Heterozygous		
1528	F	66	III	4	1.39	0	GCB	*ITPKB*	NM_002221:exon2:c.C1583T-(p.S528L)	9.3	14/151	31.0	0.0	0/1713	2.5	Absent	1.94	7
								ITPKB	NM_002221:exon2:c.C1471G-(p.P491A)	15.2	19/125		0.0	0/3582		Absent		
								ITPKB	NM_002221:exon2:c.C1454A-(p.A485E)	15.2	19/125		0.0	0/3577		Absent		
								ITPKB	NM_002221:exon2:c.C1438G-(p.P480A)	15.5	19/123		0.0	0/3570		Absent		
								MYD88	NM_002468:exon4:c.G728A-(p.S243N)	37.8	74/196		8.5	161/1888		Heterozygous		
								TNFAIP3	NM_006290:exon9:c.T2335C-(p.C779R)	45.7	32/70		5.6	177/3152		Heterozygous		
								EZH2	NM_004456:exon16:c.T1936A-(p.Y646N)	5.9	44/741		2.1	152/7401		Heterozygous		
								KMT2D	NM_003482:exon48:c.C15142T-(p.R5048C)	36.1	122/338		5.7	207/3620		Heterozygous		
								B2M	NM_004048:exon1:c.G3A-(p.M1I)	50.0	63/126		3.2	78/2465		Heterozygous		
								CIITA	NM_000246:exon11:c.2239delT-(p.Y747fs)	39.0	30/77		0.0	0/2130		Absent		
								CIITA	NM_000246:exon18:c.T3236A-(p.V1079D)	7.7	14/183		0.0	0/9150		Absent		
								CIITA	NM_000246:exon18:c.T3241A-(p.Y1081N)	7.6	14/184		0.0	0/9162		Absent		
								GNA13	NM_006572:exon1:c.G201C-(p.Q67H)	71.0	105/148		0.0	0/2695		Absent		
								GNA13	NM_006572:exon1:c.T158C-(p.L53P)	71.0	105/148		3.1	82/2672		Heterozygous		
								BCL2	NM_000657:exon2:c.G589A-(p.G197S)	34.1	47/138		4.3	214/4929		Heterozygous		
								BCL2	NM_000633:exon2:c.C502G-(p.P168A)	32.9	45/137		4.2	209/5013		Heterozygous		
								MEF2B	NM_001145785:exon3:c.G229C-(p.E77Q)	33.3	137/411		5.9	654/11153		Heterozygous		

