Targeted next generation sequencing reveals high mutation frequency of *CREBBP*, *BCL2* and *KMT2D* in high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements

Solène M. Evrard,[1,2,3] Sarah Péricart,[1] David Grand,[1] Nadia Amara,[1] Frédéric Escudié,[1] Julia Gilhodes,[4] Pierre Bories,[5] Alexandra Traverse-Glehen,[6] Romain Dubois,[7] Pierre Brousset,[1,2,3] Marie Parrens[8] and Camille Laurent[1,2,3]

[1]Pathology and Cytology Department, CHU Toulouse, IUCT Oncopole; [2]Toulouse III Paul Sabatier University; [3]Inserm, UMR1037 Centre de Recherche en Cancerologie de Toulouse, laboratoire d'excellence TOUCAN; [4]Department of Biostatistics, IUCT-Oncopole, CHU Toulouse; [5]Regional Cancer Network Onco-occitanie, IUCT-Oncopole, Toulouse; [6]Hospices Civils de Lyon, Claude Bernard Lyon 1 University, INSERM 1052, Pierre-Bénite; [7]Institut de Pathologie, CHU Lille, Avenue Oscar Lambret and [8]Pathology, CHU Bordeaux, Inserm U1053, France.

Correspondence: SOLÈNE M. EVRARD
solene.evrard@univ-tlse3.fr
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Supplemental data

Supplemental Methods

Patients:

Twenty adult patients diagnosed with DHL or THL who harbor gene rearrangements involving *MYC* and *BCL2* or/and *BCL6* determined using the FISH test were selected[1]. This cohort includes 15 patients with rearrangements of *MYC* and *BCL2*, 2 patients with rearrangements of *MYC* and *BCL6* and 3 patients with rearrangements of *MYC*, *BCL2* and *BCL6*. The COO of each tumor was assessed using the Hans algorithm[2]. 18 patients were classified as GCB-DLBCL and 2 as non-GCB-DLBCL. All patients signed informed consent forms. Clinical, immunohistochemical and cytogenetic features of the DHL/THL patients are summarized in Table 1.

DNA extraction and quantification

Tumor DNA was extracted from a 10µm-thick section of FFPE samples. DNA extraction was performed using the Maxwell® 16 FFPE Plus LEV DNA Purification Kit (Promega) based on the manufacturers' instructions. Extracted DNA was quantified and qualified for an NGS assay as reported previously by our group[3].

Lymphopanel design

Specific exons or hotspots of 43 clinically relevant genes involved in lymphomagenesis were selected for targeted sequencing based on an extensive literature review of NGS studies in de novo or relapsed/refractory DLBCL[4–12]. These selected regions are listed in Supplementary Table S1. We used an Illumina TruSeq Custom Amplicon (TSCA dual strand, v1.5 chemistry) assay for the deep sequencing of these regions. The custom mixture of oligonucleotides generated 1224 amplicons of suitable size (mean: 159bp and median: 153bp [150bp-180bp]) covering 124 kb. Samples were prepared following the TSCA protocol and as described elsewhere[3]. Multiple indexed libraries were pooled and sequenced on the Illumina MiSeq using a V3 flow cell. After pair-end sequencing (2x150 cycles), the four FastQ files generated per sample by the MiSeq Reporter software (v 2.6.2.3, Illumina) were analyzed using Amplicon DS (v1.1.13.0, Illumina). Filter criteria used for variant calling single nucleotide variants (SNVs) and short insertions and deletions (indels) are described below.

Somatic alteration assessment and filtering

In Amplicon DS software, variant scores are computed using a Poisson model that excludes calls with scores below Q20. Variants are first called separately for each pool and are then compared and combined into a single output file. The algorithm only calls variants for bases that are covered at a depth of 300X or greater for a single amplicon. Variants were called if they were present in both libraries with a mean VAF of 5% or greater. Following variant calling, variants detected by AmpliconDS software were filtered regarding their consequence. Variants that were missense variant, frameshift, stop gained, stop lost, initiator codon, in-frame insertion, in-frame deletion and splice-site variant were kept and analyzed. Stop-gain, frameshift and/or splicing SNVs are referred to in the article as truncating mutations. Variants

having a frequency of 1% or more in the population (in database Exome Aggregation Consortium (ExAC) Variants, Variants Exome Sequencing Project (ESP) or 1000 Genomes Project) were considered polymorphisms and were excluded. Alamut visual (v2.8, Interactive-Biosoftware, Rouen, France) or Integrative Genomics Viewer (IGV, v2.3.97, Broad Institute, Cambridge, Massachusetts, USA) were used to look at the alignment when necessary.
To validate our Lymphopanel, we first re-sequenced patients #3,4,5, 6 and 7 with the same protocol previously described (Amplicon targeted NGS and Lymphopanel) and found precisely the same variants for each patient with similar allele frequency (Table S3). Secondly, we sequenced these 5 patients by capture hybridization sequencing using a similar panel allowing us to find the same variants (Table S3). Briefly, DNA probe sets corresponding to genomics regions of interest were designed using the NimbleGen proprietary tool. A total of 100ng of DNA was sheared with a Covaris ME220 system to provide average fragments of 200bp. The sample preparation and target enrichment were performed according to the Roche SeqCap EZ Hypercap Workflow (ROCHE). Samples were sequenced on an Illumina MiseqDX system with the Illumina V3 chemistry cartridge reagent. The variants are called with Illumina's Enrichment workflow (v0.0.0.0).

