Supplementary data Natalia Palazón-Carrión et al.

Supplementary figures

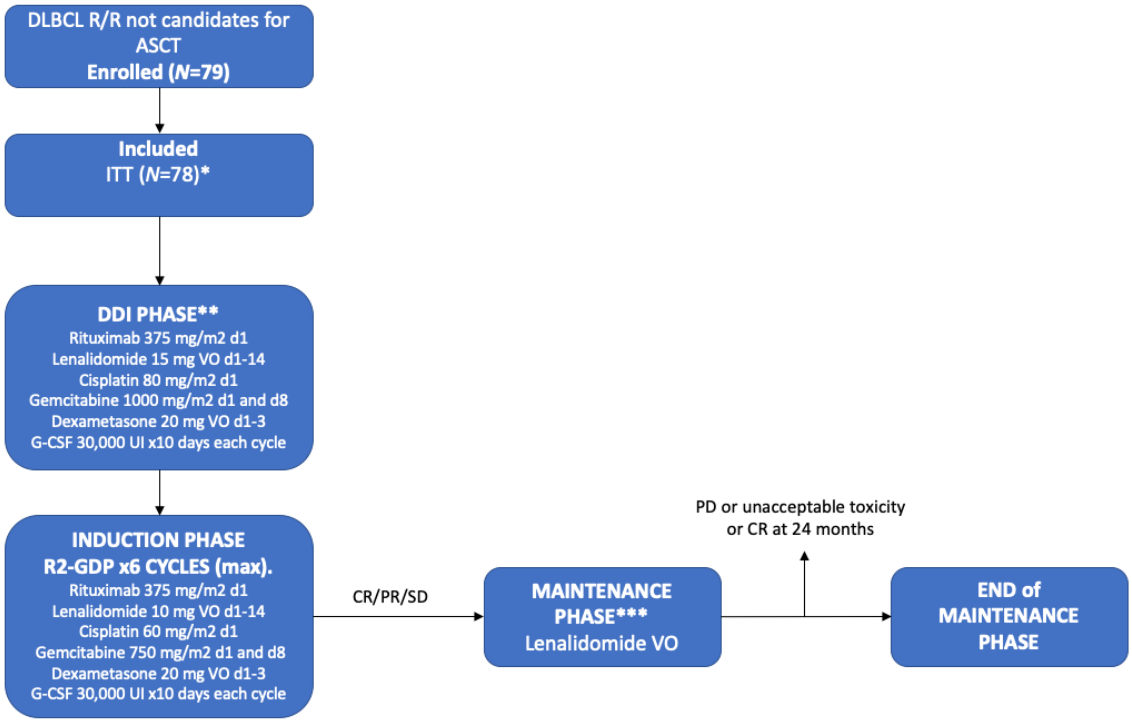

Figure S1. Study design. * Voluntary withdrawal of one patient. **R2-GDP as schedule not previously tested. Initial run in phase (DDI) period to evaluate toxicity and dose modifications. *** PO Lenalidomide 10 mg on days 1–21 for 28 days (or last lenalidomide dose received in induction phase). *DLBCL*= diffuse large B cell lymphoma. *R/R= relapsed/refractory. ASCT*=autologous stem cell transplant. ITT=intention to treat. PPP=per-protocol-population. DDI=drug-drug interaction.

