# Genetic evolution of *in situ* follicular neoplasia to aggressive B-cell lymphoma of germinal center subtype

**Antonio Vogelsberg,[1] Julia Steinhilber,[1] Barbara Mankel,[1] Birgit Federmann,[1] Janine Schmidt,[1] Ivonne A. Montes-Mojarro,[1] Katrin Hüttl,[2] Maria Rodriguez-Pinilla,[3] Praveen Baskaran,[4] Sven Nahnsen,[4] Miguel A. Piris,[3] German Ott,[2] Leticia Quintanilla-Martinez,[1] Irina Bonzheim,[1#] and Falko Fend[1#]**

[1]Institute of Pathology and Neuropathology, University Hospital and Comprehensive Cancer Center Tübingen, Tübingen, Germany; [2]Department of Clinical Pathology, Robert-Bosch-Krankenhaus, and Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany; [3]Department of Pathology, Fundación Jiménez Díaz, Madrid, Spain and [4]Quantitative Biology Center, University of Tübingen, Tübingen, Germany

*[#]IB and FF contributed equally as co-senior authors.*

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Correspondence: *FALKO FEND* - falko.fend@med.uni-tuebingen.de

*IRINA BONZHEIM* - irina.bonzheim@med.uni-tuebingen.de

**Supplementary Information**

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## Supplementary methods

### Diagnosis of *in situ* follicular neoplasia (ISFN)

The diagnosis of ISFN was based on the criteria published in the update of the 4th Edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.[1] Specifically, a diagnosis of ISFN was made when (1) the lymph node architecture was completely preserved, with normally sized follicles, and routine hematoxylin and eosin (H&E) stains gave no evidence of FL involvement, (2) all germinal centers involved by ISFN, as evidenced by strongly BCL2+ and CD10+ centrocytes, had a clearly preserved and well-delineated mantle zone, and (3) BCL2 and CD10 stains failed to show any indication of extrafollicular spread of ISFN cells.

### Microdissection and DNA isolation

Laser microdissection of ISFN samples was performed from 10 to 20 serial H&E sections with the first and every sixth slide stained for BCL2 to localize the ISFN lesions. After microdissection of between 13 and 65 germinal centers per slide, the tissue was pooled and digested with proteinase K (Merck, Darmstadt, Germany) and DNA extracted applying standard phenol/chloroform purification procedures.[2] If macrodissection of paraffin sections was performed, DNA was extracted using the Maxwell 16 MDx Instrument (Promega, Mannheim, Germany) according to the manufacturer's instructions.

### Immunohistochemistry and fluorescence *in situ* hybridization (FISH)

Immunohistochemistry was performed on an automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA). All ISFN samples were stained for BCL2, CD20, MIB1 (DAKO,

Hamburg, Germany) and CD10 (Novocastra, Wetzlar, Germany). High-grade B-cell lymphoma (HGBL), diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) samples were additionally stained for CD3 (DCS, Hamburg, Germany), BCL6 (Zytomed, Berlin, Germany), MUM1 (DAKO), MYC (Roche, Penzberg, Germany) and P53 (Novacastra). All aggressive B-cell lymphomas (BCLs) were sub-classified according to the Hans algorithm.[3] Images were taken with the Axioskop 2 Plus microscope (Zeiss, Oberkochen, Germany) and the Jenoptik ProgRes C10 Plus camera and software (Jenoptik, Jena, Germany). Objectives used were Plan-Neofluar x1.25/0.035, x2.5/0.075, x10/0.30, x20/0.50 and x40/0.75 (Zeiss). FISH analysis was performed using Vysis LSI BCL2, LSI BCL6 and LSI MYC Dual Color Break Apart Rearrangement Probes (Abbott Molecular, Wiesbaden, Germany) for the detection of *BCL2*, *MYC* and *BCL6* translocations, respectively. Case 5 was also analyzed with the Vysis LSI IGH/BCL2 Dual Color Dual Fusion Translocation Probe (Abbott Molecular) and the ZytoLight SPEC IGH Dual Color Break Apart Probe (ZytoVision, Bremershaven, Germany). For ISFN lesions, FISH for *BCL6* and *MYC* was performed only if the respective rearrangement had been detected in the paired aggressive BCL. Samples that carried a *TP53* mutation were analyzed with the Vysis LSI TP53 SpectrumOrange/CEP 17 SpectrumGreen Probe (Abbott Molecular) to investigate a loss of the second *TP53* allele.

**PCR and Sanger sequencing of the t(14;18) breakpoint region**

Forward primers used were 5’ TTAGAGAGTTGCTTTACGTGGCCTG 3’ for the major breakpoint region (MBR)[4], 5’ TCGTTCTCAGTAAGTGAGAGTGC 3’ for the intermediate cluster region (ICR)[5] and 5’ CGTGCTGGTACCACTCCTG 3’ for the minor cluster region (MCR)[6] as well as eight additional primers that cover a region of about 1 kilobase downstream of the MCR primer. The joining region consensus primer 5’ CTTACCTGAGGAGACGGTGACC 3’ was used as the reverse primer.[7] PCR was performed with 100 ng of purified DNA in a final volume of 25 µl using 0.4 mM dNTPs, 1.5 mM $MgCl_2$, 0.4 µM of each primer and 1.25 U Taq polymerase (AmpliTaq Gold DNA Polymerase; Applied Biosystems, Foster City, CA, USA). Cycling involved an initial denaturation at 95°C for 5 min followed by 45 cycles of denaturation (95°C for 45 s), annealing (60 s at 57°C for

MBR and MCR, 60 s at 56°C for the ICR) and elongation (72°C for 60 s), with a final elongation at 72°C for 10 min. To increase the detection rate, we additionally used the IdentiClone BCL2/JH Translocation Assay, which was performed according to the manufacturer's instructions (Invivoscribe, San Diego, CA, USA). PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA) and mixed with 1 µl of the respective primer (10 µM) and 2 µl of the GenomeLab DTCS-Quick Start Kit (Beckman Coulter) to a final volume of 10 µl for the sequencing reaction according to the manufacturer's protocol. Sequencing reactions were purified (CleanSEQ; Beckman Coulter), analyzed in a GenomeLab GeXP Genetic Analysis System (Beckman Coulter) and evaluated by the GenomeLab GeXP software 11.0 (Beckman Coulter) to investigate the t(14;18) breakpoint sequence.

For the ISFN samples of cases 3 and 9, primers specific to the breakpoint of the corresponding aggressive BCL were designed using the Primer3web software 4.1.0 (http://primer3.ut.ee/), with primers binding to the respective *BCL2* and t(14;18) *de novo* sequences.[8] Forward *BCL2* primers used were 5' AACACAGACCCACCCAGAG 3' (Case 3) and 5' GCTTTCTCATGGCTGTCCTT 3' (Case 9). Reverse *de novo* sequence primers used were 5' ATACCGTACGTCCGAAAGCA 3' (Case 3) and 5' GGGACCACATCGAGAAGC 3' (Case 9). PCR was performed with 100 ng of genomic DNA and modified annealing temperatures (54°C and 53°C). A successful amplification in the respective ISFN lesion was seen as evidence of the same t(14;18) breakpoint. Primer specificity was ensured using clonally unrelated t(14;18)+ samples as negative controls. Additionally, all PCR products were sequenced as stated above.

**Clonality analysis**

PCRs for the detection of immunoglobulin gene rearrangements were performed in duplicate with two different concentrations of genomic DNA using 1 U AmpliTaq Gold DNA polymerase (Applied Biosystems) and BIOMED-2 FR2, FR3, JH, and Vk, Jk, IntronRSS and Kde primers.[7] Modified amplification conditions were carried out with an initial denaturation step of 95°C (7 min), 40 cycles (95°C for 30 s, 60°C for 45 s, 72°C for 45 s) and a final step of 72°C

for 4 min. The JH, JK1-4, JK5 and Kde primers were modified with D4 fluorescent dyes (Sigma-Aldrich, St. Louis, MO, USA). For cases 6 and 10, clone-specific primers were designed based on the respective DLBCL framework region and complementarity-determining region 3 using the Primer3web software 4.1.0 (http://primer3.ut.ee/).[8] Forward primers used were 5' GAATATGCTGCGTCGGTGAA 3' (Case 6) and 5' ATGGAGTTGAGGAGGCTGAC 3' (Case 10). Reverse primers used were 5' TGTGGCTACGGACCTCTCTA 3' (Case 6) and 5' GCCCCAGACGTCCATAACAT 3' (Case 10). Reverse primers were modified with D4 fluorescent dyes (Sigma-Aldrich) and PCR was performed with 100 ng of genomic DNA and modified annealing temperatures (54°C and 53°C). For GeneScan analysis 1 µl of the PCR products were mixed with sample loading solution containing 30 µl DNA Size Standard 400 (Beckman Coulter). The products were separated by capillary electrophoresis on the GenomeLab GeXP Genetic Analysis System and analyzed by the GenomeLab GeXP software 11.0 (Beckman Coulter).

