Supplement

CD19 target evasion as a mechanism of relapse in large B-cell lymphoma treated with axicabtagene ciloleucel

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Methods

Patient sample collection and preparation

In ZUMA-1 (NCT02348216), patients received axicabtagene ciloleucel (axi-cel) at a target dose of $2.0 \times 10^6$ chimeric antigen receptor (CAR) T-cells/kg.\textsuperscript{1,2} Tumor biopsies from patients were performed at pretreatment (at screening/diagnosis or at baseline/on-study, before conditioning chemotherapy [prelymphodepletion] and axi-cel infusion) or later at relapse (defined as patients who achieved a complete or partial response and later experienced disease progression). A total of 100 pretreatment and 20 post-relapsed biopsies from patients with large B-cell lymphoma (LBCL) were analysed in this study. Of 20 patients with relapse, 18 had paired biopsies at pretreatment and post-relapse that were tested using immunohistochemistry (IHC). 22 unpaired pretreatment biopsies, 6 paired and 3 unpaired progression biopsies also had additional tissues that were successfully evaluated by RNA sequencing (RNAseq). Ongoing response referred to complete or partial responders who had not progressed in at least 2 years (ZUMA-1, Cohorts 1-2) or at least 6 months (ZUMA-1, Cohort 3) post-axi-cel infusion. Relapse included patients who achieved complete or partial response and subsequently experienced disease progression.\textsuperscript{1,2} Non-responders were patients who achieved stable disease or progressive disease as best response. Samples from formalin-fixed paraffin-embedded (FFPE) blocks or slides from both pretreatment and post-relapse biopsies were used for hematoxylin and eosin (H&E) staining, as indicated below. Number in the scheme represents number of biopsy tissues tested.
CAR T-cell detection

CAR T cells were quantified using a TaqMan-based quantitative polymerase chain reaction (qPCR; Thermo Fisher Scientific) as previously described.\textsuperscript{1-3} To report frequencies of CAR-positive cells in blood, CAR T cells per microliter were calculated by normalizing CAR gene expression to actin expression in peripheral blood mononuclear cells, followed by normalization to absolute lymphocyte counts.\textsuperscript{4}

Immunohistochemistry

H&E staining allowed FFPE tissue evaluation for tumor content and block quality controls. Slides were scanned with the Nanozoomer XR to generate digital images (20×). A trained pathologist identified the tumor area and provided qualitative and semiquantitative assessments. IHC staining was performed using tissue sections and an automated immunostainer (DAKO, Carpenteria, CA, USA). IHC staining for CD19 (LE-CD19, cytoplasmic domain), CD20 (L26, cytoplasmic domain), CD22 (FCP1, surface domain), CD79a (SP18, surface domain), and PAX5 (SP34. nuclear stain) was scored by composite H-score. H-scores were calculated as a product of IHC intensity (scale 1-3) multiplied by the percentage of tumor cells at a given intensity (0-100\%) by central pathology review. IHC staining with H-score 0 – 5 was assigned as “negative”; 6 – 300 was assigned as “positive” for the purpose of data quantification. Protein expression of B-cell lineage markers was assessed centrally by IHC using the same markers and reagents for B-cell lineage antigens as described above. Additional antibodies used in Figure 1D and Figure S4 were specific for Ki-67 (clone MIB-1) and PAX-5 (Clone 1EW from Transduction Labs, San Diego, CA). Briefly, all tissue sections underwent heat-induced epitope retrieval in pH 6.0 citrate buffer (Dako, Carpinteria, CA). Endogenous peroxidase was blocked by 3% H₂O₂ solution for 10 min. Detection was performed using the LSAB plus–streptavidin–HRP system (Dako).

RNA isolation, reverse transcriptase, PCR, and qPCR to study CD19 splice isoforms

Total RNA from tumor cells of patients with lymphoma were isolated by RNeasy Mini Kit (QIAGEN) and quantified by Nanodrop 2000 (Thermo Fisher). Complementary DNA (cDNA) was prepared with MultiScribe™ Reverse Transcriptase and random primers (Applied Biosystems). Reverse transcription (RT)-PCR was performed to amplify CD19 messenger RNA (mRNA) isoforms using Q5® Hot Start High-
Fidelity 2X Master Mix (New England Biolabs) and visualized using 1.5% agarose gels. Primers used for each CD19 mRNA isoform and expected PCR sizes are listed in **Supplementary Table 1**. When required, individual bands were gel purified (QIAquick Gel Extraction Kit; QIAGEN) and Sanger sequenced. Using qPCR primers and SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad), qPCR was performed and analyzed using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad) according to the manufacturer’s protocol. Experiments were performed in triplicate. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as internal control. The relative quantification of gene expression was obtained by comparison with the relative expression of GAPDH using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are shown in **Supplementary Table 3**.

**Western blotting**

Whole-cell protein lysates from lymphoma tumor cells isolated from patients treated with commercial axi-cel were prepared in radioimmunoprecipitation assay (RIPA) buffer (Bio-Rad) supplemented with Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific). Protein concentrations were measured by using a Pierce™ BCA Protein Assay kit (Thermo Fisher Scientific). For each sample, 20 µg of protein was separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using Tris–HCl gels (Ready Gel, Invitrogen), followed by transfer to polyvinylidene difluoride membranes (Immobilon-P, EMD Millipore). Membranes were immunoblotted with primary antibody against CD19 N-terminus (OriGene, TA506234) and the appropriate horseradish peroxidase-conjugated secondary antibody (Cell Signaling). Anti-GAPDH (Cell Signaling, #2118) was used as the loading control. Signals were detected by enhanced chemiluminescence using Immobilon™ Western HRP Substrate (EMD Millipore). Immunoblots were analyzed using ImageJ software (National Institutes of Health).

**Lentiviral constructs and infections**

A CD19-negative diffuse LBCL (DLBCL) clone, 1G7, was derived by limiting dilution from the U2932 DLBCL cell line. Lentiviral vector expressing pLenti-C-mGFP-CD19 was purchased from OriGene. To generate CD19 Δex2– and CD19 Δex5–6-expressing vectors, CD19 cDNA fragments were amplified by PCR from pLenti-C-mGFP-CD19 cDNA using Q5® Hot Start High-Fidelity 2X Master Mix (New England
Blood

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Primers for amplification of CD19 Δex2 and CD19Δex5–6 variants and the expected PCR size are listed in **Supplementary Table 4**. The specific PCR bands were visualized in 1.5% agarose gels, purified with QIAquick Gel Extraction Kit (QIAGEN), digested with MluI and Sgfl enzymes (New England Biolabs), and cloned into pLenti-C-mGFP-backbones via MluI/Sgfl cloning sites. Constructs were further confirmed by DNA Sanger sequence. Lentiviral particles were generated by transfection of 293T cells with Lipofectamine™ 3000 Transfection Reagent (Invitrogen). Viral supernatants were harvested 36 and 48 hours after transfection and were used to infect the CD19-negative DLBCL clone 1G7 in the presence of polybrene (4 µg/mL). Where indicated, selection of infected cells was done by flow cytometric green fluorescent protein (GFP) cell sorting.

