Molecular Profiling of cell-free DNA and RNA in the Blood of Patients with Non-Small Cell Lung Cancer

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Abstract #6237

Abstract

Lung cancer is the leading cause of cancer-related deaths in the United States with Non-Small Cell Lung Cancer (NSCLC) being the most commonly diagnosed subtype. However, up to 30% of advanced NSCLC patients are not eligible for tissue biopsy. If a tissue biopsy is performed it is invasive, does not reflect tumor heterogeneity, and may not yield enough quality nucleic acid for the identification of actionable targets of treatment. As a supplement, liquid biopsies are becoming increasingly utilized in clinical testing as they are minimally invasive and overall represent a decreased risk to patients. Cell-free total nucleic acid (cfTNA) was isolated from donor patient plasma specimens collected and shipped at ambient temperature in blood collection tubes to the centralized CAP/CLIA laboratory at Biodesix. Following cfTNA extraction, specimens underwent library preparation and were profiled using the Oncomine™ Pan-Cancer Cell-Free Total Nucleic Acid (cfTNA) Assay. Library templating and sequencing were performed on the Ion Chef™ and the Ion GeneStudio™ S5 Plus, respectively, and a bioinformatics workflow was used to make variant calls. Droplet digital™ PCR (ddPCR) was used as an orthogonal method for testing the clinical specimens included in this study. A high level of concordance was observed between NGS and ddPCR using analytical materials and eight clinical specimens. Slightly lower concordance was observed with the low frequency analytic samples (R^2=0.87). The Pan-Cancer cfTNA assay also demonstrated copy number variation gains for ERBB2 and MET (>2 fold-change each) as well as detection of RNA fusions. The Oncomine Pan-Cancer cfTNA assay interrogated in this study demonstrated high inter-run, inter-chip, and inter-assay (ddPCR and NGS) concordance. Validation studies are currently underway to expand the small subset of clinical specimens to a larger cohort of patients diagnosed with NSCLC.

Materials and Methods

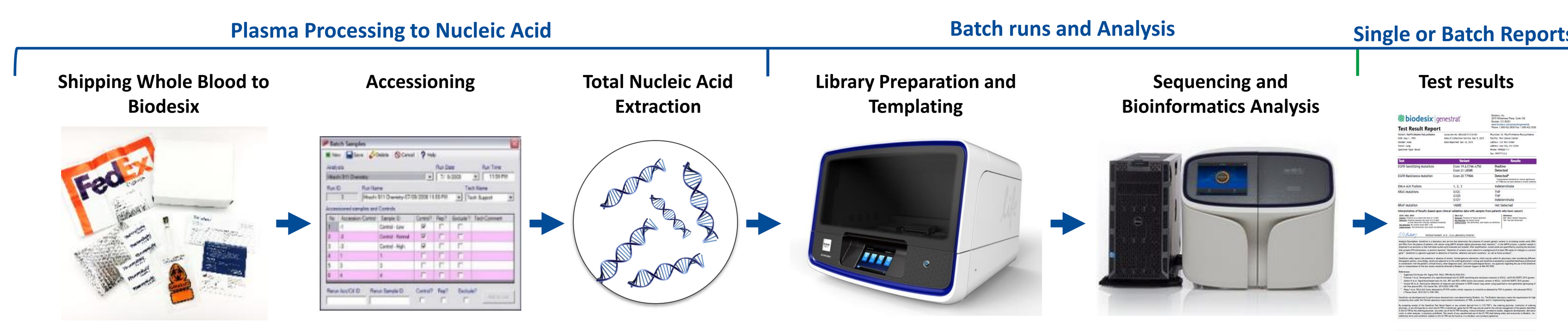


Figure 1. Next Generation Sequencing Workflow. The test process begins with a blood draw shipped at ambient temperature to the Clinical Laboratory. Test samples are accessioned and processed to isolate circulating nucleic acids, both cfDNA and cfRNA. Following nucleic acid extraction and cDNA synthesis, samples undergo library preparation and quantification using the Oncomine™ Pan-Cancer Cell-Free Total Nucleic Acid (cfTNA) Assay according to the manufacturers guidelines. Templating occurs overnight on the Ion Chef™ System. Sequencing is then performed on the Ion GeneStudio™ S5 Plus and analysis is completed with a bioinformatics workflow to make variant calls.

