Enspyre mrd: a novel enrichment approach for ultra-low variant detection with reduced sequencing requirements

Abstract Submitter: Barnaby Balmforth, United Kingdom*

Co-Authors: Katarzyna Anton, Timon Heide, Paulina Powalowska-Pickton, Ernesto Lowy-Gallego, Amy Lovell, Jeffrey Gregg, Sophie Hackinger, Magdalena Stolarek-Januszkiewicz, Robert Osborne

*Biofidelity Ltd.

Abstract

Background

The ability to detect minimal residual disease (MRD) in cancer patients post-treatment and in the surveillance setting offers the potential for earlier recurrence detection and improved patient care. To date, next-generation sequencing (NGS)-based liquid biopsy MRD tests either suffer from limited sensitivity or high cost due to the amount of sequencing data required.

Objective

Enspyre (Enrichment by selective pyrophosphorolysis and release) is a novel hybridisation and capture technology enabling selective enrichment of mutant molecules prior to sequencing, thus increasing sensitivity while simultaneously decreasing sequencing needs. We present here proof-of-concept data of an Enspyre MRD workflow, capable of correctly calling MRD status down to 10 parts per million (ppm) with only 5 million read pairs per sample.

Methods

Selective enrichment is achieved through a bespoke workflow utilizing custom capture probes designed to match the target variant. Separation of mutant and wild-type DNA fragments prior to sequencing is achieved by pyrophosphorolysis, a reverse polymerase reaction digesting fully complementary probes, but halting at a base mismatch.

To emulate clinical cell-free DNA dynamics, DNA from cell line reference samples was fragmented and diluted into background DNA with a different genotype. Mutations present in the target sample, but not in the background, were treated as the “somatic” target variants. A proprietary machine learning tool was used to design probes against these target mutations. The resulting probe pools were tested on sample dilutions ranging from 10 to 100,000 ppm, as well as blank (0 ppm) samples.

Results

Using up to 1,995 sample-specific probes and a proprietary statistical model, we were able to call positive samples and accurately quantify mutant DNA levels down to 10 ppm, with zero false-positive calls in blank samples. Additionally, Enspyre successfully called individual mutations down to less than 1% variant allele frequency. This was achieved using only 20 ng of input DNA and 5 million paired-end sequencing reads per sample.

Conclusion

By enabling ultra-low mutant DNA detection down to 10 ppm or better with just 5 million reads, Enspyre has the potential to transform MRD testing—making it more accessible, scalable, and practical for routine clinical use.

Do you have any conflicts of interest?

Yes, I have a conflict of interest.

Authors KA, TH, PP-P, EL-G, AL, SH, MS-J, RO and BB are or were employed by the company Biofidelity Ltd at the time of the study, a privately held company. Author JG is employed by the company Biofidelity Inc. All authors may hold stock or stock options.