scientific reports

OPEN

Incorporation of next-generation sequencing in clinical practice using solid and liquid biopsy for patients with non-Hodgkin's lymphoma

Mariana Bastos-Oreiro[1,2,7]✉, Julia Suárez-González[2,3,7], Cristina Andrés-Zayas[2,3], Natalia Carolina Carrión[3], Solsiré Moreno[4], Diego Carbonell[1,2], María Chicano[1,2], Paula Muñiz[1,2], Laura Sanz[1,2], Francisco Javier Diaz-Crespo[4], Javier Menarguez[2,4], José Luis Diez-Martín[1,2,5], Ismael Buño[1,2,3,6,7] & Carolina Martínez-Laperche[1,2,7]

Although next-generation sequencing (NGS) data on lymphomas require further validation before being implemented in daily practice, the clinical application of NGS can be considered right around the corner. The aim of our study was to validate an NGS lymphoid panel for tissue and liquid biopsy with the most common types of non-Hodgkin's lymphoma [follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL)]. In this series, 372 somatic alterations were detected in 93.6% (44/47) of the patients through tissue biopsy. In FL, we identified 93 somatic alterations, with a median of 7.4 mutations per sample. In DLBCL, we detected 279 somatic variants with a median of 8.6 mutations (range 0–35). In 92% (24/26) of the cases, we were able to detect some variant in the circulating tumor DNA. We detected a total of 386 variants; 63.7% were detected in both types of samples, 13.2% were detected only in the circulating tumor DNA, and 23% were detected only in the tissue biopsy. We found a correlation between the number of circulating tumor DNA mutations, advanced stage, and bulky disease. The genetic alterations detected in this panel were consistent with those previously described at diagnosis. The liquid biopsy sample is therefore a complementary tool that can provide new genetic information, even in cases where a solid biopsy cannot be performed or an insufficient sample was obtained. In summary, we describe and analyze in this study the findings and difficulties encountered when incorporating liquid biopsy into clinical practice in non-Hodgkin's lymphoma at diagnosis.

Lymphoproliferative disorders are a large and heterogeneous group of hematological malignancies. Mature B-cell lymphoproliferative syndromes comprise 80% of all lymphomas[1]. Diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) are the two most common types of lymphoma[1]. The diagnosis, prognosis assessment and efficacy evaluation mainly depend on tissue biopsy, laboratory data, and imaging tests. Classically, the biopsy assessment includes the immunohistochemical detection of markers and fluorescence in situ hybridization studies (*MYC*, *BCL6*, and *BCL2* rearrangements)[2–6]. Continuous efforts are being made to identify genomic biomarkers to better understand the behavior of lymphomas, predict their evolution and bring solutions to clinical practice. Next-generation sequencing (NGS), which allows for massive, parallel, high-throughput DNA sequencing, has emerged over the past decade and has provided new insights into the genomic and transcriptomic characterization of mature B-cell malignancies[8]. NGS has become a useful tool for the complete characterization of the spectrum of genetic variants in non-Hodgkin's lymphoma (NHL). Research on molecular profiles in NHL has advanced significantly in recent years. Various groups have attempted to establish prognostic scores[3]

[1]Department of Hematology, Gregorio Marañón General University Hospital, C/ Doctor Esquerdo 46, 28007 Madrid, Spain. [2]Gregorio Marañón Health Research Institute (IiSGM), Madrid, Spain. [3]Genomics Unit, Gregorio Marañón General University Hospital, Gregorio Marañón Health Research Institute (IiSGM), Madrid, Spain. [4]Deparment of Pathology, Gregorio Marañón General University Hospital, Madrid, Spain. [5]Department of Medicine, School of Medicine, Complutense University of Madrid, Madrid, Spain. [6]Department of Cell Biology, School of Medicine, Complutense University of Madrid, Madrid, Spain. [7]These authors contributed equally: Mariana Bastos-Oreiro, Julia Suárez-González, Ismael Buño and Carolina Martínez-Laperche. ✉email: marianabeatriz.bastos@salud.madrid.org

and genetic risk clusters based on genetic characteristics[4] or by combining the characteristics with clinical and analytical data[5,6]. The results of these studies are promising; however, the means to apply these technologies are still limited in most centers, and validation is required for implementation into clinical practice. Thus, while NGS lymphoid panels should be implemented in clinical practice, there is as yet no standard approach, and features such as gene selection, sequencing platform, read depth, and variant analysis can differ among laboratories.

Although tissue biopsy is the gold standard for identifying genetic variants, it might not reflect the entire molecular complexity of every patient with lymphoma[7,8]. Once the diagnosis of lymphoma is reached based on a tissue biopsy, a liquid biopsy can be applied to complement the tissue findings. Liquid biopsy, which is non-invasive, can also be used to explore the entire mutational landscape of the lymphoma, given that this approach has the potential for collecting the tumor-circulating tumor DNA (ctDNA) derived from most, and potentially all, tumor locations in the body. Liquid biopsy has thereby progressively transformed cancer diagnoses and prognoses, as well as oncologic therapy in general and lymphoma in particular[9]. This technique is expected to lead to important improvements in initial risk stratification, response evaluation at the end of induction therapy, and in surveillance strategies and target therapy selection in patients with lymphoma.

The aim of our study was to validate an NGS lymphoid panel for solid and liquid biopsy in the most common NHLs (DLBCL and FL) and to assess the concordance between genetic mutations detected in solid and liquid biopsies.

Patients and methods

Clinical cohort. The study included 47 nonconsecutive patients diagnosed with NHL 32 DLBCL [20 DLBCLs-not otherwise specified (NOS), 4 high-grade double-hit lymphomas, 5 high-grade NOS lymphomas and 3 primary mediastinal B-cell lymphomas] and 15 FL from 2014 to 2019 in Gregorio Marañón General University Hospital. The study also included formalin-fixed paraffin-embedded (FFPE) tissue samples from the time of diagnosis, along with matched same-day plasma samples from 26 of these patients (14 DLBCL-NOS, 4 high grade) (Supplementary Tables 1 and 2).

Ethics approval was obtained from the Research Ethics Committee of Gregorio Marañón General University Hospital. All patients provided written informed consent according to the principles of the Declaration of Helsinki.

DNA extraction. All FFPE sections (n = 47) were subjected to DNA extraction with the QIAGEN Generead DNA FFPE Kit (QIAGEN, Germany) according to the manufacturer's guidelines. Peripheral blood samples were collected from 26 patients, placed in 10 mL EDTA tubes and centrifuged at 1800 × g for 10 min to isolate plasma, which was aliquoted into 1.5–2-mL tubes and stored at − 80 °C. Cell free DNA (ctDNA) was extracted with a QIAamp circulating nucleic acid kit (QIAGEN, Germany). FFPE and ctDNA were quantified using a Qubit dsDNA BR Assay (THERMO FISHER SCIENTIFIC, Waltham, MA, USA).

NGS experiments and data analysis. We selected The Lymphoma Solution (SOPHIA GENETICS, Switzerland) targeted panel, given that it targets 54 relevant genes in lymphomagenesis (193 kb) (Supplementary Table 3). For each FFPE tissue sample, 32–100 ng of total DNA was used to prepare the library according to the manufacturer's protocol. Pools of up to 12 purified libraries were captured. For each circulating tumour DNA (ctDNA) sample, 2.5–55 ng of circulating DNA was used to prepare the library. Due to the intrinsic characteristics of the ctDNA samples, adapter ligation was performed directly without initial DNA fragmentation, followed by hybridization with the capture probes, also in pools of up to 12 purified libraries. Lastly, two capture pools (24 samples) were sequenced on a NextSeq platform (ILLUMINA, US; Paired-end 2 × 151 bp; mid-output kit).

We used the Sophia DDM platform (SOPHIA GENETICS, Switzerland) to analyze single nucleotide variants and small insertions and deletions. FASTQ files were uploaded to the data portal and aligned with the human reference genome (GRCh37/hg19). After annotation in DDM, non-synonymous variants located in exonic or ± 1.2 intronic splice regions were retained, and variants with a minor allele frequency < 0.01 (based on ExAC, GnomAD and 1000 Genomes databases), were selected for the downstream analysis. Currently, there is no standardization to establish which is the best cut-off point for VAF. In this sense, we decided to set the percentage at 5% in the FFPE since there are a high percentage of tumor in these samples, in an attempt to avoid false positives. However, the cut-off was reduced to 1% in the plasma samples where there is a lower percentage of tumor and we could lose mutations.

We used an Integrative Genomics Viewer (Broad Institute, USA) to visualize the variants aligned against the reference genome to confirm the accuracy of the variant calls by checking for possible strand biases and sequencing errors. Copy number variations (CNVs) were not analyzed in this study.

The ctDNA concentrations were expressed in haploid genome equivalents (hGE) per mL of plasma (hGE/mL) and were calculated by multiplying the mean VAF for all mutations used for detection calling by the concentration of cfDNA (pg/mL of plasma) and dividing by 3.3, using the assumption that each haploid genomic equivalent weighed 3.3 pg, as previously described by Scherer et al. (Supplementary Table 5).

Test validation. For technical validation, input DNA requirements, library generation and sequencing, two rounds of validation were performed consecutively. Three previously characterized samples with known single nucleotide variants and/or indels, as in the 24 FFPE tissue samples, were analyzed. Multiple intercapture and intracapture replicates, as well as inter-run and intra-run replicates were included (data not shown).

Statistical analysis. The patient characteristics are presented as frequencies (n) and percentages (%) for categorical variables or as medians and ranges for continuous variables. Categorical data were compared with

Fisher's exact or chi-squared test, when appropriate, and continuous data were compared using a two-tailed paired Mann Whitney U test. R Statistical Software was used for all statistical tests. Probability values < 0.05 were considered significant.

Results

Gene panel features.
A total of 73 samples (47 FFPE and 26 ctDNA) were sequenced, resulting in a median of 8,290,518 reads in the FFPE samples and 11,071,271 in the ctDNA samples. The median percentage of mapped reads was 97% in both types of samples (Supplementary Table 4). The median percentage of mapped base pairs on-target was 83% in the FFPE samples and 73% in the ctDNA samples. The median percentage of duplicate fragments per sample was 35% in the FFPE samples and 62% in the ctDNA samples. The median deep coverage of target regions was 2101x (range 231x–6518x) in the FFPE samples (median coverage heterogeneity of 0.04%) and 3678x (range 1906x–9270x) in the ctDNA samples (median coverage heterogeneity of 0.24%) (Supplementary Table 4).

Mutational data from the FFPE samples (n = 47).
The gene panel was performed on 47 patients with NHL; 93.6% (44/47) presented at least one variant in the FFPE tissue samples with VAF ≥ 5%. In total, 372 somatic alterations were detected (Table 1). The patients presented a median of 6 mutations per sample (range 0–37). Missense mutations were the most frequent at 253/372 (67.6%), followed by 48/372 (12.9%) frameshift mutations, and 34/372 (9.1%) nonsense mutations (Table 1). Figure 1 and Supplementary Figs. 1 and 2 present the gene frequencies by NHL subtype detected in the total cohort.

In FL, 83% of the patients presented *BCL2* rearrangement and a total of 93 somatic alterations, with a median of 7.4 mutations per sample (range 2–22). The most frequently mutated genes were *KMT2D* (80%), *TNFRSF14* (48%), *CREBBP* (40%), *BCL2* (40%), *TNFAIP3* (32%), *SOCS1* (32%), *CARD11* (28%) and *EZH2* (28%) (Fig. 1). A total of (13/15) 87% FL samples presented mutations in epigenetic modifiers genes.

In contrast, 28% of the patients with DLBCL presented *BCL6* rearrangement, 25% presented c-*MYC* rearrangement, and 16% presented *BCL2* rearrangement, with 16% presenting double-hit lymphomas. Furthermore, the patients presented a total of 279 somatic variants with a median of 8.6 mutations (range 0–35). In the overall cohort (n = 32), the most frequently mutated genes were *SOCS1* (40%), *KMT2D* (40%), *EP300* (40%), and c-*MYC* (32%) (Fig. 1). Sixty-eight percent (22/32) of the patients presented mutations in epigenetic modifier genes.