Comparator cases of DLBCL NOS and GCB-DLBCL

Comparator cases of DLBCL NOS patients were selected from recent studies of whole exome sequencing or targeted sequencing[4–10,13–17]. From these 12 studies of whole exome sequencing or targeted sequencing in DLBCL NOS patients, 7 studies clearly set out their data with the possibility for each case to link its mutational profile to its COO and have been selected as comparator cases of GCB-DLBCL[8–10,13-15,17]. Likewise, 3 of 12 studies including patient clinical outcome have been selected to compare the overall survival (OS) of DLBCL NOS to that of 19 DHL/THL patients with clinical outcome available[15–17].

AID mutation analysis

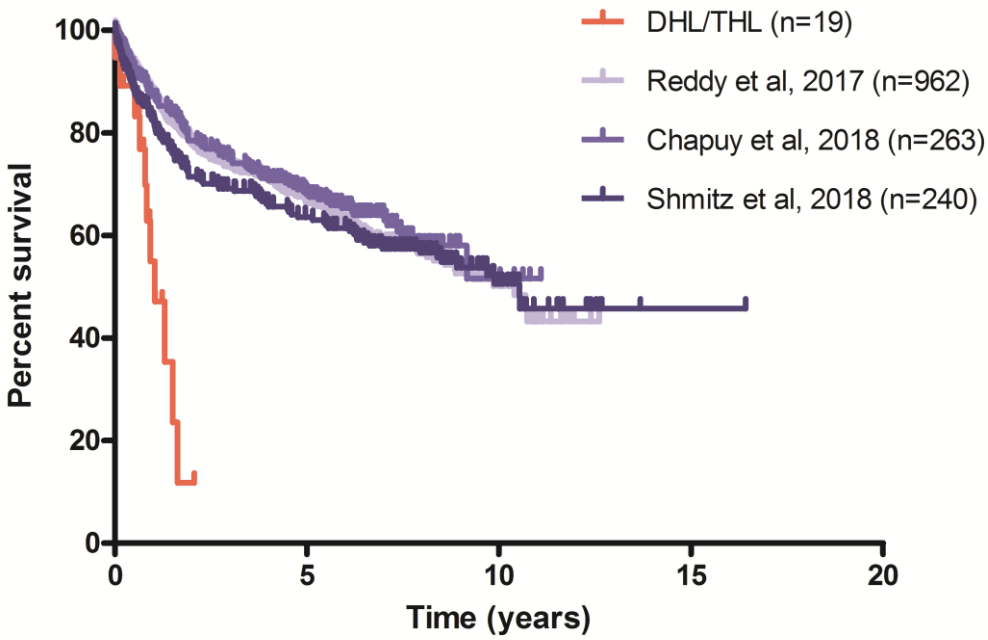
For AID mutation analysis, synonymous variants were also included, and the filter regarding the consequence of the variants was not applied. Preferential DGYW/WRCH AID target sites were targeted.

Statistical analysis

Mutation frequencies in literature were estimated by pooling data from studies of WES or targeted NGS or high-throughput sequencing in DLBCL NOS [4–10,13–17] or in GCB-DLBCL [8–10,13-15,17]. Differences in mutation frequencies were assessed using Fisher's exact test for count data. Differences in mutation pathway frequencies were assessed using the χ^2 test. All reported p-values were two-sided. Kaplan Meier survival curves were used for survival analysis. Differences in survival curves were assessed using the log-rank test. For all statistical tests, differences were considered significant at the 5% level. Statistical analyses were conducted using R 3.4.3 software or GraphPad Prism version 5.00 (GraphPad Software).

Supplemental Figure

Figure S1: Overall survival (OS) of DHL/THL patients compared to OS of DLBCL NOS patients



Supplemental Tables

Table S1: Overview of the lymphopanel used for NGS analysis.

Gene	Exons/Hotspots	Pathway
B2M	1 and 2	Immunity
BCL2	1	Apoptosis/cell cycle
BCL6	1 to 8	Immunity
BIRC3	6 to 9	Apoptosis/cell cycle
BRAF	15	MAPK
BTK	14 to 16	NFκB
CARD11	4 to 9	NFκB
CD58	1 to 3	Immunity
CD79A	2 to 4	BCR
CD79B	2 to 6	BCR
CDKN2A	1 to 3	Apoptosis/cell cycle
CDKN2B	1	Apoptosis/cell cycle
CIITA	1 to 19	Immunity
CREBBP	1 to 31	Epigenetic
DUSP2	2 to 4	MAPK
EP300	1 to 31	Epigenetic
EZH2	12, 16, 18	Epigenetic
FOXO1	1 and 2	Apoptosis/cell cycle
GNA13	1 to 4	Apoptosis/cell cycle
HIST1H1C	1	Epigenetic
ID3	1 and 2	BCR
IGLL5	1	Immunity
IRF4	1 to 8	NFκB
ITPKB	1	BCR
KMT2A	1 to 27	Epigenetic
KMT2D	1 to 54	Epigenetic
MEF2B	1 to 8	Epigenetic
MFHAS1	1	Apoptosis/cell cycle
MYC	1 to 3	Apoptosis/cell cycle
MYD88	1 to 5	NFκB
NOTCH1	26, 27, 28 and 34	NOTCH
NOTCH2	26, 27, 28 and 34	NOTCH
PIM1	1 to 6	NFκB
PLCG2	D1140G_D334H_L845Y_R665W_S707Y_R742P	BCR
PRDM1	1 to 7	NFκB
SOCS1	1	JAK/STAT
STAT6	9 to 14	JAK/STAT
TCF3	1 to 18	BCR
TNFAIP3	1 to 8	NFκB
TNFRSF14	1 to 8	Immunity
TP53	1 to 9	Apoptosis/cell cycle
TRAF3	5	NFκB
XPO1	12, 13, 15, 16, 17 and 18	Apoptosis/cell cycle

