Liquid biopsy for the identification of intravascular large B-cell lymphoma

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Timing of sample collection

Among 9 patients, whose cell-free DNAs (cfDNAs) were analyzed in targeted sequencing of 8 genes, only one patient (IVL4) had received 28 days of corticosteroid therapy prior to blood sample collection (Figure S4). Samples were collected at the timing of relapse in one patient (IVL5), when 3 years had passed since last chemotherapy including corticosteroid. The other cases did not receive any corticosteroid therapy before the first blood samples preservation. In four patients, samples were additionally preserved during the courses of chemotherapy (Figure 2A-D).

DNA extractions and analysis

Tissue-derived DNAs (tdDNAs) were extracted from archived formalin-fixed paraffin-embedded (FFPE) specimens and/or cryo-preserved bone marrow samples, in which the existence of lymphoma cells had been pathologically confirmed, using GeneRead FFPE kit (Qiagen) and/or Gentra Puregene Blood Kit (Qiagen), respectively.

Paired reference genomic DNAs (gDNAs) were extracted from buccal cells or archived FFPE specimens in which the absence of lymphoma cells had been pathologically confirmed using a DNA mini kit (Qiagen) and/or GeneRead FFPE kit (Qiagen). Three to 4 sections of each FFPE specimen were sliced at 10-micrometer thickness for DNA extraction. The cfDNAs were extracted from 1 ml of serum or plasma, obtained at various time points (e.g., prior to chemotherapy, at complete remission, at relapse), using a QIaAmp circulating nucleic acid kit (Qiagen). The serum and plasma were separated within 3 and 6 hours, respectively, after blood collection. Blood samples were collected into tubes with serum separator or tubes with heparin and centrifuged at 1750×*g* for 15 minutes at room temperature. Serum and plasma were transferred into cryogenic vials (Corning) at -80°C until DNA extraction. Quantification of the extracted DNAs was performed using a Qubit fluorometer (Thermo Fisher Scientific) or Nanodrop (ThermoFishser Scientific). A Qubit dsDNA HS Assay Kit was used for tdDNAs from FFPE specimens and cfDNAs. Extracted DNAs were stored at -20°C until analysis. Kits were performed according to the manufacturer's instructions.

Primer designs, constructing libraries, and variant calling pipelines in targeted sequencing

The Ion Ampliseq Custom DNA panel for 8 genes of coding sequences and its flanking regions was designed using online Ion Ampliseq Designer (Thermo Fisher Scientific). The gene panel consisted of 162 amplicons, with sizes ranging from 125 to 175bp, totaling 16.21 kilo-bases. Coverage of all targeted regions was 90.1%. Libraries were prepared using an Ion Ampliseq Library Kit 2.0 (Thermo Fisher Scientific). A median of 2.6 ng (range, 0.57-3.0 ng) of DNA was amplified by multiplex polymerase chain reaction (PCR) according to the manufacturer's instructions. Sequencing of cfDNAs and paired reference gDNAs to ≥ 100× coverage and tdDNA samples to ≥ 500× coverage was planned because preliminary results indicated that variant allele frequencies (VAFs) in cfDNAs were higher than those in tdDNAs. Torrent Suite version 5.2.2 (Thermo Fisher Scientific) software was used to perform signal processing, base calling, and sequence alignment to the reference genome (hg19), and the Binary Alignment Map (BAM) files were generated. The Tumor-Normal pair workflow of Ion Reporter Software version 5.6 (Thermo Fisher Scientific) was used to call somatic

mutations by uploading paired BAM files of cfDNA samples and reference gDNA samples plus those of tdDNA samples and reference gDNA samples (Figure S3). With IVL23, which had no paired reference gDNA sample, we used single sample workflow by uploading BAM files for cfDNA and tdDNA samples as an exception. In the Ion Reporter Software, the filters to call variants were set as follows: (1) P value<= 0.012 (5 x e^{-6}); (2) Allele read count >=4; (3) Variant allele frequencies >=10%; (4) Exclude synonymous SNV. As for (1), the workflow performed a statistical evaluation of the probability that the cfDNA or tdDNA variant allele was not present in the paired reference DNA and calculated a P-value representing the statistical confidence of the variant call. After completing the filtering, all somatic variants were validated by Sanger sequencing. Validated variants in cfDNA samples were explored in tdDNA samples by single sample workflow with the VAF filter turned down to 1%. The same procedure was applied for validated variants in tdDNA.

Sanger Sequencing

In order to obtain amplicons of interest, PCR was performed using KOD Plus Neo (Toyobo) or Amplitaq Gold 360 Master Mix (Thermo Fisher Scientific) according to

the manufacturer's instructions. The median amount of input DNA was 10 ng (range, 2.7-10.0 ng). Sanger Sequencing was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific)) and ABI 3130xl genetic analyzer (Thermo Fisher Scientific). The used primers are listed in Table S9.

Longitudinal analysis of cfDNA

To evaluate the utility of cfDNA analysis in assisting diagnosis and a monitoring context, we sequenced known variants for serial cfDNAs in each patient. Used primers are listed in Table S10. Libraries were constructed using the Ion Plus Fragment Library kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

Comparison analysis of cfDNAs extracted from paired serum and plasma samples

To assess the impact of the different cfDNA source (serum or plasma), we sequenced known variants for 6 pairs of cfDNAs extracted from paired serum and plasma samples in 3 patients (IVL2, IVL4, and IVL5). Used primers are listed in Table S10. Libraries were constructed in the same way as longitudinal analysis.

Droplet digital PCR analysis

Pre-designed primers and TaqMan probes with FAM and HEX were used for the detection of c.794T>C (L265P) *MYD88* variant (Bio-Rad, Assay ID: dHsaMDS2516944). Distilled water controls were run to check contamination and background probe signals. The assay was performed using the QX200 droplet digital PCR system (Bio-Rad). PCR amplification was performed as follows: initial enzyme activation at 95°C for 10 minutes, 40 cycles of denaturation and annealing/extension at 94°C for 30 seconds, hold at 56°C for 1 minute, and then enzyme deactivation at 98°C for 10 minutes. The ramp rate was at 2°C/second throughout the entire amplification process. Results were analyzed using QuantaSoft version 1.7.4. (Bio-Rad). Sensitivity thresholds were established by a dilution study with the lower limit of quantification and that of detection found to be 0.025% and 0.006% with a fractional abundance of 0.1% and 0.07%[1].

