Molecular measurable residual disease by immunoglobulin gene rearrangements on circulating tumor DNA predicts outcome in diffuse large B-cell lymphoma

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Supplementary Data

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SUPPLEMENTARY METHODS

Plasma cfDNA extraction

Peripheral blood (30 ml) was collected in EDTA tubes (Becton Dickinson, Franklin Lakes, NJ), since plasma processing was performed promptly, with separation completed within 4 hours at +4 °C. Tubes were centrifuged at 800 g for 10 min using a refrigerated centrifuge to separate plasma from cells. Plasma was then removed into new 1.5 ml tubes without disturbing the buffy coat and further centrifuged at 13,000 rpm for 10 min using a refrigerated centrifuge to remove any remaining cells. Plasma was stored in 1 ml aliquots at -80°C until DNA extraction. A rapid processing of blood samples soon after the venipuncture avoided cfDNA contamination from gDNA that may occur due to nucleated cell lysis and allowed to avoid dedicated tubes.

cfDNA was extracted from a total of 4 ml aliquots of plasma immediately after thawing by using the Maxwell RSC LV ccfDNA kit (Promega) and quantified by using the Qubit Fluorometer 3.0 (Thermo Fisher). cfDNA purity (as referred to absence of gDNA contamination) was established by capillary electrophoresis using an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA) equipped with the Expert 2100 software, in combination with a high sensitivity (HS) DNA microchip and HS DNA kit (Agilent Technologies). The assay was performed according to the instructions provided by the manufacturer.

NGS for clonality assessment and MRD analysis

Each LymphoTrack assay panel has a single multiplex master mix that targets conserved regions in the IGH or IGK genes. The LymphoTrack IGH FR1/2/3 assay panel uses primers targeting the IG framework regions (FR) to amplify V(D)J rearrangements. Each single FR multiplex master mix for IGH contains forward primers targeting one of the conserved framework regions (FR1, FR2, or FR3), as well as several consensus reverse primers targeting the JH region. Targeting all three framework regions significantly reduces the risk of not detecting the presence of clonality, as somatic hypermutations in the primer binding sites of the involved VH gene segments can impede DNA amplification.

On cfDNA samples, FR1 multiplex master mixes were excluded due to the small fragment size of cfDNA of ~166 bp.

The LymphoTrack IGK assay panel contains forward primers targeting the conserved VK region and intron sequences, with reverse primers targeting the JK and KDE regions. In 1-step PCR amplicons are generated and indexed, allowing the simultaneous sequencing of up to 24 samples in a single run. Each of these 24 indices can be considered to act as a unique barcode that allows amplicons from

individual samples to be pooled together after PCR amplification to generate the sequencing library. All baseline samples were sequenced using 150 ng of gDNA. Positive and negative controls for clonality were also included. Amplicons purity and quantity were assessed using the KAPA Library Quantification Kits for Illumina platforms (KAPA Biosystems, Boston, MA). Calculating the concentration of PCR amplicons allowed equal amplicon representation in the final pooled library. After demultiplexing, bioinformatics analysis was done by processing FASTQ files, generated during NGS, with the LymphoTrack Software-MiSeq v2.4.3 (Invivoscribe) to retrieve sequences from virtually every clonal B cell in the samples. Tumor associated clones at diagnosis were identified following three criteria: (1) 20,000 or more total reads for each sample; (2) at least 1, but not more than 2, merged top reads with 2.5% or greater (for IGH) and 5.0% or greater (for IGK) of total reads; and (3) top first or second merged reads at least two times more abundant than the third most abundant read to be considered clonotypic (for IGH), top first or second merged reads at least two times more abundant than the fifth most frequent merged sequence if there is at least ore INTR-Kde rearrangement detected in the four most frequent merged sequences or the third if there are no INTR-Kde detected (for IGK). The result of each assay was called as clonal, non-clonal or indeterminate (i.e., too few reads for evaluation). Data from the run were considered invalid if either the % cluster passing filter or the % base calls above Q30 were below 75%.

