PositiveSelect™

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

SAMPLE							
Diagnosis :	Breast Cancer	Sample Type :	Blood (ctDNA)	Sample Collection Date :	06/Nov/2016	Sample ID :	58298840644
Test :	PositiveSelect Match	Technology :	Illumina NGS	Coverage :	1000x	Report Date :	22/Dec/2016

✓ THERAPIES WITH POTENTIAL BENEFIT

Drugs	Gene	Result	Targeted Pathways
mTOR inhibitors [Everolimus] N	PTEN Loss	Positive	MTOR signaling pathway
Olaparib	BRCA1 [A433P fs]	Positive	Mismatch Repair
Platinum-based chemotherapy [Paclitaxel and Carboplatin] N	BRCA1 [A433P fs]	Positive	Mismatch Repair

✗ THERAPIES WITH POTENTIAL LACK OF BENEFIT

Drugs	Gene	Result	Targeted Pathways
Tamoxifen, Letrozole N	ESR1 [D538G]	Positive	Estrogen signaling pathway
Trastuzumab N	PTEN Loss	Positive	Receptor tyrosine kinase

N- NCCN approved drugs

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.

preTADME [Toxicity, Absorption, Distribution, Metabolism and Excretion]

Drugs	Gene-Genotype	Inference
Platinum-based chemotherapy [Paclitaxel and Carboplatin]	ABCB1 - AA	Higher risk of toxicity
Capecitabine	DPYD- CC	Decreased likelihood of developing grade 3 hand-foot syndrome

MUTATION STATUS [NCCN Recommended Genes]

Gene	Genetic Alteration	Result
BRCA1	A433P fs	Positive
BRCA2	No alteration detected	Negative
ERBB2	Amplification	Positive
ESR1	D538G	Positive
TP53	No alteration detected	Negative

MUTATION SIGNATURE

Gene	Genetic Alteration	Germline	True Somatic
BRCA1	A433P fs	✓	
ESR1	D538G		✓
ABCB1	C3435T	✓	
DPYD	D974V	✓	

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

THERAPEUTIC INFERENCE

BRCA1: Breast cancer 1, early onset (BRCA1), which functions as a tumor suppressor, is a multifunctional ubiquitin E3 ligase. It has many cellular functions including transcription, protein ubiquitination, cell cycle regulation and DNA damage response with a particularly important role in the DNA double strand break repair pathway, homologous recombination. BRCA1 mutations confer a 70-80% lifetime risk of breast cancer, a 50% lifetime risk of ovarian cancer and an increased risk of prostate cancer in patients with Ashkenazi Jewish BRCA1 founder mutations. BRCA1 is a tumor suppressive gene; if one copy of the gene is mutated in the germ line, the result is hereditary breast and ovarian cancer (HBOC) syndrome, an autosomal dominant disease. BRCA1 was also recently identified as a definitive Fanconi anemia susceptibility gene in FANCS, a rare Fanconi anemia subtype that results from biallelic mutations in the gene. BRCA1 has three major domains: the N-terminal RING (Really Interesting New Gene) domain(exons 2-7) that contains an active zinc finger motif, exons 11-13, and the BRCT (BRCA1 C-terminal) domain. Many clinically significant mutations of BRCA1 are located in the zinc finger motif of the RING domain, which is important for many protein-protein interactions. Exons 11-13 contain binding sites for proteins including RB, c-MYC, RAD50, and RAD51. Despite the fact that exons 11-13 contain many clinically significant BRCA1 mutations, little is known about the structure or function of this region when compared to the RING or BRCT domains. The BRCT domain is essential to the tumor suppressor function of the BRCA1 protein via its crucial role in transcriptional regulation and DNA repair. p53 mutations are seen almost exclusively in breast tumors with BRCA1 and BRCA2 mutations, suggesting that p53 loss of function may be a necessary step in the tumorigenesis of BRCA-associated carcinomas.

Potential Treatment: Olaparib (Lynparza, AstraZeneca) is an oral inhibitor of poly(ADP-ribose) polymerase (PARP) proteins that play a key role in DNA repair and genomic stability. Olaparib is indicated for use in treating certain patients with advanced, recurrent ovarian cancer who have mutations of the breast cancer 1 gene (BRCA1) or breast cancer 2 gene (BRCA2).

PTEN: PTEN encodes the protein Phosphatase and TENsin homolog deleted on chromosome 10 (PTEN), a tumor suppressor that is one of the most frequently mutated genes in human cancer. PTEN has several physiological functions, most notably operating as a phosphatase that converts phosphatidylinositol (3,4,5)-triphosphate (PIP3) to phosphatidylinositol (4,5)-triphosphate (PIP2) at the cell membrane. Impairment of PTEN function through multiple mechanisms, including through non-synonymous mutations, results in PIP3 accumulation and constitutive activation of catabolic downstream AKT/mTOR signaling. PTEN inactivation therefore promotes cell growth, proliferation and survival. Additionally, nuclear PTEN is thought to regulate RAD51 expression, and in this way is also associated with homologous recombination and repair of DNA strand breaks. Thus, loss of PTEN may also lead to greater genomic instability and provide a setting for the accumulation of other deleterious mutations.Germline loss-of-function PTEN mutations occur in approximately 80% of patients with the cancer predisposition syndrome .

Potential Treatment: Loss of PTEN causes persistent activation of the AKT serine-threonine kinase which promotes cell growth and proliferation, disables apoptosis, and controls metabolism by directly phosphorylating numerous downstream targets [MDM2, MTOR and FOXO]. Therefore the use of mTOR inhibitors can be a better option.

REFERENCES

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4. Jeselsohn, Rinath, et al. "Emergence of constitutively active estrogen receptor- mutations in pretreated advanced estrogen receptor positive breast cancer." Clinical Cancer Research 20.7 (2014): 1757-1767.

5. Nagata, Yoichi, et al. "PTEN activation contributes to tumor inhibition by Trastuzumab, and loss of PTEN predicts Trastuzumab resistance in patients." Cancer cell 6.2 (2004): 117-127.

6. Bergmann, Troels K., et al. "Impact of ABCB1 variants on neutrophil depression: a pharmacogenomic study of paclitaxel in 92 women with ovarian cancer." Basic & clinical pharmacology & toxicology 110.2 (2012):199-204.

7. Wu, Chaohui, et al. "Prognostic role of microRNA polymorphisms in patients with advanced esophageal squamous cell carcinoma receiving platinum-based chemotherapy." Cancer chemotherapy and pharmacology 73.2 (2014): 335-341.

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About PositiveSelect Match

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 Match: PositiveSelect Match 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 Match detects alterations to specific cancer associated genes and matches them to targeted therapies and clinical trials.This matched-normal test ensures reporting on true somatic and germline variants to identify genomic alterations driving cancer. Reporting on germline variants also aid in identifying inherited risks.

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