**Immunoglobulin sequence analysis**

Next generation sequencing (NGS) of the immunoglobulin genes was performed with the LymphoTrack Dx IGH FR1, FR2 and FR3 Assay – PGM (Invivoscribe) according to the manufacturer's instructions. Libraries were purified and quantified applying Agencourt AMPure XP (Beckman Coulter) magnetic beads and the Ion Library Quantitation Kit (Thermo Fisher Scientific, Waltham, MA, USA) on the LightCycler 480 real-time PCR system (Roche Molecular Systems, Pleasanton, CA, USA). Generated libraries were run on the Ion Torrent Personal Genome Machine (PGM; Thermo Fisher Scientific). NGS data were analyzed with the LymphoTrack Dx Software – PGM (Invivoscribe) and interpreted according to the manufacturer's protocol, which allows the detection of clonal immunoglobulin rearrangements with variable and joining gene usage and sequence information. Clonal sequences were submitted to IMGT/V-QUEST (http://www.imgt.org/ IMGT_vquest/vquest) and IgBlast (https://www.ncbi.nlm.nih.gov/igblast/) for analysis.[9,10] N-glycosylation motifs were identified by the consensus sequence Asn-X-Ser/Thr, where X can be any amino acid except proline.[11] To investigate intraclonal heterogeneity, the ten most prevalent clone-

specific sequences (i.e. subclones) of each sample were identified through the alignment of their sequence with that of the respective dominant rearrangement. Clear-cut sequencing artifacts, i.e. insertions/deletions (InDels) in homopolymer regions, InDels at the beginning of a sequence and changes of the first nucleotide were manually corrected to the sequence of the major clone. To calculate the share of each subclone, the sequence count was divided by the total number of clone-specific reads.

**Phylogenetic tree construction**

Phylogenetic trees for cases 1, 2, 4, 7, and 9 were built using the ten most prevalent subclones of the respective samples and the corresponding unmutated VDJ germline sequence, which was determined with IMGT/V-QUEST (http://www.imgt.org/IMGT_vquest/vquest) and IgBlast (https://www.ncbi.nlm.nih.gov/igblast/).[9,10] Multiple sequence alignments were generated using MAFFT (Version 7.4) with localpair alignment mode and max iteration of 1000.[12] jModelTest (Version 2.1) was used to find the best-fit substitution model for each multiple sequence alignment based on Bayesian information criteria strategy.[13] In summary, JC69 (Case 2), K80 + I (Case 4), and K80 (Cases 1, 7, and 9) were determined as most suitable. The construction of phylogenetic trees was done using the maximum likelihood method implemented in RAxML (Version 8.2).[14] The corresponding plots were generated in R (Version 3.4) (http://www.R-project.org/) using the "ape" and "phytools" packages.[15-17]

**Library preparation and sequencing**

Amplicon library preparation and semiconductor sequencing were performed according to the manufacturer's instructions (Thermo Fisher Scientific). For each reaction, 10 ng of DNA were mixed with AmpliSeq HiFi Mix (Thermo Fisher Scientific) and the respective primer pool to amplify the target regions. Subsequently, primer end sequences were partially digested using FuPa reagent (Thermo Fisher Scientific), followed by the ligation of barcoded sequencing adapters (Ion Xpress Barcode Adapters; Thermo Fisher Scientific). The final libraries were purified and quantified as described in "Immunoglobulin sequence analysis".

Libraries were diluted to 100 pM each and pooled. In the next step, DNA fragments were attached to Ion Sphere Particles (ISPs) and clonally amplified using the Ion PGM Hi-Q OT2 Kit (Thermo Fisher Scientific) and the Ion OneTouch Instrument (Thermo Fisher Scientific). The amount of template-positive ISPs was determined with the Qubit 3.0 Fluorometer (Life Technologies, Darmstadt, Germany) and the Ion Sphere Quality Control Kit (Thermo Fisher Scientific). Afterwards, the Ion OneTouch ES (Thermo Fisher Scientific) was used to enrich template-positive ISPs. In a last step, sequencing primers were attached to the DNA fragments bound to the ISPs, which were subsequently loaded on a semiconductor chip (Ion 318 Chip Kit; Thermo Fisher Scientific). Finally, sequencing was performed using the Ion PGM Hi-Q Sequencing Kit and the Ion Torrent PGM platform (Thermo Fisher Scientific).

**Targeted NGS data analysis**

Detection of variants in comparison to the human reference sequence (hg19) was performed using the Torrent Suite (Version 5.6.0) and the Ion Torrent Variant Caller (5.8.0.19) (Thermo Fisher Scientific). Detection thresholds were set at an allele frequency of 5%. Variants were annotated and filtered against the dbSNP and COSMIC databases using the Annotate variants single sample workflow of the Ion Reporter Software (Version 5.6) (Thermo Fisher Scientific). The Integrative Genomics Viewer (Version 2.3.94) (Broad Institute, Cambridge, MA, USA) software was used to inspect each detected variant to exclude possible artifacts.[18] Variants considered to be artifacts were those only detected in one sequencing direction and InDels at sites of homopolymer regions.[19] Caution was also exercised when variants occurred in regions with low coverage, especially concerning CG>TA transitions and/or alterations with VAFs <10%.[20] All sequences that harbored an alteration in at least one sample of a case were specifically reviewed in paired samples, even when not called by the Ion Reporter Software. If the mutation could not be detected in a paired sample, but the coverage was low (<100 reads), the location was reevaluated with bidirectional single amplicon sequencing to avoid a false negative result. Prediction of the deleteriousness of variants was done using the Combined Annotation Dependent Depletion (CADD) predictor (http://cadd.gs.washington.edu/home).[21] For the construction of clonal evolution patterns,

*s*ynonymous and 5' untranslated region (5'UTR) mutations of *BCL2* were taken into account as additional markers (Supplementary Table S5). Sequencing data are deposited in the European Nucleotide Archive (Accession number PRJEB34446).

**Variant validation and single amplicon sequencing**

To further exclude sequencing artifacts, the majority of variants were validated (see Supplementary Table S4). If *TP53* was mutated in an aggressive BCL, single amplicon sequencing was used to investigate if the mutation could be detected in paired ISFN and FL samples. Single amplicons were prepared following the Ion Amplicon Library Preparation Fusion Method protocol (Thermo Fisher Scientific). Primers were designed using the primer3 software 4.1.0 (http://primer3.ut.ee/).[8] The primers were composed of either the A adapter or the trP1 adapter, the barcode sequence and barcode adapter sequence, and the target primer sequence (Supplementary Table S2). Each gene region was amplified using two primer pairs (A Forward and trP1 Reverse or A Reverse and trP1 Forward) to enable bidirectional sequencing. Library preparation was done according to the manufacture's protocol (Thermo Fisher Scientific).

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## Supplementary tables

**Supplementary Table S1. Genes analyzed with AmpliSeq Custom Panels.**

| Gene | Position (GRCh37/hg19) | Exon(s) | Amplicons |
|---|---|---|---|
| *BCL2* | chr18: 60,795,858 - 60,985,965 | CDS | 9 |
| *BCL6* | chr3: 187,440,246 - 187,451,481 | CDS | 27 |
| *BTG1* | chr12: 92,537,856 - 92,539,311 | CDS | 7 |
| *BTG2* | chr1: 203,274,735 - 203,276,566 | CDS | 6 |
| *CARD11* | chr7: 2,946,272 - 2,998,140 | CDS | 54 |
| *CD79B* | chr17: 62,006,586 - 62,009,621 | CDS | 11 |
| *CREBBP* | chr16: 3,777,719 - 3,929,917 | CDS | 96 |
| *EP300* | chr22: 41,489,009 - 41,574,960 | CDS | 63 |
| *EZH2* | chr7: 148,508,712 - 148,508,817 | 16 | 1 |
| *FOXO1* | chr13: 41,133,660 - 41,240,349 | CDS | 10 |
| *GNA13* | chr17: 63,010,375 - 63,052,711 | CDS | 8 |
| *HIST1H1B* | chr6: 27,834,627 - 27,835,307 | CDS | 6 |
| *HIST1H1C* | chr6: 26,056,015 - 26,056,656 | CDS | 6 |
| *HIST1H1D* | chr6: 26,234,496 - 26,235,161 | CDS | 6 |
| *HIST1H1E* | chr6: 26,156,619 - 26,157,278 | CDS | 5 |
| *IGLL5* | chr22: 23,230,234 - 23,237,874 | CDS | 8 |
| *KMT2D* | chr12: 49,415,563 - 49,449,107 | CDS | 120 |
| *IRF4* | chr6: 393,153 - 407,598 | CDS | 18 |
| *MEF2B* | chr19: 19,256,503 - 19,261,544 | CDS | 11 |
| *MYD88* | chr3: 38,181,350 - 38,182,777 | 2-5 | 11 |
| *PIM1* | chr6: 37,138,079 - 37,141,867 | CDS | 16 |
| *PRDM1* | chr6: 106,534,429 -106,555,361 | CDS | 29 |
| *TBL1XR1* | chr3: 176,743,286 - 176,782,765 | CDS | 32 |
| *TNFAIP3* | chr6: 138,192,365 - 138,202,456 | CDS | 29 |
| *TNFRSF14* | chr1: 2,488,104 - 2,494,712 | CDS | 11 |