The same assay panels were used to assess MRD following similar methods as described above. Testing was done using only the primer sets that successfully characterized the diagnostic clone. To maximize the probability of detecting clonality in cfDNA samples, testing was conducted in triplicate reactions utilizing the maximum amount of cfDNA allowed by the protocol (10 µl). Each run included a no-template control and a low positive control (LPC, MRD level = 1E-04). However, a spike-in for MRD level quantification was omitted to prioritize the detection of clonal sequences in ctDNA, maximizing read numbers. Multiplexed libraries were sequenced using 500-bp paired-end runs on a MiSeq sequencer (Illumina). The FASTQ files were analyzed using the LymphoTrack Software-MiSeq v2.4.3 (Invivoscribe), and MRD analysis was performed with the bioinformatics LymphoTrack MRD Software v2.0.2 (Invivoscribe) tool considering the number of replicates, the amount of DNA (ng) for each replicate, the "Unique Reads" file generated with the LymphoTrack Software-MiSeq v2.4.3 (Invivoscribe) and the number of total reads. The MRD Software generates an "output.tsv" file with the full analysis of each sequence and a PDF report with the MRD results for each PCR replicate analyzed. For a "MRD Detected" result, the software reports the number of reads and cumulative frequencies of exact matched sequences and similar sequences (up to two mismatched nucleotides). For a "MRD Not Detected" result, the software reports the number of reads and cumulative frequencies of exact matched sequence and similar sequences (up to two mismatched

nucleotides). The detection limit and the relative % confidence of each MRD experiment was calculated on the basis of the number of replicates, the amount of DNA (ng) for each replicate, and the number of total reads.

Sequencing results were considered invalid when fewer than 20.000 total reads were retrieved.

Limit of detection of IGH and IGK assays on plasma

The limit of detection (LOD) of the LymphoTrack IGH and IGK assays on plasma was evaluated by analyzing the 23 positive MRD samples.

For each sample, the total number of reads of the clonotype sequence for all replicates (allowing up to 2 mismatch) was divided by the total number of sequencing reads generated for that sample and expressed as a percentage. This percentage was then multiplied by the amount of input cfDNA (expressed as ng) loaded for all PCR replicates, resulting in the Cumulative Target Read Count ($CTRC_{sample}$). The same calculation was performed for the LPC, which was included in each MRD experiment ($CTRC_{LPC}$). To estimate the expected read frequency for each sample, the $CTRC_{sample}$ and $CTRC_{LPC}$ values were related through a proportion, assuming that the LPC corresponds to an average expected read frequency of 0.0001, as per the application guide.

The formula used was:

$X_{sample} = 0.0001 \times CTRC_{sample} / CTRC_{LPC}$

where X_{sample} represents the expected read frequency of the sample.

Thus, a read frequency detection gradient was defined, relative to the percentage of target reads identified compared to a standard. The MRD experiments showed a $LOD_{maximum}$ of 5.3E-05 and a $LOD_{reproducible}$ of 6.3E-04.

Comparative Analysis of NGS and PCR/ddPCR in MRD Sample Detection

A comparative analysis between NGS and PCR/ddPCR was conducted on 8 MRD samples (4 at interim and 4 at EOT). Two of these samples were analyzed using both ddPCR and NGS, with results showing 100% concordance for both positive and negative detections. Due to the insufficient sensitivity and specificity of the derived allele-specific primers, the remaining 6 samples were analysed using conventional PCR and NGS. Among these 6 samples, a 67% concordance rate (4/6 samples) was observed between the methods: 2 samples resulted MRD positive by PCR and negative by NGS.

Statistical analysis

A ROC curve was employed to determine a cut-off for continuous variables.

SUPPLEMENTARY RESULTS

IG analysis of gDNA from FFPE

Compared with the reference germline sequence, most IG clones showed high somatic hypermutation rates, with a median rate of 7.4% (range 0.0% to 35.8%).