Sequencing Metrics

Figure 2. Sequencing Metrics. Sequencing metrics generated using the TorrentSuite™ v.5.10.1 software are shown. For this study, library preparation was done manually, templating was performed on the Ion Chef™, and sequencing on the Ion Torrent GeneStudio™ S5 Plus system. All QC metrics passed and the No Template Control (NTC) was negative. Clinical specimens demonstrated the appropriate library peak around 100 bp in length (read length histogram) and read depths of greater than 17 million reads each.

Sample	Bases	≥Q20 Bases	Reads	Read Length Histogram
No Template Control	414,622,651	395,738,479	12,436,072	
Clinical Specimen 1	2,989,443,265	2,774,122,750	30,724,722	
Clinical Specimen 2	1,453,096,295	1,361,152,118	17,627,002	
Clinical Specimen 3	1,810,577,305	1,696,671,307	20,591,433	
Clinical Specimen 4	2,176,931,117	2,044,445,529	23,573,947	
Clinical Specimen 5	2,102,692,104	1,959,381,247	24,264,622	
Clinical Specimen 6	1,366,565,251	1,280,611,439	17,236,136	
Clinical Specimen 7	1,287,639,375	1,206,410,865	17,053,224	
Clinical Specimen 8	1,240,096,936	1,158,341,513	17,299,458	