Table S2: **List of variants detected in DHL/THL patients** *(VAF: Variant allele frequency)*

Patient #	Gene	Transcript	cDNA alteration	protein alteration	VAF	Total depth
10	*B2M*	NM_004048.2	c.20T>G	p.Leu7Ter	10%	4854
12	*B2M*	NM_004048.2	c.128T>G	p.Leu43Arg	42%	4412
1	*BCL2*	NM_000633.2	c.60T>A	p.His20Gln	14%	1284
1	*BCL2*	NM_000633.2	c.31A>T	p.Asn11Tyr	25%	1872
1	*BCL2*	NM_000633.2	c.361C>G	p.Leu121Val	14%	1810
3	*BCL2*	NM_000633.2	c.585+4G>A	splice site	31%	2216
4	*BCL2*	NM_000633.2	c.386G>A	p.Arg129His	19%	1196
4	*BCL2*	NM_000633.2	c.261C>G	p.Ser87Arg	31%	804
6	*BCL2*	NM_000633.2	c.422G>A	p.Gly141Glu	46%	2224
6	*BCL2*	NM_000633.2	c.221C>A	p.Thr74Asn	50%	906
6	*BCL2*	NM_000633.2	c.179C>T	p.Ala60Val	51%	906
6	*BCL2*	NM_000633.2	c.175C>T	p.Pro59Ser	51%	906
7	*BCL2*	NM_000633.2	c.181G>A	p.Ala61Thr	19%	1224
7	*BCL2*	NM_000633.2	c.256C>T	p.Leu86Phe	18%	1224
7	*BCL2*	NM_000633.2	c.203G>A	p.Arg68Lys	18%	1224
7	*BCL2*	NM_000633.2	c.372C>A	p.Phe124Leu	17%	2442
7	*BCL2*	NM_000633.2	c.386_387delGCinsAG	p.Arg129Gln	11%	2444
7	*BCL2*	NM_000633.2	c.386G>A	p.Arg129His	6%	2438
8	*BCL2*	NM_000633.2	c.191A>G	p.Asp64Gly	5%	878
8	*BCL2*	NM_000633.2	c.20C>T	p.Thr7Ile	50%	3046
11	*BCL2*	NM_000633.2	c.32A>C	p.Asn11Thr	34%	1442
12	*BCL2*	NM_000633.2	c.31A>T	p.Asn11Tyr	11%	1468
12	*BCL2*	NM_000633.2	c.174_175delTCinsAT	p.HisPro58GlnSer	37%	1060
13	*BCL2*	NM_000633.2	c.495G>C	p.Glu165Asp	45%	2420
13	*BCL2*	NM_000633.2	c.175C>G	p.Pro59Ala	40%	692
14	*BCL2*	NM_000633.2	c.524T>A	p.Leu175Gln	49%	3776
14	*BCL2*	NM_000633.2	c.107G>A	p.Gly36Asp	54%	2454
18	*BCL2*	NM_000633.2	c.13G>A	p.Gly5Arg	9%	2698
18	*BCL2*	NM_000633.2	c.152C>T	p.Ser51Phe	20%	1820
19	*BCL2*	NM_000633.2	c.338C>G	p.Ala113Gly	32%	3290
1	*BCL6*	NM_001706.4	c.1268T>C	p.Leu423Pro	32%	3480
10	*BCL6*	NM_001706.4	c.1939A>G	p.Ser647Gly	42%	4844
14	*BCL6*	NM_001706.4	c.1756C>G	p.Pro586Ala	49%	3714
19	*BIRC3*	NM_001165.4	c.1681A>G	p.Met561Val	43%	4434
16	*BRAF*	NM_004333.4	c.1790T>A	p.Leu597Gln	46%	2608
20	*BRAF*	NM_004333.4	c.1780G>A	p.Asp594Asn	15%	438
13	*BTK*	NM_000061.2	c.1186delG	p.Glu396LysfsTer7	76%	1890
4	*CARD11*	NM_032415.4	c.758T>C	p.Leu253Pro	32%	6990
4	*CD79A*	NM_001783.3	c.670G>T	p.Glu224Ter	40%	2292
17	*CIITA*	NM_000246.3	c.1504G>A	p.Glu502Lys	47%	1778
9	*CIITA*	NM_000246.3	c.3311C>T	p.Thr1104Met	38%	4626
19	*CIITA*	NM_000246.3	c.874C>T	p.Pro292Ser	41%	3378
20	*CIITA*	NM_000246.3	c.1136A>C	p.Glu379Ala	20%	2326