Pre-treatment ctDNA IG analysis

The clonal IG rearrangements identified in ctDNA were matched with the lymphoma-specific clonotype. Most cases had clonal rearrangements identified by the same assay (67.3%, 35/52), in 10 cases (19.2%, 10/52) a slight difference in the assay was observed (5 tumor biopsies were characterized by all three IGH assays and only by one or two IGH assays on ctDNA, and 5 biopsies were characterized by IGH-FR2/3 and only by one IGH assay on ctDNA), in the remaining 7/52 samples (13.5%) a different clonality characterized the two compartments (n=2) or no clonality was identified on ctDNA (n=5).

Correlations of baseline cfDNA levels with outcome and clonality

CfDNA concentrations at baseline were measured and correlated to clinical outcome, as reported in the results. CfDNA concentrations were also correlated with the presence or absence of clonality in the plasma. Cases with different clonal markers between the tumor biopsy and plasma were also examined. The rate of clonality detection on pre-treatment ctDNA correlated with the relative concentrations of cfDNA. The optimal cut-off for cfDNA concentration was 0.6 ng/µL (p=0.027, OR 2.35, range 1.17-4.79).

Discordant cases

In 11/52 (21%) patients with a different clonality identified in each compartment (diagnostic FFPE tissue and cfDNA), further analyses were conducted to enhance the characterization of these cases. The median amount of cfDNA was 0.692 ng/µL (range 0.27-4.73).

As a first step, the tumor biopsy site was evaluated (whether lymph node or extra nodal site). Out of the 11 patients, 6 biopsies were derived from extra-nodal tissue and 5 from lymph nodes. Therefore, there was no significant difference in the site of biopsy that could justify a discordant clonality. Additionally, 8 out of the 11 patients were studied through a mutation analysis approach. The LyV4.0 CAncer Personalized Profiling by deep Sequencing Assay (CAPP-seq) was utilized and conducted at the Novara center. A targeted resequencing gene panel, including coding exons and splice sites of 59 genes that are recurrently mutated in DLBCL and in other B cell malignancies, has been specifically

designed. Mutational analysis revealed somatic non-synonymous mutations in 100% (8/8) of tumor biopsies and 75% (6/8) of ctDNA samples, with notable heterogeneity across compartments in some patients. In certain cases, distinct mutations were detected in ctDNA compared to tumor biopsies, underscoring potential compartmental difference. See Supplementary Table S3.

In 2 out of 8 patients, different mutated genes were detected in the ctDNA and tumor biopsy, mirroring our findings on IG-NGS clonality. In 2 further patients several mutations were identified only in the biopsied tumor tissue, with no mutations found in the ctDNA. For 2 other patients, only one mutated gene was detected in the ctDNA, and the identical mutation was present in the tumor biopsy, alongside with additional mutations exclusively detected in the biopsy. In the remaining 2 patients, the same mutated genes were identified both in the ctDNA and tumor biopsy but represented only a fraction of the mutations identified in the latter.

Therefore, gene mutations analysis also reveals a heterogeneity across compartments in these patients, although differences in the IG-NGS and CAPP-Seq assays does not allow to drive further conclusions.

Plasma IG analysis of healthy donors

The cfDNA of 7 healthy donors was analyzed with the IGH (FR2/FR3) and IGK assay panels and subjected to identical analysis as the patients' pre-treatment samples. The median plasma cfDNA concentration was 4.2 ng/mL (range 2.7-7.8). Clonality was detected in 1/7 donors using both FR2 and FR3 assays. In 4/7 donors, clonality was detected, albeit with a "borderline" percentage of total reads: in 3/4 only with the FR2 assay and in 1/4 only with the FR3 assay. The remaining 2/7 donors exhibited a polyclonal pattern. The expansion of these minor clones in the plasma of healthy donors does not imply the presence of a malignancy, rather parallels what seen in the background amplification of the RQ-PCR assay, that is the non-specific amplification of comparable IG gene rearrangements presents in normal cells.

