PositiveSelect™

Positive Bioscience

PATIENT		PHYSICIAN	
Name :	XXXXXXXXXXXXX	Name :	Dr. XXXXX
Gender :	M	Date of Birth :	08/Dec/1962
		Institute :	XXXXXXX

SAMPLE

Diagnosis :	Non-Small Cell Lung Cancer	Sample Type :	Blood (ctDNA)	Sample Collection Date :	06/Nov/2016	Sample ID :	58298840644
Test :	PositiveSelect Lite	Technology :	Illumina NGS	Coverage :	1000x	Report Date :	22/Dec/2016

GENOMIC ALTERATIONS WITH THERAPEUTIC IMPLICATIONS

Drugs	Gene	Result	Targeted Pathways	Recommendation
Osimertinib	EGFR Exon 20 mutation [T790M]	Positive	Receptor tyrosine kinase	✓
MEK inhibitors [Sorafenib]	KRAS Exon 2 mutation [G12C]	Positive	Receptor tyrosine kinase	✓
Gefitinib, Erlotinib[N]	EGFR Exon 20 mutation [T790M]	Positive	Receptor tyrosine kinase	✗
Cetuximab, Panitumumab[N]	KRAS Exon 2 mutation [G12C]	Positive	Receptor tyrosine kinase	✗

N- NCCN approved drugs
T- Toxicity data

Note: Though all the genes mentioned in the appendix have been analyzed, only those which have clinically actionable information have been highlighted in this report.

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MUTATION STATUS

Gene	Genetic Alteration	Result
AKT1	No alteration detected	Negative
ALK	No alteration detected	Negative
BRAF	No alteration detected	Negative
DDR2	No alteration detected	Negative
ERBB2	No alteration detected	Negative
EGFR	Exon 20 mutation [T790M]	Positive
FGFR1	No alteration detected	Negative
PTEN	No alteration detected	Negative
KRAS	Exon 2 mutation [G12C]	Positive
MAP2K1	No alteration detected	Negative
MET	No alteration detected	Negative
NRAS	No alteration detected	Negative
PIK3CA	No alteration detected	Negative
PTEN	No alteration detected	Negative
RET	No alteration detected	Negative
ROS1	No alteration detected	Negative

Note: All the genomic alterations relevant to the cancer type and the associated genesas per NCCN and mycancergenome.org are reported here.

THERAPEUTIC INFERENCE

EGFR: EGFR encodes the Epidermal Growth Factor Receptor. Binding of EGFR by its ligands, which includes Epidermal Growth Factor (EGF) and Transforming Growth Factor-alpha (TGF-alpha), activates downstream signaling pathways including the canonical oncogenic MAPK and PI3K/AKT/mTOR signaling cascades, ultimately resulting in changes in cellular proliferation, migration and differentiation. While in normal adult tissues EGFR usually is expressed at low levels, hyperactivation of this receptor by mutations and/or amplification of the EGFR gene is found in many cancer types such as lung, brain, colorectal and head and neck cancer. In brain and colorectal cancers, copy number amplification of the EGFR gene results in receptor overexpression.

Potential Treatment: AZD9291 is a selective, third generation EGFR-TKI, effective against both EGFR-TKI sensitizing and resistance T790M mutations in Clinical trials.

KRAS: KRAS is a member of the RAS family of small GTPases, which catalyze the hydrolysis of GTP to GDP. Under physiologic conditions, these RAS proteins cycle between an active (GTP-bound) and an inactive (GDP-bound) state, to activate the MAPK and PI3K oncogenic pathway signaling downstream of Receptor Tyrosine Kinases (RTKs). Once activated, RAS mediates the regulation of cellular proliferation and other cellular functions through the activation of distinct intracellular signaling pathways, including the RAF/MEK/ERK and PI3K/AKT/mTOR pathways.

Potential Treatment: Constitutional activation of K-Ras leads to signaling through the Raf-Mek-Erk pathway, which is implicated in cellular growth and survival pathways. Sorafenib has been evaluated in unselected advanced patients with NSCLC both as a single agent and in conjuncture with platinum doublet chemotherapy as first-line treatment.

REFERENCES

1. Janne, Pasi A., et al. "Clinical activity of the mutant-selective EGFR inhibitor AZD9291 in patients (pts) with EGFR inhibitorresistant non-small cell lung cancer (NSCLC)." (2014): 8009-8009.

2. Herbst, R. S., et al. "Sorafenib treatment efficacy and KRAS biomarker status in the Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) trial." Journal of Clinical Oncology 28.15suppl (2010): 7609-7609.

3. Kim, Dong-Wan, et al. "Clinical activity and safety of HM61713, an EGFR-mutant selective inhibitor, in advanced non-small cell lung cancer (NSCLC) patients (pts) with EGFR mutations who had received EGFR tyrosine kinase inhibitors (TKIs)." (2014): 8011-8011.

