PositiveSelect™

Positive Bioscience

PATIENT			PHYSICIAN	
Name :	xxx		Name :	Dr. XXXXXXXXXXXXXX
Gender :	M	Date of Birth : 08/Dec/1962	Institute :	Manipal Hospital, Bangalore

SAMPLE

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

EXPERT COMMENTARY

The pathogenic variation (1810C>T) of NTRK1 affects the function of TK domain which leads to phosphorylation of the NTRK1 receptor. Several studies have highlighted better response to NTRK1 inhibitors (Entrectinib) among neuroblastoma cases with this variation.
The pathogenic variation (Leu557Ter) of BRCA2 results in truncated non-functional protein. A pre- clincal study highlighted Mitomycin C to lead to regression in BRCA2 mutated cell lines. Therefore, patient may show better response to Mitomycin C and platinum based therapy.

Note: Complimentary call for consultation is available.

GENOMIC HIGHLIGHTS

2 Pathways driving cancer	• Receptor tyrosine kinase • DNA Repair
2 Genomic alterations with clinical actionability	• 1810C>T • Leu557Ter
1 Genomic alteration with clinical lack of benefit	• E1286G
0 Clinical trials	

IMPLICATIONS TO IMMUNOTHERAPY

Microsatellite status	MS-Stable
Tumor Mutation Burden	TMB-Low

Note: TMB-Low :- <19 mutations/MB, TMB-High:- >20 mutations/MB; *MS-Stable <2% unstable sites, MS-Unstable >2% unstable sites*

✓ THERAPIES WITH POTENTIAL BENEFIT

Drugs	Gene	Result	Targeted Pathways
Cetuximab or Panitumumab[N,F]	BRAF, KRAS , EGFR [BRAF/KRAS- wild-type] [EGFR- Amplification]	Positive [Negative]	Receptor tyrosine kinase
Entrectinib	NTRK1 [1810C>T]	Positive	Receptor tyrosine kinase
Mitomycin C	BRCA2 [Leu557Te]	Positive	DNA Repair
Platinum	BRCA2 [Leu557Te]	Positive	DNA Repair

✗ THERAPIES WITH POTENTIAL LACK OF BENEFIT

Drugs	Gene	Result	Targeted Pathways
Fluorouracil	APC [E1286G]	Positive	WNT Signaling

preTADME

Drugs	Gene-Genotype	Inference
Carboplatin, Cisplatin, Oxaliplatin, Platinum compounds	GSTP1 - AA ERCC1 - GG	Higher risk of toxicity Decreased toxicity with increased survival
Irinotecan	UGT1A1 - GA UGT1A1 - GG	Increased risk of neutropenia Decreased severity of diarrhea
Paclitaxel	TP53 - CC	Decreased toxicity with increased survival
Capecitabine	PTGS2- CC	Decreased likelihood of developing grade 3 hand-foot syndrome

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.

MUTATION STATUS

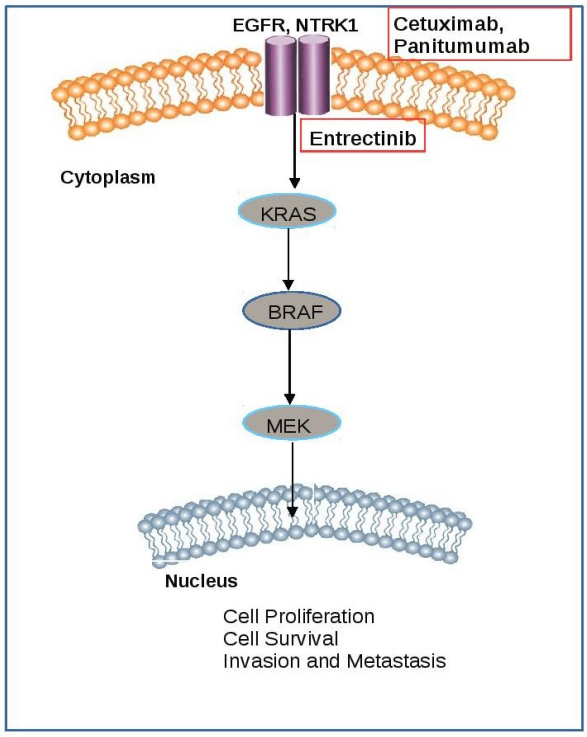
Gene	Genetic Alteration	Result
AKT1	49 G>A (E17K)	Negative
BRAF	1799 T>A (V600E)	Negative
PTEN	No alteration detected	Negative
PIK3CA	3140 A>T (H1047L) 1636 C>G (Q546E)	Negative
KRAS	37 G>A (G13S) 182 A>T (Q61L)	Negative
NRAS	No alteration detected	Negative