MRD positivity frequency according to the type of rearrangement

Among the patients who underwent MRD analysis on plasma samples, 27 were found suitable for monitoring with the FR3, 20 with the FR2 and 12 with the IGK rearrangement. The frequency of MRD positivity was then analyzed in relation to the type of rearrangement tracked in plasma. Among 53 samples analyzed by FR3, 15 were positive (28.3%); for the 35 samples analyzed by FR2, 8 were positive (22.9%), with no significant difference (p=0.63). Out of 18 samples analyzed by IGK, 2 were positive (11.1%). The lower number of tests with IGK is attributed to the limited number of patients monitored with this rearrangement.

Supplementary Table S1. This table presents the results of IGH and IGK gene rearrangements identified through NGS analysis conducted as part of this study.

N.	Assay on tumor biopsy	Clone 1	Clone 2	Assay on ctDNA	Clone 1	Clone 2
1	IGK	V2-30*01-J2*04		IGK	V2-30*01-J2*04	
2	IGK	V4-1*01-J3*01		IGK	V4-1*01-J3*01	
3	FR1+FR2+FR3	V3-30-5*02-J6*02	V3-30-5*02-J6*02	FR3	V3-30-5*02-J6*02	V3-30-5*02-J6*02
4	FR3	V4-34*13-J4*02	V4-61*07-J4*02	FR3	V4-34*13-J4*02	V4-61*07-J4*02
5	FR2+FR3	V3-35*01-J3*02		FR2+FR3	V3-35*01-J3*02	
6	FR3	V3-72*01-J5*02		FR3	V3-72*01-J5*02	
7	IGK	V4-1*01-J2*01		IGK	V4-1*01-J2*01	
8	FR1+FR2+FR3	V3-21*02-J4*02		FR2	V3-21*02-J4*02	
9	FR2	V1-8*01-J6*02		FR2	V1-8*01-J6*02	
10	FR1+FR2+FR3	V4-34*02-J4*02		FR3	V4-34*02-J4*02	
11	FR2	V2-5*08-J4*02		FR2	V2-5*08-J4*02	
12	FR2	V3-33*06-J4*02		FR2	V3-33*06-J4*02	
13	FR2+FR3	V3-48*03-J4*02		FR2+FR3	V3-48*03-J4*02	
14	FR2+FR3	V4-39*02-J6*02		FR2+FR3	V4-39*02-J6*02	
15	FR1+FR2+FR3	V1-46*01-J6*02	V4-28*01-J6*02	FR2+FR3	V1-46*01-J6*02	V4-28*01-J6*02
16	FR2	V3-64*04-J3*02		FR2	V3-64*04-J3*02	
17	FR3	V3-49*05-J4*02		FR3	V3-49*05-J4*02	
18	FR2+FR3	V1-2*03-J4*02		FR2+FR3	V1-2*03-J4*02	
19	FR2	V1-46*02-J4*02		FR2	V1-46*02-J4*02	
20	FR3	V1*01-J4*02		FR3	V1*01-J4*02	
21	FR2+FR3	V4-34*02-J4*02		FR3	V4-34*02-J4*02	
22	FR2+FR3	V1-46*02-J4*02		FR2	V1-46*02-J4*02	
23	FR3	V1-1*01-J4*02		FR3	V1-1*01-J4*02	
24	FR3	V2-70*11-J4*02		FR3	V2-70*11-J4*02	
25	FR2+FR3	V3-48*03-J4*02		FR2+FR3	V3-48*03-J4*02	
26	IGK	INTR-Kde		IGK	INTR-Kde	
27	IGK	V2-30*01-J5*01		IGK	V2-30*01-J5*01	
28	IGK	V2-24*01-J4*01		IGK	V2-24*01-J4*01	
29	IGK	V2-29*01-J5*01	V1-27*01-J5*01	IGK	V2-29*01-J5*01	V1-27*01-J5*01
30	FR2	V3-74*03-J4*02		FR2	V3-74*03-J4*02	
31	IGK	V2-29*03-J4*01		IGK	V2-29*03-J4*01	
32	FR2+FR3	V4-34*12-J5*02		FR2+FR3	V4-34*12-J5*02	
33	IGK	V4-1*01-J4*01		IGK	V4-1*01-J4*01	
34	FR2	V5-51*04-J5*02		FR2	V5-51*04-J5*02	
35	FR2	V1-2*04-J5*02		FR2	V1-2*04-J5*02	
36	FR2	V4-59*05-J3*01		FR2	V4-59*05-J3*01	
37	FR1+FR2+FR3	V4-34*13-J3*01		FR2+FR3	V2-5*02-J4*02	
38	FR2+FR3	V4-61*08-J6*02		FR3	V3-23*01-J4*02	
39	FR1	V3-30-3*02-J1*01	V3-30-3*02-J1*01	FR2	V3-15*01-J3*01	
40	IGK	V3-15*01-J4*01		IGK	V5-2*01-J1*01	V3-7*04-J4*01
41	IGK	INTR-Kde		IGK	V2-28*01-Kde	
42	FR2	V3-9*01-J5*02	V3-30*18-J4*02	FR2	V3-23*04-J6*03	V1-2*03-J4*02