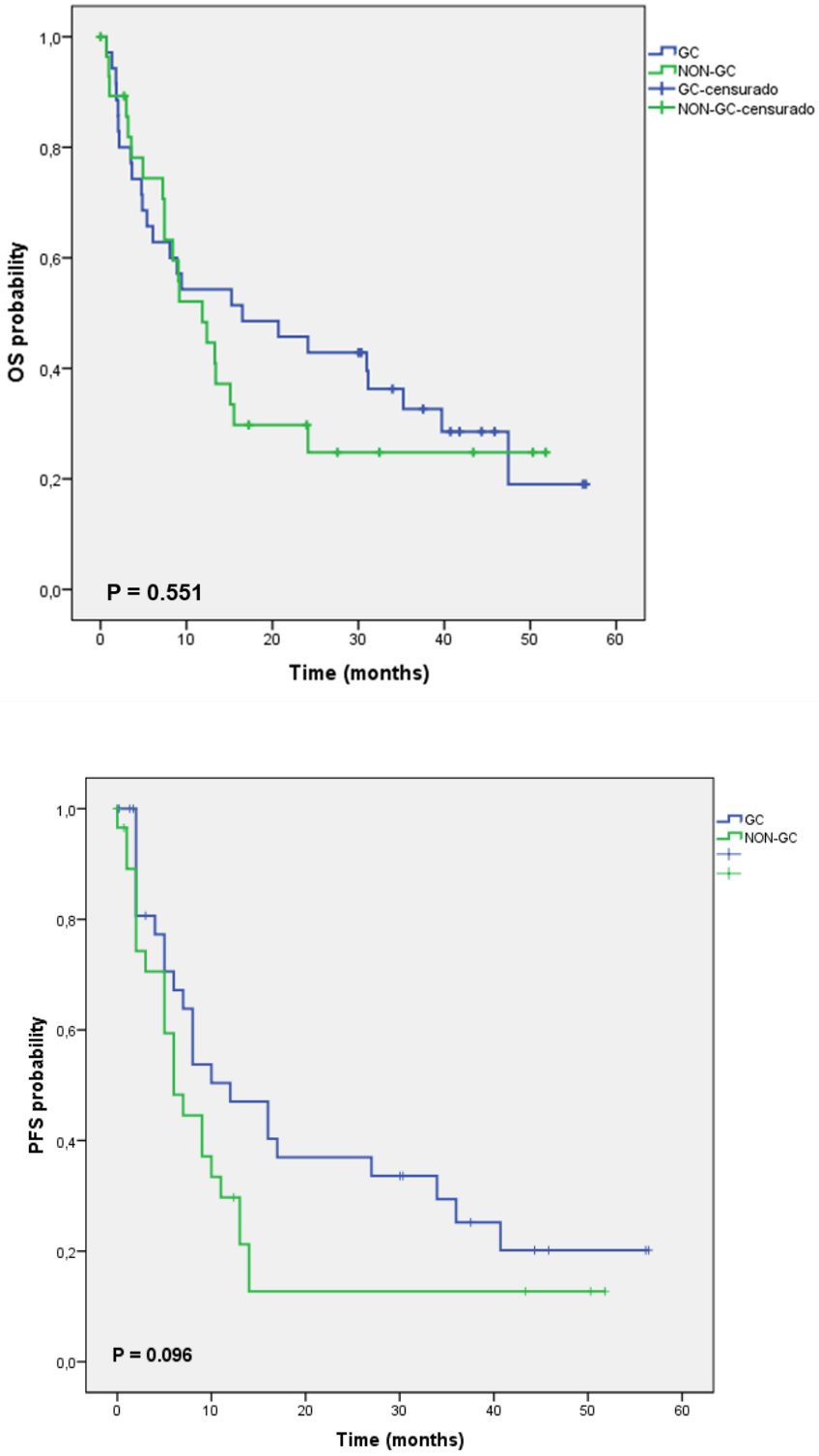

Figure S2. Kaplan-Meier plots of overall survival (OS) and progression-free survival (PFS) according to the cell-of-origin (COO). The vertical bar represents the PFS probability, while the horizontal bar represents the tumor follow-up time in months.

