Utility of a New Targeted Next Generation Sequencing Test for Liquid Biopsy Samples from Patients with NSCLC

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Background
Blood-based methods for profiling tumor-sourced nucleic acids have become increasingly important in the diagnostic workup for cancer patients, particularly in non-small cell lung cancer (NSCLC). Blood-based assays address several limitations of tissue-based testing approaches such as inaccessible lesions, limited tissue, extended time to results, and invasive specimen collection procedures. Broad-profiling next-generation sequencing (NGS) methods are used to detect rare variants in blood samples from cell-free nucleic acid (cfNA). Here we describe the utility of a newly developed GeneStrat52 (GS52) NGS test, which uses the GeneStudio™ S5 Prime platform. We report on performance verification of the assay using contrived clinical specimens. We also present a case study which demonstrates that assay’s use in during GeneStrat52 NGS test, which uses the GeneStudio™ S5 Prime platform. We report on performance verification of Blood-based methods for profiling tumor-sourced nucleic acids have become increasingly important in the diagnostic monitoring. These studies add to the growing body of knowledge supporting the usefulness of molecular detection of invasive specimen collection procedures. Broad-profiling next-generation sequencing (NGS) methods are used to detect EGFR Del19, T790M, and C797S mutations over 540-days of osimertinib treatment.

Materials and Methods

Figure 1. GeneStrat52 Next Generation Sequencing Test Workflow. The test process begins when whole blood arrives at the Biodesix laboratory. Test samples are accessioned and processed to isolate circulating nucleic acids, both cfDNA and cfRNA. Following nucleic acid extraction and cDNA synthesis, 20ng input is entered into library preparation using the Oncomine™ Pan-Cancer Cell-Free Total Nucleic Acid Assay according to the manufacturer’s guidelines. Templating occurs overnight on the Ion Chef™ system. Sequencing is then performed on the Ion Genestudio™ S5 Prime, and analysis is completed with the Torrent Suite™/Ion Reporter™ bioinformatics workflow to make variant calls.

Accuracy Analyses

Figure 5. Graphical representation of the range of allele frequencies detected with ddPCR (x-axis) and NGS (y-axis). High concordance (R=0.988) was observed between the two methods.

Table 1. Concordance between ddPCR and NGS at the variant level. The %MVF for the GeneStrat® Test1 was compared to the ddPCR reference result for each variant were compared to the %MVF results using the GeneStrat® NGS test on the Ion Torrent GeneStudio S5 Prime.

Conclusions

101 cfDNA (cell free nucleic acid) specimens were evaluated from donors previously diagnosed with NSCLC.

84 variants for EGFR (E746-A750del, T790M, L858R, G719X, C797S), BRAF V600E or KRAS G12X were detected with allele frequencies ranging from 0.85-96.16% using the GeneStrat52 NGS test. An additional 37 negative specimens were tested.

Results were highly concordant between the GeneStrat® ddPCR Test and GeneStrat52 NGS (R2=0.986) with 91.7% sensitivity and 100% specificity.

Sensitivity: 91.7% Specificity: 100%

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