43	FR2	V4-59*08-J3*02	V3-30-3*01-J4*02	FR3	V2-5*01-J5*02
44	FR3	V1-46*01-J6*02		FR3	V1-3*01-J4*02
45	FR2+FR3	V3-30*18-J4*02		FR3	V1-18*01-J3*02
46	FR3	V4-59*10-J3*02	V5-51*02-J4*02	FR3	V1-3*04-J6*02
47	FR2+FR3	V4-34*13-J4*02		FR3	V4-28*06-J6*02
48	FR3	V1-2*04-J6*02		-	
49	FR3	V1-46*01-J4*02		-	
50	FR1+FR2	V3-66*02-J6*02		-	
51	FR2+FR3	V4-34*01-J6*03		-	
52	FR2	V3-49*02-J6*03	V3-49*02-J6*03	-	

The 36 cases with concordant IG rearrangements are highlighted in bold.
Abbreviations: ctDNA, circulating tumor DNA; IGK, immunoglobulin kappa light chain gene rearrangement; FR, framework region.

Supplementary Table S2. This table presents the comparison of baseline clinical characteristics among three groups of patients categorized based on their clonality status.

		Group A	Group B	Group C	*p*
Age	<65y	4 (80.0)	7 (63.6)	21 (40.4)	.14 [a]
	≥65y	1 (20.0)	4 (36.4)	31 (59.6)	.18 [b]
Stage	I-II	4 (80.0)	6 (54.5)	20 (38.5)	.15 [a]
	III-IV	1 (20.0)	5 (45.5)	32 (61.5)	.32[b]
IPI risk	Low	5 (100.0)	7 (63.6)	15 (28.8)	
	Low-intermediate	0 (0.0)	3 (27.3)	9 (17.3)	.012[a]
	High-intermediate	0 (0.0)	0 (0.0)	11 (21.2)	.05[b]
	High	0 (0.0)	1 (9.1)	17 (32.7)	
ECOG PS	0-1	5 (100.0)	11 (100.0)	43 (82.7)	.23[a]
	2-4	0 (0.0)	0 (0.0)	9 (17.3)	.15[b]
B symptoms	No	5 (100.0)	11 (100.0)	39 (75)	.07[a]
	Yes	0 (0.0)	0 (0.0)	13 (25)	.05[b]
Extra nodal disease	No	4 (80.0)	3 (27.3)	18 (34.6)	.1[a]
	Yes	1 (20.0)	8 (72.7)	34 (65.4)	.6[b]
Elevated LDH	Yes	0 (0.0)	3 (27.3)	32 (61.5)	.006[a]
	No	5 (100.0)	8 (72.7)	20 (38.5)	.03[b]

NOTE: Data presented as No. (%) unless otherwise indicated.

These analyses consider the comparison of baseline clinical characteristics among three groups of patients. (**Group A**) Patients with undetectable clonality on plasma ctDNA at baseline. (**Group B**) Patients with detectable clonality on plasma ctDNA at baseline but different from the clonality assessed on the tumor biopsy. (**Group C**) Patients with the same clonality detected on both plasma ctDNA and tumor biopsy at baseline.

