Molecular characterization and clinical outcome of B-cell precursor acute lymphoblastic leukemia with *IG-MYC* rearrangement

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Supplementary Methods

Conventional cytogenetics and fluorescence *in situ* hybridisation

Briefly, *IGH (*CytoCell (Oxford Gene Technologies, Begbroke, Oxfordshire, UK) IGH breakapart, Vysis (Abbott Laboratories. Abbott Park, Illinois, U.S.A) LSI IGH Dual Color, Break Apart Rearrangement Probe)*, MYC* (CytoCell MYC breakapart, V*ysis MYC* Dual Color, Break Apart Rearrangement Probe) *, BCL2* (CytoCell BCL2 breakapart) and *BCL6* (CytoCell BCL6 breakapart) fluorescence *in situ* hybridisation (FISH) probes were mixed 1:1 with hybridisation buffer and denatured at 75°C for five minutes followed by hybridisation at 37°C overnight. Slides were washed for two-minutes in 0.02% SSC with 0.003% NP40 at 72°C followed by two minute room temperature incubation in 0.1% SSC. Slides were mounted with 10µl DAPI (Vector laboratories, California, USA). Manual visualisation and scoring were performed using an Olympus BX-61 fluorescence microscope with a x100 oil objective (Leica Microsystems, Gateshead, UK). Where possible, more than 100 interphase nuclei were scored for each FISH test by two independent analysts. A cut-off threshold of >5% was used for all probes to allow for interference and obscuring of signals (false positives). This cut-off level was established by counting the number of abnormal signals generated when the FISH probes were hybridised to normal cells.

Whole exome sequencing

Data analysis

Quality reports were generated using Java base tool FastQC (Babraham Bioinformatics, Cambridge, UK) and read pairs were mapped to human genome reference (hg19) (ftp://ftp.broadinstitute.org/bundle/hg19/)), using default settings from BWA-MEM (Burrows-Wheel Aligner, 0.7.17-r1188 version). Broad Institute best practices PICARD (2.19.0 version, https://broadinstitute.github.io/picard/) tools were applied for the pre-processing (merging the read groups, coordinate sorting, marking duplicates and correction of technical biases) of aligned bam files.

SNVs and Indels in tumour samples were identified using an in-house NGS data analysis pipeline. A separate analysis was run for the generation of a panel of normal (PON) from high quality 61 constitutional/normal exome samples generated using the same exome preparation methods, software and sequencing technologies to capture recurrent technical artefacts in order to improve the results of the somatic variant calling. Initial genomics variants for SNVs were identified using tumour only algorithm of MuTect2 caller of GATK (Genome Analysis Tool Kit, version 3.8), followed by extensive review of corresponding genomic positions in the PON database built in the previous step. Variant call sets were filtered after calculating the new quality scores using the VQSR (variant quality score recalibration) method. All putative somatic variants were annotated using Ensembl Variant Effect Predictor (VEP, version 90). Candidate variants

coding for synonymous consequences and allelic frequency > 1% in gnomAD (version 2.1) were discarded from further analysis.

Due to the absence of paired constitutional DNA samples, we took a targeted approach to identify common BCP-ALL and BL mutations. Genes included in the BCP-ALL panel were *ANK3, ARID1A, ASMTL, ASXL3, ATM, ATRX, BIRC6, CBL, CBLB, CHD4, CREBBP, CSF3R, CTCF, EBF1, EP300, ERG, ETV6, FAT1, FAT2, FAT4, FBXW7, FLT3, IKZF1, IKZF2, IKZF3, IL7R, JAK1, JAK2, JAK3, KAT6B, KDM6A, KIT, KRAS, KMT2A, KMT2B, KMT2C, NCL, NF1, NOTCH2, NRAS, PAX5, PDGFRA, PTPN11, RUNX1, RUNX2, SEMA7A, SF3B1, TET1, TET2, TP53, TUSC3, WHSC1, WT1* and *ZEB2*. Genes included in the BL panel were *FOXO1, MYC, ID3, TP53, DDX3X, TCF3, SMARCA4, GNA13, CCND3, ARID1A, RET, RHOA, PIK3R1, KMT2C, KMT2D and PTEN*.