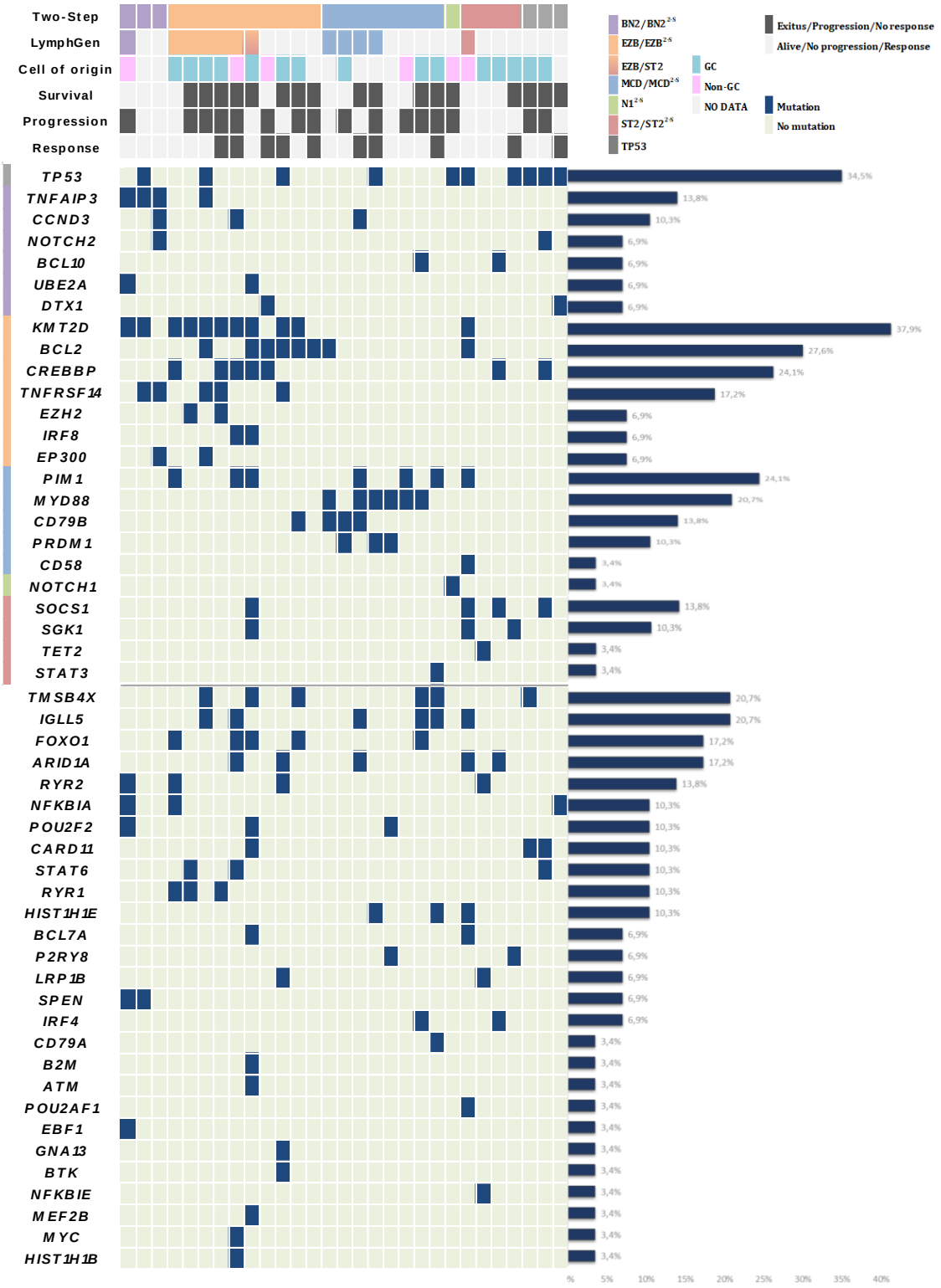

Figure S3. Distribution of the mutated genes in the 29 DLBCL cases analyzed. Each column represents an individual patient, and each row denotes a specific gene. The genetic classification in the six defined genetic subtypes of DLBCL, performed using the two-step (Pedrosa et al.) and LymphGene (Wright et al.) classifiers, and the GC/non-GC cell-of-origin classification based on Hans, are represented at the top. The bar graph shows the frequency of mutations found in each gene. GC: germinal center.

