Laboratory Investigation Report

Patient Name	Centre
Age/Gender	OP/IP No/UHID
MaxID/Lab ID	Collection Date/Time
Ref By	Reporting Date/Time

SIN No:11070

TEST REQUESTED: NGS EGFR, KRAS, NRAS, HRAS, BRAF Panel

METHOD USED

Next Generation Sequencing

CLINICAL INFORMATION

As per histopathology impression, features are suggestive of Poorly differentiated adenocarcinoma with focal signet ring cell morphology in growth caecum, endoscopic biopsy.

SAMPLE INFORMATION

FFPE Block (Block No.: S-7802/24, Tumor Content: ~20%-25%)

TARGETED GENES

EGFR	*KRAS*	*NRAS*	*HRAS*	*BRAF*
Not Detected	**Detected**	Not Detected	Not Detected	Not Detected

PRIMARY FINDINGS

Gene	CDS Variant	Amino Acid Change	Exon	Allele Frequency	Coverage	dbSNP ID	Pathogenicity (Clinvar/Varsome)
KRAS	NM_004985.5:c.38G>A	p.Gly13Asp	2	10%	1998	rs112445441	Conflicting interpretations of pathogenicity [*Pathogenic(6); Uncertain significance(1)*]

SAMPLE STATISTICS

Coverage	100%
Depth	8,285X

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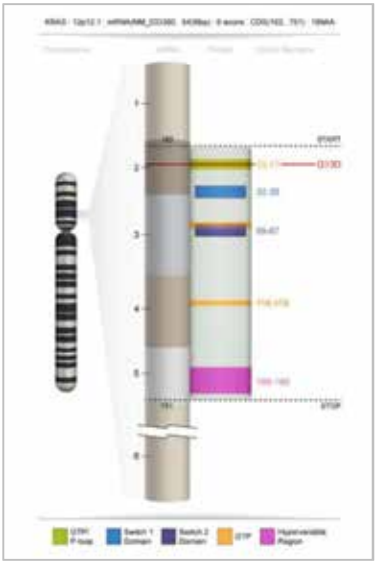
VARIANT INTERPRETATION

This alteration was characterized to be functionally activating. G13D is the most common mutation at codon 13 in KRAS and the third most common KRAS mutation in colon cancer (PMID: 12727799, 19679400, 31390567)). This alteration is located within one of the phosphate-binding loops of KRAS (amino acids 10- 18, UniProt). The phosphate-binding loops of KRAS protein, spanning amino acids 10-18 (UniProt) and amino acids 59-60 (UniProt), interact with the phosphate groups of GTP and are responsible for the GTPase function of KRAS (PMID: 31409810). KRAS G13D is located within exon 2 of KRAS gene and most mutations in this region are specifically contraindicated for treatment with cetuximab and panitumumab in metastatic colorectal patients. However, several preclinical and clinical evidence has showed that G13D may sensitize cells and tumors to cetuximab or gefitinib in CRC (PMID: 20978259, 26623049, 26371285). In contrast, other clinical studies have demonstrated that G13D mutations doesn't show the benefit of cetuximab or panitumumab treatment and is no more likely to response to EGFR inhibitors than other KRAS mutations (PMID: 26371285, 27114605). A recent research demonstrated that G13D murine colonic organoids were more sensitive to an Erk inhibitor, SCH772984, than the wild type (PMID: 31390567).



ADDITIONAL FINDINGS

No other variant that warrants to be reported was detected

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TEST METHODOLOGY

Background

Multi gene analysis through next generation sequencing allows the identification of variants to understand their prognostic and therapeutic implications in different cancer types, if any. Targeted application of next-generation sequencing (NGS) technology allows detection of specific mutations that can provide treatment opportunities to the patients. This panel with improved primer design and as little as 10 ng of DNA enable researchers to sequence challenging samples such as Formalin fixed, paraffin embedded (FFPE) tissue which exhibit variable quality. Additionally, even degraded samples can be used to generate reliable data using this panel as the primers are designed to produce, on average, 154 bp amplicons.

Method

The Ion AmpliSeq™ Cancer Hotspot Panel v2 was used to carry out next generation sequencing. After sequencing, automated analysis was performed with Torrent Suite™ Software. Variant annotations were then done using Ion Reporter™ Software. Clinically relevant mutations were also checked using published literature and databases.

Limitations

The accuracy and completeness may vary due to variable information available in different databases. The classification of variants of unknown significance can change over time. Synonymous mutations were not considered while preparing this report. The mutations have not been confirmed using Sanger sequencing and/or alternate technologies. To rule out germ line mutations i.e. variant with variant allele frequency at nearly 50% or 100%, whole blood sample is recommended to process along with tissue sample.

DISCLAIMER

A Negative result implying non-detection of mutation/deletion indicates a Benign/likely Benign polymorphism. A negative test result may also be due to the inherent technical limitations of the assay. Results obtained should be interpreted with consideration of the overall picture obtained from clinical, laboratory, and pathological findings. Rare polymorphisms may lead to false negative or positive results. False negative results may be due to sampling error/errors in sample handling as well as clonal density below the limit of detection. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. Identification of a mutation in one or more of these genes does not guarantee activity of the drug in a given indication due to the presence of contraindicated mutation in the gene not covered by the panel.

The accuracy and completeness may vary due to variable information available in different databases. Classification of the variant may change overtime. An updated variant classification may be obtained on request. Insertions and deletions greater than 20bp in size may not be detected by this assay. The scope of this assay limits to SNVs, MNVs and short deletions/duplications. Due to poor quality of FFPE DNA, indeterminate result due to low gene coverage or low variant depth cannot be ruled out. The sensitivity of the assays depends on the quality of the block, and tumor content.

The information provided should only be utilized as a guide or aid and the decision to select any therapy option based on the information reported here resides solely with the discretion of the treating physician. Patient care and treatment decisions should only be made by the physician after taking into account all relevant information available including but not limited to the patient's condition, family history, findings upon examination, results of other diagnostic tests, and the current standards of care. This report should only be used as an aid and the physician should employ sound clinical judgment in arriving at any decision for patient care or treatment.

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