When comparing the germinal center B-cell (GCB) DLBCLs (n = 17) with the activated B-cell (ABC) DLBCLs (n = 9), *PIM1* mutations were present only in the patients with GCB DLBCL (41% vs. 0%; p = 0.03), and *XPO1* mutations were present only in the patients with ABC DLBCL (22% vs. 0%; p = 0.08), with statistically significant differences (Supplementary Fig. 1).

When we analyzed the patients with high-grade DLBCLs-NOS (n = 5), those with double-hit/triple-hit (n = 4) DLBCLs, and those with DLBCL-NOS (n = 20), c-*MYC* and *TCF3* were more present in the high-grade DLBCLs-NOS than in the DLBCLs-NOS (44% vs. 15%, p = 0.1; and 22% vs. 0%, p = 0.089, respectively). Mutations in *EZH2* and *MAL* were more frequent in the high-grade double-hit DLBCLs (50% vs. 4%, p = 0.04; 75% vs. 12%, p = 0.02). Mutations in *TP53*, *TCF3*, and *CD58* were more frequent in the high-grade DLBCLs-NOS (60% vs. 20%, p = 0.11; 40% vs. 0%, p = 0.025; 40% vs. 8%, p = 0.12) (Supplementary Fig. 2).

When we compared the mutations in FL (n = 15) versus those in DLBCL (n = 32), we found that the variants in the following genes were more frequently present in FL than in DLBCL: *BCL2* (p = 0.003), *CREBBP* (p = 0.003), *KMT2D* (p = 0.012), and *TNFRS14* (p = 0.015), with significant differences. In contrast, *PIM1* variants (p = 0.033) were more frequent in DLBCLs (Fig. 1).

Recurrent mutations (1–3) were found in *ARID1A, B2M, BCL2, CIITA, CREBBP, EP300, EZH2, FOXO1, KMT2D, MAL, MYD88, NFKBIE, PIM1, SOCS1, STAT6, TP53,* and *XPO* (Table 1). Only *EZH2* (p.Tyr641Asn/His/Phe), *CIITA* (p.Ser781_Val782delinsLeuAla), *EP300* (p.Gly211Ser), and *MAL* (p.Phe33Cys) presented more than 4 recurrent mutations (Supplementary Fig. 3).

The presence of more than 1 mutation in the same gene was detected in several genes including *MYC, SOCS1, PIM1, CIITA, KMT2D,* and *BCL2* (Table 1). Nine patients presented mutations in the c-*myc* protooncogene, 4 presented more than 1 mutation and concomitant with *MYC* translocation. Eighty-three percent (27/33) of the *MYC* mutations occurred in exon 2. The other genes with more than one variant were *SOCS1* (59 mutations in 17 patients), *PIM1* (28 mutations in 6 patients), *CIITA* (12 mutations in 8 patients), and *KMT2D* (36 mutations in 22 patients); all of these patients were diagnosed with DLBCL. Also, 4 patients with FL presented more than 2 mutations in *BCL2*, all with *BCL2* rearrangement.

Mutational data in FFPE and cfDNA (n = 26).
The cfDNA samples collected from the study patients at diagnosis were subjected to targeted sequencing (n = 26) (Table 2). In 92% (24/26) of the samples, we detected some variant in the free DNA in plasma. A total of 386 variants were detected (174 in the ctDNA samples and 212 in the FFPE samples). Of the total variants, 123 mutations (63.7%) were detected in both types of samples, 51 mutations were detected only in the ctDNA samples (13.2%), and 89 mutations were detected only in the FFPE samples (23%) (Fig. 2). Those variants that were detected in both types of samples had higher VAFs (28%) in the FFPE samples than in the ctDNA samples (17.9%). When considering only those mutations with VAFs > 10% in the FFPE samples, the percentage of mutations identified in both samples was 86%; specifically, the ctDNA samples that had a percentage of mutations < 50% had an input ctDNA concentration < 0.5 ng/μL (Supplementary Table 4). Overall, 96% (25/26) of the patients had at least one alteration observed in the ctDNA sample that was identical to that in the FFPE tissue sample.

UPN	Type of sample	Diagnosis	Gene	c.DNA	Protein	VAF	Consequence
1	FFPE	FL	B2M	c.20T > G	p.(Leu7*)	62.8	Nonsense
			CREBBP	c.4445A > G	p.(Lys1482Arg)	30.2	Missense
			PAX5	c.491_577del	p.(Val164_Ala192del)	19.6	Inframe
			REL	c.1265_1273dupATTTAAATG	p.(Asp422_Ans-424dup)	13.2	Inframe
			SOCS1	c.3_13delGGTAGCACACA	p.(Met?)	28.4	No-start
			SOCS1	c.180_181delGC	p.(His61Argfs*55)	30.3	Frameshift
2	FFPE	DLBCL	CARD11	c.746A > C	p.(Gln249Pro)	43	Missense
			CREBBP	c.4393T > G	p.(Tyr1465Asp)	89.4	Missense
			EZH2	c.1921T > C	p.(Tyr641His)	42.1	Missense
			TNFRSF14	c.602G > A	p.(Trp201*)	79.1	Nonsense
			ID3	c.256G > C	p.(Glu86Gln)	46.4	Missense
			KMT2D	c.8607_8608insAAGGC	p.(Gly2870Lysfs*42)	27.7	Frameshift
3	FFPE	FL	TP53	c.743G > T	p.(Arg248Leu)	35.1	Missense
			CD79B	c.600delC	p.(Asp200Glufs*11)	72.6	Frameshift
			NFKBIE	c.1094delT	p.(Leu365Argfs*66)	13.4	Frameshift
			EP300	c.3014G > A	p.(Cys1005Tyr)	43.1	Missense
			KMT2D	c.4569C > A	p.(Cys1523*)	29.3	Nonsense
4	FFPE	FL	BCL2	c.140G > A	p.(Gly47Asp)	28	Missense
			BCL2	c.175C > A	p.(Pro59Thr)	28.4	Missense
			BCL2	c.151T > G	p.(Ser51Ala)	28.8	Missense
			EZH2	c.1922A > T	p.(Tyr641Phe)	30.5	Missense
			STAT6	c.1256A > G	p.(Asp419Gly)	5.3	Missense
			CD58	c.254C > G	p.(Thr85Ser)	47.9	Missense
			MEF2B	c.170A > G	p.(Tyr57Cys)	16.8	Missense
			KMT2D	c.3931A > T	p.(Arg1311*)	42.2	Nonsense
			KMT2D	c.13893 + 2T > A	p.(?)	22.4	Splice_donor_+2
5	FFPE	DLBCL	TP53	c.715A > G	p.(Asn239Asp)	5.9	Missense
			TP53	c.536A > C	p.(His179Pro)	7.9	Missense
			NOTCH2	c.4568A > G	p.(Asn1523Ser)	46.8	Missense
			TCF3	c.1634G > A	p.(Arg545Gln)	49.1	Missense
			TCF3	c.1670T > A	p.(Val557Glu)	6.2	Missense
			MYC	c.55A > C	p.(Val19Leu)	7.8	Missense
6	FFPE	DLBCL	–	–	–	–	–
7	FFPE	DLBCL	BCL6	c.1753C > T	p.(Arg585Trp)	12.2	Missense
			BCL6	c.1853G > A	p.(Arg618His)	11.9	Missense
			CREBBP	c.4925_4927del	p.(Ser1642del)	15	Inframe
			CCND3	c.568dupC	p.(Arg190Profs*)	36.4	Frameshift
			CD58	c.493C > T	p.(Gln165*)	53.8	Nonsense
			CDKN2A	c.172C > T	p.(Arg58*)	52.2	Nonsense
			CIITA	c.1801C > T	p.(Arg601Trp)	32.3	Missense
			EP300	c.4879C > T	p.(Arg1627Trp)	12.3	Missense
			GNA13	c.220C > T	p.(Gln74*)	71.8	Nonsense
8	FFPE	FL	SOCS1	c.299C > T	p.(Thr100Ile)	11.8	Missense
			MAL	c.271T > C	p.(Tyr91His)	61.7	Missense
			KMT2D	c.3535_3539delGGCTinsAACCATGTGAAGA	p.(Gly1179Asn Fs*36)	13	Frameshift
9	FFPE	DLBCL	CDKN2A	c.329G > A	p.(Trp110*)	6.9	Nonsense
			EP300	c.631G > A	p.(Gly211Ser)	43.3	Missense
			KMT2D	c.14450T > G	p.(Val4817Gly)	51.3	Missense
10	FFPE	DLBCL	CARD11	c.1070A > T	p.(Asp357Val)	12.9	Missense
			XPO1	c.1711G > A	p.(Glu571Lys)	13	Missense
			TNFRSF14	c.608G > A	p.(Trp203*)	12.4	Nonsense
			CCND3	c.605C > T	p.(Thr202Ile)	11.3	Missense
			CHD2	c.1397G > A	p.(Arg466Gln)	12.7	Missense
			EP300	c.4454A > T	p.(Asp1485Val)	8.7	Missense
Continued							

UPN	Type of sample	Diagnosis	Gene	c.DNA	Protein	VAF	Consenquence
11	FFPE	FL	BCL2	c.392C > G	p.(Ala131Gly)	11.7	Missense
			BCL2	c.517A > G	p.(Ile173Val)	9.4	Missense
			CARD11	c.748T > C	p.(Ser250Pro)	14.5	Missense
			CREBBP	c.4394A > G	p.(Tyr1465Cys)	32.1	Missense
			CCND3	c.531_532delCTinsTG	p.(Ser178Ala)	99.7	Missense
			KMT2D	c.6664C > T	p.(Gln2222*)	12.2	Nonsense
			KMT2D	c.5335A > T	p.(Lys1779*)	14.5	Nonsense
12	FFPE	FL	CARD11	c.864 + 1G > C	p.(?)	34.8	Splice_donor_+1
			CREBBP	c.4336T > G	p.(Phe1446Val)	36.7	Missense
			STAT6	c.1256A > C	p.(Asp419Ala)	16.2	Missense
			TNFAIP3	c.1706G > A	p.(Arg569Gln)	33.3	Missense
			CIITA	c.2342_2345delCGGTinsTGGC	p.(Ser781_Val-782delinsLeuAla)	20.7	Missense
			KMT2D	c.11456_11474del	p.(Gly3819AspFs*15)	13	Frameshift
			KMT2D	c.9781C > T	p.(Gln3261*)	13.6	Nonsense
13	FFPE	DLBCL	TP53	c.490A > T	p.(Lys164*)	31.8	Nonsense
			B2M	c.2T > G	p.(Met1?)	52.4	No-start
			PIM1	c.676G > A	p.(Glu226Lys)	35.5	Missense
			PIM1	c.370C > T	p.(Pro124Ser)	62.1	Missense
			PIM1	c.434G > A	p.(Arg145His)	6.7	Missense
			PIM1	c.202C > T	p.(His68Tyr)	23.3	Missense
			SOCS1	c.8C > T	p.(Ala3Val)	30.3	Missense
			FOXO1	c.435del	p.(Ala146Argfs*187)	17.9	Frameshift
			MEF2B	c.78C > G	p.(Phe26Leu)	52.5	Missense
			MYD88	c.818T > C	p.(Leu273Pro)	33	Missense
14	FFPE	FL	BCL2	c.191A > C	p.(Asp64Ala)	12	Missense
			BCL2	c.93T > C	p.(Asp31Glu)	9	Missense
			TNFAIP3	c.2014G > T	p.(Gly672*)	8.8	Nonsense
			TNFRSF14	c.463delA	p.(Thr155Profs*)	13.3	Frameshift
			KMT2D	c.172 + 2T > C	p.(?)	12.4	Splice_donor_+2
15	FFPE	FL	CREBBP	c.4382T > C	p.(Leu1461Pro)	29.1	Missense
			SOCS1	c.630G > C	p.(Gln210His)	47.6	Missense
			KMT2D	c.16489_16491delATC	p.(Ile5479del)	32.4	Inframe
			KMT2D	c.9019delG	p.(Glu3007Lysfs*22)	26.1	Frameshift
16	FFPE	FL	CCND1	c.31G > T	p.(Glu11*)	44.9	Nonsense
			TNFRSF14	c.29-1G > A	p.(?)	40.7	Splice_acceptor_-1
			KMT2D	c.15143G > A	p.(Arg5048His)	27	Missense
			KMT2D	c.8401C > T	p.(Arg2801*)	26.4	Nonsense
			PRDM1	c.351A > G	p.(Ile117Met)	46.7	Missense
17	FFPE	FL	BCL2	c.19_21delinsGCG	p.(Thr7Ala)	24.7	Missense
			CARD11	c.752T > C	p.(Leu251Pro)	32.7	Missense
			CREBBP	c.4925_4927del	p.(Ser1642del)	21.1	Inframe
			EZH2	c.1921T > A	p.(Tyr641Asn)	36.4	Missense
			PTPN11	c.1165A > C	p.(Lys389Gln)	7.6	Missense
			ARID1A	c.60_62del	p.(Pro21del)	7.3	Inframe
Continued							