**Flow cytometry of patient biopsies**

Staining was performed using BD Sample Prep Assistant (SPA) with the correct panel of antibodies. The antibodies tested are CD19-PE (clone SJ25C1 – BD #340720), CD22-PerCp-Cy5.5 (BD #32487) and CD79b-APC (clone SN8 – BD #335817). The staining protocol is set up in Helix, and the data was acquired in BD Canto (BD biosciences). The data are analyzed using FCS Express 6.

**Immunofluorescence of FFPE patient biopsies and confocal microscopy**

Staining was performed on BOND RX fully automated stainers (Leica Biosystems). Tissue sections of 5 μm-thick FFPE were baked for 1 hour at 60°C before loading into the BOND RX. Slides were deparaffinized (BOND Dewax Solution, Leica Biosystems) and rehydrated with a series of graded ethanol to deionized water. Antigen retrieval was performed in BOND Epitope Retrieval Solution 2 (Leica Biosystems) at pH 9 for 20 minutes at 98°C. Deparaffinization, rehydration, and antigen retrieval were all preprogrammed and executed by the BOND RX.

For detection of CD20 membrane localization, slides were stained with primary antibodies to CD20 (Thermo Fisher Scientific, #53-0202-82) and to the plasma membrane marker Na+K+ ATPase (Abcam, ab198367), both diluted to 10 µg/mL. Slides were counterstained with Hoechst 33342 (Thermo Fisher
Scientific, #62249), mounted using ProLong™ Gold Antifade Mountant (Thermo Fisher Scientific, #P36930), and imaged using Leica TCS SP8 confocal laser scanning microscope (Leica Biosystems).

For CD19 and CD20 colocalization, the slides were first stained with Na+K+ ATPase (Abcam, ab76020), diluted 1:4000 prior to detection using CF®750 tyramide signal amplification (TSA; Biotum, 96052). Slides were then treated with SDS-glycine to strip off the primary antibody and then stained with a cocktail of anti-CD19 (EMD Millipore, 05-1573; clone: EPR5906; dilution: 1:75), anti-CD20 (Novus Biologicals, NBP2-44746; clone: SPM618; dilution: 1:200), and anti-PAX5 (EMD Millipore, 05-1573; clone: 1H9; dilution: 1:75). Nuclei were detected using SYTOX™ Orange (Thermo Fisher Scientific, S11368; dilution: 1:200,000). Slides were mounted using ProLong™ Gold Antifade Mountant before imaging using Leica Aperio VERSA digital scanner (Leica Biosystems).

**Colocalization cell culture studies**

Cells expressing CD19-GFP fusion variants were fixed for 15 minutes on ice with 4% paraformaldehyde; cells were subsequently washed and permeabilized with 0.5% Tween20, 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 30 minutes at room temperature. Primary anti-CD19 antibody (OriGene) was incubated overnight at 4°C. Secondary Alexa Fluor 594–anti-mouse antibody was incubated for 1 hour at room temperature. The cells were washed and mounted on precharged glass microscope slides with 4,6-diamidino-2-phenylindole (DAPI)-containing medium (Vectashield; Vector Laboratories, H-1200-10) and visualized under a Leica TCS SP8 STED 3 super resolution confocal system (HC PL APO CS2 63/1.40-numerical-aperture oil 63 objective). Images were acquired using 4,184 × 4,184 resolution with limited signal saturation. Colocalization was quantified by Pearson correlation coefficient. For each CD19 construct, 6 images containing 100 cells on average were analyzed with BioImageXD and FIJI Coloc2 plugin software.

**Cytotoxicity assay**

An isogenic cell line of SUDHL6 DLBCL cells with CD19 knock out (KO) was generated by CRISPR/Cas9. The CD19 KO cells were transduced with CD19 full-length (FL), CD19 Δex2, or CD19
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Δex5–6 and used as targets for T-cell cytotoxicity assay. Briefly, target cells (T) were incubated with effector (E) T cells (CAR T19) at the indicated E:T ratios for 24 hours. Live cells were selected by staining with LIVE/DEAD™ Fixable Aqua Dead fluorescent reactive dye (Invitrogen) and analyzed using a BD™ LSR II cytometer (BD Biosciences).

RNAseq sample analysis of patient biopsies
TruSight RNA Pan-Cancer panel was used to assess up to 1385 RNA targets (Illumina). The assay was optimized and validated at the clinical diagnostic laboratory at NeoGenomics (Aliso Viejo, CA). Total RNA (20-100 ng) was extracted by RNeasy FFPE Kit (QIAGEN) from FFPE samples. cDNA was generated from cleaved RNA fragments using random priming. Sequencing adapters were ligated to double-stranded cDNA fragments and cDNAs were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter). Subsequently, coding regions of expressed cancer-associated genes were captured using sequence-specific probes for sequencing library formation. Libraries were loaded on the Illumina MiSeq platform using MiSeq Reagent Kit v3 (150-cycle, MS-102-3001).

RNAseq splice variants detection
The algorithm rMATS (replicate multivariate analysis of transcript splicing)⁶ was applied to RNAseq data to screen alternative splicing events. Reads were aligned to human reference genome hg19, and annotation of CD19 isoforms was downloaded from GENCODE v19.⁷ The false discovery rate (FDR) q-value cutoff was set to 0.05. Sashimi plots were generated using Integrative Genomics Viewer (IGV) version 2.4.19.

CD19 mutation analysis
The RNAseq BAM files were queried for known CD19 mutations. The reads with wildtype and mutant alleles were counted, based on which variant allele fraction was calculated for each mutation. Mutations were then annotated using the Ensembl Variant Effect Predictor (VEP)⁸ and visually verified by loading the BAM files to the Integrated Genomics Viewer (IGV).
**Statistical analyses**

Nonparametric Wilcoxon rank sum tests were used to explore the associations between 2 groups. Nonparametric Kruskal-Wallis tests were conducted for comparisons of 3 or more groups, followed by pairwise comparisons using the Dunn test with the Holm adjustment method. Spearman rank-order correlation was performed to evaluate association between any 2 covariates. Logistic regression analysis was used to assess the relationship between a covariate and clinical response. FDR q-values reported by rMATS were used to select significant alternative splicing events.

**NanoString gene expression analysis**

RNA extraction from frozen or fixed biopsies was performed using QIAGEN RNeasy kit and QIAGEN FFPE RNeasy Extraction kit, respectively. Annotations from the pathologist performing H&E staining were used to guide removal of normal tissue from the slides by macrodissection prior to RNA extraction, and after tissue deparaffinization and lysis. After extraction, RNA quantification was performed with Nanodrop and qualification was performed with the Agilent Bioanalyser. One RNA QC sample was included in each testing run as a positive control for extraction.

RNA expression profiling was performed using 3 NanoString datasets (Supplementary Table 7). The heatmap of transcript expression was generated by Spotfire 7.12.0 (TIBCO Software).
Supplementary Figures

Supplementary Figure 1.