[a] refers to univariate analysis of patients with undetectable clonality on ctDNA

[b] refers to univariate analysis of patients with a different clonality between ctDNA and tumor biopsy

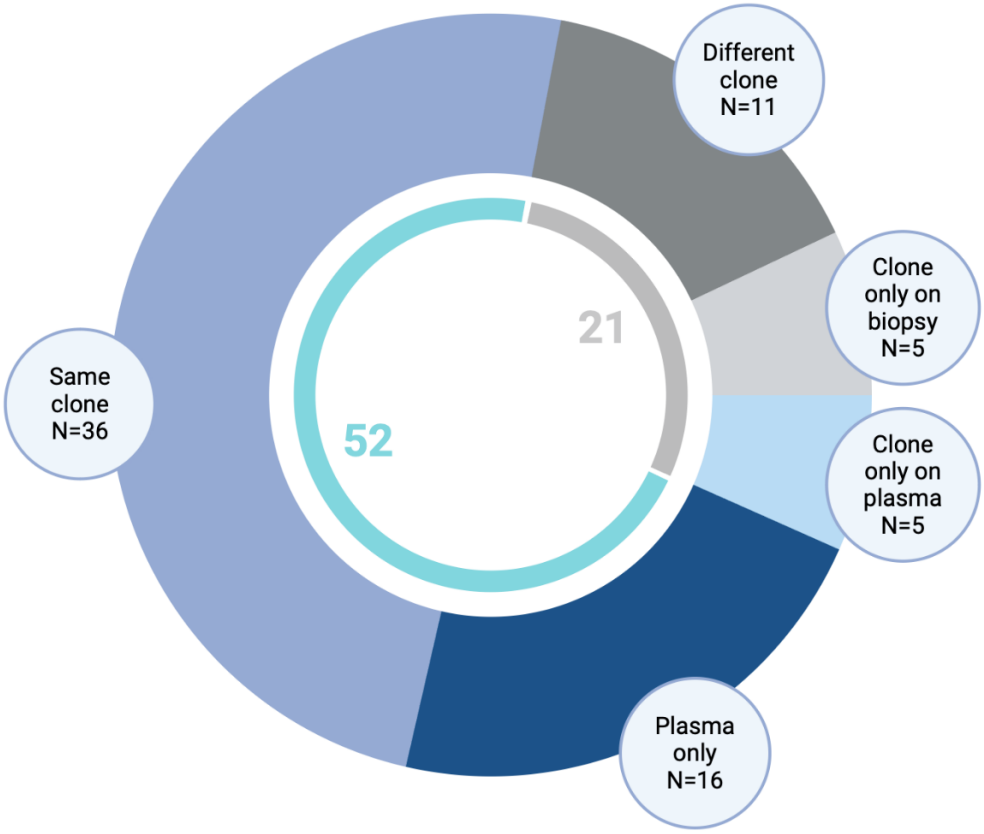
Abbreviations: ctDNA, circulating tumor DNA; IPI, international prognostic index; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase.

Supplementary Table S3. Gene mutations identified by CAPP-seq in 8 of the discordant cases. The specific gene mutations with variant allele frequency (VAF) and number of reads are shown in detail.