UPN	Type of sample	Diagnosis	Gene	c.DNA	Protein	VAF	Consenquence
18	FFPE	DLBCL	NFKBIE	c.668_671delTGCTinsAGCG	p.(Leu223_Leu224delins*Arg)	20.6	Missense
			SOCS1	c.7G > A	p.(Ala3Thr)	14	Missense
			SOCS1	c.374G > C	p.(Ser125Thr)	19.1	Missense
			SOCS1	c.407A > C	p.(His136Pro)	18	Missense
			SOCS1	c.428G > A	p.(Ser143Asn)	13.5	Missense
			SOCS1	c.398delG	p.(Gly133Alafs*72)	10.6	Frameshift
			SOCS1	c.55C > T	p.(Pro19Ser)	14.6	Missense
			SOCS1	c.412G > C	p.(Asp138His)	15.9	Missense
			SOCS1	c.391C > G	p.(Gln131Glu)	16.4	Missense
			SOCS1	c.391C > T	p.(Gln131*)	16.4	Nonsense
			SOCS1	c.318C > G	p.(Ser106Arg)	8.7	Missense
			SOCS1	c.528G > C	p.(Glu176Asp)	10.6	Missense
			ARID1A	c.5012G > A	p.(Arg1671Gln)	18.4	Missense
			EP300	c.2359G > A	p.(Gly787Ser)	44.2	Missense
			MYC	c.482C > T	p.(Ser161Leu)	10.9	Missense
			MYC	c.218_219delCCinsTA	p.(Thr73Ile)	11.2	Missense
			MYC	c.1164C > G	p.(Ser388Arg)	18.7	Missense
			MYC	c.557G > C	p.(Cys186Ser)	14.5	Missense
			MYC	c.895G > C	p.(Ala299Pro)	18.6	Missense
			MYC	c.910_999dup	p.(Lys304_Asp-333dup)	40.6	Inframe
			MYC	c.654C > G	p.(Ser218Arg)	15.1	Missense
			MYC	c.785C > T	p.(Thr262Ile)	15.8	Missense
			MYC	c.68_71delinsGCAG	p.(Phe23Cys)	9.7	Missense
			MYC	c.63C > G	p.(Ser21Arg)	9.6	Missense
			MYC	c.162G > C	p.(Glu54Asp)	9	Missense
			MYC	c.144G > A	p.(Asp48Glu)	7.9	Missense
			MYC	c.358_361delinsTTGT	p.(Asp120Leu)	11.8	Missense
19	FFPE	FL	CARD11	c.1202A > T	p.(Asp401Val)	19.9	Missense
			SOCS1	c.4G > T	p.(Val2Leu)	18.1	Missense
			SOCS1	c.14A > G	p.(Asn5Ser)	20	Missense
			SOCS1	c.134_139dupTCCCGG	p.(Val45_Pro46dup)	43.5	Inframe
			TNFAIP3	c.1035C > A	p.(Tyr345*)	28.9	Nonsense
			TNFRSF14	c.70G > T	p.(Val24Leu)	28.1	Missense
Continued							

UPN	Type of sample	Diagnosis	Gene	c.DNA	Protein	VAF	Consequence
20	FFPE	DLBCL	XPO1	c.1711G > A	p.(Glu571Lys)	7	Missense
			NFKBIE	c.759_762delTTAC	p.(Tyr254Serfs*13)	17.9	Frameshift
			PAX5	c.548G > C	p.(Gly183Ala)	7.6	Missense
			PIM1	c.3G > A	p.(Met1?)	7.3	No-start
			PIM1	c.111G > T	p.(Gln37His)	7.6	Missense
			PIM1	c.224C > T	p.(Ser75Phe)	7.3	Missense
			PIM1	c.290G > C	p.(Ser97Thr)	9.8	Missense
			PIM1	c.379C > A	p.(Gln127Lys)	6.3	Missense
			PIM1	c.73C > G	p.(Leu25Val)	6.3	Missense
			SOCS1	c.195_197delinsACC	p.(Arg66Pro)	5.5	Missense
			SOCS1	c.275G > C	p.(Arg92Pro)	5.1	Missense
			SOCS1	c.17A > C	p.(Gln6Pro)	5.3	Missense
			SOCS1	c.46_49delinsTCAA	p.(Ala16_Ala17delinsSerThr)	7	Missense
			SOCS1	c.387C > G	p.(His129Gln)	5.7	Missense
			SOCS1	c.416G > C	p.(Gly139Ala)	6.9	Missense
			SOCS1	c.356T > C	p.(Met119Thr)	5.8	Missense
			ARID1A	c.3999_4001del	p.(Gln1334del)	7.5	Inframe
			CIITA	c.52 + 1G > T	p.(?)	10.7	Splice_donor_ + 1
			CIITA	c.2342_2345delCGGTinsTGGC	p.(Ser781_Val782delinsLeuAla)	35.8	Missense
			CIITA	c.3127_3134del	p.(Ala1043Profs*9)	8.4	Frameshift
			MYC	c.680A > C	p.(Asp227Ala)	10	Missense
21	FFPE	DLBCL	B2M	c.176T > A	p.(Leu59*)	80.3	Nonsense
			ATM	c.8284C > T	p.(Gln2762*)	54.3	Nonsense
			NFKBIE	c.1147_1153delCAACCAC	p.(Gln383Serfs*46)	31.3	Frameshift
			NFKBIE	c.759_762delTTAC	p.(Tyr254Serfs*13)	33.4	Frameshift
			PRDM1	c.75delG	p.(Arg25Serfs*13)	41.8	Frameshift
			SOCS1	c.358_361delGCCTinsCC	p.(Ala120Profs*?)	39.7	Frameshift
			SOCS1	c.434_437delACTG	p.(Asp145Alafs*59)	39.4	Frameshift
			TNFAIP3	c.2350C > T	p.(Gln784*)	64	Nonsense
			TNFAIP3	C.295 + 2T > C	p.(?)	67.5	Splice_donor_ + 2
			CDKN2A	c.394G > A	p.(Ala132Thr)	32.4	Missense
			CIITA	c.34_46delTACCTGTCAGAGC	p.(Tyr12Profs*15)	36.4	Frameshift
			CIITA	c.1652delG	p.(Gly551Alafs*7)	35.9	Frameshift
			CIITA	c.3262G > A	p.(Gly1880Arg)	5.3	Missense
			FOXO1	c.61C > T	p.(Arg21Cys)	39.4	Missense
			GNA13	c.179A > G	p.(Asp60Gly)	81.6	Missense
			MYC	c.25A > G	p.(Asn9Asp)	43.3	Missense
22	FFPE	FL	BCL6	c.1752C > A	p.(Asn584Lys)	27.8	Missense
			CCND3	c.613G > A	p.(Asp205Asn)	5.3	Missense
			GNA13	c.243_244del	p.(Glu82Glyfs*19)	5	Frameshift
			MYC	c.154_156del	p.(Lys51Del)	5.4	Inframe
23	FFPE	DLBCL	TP53	c.839G > A	p.(Arg280Lys)	28.2	Missense
			NFKBIE	c.759_762delTTAC	p.(Tyr254Serfs*13)	12.3	Frameshift
			CCND3	c.626T > C	p.(Ile209Thr)	23.1	Missense
			CCND3	c.604A > C	p.(Thr202Pro)	24.4	Missense
			KMT2D	c.10919G > A	p.(Gly3640Glu)	58.5	Missense
24	FFPE	DLBCL	BCL2	c.17G > A	p.(Arg6Lys)	5.9	Missense
			B2M	c.16G > C	p.(Ala6Pro)	49.4	Missense
			TNFRSF14	c.49_50delinsCAG	p.(Lys17Glnfs*60)	8.9	Frameshift
			CXCR4	c.1025C > A	p.(Ser342*)	5	Nonsense
25	FFPE	DLBCL	TP53	c.743G > A	p.(Arg248Gln)	66.3	Missense
			SOCS1	c.8C > T	p.(Ala3Val)	38.8	Missense
			EP300	c.631G > A	p.(Gly211Ser)	49.5	Missense
			KMT2D	c.13139delC	p.(Pro4380Glnfs*4)	42.3	Frameshift
			MYD88	c.818T > C	p.(Leu273Pro)	76.3	Missense
Continued							

UPN	Type of sample	Diagnosis	Gene	c.DNA	Protein	VAF	Consequence
26	FFPE	DLBCL	TP53	c.725G > T	p.(Cys242Phe)	76.8	Missense
			TCF3	c.1688G > A	p.(Arg563His)	17.1	Missense
27	FFPE	DLBCL	XPO1	c.1711G > C	p.(Glu571Lys)	8.6	Missense
			KMT2D	c.7547C > G	p.(Pro2516Arg)	45.2	Missense
			KMT2D	c.7604G > A	p.(Arg2535His)	39.2	Missense
28	FFPE	DLBCL	CHD2	c.1281G > A	p.(Trp427*)	18.6	Nonsense
			MAL	c.98T > G	p.(Phe33Cys)	6	Missense
			MYC	c.1148A > G	p.(Asn383Ser)	22.9	Missense
			MYC	c.490C > T	p.(Pro164Ser)	20.6	Missense
			MYD88	c.818T > C	p.(Leu273Pro)	18.4	Missense
			MYD88	c.797C > T	p.(Pro266Leu)	19.1	Missense
29	FFPE	DLBCL	PIM1	c.403G > T	p.(Glu135*)	36.6	Nonsense
			PIM1	c.3G > T	p.(Met?)	34.8	No-start
			PIM1	c.544C > G	p.(Leu182Val)	33.7	Missense
			PIM1	c.111G > C	p.(Gln37His)	34.7	Missense
			PIM1	c.550C > G	p.(Leu184Val)	33.8	Missense
			PIM1	c.455T > A	p.(Leu152Gln)	38	Missense
			PIM1	c.382G > A	p.(Asp128Asn)	36.4	Missense
			PIM1	c.300C > G	p.(Phe100Leu)	35.7	Missense
			PIM1	c.83G > T	p.(Gly28Val)	30.5	Missense
			PIM1	c.424_427delGAGCinsCAGG	p.(Glu142_Leu143delinsGlnVal)	31.3	Missense
			SOCS1	c.564_565delCG	p.(Asn190Profs*?)	25.4	Frameshift
			SOCS1	c.213_220delCGCGCTCCinsT	p.(Ala72Trpfs*11)	44.8	Frameshift
			SOCS1	c.523C > T	p.(Gln175*)	23.5	Nonsense
			SOCS1	c.264_437del	p.(Ala89_Cys146del)	23	Inframe
			SOCS1	c.18G > C	p.(Gln6His)	44.9	Missense
			SOCS1	c.318C > A	p.(Ser106Arg)	30.4	Missense
			SOCS1	c.4_7delGTAGinsCTAC	p.(Val2_Ala3delinsLeuPro)	22.8	Missense
			SOCS1	c.195_197delGCGinsACA	p.(Arg66His)	23	Missense
			SOCS1	c.237C > G	p.(Phe79Leu)	42.9	Missense
			SOCS1	c.255C > G	p.(Ser85Arg)	38.2	Missense
			SOCS1	c.529C > G	p.(Leu177Val)	23.9	Missense
			SOCS1	c.254G > C	p.(Ser85Thr)	28.4	Missense
			SOCS1	c.176G > C	p.(Arg59Pro)	23.2	Missense
			SOCS1	c.391C > G	p.(Gln131Glu)	28.2	Missense
			SOCS1	c.4_6delGTAinsTTG	p.(Val2Leu)	43.3	Missense
			CD58	c.23_24dup	p.(Arg9Glyfs*34)	7.1	Frameshift
			CHD2	c.3976G > A	p.(Glu1326Lys)	43	Missense
			MYC	c.214C > T	p.(Pro72Ser)	34.5	Missense
			MYC	c.490C > G	p.(Leu164Val)	49.6	Missense
			MYC	c.245_246delCCinsTA	p.(Ser82Leu)	45.6	Missense
			MYC	c.223C > G	p.(Pro75Ala)	35.3	Missense
			MYC	c.963G > C	p.(Gln321His)	47.5	Missense
			MYC	c.569G > C	p.(Ser190Thr)	50.4	Missense
			MYC	c.358C > G	p.(Leu120Val)	47.5	Missense
			MYC	c.763C > T	p.(Leu255Phe)	48.4	Missense
			MYC	c.221_223delinsAGG	p.(Tyr74*)	8.3	Nonsense
			MYC	c.474G > A	p.(Asp158Glu)	48.3	Missense
30	FFPE	DLBCL	MAL	c.98T > C	p.(Phe33Cys)	6.9	Missense
Continued							