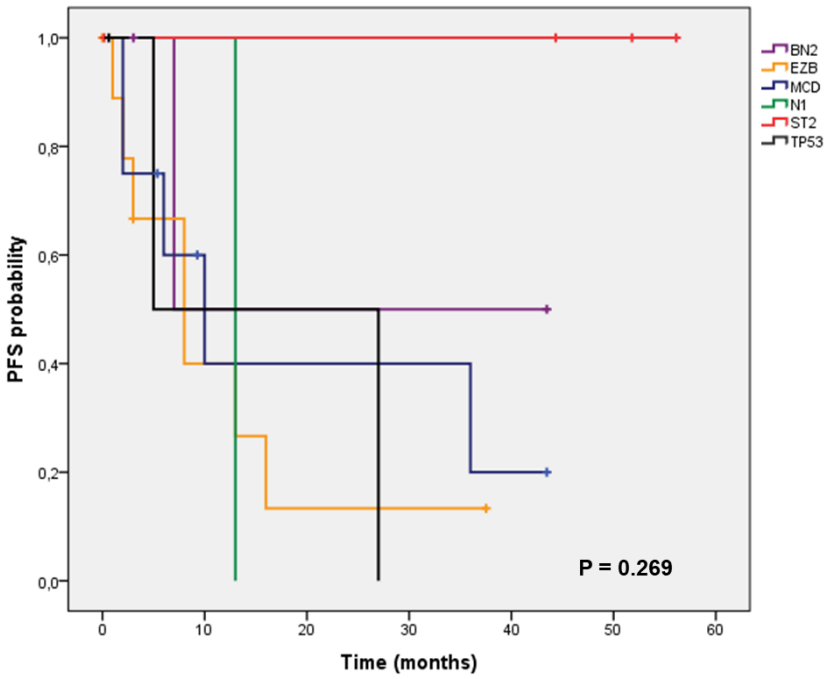

Figure S4. Kaplan-Meier plots of progression-free survival (PFS) according to genetic subtypes determined by the two-step classifier. The vertical bar represents the PFS probability, while the horizontal bar represents the tumor follow-up time in months.

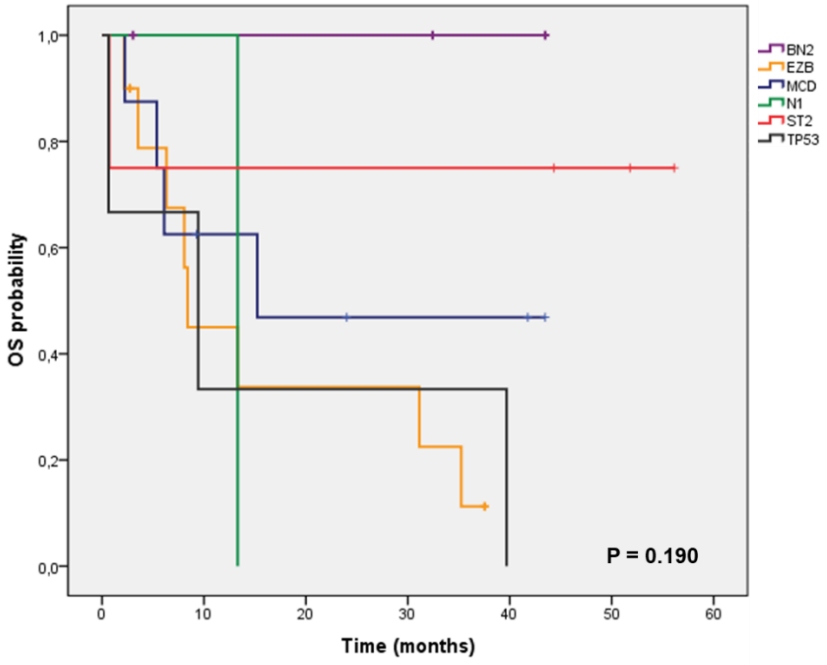

Figure S5. Kaplan-Meier plots of overall survival (OS) according to genetic subtypes determined by the two-step classifier. The vertical bar represents the OS probability, while the horizontal bar represents the tumor follow-up time in months

Supplementary tables

Table S1. Response rates (%) ITT	R/R DLBCL *N*=78	Primary Refractory DLBCL *N*=33
ORR	60.2	45.5
CR	37.1	21.2
PR	23.1	24.3
SD	7.8	12.1
PD	32.0	42.4

ITT=intention to treat. R/R= relapsed or refractory. DLBCL=diffuse large B-cell lymphoma. ORR= overall response rate. CR= complete response. PR= partial response. SD= stabilization of disease. PD= progression of disease.

