Validation of Target Selector™ Next Generation Sequencing Lung Panel for the Detection of Circulating Tumor TNA Alterations

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Completing the Answer™

Introduction

Lung cancer is one of the most common cancers and the leading cause of cancer-related deaths among both men and women.[1] Early detection of actionable biomarkers associated with cancer are critical to detect at an early stage. Traditional diagnosis and genotyping of cancer is dependent on a tissue biopsy. These invasive tissue biopsies are often associated with high costs, long turnaround times, discomfort, and complications. The use of liquid biopsies is a vital approach in the detection and monitoring of cancer biomarkers that is enabled through a simple blood sample. Further, next generation sequencing (NGS) has proven to be an invaluable tool for molecular profiling and biomarker discovery across multiple studies.[2-3] Biocept's Target Selector™ NGS Lung Panel Powered by Oncomine from ThermoFisher is a comprehensive liquid biopsy workflow that employs NGS to detect mutations in a panel of 12 genes that are relevant to non-small cell lung cancer, focusing on cancer biomarkers that are clinically actionable. Here we outline analytical and clinical validation of the Target Selector™ NGS Lung Panel for the detection of actionable circulating biomarkers associated with lung cancer.

Methods

More than 100 samples were included in the validation, including more than 50 unique patient samples. Well-characterized circulating tumor reference samples were used to establish limits of detection through analytical validation. For the clinical samples, cell-free total nucleic acid (cfTNA) was extracted from blood collected in Biocept's CEE-Sure™ or K_2 EDTA tubes. cfTNA was then used to prepare amplicon-based NGS libraries to detect somatic alterations in 12 clinically-relevant lung cancer genes. Libraries were templated and sequenced using the Ion Chef and S5 XL Ion Torrent systems. Data was analyzed using Torrent Suite and Ion Reporter software. Reporting and annotation was accomplished using Ion Reporter and Oncomine Knowledgebase software.

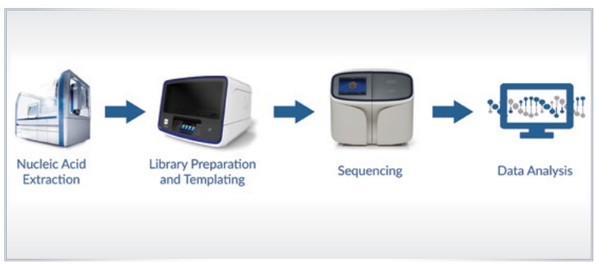

Figure 1. Workflow of the Target Selector™ NGS Lung Panel

Results

Gene List and Content

Target Selector™ NGS Lung Panel Gene List					
Hotspot Genes			CNVs	Fusions	Exon Variants
ALK	*KRAS*	*PIK3CA*		*ALK*	
BRAF	*MAP2K1*	*ROS1*	*MET*	*RET*	*MET exon 14 skipping*
EGFR	*MET*	*TP53*		*ROS1*	
ERBB2	*NRAS*				

All genes in red font are referenced in NCCN Guidelines and/or are associated with FDA-approved therapy.

Target Selector™ NGS Lung Panel Content	
Assay input	DNA + RNA
Hotspot SNV/indel LOD	0.1% MAF
De novo LOD	0.5% MAF
CNV LOD	1.12X
Fusion/exon skipping LOD	3 molecular counts

Table 1. Target Selector™ NGS Lung Panel coverage and content

Analytical Validation

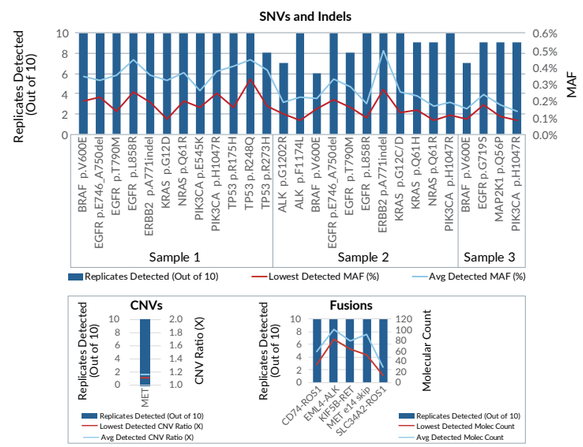

Figure 2. Graph illustrating analytical validation of the Target Selector™ NGS Lung Panel