UPN	Type of sample	Diagnosis	Gene	c.DNA	Protein	VAF	Consenquence
31	FFPE	DLBCL	BRAF	c.1780G > A	p.(Asp594Asn)	29.4	Missense
			EZH2	c.1921T > A	p.(Tyr641Asn)	34.4	Missense
			STAT6	c.1255G > T	p.(Asp419Tyr)	52.4	Missense
			PIM1	c.409G > T	p.(Gly137*)	34	Nonsense
			PIM1	c.285G > C	p.(Lys95Asn)	31.8	Missense
			SOCS1	c.220C > G	p.(Leu74Val)	30.7	Missense
			SOCS1	c.178T > C	p.(Ser60Pro)	28.6	Missense
			CIITA	c.2342_2345delCGGTinsTGGC	p.(Ser781_Val-782delinsLeuAla)	43.7	Missense
			EP300	c.6091C > T	p.(Pro2031Ser)	48.4	Missense
			KMT2D	c.14843C > G	p.(Ser4948*)	40.3	Nonsense
			KMT2D	c.7586delG	p.(Gly-2529Alafs*14)	22.7	Frameshift
32	FFPE	DLBCL	NOTCH1	c.7541_7542delCT	p.(Pro2514Argfs*4)	34.7	Frameshift
			ARID1A	c.4540_4543delACGGinsCCGT	p.(Thr1514_Gly1515delinsPro-Cys)	10.1	Missense
			MAL	c.98T > C	p.(Phe33Cys)	6	Missense
			KMT2D	c.2886_2887delTGinsCA	p.(Ala963Thr)	11.5	Missense
33	FFPE	DLBCL	NFKBIE	c.98C > T	p.(Ser33Phe)	63	Missense
			CCND3	c.544_554dupTCCAGCCCAGC	p.(Lys187Alafs*?)	63.7	Frameshift
			CXCR4	c.1012C > T	p.(Arg338*)	45.1	Nonsense
			EP300	c.6316delA	p.(Met-2106Cysfs*28)	11.1	Frameshift
			EP300	c.6329_6330insT	p.(Gln2110His-fs*100)	9.9	Frameshift
			MAL	c.98T > G	p.(Phe33Cys)	8.7	Missense
			KMT2D	c.2886_2887delTGinsCA	p.(Ala963Thr)	13.6	Missense
			MYC	c.77_78delACinsGT	p.(Asn26Ser)	67.4	Missense
			MYC	c.63C > G	p.(Ser21Arg)	67.6	Missense
			MYC	c.214C > A	p.(Pro72Thr)	68.3	Missense
			MYC	c.175G > A	p.(Ala59Thr)	67.7	Missense
34	FFPE	FL	BCL2	c.256C > T	p.(Leu86Phe)	25.8	Missense
			BCL2	c.20C > A	p.(Thr7Lys)	20.2	Missense
			BCL2	c.185C > G	p.(Ser62Cys)	25	Missense
			BCL2	c.133G > A	p.(Ala45Thr)	20.8	Missense
			BCL2	c.469A > C	p.(Met157Leu)	25.2	Missense
			EZH2	c.1921T > A	p.(Tyr641Asn)	22.5	Missense
			NOTCH2	c.4609G > T	p.(Asp1537Tyr)	48.7	Missense
			SOCS1	c.416_418delinsCCG	p.(Gly139_ser140delin-sAlaGly)	29.3	Missense
			SOCS1	c.348C > G	p.(Ser116Arg)	30.5	Missense
			TNFRSF14	c.3G > T	p.(Met1?)	22.4	No-start
			TNFRSF14	c.178 + 1G > T	p.(?)	10.5	Splice_donor_+1
			TNFRSF14	c.433_434dup	p.(Ser145Argfs*)	8.9	Frameshift
			EP300	c.4115G > A	p.(Cys1372Tyr)	10.7	Missense
			FOXO1	c.1A > T	p.(Met1?)	24.1	No-start
			FOXO1	c.358C > G	p.(Pro120Ala)	20.9	Missense
			GNA13	c.1A > T	p.(Met1?)	21.5	No-start
			GNA13	c.841C > G	p.(Leu281Val)	5	Missense
			KMT2D	c.15088C > T	p.(Arg5030Cys)	23.6	Missense
			KMT2D	c.8311C > T	p.(Arg2771*)	24.4	Nonsense
Continued							

UPN	Type of sample	Diagnosis	Gene	c.DNA	Protein	VAF	Consenquence
35	FFPE	DLBCL	NRAS	c.38G > T	p.(Gly13Val)	6.1	Missense
			EZH2	c.2060C > T	p.(Ala687Val)	35.4	Missense
			REL	c.392A > G	p.(Asn131Ser)	57.9	Missense
			ARID1A	c.2668A > G	p.(Met890Val)	31.8	Missense
			ARID1A	c.4540_4543delinsCCGT	p.(Thr1514_Gly1515delinsPro-Cys)	9.7	Missense
			EP300	c.6329_6330insT	p.(Gln2110Hisfs*100)	22.2	Frameshift
			EP300	c.6323A > T	p.(Gln2108Leu)	22.7	Missense
			EP300	c.6316del	p.(Met2106Cysfs*28)	25.2	Frameshift
			FOXO1	c.62G > T	p.(Arg21Leu)	28.8	Missense
			FOXO1	c.118T > C	p.(Ser40Pro)	30.8	Missense
			MAL	c.98T > G	p.(Phe33Cys)	13.5	Missense
			MEF2B	c.32T > C	p.(Ile11Thr)	27.5	Missense
			KMT2D	c.6221_6224dupACAA	p.(Val2076Glnfs*7)	23.9	Frameshift
			KMT2D	c.12204_12207delACTC	p.(Ser4070Glyfs*25)	37.6	Frameshift
			KMT2D	c.2876delA	p.(Tyr959Serfs*41)	10.2	Frameshift
			KMT2D	c.10192A > G	p.(Met3398Val)	40.7	Missense
			KMT2D	c.2886_2887delTGinsCA	p.(Ala963Thr)	22.6	Missense
36	FFPE	FL	EZH2	c.1921T > A	p.(Tyr641Asn)	32.4	Missense
			TNFRSF14	c.42delC	p.(Thr15Profs*7)	29.5	Frameshift
			ARID1A	c.4899delC	p.(Met1634fs*1)	30.6	Frameshift
			EP300	c.631G > A	p.(Gly211Ser)	50.6	Missense
			KMT2D	c.5188_5782 + 1del	p.(?)	19.8	Splice_donor_ + 1
			KMT2D	c.15583C > T	p.(Gln5195*)	21	Nonsense
37	FFPE	DLBCL	BCL6	c.1760C > G	p.(Ala587Gly)	29.2	Missense
			PLCG2	c.2009T > G	p.(Leu670Arg)	7.1	Missense
			POT1	c.1315_1317del	p.(Ala439del)	6.4	Inframe
			SOCS1	c.195_206delGCGCATCACGCG	p.(Arg66_Arg69del)	34.7	Inframe
			ARID1A	c.4540_4543delACGGinsCCGT	p.(Thr1514_Gly1515delinsPro-Cys)	16.6	Missense
			CIITA	c.2342_2345delCGGTinsTGGC	p.(Ser781_Val782delinsLeuAla)	45.5	Missense
			EP300	c.6329_6330insT	p.(Gln2110Hisfs*100)	18.2	Frameshift
			EP300	c.6316delA	p.(Met2106Cysfs*28)	18.7	Frameshift
			EP300	c.6323A > T	p.(Gln2108Leu)	19.1	Missense
			FOXO1	c.1478G > C	p.(Gly493Ala)	7.2	Missense
			MAL	c.98T > G	p.(Phe33Cys)	19.3	Missense
			KMT2D	c.13753_13757delinsTTGAC	p.(Val4585_Asn4586delinsLeuThr)	5.4	Missense
			KMT2D	c.2886_2887delTGinsCA	p.(Ala963Thr)	37.6	Missense
38	FFPE	DLBCL	B2M	c.2T > A	p.(Met1?)	31.9	No-start
			NFKBIE	c.1108 + 2T > A	p.(?)	25.1	Splice_donor_ + 2
			NFKBIE	c.759_762delTTAC	p.(Tyr254Serfs*13)	24.6	Frameshift
			PRDM1	c.1142A > G	p.(Tyr381Cys)	49.5	Missense
			TNFRSF14	c.632T > A	p.(Val211Asp)	32	Missense
			CD58	c.70 + 2T > G	p.(?)	34.2	Splice_donor_ + 2
39	FFPE	DLBCL	BRAF	c.1799T > A	p.(Val600Glu)	26.4	Missense
			SOCS1	c.49_52delGCAG	p.(Ala17Serfs*67)	26	Frameshift
			CIITA	c.2342_2345delCGGTinsTGGC	p.(Ser781_Val782delinsLeuAla)	45.6	Missense
			KMT2A	c.627G > T	p.(Lys209Asn)	24.8	Missense
			KMT2D	c.11180G > A	p.(Arg3727His)	50.7	Missense
40	FFPE	DLBCL	–	–	–	–	–
Continued							