Statistical analysis

Statistical analysis was performed with EZR version 1.3.2.[2]. Paired t-test was used to

compare VAFs, and concentrations of cfDNA in paired samples. The enrichment and significance value of *PIM1* variants for the motif (WRCY), transition, and C:G base specific SNVs were calculated by Fisher's exact test (Table S7) as previously reported[3]. All *P* values were calculated by two-sided testing. The difference was considered statistically significant when the *P* value was less than 0.05.

Supplementary References

1. Hattori K, Sakata-Yanagimoto M, Suehara Y, et al. Clinical significance of disease-specific MYD88 mutations in circulating DNA in primary central nervous system lymphoma. Cancer science. 2018;109(1):225-230.
2. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone marrow transplantation. 2013;48(3):452-458.
3. Khodabakhshi AH, Morin RD, Fejes AP, et al. Recurrent targets of aberrant somatic hypermutation in lymphoma. Oncotarget. 2012;3(11):1308-1319.

Table S1. List of genes sequenced in targeted sequencing

Gene	Transcript reference	Location	References
B2M	NM_004048	15q21–q22.2	Challa-Malladi, Cancer Cell, 2011; Morin, Nature, 2011
BTG2	NM_006763	1q32	Morin, Nature, 2011
CARD11	NM_032415	7p22	Lenz, Science, 2008; Davis, Nature, 2010
CD79B	NM_000626	17q23	Davis, Nature, 2010
MYD88	NM_002468	3p22	Ngo, Nature, 2011
PIM1	NM_002648	6p21.2	Pasqualucci, Nature, 2001
PRDM1	NM_1198	6q21–q22.1	Pasqualucci, JEM, 2006
TNFAIP3	NM_001270507	6q23	Compagno, Nature, 2009

Table S2. Case description of 27 patients with intravascular large B-cell lymphoma

ID	IVL1	IVL2	IVL3	IVL4	IVL5	IVL6	IVL7	IVL8	IVL9	IVL10	IVL11
Age, Sex	38, F	55, F	62, M	71, M	74, F	63, F	74, F	33, M	28, F	76, M	63, F
Fever	+	+	+	+	+	+	−	+	+	−	−
Neulological symptoms	−	−	−	−	−	−	convulsion, left motor hemiparesis	−	convulsion, hemiplegia, loss of consciousness	gait disturbance, impaired speech	−
Other symptoms	−	fatigue, appetite loss	fatigue, appetite loss	−	−	−	−	cough	−	−	abdominal pain, hemato-chezia
Oxygen Saturation (%)	98	94	89	96	95	96	98	97	97	97	99
Leukocytopenia†	+	+	−	−	−	−	−	−	−	−	−
Anemia‡	+	+	−	+	+	+	+	+	−	−	−
Thrombocytopenia*	−	−	+	+	+	−	−	−	−	−	−
Splenomegaly	+	+	+	+	+	+	−	+	+	+	+
Lactate dehydrogenase [U/l]	1707	632	1638	721	917	1104	377	644	425	542	555
Soluble interleukin 2 receptor [U/ml]	6860	8320	1510	7480	8700	6020	595	2920	3200	3500	718
C-reactive protein [mg/dl]	18	5.4	3.77	6.2	10.3	7.7	0.03	23	5.36	0.03	2.35
Albumin [g/dl]	2.6	2.2	3.5	1.7	2	2.2	3.7	2.4	2.4	3.3	3.7
Bone marrow aspiration	+	(−)→(+)	(−)→(+)	−	−	−	−	−	−	NA	−
Bone marrow biopsy	−	(−)→(+)	(−)→(+)	+	+	−	−	−	−	+	−
Random skin biopsy	+	NA→(+)	+	−	+	+	+	+	−	+	NA
Other biopsy-proven site	breast (+, relapse)	NA	NA	NA	NA	kidney (+), liver (+)	NA	lung (+), liver (−), spleen (−)	brain (+), liver (−)	NA	mesentery (+)
18F-fluorodeoxyglucose-positron emission tomography/computed tomography	BM, subcutaneous tissue (left abdomial wall and left breast), uterus	negative	spleen	NA	BM, liver	BM, kidney, liver	NA	lungs	NA	NA	NA
Hemophagocytic Lymphohistiocytosis§	−	−	−	−	+	+	−	+	−	−	−
Spontaneous regression episode prior to diagnosis	−	+	−	−	−	−	−	−	−	−	−

IVL13	IVL15	IVL16	IVL17	IVL18	IVL19	IVL20	IVL21	IVL22	IVL23	IVL24	IVL25	IVL26	IVL27
74, M	65, M	79, F	71, F	46, F	62, F	74, M	76, F	61, M	72, F	74, F	71, F	88, M	57, F
+	+	+	+	–	+	+	+	+	+	+	+	+	+
loss of consciousness	gait disturbance, cauda equina syndrome	–	–	–	loss of consciousness	–	–	convulsion	–	confusion	confusion	gait disturbance	–
–	–	–	fatigue, appetite loss	incidental	–	cough, dyspnea	fatigue, appetite loss	–	cough	appetite loss	dyspnea	–	diarrhea, arthralgia
86	92	95	96	98	95	94	NA	NA	93	87	NA	NA	95
–	–	–	+	–	–	+	+	–	–	–	–	+	–
+	–	+	–	+	+	–	+	+	+	+	+	–	–
+	–	+	+	–	–	–	+	–	–	+	+	–	+
+	+	+	–	–	+	+	+	–	–	+	+	+	+
1255	1044	875	490	268	316	1764	223	341	699	1205	773	381	1090
2800	1740	22100	13883	5090	4205	2870	10200	1433	4739	5234	8924	2679	25012
10.04	14.36	15.86	1.16	3.32	22.02	3.8	10.2	0.7	10.01	13.88	9.55	2.9	20.65
1.8	2.3	1.3	2.9	3	1.9	3.4	1.4	1.7	2.1	2.9	2	3	2.5
+	–	+	dry tap	–	(–)→(+)	–	–	–	+	+	–	(–)→(–)	+
+	–	+	+	–	(–)→(+)	(–)→(+)	+	+	+	+	–	(–)→(+)	+
NA	+	NA	NA	NA	NA	–	+	+	+	+	+	(–)→(+)	+
NA	NA	liver (+)	NA	uterus (+), lung (+)	brain (+)	lung (+)	NA	NA	heart (+), mediastinal LN (+)	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	negative	NA	adrenal glands	heart, lungs, adrenal glands, mediastinal LN	liver, spleen	lungs	negative	BM, liver, spleen
+	–	+	+	–	–	–	+	–	–	–	+	+	+
–	–	–	–	–	–	+	–	–	–	–	–	+	–