4. Saridaki, Zenia, et al. "A let-7 microRNA-binding site polymorphism in KRAS predicts improved outcome in patients with metastatic colorectal cancer treated with salvage cetuximab/panitumumab monotherapy." Clinical Cancer Research 20.17 (2014): 4499-4510.

About PositiveSelect Lite

We at Positive Bioscience wish to ensure our clients understand the entire work flow of activities that goes around while generating each "cancer genomics" report. A genomics test involves big data analysis, the process for which begins right at the time of sample collection, DNA isolation, sequencing, processing of the generated data followed by expert personalized recommendations. The entire process has been highlighted for a better understading.

PositiveSelect Lite: PositiveSelect Lite is a liquid biopsy test, designed to aid oncologists in the identification of circulating tumor mutation, monitoring of cancer patients and treatment planning.

The ctDNA is isolated from the blood sample collected in Streck tubes using QIAamp Circulating Nucleic Acid extraction kit. An Agilent custom-designed HaloPlex^{HS} assay is used to the enrich 100 gene targets from cfDNA and subjected to 1000X NGS using the Illumina NextSeq NGS platform. This results in massive parallel sequencing of the enriched cancer-specific targeted sequences along with the flanking intronic regions.

Test Significance: PositiveSelect Lite detects all alterations associated with patientstumor type as per NCCN guidelines in addition to off-labels drugs and clinical trials as per genomic altrations.

1 **Sample Requirement**: Whole blood or FFPE block. Three tubes of whole blood; Two in Streak tube and another in lavendar top EDTA tube or one FFPE block with whole blood in lavendar top EDTA tube.

2 **Sample Storage**: Whole blood tubes in between prefrozen gel packs FFPE blocks well sealed in air-tight containers

3 **DNA Extraction**: QIAamp Circulating Nucleic Acid Kit, QIAgen, Germany

4 **QC Analysis**: PicoGreen/Bioanalyzer

5 **Bioinformatics Analysis**: Positive Bioscience trademark TEST pipeline

6 **Databases**: COSMIC70, ClinVar, dbSNP, OMIM, TCGA, SIFT, PolyPhen, FATHMM & PositiveMD

7 **Analysis and Reporting**: Analysis and mapping of variants at six different levels followed by reporting using the trademark database; PositiveMD to report on clinically actionable variants

Analyzed by: Verified by: Approved by:

Disclaimer: The information in this report is meant for medical professionals only. This report should not be construed as personal medical advice and is not intended to replace medical advice offered by physicians. This document should not be used to establish any standard of care. Clinicians should use their own clinical judgment and not base clinical decisions solely on this document. Positive Bioscience will not be liable for any direct, indirect, consequential, special, exemplary, or other damages.

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GENES COVERED

SINGLE NUCLEOTIDE VARIATIONS

ABCB1	CYP19A1	ERCC2	JAK2	PARP1
ABCC1	CYP1A1	ERCC3	JAK3	PDCD1
ABCC2	CYP1A2	ERCC4	KDR	PDGFRA
ABCC3	CYP1B1	ERCC5	KIT	PDGFRB
ABCC4	CYP24A1	ESR1	KRAS	PGR
ABCG2	CYP27B1	EWSR1	LINS1	PIK3CA
ABL1	CYP2B6	EZH2	MAP2K1	PTEN
AKT1	CYP2C19	F2R	MAP2K2	REL
ALK	CYP2C9	FGFR1	MAPK1	RET
AR	CYP2E1	FGFR2	MET	ROS1
BCR	CYP3A4	FGFR4	MLH1	RRM1
BRAF	CYP3A5	FLT3	MSH2	STAT3
BRCA1	DCK	GSTA1	MSH6	TERT
BRCA2	DDB1	GSTP1	MTHFD1	TOP1
BTK	DDR2	HIF1A	MTHFR	TP53
CCND1	DYNC2H1	HRAS	MTOR	TSC1
CCND2	EGFR	IDH1	NF1	TSC2
CDA	EML4	IGF1R	NR1I2	VEGFA
CDK4	ERBB2	IL6	NR1I3	VHL
CDK6	ERCC1	JAK1	NRAS	XRCC1

FUSIONS	AMPLIFICATIONS		INSERTION/DELETIONS (INDELS)	
ALK	AR	BRAF	BRCA1	MTOR
FGFR2	CCND1	CDK4	EGFR	NF1
RET	CCND2	EGFR	ERBB2	PDGFRA
ROS1	ERBB2	FGFR1	KIT	PTEN
	FGFR2	KIT	MET	TP53
	KRAS	MET	MLH1	TSC1
	PIK3CA	PDGFRA		VHL