UPN	Type of sample	Diagnosis	Gene	c.DNA	Protein	VAF	Consequence
41	FFPE	FL	*MYD88*	c.909_929dup	p.(Ser304_Leu310dup)	7.1	Inframe
42			*SOCS1*	c.523C > T	p.(Gln175*)	8.9	Nonsense
			ARID1A	c.20_52del	p.(Ser11Leufs*89)	13.6	Frameshift
			EP300	c.631G > A	p.(Gly211Ser)	46	Missense
			EP300	c.3754A > G	p.(Arg1252Gly)	47.4	Missense
			GNA13	c.32T > C	p.(Leu11Pro)	24.2	Missense
			MYC	c.212_21dupTGC	p.(Leu71dup)	19.7	Inframe
43	FFPE	DLBCL	–				
44	FFPE	DLBCL	*TP53*	c.743G > A	p.(Arg248Gln)	12.6	Missense
			TP53	c.919 + 1G > A	p.(?)	12.6	Splice_donor_ + 1
			PRDM1	c.626_627del	p.(His209Leufs*25)	19.1	Frameshift
			ARID1A	c.60_62del	p.(Pro21del)	9.4	Inframe
			MYD88	c.719T > C	p.(Met240Thr)	35	Missense
45	FFPE	DLBCL	*TP53*	c.404G > T	p.(Cys135Phe)	7.2	Missense
			KRAS	c.38G > A	p.(Gly13Asp)	13.5	Missense
			CARD11	c.383C > T	p.(Thr128Met)	18.2	Missense
			PIM1	c.447G > T	p.(Trp149Cys)	15.9	Missense
			PIM1	c.451G > C	p.(Val151Leu)	16.4	Missense
			PIM1	c.242C > T	p.(Pro81Leu)	14.6	Missense
			SOCS1	c.430C > T	p.(Phe144Leu)	14.4	Missense
			SOCS1	c.534C > G	p.(Cys178Trp)	15.3	Missense
			CCND3	c.541_544dup	p.(Ser182*)	12.1	Nonsense
			EP300	c.865A > G	p.(Met289Val)	35.9	Missense
			ID3	c.203A > G	p.(Glu68Gly)	16.3	Missense
			ID3	c.243G > C	p.(Gln81His)	15.5	Missense
			ID3	c.305C > T	p.(Ala102Val)	17.2	Missense
46	FFPE	DLBCL	*TP53*	c.919 + 1G > T	p.(?)	22.7	Splice_donor_ + 1
			TP53	c.455_456delinsT	p.(Pro152Leufs*18)	11	Frameshift
			TP53	c.743G > A	p.(Arg248Gln)	8.3	Missense
			PRDM1	c.695G > A	p.(Ser232Asn)	20.8	Missense
			SOCS1	c.248_280del	p.(Pro83_Leu-93del)	5.7	Inframe
			SOCS1	c.120_122delinsACG	p.(Pro41Arg)	8.4	Missense
			SOCS1	c.299C > T	p.(Thr100Ile)	7	Missense
			SOCS1	c.140C > T	p.(Ala47Val)	9.3	Missense
			SOCS1	c.347G > A	p.(Ser116Asn)	5.6	Missense
			SOCS1	c.233G > A	p.(Gly78Glu)	7.9	Missense
			CD58	c.66C > A	p.(Cys22*)	8.4	Nonsense
			CIITA	c.3344G > A	p.(Ser1115Asn)	15.2	Missense
			EP300	c.631G > A	p.(Gly211Ser)	6.4	Missense
47	FFPE	DLBCL	*PIM1*	c.72G > C	p.(Lys24Asn)	11.1	Missense
			PIM1	c.61C > T	p.(His21Tyr)	30.1	Missense
			PIM1	c.4C > G	p.(Leu2Val)	30.1	Missense
			CCND3	c.568dupC	p.(Arg190Profs*)	30.5	Frameshift
			EP300	c.631G > A	p.(Gly211Ser)	41.7	Missense
			FOXO1	c.290C > G	p.(Ala97Gly)	6.2	Missense
			KMT2D	c.14782C > A	p.(Pro4928Thr)	49.1	Missense
			MYD88	c.818T > C	p.(Leu273Pro)	32.8	Missense

Table 1. Mutational analysis of formalin-fixed paraffin-embedded tissue samples.

We found that the median number of mutations detected in ctDNA was higher among the stage III and IV patients than the early-stage patients (6 vs. 2.5 mutations, $p = 0.05$) and in the patients with bulky disease (7 vs. 3 mutations, $p = 0.04$) (Supplementary Fig. 4).

For the 51 variants detected only in ctDNA (12 patients, 9 DLBCL and 3 FL), the median VAF was lower than those that were also identified in the FFPE samples (2.5 vs. 9.1%). Interestingly, there were 5 patients harboring
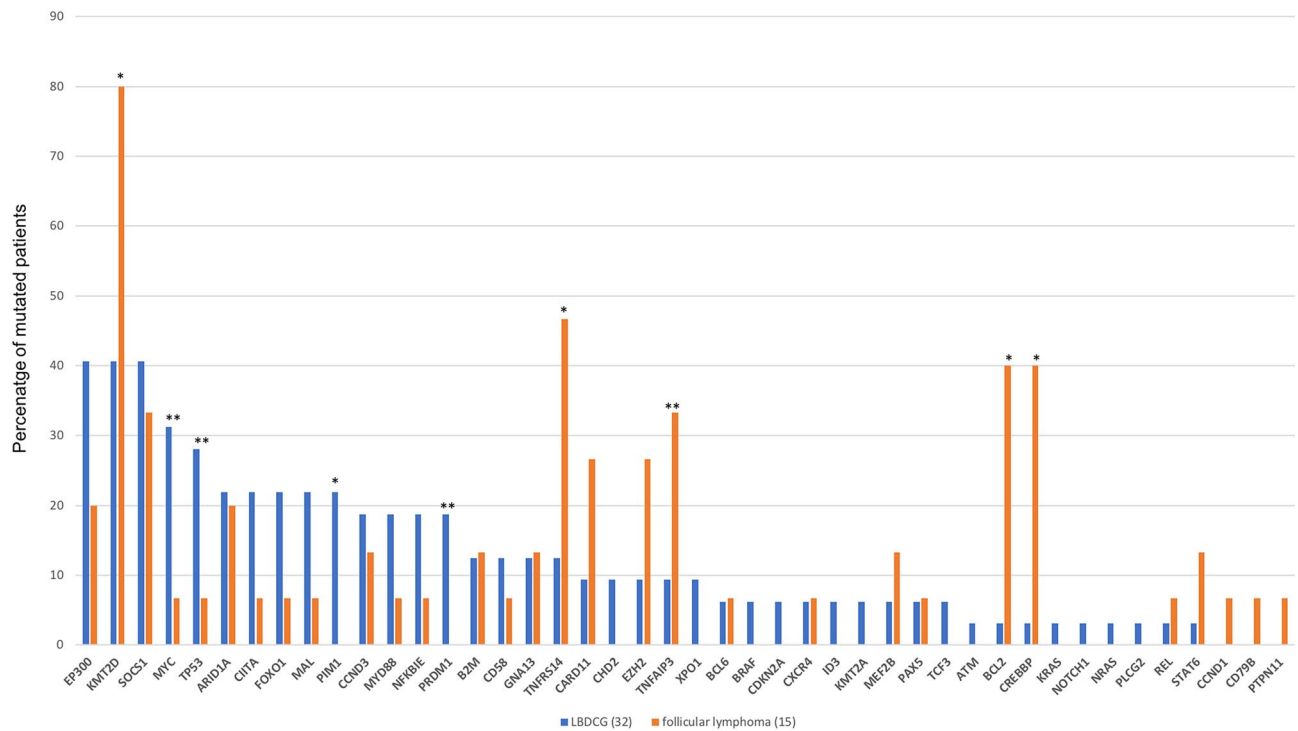
Figure 1. Frequencies of mutated genes in the cohort (n = 47). Significant differences between follicular lymphoma and diffuse large B-cell lymphoma ($p < 0.05^*$) ($p < 0.1^{**}$).

more than 2 mutations in the ctDNA samples that were not detected in their matched FFPE samples (UPN of 19, 24, 28, 43, 46). Four of these patients presented bulky disease and were stage III at diagnosis.

The mean baseline ctDNA concentration was 42.803 hGE/mL (range 0–635.152) at diagnosis (Supplementary Table 5). Higher ctDNA levels were also correlated with bulky disease (4.369 vs. 15.852 hGE/mL, $p = 0.016$). There were no differences based on the stage (Supplementary Fig. 4).

Discussion

The optimal assessment of NHL includes morphological and immunophenotypic studies and chromosome and molecular analyses. NGS techniques provide relevant additional data for diagnosis, prognosis, and therapeutic management. Although NGS data on lymphomas require further validation before being implemented in daily practice, their clinical application is just around the corner. Numerous studies over the past decade have analyzed hundreds of tumor genomes of DLBCLs and FLs to better understand the molecular pathogenesis of these diseases[3–5,11–13]. In this study, we validated an NGS panel for DLBCL and FL in FFPE and ctDNA samples at diagnosis.

As one might expect of a cancer derived from cells and an environment of combinatorial diversity, heterogeneity is a defining characteristic of FL and DLBCL. We detected 372 pathogenic variants in 54 genes in 47 of the FFPE samples (93 in FL [median of 7.4 variants] and 279 in DLBCL [median of 8.6 variants]). In our study, 83% of the patients with FL presented BCL2 rearrangements, and the variants most frequently detected were *KMT2D, TNFRSF14, CREBBP, BCL2, TNFAIP3, SOCS1, CARD11,* and *EZH2*. Eighty-seven percent FL samples presented mutations in epigenetic modifier genes. These results agree with those from previous studies in the literature[14–16]. Twenty-eight percent of the patients with DLBCL presented *BCL6* rearrangement, 25% presented c-*MYC* rearrangement, and 16% presented *BCL2* rearrangement, with 16% presenting double-hit lymphomas. However, c-MYC rearrangement might be over-represented in our cohort compared with that described in the literature, given that the cases were not selected consecutively[17,18]. The variants most frequently detected were present in *SOCS1, KMT2D, EP300, c-MYC* and *TP53*, and 68% of the samples presented mutations in epigenetic modifier genes.

The mutational profile of DLBCL differs depending on the cell of origin. While GCB DLBCL is characterized by frequent translocations of *BCL2* and mutations of the epigenetic modifiers *CREBBP* and *EZH2*, these abnormalities are rare in ABC DLBCL. In contrast, mutations in genes encoding proteins implicated in B-cell receptor signaling and the nuclear factor kappa-light-chain-enhancer of activated B cells pathway (such as *CD79b* and *MYD88*) and genes involved in the regulation of the cell cycle (such as *CDKN2A*) contribute to the molecular pathogenesis of ABC DLBCL[5,19,20]. Our study found differentiated genetic profiles according to the GCB and ABC subtype. *BCL2* rearrangement, *EZH2, PIM1, CD58,* and *NFKBIE* were present only in the GCB subtype while *XPO1* was present only in ABC. Also, different profiles were observed in those patients classified as having high-grade lymphomas, where mutations in *EZH2* and *MAL* were more frequent in high-grade double-hit lymphomas and mutations in *TP53, TCF3* and *CD58* in high-grade NOS lymphomas. More extensive and complex panels