Patient #	Gene	Transcript	cDNA alteration	protein alteration	VAF	Total depth
1	*CREBBP*	NM_004380.2	c.3780-2A>C	splice site	49%	3252
2	*CREBBP*	NM_004380.2	c.1941+2T>G	splice site	76%	2678
3	*CREBBP*	NM_004380.2	c.3195T>G	p.Ser1065Arg	24%	4092
3	*CREBBP*	NM_004380.2	c.3710G>A	p.Cys1237Tyr	46%	6478
4	*CREBBP*	NM_004380.2	c.904_905delAG	p.Ser302HisfsTer47	19%	4126
5	*CREBBP*	NM_004380.2	c.1571dupT	p.Asn526LysfsTer2	43%	3692
5	*CREBBP*	NM_004380.2	c.4559A>T	p.Lys1520Met	46%	1898
7	*CREBBP*	NM_004380.2	c.4709C>T	p.Ala1570Val	11%	1512
8	*CREBBP*	NM_004380.2	c.4336C>T	p.Arg1446Cys	46%	5374
8	*CREBBP*	NM_004380.2	c.3375T>G	p.Tyr1125Ter	48%	4888
8	*CREBBP*	NM_004380.2	c.3377T>A	p.Phe1126Tyr	48%	4888
9	*CREBBP*	NM_004380.2	c.1941+1G>T	splice site	61%	9558
9	*CREBBP*	NM_004380.2	c.1934A>G	p.Asn645Ser	71%	8104
9	*CREBBP*	NM_004380.2	c.1921T>A	p.Tyr641Asn	71%	8102
10	*CREBBP*	NM_004380.2	c.4303G>T	p.Asp1435Tyr	46%	4206
11	*CREBBP*	NM_004380.2	c.712G>C	p.Val238Leu	27%	6156
11	*CREBBP*	NM_004380.2	c.4628A>T	p.Asp1543Val	61%	2544
11	*CREBBP*	NM_004380.2	c.85+2T>G	splice site	39%	528
12	*CREBBP*	NM_004380.2	c.4395-2A>C	splice site	31%	4638
13	*CREBBP*	NM_004380.2	c.3780-1G>A	splice site	37%	6232
13	*CREBBP*	NM_004380.2	c.5039_5041delCCT	p.Ser1680del	41%	1494
13	*CREBBP*	NM_004380.2	c.458C>T	p.Pro153Leu	48%	3638
14	*CREBBP*	NM_004380.2	c.3609+2T>C	splice site	53%	6904
18	*CREBBP*	NM_004380.2	c.4445A>G	p.Tyr1482Cys	83%	2058
18	*CREBBP*	NM_004380.2	c.4447A>T	p.Ile1483Phe	72%	908
19	*CREBBP*	NM_004380.2	c.4427C>T	p.Pro1476Leu	80%	5518
20	*CREBBP*	NM_004380.2	c.5666C>T	p.Pro1889Leu	69%	852
20	*CREBBP*	NM_004380.2	c.4685A>G	p.Glu1562Gly	21%	2236
6	*DUSP2*	NM_004418.3	c.388+5G>C	splice site	35%	2190
6	*DUSP2*	NM_004418.3	c.389-7C>T	splice site	39%	2190
11	*DUSP2*	NM_004418.3	c.388+5_388+6delGCinsTG	splice site	50%	1916
20	*DUSP2*	NM_004418.3	c.532C>T	p.Pro178Ser	16%	1484
8	*EP300*	NM_001429.3	c.6687T>G	p.His2229Gln	9%	1112
18	*EP300*	NM_001429.3	c.2773C>A	p.Pro925Thr	48%	1624
19	*EP300*	NM_001429.3	c.6687T>G	p.His2229Gln	10%	1002
1	*EZH2*	NM_004456.4	c.1937A>T	p.Tyr646Phe	15%	6954
2	*EZH2*	NM_004456.4	c.1936T>A	p.Tyr646Asn	39%	5320
5	*EZH2*	NM_004456.4	c.1937A>T	p.Tyr646Phe	30%	8736
10	*EZH2*	NM_004456.4	c.1852-5T>C	splice site	48%	7402
12	*EZH2*	NM_004456.4	c.1937A>T	p.Tyr646Phe	29%	6904
13	*EZH2*	NM_004456.4	c.1937A>T	p.Tyr646Phe	36%	5952
14	*EZH2*	NM_004456.4	c.1937A>T	p.Tyr646Phe	49%	8032
15	*EZH2*	NM_004456.4	c.2075C>T	p.Ala692Val	25%	3928
2	*FOXO1*	NM_002015.3	c.70A>G	p.Thr24Ala	80%	432
3	*FOXO1*	NM_002015.3	c.55C>T	p.Arg19Trp	26%	712