Analytical Results

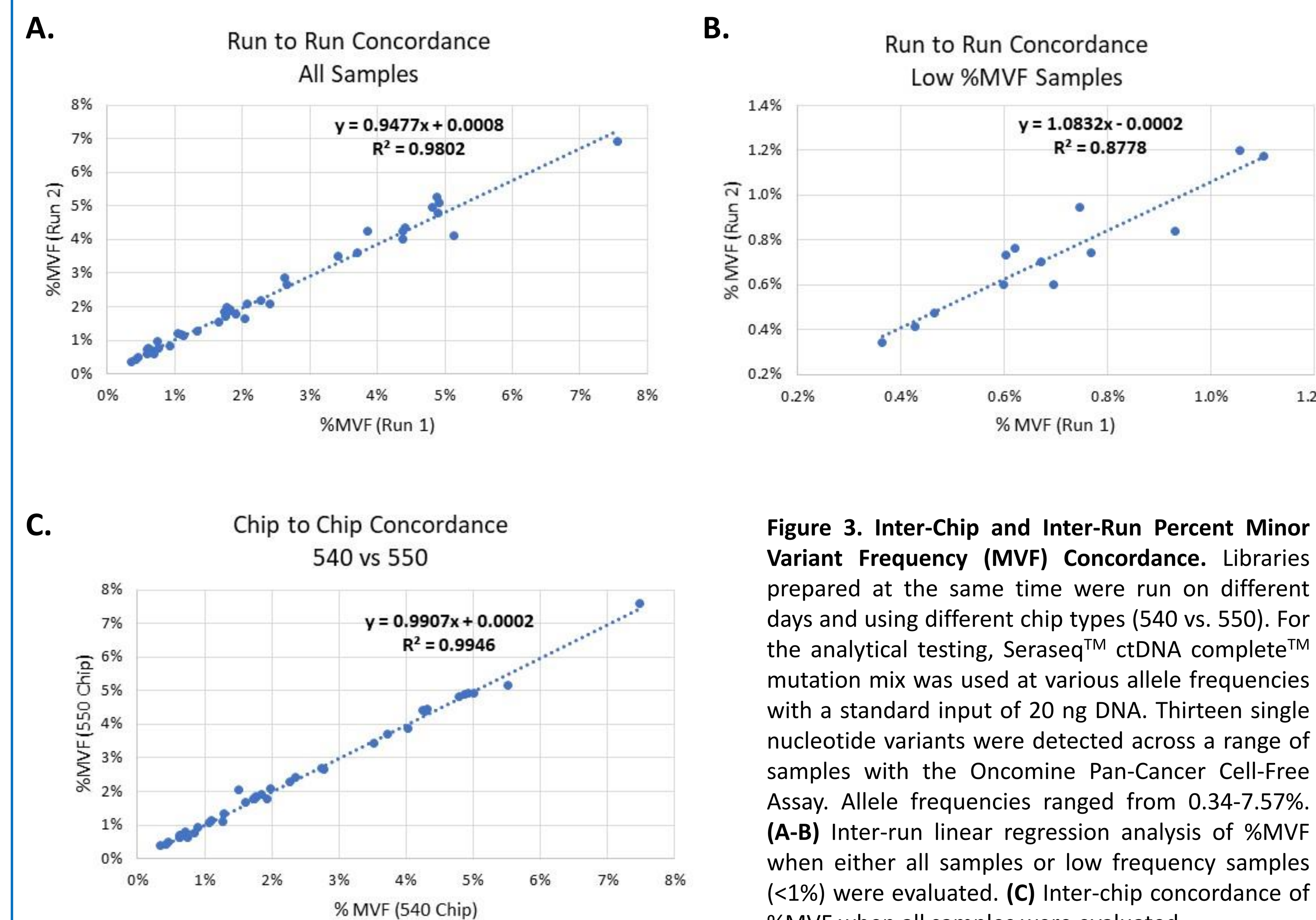
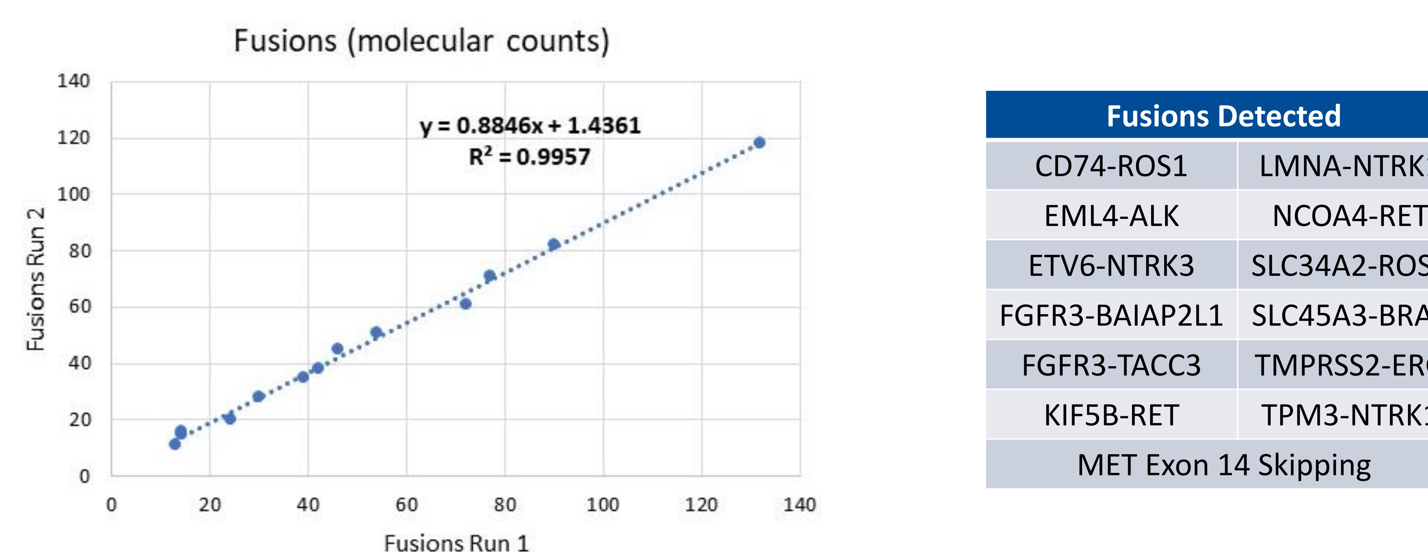