(A) Paired CD19 and CD20 H-scores at pretreatment in ZUMA-1 patients (N=100) by CD19 H-score (0-100, 101-200, and 201-300). *10 patients in the H-score 1-100 range were CD19-negative at pretreatment; 2 patients were CD19- and CD20-negative at pretreatment. (B) Association of pretreatment CD19 and CD20 H-score (same patients as in A) exhibiting statistical significance. (C) RT-PCR and (D) Western blot analyses in primary LBCL tumors from CD19 CAR T-cell-naïve patients showing variable CD19 transcript and protein levels. CAR, chimeric antigen receptor; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; LBCL, large B-cell lymphoma; RT-PCR, reverse transcription polymerase chain reaction.
Supplementary Figure 2.

(A) Association of pretreatment CD19 H-score with engraftment index (CAR T Peak) and best response. (B) Time to progression by positive versus negative CD19 H-score and tumor burden (SPD) (C) CAR T-cell peak by CD19 H-score and SPD showing that CAR T-cell peak and SPD are trending inversely in the CD19 negative group. (D) Comparison of product attributes (infused CD8 T cells and naive cells) between CD19 negative and CD19 positive relapses. (E) CAR T cells in circulation over time (left) and peak level (right). Consistent with lack of association between persistence and durable response to axi-cel, no difference was observed between CD19-positive and negative relapse patients. (F) CAR T cells in circulation over time (left) and peak level (right), showing negative trend with H-score at progression in evaluable CD19 positive patients with relapse. Of note, the association between CD19 expression at relapse and CAR peak is not monotonic. †A small constant (0.01) in (E) and (F) was added to the CAR T-cell concentration to avoid the loss of zero values due to the logarithmic transformation.
Supplementary Figure 3.

Representative PET scan and IHC images from patients with CD19-positive relapse. (A) PET scan images from Patient 10 who relapsed with CD19-positive disease. (B) IHC images from Patient 10 showing CD19 staining at pretreatment and at progression (3 months post-infusion). HGBCL, high grade B-cell lymphoma; IHC, immunohistochemistry; PD, progressive disease; PET, positron emission tomography; PR, partial response.
Supplementary Figure 4.

Representative PET scan and IHC images for patients with CD19-negative relapse. (A) PET scan images from Patient 21, who also appears in IHC images evaluated locally in the main Figure 1, showing CD19-negative relapse in a patient with CD19-positive tumor pretreatment. (B and C) PET scan images from Patients 17 and 18, showing CD19-negative relapse in patients with CD19-positive tumor at baseline (pretreatment). Axi-cel, axicabtagene ciloleucel; CR, complete response; IHC, immunohistochemistry; PD, progressive disease; PET, positron emission tomography; PR, partial response.
Supplementary Figure 5.

CD20 expression and other B-cell antigens were present in relapse biopsies even in tumors with low CD19 expression. (A) Preservation of B-cell lineage markers in most patients with relapse, even those that lost CD19. (B) Pie chart demonstrating distribution of CD19/CD20 expression within evaluable patients shown in (A). (C) Associations among B-cell antigens. R² values represent Pearson correlation coefficients. (D) Representative immunohistochemistry images of pre-treatment and progression biopsies depicting expression of CD19, CD20, CD79a, CD22 and PAX5 for Pt 18, demonstrating preservation of B-cell antigens in a relapse patient with CD19-negative biopsy. IHC data on CD22 and PAX5 were only generated for progression biopsies. PAX5, paired box 5. (E) Representative flow cytometry plots from Patient 18 progression biopsy (shown in D), confirming CD19-negative relapse with antibody staining the surface domain.
D

Patient 18

Pre-treatment

Progression

CD19  CD20  CD79a  CD22  PAXS

E

CD706 APC-A

CD19 PE-Cy7-A

CD79b APC-A

CD19 PE-Cy7-A

CD22 PE-Cy5-A

CD19 PE-Cy7-A

CD22 PE-Cy5-A

CD19 PE-Cy7-A

CD22 PE-Cy5-A
Supplementary Figure 6.

(A) Representative CD19 and CD20 IHC images from paired biopsies at the time of screening (pretreatment) and progression along with additional CD19-positive images at pretreatment from other patients. (B) Representative CD19 IHC images from progression biopsies in 3 patients. (C) Representative immunofluorescence confocal microscopy in CD19-positive progression biopsy from Patient 6. Left image showing CD19 and CD20 co-localization and coverage in case of dual targeting. Right image showing majority of PAX5-positive cells are also CD19-positive, matching high CD19 H-score stain with an antibody targeting the cytoplasmic domain from this patient’s progression biopsy (H-score=230). CD19 is localized to the cell surface, as demonstrated by confocal microscopy. (D) Representative image from Patient 16 by immunofluorescence confocal microscopy, showing cell membrane localization of CD20 (n=10 tumors) by co-staining with Na+K+ ATPase. IHC, immunohistochemistry; PAX5, paired box 5.
Patient 6 post-progression CD19 staining
H-score: 230 (68.5% PAX5+ cells are CD19+)

Patient 16

Na+/K+ ATPase and CD20

CD20

Na+/K+ ATPase

Hoechst (DNA)
Supplementary Figure 7.

(A) Heatmap of selected hematopoietic genes in patients (N=12) with pretreatment biopsies analyzed by NanoString and progression biopsies evaluated by IHC. Unsupervised hierarchical clustering revealed the association between transcript expression at pretreatment and protein expression at progression. Patients 20, 41, and 42 show no protein expression of CD19, CD20 or CD22, although all progression biopsies contain LBCL tumor tissue. NanoString analyses of pretreatment biopsies show relatively lower gene expression of B-cell antigens and enrichment of myeloid markers, including CD33, FCGR3A, MRC1 and CD163, in Patients 20, 41, and 42, compared with others. In all biopsies tested, the presence of tumor LBCL tissue was confirmed. (B) Representative IHC images of B-cell antigens in Patient 20 that showed positive staining for CD19, CD20 and CD79a at pretreatment and loss absence of all stained B-cell antigens in progression biopsies. IHC data on CD22 and PAX5 were only generated for progression biopsies. IHC, immunohistochemistry; LBCL, large B-cell lymphoma; PAX5, paired box 5.
Supplementary Figure 8.

The CD19 Δex2 and Δex5-6 isoforms in lymphoma cells are associated with absence of surface CD19 and escape from anti-CD19 CAR T-cell killing. (A) Immunoblotting analysis for CD19 expression in primary DLBCL biopsies using CD19 N-terminal antibody (anti-CD19-N). Data show variable expression of full-length CD19 across various samples and also expression of CD19 Δex2 variant in all samples tested. (B) CD19 Δex2 (exon 2 deletion), and CD19 Δex5-6 (exon 5-6 deletion), isoforms result in cell surface localization defect. Immunofluorescence of 1G7 CD19-negative lymphoma cells expressing CD19 full-length- (1G7-pCD19), Δex2-, and Δex5-6-GFP fusion proteins. Cells were stained with anti-CD19 (Origen)/anti-mouse antibody-Alexa Fluor 594. The full-length CD19 (red) colocalizes with GFP (green) exclusively to the plasma membrane. CD19 Δex2–GFP is found in the membrane as well as the cytoplasmic compartment. CD19 Δex5-6–GFP is cytosolic and does not localize to the membrane. (C) Flow cytometry was performed on 1G7 CD19-negative DLBCL clone that was transduced with CD19 full-length-, Δex2 (blue)- or Δex5-6 (yellow)-containing lentiviral vectors. CD19 Δex2 and Δex5-6 variants are associated with absence of surface expression of CD19. (D) SU-DHL6 CD19KO cells were transduced with CD19 full-length-, Δex2-, or Δex5-6-GFP fusion proteins and then incubated for 24 hours with anti-CD19 CAR T cells at indicated ratios of effector:target (E:T) ratios. Viable tumor cells were determined by flow cytometry after staining with aqua and then percentage of cytotoxicity was calculated. CAR, chimeric antigen receptor; DLBCL, diffuse large B-cell lymphoma; E, effector; GFP, green fluorescent protein; T, target. Patients shown in Figure S8 and Figure S9 are CAR-naïve DLBCL patients.
Supplementary Figure 9.

