TARGETED LOCUS AMPLIFICATION & NEXT GENERATION SEQUENCING FOR THE DETECTION OF RECURRENT AND NOVEL GENE FUSIONS FOR IMPROVED TREATMENT DECISIONS IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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INTRODUCTION
Despite developments in targeted and whole genome gene sequencing, the robust detection of all genetic variation, including structural variants, in and around genes of interest and in an allele-specific manner remains a challenge. Targeted Locus Amplification (TLA) [de Vree et al., Nature Biotechnology 2014;32:1019-25] is a strategy to selectively amplify and sequence entire genes on the basis of the crosslinking of physically proximal sequences. Unlike other targeted re-sequencing methods, TLA works without detailed prior locus information, as one or a few primer pairs are sufficient for sequencing tens to hundreds of kilobases of surrounding DNA. TLA enables robust detection of all single nucleotide variants, structural variants and gene fusions in genes of interest. In addition, TLA enables the haplotyping of sequenced regions.

We describe the use of TLA and NGs to detect fusion genes and sequence mutations relevant for stratification of B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Genomic profiling of BCP-ALL in the last few years has substantially extended the number of risk factors that can be used for risk stratification. However, conventional tests provide incomplete sequence information and can therefore miss clinically relevant information. In addition the opportunities TLA presents in the detection of breakpoint sequences promise to empower breakpoint specific minimal residual disease tests.

TLA TECHNOLOGY
A total of 31 primer sets targeting 19 recurrently affected genes were designed and multiplexed, including the ‘classical’ players MLL, RUNX1, TCF3, and IKZF1, the tyrosine kinase genes ABL1, ABL2, PDGFRB, CSF1R, JAK1, JAK2, JAK3, FLT3, and TYK2, and the tyrosine signaling genes CRLF2, EPOR, IL7R, TSLP, SH2B3, and IL2RB. Primer sets were chosen such that the most relevant regions were sufficiently covered. Viable cells from 47 selected BCP-ALL samples were analysed. TLA prep was performed. TLA amplicons were library preppe using Nextera and sequenced on an Illumina NextSeq (D3 patient samples were analysed per High Output PE150 NextSeq Run).

RESULTS
All 20 rearrangements known to be present in these samples were detected by TLA, including rearrangements in ETV6-RUNX1 (n=5), MLL (n=2), TEF3-PBX1 (n=3), CRLF2 (n=3), EBFB1-PDGFB (n=2), BCR-ABL1 (n=1), RCSD1-ABL2 (n=1), SSBP2-CSF1R (n=1) and iAMP21 (n=2). For 14 of the fusions sequencing depth was sufficient to extract breakpoint-spanning sequences directly. For two cases with known JAK2 fusions with an unknown partner, the fusion gene was identified (TERF2 and BCR), as was the case for an unknown ABL1 fusion (FDXPO). New fusions were identified in 8 cases, including previously described IGHD-EPOR, TCF3-ZNF384, MLL, and CRLF2 fusions, and novel gene fusion of TCF3-CRD6.1L1 and HDAC9-FLT3. In addition we identified deletion breakpoint fusions in IKZF1, and sequence mutations in JAK2.

CONCLUSION
We conclude that TLA is an effective method for the reliable detection of sequence mutations and structural variations that are relevant for disease prognosis and/or could be targeted by approved kinase inhibition.

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