**CDS, coding sequence.**

**Supplementary Table S2. Primer sequences for targeted resequencing including the sequences of the A or trP1 adapter and the barcodes.**

| Primer | Sequence 5'-3' |
|---|---|
| GNA13 Ex4 326 BC50 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGACAATGGCGATTCCCCACTGCTTAAGAGACG |
| GNA13 Ex4 326 trP1F | CCTCTCTATGGGCAGTCGGTGATTCCCCACTGCTTAAGAGACG |
| GNA13 Ex4 326 BC50 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGACAATGGCGATTCCGTGTTGATAGCAGTGGT |
| GNA13 Ex4 326 trP1R | CCTCTCTATGGGCAGTCGGTGATTCCGTGTTGATAGCAGTGGT |
| TP53 Ex8 273 BC51 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGAGCCTATTCGATTTGCTTCTCTTTTCCTATCCTGA |
| TP53 Ex8 273 trP1F | CCTCTCTATGGGCAGTCGGTGATTTGCTTCTCTTTTCCTATCCTGA |
| TP53 Ex8 273 BC51 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGAGCCTATTCGATTCTTGCGGAGATTCTCTTCCT |
| TP53 Ex8 273 trP1R | CCTCTCTATGGGCAGTCGGTGATTCTTGCGGAGATTCTCTTCCT |
| CREBBP Ex7 551 BC52 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGCATGGAACGATTCCAATGAACATTCCAGCAGG |
| CREBBP Ex7 551 trP1F | CCTCTCTATGGGCAGTCGGTGATTCCAATGAACATTCCAGCAGG |
| CREBBP Ex7 551 BC52 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGCATGGAACGATCAGGGTCTTACTTTGTGGCC |
| CREBBP Ex7 551 trP1R | CCTCTCTATGGGCAGTCGGTGATCAGGGTCTTACTTTGTGGCC |
| CREBBP Ex30 1680 BC60 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTAGCTCTTCGATCTTCCTCACCCTCGCCAG |
| CREBBP Ex30 1680 trP1F | CCTCTCTATGGGCAGTCGGTGATCTTCCTCACCCTCGCCAG |
| CREBBP Ex30 1680 BC60 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTAGCTCTTCGATATGCAGAGCGTGGACCAC |
| CREBBP Ex30 1680 trP1R | CCTCTCTATGGGCAGTCGGTGATATGCAGAGCGTGGACCAC |
| KMT2D Ex31 2623 BC61 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACTCGGATCGATTGTCCCCACTACGCCCTC |