IVL28	IVL29
61, F	81, M
+	+
−	loss of consciousness
arthralgia	fatigue
91	98
−	+
+	+
+	+
+	+
5199	511
10769	8998
22.47	4.39
2.3	2.6
−	+
+	+
−	+
NA	NA
BM, liver, dura mater	BM,sternal bone, rib, spleen
+	+
−	−

†WBC count < 4000/μL; ‡Hemoglobin < 11g/dl; *Platelet count < 10×104/μL; § Diagnosis was based on HLH 2004 criteria; F, Female; M, Male; NA, not available; BM, bone marrow; LN, lymph node; CNS, central nervous system; +, positive; −, negative

Table S3. Results of immunohistochemistry

ID	Specimen	Immunohistochemistry			Hans classification
		CD10	BCL6	MUM-1	
IVL1	breast	−	−	+	non-GCB
IVL2	skin	−	−	+	non-GCB
IVL3	skin	NA	NA	NA	NA
IVL4	skin	NA	NA	NA	NA
IVL5	skin	−	NA	+	non-GCB
IVL6	skin	−	−	+	non-GCB
IVL7	skin	−	+	+	non-GCB
IVL8	lung	−	+	+	non-GCB
IVL9	brain	−	+	+	non-GCB
IVL10	skin	−	+	+	non-GCB
IVL11	mesentery	−	−	+	non-GCB
IVL13	bone marrow clot	NA	NA	NA	NA
IVL15	skin	−	+	+	non-GCB
IVL16	bone marrow clot	−	+	+	non-GCB
IVL17	bone marrow	−	+	+	non-GCB
IVL18	lung	−	−	+	non-GCB
IVL19	brain	−	+	+	non-GCB
IVL20	lung	−	+	+	non-GCB
IVL21	bone marrow	−	+	+	non-GCB
IVL22	skin	NA	+	+	NA
IVL23	skin	−	−	NA	non-GCB
IVL24	skin	−	+	+	non-GCB
IVL25	skin	−	+	+	non-GCB
IVL26	skin/bone marrow	NA	NA	NA	NA
IVL27	bone marrow	−	NA	+	non-GCB
IVL28	lung	−	+	+	non-GCB
IVL29	skin	NA	NA	NA	NA

NA, not available; non-GCB, non-germinal center B-type; +, positive; −, negative

Table S4. Concentration of cell-free DNA

	ID	Event	Sample collection (counted from the day of diagnosis)	Prior chemotherapy	Prior glucocorticoid	Concentration (ng/ml)	Sample
Samples for targeted sequencing (8 genes)	IVL2	Diagnosis	day 0	no	no	147	plasma
	IVL3	Diagnosis	day 0	no	no	2900	plasma
	IVL4	Diagnosis	day 0	no	yes (since 28 days before)	179	plasma
	IVL5	Relapse (IVLBCL)	day1022	yes (3 years ago)	yes (3 years ago)	157	plasma
	IVL6	Diagnosis	day 0	no	no	56.7	plasma
	IVL21	Diagnosis	day 0	no	no	211.7	serum
	IVL23	Diagnosis	day 0	no	no	666.7	serum
	IVL24	Diagnosis	day 0	no	no	2170	serum
	IVL25	Diagnosis	day 0	no	no	422.5	serum
Samples for longitudinal analysis/ Samples for comparative analysis of serum and plasma	IVL1	Complete remission	day 496	yes	yes	30.7	serum
	IVL1	Regular visits	day 852	yes	yes	67.5	serum
	IVL1	Regular visits	day 908	yes	yes	79.5	serum
	IVL1	Regular visits	day 971	yes	yes	69.5	serum
	IVL1	Regular visits	day 999	yes	yes	88	serum
	IVL1	Relapse (cecum)	day 1033	yes	yes	88.5	serum
	IVL2	Before diagnosis	day −117	no	no	189	serum
	IVL2	Before diagnosis	day −101	no	no	68	serum
	IVL2	Before diagnosis	day −84	no	no	19.1	serum
	IVL2	Before diagnosis	day −7	no	no	185.5	serum
	IVL2	Before diagnosis	day −7	no	no	73.2	plasma
	IVL2	Before diagnosis	day −3	no	no	169	serum
	IVL2	Before diagnosis	day −3	no	no	83.1	plasma
	IVL2	Diagnosis	day 0	no	no	515	serum
	IVL2	Complete remission	dat 168	yes	yes	22.25	serum
	IVL2	Regular visits	day 512	yes	yes	25.2	serum
	IVL4	Before diagnosis	day −5	no	yes (since 23 days before)	207.5	serum
	IVL4	Before diagnosis	day −5	no	yes (since 23 days before)	58.1	plasma
	IVL4	Before diagnosis	day −3	no	yes (since 25 days before)	189	serum
	IVL4	Before diagnosis	day −3	no	yes (since 25 days before)	70.6	plasma
	IVL5	Complete remission	day 742	yes	yes	80	serum
	IVL5	Regular visits	day 805	yes	yes	44.15	serum
	IVL5	Regular visits	day 944	yes	yes	490	serum
	IVL5	Relapse (IVLBCL)	day 1005	yes	yes	423	serum
	IVL5	Relapse (IVLBCL)	day 1005	yes	yes	295	plasma
	IVL6	Regular visits	day 208	yes	yes	81.5	serum
	IVL6	Regular visits	day 271	yes	yes	116.5	serum
	IVL6	Regular visits	day 341	yes	yes	89	serum
	IVL6	Relapse (CNS)	day 393	yes	yes	93.5	serum