The CD19 splice isoforms CD19 Δex2 and Δex5–6 are present in primary DLBCL tumor samples. (A, B) RT-PCR of cDNA from lymphoma samples was performed corresponding to exon 1–4 (A) or exon 4–8 (B) of CD19 and visualized by agarose gel electrophoresis. Arrows indicate full-length (FL), partial deletion (Δex2part), and the Δex2 or Δex5–6 isoforms. (C-E) Sanger sequencing was performed on gel-purified band samples shown in (A) and (B). Shown here are exon 2/3 or exon 4/5 junction (representing CD19 wild type), exon 1/3 or exon 4/7 junction (representing CD19 whole exon 2), and exon 5–6 deletion. CD19 partial exon 2 deletion, which deletes 130bp nucleotides, is shown in (D). (F) Schematic representation of CD19 wild type, CD19 exon 2 deletion, exon 2 partial deletion, or exon 5–6 deletion sequence. Each box indicates corresponding exons (not drawn to scale). (G) Representative sequences for CD19 Δex2, partial Δex2, and Δex5-6 isoforms. Patients shown in Figures S8 and S9 are CAR-naïve DLBCL patients. CAR, chimeric antigen receptor; cDNA, complementary deoxyribonucleic acid; DLBCL, diffuse large B-cell lymphoma.
Supplementary Figure 10.

(A) Splicing events in normal B cells in healthy individuals. The first 2 variants are found in UniProt and differ by one amino acid. The next 3 variants do not express due to retained introns. Splice variants are taken from Ensembl splice variant database (release 100), which uses the human genome assembly, GRCh38.p13. (B) Frequency of different variants in normal B cells from healthy individuals (https://uswest.ensembl.org/Homo_sapiens/Info/Annotation).

| Name   | bp    | Protein | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|--------|-------|---------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|
| CD19-201 | 1922  | 557aa   |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |
| CD19-202 | 1918  | 556aa   |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |
| CD19-203 | 2322  | No      |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |
| CD19-204 | 847   | No      |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |
| CD19-205 | 590   | No      |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |
Supplementary Figure 11.

Quantification of splice variants shown in Figure 2B, including their respective exon locations sorted by CD19 H-score (A) or sorted by best response (B) or ongoing response (C) and ranked based on H-score in descending order. Numbers within bars denote impacted exons. A3SS, alternative 3' splice site; Ctrl, control; CR, complete response; MXE, mutually exclusive exons; PD, progressive disease; PR, partial response; Pt, patient; RI, retained intron; SD, stable disease, SE, skipped exon. The numbers on the bars in A represent exons where the splice variant event occurs.

A

B

C
Supplementary Figure 12.

(A) Sashimi plots showing examples of different splice variant events at pretreatment. Patient 4 is shown as control. (B) Sashimi plots showing splice variants at pretreatment and relapse in 6 patients (H-score pretreatment->relapse). Patients 4 and 10: Exon 2 and exon 5 skip events are common in CD19 so may not affect overall protein levels. Patient 6: Exon 6 skip is a rare event, unlikely to affect the structure of CD19. Patient 16: Since mRNA expression was low, it is difficult to assess the impact on CD19 protein expression. Same assessment should be considered for Patients 5 and 10. Patient 5: Although exon 10 skip event was observed, the coverage was low. Patient 17: Exon 10 intron retain events were observed; however, a low percentage of transcripts is impacted by intron retain events, implying the impact on protein may be negligible. Further, the level of impact from subclonal populations may not be apparent because the bulk sample was analyzed. Numbers represent H scores and (left: H-score at pretreatment, right: H-score at relapse). (C) CD19 splice variants in 3 unpaired ZUMA-1 progression biopsies; numbers denote impacted exons (left) and prevalence of splice variants at progression (right). Of note, Patients 13 and 14 are relapse subjects but patient 43 is a non-responder but for the latter, a biopsy taken after axi-cel infusion was analyzed by RNAseq (D) CD19 gene and protein expression of paired samples. Expression shown in fragments per kilobase of transcript per million mapped reads (FPKM), Ctrl2: Patient 22. (E) Representative (Patient 16) of downregulation of CD19 mRNA transcript and CD19 H-score in progression biopsies, Expression shown in transcript per million (TPM). (F) CD19 mutations in relapse biopsy samples, compared with pretreatment in Patient 6 and 17. CD19 mutation in Patient 10 is shown in Figure 2E. (G) Exon 3 shows residues involved in mutations. Left: Wild-type structure (pretreatment); right: Substitution with Ser173 disrupts disulfide formation (pretreatment), as shown in Figure 2E, Patient 10. (H) Structural models of splicing events in biopsy samples at pretreatment (*), relapse (**), or both time points. The 3D model depicts disulfide bonds (black), exons of the known structure (colored indicated), and FMC63 binding region (orange). Common mutation C173 (purple arrow) and FMC63 binding domain (yellow arrow) are indicated. Exon 5 covers the last extracellular amino acids, transmembrane helix, and first intracellular amino acids. Exon 6 is the first full intracellular exon, followed by exons 7 through 14, that covers the C-terminal region. An exon 5 deletion would prevent the protein from being anchored in the membrane. Exon deletions after exon 5 have unknown consequences but could still allow proper CD19 extracellular presentation and FMC63 binding site. Beta strand swapping occurs between the 2 sheets that comprise the extended sandwich architecture of CD19. Consequently, deletion of exon 2 or 4 would affect proper beta strand formation. FMC63bs, FMC63 axi-cel binding site.
A

Sashimi plots for SE events

| Patient 4 | Patient 34 | Patient 41 | Patient 29 | Patient 40 |
|-----------|------------|------------|------------|------------|
| Exon 5 skip | Exon 6 skip | Exon 5 skip | Exon 6 skip | Exon 11 skip |

Sashimi plots for RI events

| Patient 4 | Patient 29 | Patient 40 | Patient 32 | Patient 38 | Patient 35 |
|-----------|------------|------------|------------|------------|------------|

Sashimi plots for A3SS events

| Patient 4 | Patient 41 | Patient 28 |
|-----------|------------|------------|
| Exon 13 A3SS | Exon 10 A3SS | Exon 13 A3SS |

Sashimi plots for MXE events

| Patient 4 | Patient 34 |
|-----------|------------|
| Exon 5 MXE | Exon 5,6 MXE |

B

| Pre-treatment | Relapse |
|---------------|---------|
| Patient 5     |         |
| Pt. 5 260->270 | Low exp |
| Patient 4     |         |
| Pt. 4 280->270 |         |
| Patient 10    |         |
| Pt. 10 70->70 | Low exp |
| Patient 6     |         |
| Pt. 6 280->270 |         |
| Patient 16    |         |
| Pt. 16 300->0 | Low exp |

CD19
Tables

Supplementary Table 1.