| | |
|---|---|
| KMT2D Ex31 2623 trP1F | CCTCTCTATGGGCAGTCGGTGATTGTCCCCACTACGCCCTC |
| KMT2D Ex31 2623 BC61 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACTCGGATCGATGATCGCTGTGAGGCTCCAT |
| KMT2D Ex31 2623 trP1R | CCTCTCTATGGGCAGTCGGTGATGATCGCTGTGAGGCTCCAT |
| KMT2D Ex22 1739 BC62 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCTGCTTCACGATACACTTCCGTTCTGTCCACA |
| KMT2D Ex22 1739 trP1F | CCTCTCTATGGGCAGTCGGTGATACACTTCCGTTCTGTCCACA |
| KMT2D Ex22 1739 BC62 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCTGCTTCACGATTCTTCTCATCCCCTTCAGCT |
| KMT2D Ex22 1739 trP1R | CCTCTCTATGGGCAGTCGGTGATTCTTCTCATCCCCTTCAGCT |
| TP53 Ex5 150 BC63 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTAGAGTTCGATGCCAAGACCTGCCCTGTG |
| TP53 Ex5 150 trP1F | CCTCTCTATGGGCAGTCGGTGATGCCAAGACCTGCCCTGTG |
| TP53 Ex5 150 BC63 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTAGAGTTCGATCATGTGCTGTGACTGCTTGT |
| TP53 Ex5 150 trP1R | CCTCTCTATGGGCAGTCGGTGATCATGTGCTGTGACTGCTTGT |
| BCL2 Ex2 101-113 BC64 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGAGTTCCGACGATACCTGACCCTCCGCCA |
| BCL2 Ex2 101-113 trP1F | CCTCTCTATGGGCAGTCGGTGATACCTGACCCTCCGCCA |
| BCL2 Ex2 101-113 BC64 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGAGTTCCGACGATGGTGAAGGGCGTCAGGT |
| BCL2 Ex2 101-113 trP1R | CCTCTCTATGGGCAGTCGGTGATGGTGAAGGGCGTCAGGT |
| BCL2 Ex2 33 BC65 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTGGCACATCGATCAGAGGGGCTACGAGTGG |
| BCL2 Ex2 33 trP1F | CCTCTCTATGGGCAGTCGGTGATCAGAGGGGCTACGAGTGG |
| BCL2 Ex2 33 BC65 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTGGCACATCGATGGGCTGGGAGGAGAAGATG |
| BCL2 Ex2 33 trP1R | CCTCTCTATGGGCAGTCGGTGATGGGCTGGGAGGAGAAGATG |
| BCL2 Ex2 6 BC66 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGCAATCATCGATGCGAGAGGTGCCGTTG |
| BCL2 Ex2 6 trP1F | CCTCTCTATGGGCAGTCGGTGATGCGAGAGGTGCCGTTG |
| BCL2 Ex2 6 BC66 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGCAATCATCGATACTTCATCACTATCTCCCGGT |
| BCL2 Ex2 6 trP1R | CCTCTCTATGGGCAGTCGGTGATACTTCATCACTATCTCCCGGT |
| CREBBP Ex27 1503 BC57 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTGGCAACGGCGATATTGCCACCCACCTGATCAA |
| CREBBP Ex27 1503 trP1F | CCTCTCTATGGGCAGTCGGTGATATTGCCACCCACCTGATCAA |
| CREBBP Ex27 1503 BC57 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTGGCAACGGCGATGGATGATCCGCTCTGCAAAC |
| CREBBP Ex27 1503 trP1R | CCTCTCTATGGGCAGTCGGTGATGGATGATCCGCTCTGCAAAC |
| CARD11 Ex20 871 BC58 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTAGAACACGATAGGGCCTGACTGATTGATAAAT |
| CARD11 Ex20 871 trP1F | CCTCTCTATGGGCAGTCGGTGATAGGGCCTGACTGATTGATAAAT |
| CARD11 Ex20 871 BC58 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTAGAACACGATCTGAAGGAGCTGGCCAAAA |
| CARD11 Ex20 871 trP1R | CCTCTCTATGGGCAGTCGGTGATCTGAAGGAGCTGGCCAAAA |
| TP53 Ex5 179 BC70 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTACTGGTCGATCAAGCAGTCACAGCACATGA |
| TP53 Ex5 179 trP1F | CCTCTCTATGGGCAGTCGGTGATCAAGCAGTCACAGCACATGA |
| TP53 Ex5 179 BC70 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTACTGGTCGATCTGCTCACCATCGCTATCTG |
| TP53 Ex5 179 trP1R | CCTCTCTATGGGCAGTCGGTGATCTGCTCACCATCGCTATCTG |
| BCL2 Ex2 20-43 BC55 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCACCTCCTCGATCCCCGTTGCTTTTCCTCTG |
| BCL2 Ex2 20-43 trP1F | CCTCTCTATGGGCAGTCGGTGATCCCCGTTGCTTTTCCTCTG |
| BCL2 Ex2 20-43 BC55 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCACCTCCTCGATGGGCTGGGAGGAGAAGATG |
| BCL2 Ex2 20-43 trP1R | CCTCTCTATGGGCAGTCGGTGATGGGCTGGGAGGAGAAGATG |
| CD79B Ex5 196 BC56 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGCATTAATTCGATGATCTCCATCCCTCTCCGC |
| CD79B Ex5 196 trP1F | CCTCTCTATGGGCAGTCGGTGATGATCTCCATCCCTCTCCGC |
| CD79B Ex5 196 BC56 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGCATTAATTCGATCCCAACCACACCAGCAGATA |
| CD79B Ex5 196 trP1R | CCTCTCTATGGGCAGTCGGTGATCCCAACCACACCAGCAGATA |
| KMT2D Ex39 4473 BC57 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTGGCAACGGCGATGAACCAGCAACCACCTCCT |
| KMT2D Ex39 4473 trP1F | CCTCTCTATGGGCAGTCGGTGATGAACCAGCAACCACCTCCT |
| KMT2D Ex39 4473 BC57 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTGGCAACGGCGATAATGTGCCCGTTGATCTCAG |
| KMT2D Ex39 4473 trP1R | CCTCTCTATGGGCAGTCGGTGATAATGTGCCCGTTGATCTCAG |
| GNA13 Ex4 203 BC58 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTAGAACACGATCGCATTACTTCGGGATTAATAGG |
| GNA13 Ex4 203 trP1F | CCTCTCTATGGGCAGTCGGTGATCGCATTACTTCGGGATTAATAGG |
| GNA13 Ex4 203 BC58 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTAGAACACGATTCTGACCACCTACATCAACCA |
| GNA13 Ex4 203 trP1R | CCTCTCTATGGGCAGTCGGTGATTCTGACCACCTACATCAACCA |
| TNFRSF14 Ex6 187 BC59 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTTGATGTTCGATTGCCTCTCCCACGTCCTC |
| TNFRSF14 Ex6 187 trP1F | CCTCTCTATGGGCAGTCGGTGATTGCCTCTCCCACGTCCTC |
| TNFRSF14 Ex6 187 BC59 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTTGATGTTCGATTGTGGAGCAAACAATGACGA |
| TNFRSF14 Ex6 187 trP1R | CCTCTCTATGGGCAGTCGGTGATTGTGGAGCAAACAATGACGA |
| CARD11 Ex23 1046 BC60 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTAGCTCTTCGATCTCAGAAGGCAGAAGACGGA |
| CARD11 Ex23 1046 trP1F | CCTCTCTATGGGCAGTCGGTGATCTCAGAAGGCAGAAGACGGA |
| CARD11 Ex23 1046 BC60 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTAGCTCTTCGATCATCCAACCTCCCAGTCCC |
| CARD11 Ex23 1046 trP1R | CCTCTCTATGGGCAGTCGGTGATCATCCAACCTCCCAGTCCC |
| CREBBP Ex27 1482 BC54 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGAGAATCGCGATCCTGTCCTCCAAGTGAAGGA |
| CREBBP Ex27 1482 trP1F | CCTCTCTATGGGCAGTCGGTGATCCTGTCCTCCAAGTGAAGGA |
| CREBBP Ex27 1482 BC54 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGAGAATCGCGATCAGTCGTTTTGGCTTGGGTA |
| CREBBP Ex27 1482 trP1R | CCTCTCTATGGGCAGTCGGTGATCAGTCGTTTTGGCTTGGGTA |
| BCL2 Ex2 79-90 BC57 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTGGCAACGGCGATCATCTTCTCCTCCCAGCCC |
| BCL2 Ex2 79-90 trP1F | CCTCTCTATGGGCAGTCGGTGATCATCTTCTCCTCCCAGCCC |
| BCL2 Ex2 79-90 BC57 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTGGCAACGGCGATGTAGCGGCGGGAGAAGTC |
| BCL2 Ex2 79-90 trP1R | CCTCTCTATGGGCAGTCGGTGATGTAGCGGCGGGAGAAGTC |
| PIM1 Ex1 1 BC61 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACTCGGATCGATTGCGCCGACATCCTGGA |
| PIM1 Ex1 1 trP1F | CCTCTCTATGGGCAGTCGGTGATTGCGCCGACATCCTGGA |
| PIM1 Ex1 1 BC61 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACTCGGATCGATTTGGTGGCGTGCAGGTC |
| PIM1 Ex1 1 trP1R | CCTCTCTATGGGCAGTCGGTGATTTGGTGGCGTGCAGGTC |
| KMT2D Ex10 468-477 BC62 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCTGCTTCACGATGGAATCACCCACGTCCCC |
| KMT2D Ex10 468-477 trP1F | CCTCTCTATGGGCAGTCGGTGATGGAATCACCCACGTCCCC |
| KMT2D Ex10 468-477 BC62 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCTGCTTCACGATAGGTGCAATGCCTCAGGA |
| KMT2D Ex10 468-477 trP1R | CCTCTCTATGGGCAGTCGGTGATAGGTGCAATGCCTCAGGA |
| GNA13 Ex1 53-54 BC63 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTAGAGTTCGATTATGTGAAGCGGCTGGTGAA |
| GNA13 Ex1 53-54 trP1F | CCTCTCTATGGGCAGTCGGTGATTATGTGAAGCGGCTGGTGAA |
| GNA13 Ex1 53-54 BC63 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTAGAGTTCGATTCCTGCCCGTGGATGATC |
| GNA13 Ex1 53-54 trP1R | CCTCTCTATGGGCAGTCGGTGATTCCTGCCCGTGGATGATC |
| GNA13 Ex4 222 BC64 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGAGTTCCGACGATGAAGACCCACCAAAGGCATC |
| GNA13 Ex4 222 trP1F | CCTCTCTATGGGCAGTCGGTGATGAAGACCCACCAAAGGCATC |
| GNA13 Ex4 222 BC64 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGAGTTCCGACGATTCCTTTCTGATCTCTGACCACC |
| GNA13 Ex4 222 trP1R | CCTCTCTATGGGCAGTCGGTGATTCCTTTCTGATCTCTGACCACC |