IVLBCL, intravascular large B-cell lymphoma; CNS, central nervous system

Table S5. List of variants in targeted sequencing

Position (hg19)	variant classification	VAF_ cfDNA (%)	VAF_ tdDNA (%)	genes	Ref.	Var.	COSMIC	dbSNP	Exon	cDNA change	Protein change	coverage _cfDNA	coverage _tdDNA	ID
chr3:38182641	nonsynonymous SNV	46.18	5.2	*MYD88*	T	C	COSM85940	rs387907272	5	c.794T>C	p.Leu265Pro	1047	3526	IVL2
chr6:106553712	Stop gain	54.82	2.6	*PRDM1*	C	A	–	–	5	c.1677C>A	p.Tyr559Ter	197	2269	IVL2
chr6:37138644	nonsynonymous SNV	41.53	6.5	*PIM1*	G	A	–	–	2	c.178G>A	p.Asp60Asn	118	356	IVL2
chr6:37138925	nonsynonymous SNV	41.07	2.4	*PIM1*	G	A	–	–	4	c.265G>A	p.Glu89Lys	56	352	IVL2
chr6:37139110	nonframeshift substitution	52.83	4	*PIM1*	GG	AA	–	–	4	c.450_451GG>AA	p.Val151Met	265	2768	IVL2
chr1:203274762	nonsynonymous SNV	14.15	no call	*BTG2*	C	G	–	–	1	c.28C>G	p.Leu10Val	424	3010	IVL21
chr3:38182641	nonsynonymous SNV	26.73	no call	*MYD88*	T	C	COSM85940	rs387907272	5	c.794T>C	p.Leu265Pro	549	9372	IVL21
chr6:37138761	nonsynonymous SNV	20.61	no call	*PIM1*	C	A	–	–	3	c.194C>A	p.Ala65Asp	427	2248	IVL21
chr6:37139045	nonsynonymous SNV	45.45	no call	*PIM1*	C	T	–	–	4	c.385C>T	p.Leu129Phe	11	1950	IVL21
chr6:37139098	nonsynonymous SNV	12.35	no call	*PIM1*	C	A	–	–	4	c.438C>A	p.Ser146Arg	429	5174	IVL21
chr6:37140800	nonsynonymous SNV	21.44	no call	*PIM1*	G	A	–	–	5	c.636G>A	p.Trp212Ter	1617	14392	IVL21
chr17:62006799	nonsynonymous SNV	70.96	no call	*CD79B*	A	G	COSM220734	–	5	c.586T>C	p.Tyr196His	834	5101	IVL23
chr3:38182641	nonsynonymous SNV	70.18	no call	*MYD88*	T	C	COSM85940	rs387907272	5	c.794T>C	p.Leu265Pro	1054	9814	IVL23
chr6:37138804	nonsynonymous SNV	45.08	no call	*PIM1*	G	C	COSM220741	–	3	c.237G>C	p.Glu79Asp	578	4193	IVL23
chr6:37138950	nonsynonymous SNV	19.12	no call	*PIM1*	G	A	COSM220740	rs562319987	4	c.290G>A	p.Ser97Asn	68	1069	IVL23
chr1:203274817	nonsynonymous SNV	18.66	6.4	*BTG2*	G	A	COSM5946364	rs55906353	1	c.83G>A	p.Gly28Asp	418	4139	IVL24
chr17:62006799	nonsynonymous SNV	22.72	5.7	*CD79B*	A	G	COSM220734	–	5	c.586T>C	p.Tyr196His	625	5994	IVL24
chr3:38182641	nonsynonymous SNV	31.24	7.8	*MYD88*	T	C	COSM85940	rs387907272	5	c.794T>C	p.Leu265Pro	813	10142	IVL24
chr6:37138804	nonsynonymous SNV	28.34	5.9	*PIM1*	G	C	COSM220741	–	3	c.237G>C	p.Glu79Asp	437	3871	IVL24
chr6:37138955	nonsynonymous SNV	33.09	8.9	*PIM1*	G	A	–	–	4	c.295G>A	p.Gly99Ser	136	940	IVL24
chr6:37139097	nonsynonymous SNV	20.82	4.8	*PIM1*	G	A	–	–	4	c.437G>A	p.Ser146Asn	293	4082	IVL24
chr6:37139150	nonsynonymous SNV	41.3	9.6	*PIM1*	C	T	COSM220738	–	4	c.490C>T	p.Leu164Phe	293	4089	IVL24
chr17:62006799	nonsynonymous SNV	50	3.3	*CD79B*	A	G	COSM220734	–	5	c.586T>C	p.Tyr196His	322	1287	IVL25
chr3:38182641	nonsynonymous SNV	49.07	1.3	*MYD88*	T	C	COSM85940	rs387907272	5	c.794T>C	p.Leu265Pro	222	1899	IVL25
chr6:106554273	nonsynonymous SNV	38.1	no call	*PRDM1*	C	T	–	–	6	c.1801C>T	p.Arg601Trp	735	2745	IVL3
chr17:62006798	nonsynonymous SNV	40.57	no call	*CD79B*	T	C	COSM220736	–	5	c.587A>G	p.Tyr196Cys	838	3440	IVL4
chr6:37138805	nonsynonymous SNV	38.5	no call	*PIM1*	C	A	COSM5948425	–	3	c.238C>A	p.Leu80Met	813	3607	IVL4
chr17:62006798	nonsynonymous SNV	84.03	4.6	*CD79B*	T	C	COSM220736	–	5	c.587A>G	p.Tyr196Cys	744	2108	IVL5
chr6:106543541	nonsynonymous SNV	77.41	3.7	*PRDM1*	C	A	–	–	3	c.343C>A	p.Pro115Thr	239	3107	IVL5
chr17:62006680	nonsynonymous SNV	53.35	1.5	*CD79B*	A	G	COSM1737940	–	6	c.596T>C	p.Leu199Pro	1015	3457	IVL6
chr6:106552836	Stop gain	41.2	1.9	*PRDM1*	C	A	–	–	5	c.801C>A	p.Tyr267Ter	267	2472	IVL6
chr6:37138805	nonsynonymous SNV	34.36	no call	*PIM1*	C	G	–	–	3	c.238C>G	p.Leu80Val	806	2350	IVL6
chr6:37139111	nonsynonymous SNV	27.44	no call	*PIM1*	G	C	–	–	4	c.451G>C	p.Val151Leu	696	2758	IVL6

VAF, Variant allele frequencies; SNV, single nucleotide variation; MNV, multiple nucleotide variation; Ref., Reference allele; Var., Variant allele; cfDNA, cell-free DNA; tdDNA, tissue-derived DNA

Table S6. Coverage analysis of targeted regions

ID	target region coverage		
	≥×100 in cfDNA (%)	≥×500 in tdDNA (%)	≥×100 in normal gDNA (%)
IVL2	83.8	85.13	89.14
IVL3	86	86.54	83.14
IVL4	85.38	86.4	89.03
IVL5	86.55	87.97	89.62
IVL6	88.21	87.69	88.98
IVL21	87.63	90.47	91.54
IVL23	88.45	90.6	NA
IVL24	88.81	89.51	91.03
IVL25	78.26	82.88	90.27

cfDNA, cell-free DNA; tdDNA, tissue-derived DNA; gDNA, genomic DNA; NA, not available

Table S7. Three measures indicating aberrant somatic hypermutations in *PIM1*

Mutation enrichment in WRCY (*P* value)	Mutation enrichment in transition (*P* value)	Mutation enrichment in C:G (*P* value)
3.18 (0.0005)	1.29 (0.285)	1.68 (0.0004)

The variation enrichment value was calculated as previously reported (supplementary reference 2). WRCY, W denotes A or T, R denotes A or G, Y denotes C or T.