Sample ID	Gene	Nucleotide Change	AA Change	VAF Tumor	READS Tumor	VAF Plasma	READS Plasma
3	ACTB	c.241G>A	p.D81N	11.75%	78/664	9.12%	33/362
	CREBBP	c.3233C>A	p.S1078*	5.33%	44/826	12.73%	280/2199
	CREBBP	c.4849C>A	p.L1617M	-	-	0.83%	23/2784
	HIST1H1E	c.319C>T	p.L107F	-	-	1.93%	66/3416
	HIST1H2BK	c.31C>T	p.P11S	8.22%	496/6034	5.99%	74/1236
	KMT2D	c.11069delG	p.G3690fs*59	-	-	0.73%	18/2467
	PIM1	c.449C>A	p.S150Y	5.78%	160/2766	3.28%	51/1554
	PIM1	c.475C>T	p.H159Y	6.18%	144/2331	3.46%	62/1793
	PIM1	c.710G>A	p.S237N	7.31%	388/5305	2.53%	47/1858
	PIM1	c.847C>G	p.L283V	5.21%	622/11934	3.57%	56/1568
	PIM1	c.334C>T	p.H112Y	-	-	4.89%	48/982
	PIM1	c.384G>T	p.Q128H	-	-	5.04%	75/1489
	PIM1	c.511C>A	p.L171M	-	-	2.89%	51/1767
	PIM1	c.823C>T	p.L275F	-	-	3.09%	52/1681
	SPEN	c.8075T>A	p.V2692D	-	-	0.49%	13/2679
	TNFAIP3	c.805+1_805+2insTGA	-	6.57%	39/594	9.44%	162/1716
	TNFAIP3	c.2080G>T	p.E694*	6.91%	39/564	7.83%	141/1801
	TNFAIP3	c.2350C>T	p.Q784*	-	-	2.45%	43/1756
	TP53	c.839G>A	p.R280K	5.91%	200/3384	8.68%	216/2488
	TP53	c.404G>A	p.C135Y	11.14%	142/1275	10.06%	175/1739
6	ACTB	c.142G>A	p.G48S	17.08%	2117/12396	1.66%	60/3616
	BTG1	c.92T>G	p.L31R	11.89%	240/2018	-	-
	BTG1	c.233C>T	p.P78L	19.02%	336/1767	-	-
	BTG1	c.138G>C	p.E46D	20.08%	200/996	-	-
	BTG1	c.108G>C	p.Q36H	20.97%	330/1574	-	-
	CD58	c.284T>G	p.L95*	20.09%	311/1548	-	-
	CD83	c.137_138insG	p.V47fs*11	14.82%	542/3656	-	-
	CD83	c.104G>A	p.C35Y	14.85%	598/4028	-	-
	HIST1H1E	c.140C>T	p.A47V	18.50%	810/4378	-	-
	HIST1H2AC	c.253C>T	p.Q85*	18.00%	770/4277	-	-
	HIST1H2AC	c.99delC	p.L34fs*23	21.44%	450/2099	-	-
	HIST1H2AM	c.193G>T	p.E65*	18.96%	229/1208	-	-
	HIST1H2BK	c.133G>C	p.V45L	12.88%	467/3625	-	-
	HIST1H2BK	c.148C>T	p.H50Y	13.02%	375/2881	-	-
	NFKBIA	c.205C>T	p.Q69*	15.71%	386/2457	-	-
	NFKBIA	c.185delG	p.G62fs*28	23.21%	688/2964	-	-
	TNFAIP3	c.982G>C	p.A328P	8.94%	68/761	-	-
	ZFP36L1	c.587C>A	p.P196H	12.22%	290/2374	-	-
84	ACTB	c.94C>T	p.P32S	28.03%	97/346	11.68%	48/411
	BCL6	c.1675G>C	p.G559R	50.89%	1976/3883	14.26%	937/6572
	BCL6	c.128T>G	p.F43C	49.04%	1256/2561	31.01%	1536/4954
	HIST1H2AM	c.346C>G	p.L116V	-	-	14.03%	452/3222
	HIST1H2BK	c.260G>C	p.R87P	-	-	7.62%	118/1548
	IRF8	c.131G>C	p.G44A	27.63%	334/1209	3.92%	138/3519
	IRF8	c.272A>C	p.D91A	44.04%	839/1905	12.66%	555/4383
	IRF8	c.313G>A	p.E105K	42.70%	798/1869	11.63%	450/3869
	MYD88	c.656C>G	p.S219C	44.00%	1534/3486	-	-
	MYD88	c.719T>C	p.M240T	-	-	17.46%	838/4799
	SF3B1	c.28+1G>T	-	5.85%	147/2511	-	-
	SGK1	c.1023G>T	p.E341D	-	-	5.47%	188/3439
	STAT3	c.1229A>C	p.H410P	23.01%	249/1082	-	-
	STAT3	c.1840A>C	p.S614R	-	-	11.92%	446/3742
	TMEM30A	c.281G>T	p.C94F	-	-	4.19%	100/2389
	TP53	c.614A>C	p.Y205S	64.85%	666/1027	-	-
	TP53	c.400T>A	p.F134I	-	-	11.54%	262/2271
	ZFP36L1	c.57+1G>A	-	45.91%	1004/2187	13.06%	217/1661
87	PLCG2	c.3431A>G	p.D1144G	29.21%	434/1486	-	-
34	BRAF	c.715C>T	p.R239*	3.87%	18/465	-	-
	HIST1H1C	c.523A>C	p.K175Q	-	-	8.4%	180/2148
	HIST1H1C	c.362A>C	p.K121T	-	-	1.6%	33/2043
	KMT2D	c.13081_13082insTG	p.A4361fs*24	-	-	12.4%	293/2356