| | |
|---|---|
| MEF2B Ex5 77 BC65 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTGGCACATCGATCCAACCGCCTCTTCCAGTAT |
| MEF2B Ex5 77 trP1F | CCTCTCTATGGGCAGTCGGTGATCCAACCGCCTCTTCCAGTAT |
| MEF2B Ex5 77 BC65 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTGGCACATCGATGAGGATGTCAGTGTTGGTGC |
| MEF2B Ex5 77 trP1R | CCTCTCTATGGGCAGTCGGTGATGAGGATGTCAGTGTTGGTGC |
| HIST1H1D Ex1 77 BC50 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGACAATGGCGATTCTGGCCGCGCTTAAGAAA |
| HIST1H1D Ex1 77 trP1F | CCTCTCTATGGGCAGTCGGTGATTCTGGCCGCGCTTAAGAAA |
| HIST1H1D Ex1 77 BC50 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGACAATGGCGATTTGAGGCCAAGCTTGATACG |
| HIST1H1D Ex1 77 trP1R | CCTCTCTATGGGCAGTCGGTGATTTGAGGCCAAGCTTGATACG |
| EP300 Ex5 415 BC51 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGAGCCTATTCGATCTCGACAAATCATTTCACACTGG |
| EP300 Ex5 415 trP1F | CCTCTCTATGGGCAGTCGGTGATCTCGACAAATCATTTCACACTGG |
| EP300 Ex5 415 BC51 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGAGCCTATTCGATTCACTTACGCTGTTGATTTCTCT |
| EP300 Ex5 415 trP1R | CCTCTCTATGGGCAGTCGGTGATTCACTTACGCTGTTGATTTCTCT |
| BCL2 Ex2 76 BC52 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGCATGGAACGATCCCCATCCAGCCGCAT |
| BCL2 Ex2 76 trP1F | CCTCTCTATGGGCAGTCGGTGATCCCCATCCAGCCGCAT |
| BCL2 Ex2 76 BC52 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGCATGGAACGATTGGCGGAGGGTCAGGT |
| BCL2 Ex2 76 trP1R | CCTCTCTATGGGCAGTCGGTGATTGGCGGAGGGTCAGGT |
| TNFRSF14 Ex1 12 BC70 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTACTGGTCGATTGCCGGTCTGAGCCTGAG |
| TNFRSF14 Ex1 12 trP1F | CCTCTCTATGGGCAGTCGGTGATTGCCGGTCTGAGCCTGAG |
| TNFRSF14 Ex1 12 BC70 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTACTGGTCGATAGCCTCAAGACGTCGGTTTT |
| TNFRSF14 Ex1 12 trP1R | CCTCTCTATGGGCAGTCGGTGATAGCCTCAAGACGTCGGTTTT |
| TNFRSF14 Ex6 219 BC61 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACTCGGATCGATATGGTGGTTTCTCTCAGGGA |
| TNFRSF14 Ex6 219 trP1F | CCTCTCTATGGGCAGTCGGTGATATGGTGGTTTCTCTCAGGGA |
| TNFRSF14 Ex6 219 BC61 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACTCGGATCGATCCCCTTGGCTTTCTTCTTTTCA |
| TNFRSF14 Ex6 219 trP1R | CCTCTCTATGGGCAGTCGGTGATCCCCTTGGCTTTCTTCTTTTCA |
| KMT2D Ex48 4987 BC62 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCTGCTTCACGATCTCCTCGCCTCAAGAAATGG |
| KMT2D Ex48 4987 trP1F | CCTCTCTATGGGCAGTCGGTGATCTCCTCGCCTCAAGAAATGG |
| KMT2D Ex48 4987 BC62 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCTGCTTCACGATCTTCCCGCTCATCCTCCTG |
| KMT2D Ex48 4987 trP1R | CCTCTCTATGGGCAGTCGGTGATCTTCCCGCTCATCCTCCTG |
| EZH2 Ex16 646 BC63 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTAGAGTTCGATTATTGCTGGCACCATCTGAC |
| EZH2 Ex16 646 trP1F | CCTCTCTATGGGCAGTCGGTGATTATTGCTGGCACCATCTGAC |
| EZH2 Ex16 646 BC63 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTAGAGTTCGATTGAATACAGGTTATCAGTGCCTT |
| EZH2 Ex16 646 trP1R | CCTCTCTATGGGCAGTCGGTGATTGAATACAGGTTATCAGTGCCTT |
| BCL2 Ex2 8 BC64 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGAGTTCCGACGATCTCTGGGAAGGATGGCGC |
| BCL2 Ex2 8 trP1F | CCTCTCTATGGGCAGTCGGTGATCTCTGGGAAGGATGGCGC |
| BCL2 Ex2 8 BC64 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGAGTTCCGACGATCACTCGTAGCCCCTCTGC |
| BCL2 Ex2 8 trP1R | CCTCTCTATGGGCAGTCGGTGATCACTCGTAGCCCCTCTGC |
| PIM1 Ex3 79 BC65 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTGGCACATCGATCGTGGAGAAGGACCGGATTT |
| PIM1 Ex3 79 trP1F | CCTCTCTATGGGCAGTCGGTGATCGTGGAGAAGGACCGGATTT |
| PIM1 Ex3 79 BC65 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTGGCACATCGATCTCACCCCACCCACTCATC |
| PIM1 Ex3 79 trP1R | CCTCTCTATGGGCAGTCGGTGATCTCACCCCACCCACTCATC |
| TNFRSF14 Ex8 266 BC66 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGCAATCATCGATCACAGCGGAAAAGACAGGAG |
| TNFRSF14 Ex8 266 trP1F | CCTCTCTATGGGCAGTCGGTGATCACAGCGGAAAAGACAGGAG |
| TNFRSF14 Ex8 266 BC66 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGCAATCATCGATTCAGTGGTTTGGGCTCCTC |
| TNFRSF14 Ex8 266 trP1R | CCTCTCTATGGGCAGTCGGTGATTCAGTGGTTTGGGCTCCTC |
| BCL2 Ex2 10-11 BC53 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGGCAATCCTCGATCCGTTGCTTTTCCTCTGGG |
| BCL2 Ex2 10-11 trP1F | CCTCTCTATGGGCAGTCGGTGATCCGTTGCTTTTCCTCTGGG |
| BCL2 Ex2 10-11 BC53 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGGCAATCCTCGATCATCTCCCGCATCCCACTC |
| BCL2 Ex2 10-11 trP1R | CCTCTCTATGGGCAGTCGGTGATCATCTCCCGCATCCCACTC |
| CARD11 Ex5 250 BC54 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGAGAATCGCGATGGAGGAGGAATGTAAGCTGGA |
| CARD11 Ex5 250 trP1F | CCTCTCTATGGGCAGTCGGTGATGGAGGAGGAATGTAAGCTGGA |
| CARD11 Ex5 250 BC54 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGAGAATCGCGATCCTTCTTGGGCCGATTTTCA |
| CARD11 Ex5 250 trP1R | CCTCTCTATGGGCAGTCGGTGATCCTTCTTGGGCCGATTTTCA |
| HIST1H1B Ex1 107 BC55 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCACCTCCTCGATCTTCTAAGGAGCGCAATGGC |
| HIST1H1B Ex1 107 trP1F | CCTCTCTATGGGCAGTCGGTGATCTTCTAAGGAGCGCAATGGC |
| HIST1H1B Ex1 107 BC55 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCACCTCCTCGATAGGCCGCCTTCTTGTTGA |
| HIST1H1B Ex1 107 trP1R | CCTCTCTATGGGCAGTCGGTGATAGGCCGCCTTCTTGTTGA |
| BCL2 Ex2 135 BC56 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGCATTAATTCGATTTCGCCGAGATGTCCAGC |
| BCL2 Ex2 135 trP1F | CCTCTCTATGGGCAGTCGGTGATTTCGCCGAGATGTCCAGC |
| BCL2 Ex2 135 BC56 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGCATTAATTCGATCCGAACTCAAAGAAGGCCAC |
| BCL2 Ex2 135 trP1R | CCTCTCTATGGGCAGTCGGTGATCCGAACTCAAAGAAGGCCAC |
| KMT2D Ex10 831 BC59 AF | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTTGATGTTCGATCCTGTCTCCTGTGCCTGAG |
| KMT2D Ex10 831 trP1F | CCTCTCTATGGGCAGTCGGTGATCCTGTCTCCTGTGCCTGAG |
| KMT2D Ex10 831 BC59 AR | CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTTGATGTTCGATTCAGGGGACAGATGCGATT |
| KMT2D Ex10 831 trP1R | CCTCTCTATGGGCAGTCGGTGATTCAGGGGACAGATGCGATT |

**Supplementary Table S3. Immunohistochemical findings of FL and aggressive BCL samples.**

| Case | Diagnosis | CD10 | BCL6 | MUM1 | BCL2 | MIB-1 (%) | CD20 | P53* | MYC# (%) |
|---|---|---|---|---|---|---|---|---|---|
| De novo aggressive B-cell lymphoma | | | | | | | | | |
| 1 | HGBL-TH | + | + | - | + | 70 | + | + | 40 (w) |
| 2 | DLBCL | + | + | - | + | 40 | + | -˚ | - |
| 3 | DLBCL | + | + | - | + | 70 | + | + | - |
| | DLBCL | + | + | - | + | 70 | + | + | - |
| 4 | DLBCL | + | + | - | + | 70 | + | - | 30 (h) |
| 5 | DLBCL | + | + | - | + | 40 | + | - | 5 (s) |
| 6 | DLBCL | + | + | - | + | 30 | + | - | - |
| Transformed FL | | | | | | | | | |
| 7 | FL | + | + | - | + | 10 | + | - | - |
| | DLBCL | + | + | + | + | 90 | + | - | 30 (s) |
| 8 | HGBL-DH | + | + | - | + | 80 | + | - | 60 (s) |
| 9 | FL | + | + | - | + | 5 | + | - | - |
| | HGBL-DH | + | + | - | + | 70 | + | - | 40 (h) |
| 10 | FL | + | + | - | + | N/A | + | - | - |
| | DLBCL | + | + | - | + | 50 | + | - | 15 (s) |

DH, Double-hit; N/A, Not available; TH, Triple-hit. *Only samples with a strong staining of ≥ 20% of neoplastic cells were considered positive. #Percentages represent the share of positive lymphoma cells with strong (s), heterogeneous (h) or weak (w) staining. ˚Complete loss in the neoplastic cells.