Primer sequences for RT-PCR amplification of CD19 cDNA followed by visualization on agarose gel. The same primers were used for sequencing after gel purification of specific bands. cDNA, complementary deoxyribonucleic acid; NA, not applicable; RT-PCR, reverse transcription polymerase chain reaction.

| Region     | Direction | Sequence (5’ → 3’)                  | Full Length | Δex2 | Δex5–6 |
|------------|-----------|-------------------------------------|-------------|------|--------|
| Exon 1–4   | Forward   | GGAGAGTCTGACCACCATGC                | 640 bp      | 374 bp | NA     |
|            | Reverse   | GGACACAGAGTCAGGGGTA                 |             |      |        |
| Exon 4–8   | Forward   | AAGGGGCCTAAGTCATTGCT                | 490 bp      | NA   | 331 bp |
|            | Reverse   | TGCTCGGGTTTCCATAAGAC                |             |      |        |
Supplementary Table 2.

Baseline characteristics and clinical parameters of 43 patients treated in ZUMA-1. Patients 2-13, 15-20 have IHC data from paired pretreatment-progression biopsies; Patients 1, 14 have IHC data at progression only; Patients 4-6, 10, 16, 17 have paired pretreatment-progression RNAseq data; Patients 13, 14 and 43 have RNAseq data only from progression biopsy; Patients 21-42 have only pretreatment but no evaluable RNAseq from their progression biopsies. Patient 43 is a non-responder, but a biopsy was excised post axi-cel and tested with RNAseq. CR, complete response; CRS, cytokine release syndrome; DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; N, no; NE, neurologic event; PD, progressive disease; PR, partial response; SD, stable disease; SPD, sum of product diameters (pretreatment tumor burden measured in mm²); Sys, systemic; TFL, transformed follicular lymphoma; Y, yes.

| Patient ID | Disease Type | SPD (mm²) | CD19 status by IHC (Pretreatment) | CD19 status by IHC (Progression) | Best Response | Ongoing response |
|------------|--------------|-----------|-----------------------------------|----------------------------------|---------------|-----------------|
| 1          | TFL          | 7566      | Positive                          |                                  | CR            | RELAPSED        |
| 2          | DLBCL        | 431       | Positive                          | Positive                         | PR            | RELAPSED        |
| 3          | DLBCL        | 3648      | Positive                          | Positive                         | PR            | RELAPSED        |
| 4          | DLBCL        | 4433      | Positive                          | Positive                         | PR            | RELAPSED        |
| 5          | TFL          | 23297     | Positive                          | Positive                         | PR            | RELAPSED        |
| 6          | DLBCL        | 9027      | Positive                          | Positive                         | PR            | RELAPSED        |
| 7          | DLBCL        | 6167      | Positive                          | Positive                         | CR            | RELAPSED        |
| 8          | DLBCL        | 9772      | Positive                          | Positive                         | PR            | RELAPSED        |
| 9          | TFL          | 9371      | Positive                          | Positive                         | CR            | RELAPSED        |
| 10         | DLBCL        | 5490      | Positive                          | Positive                         | PR            | RELAPSED        |
| 11         | DLBCL        | 8877      | Positive                          | Positive                         | PR            | RELAPSED        |
| 12         | DLBCL        | 13936     | Positive                          | Positive                         | CR            | RELAPSED        |
| 13         | DLBCL        | 4183      | Positive                          | Positive                         | CR            | RELAPSED        |
| 14         | DLBCL        | 2935      | Negative                          | Positive                         | PR            | RELAPSED        |
| 15         | DLBCL        | 10878     | Positive                          | Negative                         | CR            | RELAPSED        |
| 16         | DLBCL        | 3719      | Positive                          | Negative                         | CR            | RELAPSED        |
| 17         | DLBCL        | 1008      | Positive                          | Negative                         | PR            | RELAPSED        |
| 18         | DLBCL        | 7991      | Positive                          | Negative                         | CR            | RELAPSED        |
| 19         | DLBCL        | 784       | Positive                          | Positive                         | CR            | RELAPSED        |
| 20         | PMBCl        | 5056      | Positive                          | Negative                         | PR            | RELAPSED        |
| 21         | DLBCL        | 4205      | Negative                          | Positive                         | PR            | RELAPSED        |
| 22         | DLBCL        | 6262      | Positive                          | CR                               | RELAPSED      |
| 23         | DLBCL        | 2200      | Positive                          | CR                               | ONGOING       |
| 24         | DLBCL        | 2457      | Positive                          | CR                               | RELAPSED      |
| 25         | DLBCL        | 6062      | Negative                          | SD                               | NON-RESPONDER |
| 26         | DLBCL        | 600       | Positive                          | CR                               | RELAPSED      |
| 27         | PMBCl        | 2244      | Negative                          | CR                               | ONGOING       |
| 28         | DLBCL        | 2564      | Positive                          | CR                               | ONGOING       |
| 29         | DLBCL        | 2039      | Positive                          | CR                               | ONGOING       |
| 30         | DLBCL        | 320       | Positive                          | CR                               | ONGOING       |
| 31         | PMBCl        | 4872      | Positive                          | CR                               | ONGOING       |
| 32         | DLBCL        | 8436      | Positive                          | CR                               | ONGOING       |
| 33         | DLBCL        | 3790      | Positive                          | CR                               | OTHERS        |
| 34         | DLBCL        | 14354     | Positive                          | SD                               | NON-RESPONDER |
| 35         | DLBCL        | 3014      | Negative                          | PR                               | OTHERS        |
| 36         | DLBCL        | 7552      | Positive                          | SD                               | NON-RESPONDER |
| 37         | TFL          | 2847      | Positive                          | SD                               | NON-RESPONDER |
| 38         | DLBCL        | 3245      | Positive                          | CR                               | RELAPSED      |
| 39         | DLBCL        | 7141      | Positive                          | PR                               | RELAPSED      |
| 40         | DLBCL        | 7868      | Positive                          | PR                               | RELAPSED      |
| 41         | DLBCL        | 1921      | Negative                          | Negative                         | PD            | NON-RESPONDER   |
| 42         | DLBCL        | 11042     | Negative                          | Negative                         | PD            | NON-RESPONDER   |
| 43         | DLBCL        | 6760      | Positive                          | PD                               | NON-RESPONDER |
Supplementary Table 3.
Primer sequences used for quantitative polymerase chain reaction analysis. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

| Gene name                        | Forward Primer          | Reverse Primer          |
|----------------------------------|-------------------------|-------------------------|
| CD19 exon 1–exon 2               | GGAGAGTCTGACCAATGC      | ACTGCACAGACGTTATCT      |
| CD19 exon 3–exon 4               | GAGCCCAAGCTGTATGTG      | GGACACAGAGTCAGGGGTA     |
| CD19 junction exon 1/3           | GCCTCCTCTTCTCTCTCTCT    | CCGGAACAGCTCCCTCCACCTTC |
| CD19 exon 4–5                    | AAGGGGCCTAAGTCTTGCT     | CAGCAGCCAGTGCCATAGTA    |
| CD19 junction exon 6/7–exon 8    | CCCCACCGGAGATTCTTCA     | TGCTCGGGTTCCATAAGAC     |
| CD19                             | GCCAACCTGACCATGTCATT    | TCACAGCTGAGCCTCCAG      |
| GAPDH                            | AAGTGAAAGTGCGGAGTCAA    | AATGAGGCTCATTAGGG       |
**Supplementary Table 4.**