Table S2*. Response according to previous chemotherapy.*

	SD (N=6)	***PD (N=25)***	***CR (N=29)***	***PR (N=18)***	***Total (N=78)***	***p value****
Chemotherapy (number of lines)						***0.356***
1	3 (50.0%)	14 (56.0%)	18 (62.1%)	8 (44.4%)	43 (55.1%)	
2	3 (50.0%)	7 (28.0%)	6 (20.7%)	9 (50.0%)	25 (32.1%)	
3	0 (0.0%)	3 (12.0%)	1 (3.4%)	0 (0.0%)	4 (5.1%)	
4	0 (0.0%)	1 (4.0%)	2 (6.9%)	0 (0.0%)	3 (3.8%)	
5	0 (0.0%)	0 (0.0%)	2 (6.9%)	0 (0.0%)	2 (2.6%)	
6	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.6%)	1 (1.3%)	

***Table S3**. Response according to previous chemotherapy (primary refractory disease only).*

	SD (N=3)	***PD (N=14)***	***CR (N=10)***	***PR (N=6)***	***Total (N=33)***	***p value****
Chemotherapy (number of lines)						***0.210***
1	2 (66.7%)	10 (71.4%)	7 (70.0%)	1 (16.7%)	20 (60.6%)	
2	1 (33.3%)	3 (21.4%)	1 (10.0%)	5 (83.3%)	10 (30.3%)	
3	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	1 (3.0%)	
4	0 (0.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)	1 (3.0%)	
5	0 (0.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)	1 (3.0%)	
6	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	

* Chi-square test. SD: stabilization of the disease; PD: progression of the disease; CR; complete response; PR; partial response.

***Table S4**. Response according to IPI score.*

	UK (N=16)	***Low (N=13)***	***Intermediate (N=29)***	***High (N=20)***	***Total (N=78)***	***p value****
Response						***0.118***
SD	3 (18.8%)	0 (0.0%)	3 (10.3%)	0 (0.0%)	6 (7.7%)	
PD	4 (25.0%)	3 (23.1%)	7 (24.1%)	11 (55.0%)	25 (32.1%)	
CR	5 (31.2%)	8 (61.5%)	12 (41.4%)	4 (20.0%)	29 (37.2%)	
PR	4 (25.0%)	2 (15.4%)	7 (24.1%)	5 (25.0%)	18 (23.1%)	

* Chi-square test. SD: stabilization of the disease; PD: progression of the disease; CR; complete response; PR; partial response. Low: 0-1; Intermediate:2-3; High:4-5.

***Table S5**. Response according to IPI scale in primary refractory disease.*

	UK (N=16)	***Low (N=13)***	***Intermediate-low (N=16)***	***Intermediate-high (N=13)***	***High (N=20)***	***Total (N=78)***	***p value****
Response							***0.242***
SD	3 (18.8%)	0 (0.0%)	1 (6.2%)	2 (15.4%)	0 (0.0%)	6 (7.7%)	
PD	4 (25.0%)	3 (23.1%)	4 (25.0%)	3 (23.1%)	11 (55.0%)	25 (32.1%)	
CR	5 (31.2%)	8 (61.5%)	7 (43.8%)	5 (38.5%)	4 (20.0%)	29 (37.2%)	
PR	4 (25.0%)	2 (15.4%)	4 (25.0%)	3 (23.1%)	5 (25.0%)	18 (23.1%)	

* Chi-square test. SD: stabilization of the disease; PD: progression of the disease; CR; complete response; PR; partial response. Low: 0-1; Intermediate-low:2; Intermediate-high;3; High:4-5.

Table S6. *Response according to COO subtype.*

	GC (N=29)	***Non-GC (N=45)***	***Total (N=74)***	***p value****
Response				***0.644***
SD	3 (10.3%)	3 (6.7%)	6 (8.1%)	
PD	8 (27.6%)	17 (37.8%)	25 (33.8%)	
CR	13 (44.8%)	15 (33.3%)	28 (37.8%)	
PR	5 (17.2%)	10 (22.2%)	15 (20.3%)	

* Chi-square test. SD: stabilization of the disease; PD: progression of the disease; CR; complete response; PR; partial response.

Table S8. *Response according to Lenalidomide dose.*

	10 mg Lena (N=44)	***5 mg Lena (N=34)***	***Total (N=78)***	***p value ****
Response				***0.058***
SD	2 (4.5%)	4 (11.8%)	6 (7.7%)	
PD	19 (43.2%)	6 (17.6%)	25 (32.1%)	
CR	16 (36.4%)	13 (38.2%)	29 (37.2%)	
OR	7 (15.9%)	11 (32.4%)	18 (23.1%)	

* Chi-square test. SD: stabilization of the disease; PD: progression of the disease; CR; complete response; PR; partial response.

