Ultrasensitive amplicon based detection of circulating tumour DNA for identification of minimal residual disease

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Background & objectives

Detecting rare variant alleles is becoming increasingly relevant for developing novel diagnostic tools in diverse fields, such as oncology, prenatal diagnostics and transplantation medicine. Significant challenges in analysing cell-free DNA from blood plasma are limited amounts of material, highly degraded source material, and the presence of variants of interest at extremely low variant allele frequency. Here we present a highly optimised workflow for creating tumour-guided personalised ultrasensitive sequencing assays for cancer detection from liquid biopsies based on SiMSen-Seq.

Methods

We used personalised amplicon-based sequencing panels to detect ultra-rare variants. We develop multiplex assays using an advanced, dedicated, in silico assay design software, a carefully optimised reaction chemistry and a machine-learning enhanced variant calling algorithm.

Results

These enhancements significantly increase the dynamic range and sensitivity to detect rare mutations and recover target molecules. We also developed tools for the analysis of sequencing data containing UMI. Our results show the benefit of using a personalised approach to detect minimal residual disease in cancer patients, as this is providing extremely high sensitivity and specificity, irrespective of cancer type. Our data also demonstrates the ability of personalised assays to predict the treatment response of cancer patients during treatment.

Conclusion

Thus, SiMSen-seq facilitates highly accurate variant detection in challenging sample types such as liquid biopsies. Lastly, we demonstrate assay performance using real-world data from clinically relevant material.