PCR primers for generating CD19 exon 2 deletion or exon 5–6 deletion fragments. PCR, polymerase chain reaction.

| Region                        | Primers | Sequence (5' → 3') | PCR Size |
|-------------------------------|---------|--------------------|----------|
| **CD19 exon 2 deletion**      | P1 forward | CCATGGAAGTCAGGCCCGAGGAACCTCTAGTGGT GAAGTGGAGGGGAGCTGTTCCGGTGGAATG | P1 + P2 1356 bp |
|                               | P2 reverse | CGTACCGGTCTCTGGTGCTCCAGGTGCCCATGCAGGCCCCCTC |          |
|                               | P3 forward | CGCGATCGCATGCCACCTCCTCGCCCTCCTTTCTTCTCT | P3 + P2 1410 bp |
|                               |          | CCTCTTTTCCTCACCCCCCATGGAAGTCAGGCCCCGAG |          |
| **CD19 exon 5–6 deletion**    | P4 forward | CTGGAGATCAGTCCTGAGCCACATTTCTTTCTAAGTGAAGCCTCC | P4 + P2 866 bp (A) |
|                               | P5 reverse | GGAGCGTCATTTGAAGAATCTGGCCGAGCAGTGCTCTCCAG | P6 + P5 644 bp (B) |
|                               | P6 forward | CGCGATCGCATGCCACCTCCTCGCCCTCCTTTCTTCC | P6 + P2 (A + B) 1517 bp |
**Supplementary Table 5.**

Cumulative splicing score versus best response. CAR, chimeric antigen receptor; CR, complete response; PR, partial response; SPD, sum of product diameters.

| Patient | Splice Variant Annotation | Mutations | Phase Cohort | Best Response | Peak CAR T-Cell Levels | CD19 H-Score at Pretreatment | CD19 at Progression | SPD |
|---------|---------------------------|-----------|--------------|---------------|------------------------|-------------------------------|---------------------|-----|
| 5       | Exon 2, 5, 6, 10, 13      | No        | Phase 2 Cohort 2 | PR            | 1.53                   | 260                           | 270                 | 6208|
| 4       | Exon 2, 5, 10             | No        | Phase 2 Cohort 1 | PR            | 66.10                  | 280                           | 270                 | 9309|
| 10      | Exon 2, 13                | Yes       | Phase 2 Cohort 1 | PR            | 95.35                  | 70                            | 70                  | 5876|
| 6       | Exon 5, 10, 13            | Yes       | Phase 2 Cohort 1 | PR            | 25.92                  | 280                           | 270                 | 9371|
| 16      | Exon 2                    | No        | Phase 2 Cohort 1 | CR            | 403.96                 | 300                           | 0                   | 5766|
| 17      | Exon 10, 11               | Yes       | Phase 2 Cohort 1 | PR            | 146.14                 | 140                           | 0                   | 732 |
Supplementary Table 6.

CD19 mutations detected at relapse.

| Patient | Site Position         | Nucleotide Change | Amino Acid Change | Exon |
|---------|-----------------------|-------------------|-------------------|------|
| 10      | Chr16:28944394-28944394 | G/C               | C173S             | 3    |
| 6       | Chr16:28944393-28944394 | TC/-              | S173X             | 3    |
| 17      | Chr16:28944403-28944404 | CG/-              | P176X             | 3    |
Supplementary Table 7.

NanoString data table (N=12)

| Data presented here are normalized count |
|-----------------------------------------|

| Patient ID | BCL2 | CCR7 | CD163 | CD19 | CD32 | CD27 | CD33 | CD79A | CD79B | FCGR3A | MRC1 | M8A1 | PAX5 | PDCD1LG2 | SLAMF7 | TNFRSF13C |
|------------|------|------|-------|------|------|------|------|-------|-------|--------|------|------|------|-----------|---------|-----------|
| 2          | 1180.11 | 70.31 | 70.31 | 3456.48 | 7961.49 | 2820.86 | 44.02 | 9797.02 | 9373.83 | 64.63 | 43.32 | 13897.64 | 1219.16 | 294.73 | 150.56 | 1323.79 |
| 4          | 300.98 | 108.13 | 1026.67 | 5200.2 | 2095.77 | 3328.05 | 48.89 | 8803.11 | 6789.78 | 345.73 | 400.71 | 9259.56 | 1867.26 | 255.79 | 124.48 | 3795.09 |
| 7          | 1184.17 | 446.85 | 154.68 | 1235.14 | 1908.89 | 1027.6 | 0 | 5710.44 | 9693.34 | 472.63 | 0 | 3286.94 | 2145.97 | 167.57 | 432.89 | 1993.42 |
| 8          | 3202.51 | 26.04 | 467.78 | 3730.73 | 1685.35 | 1747.35 | 3541.5 | 0 | 3424.68 | 9115.05 | 381.15 | 189.62 | 5110.71 | 1797.99 | 0 | 26.64 | 1432.74 |
| 10         | 453.71 | 131.76 | 184.15 | 4957.37 | 2127.24 | 187.32 | 0 | 3813.15 | 16935.33 | 130.17 | 65.09 | 2522.52 | 1447.79 | 31.75 | 0 | 966.78 |
| 11         | 2010.53 | 36.07 | 1206.31 | 1909.82 | 2470.64 | 3123.07 | 41.7 | 5681.53 | 8101.35 | 1424.86 | 251.14 | 5084.37 | 1359.54 | 110.96 | 632.58 | 388.59 |
| 17         | 2579.48 | 31.8 | 658.42 | 2097.15 | 4375.7 | 2113.04 | 28.27 | 7944.64 | 12997.05 | 353.88 | 409.89 | 6335.63 | 1694.52 | 60.07 | 47.11 | 376.91 |
| 19         | 5030.23 | 187.64 | 429.25 | 3549.77 | 2423.87 | 3665.36 | 0 | 4824.6 | 20545.05 | 573.19 | 370.13 | 3593.39 | 1256.52 | 185.07 | 1115.55 | 2580.66 |
| 20         | 530.56 | 754.33 | 2465.1 | 1320.97 | 570.26 | 274.3 | 140.78 | 970.86 | 3417.93 | 1962.09 | 1533.46 | 812.07 | 584.69 | 544.95 | 555.82 | 602.74 |
| 35         | 2417.32 | 0 | 2429.4 | 1756.17 | 3713.09 | 2267.21 | 61.28 | 11591.94 | 6962.38 | 1476.97 | 864.44 | 10487.16 | 2133.04 | 351.12 | 196.84 | 2077.42 |
| 41         | 610.76 | 492.75 | 1488.99 | 1281.56 | 1366.44 | 403.72 | 84.86 | 3171.8 | 3070.96 | 1573.48 | 1650.08 | 1113.86 | 569.35 | 432.71 | 722.56 | 472.04 |
| 42         | 359.41 | 488.84 | 2110.65 | 969.1 | 649.37 | 281.57 | 40.44 | 1933.61 | 1872.57 | 2362.54 | 570.12 | 0 | 5674.86 | 656.16 | 798.25 | 1228.54 |
Supplementary Table 8.

