Validation of InVision ctDNA NGS Profiling via dPCR Testing in Patients with Non-Small Cell Lung Cancer (NSCLC)

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INTRODUCTION

Tumor tissue based molecular profiling is widely utilized to guide therapy in advanced NSCLC and recently, circulating tumor DNA (ctDNA) assays have been developed to detect actionable alterations in a non-invasive manner. However, there are frequent reports of discordance between analysis platforms and here we compare the Invivata InVisionFirst Next Generation Sequencing (NGS) ctDNA assay with digital PCR (dPCR) based ctDNA analysis. InVisionFirst, an amplicon based NGS method, provides a comprehensive genomic profile of key therapeutically important genomic alterations. (Figure 1 & 2)

METHODS

• A cohort of newly diagnosed, late-stage NSCLC patients (n=52) underwent ctDNA analysis by InVisionFirst
• 36 of the cohort were tested with dPCR assays available from a CLIA/CAP commercial service (Biodiesexs Genestrat) for 9 actionable gene alterations (EGFR L858R, Exon19Del & T790M, EML4-ALK fusions, ROS1 fusions, KRAS G12C, G12D & G12V and BRAF V600E) as part of the routine clinical care provided to the patient
• 16 patients were tested with dPCR with a subset of above gene panel (EGFR L858R & Ex19del, KRAS G12G & G12D) at a reference laboratory
• Tissue analysis in 21 patients was tested to arbitrate any discordance between the results of the 2 liquid biopsy techniques (Caris Molecular Intelligence CGP).

RESULTS

• Across the 9 specific genetic alterations investigated by both ctDNA platforms, 26 alterations were detected by the InVision platform and 23 were detected by the dPCR platform.
• Comparing the liquid biopsy platforms, the overall concordance of gene alterations was 98.5% (338/343) with positive agreement of 95.7% and negative agreement of 98.8%. (Table 1)
• Discordance was observed in 6 detected gene alterations.

DISCUSSION & CONCLUSION

The excellent positive and negative agreement and more accurate variant detection support the use of the InVisionFirst assay as an alternative to dPCR in non-invasive “liquid biopsy” for molecular profiling.

- The use of target specific dPCR tests in liquid biopsy testing has been used for patient care over the past few years which provide the physician with a rapid read-out of potential therapeutic targets. The introduction of plasma-based NGS provides a comprehensive genomic profile allowing for more efficient use of the plasma volume and provides a more accurate read-out of the patient’s specific somatic mutations.
- In this orthogonal comparison study with late-stage NSCLC patients, excellent concordance of the InVision ctDNA NGS assay with dPCR based ctDNA is demonstrated via blinded testing performed by independent laboratories.
- False positive variant detection could occur with dPCR with targets which allow for the PCR primers to hybridize to the patient’s DNA as seen with the KRAS G12D call from the dPCR test.
- The excellent positive and negative agreement and more accurate variant detection support the use of the InVisionFirst assay as an alternative to dPCR in non-invasive “liquid biopsy” for molecular profiling.

Table 1. 2x2 Table for comparison of dPCR to NGS results. For this analysis the KRAS G12A variant was not included.

Table 2. discordant ctDNA testing results

Figure 1. The InVisionFirst assay (Invivata) is a ctDNA NGS assay for detection of genomic alterations in 36 genes commonly mutated in NSCLC and other cancer types.

Figure 2. The InVisionFirst assay utilizes enhanced Tagged Amplicon Sequencing (eTAmSeq) NGS technology which measures somatic Single Nucleotide Variants (SNV), Copy Number Variants (CNV), Insertions and Deletions (InDels) and Gene Fusions.

Figure 3. Mutations not detected by dPCR but detected by InVisionFirst (in white fraction) vary from 0.83% to 1.03%.