Patient #	Gene	Transcript	cDNA alteration	protein alteration	VAF	Total depth
4	*FOXO1*	NM_002015.3	c.56G>A	p.Arg19Gln	20%	978
7	*FOXO1*	NM_002015.3	c.1571A>T	p.His524Leu	6%	5106
13	*FOXO1*	NM_002015.3	c.62G>C	p.Arg21Pro	63%	600
19	*FOXO1*	NM_002015.3	c.70A>G	p.Thr24Ala	38%	902
5	*GNA13*	NM_006572.4	c.661G>C	p.Val221Leu	11%	5824
5	*GNA13*	NM_006572.4	c.203T>A	p.Met68Lys	78%	1086
6	*GNA13*	NM_006572.4	c.143T>C	p.Leu48Pro	35%	3540
6	*GNA13*	NM_006572.4	c.91_96dupTCCAAG	p.Ser31_Lys32dup	32%	4562
12	*GNA13*	NM_006572.4	c.82C>T	p.Gln28Ter	34%	1302
5	*HIST1H1C*	NM_005319.3	c.203C>T	p.Ala68Val	85%	2440
6	*HIST1H1C*	NM_005319.3	c.565G>A	p.Ala189Thr	37%	7602
6	*HIST1H1C*	NM_005319.3	c.139G>T	p.Ala47Ser	62%	2950
16	*ID3*	NM_002167.4	c.167C>T	p.Pro56Leu	43%	2970
2	*IGLL5*	NM_001178126.1	c.174C>A	p.Ser58Arg	79%	690
2	*IGLL5*	NM_001178126.1	c.167T>C	p.Val56Ala	78%	690
4	*IGLL5*	NM_001178126.1	c.206G>C	p.Arg69Thr	7%	508
4	*IGLL5*	NM_001178126.1	c.46C>G	p.Leu16Val	33%	486
4	*IGLL5*	NM_001178126.1	c.93_95delGGCinsAAA	p.Ala32Asn	32%	486
6	*IGLL5*	NM_001178126.1	c.206_206+1delGGinsAA	splice site	64%	450
16	*IGLL5*	NM_001178126.1	c.125T>A	p.Met42Lys	82%	588
17	*IGLL5*	NM_001178126.1	c.176G>C	p.Ser59Thr	54%	1512
17	*IGLL5*	NM_001178126.1	c.169G>A	p.Gly57Arg	38%	1512
17	*IGLL5*	NM_001178126.1	c.206+4A>C	splice site	38%	1508
12	*IGLL5*	NM_001178126.1	c.125T>G	p.Met42Arg	26%	1134
12	*IGLL5*	NM_001178126.1	c.176_177delGCinsCT	p.Ser59Thr	30%	1134
13	*IGLL5*	NM_001178126.1	c.195_196delCCinsTG	p.Leu66Val	65%	728
13	*IGLL5*	NM_001178126.1	c.196C>G	p.Leu66Val	51%	728
14	*IGLL5*	NM_001178126.1	c.166G>A	p.Val56Ile	49%	1754
4	*ITPKB*	NM_002221.3	c.1229G>A	p.Ser410Asn	15%	1368
6	*ITPKB*	NM_002221.3	c.353C>T	p.Thr118Ile	45%	1380
5	*KMT2A*	NM_001197104.1	c.11620C>T	p.Arg3874Trp	35%	2520
2	*KMT2D*	NM_003482.3	c.16048_16051delAAAC	p.Lys5350GlyfsTer5	58%	4360
2	*KMT2D*	NM_003482.3	c.6595delT	p.Tyr2199IlefsTer65	33%	2706
2	*KMT2D*	NM_003482.3	c.6594delC	p.Tyr2199IlefsTer65	12%	4760
4	*KMT2D*	NM_003482.3	c.11649_11650delCA	p.His3883GlnfsTer128	33%	2462
5	*KMT2D*	NM_003482.3	c.5279A>G	p.Lys1760Arg	47%	2394
8	*KMT2D*	NM_003482.3	c.2772_2774delCTT	p.Leu925del	46%	3076
17	*KMT2D*	NM_003482.3	c.9884dupT	p.Met3295IlefsTer6	71%	11666
10	*KMT2D*	NM_003482.3	c.16455delC	p.Val5486Ter	48%	3874
11	*KMT2D*	NM_003482.3	c.7933C>T	p.Arg2645Ter	45%	4038
13	*KMT2D*	NM_003482.3	c.13040_13041delAG	p.Gln4347ArgfsTer24	25%	1972
14	*KMT2D*	NM_003482.3	c.2173dupC	p.Leu725ProfsTer6	46%	4138
15	*KMT2D*	NM_003482.3	c.5994T>A	p.Tyr1998Ter	28%	1318
15	*KMT2D*	NM_003482.3	c.10378C>T	p.Gln3460Ter	49%	5750
18	*KMT2D*	NM_003482.3	c.8727_8730delAAGT	p.Ser2910ArgfsTer32	74%	680