UPN	Diagnosis	Gene	c.DNA	Protein	FPPE VAF	cfDNA VAF	Consenquence
4	FL	BCL2	c.140G > A	p.(Gly47Asp)	28	2	Missense
		BCL2	c.175C > A	p.(Pro59Thr)	28.4	2.2	Missense
		BCL2	c.151T > G	p.(Ser51Ala)	28.8	2.6	Missense
		EZH2	c.1922A > T	p.(Tyr641Phe)	30.5	4	Missense
		STAT6	c.1256A > G	p.(Asp419Gly)	5.3	–	Missense
		CD58	c.254C > G	p.(Thr85Ser)	47.9	49.4	Missense
		MEF2B	c.170A > G	p.(Tyr57Cys)	16.8	4	Missense
		KMT2D	c.3931A > T	p.(Arg1311*)	42.2	7.6	Nonsense
		KMT2D	c.13893 + 2T > A	p.(?)	22.4	4.5	Splice_donor_ + 2
		TNFRSF14	c.139T > A	p.Tyr47Asn	–	1.1	Missense
9	DLBCL	CDKN2A	c.329G > A	p.(Trp110*)	6.9	–	Nonsense
		KMT2D	c.14450T > G	p.(Val4817Gly)	51.3	50	Missense
11	FL	BCL2	c.392C > G	p.(Ala131Gly)	11.7	2.5	Missense
		BCL2	c.517A > G	p.(Ile173Val)	9.4	1.6	Missense
		CARD11	c.748T > C	p.(Ser250Pro)	14.5	–	Missense
		CREBBP	c.4394A > G	p.(Tyr1465Cys)	32.1	4.5	Missense
		CCND3	c.531_532delCTinsTG	p.(Ser178Ala)	99.7	97.3	Missense
		KMT2D	c.6664C > T	p.(Gln2222*)	12.2	–	Nonsense
		KMT2D	c.5335A > T	p.(Lys1779*)	14.5	–	Nonsense
		CXCR4	c.1025C > G	p.(Ser342*)	–	1.5	Nonsense
		KMT2A	c.6664C > T	p.(Gln2222*)	–	1	Nonsense
13	DLBCL	TP53	c.490A > T	p.(Lys164*)	31.8	–	Nonsense
		B2M	c.2T > G	p.(Met1?)	52.4	–	No-start
		PIM1	c.676G > A	p.(Glu226Lys)	35.5	–	Missense
		PIM1	c.370C > T	p.(Pro124Ser)	62.1	–	Missense
		PIM1	c.434G > A	p.(Arg145His)	6.7	–	Missense
		PIM1	c.202C > T	p.(His68Tyr)	23.3	–	Missense
		SOCS1	c.8C > T	p.(Ala3Val)	30.3	–	Missense
		FOXO1	c.435del	p.(Ala146Argfs*187)	17.9	–	Frameshift
		MEF2B	c.78C > G	p.(Phe26Leu)	52.5	–	Missense
		MYD88	c.818T > C	p.(Leu273Pro)	33	2.1	Missense
14	FL	BCL2	c.191A > C	p.(Asp64Ala)	12	–	Missense
		BCL2	c.93T > C	p.(Asp31Glu)	9	–	Missense
		TNFAIP3	c.2014G > T	p.(Gly672*)	8.8	–	Nonsense
		TNFRSF14	c.463delA	p.(Thr155Profs*)	13.3	–	Frameshift
		KMT2D	c.172 + 2T > C	p.(?)	12.4	–	Splice_donor_ + 2
15	FL	CREBBP	c.4382T > C	p.(Leu1461Pro)	29.1	5.6	Missense
		KMT2D	c.16489_16491delATC	p.(Ile5479del)	32.4	4	Inframe
		KMT2D	c.9019delG	p.(Glu3007Lysfs*22)	26.1	4.5	Frameshift
Continued							

UPN	Diagnosis	Gene	c.DNA	Protein	FPPE VAF	cfDNA VAF	Consenquence
18	DLBCL	NFKBIE	c.668_671delTGCTinsAGCG	p.(Leu223_Leu224delins*Arg)	20.6	9.8	Missense
		SOCS1	c.7G > A	p.(Ala3Thr)	14	6.4	Missense
		SOCS1	c.374G > C	p.(Ser125Thr)	19.1	7.7	Missense
		SOCS1	c.407A > C	p.(His136Pro)	18	7.2	Missense
		SOCS1	c.428G > A	p.(Ser143Asn)	13.5	7.2	Missense
		SOCS1	c.398delG	p.(Gly133Alafs*72)	10.6	7.2	Frameshift
		SOCS1	c.55C > T	p.(Pro19Ser)	14.6	8.3	Missense
		SOCS1	c.412G > C	p.(Asp138His)	15.9	7	Missense
		SOCS1	c.391C > G	p.(Gln131Glu)	16.4	7.3	Missense
		SOCS1	c.391C > T	p.(Gln131*)	16.4	6.3	Nonsense
		SOCS1	c.318C > G	p.(Ser106Arg)	8.7	6.2	Missense
		SOCS1	c.528G > C	p.(Glu176Asp)	10.6	6.3	Missense
		ARID1A	c.5012G > A	p.(Arg1671Gln)	18.4	8.3	Missense
		MYC	c.482C > T	p.(Ser161Leu)	10.9	5.2	Missense
		MYC	c.218_219delCCinsTA	p.(Thr73Ile)	11.2	3.6	Missense
		MYC	c.1164C > G	p.(Ser388Arg)	18.7	3.5	Missense
		MYC	c.557G > C	p.(Cys186Ser)	14.5	–	Missense
		MYC	c.895G > C	p.(Ala299Pro)	18.6	3.8	Missense
		MYC	c.910_999dup	p.(Lys304_Asp333dup)	40.6	–	Inframe
		MYC	c.654C > G	p.(Ser218Arg)	15.1	3.5	Missense
		MYC	c.785C > T	p.(Thr262Ile)	15.8	2.9	Missense
		MYC	c.68_71delinsGCAG	p.(Phe23Cys)	9.7	2.4	Missense
		MYC	c.63C > G	p.(Ser21Arg)	9.6	2.5	Missense
		MYC	c.162G > C	p.(Glu54Asp)	9	3.1	Missense
		MYC	c.144G > A	p.(Asp48Glu)	7.9	3.1	Missense
		MYC	c.358_361delinsTTGT	p.(Asp120Leu)	11.8	–	Missense
		REL	c.868A > G	p.(Lys290Glu)	–	5.7	Missense
		MYC	c.361C > T	p.(Asp121Tyr)	–	2.7	Missense
19	FL	CARD11	c.1202A > T	p.(Asp401Val)	19.9	13.1	Missense
		SOCS1	c.4G > T	p.(Val2Leu)	18.1	11.6	Missense
		SOCS1	c.14A > G	p.(Asn5Ser)	20	46.6	Missense
		SOCS1	c.134_139dupTCCCGG	p.(Val45_Pro46dup)	43.5	11.3	Inframe
		TNFAIP3	c.1035C > A	p.(Tyr345*)	28.9	14.8	Nonsense
		TNFRSF14	c.70G > T	p.(Val24Leu)	28.1	16.6	Missense
		B2M	c.1A > G	p.(Met1?)	–	6.9	No-start
		B2M	c.346 + 2T > A	p.(?)	–	1.5	Splice_donor_ + 2
		B2M	c.35T > C	p.(Leu12Pro)	–	3.3	Missense
		CREBBP	c.4406T > C	p.(Leu1469Pro)	–	1.9	Missense
		KMT2D	c.10867C > T	p.(Gln3623*)	–	2.3	Nonsense
		MYD88	c.719T > C	p.(Met240Thr)	–	4.7	Missense
Continued							

UPN	Diagnosis	Gene	c.DNA	Protein	FPPE VAF	cfDNA VAF	Consequence
21	DLBCL	B2M	c.176T > A	p.(Leu59*)	80.3	16.4	Nonsense
		ATM	c.8284C > T	p.(Gln2762*)	54.3	16.5	Nonsense
		NFKBIE	c.1147_1153delCAACCAC	p.(Gln383Serfs*46)	31.3	6.5	Frameshift
		NFKBIE	c.759_762delTTAC	p.(Tyr254Serfs*13)	33.4	.6	Frameshift
		PRDM1	c.75delG	p.(Arg25Serfs*13)	41.8	49.5	Frameshift
		SOCS1	c.358_361delGCCTinsCC	p.(Ala120Profs*?)	39.7	7.6	Frameshift
		SOCS1	c.434_437delACTG	p.(Asp145Alafs*59)	39.4	5.4	Frameshift
		TNFAIP3	c.2350C > T	p.(Gln784*)	64	8.7	Nonsense
		TNFAIP3	C.295 + 2T > C	p.(?)	67.5	6.1	Splice_donor_+2
		CDKN2A	c.394G > A	p.(Ala132Thr)	32.4	1.1	Missense
		CIITA	c.34_46delTACCTGTCAGAGC	p.(Tyr12Profs*15)	36.4	7	Frameshift
		CIITA	c.1652delG	p.(Gly551Alafs*7)	35.9	–	Frameshift
		CIITA	c.3262G > A	p.(Gly1880Arg)	5.3	–	Missense
		FOXO1	c.61C > T	p.(Arg21Cys)	39.4	6.1	Missense
		GNA13	c.179A > G	p.(Asp60Gly)	81.6	15.2	Missense
		MYC	c.25A > G	p.(Asn9Asp)	43.3	–	Missense
		CIITA	c.3317 + 2T > C	p.(?)	–	1.2	Splice_donor_+2
		KMT2A	c.137T > G	p.(Val46Gly)	–	1.4	Missense
22	FL	BCL6	c.1752C > A	p.(Asn584Lys)	27.8	4.2	Missense
		CCND3	c.613G > A	p.(Asp205Asn)	5.3	–	Missense
		GNA13	c.243_244del	p.(Glu82Glyfs*19)	5	–	Frameshift
		MYC	c.154_156del	p.(Lys51Del)	5.4	–	Inframe
23	DLBCL	TP53	c.839G > A	p.(Arg280Lys)	28.2	8.7	Missense
		NFKBIE	c.759_762delTTAC	p.(Tyr254Serfs*13)	12.3	–	Frameshift
		CCND3	c.626T > C	p.(Ile209Thr)	23.1	9.1	Missense
		CCND3	c.604A > C	p.(Thr202Pro)	24.4	9.1	Missense
24	DLBCL	BCL2	c.17G > A	p.(Arg6Lys)	5.9	–	Missense
		B2M	c.16G > C	p.(Ala6Pro)	49.4	42.2	Missense
		TNFRSF14	c.49_50delinsCAG	p.(Lys17Glnfs*60)	8.9	–	Frameshift
		CXCR4	c.1025C > A	p.(Ser342*)	5	–	Nonsense
		MAL	c.59C > T	p.(Thr20Ile)	46.6	49.3	Missense
		B2M	c.3G > C	p.(Met1?)	–	8.5	No-start
		CREBBP	c.4829_4830del	p.(Pro1610Hisfs*11)	–	2.1	Frameshift
		NRAS	c.38G > A	p.(Gly13Asp)	–	1	Missense
		PAX5	c.979T > C	p.(Tyr327His)	–	2.1	Missense
		PIM1	c.117G > T	p.(Gln39His)	–	5.4	Missense
26	DLBCL	TP53	c.725G > T	p.(Cys242Phe)	76.8	91.4	Missense
		TCF3	c.1688G > A	p.(Arg563His)	17.1	28.1	Missense
28	DLBCL	CHD2	c.1281G > A	p.(Trp427*)	18.6	30.6	Nonsense
		MAL	c.98T > G	p.(Phe33Cys)	6	–	Missense
		MYC	c.1148A > G	p.(Asn383Ser)	22.9	–	Missense
		MYC	c.490C > T	p.(Pro164Ser)	20.6	–	Missense
		MYD88	c.818T > C	p.(Leu273Pro)	18.4	33.8	Missense
		MYD88	c.797C > T	p.(Pro266Leu)	19.1	33.4	Missense
		PIM1	c.550C > T	p.(Leu184Phe)	–	5.7	Missense
		PRDM1	c.2182G > T	p.(Glu728*)	–	2.9	Nonsense
		EP300	c.3754A > G	p.(Arg1252Gly)	–	1.4	Missense
31	DLBCL	BRAF	c.1780G > A	p.(Asp594Asn)	29.4	–	Missense
		EZH2	c.1921T > A	p.(Tyr641Asn)	34.4	–	Missense
		STAT6	c.1255G > T	p.(Asp419Tyr)	52.4	1.1	Missense
		PIM1	c.409G > T	p.(Gly137*)	34	–	Nonsense
		PIM1	c.285G > C	p.(Lys95Asn)	31.8	–	Missense
		SOCS1	c.220C > G	p.(Leu74Val)	30.7	1.4	Missense
		SOCS1	c.178T > C	p.(Ser60Pro)	28.6	1.4	Missense
		KMT2D	c.14843C > G	p.(Ser4948*)	40.3	2	Nonsense
		KMT2D	c.7586delG	p.(Gly2529Alafs*14)	22.7	–	Frameshift
Continued							