Illumina Infinium MethylationEPIC BeadChip array

Briefly, 250ng dsDNA from 17 patients was hybridised to the Illumina Infinium MethylationEPIC BeadChip Array (Illumina, San Diego, CA, USA) by Eurofins Genomics (Ebersberg, Germany). DNA was treated with sodium bisulfite using the EZ-96 DNA methylation kit (Zymo Research, Irvine, CA, USA). The bisulfite-treated DNA was analysed using the manual Illumina Infinium HD.

Methylation assay according to manufacturer's guidelines. The BeadChip was scanned with both red (Cy5) and green (Cy3) laser on an iScan instrument with the iScan Control software (Illumina) determining intensity values for each bead type. The data was analysed using Illumina's Genomestudio software. Data were quality controlled and normalised as previously reported[1]. We augmented this data with additional publicly available methylation array data (Supplementary Table 3).

RNA-sequencing

RNA integrity was assessed using the Bioanalyser with RIN scores >6 being included. 500ng of RNA was used to prepare RNA strand-specific libraries using TruSeq Stranded mRNA Library Prep Kit (Illumina) according to the manufacturer's instructions. For sequencing, pooled libraries were loaded on the cBot (Illumina) and cluster generation was performed using manufacturer's instructions. Paired-end sequencing using 100bp read length was performed on a HiSeq2500 machine (HiSeq Control Software 2.2.58) using HiSeq Flow Cell v4 and TruSeq SBS Kit v4. For processing of raw data RTA version 1.18.64 and CASAVA 1.8.4 was used to generate FASTQ-files. Data were quantified by kallisto[2]. The estimated counts were transformed using voom from the limma R package[3] and batch effect was corrected by permuted SVA[4].

Supplementary Table Legends

Supplementary Table 1. Supplementary Table 1. Patient cohort demographic, clinical and cytogenetic characteristics

Supplementary Table 2. Immunophenotype data collected from patients with IG-MYC-r