**Supplementary Table S4. Overview of non-synonymous and splice site mutations.**

| Case | Diagnosis | Gene | Transcript | Predicted protein change | cDNA change | VAF (%) | Coverage | Validation | CADD Score* |
|---|---|---|---|---|---|---|---|---|---|
| De novo aggressive B-cell lymphoma | | | | | | | | | |
| 1 | ISFN | *BCL2* | NM_000633 | p.D31N | c.91G>A | 15 | 3210 | Confirmed | 22.0 |
| | HGBL-TH | *BCL2* | NM_000633 | p.T56S | c.166A>T | 42 | 2427 | ND | 10.65 |
| | | *BCL2* | NM_000633 | p.L86S | c.256_257delinsTC | 45 | 2426 | ND | 21.6 |
| | | *TP53* | NM_000546 | p.R273C | c.817C>T | 52 | 8697 | Confirmed | 25.3 |
| | | *GNA13* | NM_006572 | p.L326R | c.977T>G | 8 | 2322 | Confirmed | 32 |
| 2 | ISFN | ***BCL2*** | **NM_000633** | **p.G33R** | **c.97G>A** | **8** | **3266** | **Confirmed** | **15.30** |
| | | *BCL2* | NM_000633 | p.G101A | c.302G>C | 7 | 7474 | Confirmed | 25.1 |
| | | ***BCL2*** | **NM_000633** | **p.A113G** | **c.338C>G** | **7** | **7482** | **Confirmed** | **13.61** |
| | | ***CREBBP*** | **NM_004380** | **p.S1680del** | **c.5039_5041del** | **28** | **588** | **Confirmed** | **22.7** |
| | DLBCL | *BCL2* | NM_000633 | p.R6G | c.16A>G | 20 | 2481 | Confirmed | 21.2 |
| | | ***BCL2*** | **NM_000633** | **p.G33R** | **c.97G>A** | **21** | **3097** | **Confirmed** | **15.30** |
| | | ***BCL2*** | **NM_000633** | **p.A113G** | **c.338C>G** | **22** | **6631** | **Confirmed** | **13.61** |
| | | ***CREBBP*** | **NM_004380** | **p.S1680del** | **c.5039_5041del** | **67** | **565** | **Confirmed** | **22.7** |
| | | *TP53* | NM_000546 | p.T150fs | c.447_459del | 52 | 9399 | ND | 28.7 |
| | | *CARD11* | NM_032415 | p.Q249P | c.746A>C | 46 | 7624 | Confirmed | 27.1 |
| 3 | ISFN | ***CREBBP*** | **NM_004380** | **p.Y1503D** | **c.4507T>G** | **16** | **1673** | **Confirmed** | **29.4** |
| | | ***CREBBP*** | **NM_004380** | **p.N1589fs** | **c.4767del** | **15** | **1171** | **ND** | **26.3** |
| | DLBCL | *BCL2* | NM_000633 | p.W214C | c.642G>T | 51 | 32774 | ND | 32 |
| | | *KMT2D* | NM_003482 | p.I5455fs | c.16365_16371del | 47 | 4421 | ND | 36 |
| | | ***CREBBP*** | **NM_004380** | **p.Y1503D** | **c.4507T>G** | **26** | **8807** | **Confirmed** | **29.4** |
| | | ***CREBBP*** | **NM_004380** | **p.N1589fs** | **c.4767del** | **45** | **1983** | **ND** | **26.3** |
| | | *TP53* | NM_000546 | p.H179N | c.535C>A | 83 | 3576 | ND | 28.2 |
| | DLBCL | *BCL2* | NM_000633 | p.W214C | c.642G>T | 60 | 14102 | ND | 32 |
| | | *KMT2D* | NM_003482 | p.I5455fs | c.16365_16371del | 28 | 491 | ND | 36 |
| | | ***CREBBP*** | **NM_004380** | **p.Y1503D** | **c.4507T>G** | **21** | **1385** | **Confirmed** | **29.4** |
| | | ***CREBBP*** | **NM_004380** | **p.N1589fs** | **c.4767del** | **42** | **1187** | **ND** | **26.3** |
| | | *TP53* | NM_000546 | p.H179N | c.535C>A | 84 | 3949 | ND | 28.2 |
| 4 | ISFN | ***BCL2*** | **NM_000633** | **p.A76D** | **c.227C>A** | **44** | **1015** | **Confirmed** | **13.25** |
| | | ***TNFRSF14*** | **NM_003820** | **p.W12*** | **c.35G>A** | **25**[#] | **7259**[#] | **Confirmed** | **35** |
| | | ***HIST1H1D*** | **NM_005320** | **p.N77K** | **c.231C>G** | **31** | **6107** | **Confirmed** | **24.8** |
| | | ***EP300*** | **NM_001429** | **p.L415P** | **c.1244T>C** | **24** | **841** | **Confirmed** | **29.3** |
| | DLBCL | *BCL2* | NM_000633 | p.P59S | c.175C>T | 24 | 5580 | ND | 13.71 |
| | | ***BCL2*** | **NM_000633** | **p.A76D** | **c.227C>A** | **23** | **5649** | **Confirmed** | **13.25** |
| | | *KMT2D* | NM_003482 | Splice site | c.10507+2T>C | 18 | 3764 | ND | 34 |
| | | *EZH2* | NM_004456 | p.Y646N | c.1936T>A | 32 | 2152 | ND | 32 |
| | | ***TNFRSF14*** | **NM_003820** | **p.W12*** | **c.35G>A** | **14** | **182** | **Confirmed** | **35** |
| | | ***HIST1H1D*** | **NM_005320** | **p.N77K** | **c.231C>G** | **13** | **8350** | **Confirmed** | **24.8** |
| | | ***EP300*** | **NM_001429** | **p.L415P** | **c.1244T>C** | **19** | **1200** | **Confirmed** | **29.3** |
| 5 | ISFN | *BCL2* | NM_000633 | p.P53A | c.157C>G | 28 | 2009 | Confirmed | 10.60 |
| | | *BCL2* | NM_000633 | p.R129C | c.385C>T | 13 | 7370 | Confirmed | 24.3 |
| | | *BCL2* | NM_000633 | p.F153L | c.457T>C | 8 | 8140 | Confirmed | 32 |
| | | ***KMT2D*** | **NM_003482** | **p.Q4473*** | **c.13417C>T** | **24** | **702** | **Confirmed** | **43** |
| | | *CREBBP* | NM_004380 | p.V1371D | c.4112T>A | 30 | 956 | ND | 29.4 |
| | | ***IGLL5*** | **NM_001256296** | **p.C3S** | **c.8G>C** | **25** | **1331** | **ND** | **0.018** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 5 | DLBCL | *BCL2* | NM_000633 | p.P59S | c.175C>T | 51 | 2893 | ND | 13.71 |
| | | *BCL2* | NM_000633 | p.A82T | c.244G>A | 50 | 3013 | ND | 12.65 |
| | | *BCL2* | NM_000633 | p.D102G | c.305A>G | 40 | 8896 | ND | 26.2 |
| | | ***KMT2D*** | **NM_003482** | **p.Q4473*** | **c.13417C>T** | **30** | **4234** | **Confirmed** | **43** |
| | | *EZH2* | NM_004456 | p.Y646F | c.1937A>T | 27 | 10985 | ND | 25.3 |
| | | ***IGLL5*** | **NM_001256296** | **p.C3S** | **c.8G>C** | **54** | **1658** | **ND** | **0.018** |
| | | *IGLL5* | NM_001256296 | p.G13W | c.37G>T | 66 | 1449 | ND | 5.721 |
| | | *GNA13* | NM_006572 | p.M68R | c.203T>G | 34 | 18503 | ND | 24.1 |
| | | *GNA13* | NM_006572 | p.D155A | c.464A>C | 35 | 9386 | ND | 27.7 |
| | | *GNA13* | NM_006572 | p.T203A | c.607A>G | 38 | 118 | Confirmed | 23.8 |
| | | *HIST1H1D* | NM_005320 | p.T93S | c.278C>G | 45 | 21464 | ND | 23.3 |