Table S8. Results of droplet digital PCR for L265P *MYD88* mutation

	MYD88 L265P variant allele frequency (%)			
ID	BM	serum/plasma	skin	others
IVL1	0.00	NA	0.68	NA
IVL2	1.74	42.58	5.01	NA
IVL3	0.00	0.00	0.00	NA
IVL4	0.00	0.00	0.00	NA
IVL5	0.00	0.00	NA	NA
IVL6	0.00	0.00	NA	NA
IVL7	0.00	NA	0.00	NA
IVL8	0.00	NA	0.00	0.00
IVL9	NA	NA	NA	4.68
IVL10	0.00	NA	0.00	NA
IVL11	NA	NA	NA	9.09
IVL13	0.00	NA	NA	NA
IVL15	NA	NA	0.00	NA
IVL16	13.28	NA	NA	NA
IVL17	14.51	NA	NA	NA
IVL18	NA	NA	NA	34.76
IVL19	NA	NA	NA	2.58
IVL20	NA	NA	NA	10.04
IVL21	NA	34.92	0.72	NA
IVL22	NA	0.00	NA	NA
IVL23	0.61	81.33	0.82	NA
IVL24	11.81	26.69	7.64	NA
IVL25	NA	60.98	2.03	NA
IVL26	0.21	NA	NA	NA
IVL27	0.00	NA	NA	NA
IVL28	9.31	NA	NA	NA
IVL29	NA	NA	0.34	NA

BM, bone marrow; NA, not available

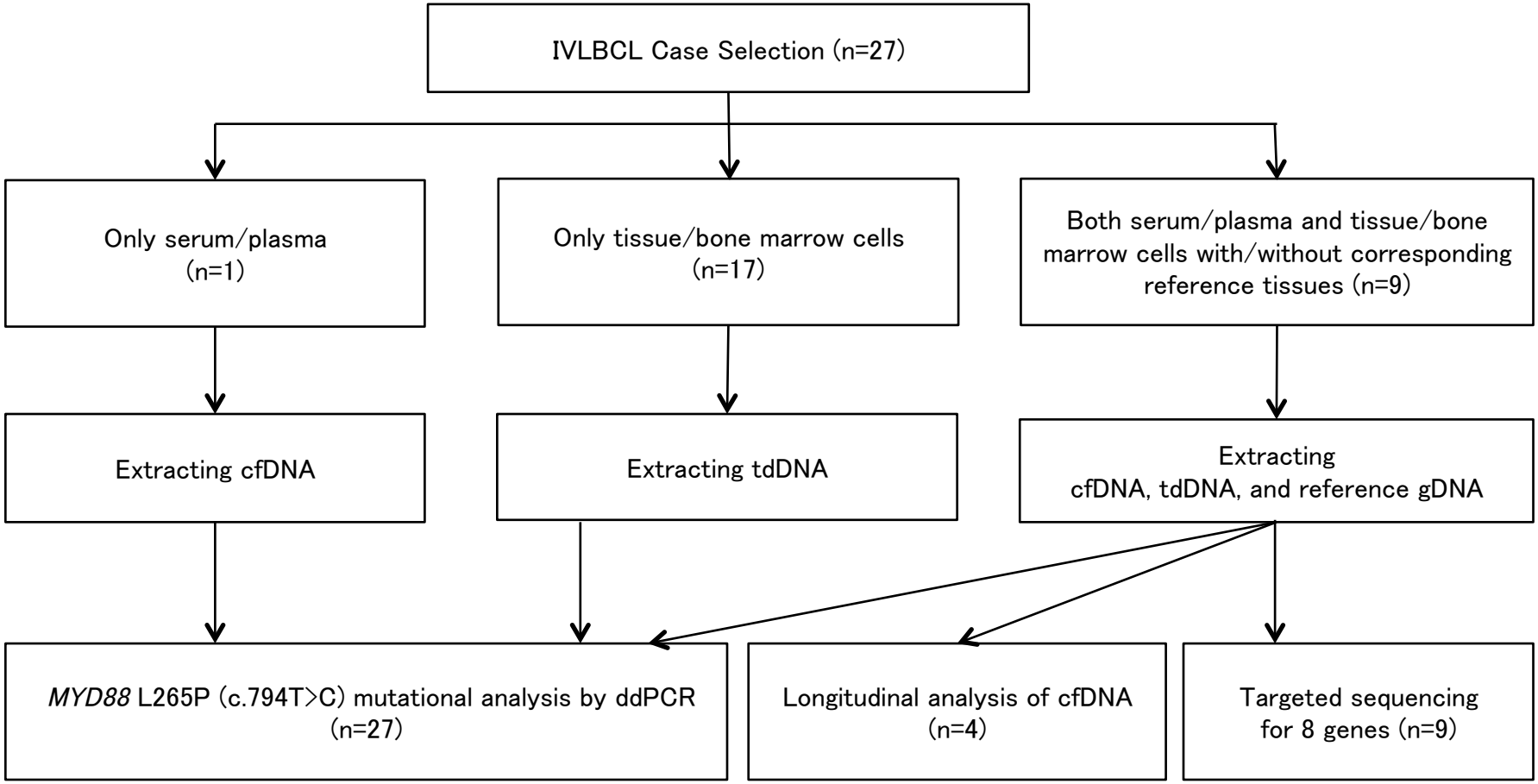
Table S9. Primers used for Sanger sequencing