Figure 3. Inter-Chip and Inter-Run Percent Minor Variant Frequency (MVF) Concordance. Libraries prepared at the same time were run on different days and using different chip types (540 vs. 550). For the analytical testing, Seraseq™ ctDNA complete™ mutation mix was used at various allele frequencies with a standard input of 20 ng DNA. Thirteen single nucleotide variants were detected across a range of samples with the Oncomine Pan-Cancer Cell-Free Assay. Allele frequencies ranged from 0.34-7.57%. **(A-B)** Inter-run linear regression analysis of %MVF when either all samples or low frequency samples (<1%) were evaluated. **(C)** Inter-chip concordance of %MVF when all samples were evaluated.

Copy Number Gain (fold-change)			
Gene	Ref. Result	Run 1	Run 2
ERBB2	3.84	2.88	2.87
MET	3.06	2.63	2.65

QC Metrics for CNV calling	
MAPD	< 0.4
Fold-change	≥ 1.15
P-value	$< 10^{-5}$

Figure 4. Copy Number Variations detected by the Pan-Cancer cfTNA assay. The Seraseq™ ctDNA complete™ mutation mix contains SNVs, INDELs, and CNVs. CNVs were detected using a reference method and compared to two independent sequencing runs performed at Biodesix. QC metrics used in the bioinformatics pipeline to accurately call CNVs are shown. Only copy number gains are detectable with this assay.



Fusions Detected	
CD74-ROS1	LMNA-NTRK1
EML4-ALK	NCOA4-RET
ETV6-NTRK3	SLC34A2-ROS1
FGFR3-BAIAP2L1	SLC45A3-BRAF
FGFR3-TACC3	TMPRSS2-ERG
KIF5B-RET	TPM3-NTRK1
MET Exon 14 Skipping	

Figure 5. Fusions detected by the Pan-Cancer cfTNA assay. Total nucleic acid is reverse transcribed prior to library preparation allowing for the detection of fusions. The Seraseq Tumor Fusion RNA Mix v3 reference material was used to detect fusions. Twelve fusion partners and Met exon 14 skipping were identified via NGS with high concordance across multiple runs.