| | | *MEF2B* | NM_001145785 | p.R3M | c.8G>T | 36 | 13251 | ND | 26.1 |
| | | *CD79B* | NM_000626 | p.Y196H | c.586T>C | 38 | 5105 | Confirmed | 24.6 |
| 6 | ISFN | *BCL2* | NM_000633 | p.G5V | c.14G>T | 14 | 3104 | Confirmed | 25.5 |
| | | *BCL2* | NM_000633 | p.A42V | c.125C>T | 15 | 8584 | Confirmed | 15.15 |
| | | *BCL2* | NM_000633 | p.S87R | c.261C>A | 26 | 1338 | Confirmed | 21.2 |
| | | *KMT2D* | NM_003482 | p.S468* | c.1403C>A | 13 | 9012 | Confirmed | 35 |
| | | *KMT2D* | NM_003482 | p.S477P | c.1429T>C | 13 | 13514 | Confirmed | 14.21 |
| | | *CREBBP* | NM_004380 | p.Y1503D | c.4507T>G | 8 | 6881 | Confirmed | 29.4 |
| | | *IGLL5* | NM_001256296 | p.P19S | c.55C>T | 16 | 694 | ND | 10.87 |
| | | *IGLL5* | NM_001256296 | p.A30V | c.89C>T | 26 | 702 | ND | 5.331 |
| | | *GNA13* | NM_006572 | p.L54* | c.159_161delinsCTA | 15 | 15333 | Confirmed | 35 |
| | | *GNA13* | NM_006572 | p.D222N | c.664G>A | 17 | 695 | Confirmed | 32 |
| | | *MEF2B* | NM_001145785 | p.E77A | c.230A>C | 14 | 1248 | Confirmed | 27.9 |
| | | ***TBL1XR1*** | **NM_024665** | **p.L198*** | **c.592_609delinsT** | **34** | **16045** | **ND** | **35** |
| | | *PIM1* | NM_002648 | p.M1I | c.3G>A | 13 | 2241 | Confirmed | 24.1 |
| | DLBCL | *BCL2* | NM_000633 | p.L86V | c.256C>G | 12 | 1902 | Confirmed | 18.04 |
| | | *BCL2* | NM_000633 | p.P90S | c.268C>T | 23 | 1911 | Confirmed | 23.9 |
| | | *BCL2* | NM_000633 | p.F153L | c.457T>C | 12 | 14816 | ND | 32 |
| | | *BCL2* | NM_000633 | p.V162D | c.485T>A | 10 | 5631 | ND | 26.5 |
| | | *EZH2* | NM_004456 | p.Y646C | c.1937A>G | 27 | 2218 | ND | 25.7 |
| | | *CREBBP* | NM_004380 | p.Y1482S | c.4445A>C | 15 | 6079 | Confirmed | 28.4 |
| | | *MEF2B* | NM_001145785 | p.D83V | c.248A>T | 22 | 1813 | ND | 26.5 |
| | | ***TBL1XR1*** | **NM_024665** | **p.L198*** | **c.592_609delinsT** | **23** | **10662** | **ND** | **35** |
| Transformed FL | | | | | | | | | |
| 7 | ISFN | ***BCL2*** | **NM_000633** | **p.G8E** | **c.23G>A** | **12** | **1398** | **Confirmed** | **28.1** |
| | | ***KMT2D*** | **NM_003482** | **p.W4987*** | **c.14960G>A** | **9** | **7593** | **Confirmed** | **45** |
| | | ***TNFRSF14*** | **NM_003820** | **p.V219G** | **c.656T>G** | **15**[#] | **46363**[#] | **Confirmed** | **1.922** |
| | FL | ***BCL2*** | **NM_000633** | **p.G8E** | **c.23G>A** | **23** | **1629** | **Confirmed** | **28.1** |
| | | ***KMT2D*** | **NM_003482** | **p.W4987*** | **c.14960G>A** | **25** | **12168** | **Confirmed** | **45** |
| | | *EZH2* | NM_004456 | p.Y646N | c.1936T>A | 14 | 334 | Confirmed | 32 |
| | | *CREBBP* | NM_004380 | p.C1237Y | c.3710G>A | 24 | 5787 | ND | 31 |
| | | *CREBBP* | NM_004380 | p.K1586fs | c.4755del | 23 | 1718 | ND | 35 |
| | | ***TNFRSF14*** | **NM_003820** | **p.V219G** | **c.656T>G** | **29** | **164** | **Confirmed** | **1.922** |
| | DLBCL | ***BCL2*** | **NM_000633** | **p.G8E** | **c.23G>A** | **50** | **2590** | **Confirmed** | **28.1** |
| | | *BCL2* | NM_000633 | p.P75L | c.224C>T | 62 | 1788 | ND | 8.790 |
| | | ***KMT2D*** | **NM_003482** | **p.W4987*** | **c.14960G>A** | **48** | **19088** | **Confirmed** | **45** |
| | | ***TNFRSF14*** | **NM_003820** | **p.V219G** | **c.656T>G** | **70** | **107** | **Confirmed** | **1.922** |
| | | *PIM1* | NM_002648 | p.E79D | c.237G>C | 32 | 4210 | Confirmed | 19.23 |
| 8 | ISFN | ***EZH2*** | **NM_004456** | **p.Y646N** | **c.1936T>A** | **32** | **715** | **Confirmed** | **32** |
| | | ***CREBBP*** | **NM_004380** | **p.L1499P** | **c.4496T>C** | **52**[#] | **56313**[#] | **Confirmed** | **24.5** |
| | | *CARD11* | NM_032415 | p.S250P | c.748T>C | 23 | 9399 | Confirmed | 23.9 |
| | HGBL-DH | *BCL2* | NM_000633 | p.D10A | c.29A>C | 37 | 4171 | Confirmed | 25.0 |
| | | *BCL2* | NM_000633 | p.N11D | c.31A>G | 32 | 4766 | Confirmed | 23.1 |
| | | ***EZH2*** | **NM_004456** | **p.Y646N** | **c.1936T>A** | **56** | **21249** | **Confirmed** | **32** |
| | | ***CREBBP*** | **NM_004380** | **p.L1499P** | **c.4496T>C** | **77** | **15559** | **Confirmed** | **24.5** |
| | | *TNFRSF14* | NM_003820 | p.S171C | c.512C>G | 68 | 5035 | ND | 24.2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 9 | ISFN | ***BCL2*** | **NM_000633** | **p.L86F** | **c.256C>T** | **15** | **110** | **Confirmed** | **19.85** |
| | | ***BCL2*** | **NM_000633** | **p.E135D** | **c.405G>C** | **8** | **801** | **Confirmed** | **18.34** |
| | FL | ***BCL2*** | **NM_000633** | **p.L86F** | **c.256C>T** | **25** | **301** | **Confirmed** | **19.85** |
| | | ***BCL2*** | **NM_000633** | **p.E135D** | **c.405G>C** | **18** | **887** | **Confirmed** | **18.34** |
| | HGBL-DH | ***BCL2*** | **NM_000633** | **p.L86F** | **c.256C>T** | **55** | **2690** | **Confirmed** | **19.85** |
| | | *EZH2* | NM_004456 | p.Y646F | c.1937A>T | 23 | 4420 | Confirmed | 25.3 |
| | | *HIST1H1B* | NM_005322 | p.S107C | c.320C>G | 32 | 1371 | Confirmed | 32 |
| 10 | ISFN | ***EZH2*** | **NM_004456** | **p.Y646F** | **c.1937A>T** | **9** | **357** | **Confirmed** | **25.3** |
| | FL | ***EZH2*** | **NM_004456** | **p.Y646F** | **c.1937A>T** | **52** | **409** | **Confirmed** | **25.3** |
| | | *KMT2D* | NM_003482 | p.S831* | c.2492C>A | 13 | 505 | Confirmed | 22.2 |
| | DLBCL | ***EZH2*** | **NM_004456** | **p.Y646F** | **c.1937A>T** | **40** | **1559** | **Confirmed** | **25.3** |
| | | *KMT2D* | NM_003482 | p.S831* | c.2492C>A | 21 | 1131 | Confirmed | 22.2 |