Results

Clinical Validation

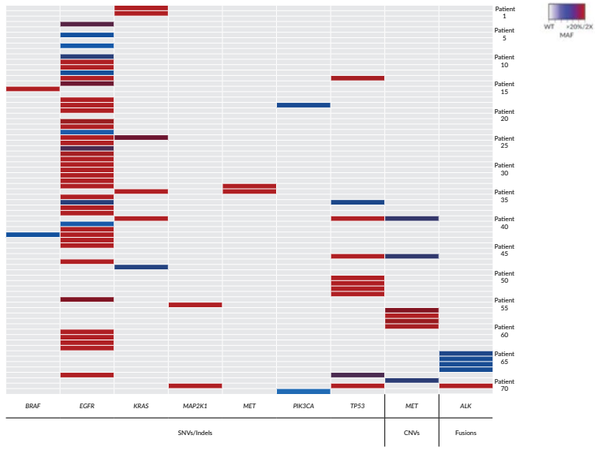

Figure 3. Diagram depicting genetic variants detected in the cohort of patient samples

Demographics | Actionable Mutations

Target Selector™ NGS Lung Panel Patient Demographics		
Age	Median age (years)	68
	Range (years)	42-94
Gender	Female	60.6%
	Male	39.4%
Stage	I-II	6.7%
	III	2.2%
	IV	91.1%
Treatment status	Pre-Treatment	56.4%
	Post Treatment	33.3%
	At Progression	5.1%
	On Treatment	5.1%

Table 2. Demographics of the patient samples included in the validation

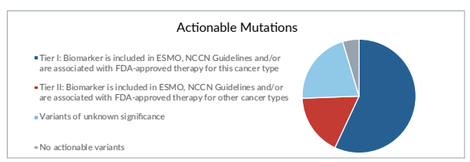

Figure 4. Graph depicting actionable mutations in the patient samples

Summary

Overall Performance Summary

Target Selector™ NGS Lung Panel Performance Summary		
Study		Results
Analytical Accuracy		99.7%
Analytical Specificity		0% error rate
Analytical Sensitivity		SNV: 91.7%
		CNV: 100%
		INDEL: 100%
		Fusions: 100%
Clinical Accuracy		95%
Clinical Precision	Intra-Assay	100%
	Inter-Assay	100%
	Inter-Operator	100%
	Inter-Instrument	100%
	Inter-Reagent	100%
PPV		93.4%
NPV		99.9%
Analytical Interferences		No interference shown by substances tested

Table 3. Table depicting the overall Target Selector™ NGS Lung Panel performance

Conclusions

- Target Selector™ NGS Lung Panel has demonstrated consistent performance for detecting actionable mutations in the circulating DNA and RNA of reference samples and cancer patients.
- Clinically significant biomarkers were detected with a limit of detection as low as 0.1% molecular allele frequency for SNVs and indels, 1.12X for CNVs, and 3 molecular counts for fusions/exon variants.

References

1. National Cancer Institute; Surveillance, Epidemiology and End Results Program. Cancer stat facts: lung and bronchus cancer. 2019. https://seer.cancer.gov/statfacts/html/lungb.html.
2. Pritchett MA, Camidge DR, Patel M, Khatri J, Boniol S, et al. (2019) Prospective Clinical Validation of the InVisionFirst-Lung Circulating Tumor DNA Assay for Molecular Profiling of Patients with Advanced Nonsquamous Non–Small-Cell Lung Cancer. JCO Precision Oncology 2019:3, 1-15.
3. Volckmar AL, Leichsenring J, Kirchner M, Christopoulos P, Neumann O, et al., (2019) Combined targeted DNA and RNA sequencing of advanced NSCLC in routine molecular diagnostics: Analysis of the first 3,000 Heidelberg cases. Int J Cancer. 2019 Aug 1;145(3):649-661.