Patient #	Gene	Transcript	cDNA alteration	protein alteration	VAF	Total depth
20	*KMT2D*	NM_003482.3	c.267delT	p.Phe89LeufsTer41	20%	3720
20	*KMT2D*	NM_003482.3	c.11260C>T	p.Gln3754Ter	37%	476
5	*MEF2B*	NM_001145785.1	c.10A>G	p.Lys4Glu	41%	7386
6	*MEF2B*	NM_001145785.1	c.1105T>G	p.Ter369GluextTer68	39%	1036
10	*MEF2B*	NM_001145785.1	c.205T>C	p.Tyr69His	48%	2944
15	*MEF2B*	NM_001145785.1	c.54+1G>T	splice site	33%	5260
6	*MFHAS1*	NM_004225.2	c.1232A>G	p.Lys411Arg	37%	1398
6	*MFHAS1*	NM_004225.2	c.663G>C	p.Glu221Asp	38%	1534
11	*MFHAS1*	NM_004225.2	c.1688A>G	p.Lys563Arg	62%	4344
1	*MYC*	NM_002467.4	c.225_227dupCCT	p.Leu76dup	25%	3136
3	*MYC*	NM_002467.4	c.62G>A	p.Ser21Asn	31%	2912
3	*MYC*	NM_002467.4	c.490C>G	p.Leu164Val	37%	1128
3	*MYC*	NM_002467.4	c.573G>C	p.Leu191Phe	38%	1128
3	*MYC*	NM_002467.4	c.232C>G	p.Pro78Ala	29%	572
4	*MYC*	NM_002467.4	c.109T>G	p.Tyr37Asp	28%	3086
4	*MYC*	NM_002467.4	c.111T>A	p.Tyr37Ter	28%	3086
7	*MYC*	NM_002467.4	c.220C>A	p.Pro74Thr	13%	4952
7	*MYC*	NM_002467.4	c.485_496delAGAAGCTGGCCT	p.Glu162_Ser166delinsAla	12%	2442
7	*MYC*	NM_002467.4	c.686G>A	p.Ser229Asn	11%	5148
16	*MYC*	NM_002467.4	c.220C>G	p.Pro74Ala	51%	2936
16	*MYC*	NM_002467.4	c.386G>C	p.Ser129Thr	54%	4224
9	*MYC*	NM_002467.4	c.229T>C	p.Ser77Pro	35%	3544
9	*MYC*	NM_002467.4	c.289C>G	p.Leu97Val	35%	3544
12	*MYC*	NM_002467.4	c.16G>C	p.Val6Leu	11%	3310
12	*MYC*	NM_002467.4	c.322T>G	p.Phe108Val	27%	3298
12	*MYC*	NM_002467.4	c.230C>T	p.Ser77Phe	6%	1600
12	*MYC*	NM_002467.4	c.301A>G	p.Asn101Asp	6%	1600
19	*MYC*	NM_002467.4	c.220C>A	p.Pro74Thr	33%	9228
20	*MYC*	NM_002467.4	c.961C>G	p.Gln321Glu	12%	5092
20	*MYD88*	NM_002468.4	c.695T>C	p.Met232Thr	21%	832
6	*PIM1*	NM_001243186.1	c.356-4C>T	splice site	34%	2638
6	*PIM1*	NM_001243186.1	c.575C>G	p.Ser192Cys	36%	3580
12	*PIM1*	NM_001243186.1	c.361G>C	p.Glu121Gln	28%	1772
20	*PIM1*	NM_001243186.1	c.802C>T	p.Leu268Phe	16%	5202
20	*PIM1*	NM_001243186.1	c.97C>G	p.Gln33Glu	45%	2528
20	*PIM1*	NM_001243186.1	c.521G>A	p.Gly174Asp	13%	6138
12	*PRDM1*	NM_001198.3	c.1061G>A	p.Ser354Asn	50%	3260
18	*PRDM1*	NM_001198.3	c.1571C>T	p.Thr524Met	34%	1458
3	*SOCS1*	NM_003745.1	c.197G>A	p.Arg66His	22%	1456
3	*SOCS1*	NM_003745.1	c.347G>A	p.Ser116Asn	17%	374
4	*SOCS1*	NM_003745.1	c.456G>C	p.Glu152Asp	18%	998
6	*SOCS1*	NM_003745.1	c.7G>C	p.Ala3Pro	45%	5272
7	*SOCS1*	NM_003745.1	c.299C>A	p.Thr100Asn	14%	2716
13	*SOCS1*	NM_003745.1	c.157A>C	p.Thr53Pro	23%	538
13	*SOCS1*	NM_003745.1	c.164delT	p.Phe55SerfsTer30	23%	540

Patient #	Gene	Transcript	cDNA alteration	protein alteration	VAF	Total depth
20	*SOCS1*	NM_003745.1	c.296G>C	p.Gly99Ala	17%	928
13	*STAT6*	NM_003153.4	c.1256A>C	p.Asp419Ala	30%	7594
10	*TCF3*	NM_001136139.2	c.1653T>A	p.Asn551Lys	47%	3424
15	*TCF3*	NM_001136139.2	c.22G>T	p.Ala8Ser	45%	1360
8	*TNFAIP3*	NM_006290.3	c.1939A>C	p.Thr647Pro	46%	4670
11	*TNFAIP3*	NM_006290.3	c.1681C>T	p.Gln561Ter	90%	700
2	*TNFRSF14*	NM_003820.2	c.263A>T	p.Asn88Ile	42%	1550
9	*TNFRSF14*	NM_003820.2	c.269T>C	p.Leu90Pro	27%	1806
9	*TNFRSF14*	NM_003820.2	c.1A>G	p.Met1?	32%	2598
11	*TNFRSF14*	NM_003820.2	c.219_220delAG	p.Cys75Ter	90%	888
14	*TNFRSF14*	NM_003820.2	c.460+1G>T	splice site	45%	3464
14	*TNFRSF14*	NM_003820.2	c.467_470delAGAG	p.Glu156ValfsTer33	50%	4728
7	*TP53*	NM_000546.5	c.261_270delAGCCCCCTCC	p.Ala88GlyfsTer32	23%	990
7	*TP53*	NM_000546.5	c.773A>T	p.Glu258Val	11%	5186
7	*TP53*	NM_000546.5	c.715A>G	p.Asn239Asp	10%	5948
8	*TP53*	NM_000546.5	c.712T>A	p.Cys238Ser	81%	4790
17	*TP53*	NM_000546.5	c.273G>A	p.Trp91Ter	93%	472
11	*TP53*	NM_000546.5	c.536A>C	p.His179Pro	92%	744
19	*TP53*	NM_000546.5	c.470T>A	p.Val157Asp	72%	1668