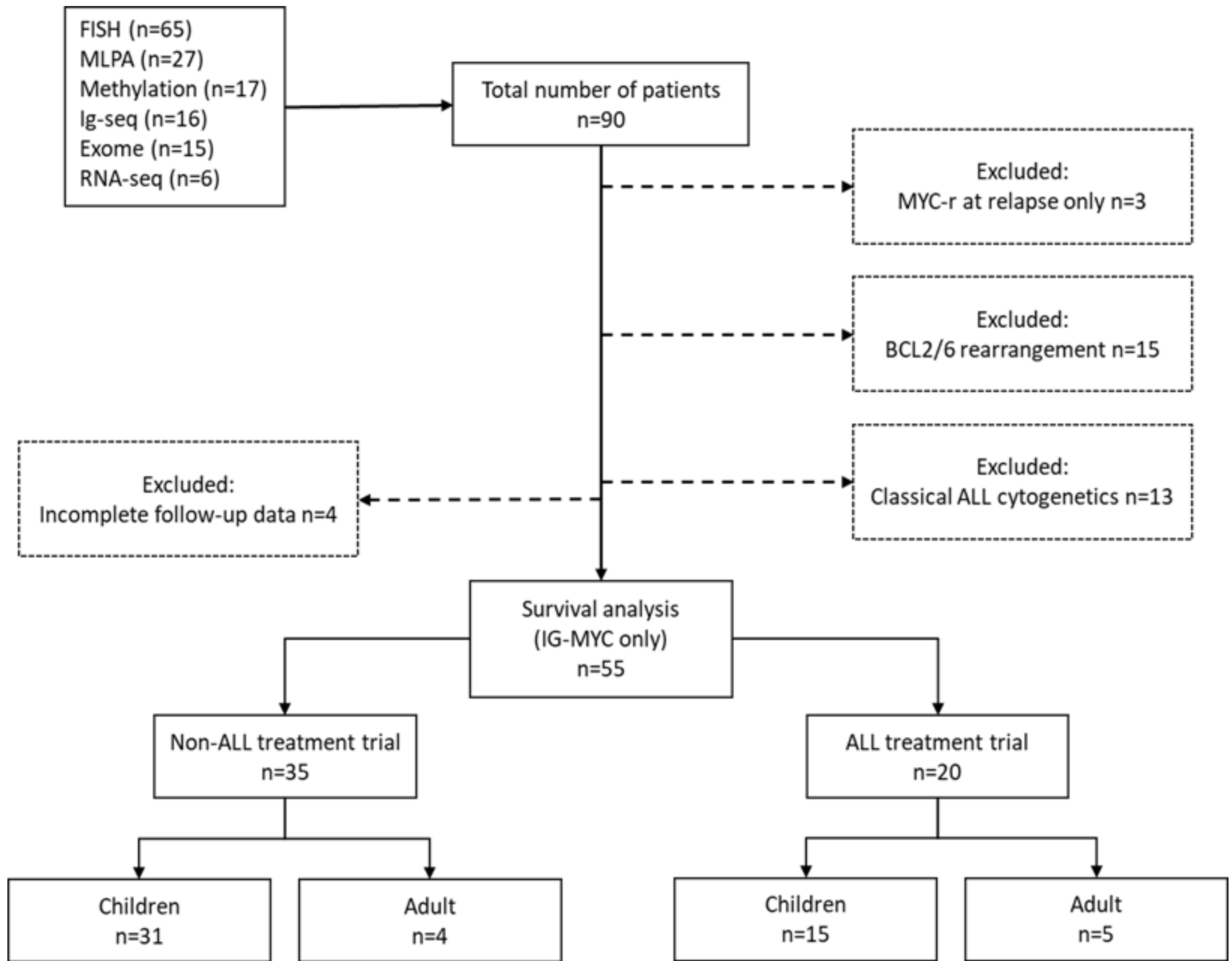
Supplementary Table 3. Publicly available methylation array datasets used in our analysis

Supplementary Table 4. Detail of translocations involving immunoglobulin and/or MYC genes detected by targeted sequencing

Supplementary Table 5. Univariate analysis of the key molecular features identified in children and adults with IG-MYC (excluding those with BCL2/6-r and established ALL-specific rearrangements).

Supplemental tables provided as excel files

Supplementary Figures



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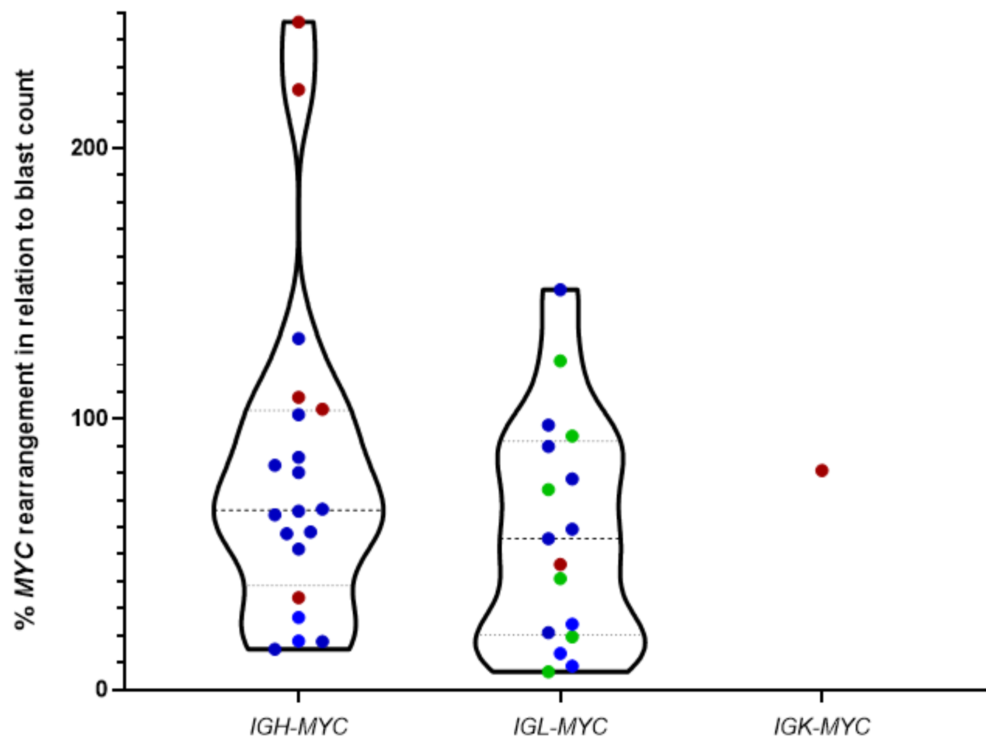

