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POSTER ABSTRACTS

618.ACUTE LYMPHOBLASTIC LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Nanopore Cas9-Targeted Long-Read Sequencing - a Fast and Flexible Diagnostic Tool for the Identification of B-Cell Acute Lymphoblastic Leukemia Associated Gene Rearrangements

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B-cell acute lymphoblastic leukemia (B-ALL) is characterized by a multitude of recurrent genetic alterations, which drive malignant transformation. Besides their essential role in leukemia biology these aberrations also serve as predictors of treatment response and survival, making their reliable detection a prerequisite for risk stratification in state-of-the-art clinical trials. Currently, the genetic characterization of B-ALL in most diagnostic laboratories still relies on an integrative approach combining conventional cytogenetics, reverse transcription polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH) assays as well as single nucleotide polymorphism (SNP) array analysis. However, these conventional diagnostic tools fail to identify a considerable number of genetic alterations, particularly cryptic rearrangements such as *IGH::DUX4*, *IGH::EPOR* or *MEF2D* involving fusions. Although next generation DNA- and RNA-based sequencing (NGS) assays have significantly improved the reliable detection of fusion genes, the need for large sample numbers to achieve acceptable turnaround times and cost effectiveness impairs their application in small- to medium-sized diagnostic laboratories.

To address these issues, we developed a targeted sequencing assay, based on the commercially available combination of Cas9-mediated target enrichment and Nanopore long-read sequencing, which facilitates the identification of B-ALL associated gene rearrangements on the DNA level in a single sample format.

The targeted panel design included an initial review of the literature to define the breakpoint-spanning regions involved in fusion gene formation followed by the design of specific guide RNAs (gRNA) to facilitate Cas9-mediated enrichment of these regions of interest (ROIs). We first conducted ROI enrichment and sequencing with a limited number of gRNAs targeting only *ETV6*, *KMT2A* and *DUX4* fusions. Then we progressively extended the pool of gRNAs with each sequencing run until it comprised 91 gRNAs covering most B-ALL-associated rearrangements. This stepwise approach allowed us to identify negative effects of increasing panel size on overall enrichment efficacy and simplified the identification and replacement of poor performing gRNAs.

Our initial cohort consisted of 12 well-characterized samples harboring 13 gene rearrangements, including *ETV6::RUNX1*, *KMT2A::AFF1*, *MEF2D::BCL9*, *ZMIZ1::ABL1*, *SSBP2::CSF1R*, *PDGFRB::EBF1*, *IGH::EPOR*, *IGH::DUX4*, *IGH::CRLF2*, *IGH::CEBPE*, *IGH::MYC*, and *IGH::BCL2*. To ensure easy implementation in diagnostic workflows, we used genomic DNA, isolated according to standard protocols. Cas9-based target enrichment and long-read sequencing on MinION flow cells (v9.4.1) were performed according to protocols provided by Oxford Nanopore and each sample was sequenced for 72 hours. On average, each run yielded 3.72±1.21 Gb, a mean read length of 7.40±1.00 kb with an N50 value of 12.87±1.71 kb and an average read quality (Q) score of 14.72±0.52. For data processing and analysis, we adapted the existing nextflow nf-core bioinformatics pipeline, nanoseq, to include publicly available structural variation callers and quality control tools.

Our approach correctly identified 92.3% (12/13) of the rearrangements with a mean of 42 (range 2-97) breakpoint-supporting reads (BSR) per alteration. Only the *IGH::MYC* translocation was missed as the breakpoint was located outside of our predefined ROIs for MYC. To corroborate these results, we sequenced an additional cohort of 14 B-ALL samples, including

ZNF384::DUX4, *KMT2A::MLLT10*, *KMT2A::USP2*, *PAX5::SOX5*, *EP300::ZNF384*, *P2RY8::CRLF2*, and *IGH::DUX4* positive cases and were able to correctly identify 100% (14/14) of the fusion genes with an average of 83 (range 7-367) BSR.
In conclusion, our newly developed targeted sequencing panel correctly identified 96.3% (26/27) analyzed gene rearrangements in B-ALL, including alterations evading standard diagnostic methodologies such as *IGH::DUX4* or *IGH::EPOR*. Importantly, the assay allows individual analysis in a single sample format, thereby representing a valuable tool for B-ALL diagnostics in small to medium-sized laboratories.

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