Table S3: **Validation of the Lymphopanel.** *(VAF: Variant allele frequency)*

Sample #	Gene	cDNA alteration	Protein alteration	TSCA run 1			TSCA run2			Capture
				VAF	Depth (Library A)	Depth (Library B)	VAF	Depth (Library A)	Depth (Library B)	VAF
3	*BCL2*	c.585+4G>A		31%	1832	384	28%	1816	343	35%
	CREBBP	c.3195T>G	p.Ser1065Arg	24%	1426	2666	21%	1970	2436	27%
	CREBBP	c.3710G>A	p.Cys1237Tyr	46%	3634	2844	44%	3008	2830	45%
	FOXO1	c.55C>T	p.Arg19Trp	26%	558	154	20%	494	79	27%
	MYC	c.62G>A	p.Ser21Asn	31%	1328	1584	29%	1065	893	38%
	MYC	c.490C>G	p.Leu164Val	37%	574	554	28%	650	338	34%
	MYC	c.573G>C	p.Leu191Phe	38%	574	554	28%	649	338	27%
	MYC	c.232C>G	p.Pro78Ala	29%	64	508	30%	246	861	36%
	SOCS1	c.197G>A	p.Arg66His	22%	592	864	22%	699	392	24%
	SOCS1	c.347G>A	p.Ser116Asn	17%	246	128	29%	316	110	31%
4	*BCL2*	c.386G>A	p.Arg129His	19%	774	422	19%	648	930	21%
	BCL2	c.261C>G	p.Ser87Arg	31%	574	230	22%	300	395	22%
	CARD11	c.758T>C	p.Leu253Pro	32%	3912	3078	31%	1788	2092	33%
	CD79A	c.670G>T	p.Glu224Ter	40%	1622	670	40%	1957	1402	41%
	CREBBP	c.904_905delAG	p.Ser302HisfsTer47	19%	1906	2220	23%	1071	1483	19%
	FOXO1	c.56G>A	p.Arg19Gln	20%	844	134	15%	313	127	25%
	IGLL5	c.206G>C	p.Arg69Thr	7%	258	250	13%	278	267	28%
	IGLL5	c.46C>G	p.Leu16Val	33%	446	40	34%	244	265	30%
	IGLL5	c.93_95delGGCinsAAA	p.Ala32Asn	32%	446	40	33%	244	265	23%
	ITPKB	c.1229G>A	p.Ser410Asn	15%	1220	148	18%	798	413	22%
	KMT2D	c.11649_11650delCA	p.His3883GlnfsTer128	33%	1718	744	31%	1298	1564	31%
	MYC	c.109T>G	p.Tyr37Asp	28%	1698	1388	31%	700	1090	24%
	MYC	c.111T>A	p.Tyr37Ter	28%	1698	1388	31%	700	1090	24%
	SOCS1	c.456G>C	p.Glu152Asp	18%	738	260	20%	362	303	25%
5	*CREBBP*	c.1571dupT	p.Asn526LysfsTer2	43%	1648	2044	44%	852	1468	44%
	CREBBP	c.4559A>T	p.Lys1520Met	46%	402	1496	37%	1312	929	37%
	EZH2	c.1937A>T	p.Tyr646Phe	30%	5458	3278	26%	4194	1874	22%
	GNA13	c.661G>C	p.Val221Leu	11%	1874	3950	12%	2903	3125	13%
	GNA13	c.203T>A	p.Met68Lys	78%	826	260	75%	569	435	71%
	HIST1H1C	c.203C>T	p.Ala68Val	85%	910	1530	72%	359	981	53%
	KMT2A	c.11620C>T	p.Arg3874Trp	35%	824	1096	34%	1034	908	43%
	KMT2D	c.5279A>G	p.Lys1760Arg	47%	1476	918	45%	952	617	51%
	MEF2B	c.10A>G	p.Lys4Glu	41%	5354	2032	45%	2370	1589	44%
6	*BCL2*	c.422G>A	p.Gly141Glu	46%	1272	952	44%	1003	641	45%
	BCL2	c.221C>A	p.Thr74Asn	50%	564	342	47%	206	364	45%
	BCL2	c.179C>T	p.Ala60Val	51%	564	342	48%	206	364	46%
	BCL2	c.175C>T	p.Pro59Ser	51%	564	342	48%	206	364	46%
	DUSP2	c.388+5G>C	splice site	35%	1912	278	36%	371	960	44%
	DUSP2	c.389-7C>T	splice site	39%	1910	280	35%	371	961	37%
	GNA13	c.143T>C	p.Leu48Pro	35%	1810	1730	33%	1033	929	35%
	GNA13	c.91_96dupTCCAAG	p.Ser31_Lys32dup	32%	2404	2158	31%	1465	1173	27%
	HIST1H1C	c.565G>A	p.Ala189Thr	37%	3802	3800	36%	2671	2607	34%
	HIST1H1C	c.139G>T	p.Ala47Ser	62%	1140	1810	47%	1192	445	35%
	IGLL5	c.206_206+1delGGinsAA	splice site	64%	298	152	70%	106	372	72%
	ITPKB	c.353C>T	p.Thr118Ile	45%	194	1186	41%	609	316	32%
	MEF2B	c.1105T>G	p.Ter369GluextTer68	39%	84	952	23%	144	31	34%
	MFHAS1	c.1232A>G	p.Lys411Arg	37%	858	540	41%	861	584	38%
	MFHAS1	c.663G>C	p.Glu221Asp	38%	614	920	40%	692	707	43%
	PIM1	c.356-4C>T	splice site	34%	1078	1560	36%	1038	496	34%
	PIM1	c.575C>G	p.Ser192Cys	36%	1682	1898	42%	903	959	38%
	SOCS1	c.7G>C	p.Ala3Pro	45%	3410	1862	42%	930	1129	42%
7	*BCL2*	c.181G>A	p.Ala61Thr	19%	770	454	12%	463	484	15%
	BCL2	c.256C>T	p.Leu86Phe	18%	770	454	12%	463	485	14%
	BCL2	c.203G>A	p.Arg68Lys	18%	770	454	12%	463	483	15%
	BCL2	c.372C>A	p.Phe124Leu	17%	1514	928	8%	875	907	9%
	BCL2	c.386_387delGCinsAG	p.Arg129Gln	11%	1516	928	5%	876	907	5%
	BCL2	c.386G>A	p.Arg129His	6%	1510	928	6%	877	907	8%
	CREBBP	c.4709C>T	p.Ala1570Val	11%	1128	384	12%	1837	594	14%
	FOXO1	c.1571A>T	p.His524Leu	6%	2490	2616	7%	1506	1097	9%
	MYC	c.220C>A	p.Pro74Thr	13%	2748	2204	9%	1423	2678	12%
	MYC	c.485_496delAGAAGCTGGCCT	p.Glu162_Ser166delinsAla	12%	1206	1236	12%	683	643	9%
	MYC	c.686G>A	p.Ser229Asn	11%	414	4734	11%	822	1860	11%
	SOCS1	c.299C>A	p.Thr100Asn	14%	1258	1458	11%	674	674	14%
	TP53	c.261_270delAGCCCCCTCC	p.Ala88GlyfsTer32	23%	250	740	15%	987	865	11%
	TP53	c.773A>T	p.Glu258Val	11%	2598	2588	10%	1658	1214	12%
	TP53	c.715A>G	p.Asn239Asp	10%	1256	4692	11%	1878	2026	17%