Table S9*. Dropouts due to toxicity during the induction phase stratified by lenalidomide dose.*

	10 mg Lena (N=44)	***5 mg Lena (N=34)***	***Total (N=78)***	***p value****
Dropout				***0.353***
NO	37 (84.1%)	31 (91.2%)	68 (87.2%)	
YES	7 (15.9%)	3 (8.8%)	10 (12.8%)	

* Chi-square test.

Table S10. *Treatment-related deaths. C= Cycle, G =Grade, AEs = adverse events.*

	AA-death	***Cycle***	***Lenalidomide Dose (mg)***	***Previous Neutropenia (Cycle, grade)***
1	G5 Febrile neutropenia	Induction (C2)	15	No
2	G5 Febrile neutropenia	Induction (C3)	10	No
3	G5 Febrile neutropenia	Induction (C3)	5	Yes (C2, G3)
4	G5 Febrile neutropenia	Induction (C2)	10	No

Supplementary material and methods

Genetic subtypes: Methods of targeted sequencing

Genomic DNA was extracted from FFPET using a truXTRAC FFPE DNA Kit (Covaris, Woburn, MA, USA) following the manufacturer's instructions. A SureSelect target enrichment custom panel was designed using the SureDesign (Agilent Technologies, Santa Clara, CA, USA) web-based tool (earray.chem.agilent.com/suredesign/). The design covered coding exons of the selected genes. The genes included (61) are involved in lymphomagenesis-relevant pathways and were selected based on previous studies (1-8). The targeted regions (according to Human Assembly GRCh38/hg38) were captured using a SureSelect XT-HS (Agilent), as described in the manufacturer's instructions. Captured libraries were diluted to 8 pM for Illumina clustering, and paired-end sequencing was performed on MiSeq sequencer (Illumina Inc., San Diego, CA, USA).

List of genes included in the targeted panel:

ARID1A	IGLL5	POU2AF1
ATM	IKBKB	POU2F2
B2M	IKZF3	PRDM1
BCL10	IRF4	RYR1
BCL2	IRF8	RYR2
BCL6	KMT2D	S1PR2
BCL7A	LRP1B	SGK1
BTK	MEF2B	SOCS1
CARD11	MYC	SPEN
CCND3	MYD88	STAT3
CD58	NFKBIA	STAT6
CD79A	NFKBIE	TAGAP
CD79B	NOTCH1	TET2
CREBBP	NOTCH2	TMSB4X
DTX1	P2RY8	TNFAIP3
EBF1	PIK3CA	TNFRSF14
EP300	PIK3CD	TP53
EZH2	PIK3CG	TRAF6
FOXO1	PIK3R1	UBE2A
GNA13	PIM1	
HIST1H1E	PLCG2	

Three independent analyses were carried out for each sample. We first used the tools available in the Variant Reporter instrument (Illumina). A second variant calling was done with VarScan 2.3.9 to align the files extracted from two sources: (1) BWA Enrichment of Illumina Base Space, and (2) using the Burrows-Wheeler Aligner (BWA),

Picard, and Indel Realignment-Base Recalibration from the Genome Analysis Toolkit 3.8.1.0 (GATK).

Annotation was carried out with Annovar. All variants identified by the three complementary methods were visualized using an Integrative Genomics Viewer (Broad Institute and UC San Diego, San Diego, CA, USA. Data have been deposited in the Sequence Read Archive (SRA) (accession number GEO: PRJNA834596).

After annotation, the variants were subjected to additional, more stringent, and quality- and relevance-based filtering by the following criteria: quality read depth of bases ≥ 50; depth of variant-supporting bases ≥ 5; localization (exonic, UTRs and splice site); variant effect (non-synonymous); variant allele frequency ≥ 5% and not listed as a single nucleotide polymorphism, or listed but with an MAF < 0.01% (The Exome Aggregation Consortium, 1000 Genomes Project of the International Genome Sample Resource (IGSR), Single Nucleotide Polymorphism Database (dbSNP) v138 of the National Center for Biotechnology Information (NCBI)).

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