	KMT2D	c.5065C>T	p.R1689C	-	-	0.9%	18/1932
	NFKBIA	c.198G>A	p.W66*	-	-	8.8%	32/364
	PIM1	c.550C>T	p.L184F	-	-	11.0%	197/1789
	SPEN	c.6254_6256delTAG	p.L2085_A2086delinsS	-	-	0.8%	19/2257
	TNFAIP3	c.832_833delAG	p.D279fs*5	-	-	25.3%	437/1725
	TP53	c.725G>A	p.C242Y	-	-	33.0%	771/2337
025	ACTB	c.1021A>G	p.I341V	17.50%	28/160	-	-
	HIST1H1E	c.122delC	p.E42fs*47	-	-	1.70%	62/3653
030	CD70	c.196+1G>A	-	52.26%	602/1152	-	-
	CD79B	c.552+2T>A	-	21.99%	419/1905	-	-
	HIST1H1C	c.194C>T	p.A65V	21.42%	921/4300	-	-
	HIST1H1C	c.124G>A	p.E42K	20.94%	768/3667	-	-
	HIST1H1D	c.571G>A	p.A191T	17.36%	516/2973	-	-
	HIST1H1E	c.367G>A	p.A123T	23.58%	1410/5980	-	-
	HIST1H2AM	c.379G>T	p.A127S	20.59%	561/2724	-	-
	HIST1H2BC	c.259C>T	p.R87C	18.01%	476/2643	1.31%	17/1302
	MYD88	c.656C>G	p.S219C	24.86%	1034/4159	-	-
	PIM1	c.521G>A	p.G174D	21.71%	647/2980	-	-
	SGK1	c.751C>T	p.H251Y	19.68%	109/554	-	-
	STAT3	c.1919A>T	p.Y640F	21.68%	137/632	-	-
	ZFP36L1	c.609C>G	p.S203R	21.37%	479/2241	-	-
	ZFP36L1	c.466G>C	p.E156Q	23.67%	835/3528	-	-
	ZFP36L1	c.277G>T	p.E93*	20.85%	519/2489	-	-
021	BTG1	c.109C>G	p.L37V	14.15%	176/1244	-	-
	HIST1H1E	c.14C>T	p.A5V	50.63%	283/559	-	-
	IRF8	c.1271T>A	p.I424N	18.73%	62/331	-	-
	PLCG2	c.731A>G	p.H244R	60.27%	264/438	-	-

Supplementary Figure S1. Pie chart illustrating the number of patients in each category: patients with the same IG clone identified in tumor biopsy and plasma ctDNA, patients only studied in the plasma compartment, and subgroups of patients excluded from MRD analysis due to the absence or unsuitability of the IG marker for subsequent analysis.



Supplementary Figure S2. Kaplan-Meier curve of PFS stratified according to whether a negative MRD on ctDNA was achieved or not at (A) cycle 2, day 1 (C2D1) and (B) cycle 4, day 1 (C4D1).