<u>Table S4:</u> Comparisons of observed mutation frequencies in DHL/THL and those observed in DLBCL NOS in literature (*NA: not available*)

Genes		DLBCL NOS												Total	DHL/THL (n=20)	p value
		Lohr et al, 2012 (n=49)	de Miranda et al, 2014 (n=31)	Morin et al, 2013 (n=40)	Pasqualucci et al, 2011 (n=variable)	Zhang et al, 2013 (n=73)	Reddy et al, 2017 (n=1001)	Chapuy et al, 2017(n=304)	Schmitz et al, 2018 (n=574)	Dubois et al, 2016 (n=215)	Hung et al, 2018 (n=76)	Juskevicius et al, 2017 (n=76)	Karube et al, 2017 (n=150)			
CREBBP	mutated	8	3	3	20	3	110	52	92	41	10	12	31	385	16	**<0,001**
	unmutated	41	28	37	91	70	891	252	482	174	66	64	119	2315	4	
BCL2	mutated	11	1	8	10	3	160	52	57	22	NA	4	2	330	12	**<0,001**
	unmutated	38	30	32	101	70	841	252	517	193	NA	72	148	2294	8	
KMT2D	mutated	14	2	9	21	NA	225	76	178	88	17	26	43	699	12	**<0,01**
	unmutated	35	29	31	71	NA	776	228	396	127	59	50	107	1909	8	
MYC	mutated	4	NA	2	7	1	50	19	34	14	NA	5	3	139	9	**<0,001**
	unmutated	45	NA	38	104	72	951	285	540	201	NA	71	147	2454	11	
EZH2	mutated	7	1	9	6	1	60	21	46	19	14	3	17	204	8	**<0,001**
	unmutated	42	30	31	101	72	941	283	528	196	62	73	133	2493	12	
IGLL5	mutated	NA	4	NA	NA	NA	NA	30	NA	NA	NA	NA	NA	34	8	**<0,01**
	unmutated	NA	26	NA	NA	NA	NA	274	NA	NA	NA	NA	NA	300	20	
FOXO1	mutated	NA	NA	NA	NA	NA	40	4	43	15	6	3	16	127	6	**<0,001**
	unmutated	NA	NA	NA	NA	NA	961	300	531	200	70	73	134	2269	14	
SOCS1	mutated	3	NA	5	NA	3	90	16	75	32	NA	21	15	260	6	**<0,05**
	unmutated	46	NA	35	NA	70	911	288	499	183	NA	55	135	2222	14	

<u>Table S5</u>: Comparisons of observed mutation frequencies in GCB-DHL/THL and those observed in GCB-DLBCL in the literature (*NA: not available*)

Genes		GCB -DLBCL									
		Zhang et al, 2013 (n=17)	Reddy et al, 2017 (n=331)	Schmitz et al, 2018 (n=164)	Dubois et al, 2016 (n=83)	Hung et al, 2018 (n=33)	Juskevicius et al, 2017 (n=32)	Karube et al, 2017 (n=60)	Total GCB-DLBCL	GCB-DHL/THL (n=18)	p value
CREBBP	mutated	1	56	43	26	5	8	18	157	16	**<0.001**
	unmutated	16	275	121	57	28	24	42	563	2	
BCL2	mutated	2	80	34	20	NA	3	2	141	12	**<0.001**
	unmutated	15	251	130	63	NA	29	58	546	6	
KMT2D	mutated	NA	93	52	38	8	11	23	225	10	**<0.05**
	unmutated	NA	238	112	45	25	21	37	478	8	
MYC	mutated	NA	21	11	8	NA	2	2	44	9	**<0.001**
	unmutated	NA	310	153	75	NA	30	58	626	9	
EZH2	mutated	1	41	36	15	11	8	12	124	7	**<0.05**
	unmutated	16	290	128	68	22	24	48	596	11	
FOXO1	mutated	NA	16	20	10	4	1	11	62	6	**<0.01**
	unmutated	NA	315	144	73	29	31	49	641	12	
SOCS1	mutated	2	46	38	13	NA	9	12	120	6	0.11
	unmutated	15	285	126	70	NA	23	48	567	12	

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