MUTATION SIGNATURE

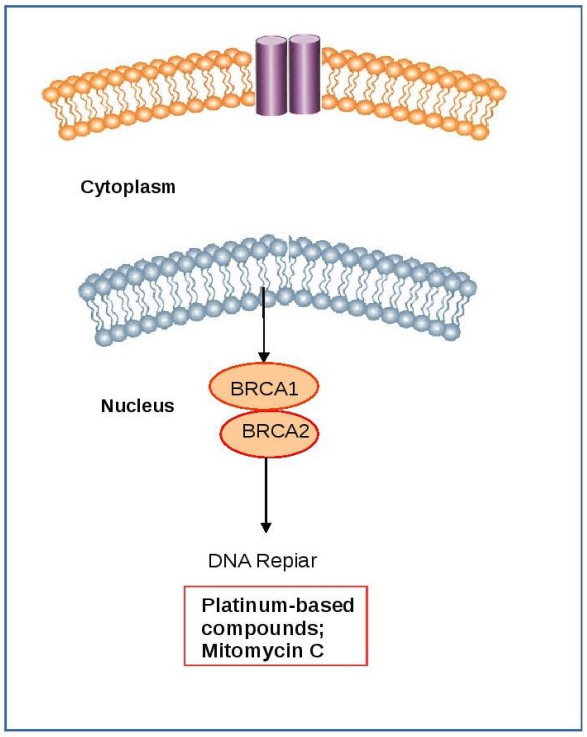
Gene	Genetic Alteration	Germline	True Somatic
BRCA2	Leu557Ter	✓	
NTRK1	1810C>T		✓

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

CANCER DRIVING PATHWAY



Active Pathway (↑)



Inactive Pathway (↓)

Interpretation:

1. Our analysis found significant mutations in PI3K-MTOR signaling pathway.

2. PI3K/MTOR pathway is a central coordinator of fundamental biological events, playing a key role in cell growth, regulation of actin cytoskeleton, gene transcription, ribosome biogenesis, mRNA translation, cell survival and lifespan. Dysregulation of PI3K/MTOR pathway occurs through activation of PI3K via mutations in PIK3CA and MTOR complex which leads to aberrant cell proliferation, transcription, and tumorigenesis.

THERAPEUTIC INFERENCE

BRAF: BRAF is a serine/threonine kinase that plays a key role in the regulation of the mitogen activated protein kinase (MAPK) cascade. which under physiologic conditions regulates the expression of genes involved in cellular functions, including proliferation. Genetic alterations in BRAF are found primarily in melanomas and thyroid cancers as well as a small fraction of lung and colorectal cancers.

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.

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: Several studies have reported that patients with metastatic colon cancer that harbor BRAF mutations do not respond to anti-EGFR antibody agents cetuximab or panitumumab in the chemotherapy-refractory setting (Bardelli and Siena 2010; Folprecht et al. 2010; Gravalos et al. 2010; Lievre, Blons, and Laurent-Puig 2010). Based on these findings, BRAF mutations were suggested to be a negative predictor of response to anti-EGFR therapy (De Roock et al. 2009; Mao et al. 2011; Rizzo et al. 2010; Sharma and Gulley 2010; Tejpar et al. 2010).

NTRK1: Pathogenic NTRK1 (1810C>T) affects conservative residue of the TK domain. This substitution changes the chemical properties of residue in conserved position which may possibly alter the function of TK domain and may lead to increase in autophosphorylation of NTRK1 receptor. As per Lipska, Beata S., et al. (2009) NTRK1 c.1810C>T mutation present in 8.7% of neuroblastoma cases was found to be an independent marker of disease recurrence (OR- 13.3; P- 0.009) associated with lower survival rates (HR- 4.45; P- 0.041).

BRCA2: Stopgain BRCA2 (Leu557Ter) mutation leads to variant allele predicted to encode a truncated non-functional protein. As per van der Heijden, et al. (2005) CAPAN1 (BRCA2-mutated), sensitivity to DNA cross-linking agents was observed compared to FANCC/BRCA2 wildtype cell lines (Su86.86, CFPAC, AsPc1, and MiaPaCa2). Mitomycin C and Platinum specifically led to regression in 8/11 xenografts of the CAPAN1 cell line compared to 2/9 in the CFPAC (BRCA2 wildtype) cell line.