Name		Sequence (5'-3')
MYD88_4	Forward	TGCCAGGGGTACTTAGATGG
	Reverse	GCGAGTCCAGAACCAAGATT
CD79B_9	Forward	CCAACCACACCAGCAGATAG
	Reverse	GCTGTTCTTGCAGAATGCAC
CD79B_11	Forward	GGTGCTCACCTACAGACCACT
	Reverse	TGGGGGACACTAACACTCTG
BTG2_1	Forward	GCCAGGGTAACGCTGTCTT
	Reverse	CTCACCTGTGAGTGCCTCCT
PRDM1_2-5	Forward	TGAAGGACAAGGCCTGTAGC
	Reverse	GCAAACGTTGCATTCGTACTT
PRDM1_3-2	Forward	CAACACTTGAGTCTTGGAGCAG
	Reverse	CCAGAAAATACTGCGCACCT
PRDM1_5-14	Forward	TCCTGTTTAGGTTATTGGAGTGA
	Reverse	GGGGATGCTGGATACTTATGG
PRDM1_6-12	Forward	TTCAGCACAAACACAGAGCA
	Reverse	GTCTCTCGATCCCGTAGGC
TNFAIP3_1	Forward	AGAAATGGCAGGAAAACAGC
	Reverse	AAGGGCTCATAGGCTTCTCC
PIM1_long1	Forward	CACTGAGTCCCCGTGCTT
	Reverse	ACTCACCAGCTCTCCCCAGT
PIM1_long3	Forward	TCATTAGGCTCCTGGACTGG
	Reverse	TCACCATCGAAGTCCGTGTA
PIM1_long4	Forward	TCCACTCTCCTTAGCCCAGA
	Reverse	CCCCTGATGATCTCTTCGTC
PIM1_long5	Forward	GCTGAAAGCAGACTGGAGGA
	Reverse	GGCAGGAGAACATCTTGCAT
PIM1_long6	Forward	CATCAAACACGTGGAGAAGG
	Reverse	TTCCGTGATGAAGTCGAAGA

Table S10. Primers used for longitudinal analysis and comparing serum/plasma analysis

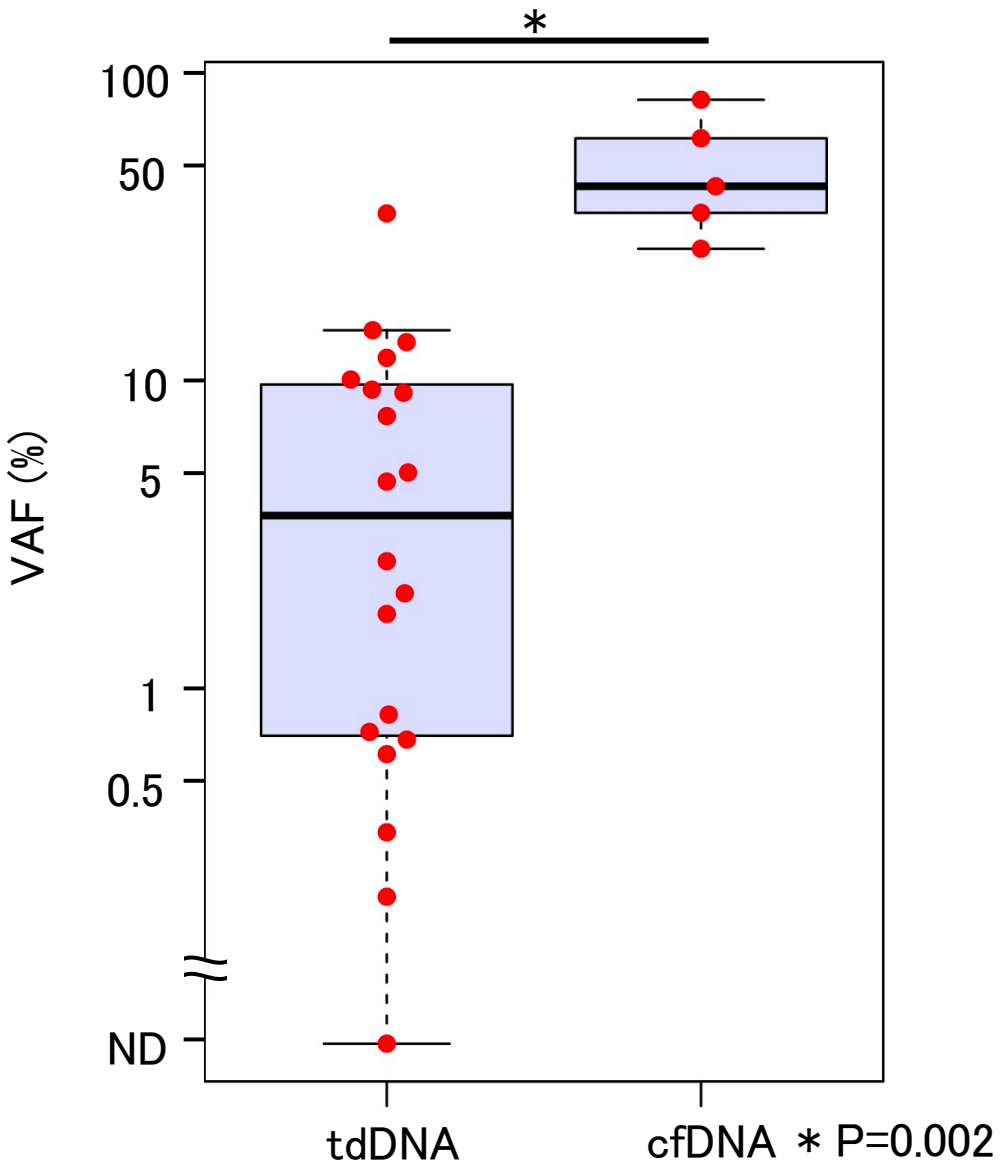
Name		Sequence (5'-3')
MYD88_2	Forward	ACTGGGCTTGTCCCACCAT
	Reverse	TCGCAGACAGTGATGAACCT
CD79B_1	Forward	GCAGCGTCACTATGTCCTCA
	Reverse	CTGAGTCCTCGGGGTCAGT
CD79B_3	Forward	CCAACCACACCAGCAGATAG
	Reverse	CCCCAGGATGACAGCAAG
PIM1_2-4	Forward	GGAGCCCTGCAAGAGGAG
	Reverse	GCGATTGAGGTCGATAAGGA
PRDM1_2-1	Forward	AGCCCAAAGCTACCTCAGC
	Reverse	GCAAACGTTGCATTCGTACT
PRDM1_5-11	Forward	TTTTTCCTGTTTAGGTTATTGGAGTGA
	Reverse	TAACATTTAATGGGTCTGAAGAAATTTCCCTTA
PRDM1_6-9	Forward	ACTCTGCCCAAAGAATGTCC
	Reverse	GGGCTCCCACGTCTTCTAA