UPN	Diagnosis	Gene	c.DNA	Protein	FPPE VAF	cfDNA VAF	Consenquence
32	DLBCL	NOTCH1	c.7541_7542delCT	p.(Pro2514Argfs*4)	34.7	1.3	Frameshift
		ARID1A	c.4540_4543delACGGinsCCGT	p.(Thr1514_Gly1515delinsProCys)	10.1	–	Missense
		MAL	c.98T>C	p.(Phe33Cys)	6	–	Missense
		KMT2D	c.2886_2887delTGinsCA	p.(Ala963Thr)	11.5	–	Missense
		MEF2B	c.928T>G	p.(Ser310Ala)	–	2	Missense
33	DLBCL	NFKBIE	c.98C>T	p.(Ser33Phe)	63	52.6	Missense
		CCND3	c.544_554dupTCCAGCCCAGC	p.(Lys187Alafs*?)	63.7	1.5	Frameshift
		CXCR4	c.1012C>T	p.(Arg338*)	45.1	–	Nonsense
		EP300	c.6316delA	p.(Met2106Cysfs*28)	11.1	–	Frameshift
		EP300	c.6329_6330insT	p.(Gln2110Hisfs*100)	9.9	–	Frameshift
		MAL	c.98T>G	p.(Phe33Cys)	8.7	–	Missense
		KMT2D	c.2886_2887delTGinsCA	p.(Ala963Thr)	13.6	–	Missense
		MYC	c.77_78delACinsGT	p.(Asn26Ser)	67.4	1	Missense
		MYC	c.63C>G	p.(Ser21Arg)	67.6	1.3	Missense
		MYC	c.214C>A	p.(Pro72Thr)	68.3	–	Missense
		MYC	c.175G>A	p.(Ala59Thr)	67.7	–	Missense
35	DLBCL	NRAS	c.38G>T	p.(Gly13Val)	6.1	–	Missense
		EZH2	c.2060C>T	p.(Ala687Val)	35.4	12.5	Missense
		REL	c.392A>G	p.(Asn131Ser)	57.9	4.6	Missense
		ARID1A	c.2668A>G	p.(Met890Val)	31.8	49.4	Missense
		ARID1A	c.4540_4543delinsCCGT	p.(Thr1514_Gly1515delinsProCys)	9.7	–	Missense
		EP300	c.6329_6330insT	p.(Gln2110Hisfs*100)	22.2	–	Frameshift
		EP300	c.6323A>T	p.(Gln2108Leu)	22.7	–	Missense
		EP300	c.6316del	p.(Met2106Cysfs*28)	25.2	–	Frameshift
		FOXO1	c.62G>T	p.(Arg21Leu)	28.8	2.2	Missense
		FOXO1	c.118T>C	p.(Ser40Pro)	30.8	2.5	Missense
		MAL	c.98T>G	p.(Phe33Cys)	13.5	–	Missense
		MEF2B	c.32T>C	p.(Ile11Thr)	27.5	4.5	Missense
		KMT2D	c.6221_6224dupACAA	p.(Val2076Glnfs*7)	23.9	–	Frameshift
		KMT2D	c.12204_12207delACTC	p.(Ser4070Glyfs*25)	37.6	9.6	Frameshift
		KMT2D	c.2876delA	p.(Tyr959Serfs*41)	10.2	–	Frameshift
		KMT2D	c.2886_2887delTGinsCA	p.(Ala963Thr)	22.6	–	Missense
		KMT2D	c.4279T>G	p.(Cys1427Gly)	–	2.6	Missense
37	DLBCL	BCL6	c.1760C>G	p.(Ala587Gly)	29.2	–	Missense
		PLCG2	c.2009T>G	p.(Leu670Arg)	7.1	–	Missense
		POT1	c.1315_1317del	p.(Ala439del)	6.4	–	Inframe
		SOCS1	c.195_206delGCGCATCACGCG	p.(Arg66_Arg69del)	34.7	–	Inframe
		ARID1A	c.4540_4543delACGGinsCCGT	p.(Thr1514_Gly1515delinsProCys)	16.6	–	Missense
		EP300	c.6329_6330insT	p.(Gln2110Hisfs*100)	18.2	–	Frameshift
		EP300	c.6316delA	p.(Met2106Cysfs*28)	18.7	–	Frameshift
		EP300	c.6323A>T	p.(Gln2108Leu)	19.1	–	Missense
		FOXO1	c.1478G>C	p.(Gly493Ala)	7.2	–	Missense
		MAL	c.98T>G	p.(Phe33Cys)	19.3	–	Missense
		KMT2D	c.13753_13757delinsTTGAC	p.(Val4585_Asn4586delinsLeuThr)	5.4	–	Missense
		KMT2D	c.2886_2887delTGinsCA	p.(Ala963Thr)	37.6	–	Missense
38	DLBCL	B2M	c.2T>A	p.(Met1?)	31.9	–	No-start
		NFKBIE	c.1108+2T>A	p.(?)	25.1	–	Splice_donor_+2
		NFKBIE	c.759_762delTTAC	p.(Tyr254Serfs*13)	24.6	–	Frameshift
		PRDM1	c.1142A>G	p.(Tyr381Cys)	49.5	48.3	Missense
		TNFRSF14	c.632T>A	p.(Val211Asp)	32	–	Missense
		CD58	c.70+2T>G	p.(?)	34.2	–	Splice_donor_+2
40	DLBCL	–	–	–	–	–	–
Continued							

UPN	Diagnosis	Gene	c.DNA	Protein	FPPE VAF	cfDNA VAF	Consenquence
43	DLBCL	TNFAIP3	c.1939A > C	p.(Thr647Pro)	50.2	51.8	Missense
		SOCS1	c.529C > G	p.(Leu177Val)	–	2.1	Missense
		SOCS1	c.174C > G	p.(Phe58Leu)	–	2.3	Missense
		SOCS1	c.598C > G	p.(Leu200Val)	–	1.8	Missense
		SOCS1	c.614G > C	p.(Ser205Thr)	–	1.9	Missense
		SOCS1	c.46_49delinsTCAA	p.(Ala16_Ala17delins-SerThr)	–	2.4	Missense
		SOCS1	c.22G > C	p.(Ala8Pro)	–	2.2	Missense
		SOCS1	c.4G > C	p.(Val2Leu)	–	2.2	Missense
		MEF2B	c.928T > G	p.(Ser310Ala)	–	1.7	Missense
44	DLBCL	TP53	c.743G > A	p.(Arg248Gln)	12.6	28.7	Missense
		TP53	c.919 + 1G > A	p.(?)	12.6	25.8	Splice_donor_ + 1
		PRDM1	c.626_627del	p.(His209Leufs*25)	19.1	46	Frameshift
		ARID1A	c.60_62del	p.(Pro21del)	9.4	–	Inframe
		MYD88	c.719T > C	p.(Met240Thr)	35	70.9	Missense
45	DLBCL	TP53	c.404G > T	p.(Cys135Phe)	7.2	1	Missense
		KRAS	c.38G > A	p.(Gly13Asp)	13.5	14.7	Missense
		CARD11	c.383C > T	p.(Thr128Met)	18.2	17.5	Missense
		PIM1	c.447G > T	p.(Trp149Cys)	15.9	14.7	Missense
		PIM1	c.451G > C	p.(Val151Leu)	16.4	15.3	Missense
		PIM1	c.242C > T	p.(Pro81Leu)	14.6	15.3	Missense
		SOCS1	c.430C > T	p.(Phe144Leu)	14.4	13.9	Missense
		SOCS1	c.534C > G	p.(Cys178Trp)	15.3	12.4	Missense
		CCND3	c.541_544dup	p.(Ser182*)	12.1	18.1	Nonsense
		EP300	c.865A > G	p.(Met289Val)	35.9	48.9	Missense
		ID3	c.203A > G	p.(Glu68Gly)	16.3	11.3	Missense
		ID3	c.243G > C	p.(Gln81His)	15.5	10.3	Missense
		ID3	c.305C > T	p.(Ala102Val)	17.2	13.7	Missense
		TP53	c.800G > T	p.(Arg267Leu)	–	2.3	Missense
		MEF2B	c.928T > G	p.(Ser310Ala)	–	1.6	Missense
Continued							

UPN	Diagnosis	Gene	c.DNA	Protein	FPPE VAF	cfDNA VAF	Consenquence
46	DLBCL	TP53	c.919 + 1G > T	p.(?)	22.7	–	Splice_donor_ + 1
		TP53	c.455_456delinsT	p.(Pro152Leufs*18)	11	–	Frameshift
		TP53	c.743G > A	p.(Arg248Gln)	8.3	–	Missense
		PRDM1	c.695G > A	p.(Ser232Asn)	20.8	–	Missense
		SOCS1	c.248_280del	p.(Pro83_Leu93del)	5.7	21.8	Inframe
		SOCS1	c.120_122delinsACG	p.(Pro41Arg)	8.4	21.4	Missense
		SOCS1	c.299C > T	p.(Thr100Ile)	7	20.7	Missense
		SOCS1	c.140C > T	p.(Ala47Val)	9.3	20.6	Missense
		SOCS1	c.347G > A	p.(Ser116Asn)	5.6	18.5	Missense
		SOCS1	c.233G > A	p.(Gly78Glu)	7.9	25.2	Missense
		CD58	c.66C > A	p.(Cys22*)	8.4	20.1	Nonsense
		CIITA	c.3344G > A	p.(Ser1115Asn)	15.2	–	Missense
		EP300	c.631G > A	p.(Gly211Ser)	6.4	49.2	Missense
		TP53	c.845G > A	p.(Arg282Gln)	–	24	Missense
		SOCS1	c.522_523delinsAT	p.(Gln175*)	–	16.3	Nonsense
		SOCS1	c.435_*98del	p.(Asp145_*212del)	–	1.9	Frameshift
		SOCS1	c.385_388delinsTATA	p.(His129Phe130insTyrIle)	–	8.3	Missense
		SOCS1	c.466_469delinsACGC	p.(Ala156_Ala157delinsThrPro)	–	14.6	Missense
		SOCS1	c.37G > C	p.(Val13Leu)	–	12.7	Missense
		SOCS1	c.363_364delinsAC	p.(Gly122Arg)	–	16.2	Missense
		SOCS1	c.528_531delinsCCTA	p.(Gly176Asp)	–	15.7	Missense
		SOCS1	c.454G > A	p.(Glu152Lys)	–	13	Missense
		SOCS1	c.574G > A	p.(Ala192Thr)	–	11.4	Missense
		SOCS1	c.374G > A	p.(Ser125Asn)	–	11.5	Missense
		SOCS1	c.484C > A	p.(Ser162Met)	–	15.5	Missense
		SOCS1	c.46_49delinsACAC	p.(Ala16_Ala17delinsThrPro)	–	13.4	Missense
		SOCS1	c.429C > G	p.(Ser143Arg)	–	9	Missense
		SOCS1	c.614_617delinsATTA	p.(Ser205_206delinsAsnTyr)	–	6.5	Missense
		SOCS1	c.541C > T	p.(Arg181Cys)	–	1.7	Missense
		SOCS1	c.622C > T	p.(Pro208Ser)	–	5.8	Missense
47	DLBCL	PIM1	c.72G > C	p.(Lys24Asn)	11.1	–	Missense
		PIM1	c.61C > T	p.(His21Tyr)	30.1	–	Missense
		PIM1	c.4C > G	p.(Leu2Val)	30.1	–	Missense
		CCND3	c.568dupC	p.(Arg190Profs*)	30.5	–	Frameshift
		FOXO1	c.290C > G	p.(Ala97Gly)	6.2	–	Missense
		KMT2D	c.14782C > A	p.(Pro4928Thr)	49.1	50.9	Missense
		MYD88	c.818T > C	p.(Leu273Pro)	32.8	–	Missense

Table 2. Mutational analysis of circulating tumor DNA and formalin-fixed paraffin-embedded tissue samples.

than the ones used in this study are needed to adequately perform the molecular classification[4,5]. However, it is not entirely clear which strategy will be the most appropriate for clinical practice: large panels of genes, exomes, or whole genomes. What is clear is that, by including genetic analyses of lymphomas, we will be able to reach a much more certain diagnosis by establishing genetic risk profiles, as is the case for other hematological neoplasms such as acute leukemia, thus bringing us closer to more personalized care.

