Monitoring breast cancer biomarkers from circulating tumor DNA using Target Selector™ NGS Breast Panel

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Introduction

The most common cancer diagnosis among women in the United States is breast cancer, which accounts for 30% of all new cancer diagnoses.[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. Liquid biopsies are a non-invasive approach to rapidly detect and monitor cancer biomarkers 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 Breast Panel Powered by Oncomine from ThermoFisher is a targeted, multi-gene liquid biopsy panel assay that detects mutations in 12 breast cancer-related genes, including hotspots in 10 genes and copy number variation in 3 genes. Here, we outline analytical and clinical validation of the Target Selector™ NGS Breast Panel for the detection and monitoring of actionable biomarkers associated with breast cancer.

Methods

A total of more than 100 samples were included in the validation, including well-characterized circulating tumor reference analytical samples and more than 20 unique patient samples. Blood collected in Biocept's CEE-Sure™ or K_2 EDTA tubes was used to extract cell-free DNA (cfDNA) for clinical samples. Using the cfDNA, amplicon-bases NGS libraries were prepared and then templated and subsequently sequenced using the Ion Torrent Ion Chef and S5 XL systems. Data analysis was performed using the Torrent Suite and Ion Reporter software. Annotation and report curation were achieved by the Ion Reporter and Oncomine Knowledgebase Reporter software.

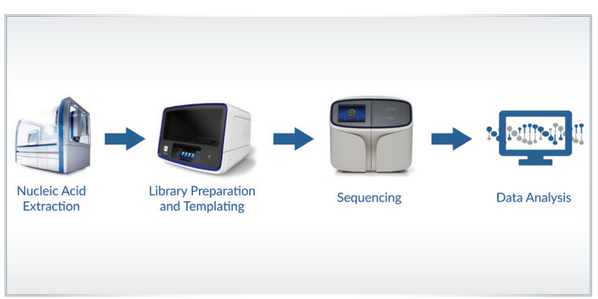

Figure 1. Workflow of the Target Selector™ NGS Breast Panel

Results

Gene List and Content

Target Selector™ NGS Breast Panel Gene List			
Hotspot Genes			CNVs
AKT1	*ESR1*	*PIK3CA*	*CCND1*
EGFR	*FBXW7*	*SF3B1*	*ERBB2*
ERBB2	*KRAS*	*TP53*	*FGFR1*
ERBB3			

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

Target Selector™ NGS Breast Panel Content	
Assay input	DNA
Hotspot SNV/short indel LOD	0.1% MAF
De novo LOD	0.5% MAF
CNV LOD	1.2X

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

Analytical Validation

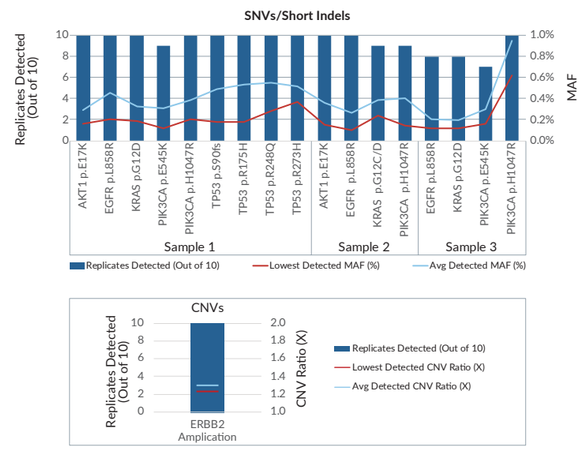

Figure 2. Graph illustrating analytical validation of the Target Selector™ NGS Breast Panel

Results

Clinical Validation

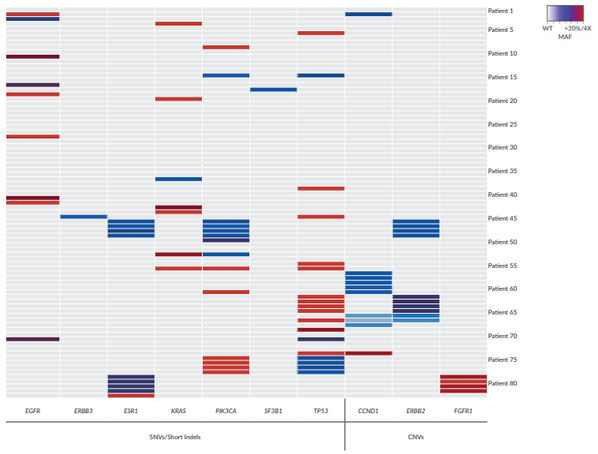

Figure 3. Diagram depicting genetic variants detected in the cohort of patient samples

Demographics | Actionable Mutations

Target Selector™ NGS Breast Panel Patient Demographics		
Age	Median age (years)	66
	Range (years)	32-95
Stage	I-II	7.7%
	III	5.1%
	IV	87.2%
Treatment status	Pre-Treatment	48.5%
	Post Treatment	48.5%
	At Progression	3.0%

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

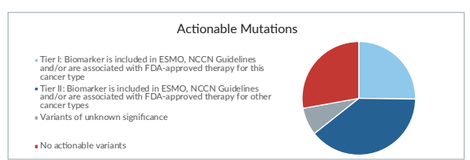

Figure 4. Graph depicting actionable mutations in the patient samples

Summary

Overall Performance Summary

Target Selector™ NGS Breast Panel Performance Summary		
Study		Results
Analytical Accuracy		99.7%
Analytical Specificity		0% error rate
Analytical Sensitivity		SNV: 94.1%
		CNV: 100%
Clinical Verification of Analytical Sensitivity		SNV: 100%
		CNV: 100%
Clinical Accuracy		99.9%
Clinical Precision	Intra-Assay	100%
	Inter-Assay	100%
	Inter-Operator	100%
	Inter-Instrument	100%
	Inter-Reagent	100%
PPV		91.8%
NPV		99.9%
Analytical Interferences		No interference shown by substances tested

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

Conclusions

- Target Selector™ NGS Breast Panel has demonstrated consistent performance for detecting actionable mutations in the ctDNA 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/short indels and 1.2x for CNVs.

References

1. Siegel RL, Miller KD, Jemal A. (2019) Cancer statistics, 2019. CA Cancer J Clin 69(1):7-34. doi: 10.3322/caac.21551.

2. Chung JH, Pavlick D, Hartmaier R, Schrock AB, Young L, et al. (2017) Hybrid capture-based genomic profiling of circulating tumor DNA from patients with estrogen receptor-positive metastatic breast cancer. Ann Oncol. 2017 Nov 1;28(11):2866-2873. doi: 10.1093/annonc/mdx490.

3. Lee J, Franovic A, Shiotsu Y, Kim ST, Kim KM, Banks KC, Raymond VM, Lanman RB (2019) Detection of *ERBB2 (HER2)* Gene Amplification Events in Cell-Free DNA and Response to Anti-HER2 Agents in a Large Asian Cancer Patient Cohort. Front Oncol. 2019 Apr 4;9:212. doi: 10.3389/fonc.2019.00212.