Figure S1. Workflow of the study



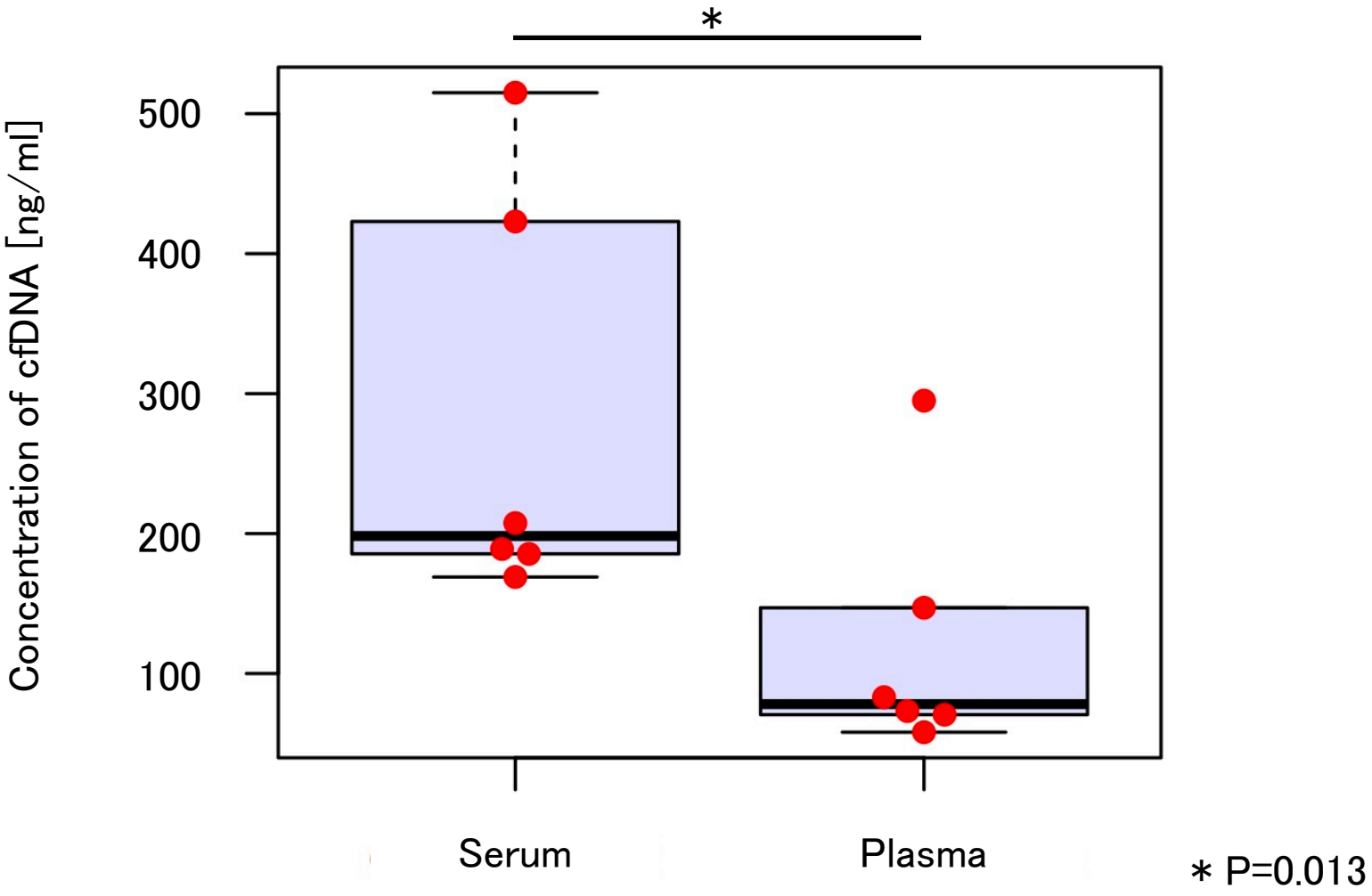
IVLBCL, intravascular large B-cell lymphoma; cfDNA, cell-free DNA; tdDNA, tissue-derived DNA; gDNA, genomic DNA; ddPCR, droplet digital PCR

Figure S2. Comparison of variant allele frequencies in tissue-derived DNA with those in cell-free DNA measured by digital droplet PCR for L265P *MYD88* among mutation-positive patients