Undoubtedly, the paradigm of lymphoma diagnosis has changed since the incorporation of ctDNA. In addition to the genetic studies already performed on solid biopsies, we have the option of performing these genetic studies on non-invasive samples such as liquid biopsies. This type of sample has been increasingly used for a variety of applications in oncology, including diagnosis, prognosis, and the identification of therapeutic targets[10]. In addition, ctDNA provides information on tumor burden and the dynamics of treatment response[21,22]. Our study assessed the utility of liquid biopsy in B-cell lymphomas in routine clinical practice through the validation of a commercial gene panel in patients with lymphoma at diagnosis. Including 26 patients, we showed that the use of liquid biopsies is feasible in routine clinical practice for DLBCL and FL. Specifically, ctDNA was detectable in 92% of the patients, and in 96% of the cases we were able to identify at least 1 alteration in ctDNA that was identical to the FFPE at diagnosis, indicating the potentially universal applicability of ctDNA. When explaining the reasons for the differences found between FFPE and plasma samples, we believe that they have to do mainly with the quality of the sample and the characteristics of the tumor. In our study, some mutations present in FFPE
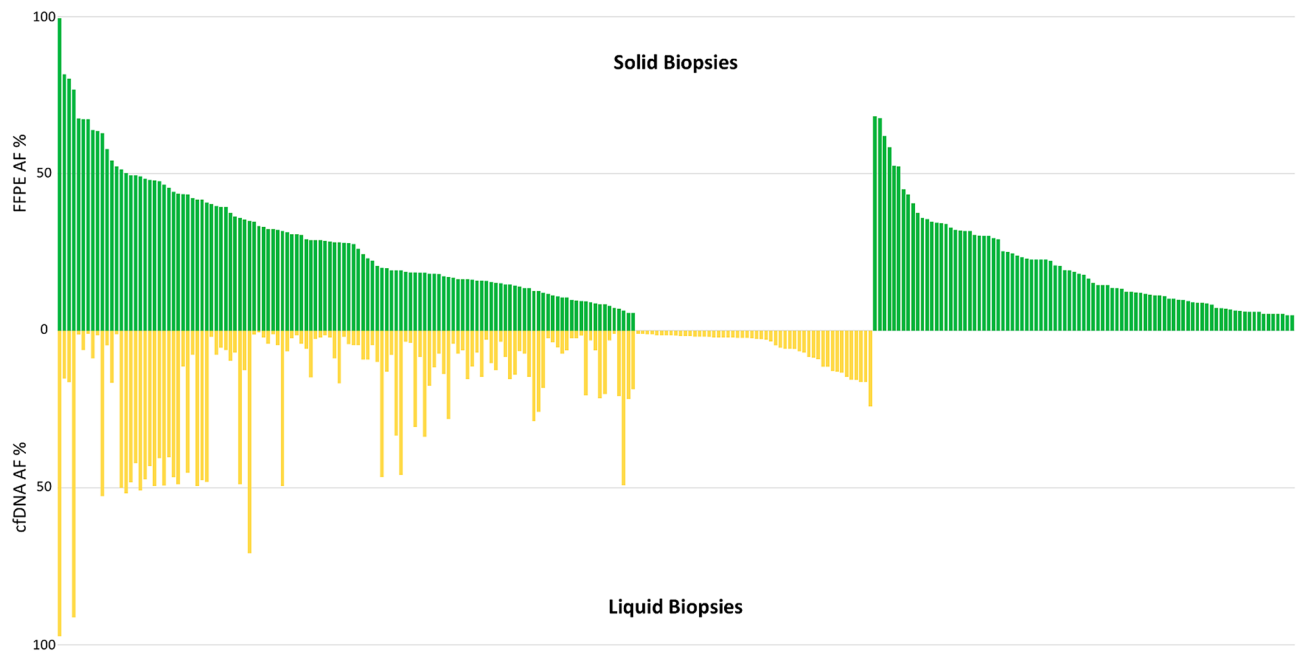
Figure 2. Concordance of mutations between solid and liquid biopsies. Allele frequencies (AF) are provided for the solid tissue biopsies (green bar plot) and for the liquid biopsies (yellow bar plot).

were not detected in plasma samples, probably due to a low total amount of plasma used (< 5 ml) and, therefore, the quantity of ctDNA obtained was insufficient in a few cases. It is also true that localized diseases or those with a low tumor burden could release a small amount of ctDNA into plasma, so we have learned that a volume of at least 10 ml of plasma should be used for optimal analysis. On the contrary, mutations detected in plasma and not in FFPE may be due to the heterogeneity of the tumor, taking into account that we are analyzing only a small fragment of tissue and not the entire tumor, so not all clones would be represented. Different is with the liquid biopsy, where from all the existing lesions DNA is being released into the bloodstream.

Although various studies have shown the usefulness of these techniques in specialized centers[8,23], particularly in clinical trials, the applicability of this technique in routine clinical practice has rarely been reported. Numerous reviews on the subject have listed the potential benefits of liquid biopsy[20,23,24], both in the diagnosis and follow-up of NHL; however, the standardization of these tools is not yet a reality.

As previously described, we found a correlation between advanced stage and bulky disease and the number of ctDNA mutations[23,25]. Our analysis also found mutations in the liquid biopsy from patients at localized stages and with low tumor burden, which means that this tool can also be used in this patient group. As previously mentioned, not less than 10 ml must be used, in order to obtain a greater amount of DNA and thus be able to identify all mutations. Moreover, we found that patients with bulky disease had more mutations found only in ctDNA (i.e., not in the FFPE samples), which could indicate that ctDNA samples better represent the tumor's genetic variability than standard biopsies. The possibility of finding a different mutational profile when comparing liquid biopsies and FFPE samples from the same patient has already been demonstrated by Sherer et al.[8], who identified transformed FL in a liquid biopsy sample from a patient with low-grade FL, which had not been previously identified in the paraffin biopsy. Liquid biopsy could therefore be a useful strategy when looking for specific mutations for target molecules, especially in patients with bulky disease.

In conclusion, our results confirm that the NGS techniques provides additional relevant data at the time of diagnosis, not only in FFPE samples but also in ctDNA, both complementary, and also the liquid biopsy provides the extra of how easy it is to obtain. These ctDNA samples are useful not only in patients with advanced stages and large masses, but also provide information in patients with localized disease and low tumor burden. Although there is still a lack of standardization today, it is important that we begin to incorporate these techniques into clinical practice, given the valuable information they can offer us about the lymphoma.

References

1. Bastos-Oreiro, M. *et al.* RELINF: Prospective epidemiological registry of lymphoid neoplasms in Spain. A project from the GEL-TAMO group. *Ann. Hematol.* **99**, 799–808 (2020).
2. Lester, J. F. *et al.* The clinical impact of expert pathological review on lymphoma management: A regional experience. *Br. J. Haematol.* **123**, 463–468 (2003).

3. Pastore, A. *et al.* Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: A retrospective analysis of a prospective clinical trial and validation in a population-based registry. *Lancet Oncol.* **16**, 1111–1122 (2015).
4. Chapuy, B. *et al.* Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nat. Med.* **24**, 679–690 (2018).
5. Schmitz, R. *et al.* Genetics and pathogenesis of diffuse large B-cell lymphoma. *N. Engl. J. Med.* **378**, 1396–1407 (2018).
6. Spina, V. *et al.* Circulating tumor DNA reveals genetics, clonal evolution, and residual disease in classical Hodgkin lymphoma. *Blood* **131**, 2413–2425 (2018).
7. Cirillo, M., Craig, A. F. M., Borchmann, S. & Kurtz, D. M. Liquid biopsy in lymphoma: Molecular methods and clinical applications. *Cancer Treat. Rev.* **91**, 102106 (2020).
8. Scherer, F. *et al.* Distinct biological subtypes and patterns of genome evolution in lymphoma revealed by circulating tumor DNA. *Sci. Transl. Med.* **8**, 364ra155 (2016).
9. Melani, C., Wilson, W. H. & Roschewski, M. Liquid biopsy in non-Hodgkin's lymphoma. *Hematol. Oncol.* **37**(Suppl 1), 70–74 (2019).
10. Lenz, G. *et al.* Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 13520–13525 (2008).
11. Zhou, M. *et al.* Discovery and validation of immune-associated long non-coding RNA biomarkers associated with clinically molecular subtype and prognosis in diffuse large B cell lymphoma. *Mol. Cancer* **16**, 16 (2017).
12. Huet, S. *et al.* A gene-expression profiling score for prediction of outcome in patients with follicular lymphoma: a retrospective training and validation analysis in three international cohorts. *Lancet Oncol.* **19**, 549–561 (2018).
13. Green, M. R. Chromatin modifying gene mutations in follicular lymphoma. *Blood* **131**, 595–604 (2018).
14. Korfi, K., Ali, S., Heward, J. A. & Fitzgibbon, J. Follicular lymphoma, a B cell malignancy addicted to epigenetic mutations. *Epigenetics* **12**, 370–377 (2017).
15. Lackraj, T., Goswami, R. & Kridel, R. Pathogenesis of follicular lymphoma. *Best Pract. Res. Clin. Haematol.* **31**, 2–14 (2018).
16. Chong, L. C. *et al.* High-resolution architecture and partner genes of MYC rearrangements in lymphoma with DLBCL morphology. *Blood Adv.* **2**, 2755–2765 (2018).
17. McPhail, E. D. *et al.* Inferior survival in high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements is not associated with MYC/IG gene rearrangements. *Haematologica* **103**, 1899–1907 (2018).
18. Pasqualucci, L. & Dalla-Favera, R. Genetics of diffuse large B-cell lymphoma. *Blood* **131**, 2307–2319 (2018).
19. Pasqualucci, L. & Dalla-Favera, R. The genetic landscape of diffuse large B-cell lymphoma. *Semin. Hematol.* **52**, 67–76 (2015).
20. Rossi, D., Spina, V., Bruscaggin, A. & Gaidano, G. Liquid biopsy in lymphoma. *Haematologica* **104**, 648–652 (2019).
21. Kurtz, D. M. *et al.* Dynamic risk profiling using serial tumor biomarkers for personalized outcome prediction. *Cell* **178**, 699-713.e19 (2019).
22. Rossi, D., Condoluci, A., Spina, V. & Gaidano, G. Methods for measuring ctDNA in lymphomas. *Methods Mol. Biol. Clifton NJ* **1881**, 253–265 (2019).
23. Kwok, M., Wu, S. P., Mo, C., Summers, T. & Roschewski, M. Circulating tumor DNA to monitor therapy for aggressive B-cell lymphomas. *Curr. Treat. Options Oncol.* **17**, 47 (2016).
24. Huet, S. & Salles, G. Potential of circulating tumor DNA for the management of patients with lymphoma. *JCO Oncol. Pract.* **16**, 561–568 (2020).
25. Kurtz, D. M. *et al.* Circulating tumor DNA measurements as early outcome predictors in diffuse large B-cell lymphoma. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* **36**, 2845–2853 (2018).

Author contributions

M.B.O., J.S.G. and C.M.L. conceived and designed the analysis. C.A.Z., N.C.C., S.M., D.C., M.C., P.M., L.S. and F.J.D.C. collected the data end/or contributed with data or analysis tools. M.B.O., J.S.G., C.M.L. wrote the main manuscript text and prepared tables and figures. J.S.G., M.B.O. and C.M.L. performed the analysis. M.B.O., J.S.G., C.M.L. and I.B. did interpretation of the data. J.M.P., J.L.D.M., and I.B. supervised and critically reviewed and edited the draft. All authors read and reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.