Splicing events annotations

| Annotation                  | FDR     | IncLevel1 | IncLevel2 | Sample     | Event Type |
|-----------------------------|---------|-----------|-----------|------------|------------|
| Exon10 and exon11           | 0.00447 | 0.009     | 0.041     | CTRL1-PT. 24 | RI         |
| Exon2 and exon3             | 1.63E-10| 0.062     | 0.23      | CTRL1-PT. 41 | RI         |
| Exon10 and exon11           | 2.41E-10| 0.009     | 0.04      | CTRL1-PT. 41 | RI         |
| Exon2 and exon3             | 0.000404| 0.062     | 0.128     | CTRL1-PT. 29 | RI         |
| Exon10 and exon11           | 4.32E-13| 0.009     | 0.048     | CTRL1-PT. 29 | RI         |
| Exon2 and exon3             | 0.002826| 0.062     | 0.2       | CTRL1-PT. 39 | RI         |
| Exon2 and exon3             | 0.024918| 0.062     | 0.107     | CTRL1-PT. 40 | RI         |
| Exon2 and exon3             | 3.41E-10| 0.009     | 0.039     | CTRL1-PT. 40 | RI         |
| Exon10 and exon11           | 1.29E-06| 0.062     | 0.215     | CTRL1-PT. 35 | RI         |
| Exon10 and exon11           | 2.04E-11| 0.009     | 0.047     | CTRL1-PT. 34 | RI         |
| Exon10 and exon11           | 5.13E-11| 0.009     | 0.26      | CTRL1-PT. 35 | RI         |
| Exon2 and exon3             | 0        | 0.062     | 0.323     | CTRL1-PT. 32 | RI         |
| Exon10 and exon11           | 3.32E-15| 0.009     | 0.054     | CTRL1-PT. 32 | RI         |
| Exon2 and exon3             | 2.59E-11| 0.062     | 0.233     | CTRL1-PT. 38 | RI         |
| Exon10 and exon11           | 9.99E-14| 0.009     | 0.047     | CTRL1-PT. 38 | RI         |
| Exon2 and exon3             | 0.000262| 0.062     | 0.233     | CTRL1-PT. 25 | RI         |
| Exon10 and exon11           | 0.00071 | 0.009     | 0.031     | CTRL1-PT. 25 | RI         |
| Exon2 and exon3             | 5.15E-13| 0.009     | 0.338     | CTRL1-PT. 27 | RI         |
| Exon10 and exon11           | 4.12E-13| 0.009     | 0.053     | CTRL1-PT. 27 | RI         |
| Exon2 and exon3             | 7.76E-14| 0.062     | 0.236     | CTRL1-PT. 33 | RI         |
| Exon10 and exon11           | 2.31E-15| 0.009     | 0.05      | CTRL1-PT. 33 | RI         |
| Exon2 and exon3             | 2.56E-11| 0.062     | 0.263     | CTRL1-PT. 9  | RI         |
| Exon10 and exon11           | 3.69E-11| 0.009     | 0.046     | CTRL1-PT. 9  | RI         |
| Exon2 and exon3             | 4.65E-08| 0.062     | 0.233     | CTRL1-PT. 23 | RI         |
| Exon10 and exon11           | 4.51E-12| 0.009     | 0.049     | CTRL1-PT. 23 | RI         |
| Exon2 and exon3             | 0        | 0.062     | 0.57      | CTRL1-PT. 37 | RI         |
| Exon10 and exon11           | 2.57E-07| 0.009     | 0.033     | CTRL1-PT. 37 | RI         |
| Exon2 and exon3             | 6.07E-16| 0.062     | 0.346     | CTRL1-PT. 36 | RI         |
| Exon10 and exon11           | 0.004682| 0.009     | 0.021     | CTRL1-PT. 36 | RI         |
| Exon2 and exon3             | 4.67E-13| 0.062     | 0.277     | CTRL1-PT. 30 | RI         |
| Exon10 and exon11           | 8.5E-10  | 0.009     | 0.039     | CTRL1-PT. 30 | RI         |
| Exon2 and exon3             | 1.08E-11| 0.062     | 0.255     | CTRL1-PT. 31 | RI         |
| Exon10 and exon11           | 0        | 0.009     | 0.084     | CTRL1-PT. 31 | RI         |
| Exon2 and exon3             | 0.023513| 0.068     | 0.04      | CTRL2-PT. 41 | RI         |
| Exon2 and exon3             | 0.012819| 0.256     | 0.128     | CTRL2-PT. 29 | RI         |
| Exon10 and exon11           | 3.26E-12| 0.068     | 0         | CTRL2-PT. 39 | RI         |
| Exon2 and exon3             | 0.003777| 0.256     | 0.107     | CTRL2-PT. 40 | RI         |
| Exon10 and exon11 | 0.019501 | 0.068 | 0.039 | CTRL2-PT. 40 | RI |
|-------------------|-----------|-------|-------|-------------|----|
| Exon10 and exon11 | 0.03111   | 0.068 | 0     | CTRL2-PT. 8 | RI |
| Exon10 and exon11 | 0.008664  | 0.068 | 0.031 | CTRL2-PT. 25| RI |
| Exon2 and exon3   | 0.000148  | 0.256 | 0.57  | CTRL2-PT. 37| RI |
| Exon10 and exon11 | 0.001942  | 0.068 | 0.033 | CTRL2-PT. 37| RI |
| Exon10 and exon11 | 3.44E-06  | 0.068 | 0.021 | CTRL2-PT. 36| RI |
| Exon10 and exon11 | 0.021251  | 0.068 | 0.039 | CTRL2-PT. 30| RI |
| Exon2 and exon3   | 0.013297  | 0.256 | 0.113 | CTRL2-PT. 28| RI |
| Exon10 and exon11 | 1.01E-06  | 0.068 | 0.017 | CTRL2-PT. 28| RI |
| Exon10 and exon11 | 0         | 0.068 | 0     | CTRL2-PT. 42| RI |