Acknowledgments

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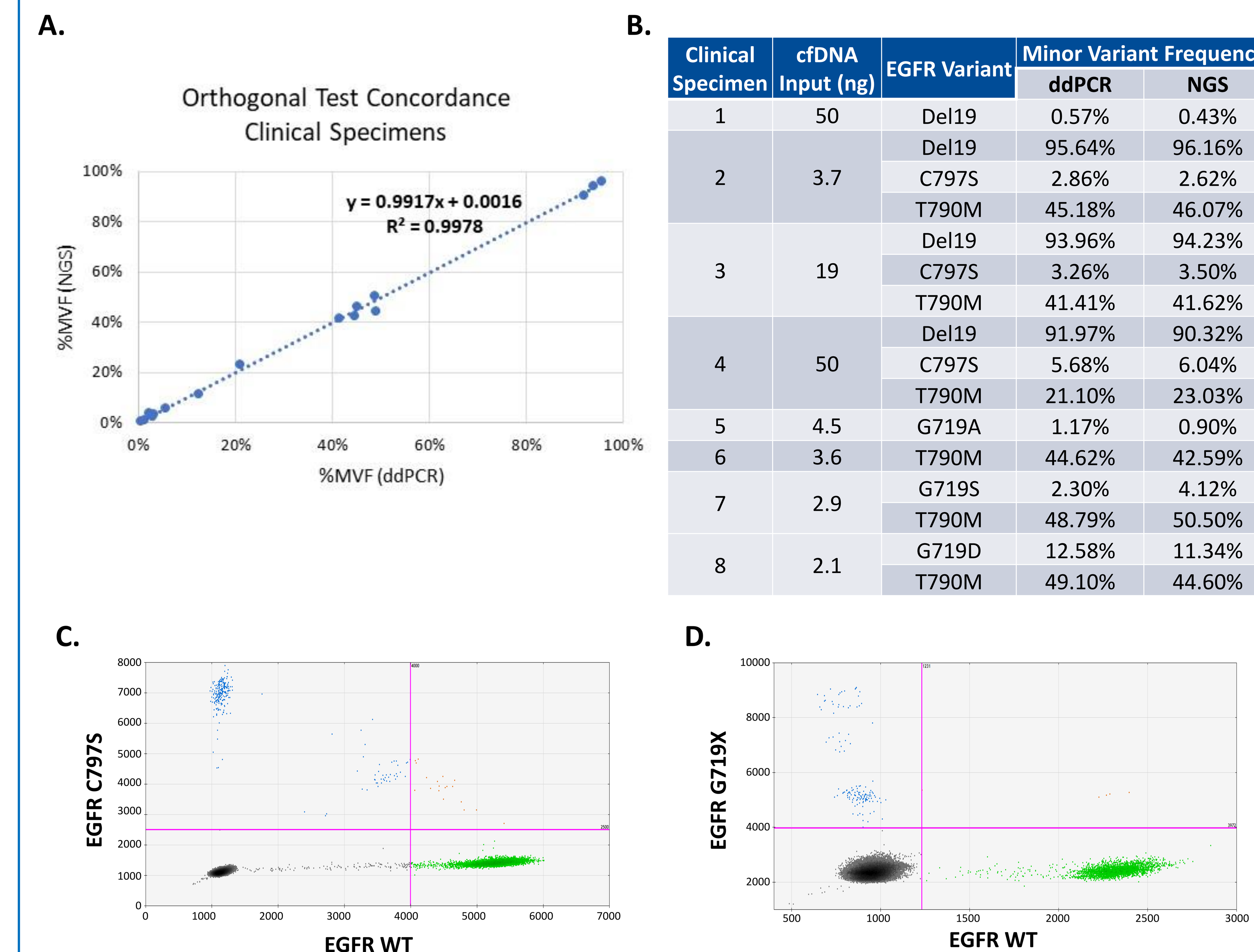
Clinical Specimen Evaluation



B.

Clinical Specimen	cfDNA Input (ng)	EGFR Variant	Minor Variant Frequency	
			ddPCR	NGS
1	50	Del19	0.57%	0.43%
2	3.7	Del19	95.64%	96.16%
		C797S	2.86%	2.62%
		T790M	45.18%	46.07%
3	19	Del19	93.96%	94.23%
		C797S	3.26%	3.50%
		T790M	41.41%	41.62%
4	50	Del19	91.97%	90.32%
		C797S	5.68%	6.04%
		T790M	21.10%	23.03%
5	4.5	G719A	1.17%	0.90%
6	3.6	T790M	44.62%	42.59%
7	2.9	G719S	2.30%	4.12%
		T790M	48.79%	50.50%
8	2.1	G719D	12.58%	11.34%
		T790M	49.10%	44.60%

Figure 6: Concordance between NGS and ddPCR of Clinical Specimens. Eight clinical specimens were selected based on their EGFR status which was previously identified with ddPCR. These specimens underwent library preparation with the Pan-Cancer cfTNA assay and unique EGFR variants were detected. ddPCR was used as an orthogonal method to confirm these variants. **(A)** Percent minor variant frequency was compared between NGS and ddPCR for all EGFR variants. **(B)** List of detected variants and their frequency. **(C-D)** QuantaSoft™ 2D plots demonstrating unique EGFR variant detection in the clinical specimens.

Conclusions

- Analytical and clinical specimens were analyzed during this study
- 100% of reference variants were detected
- Multiple variant types were identified including SNVs, CNVs, and fusions
- High inter-run, inter-chip, and inter-assay (ddPCR and NGS) concordance was observed
- Validation studies are currently underway to evaluate a larger cohort of patients diagnosed with NSCLC

References

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