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 Inivata InVisionFirst Next Generation Sequencing (NGS) ctDNA assay with digital PCR based ctDNA analysis. InVisionFirst, an amplicon based NGS method, provides a comprehensive genomic profile of key therapeutically important genomic alterations. ***(Figure 1 & 2)***

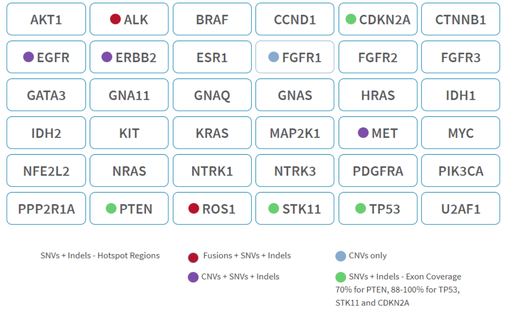

Figure 1. The InVisionFirst assay (Inivata) is a ctDNA NGS assay for detection of genomic alterations in 36 genes commonly mutated in NSCLC and other cancer types.

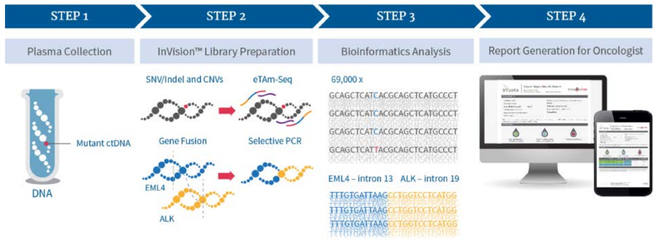

Figure 2. The InVisionFirst assay utilizes enhanced Tagged Amplicon Sequencing (eTAmSeq) NGS technology which measures somatic Single Nucleotide Variants (SNV), Copy Number Variants (CNV), Insertions and Deletions (InDels) and Gene Fusions.

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 (Biodesix 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 by dPCR with a subset of above gene panel (EGFR L858R & Ex19del, KRAS G12C & 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. ***(Table 2)***
- One EGFR L858R detected in dPCR but not in liquid NGS
- Five alterations detected in liquid NGS, at allele fractions 0.3%-3.0% ***(Figure 3)***, were not detected by dPCR. Where tissue data was available (3 cases) this confirmed detection by liquid NGS. These included: two KRAS G12C, one EML4-ALK fusion, one EFGR deletion, and one KRAS G12A mutation.
- The KRAS G12A mutation detected by liquid NGS was confirmed by tissue NGS testing. dPCR testing does not include the G12A variant, however the variant was incorrectly identified as G12D and has been included for discussion purposes.

	dPCR Positive	dPCR Negative	
NGS Positive	22	4	26
NGS Negative	1	316	317
	23	320	343

RESULT:					
Concordance:	98.5%	CI:	0.9663	to	0.9938
Positive Percent Agreement:	**95.7%**	CI:	0.7901	to	0.9923
Negative Percent Agreement:	**98.8%**	CI:	0.9683	to	0.9951

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

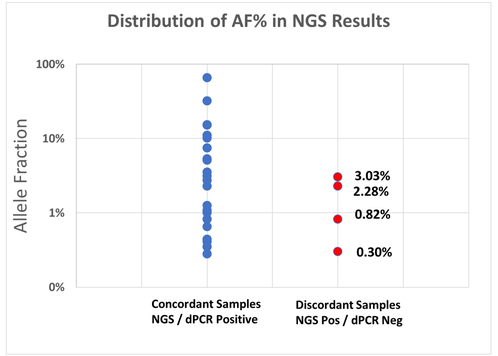

Figure 3. *Mutations not detected by dPCR but detected by InVisionFirst ranged in allele fraction (AF) from 0.30% to 3.03%*

Orthogonal Testing
Discordant Samples

InVisionFirst	*Invision First AF%*	dPCR	Tissue Confirmation
EGFR Ex19Del	*3.03%*	ND	No Tissue Available
KRAS G12C	*0.82%*	ND	No Tissue Available
KRAS G12C	*0.30%*	ND	KRAS G12C
ND	*0%*	EGFR L858R	EGFR L858R
KRAS G12A	*2.28%*	KRAS G12D†	KRAS G12A
EML4-ALK	*	ND	EML4-ALK

ND - Not Detected

* - AF% Not reported for Fusion Variants

† KRAS G12A Not Analyzed by dPCR Assay

Table 2. *Discordant ctDNA testing results*

DISCUSSION & CONCLUSION

- 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.