Bold letters indicate that mutations are shared between ISFN and FL and/or aggressive BCL. ND, Not done. *Mutations with a CADD algorithm score >15 were considered deleterious. #Bidirectional single amplicon sequencing.

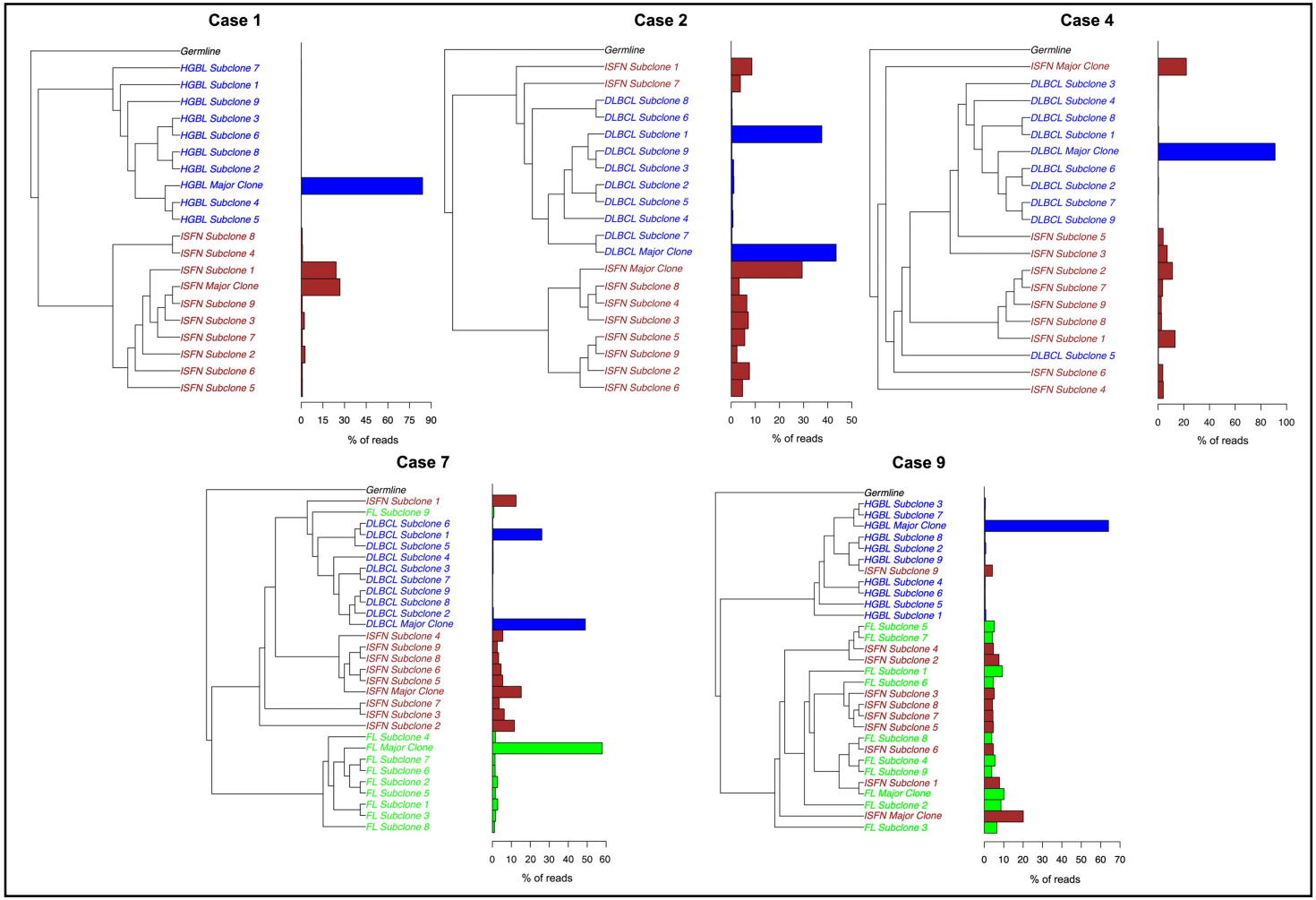
**Supplementary Table S5. Overview of synonymous and 5'UTR mutations of *BCL2*.**

| Case | Diagnosis | Protein level | cDNA change | VAF (%) | Coverage |
|---|---|---|---|---|---|
| De novo aggressive B-cell lymphoma | | | | | |
| 1 | ISFN | — | — | — | — |
| | HGBL-TH | 5'UTR | c.1-18G>A | 42 | 7049 |
| | | 5'UTR | c.1-1G>A | 42 | 7057 |
| | | Synonymous | c.207C>T | 44 | 2475 |
| | | Synonymous | c.381G>A | 30 | 27929 |
| 2 | ISFN | **5'UTR** | **c.1-2G>C** | **12** | **1516** |
| | DLBCL | 5'UTR | c.1-17C>T | 20 | 2472 |
| | | **5'UTR** | **c.1-2G>C** | **20** | **2478** |
| 3 | ISFN | **Synonymous** | **c.67C>T** | **15** | **6895** |
| | DLBCL | 5'UTR | c.1-17C>G | 47 | 5862 |
| | | **Synonymous** | **c.67C>T** | **50** | **6282** |
| | | Synonymous | c.588T>C | 48 | 18642 |
| | DLBCL | 5'UTR | c.1-17C>G | 55 | 2601 |
| | | **Synonymous** | **c.67C>T** | **55** | **3771** |
| | | Synonymous | c.588T>C | 58 | 8330 |
| 4 | ISFN | 5'UTR | c.1-49G>C | 9 | 1085 |
| | DLBCL | 5'UTR | c.1-1G>A | 15 | 8031 |
| 5 | ISFN | — | — | — | — |
| | DLBCL | Synonymous | c.186C>T | 51 | 2892 |
| | | Synonymous | c.261C>T | 52 | 3191 |
| 6 | ISFN | 5'UTR | c.1-1G>C | 21 | 3096 |
| | | Synonymous | c.67C>T | 21 | 9335 |
| | | Synonymous | c.291C>G | 16 | 26582 |
| | | Synonymous | c.355C>T | 12 | 26894 |
| | | Synonymous | c.438G>A | 17 | 12968 |
| | | Synonymous | c.456G>A | 9 | 15227 |
| | DLBCL | 5'UTR | c.1-17C>T | 26 | 5333 |
| Transformed FL | | | | | |
| 7 | ISFN | **5'UTR** | **c.1-17C>A** | **11** | **1391** |
| | | **Synonymous** | **c.24G>A** | **12** | **1398** |
| | | **Synonymous** | **c.67C>T** | **11** | **1981** |
| | FL | **5'UTR** | **c.1-17C>A** | **22** | **1609** |
| | | **Synonymous** | **c.24G>A** | **23** | **1630** |
| | | **Synonymous** | **c.67C>T** | **24** | **2201** |
| | DLBCL | **5'UTR** | **c.1-17C>A** | **50** | **2574** |
| | | **Synonymous** | **c.67C>T** | **56** | **3402** |
| | | Synonymous | c.354G>A | 25 | 8459 |
| | | Synonymous | c.408G>A | 55 | 7506 |
| | | Synonymous | c.447C>G | 54 | 7523 |
| 8 | ISFN | **5'UTR** | **c.1-49G>C** | **29** | **4229** |
| | | 5'UTR | c.1-17C>G | 14 | 4215 |
| | HGBL-DH | **5'UTR** | **c.1-49G>C** | **37** | **4151** |
| | | Synonymous | c.426G>A | 43 | 8014 |
| 9 | ISFN | **Synonymous** | **c.66G>A** | **10** | **389** |
| | | **Synonymous** | **c.258C>T** | **16** | **109** |
| | | **Synonymous** | **c.357G>A** | **9** | **1637** |
| | FL | **Synonymous** | **c.66G>A** | **23** | **618** |
| | | **Synonymous** | **c.258C>T** | **23** | **295** |
| | | **Synonymous** | **c.357G>A** | **13** | **1832** |
| | HGBL-DH | **Synonymous** | **c.66G>A** | **46** | **5918** |
| | | Synonymous | c.93T>C | 48 | 5722 |
| | | **Synonymous** | **c.258C>T** | **56** | **2682** |
| 10 | ISFN | **5'UTR** | **c.1-63G>A** | **4** | **2232** |
| | FL | **5'UTR** | **c.1-63G>A** | **29** | **3562** |
| | | 5'UTR | c.1-17C>T | 29 | 3608 |
| | DLBCL | **5'UTR** | **c.1-63G>A** | **26** | **2970** |
| | | 5'UTR | c.1-17C>T | 26 | 3013 |

All mutations refer to the NM_000633 transcript of the *BCL2* gene. Bold letters indicate that mutations are shared between ISFN and FL and/or aggressive BCL. 5'UTR, 5' untranslated region.

## Supplementary figures

**Supplementary Figure S1. Branched evolution illustrated by phylogenetic trees.** The trees were constructed using the ten most prevalent subclones of every sample and rooted to the corresponding VDJ germline sequence. Aggressive BCL is represented in blue, FL in green and ISFN in red. The bar graphs show the share of each subclonal sequence out of the total number of clone-specific reads of the respective sample.



**Supplementary Figure S2. Patterns of clonal evolution based on the distribution of private and shared mutations**. The respective evolutionary pattern is indicated in parentheses. All variants are depicted at protein level. Mutations highlighted in red were gained during the evolution. Synonymous and 5'UTR variants of *BCL2* are not shown, but were also taken into account for the construction. The existence of "Progenitor clones" was assumed based on the distribution of mutations.