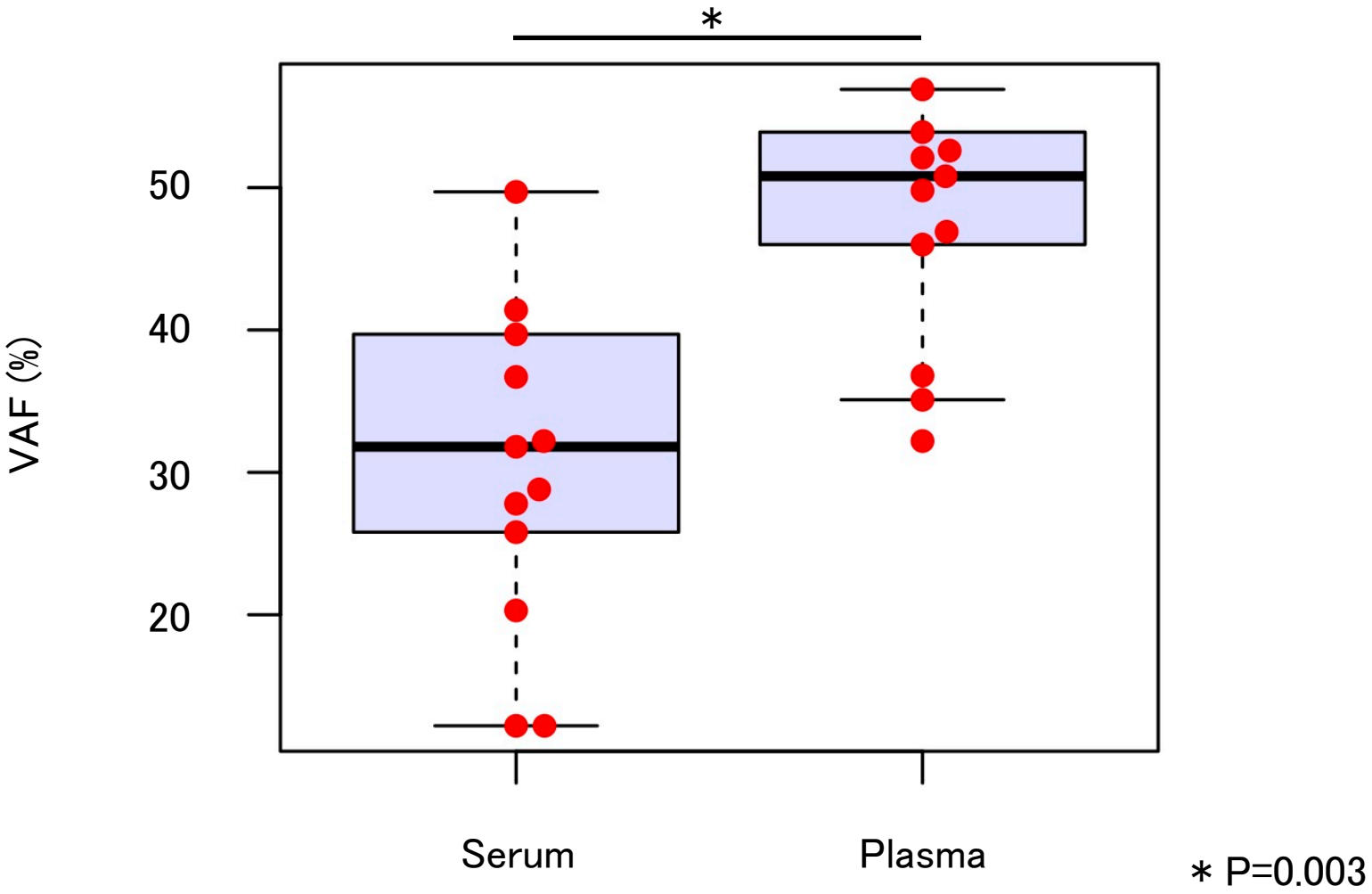
VAF, variant allele frequencies; tdDNA, tissue-derived DNA; cfDNA, cell-free DNA

Figure S3. Comparison of cell-free DNA concentrations extracted from paired serum and plasma samples



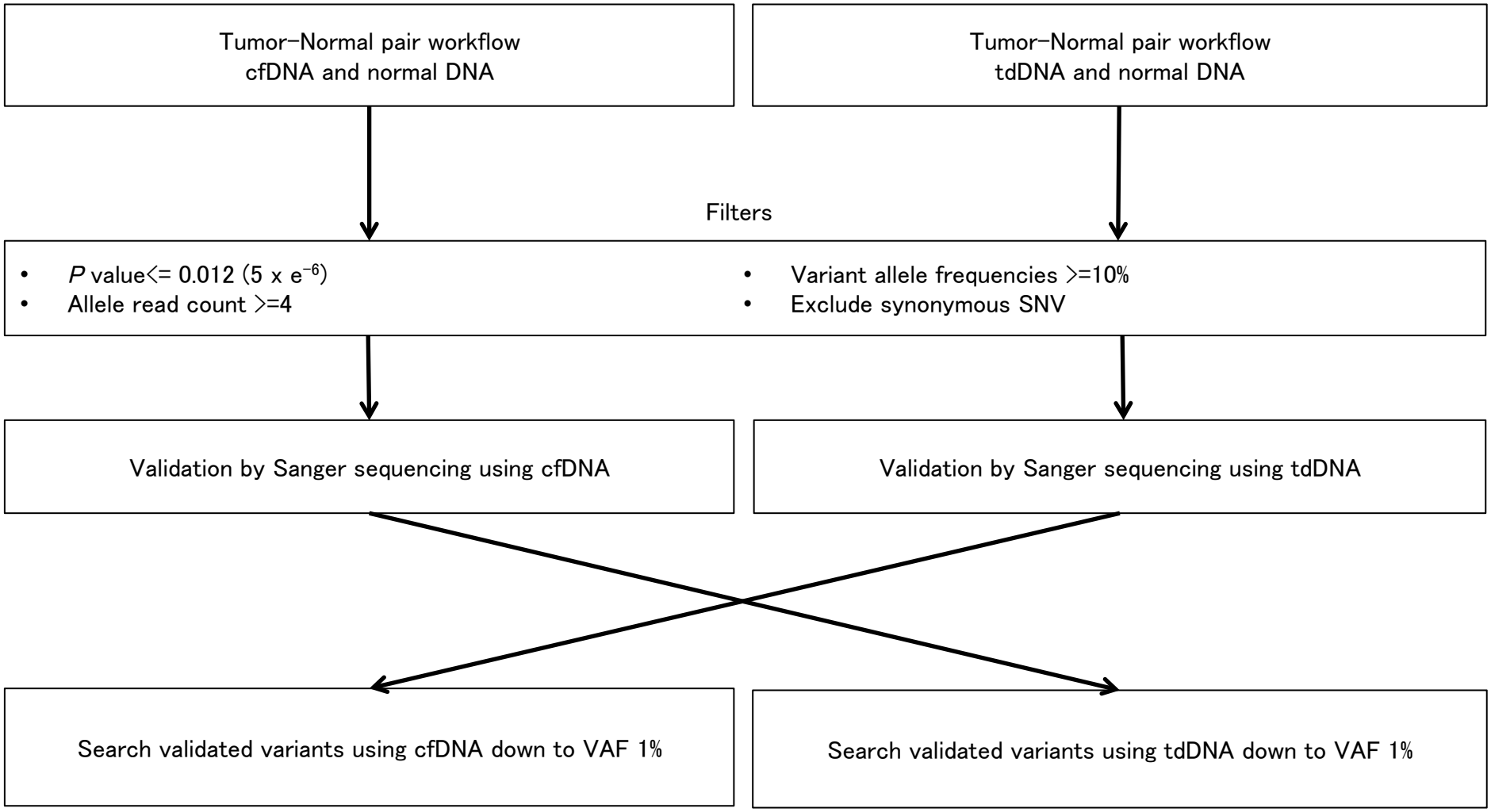
cfDNA, cell-free DNA

Figure S4. Comparison of variant allele frequencies in cell-free DNAs extracted from paired serum and plasma samples



VAF, Variant allele frequency

Figure S5. Sequencing analysis pipeline



cfDNA, cell-free DNA, tdDNA, tissue derived DNA, SNV, single nucleotide variant, VAF, Variant allele frequencies