Supplementary table 1

1559	F	83	IE	1	0.8	0	ABC	*CD58*	NM_001779:exon3:c.C454T-(p.R152X)	92.9	92/99	64.0	0.0	0/13231	<1	Absent	0.618	7
								MYD88	NM_002468:exon5:c.T778C-(p.L265P)	51.8	187/361		0.0	0/11427		Absent		
								CREBBP	NM_004380:exon30:c.G5105T-(p.R1702L)	46.3	154/333		0.0	0/5663		Absent		
								TP53	NM_000546:exon5:c.G524A-(p.R175H)	89.0	105/118		0.0	0/13012		Absent		
								CD79A	NM_001783:c.568-2A>G	52.0	122/235		0.5	20/3930		Absent		
								CD79A	NM_001783:exon5:c573_617del-(p.191_206del)	52.0	122/235		0.5	20/3932		Absent		
1586	F	62	III	2	0.85	0	NA	*TNFRSF14*	NM_003820:exon2:c.C164T-(p.P55L)	13.8	44/318	20	2.1	6/284	<1	Heterozygous	1.31	6
								EZH2	NM_004456:exon16:c.A1937T-(p.Y646F)	13.8	41/298		0.0	0/6575		Absent		
								STAT6	NM_001178078:exon12:c.A1256G-(p.D419G)	18.9	83/439		0.0	2/5517		Absent		
								CREBBP	NM_004380:exon26:c.G4283C-(p.R1428P)	33.6	49/146		1.3	65/4894		Absent		
								GNA13	NM_006572:c.561+2A>G	17.7	11/62		0.0	0/4391		Absent		
1623	M	53	IV	2	1.03	0	GCB	*TNFRSF14*	NM_003820:c.179-1G>C	34.8	146/419	18	19.3	694/3587	11	Heterozygous	0.95	6
								EZH2	NM_004456:exon16:c.A1937T-(p.Y646F)	9.9	57/575		1.8	62/3480		Heterozygous		
								STAT6	NM_001178078:exon12:c.A1256G-(p.D419G)	15.4	79/514		5.1	200/3889		Heterozygous		
								CREBBP	NM_004380:exon28:c.A4628T-(p.D1543V)	23.2	166/717		11.6	489/4222		Heterozygous		
								CREBBP	NM_004380:exon27:c.T4504C-(p.W1502R)	14.0	54/387		10.1	328/3239		Heterozygous		
								CD79B	NM_000626:exon6:c.611dupC-(p.T204fs)	17.1	83/486		18.0	839/4651		Heterozygous		
								MEF2B	NM_001145785:exon3:c.G229A-(p.E77K)	11.8	74/626		9.9	1283/12969		Heterozygous		
1631	M	64	I	2	1.54	0	ABC	*MYD88*	NM_002468:exon4:c.G728A-(p.S243K)	49.8	220/442	35	11.7	655/5586	5	Heterozygous	1.1	6
								PIM1	NM_002648:exon4:c.G290A-(p.S97N)	20.6	14/68		2.7	130/4797		Heterozygous		
								CARD11	NM_032415:exon5:c.645_647del-(p.215_216del)	47.1	99/210		5.1	154/3028		Heterozygous		
								CARD11	NM_032415:exon4:c.T343C-(p.F115L)	47.9	68/142		5.5	344/6300		Heterozygous		
								STAT6	NM_001178078:exon12:c.A1249G-(p.N417D)	18.1	96/532		4.3	570/13250		Heterozygous		
								TP53	NM_000546:exon5:c.T518A-(p.V173E)	32.4	72/222		3.9	522/13317		Heterozygous		
								CD79B	NM_000626:exon5:c.A587T-(p.Y196F)	26.7	52/195		3.3	386/11853		Heterozygous		
1639	M	69	IV	2	2.4	0	GCB	*TNFRSF14*	NM_003820:exon6:c.C620A-(p.S207X)	75.8	47/62	39	82.3	2400/2915	34	Heterozygous	10.2	6
								MYD88	NM_002468:exon3:c.C840G-(p.S219C)	27.5	133/483		7.1	1616/22541		Heterozygous		
								EZH2	M_004456:exon16:c.A1937T-(p.Y646F)	24.0	126/526		32.8	3259/9939		Heterozygous		
								KMT2D	NM_003482:exon34:c.C9838T-(p.Q3280X)	33.8	187/554		35.0	5878/16784		Heterozygous		
								KMT2D	NM_003482:exon22:c.5296_5297insGAGG-(p.D1766fs)	28.3	13/46		22.9	671/2929		Heterozygous		
								CREBBP	NM_004380:c.4560+2A>G	37.0	17/46		30.6	2918/9530		Heterozygous		
								BCL2	NM_000633:exon2:c.C176A-(p.P59Q)	45.8	11/24		31.5	223/709		Heterozygous		
								BCL2	NM_000633:exon2:C20T-(p.T7I)	53.0	98/185		28.2	2267/8032		Heterozygous		
								EP300	NM_001429:exon27:c.4373_4375del-(p.1458_1459del)	29.6	34/115		38.3	4597/11990		Heterozygous		
1768	M	83	IV	3	4.2	0	ABC	*MYD88*	NM_002468:exon5:c.T778C-(p.L265P)	62.1	216/348	52	45.0	5936/13194	34	Heterozygous	1.82	4
								IRF4	NM_002460:exon2:c.G53A-(p.S18N)	28.5	75/263		21.2	929/4374		Heterozygous		
								PIM1	NM_002648:exon3:c.237delG-(p.E79fs)	61.7	145/235		38.2	1541/4030		Heterozygous		
								PIM1	NM_002648:exon4:c.G382C-(p.D128H)	29.8	34/114		25.9	987/3812		Heterozygous		
								PRDM1	NM_001198:exon2:c.G284A-(p.S95N)	81.9	149/182		34.2	1273/3724		Heterozygous		
								PRDM1	NM_001198:exon5:c.1103_1112del-(p.368_371del)	80.7	221/274		43.4	5076/11689		Heterozygous		
								MYC	NM_002467:exon2:c.C145T-(p.Q49X)	36.4	102/280		26.8	262/979		Heterozygous		
								CD79B	NM_000626:exon5:c.A587C-(p.Y196S)	36.5	57/156		33.6	3041/9060		Heterozygous		

Supplementary Table 1: Clinical characteristics and list of somatic variants (insertion/deletion/ single nucleotide variant) detected by sequencing in tumor DNA and cell-free plasma circulating DNA.

ABC: Activated B-Cell like; GCB: Germinal Center B-cell like; In/del: insertion/deletion; LDH: Lactate dehydrogenase; IPI: International Prognosis Index; SNV: single nucleotide variant; UPN: Unique Personal Number; ULN: upper limit value; VAF: variant allele frequency