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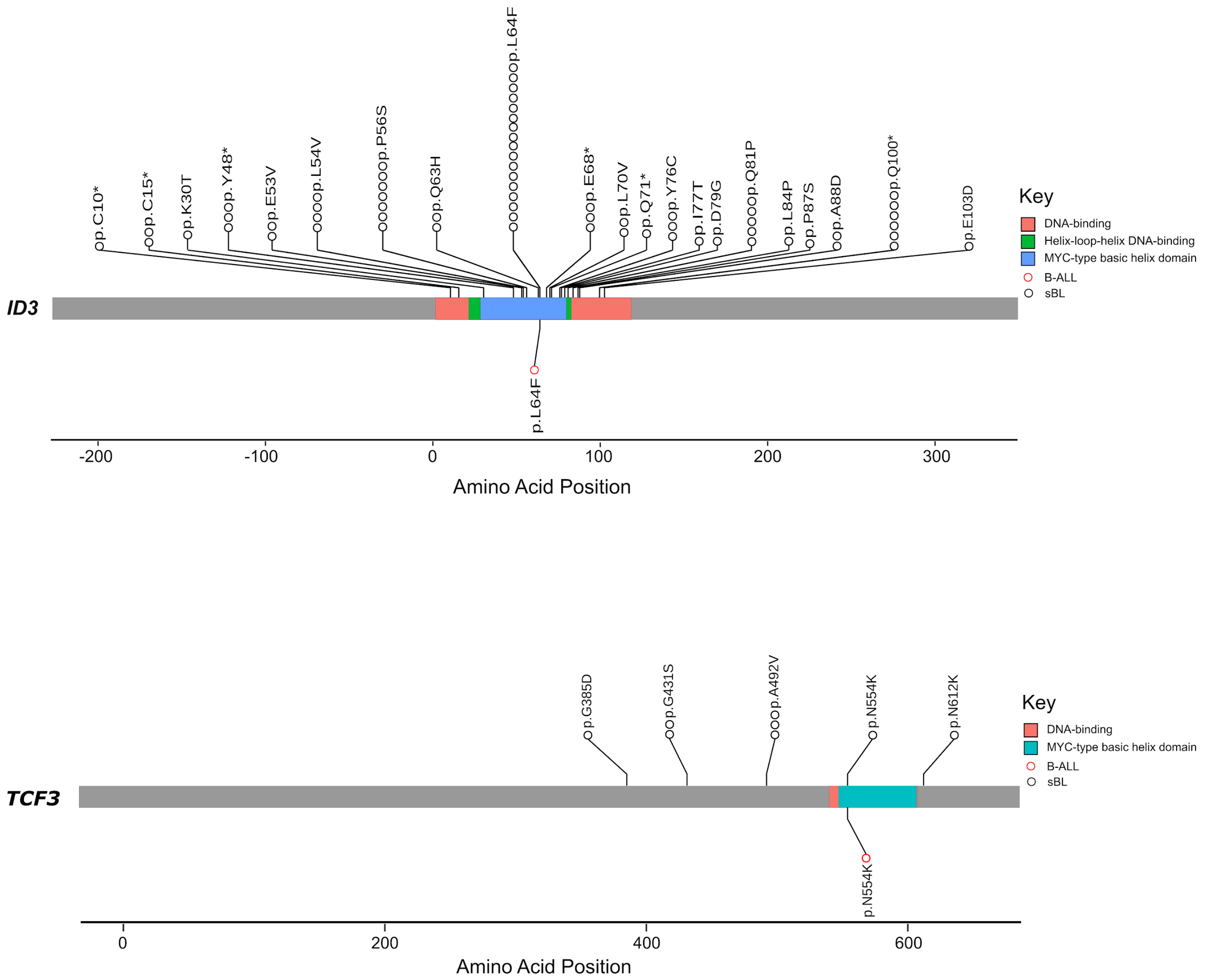