| Exon5             | 2.4E-10   | 0     | 0.655 | CTRL1-PT. 24| SE |
| Exon6             | 9.01E-08  | 1     | 0.851 | CTRL1-PT. 24| SE |
| Exon5             | 2.19E-07  | 1     | 0.956 | CTRL1-PT. 41| SE |
| Exon5             | 0         | 0     | 0.98  | CTRL1-PT. 29| SE |
| Exon6             | 0.000449  | 1     | 0.976 | CTRL1-PT. 29| SE |
| Exon2             | 0.003441  | 1     | 0.949 | CTRL1-PT. 40| SE |
| Exon11            | 4.02E-07  | 1     | 0.953 | CTRL1-PT. 40| SE |
| Exon2             | 0.00913   | 1     | 0.935 | CTRL1-PT. 35| SE |
| Exon5             | 0         | 0     | 0.938 | CTRL1-PT. 35| SE |
| Exon8             | 2.19E-05  | 1     | 0.858 | CTRL1-PT. 35| SE |
| Exon5             | 3.25E-12  | 1     | 0.922 | CTRL1-PT. 34| SE |
| Exon5             | 0         | 0     | 0.943 | CTRL1-PT. 34| SE |
| Exon6             | 0         | 1     | 0.919 | CTRL1-PT. 34| SE |
| Exon2             | 7.59E-09  | 1     | 0.891 | CTRL1-PT. 38| SE |
| Exon5             | 0         | 0     | 0.965 | CTRL1-PT. 38| SE |
| Exon2             | 0.018798  | 1     | 0.925 | CTRL1-PT. 27| SE |
| Exon5             | 0         | 0     | 0.956 | CTRL1-PT. 33| SE |
| Exon5             | 0.00303   | 1     | 0.968 | CTRL1-PT. 9 | SE |
| Exon6             | 0.016242  | 0.971| 1     | CTRL1-PT. 9 | SE |
| Exon5             | 0         | 0     | 0.957 | CTRL1-PT. 23| SE |
| Exon5             | 0         | 0     | 0.975 | CTRL1-PT. 37| SE |
| Exon8             | 9.08E-06  | 1     | 0.886 | CTRL1-PT. 37| SE |
| Exon5             | 0         | 0     | 0.826 | CTRL1-PT. 36| SE |
| Exon5             | 0.000116  | 1     | 0.655 | CTRL2-PT. 24| SE |
| Exon6             | 4.1E-07   | 1     | 0.851 | CTRL2-PT. 41| SE |
| Exon5             | 5.29E-05  | 1     | 0.956 | CTRL2-PT. 41| SE |
| Exon6             | 0.014684  | 1     | 0.965 | CTRL2-PT. 41| SE |
| Exon6             | 0.003267  | 1     | 0.976 | CTRL2-PT. 29| SE |
| Exon2             | 0.042443  | 1     | 0.949 | CTRL2-PT. 40| SE |
| Exon11            | 0.000131  | 1     | 0.953 | CTRL2-PT. 40| SE |
| Exon2             | 0.023843  | 1     | 0.935 | CTRL2-PT. 35| SE |
| Exon | OR  | p-value | Expression Value | Control | Sample |
|------|-----|---------|-----------------|---------|--------|
| Exon5 | 0.02414 | 1 | 0.938 | CTRL2-PT. 35 | SE |
| Exon8 | 0.003747 | 1 | 0.858 | CTRL2-PT. 35 | SE |
| Exon5 | 5.6E-08 | 1 | 0.922 | CTRL2-PT. 34 | SE |
| Exon5 | 0.038203 | 1 | 0.943 | CTRL2-PT. 34 | SE |
| Exon6 | 0.03055 | 1 | 0.955 | CTRL2-PT. 34 | SE |
| Exon6 | 2.54E-10 | 1 | 0.919 | CTRL2-PT. 34 | SE |
| Exon2 | 0.001843 | 1 | 0.891 | CTRL2-PT. 38 | SE |
| Exon5 | 0.006081 | 1 | 0.968 | CTRL2-PT. 9 | SE |
| Exon8 | 0.005698 | 1 | 0.886 | CTRL2-PT. 37 | SE |
| Exon5 | 1.19E-05 | 1 | 0.826 | CTRL2-PT. 36 | SE |
| Exon13 | 0.0072 | 0.061 | 0.036 | CTRL1-PT. 41 | A3SS |
| Exon13 | 2.31E-06 | 0.061 | 0.133 | CTRL1-PT. 29 | A3SS |
| Exon13 | 6.73E-08 | 0.061 | 0.005 | CTRL1-PT. 39 | A3SS |
| Exon13 | 0.01702 | 0.061 | 0.102 | CTRL1-PT. 35 | A3SS |
| Exon13 | 0.016737 | 0.061 | 0.036 | CTRL1-PT. 25 | A3SS |
| Exon13 | 5.51E-11 | 0.061 | 0.2 | CTRL1-PT. 9 | A3SS |
| Exon13 | 4.71E-08 | 0.061 | 0.153 | CTRL1-PT. 28 | A3SS |
| Exon13 | 4.33E-16 | 0.061 | 0 | CTRL1-PT. 42 | A3SS |
| Exon13 | 0.003739 | 0.073 | 0.036 | CTRL2-PT. 41 | A3SS |
| Exon13 | 0.004242 | 0.073 | 0.133 | CTRL2-PT. 29 | A3SS |
| Exon13 | 7.91E-08 | 0.073 | 0.005 | CTRL2-PT. 39 | A3SS |
| Exon13 | 1.19E-06 | 0.073 | 0.2 | CTRL2-PT. 9 | A3SS |
| Exon13 | 0.000171 | 0.073 | 0.153 | CTRL2-PT. 28 | A3SS |
| Exon13 | 7.32E-15 | 0.073 | 0 | CTRL2-PT. 42 | A3SS |
| Exon5 and exon6 | 0 | 0 | 0.438 | CTRL1-PT. 34 | MXE |
| Exon5 and exon6 | 0 | 0 | 0.378 | CTRL1-PT. 33 | MXE |
| Exon5 and exon6 | 0 | 0 | 0.455 | CTRL1-PT. 23 | MXE |
| Exon5 and exon6 | 9.65E-05 | 0.253 | 0.438 | CTRL2-PT. 34 | MXE |
| Exon5 and exon6 | 0.003629 | 0.253 | 0.378 | CTRL2-PT. 33 | MXE |
| Exon5 and exon6 | 1.25E-05 | 0.253 | 0.455 | CTRL2-PT. 23 | MXE |
| Exon5 | 0 | 0 | 0.897 | CTRL1-NTP17-010517 | SE |
| Exon2 and exon3 | 3.23E-08 | 0.062 | 0.396 | CTRL1-NTP17-010517 | RI |
| Exon10 and exon11 | 1.64E-14 | 0.009 | 0.082 | CTRL1-NTP17-010517 | RI |
| Exon2 and exon3 | 3.64E-13 | 0.062 | 0.443 | CTRL1-PT.13 | RI |
| Exon10 and exon11 | 0 | 0.009 | 0.144 | CTRL1-PT.13 | RI |
| Exon2 and exon3 | 0 | 0.062 | 0.73 | CTRL1-PT.14 | RI |
| Exon10 and exon11 | 1.64E-09 | 0.009 | 0.069 | CTRL1-PT.14 | RI |
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