REFERENCES

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About PositiveSelect Ultimate

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 Ultimate: PositiveSelect Ultimate 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 SureSelect XT assay is used to the enrich 350 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 Ultimate 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	ABCB1	ABCB1	ABCB1	ABCB1	ABCB1	ABCB1	ABCB1	ABCB1	ABCB1	ABCB1	ABCB1
ABCC1	ABCC1	ABCC1	ABCC1	ABCC1	ABCC1	ABCC1	ABCC1	ABCC1	ABCC1	ABCC1	ABCC1
ABCC2	ABCC2	ABCC2	ABCC2	ABCC2	ABCC2	ABCC2	ABCC2	ABCC2	ABCC2	ABCC2	ABCC2
ABCC4	ABCC4	ABCC4	ABCC4	ABCC4	ABCC4	ABCC4	ABCC4	ABCC4	ABCC4	ABCC4	ABCC4
ABCG2	ABCG2	ABCG2	ABCG2	ABCG2	ABCG2	ABCG2	ABCG2	ABCG2	ABCG2	ABCG2	ABCG2
ABL1	ABL1	ABL1	ABL1	ABL1	ABL1	ABL1	ABL1	ABL1	ABL1	ABL1	ABL1
AKT1	AKT1	AKT1	AKT1	AKT1	AKT1	AKT1	AKT1	AKT1	AKT1	AKT1	AKT1
AKT2	AKT2	AKT2	AKT2	AKT2	AKT2	AKT2	AKT2	AKT2	AKT2	AKT2	AKT2
AKT3	AKT3	AKT3	AKT3	AKT3	AKT3	AKT3	AKT3	AKT3	AKT3	AKT3	AKT3
ALK	ALK	ALK	ALK	ALK	ALK	ALK	ALK	ALK	ALK	ALK	ALK
ALOX12B	ALOX12B	ALOX12B	ALOX12B	ALOX12B	ALOX12B	ALOX12B	ALOX12B	ALOX12B	ALOX12B	ALOX12B	ALOX12B
AMELY	AMELY	AMELY	AMELY	AMELY	AMELY	AMELY	AMELY	AMELY	AMELY	AMELY	AMELY
APC	APC	APC	APC	APC	APC	APC	APC	APC	APC	APC	APC
AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR
ARAF	ARAF	ARAF	ARAF	ARAF	ARAF	ARAF	ARAF	ARAF	ARAF	ARAF	ARAF
ARID1A	ARID1A	ARID1A	ARID1A	ARID1A	ARID1A	ARID1A	ARID1A	ARID1A	ARID1A	ARID1A	ARID1A
ASXL1	ASXL1	ASXL1	ASXL1	ASXL1	ASXL1	ASXL1	ASXL1	ASXL1	ASXL1	ASXL1	ASXL1
ASXL2	ASXL2	ASXL2	ASXL2	ASXL2	ASXL2	ASXL2	ASXL2	ASXL2	ASXL2	ASXL2	ASXL2
ATM	ATM	ATM	ATM	ATM	ATM	ATM	ATM	ATM	ATM	ATM	ATM
ATR	ATR	ATR	ATR	ATR	ATR	ATR	ATR	ATR	ATR	ATR	ATR
ATRX	ATRX	ATRX	ATRX	ATRX	ATRX	ATRX	ATRX	ATRX	ATRX	ATRX	ATRX
AURKA	AURKA	AURKA	AURKA	AURKA	AURKA	AURKA	AURKA	AURKA	AURKA	AURKA	AURKA
AURKB	AURKB	AURKB	AURKB	AURKB	AURKB	AURKB	AURKB	AURKB	AURKB	AURKB	AURKB
AXIN1	AXIN1	AXIN1	AXIN1	AXIN1	AXIN1	AXIN1	AXIN1	AXIN1	AXIN1	AXIN1	AXIN1
AXIN2	AXIN2	AXIN2	AXIN2	AXIN2	AXIN2	AXIN2	AXIN2	AXIN2	AXIN2	AXIN2	AXIN2
AXL	AXL	AXL	AXL	AXL	AXL	AXL	AXL	AXL	AXL	AXL	AXL
B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
BAP1	BAP1	BAP1	BAP1	BAP1	BAP1	BAP1	BAP1	BAP1	BAP1	BAP1	BAP1
BARD1	BARD1	BARD1	BARD1	BARD1	BARD1	BARD1	BARD1	BARD1	BARD1	BARD1	BARD1
BBC3	BBC3	BBC3	BBC3	BBC3	BBC3	BBC3	BBC3	BBC3	BBC3	BBC3	BBC3

FUSIONS	AMPLIFICATIONS		INSERTION/DELETIONS (INDELS)		
ALK	AR	BRAF	ATM	GATA3	SMAD4
FGFR2	CCNE1	CDK4	APC	KIT	STK11
FGFR3	CCND1	CDK6	ARID1A	MET	TP53
RET	CCND2	EGFR	BRCA1	MLH1	TSC1
ROS1	ERBB2	FGFR1	BRCA2	MTOR	VHL
NTRK1	FGFR2	KIT	CDH1	NF1	
	KRAS	MET	CDKN2A	PDGFRA	
	PIK3CA	PDGFRA	EGFR	PTEN	
	MYC	RAF1	ERBB2	RB1	