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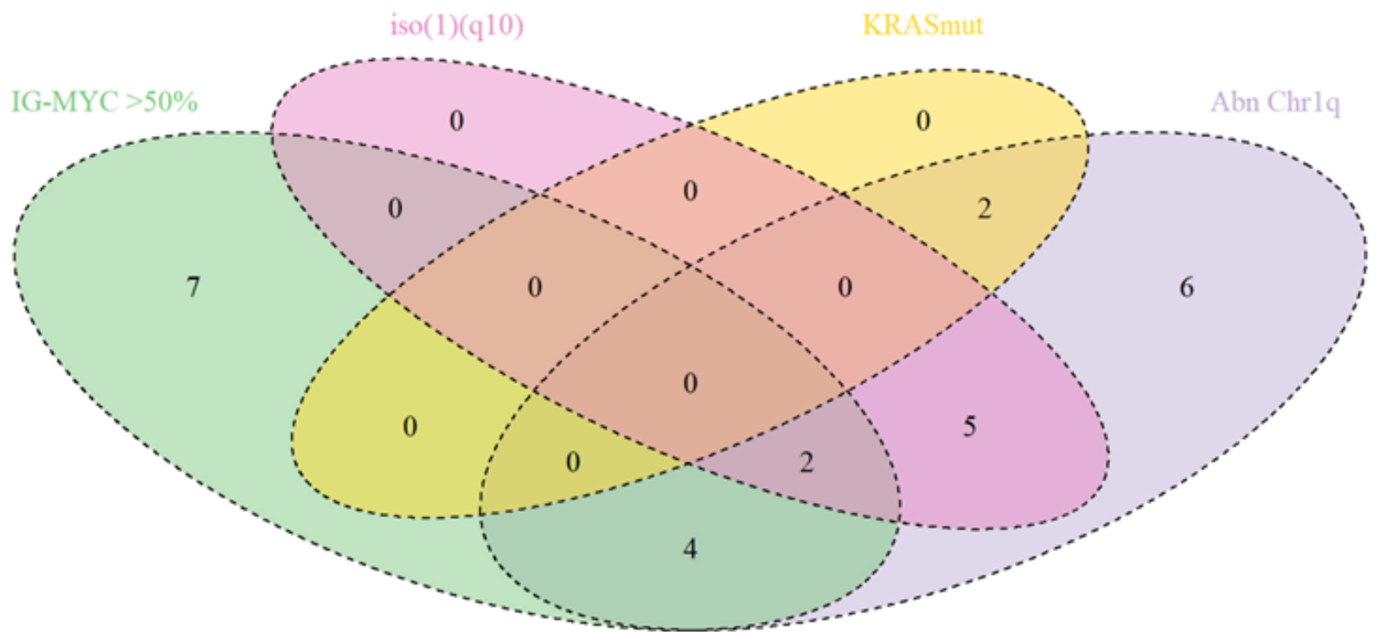

Supplementary Figure 4. Venn diagram of the key molecular features identified within children with *IG-MYC*-r BCP-ALL.

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