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Evaluation of a next generation sequencing panel for ABL1 kinase domain mutations in patients with BCR-ABL1 positive chronic myeloid leukaemia

De Jager, Tanya

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Abstract

Background

The commonest cause of failure to respond to imatinib or loss of molecular response in patients with *BCR-ABL1* positive chronic myeloid leukaemia (CML) is the presence of *BCR-ABL1* tyrosine kinase domain mutations. Next generation sequencing (NGS) can detect clinically relevant low-level mutations in this subgroup of patients thus guiding therapeutic options . We aimed to: 1. establish a novel "in-house" NGS assay to detect *ABL1* mutations. The Nextera™ XT DNA library preparation kit as well as the iSeq™ 100 Sequencing System were both selected for this application. The Nextera™ Kit allows for simultaneous fragmentation and tagmentation of the DNA fragment with unique sequencing adapters. This feature along with the iSeq™ 100 Sequencing System with its small size and cost effectiveness, allows single gene sequencing with less scientist hands-on time, faster TAT and reduction in all round cost for *ABL1* kinase domain sequencing. Our second aim was evaluate the performance of this assay in detecting mutations previously characterized by Sanger sequencing (SS).

Methods

Retrospective samples from 24 patients diagnosed with CML, who underwent *BCR-ABL1* tyrosine kinase domain mutations due to suboptimal/failed/loss of response to tyrosine kinase inhibitor (TKI) therapy were tested. All samples had previously undergone Sanger sequencing. Messenger RNA (mRNA) from forty patient samples was converted to cDNA. Two rounds of polymerase chain reaction (PCR) was performed to first select-out *BCR-ABL1* and then *ABL1*. Amplification was assessed using 2% agarose gel electrophoresis for product bands that were clearly visible and of the correct size. Twenty-four samples with the best results were chosen for NGS. NGS library preparation was performed using the Illumina ® (San Diego, CA, USA) Nextera™ Kit that uses transposase technology. Library quantification was performed using the Qubit™ (ThermoFisher, Waltham, USA), samples were pooled into a single tube and loaded onto the sequencing cartridge. Data analysis was initiated as the sequencing run progressed and the sequencing data aligned to the reference genome (Hg38) specifically using chromosome 9. The Illumina® (San Diego, CA, USA) variant caller software separated patient samples based on the

unique barcode, after which the software package Burrows-Wheeler-Aligner (BWA) was used for mapping low-divergent sequences against the reference genome. A binary Alignment Map (BAM) file is generated first and used to create the Variant Call File (VCF) that contains the data of the mutations detected during the sequencing run

Results and Discussion

Due to non-specific amplification suspected to have occurred during the first round PCR, off target gene sequences, in addition to *BCR-ABL1*, were also amplified and sequenced during the first NGS run. During analysis of the samples it was noted that amplicons were aligning to numerous other sequences in the reference genome. After trouble shooting and a new set of PCR primers, the second sequencing attempt was successful. Here we describe the design of the assay, improvements to the original design and preliminary NGS results compared to Sanger sequencing results. Sequencing of *ABL1* during the second NGS run yielded good sequencing reads on 23 out of 24 samples and detected mutations that were also identified using SS. In addition, the average depth of coverage was noted to be ~5000.

Conclusion

The Illumina® (San Diego, CA, USA) iSeq™ 100 Sequencing System for *ABL1* KD mutational analysis, using the semi nested *BCR-ABL1* PCR product was successful in detecting 23/24 mutations previously detected by SS. This study does not allow for a full validation owing to the retrospective nature of samples and a prospective assessment is planned to validate and implement NGS for this purpose.
Multiple international studies have shown NGS to be a reliable, accurate and sensitive diagnostic tool for this purpose. With both the European LeukaemiaNet and the National Comprehensive Cancer Networks recommending NGS in CML for *ABL1* KD mutational analysis, it is imperative that the SCGU laboratory, a large centre which services multiple clinical haematology oncology centres across South Africa be able to offer NGS for *ABL1* KD mutational analysis. Validation and implementation of this test will increase turnaround time, decrease scientist hands-on time, and reduce the cost per test. In addition, once suboptimal response or failure is detected, earlier detection of sensitive clones allows clinicians to switch to more appropriate therapy.