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

SIN No:DD0481620

TEST REQUESTED

Max NGS Colorectal Cancer Panel

METHOD USED

Next Generation Sequencing (NGS)- KRAS, BRAF, HRAS, NRAS, PIK3CA, PTEN, TP53

Fragment Analysis- MicroSatellite Instability Panel (MSI)

CLINICAL INFORMATION

As per clinical data, known case of rectosigmoid carcinoma with mets.

SAMPLE INFORMATION

FFPE Block (Block No.: D-1565/23 H, Tumor Content: ~40%-45%)

MUTATION IN TARGETED GENES

KRAS	*BRAF*	*HRAS*	*NRAS*	*PIK3CA*	*PTEN*	*TP53*	*MSI*
Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Stable (MSS)

INTERPRETATION

- DNA sequencing did not identify any variant from the genes mentioned in the panel.
- Fragment analysis did not identify any unstable markers in Microsatellite Stability Panel, hence sample is MSI-Stable.
- No other variant that warrants to be reported was detected. Variations with high minor allele frequencies which are benign will be given upon request.

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MOLECULAR AND BIO-MARKER DIRECTED THERAPY (As per NCCN Guidelines)		
Therapy	**Tested Markers**	**Predicted Response**
Panitumumab	KRAS, NRAS, BRAF	
Cetuximab	KRAS, NRAS, BRAF	
Bevacizumab	KRAS, NRAS, BRAF	
Cetuximab/Panitumumab plus irinotecan	KRAS, NRAS, BRAF	
Cetuximab/Panitumumab plus FOLFIRI	KRAS, NRAS, BRAF	
Encorafenib Plus Cetuximab/Panitumumab	BRAF V600E	

PREDICTED RESPONSE OF THERAPY	
Green	Good response
Red	Contraindicated response
Orange	Limited response

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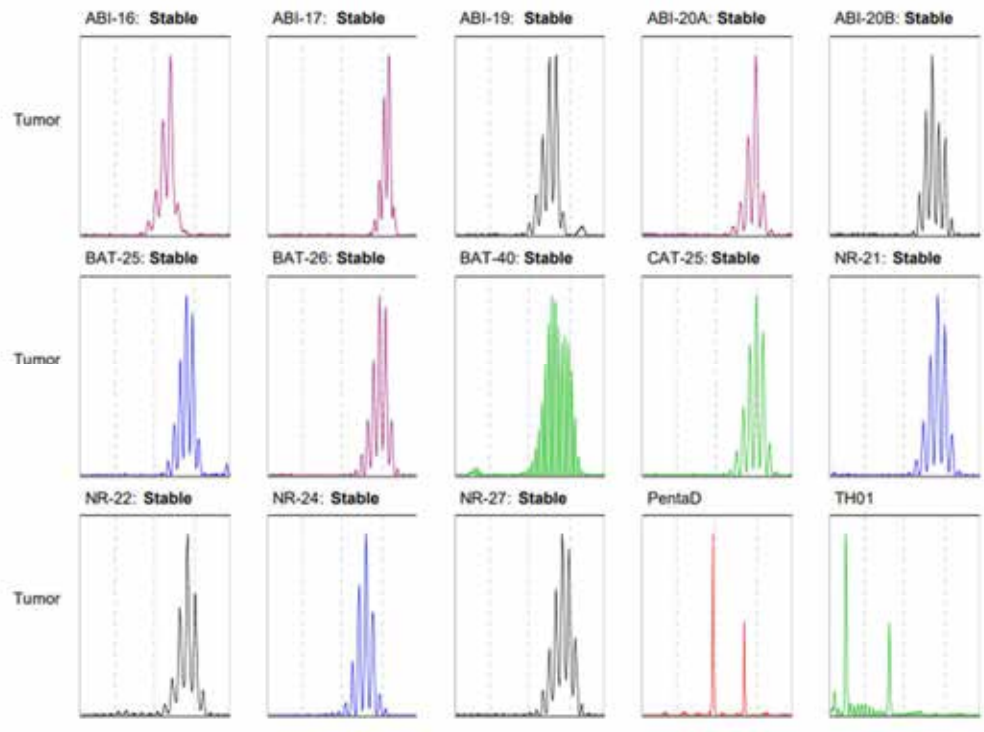
MICROSATELLITE INSTABILITY RESULT

MSS

INTERPRETATION SUMMARY	
Count of markers reported Unstable	**0**
Count of markers reported stable	**13**
Count of markers reported No Call	**0**
Reported Unstable Rate	**0%**

INTERPRETATION CRITERIA	
Minimum Unstable ratio to call MSI High (MSI-H)	**≥30%**
Minimum Unstable ratio to call MSI Low (MSI-L)	**1-29%**
Marker status for MSI Stable (MSS)	**<5% or all the markers are stable**

Marker Details



MC-2714

Patient Name	Centre
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NEXT GENERATION SEQUENCING RESULTS

SAMPLE STATISTICS

Coverage	100%
Depth	2,776X

VARIANT FINDINGS

Gene	CDS Variant	Amino Acid Change	Exon	Variant Allele Frequency	Coverage	Variant Classsification (AMP)	Pathogenicity (Clinvar/Varsome)
KRAS	NO CLINICALLY SIGNIFICANT VARIANT(S) WAS FOUND						
BRAF	NO CLINICALLY SIGNIFICANT VARIANT(S) WAS FOUND						
HRAS	NO CLINICALLY SIGNIFICANT VARIANT(S) WAS FOUND						
PIK3CA	NO CLINICALLY SIGNIFICANT VARIANT(S) WAS FOUND						
TP53	NO CLINICALLY SIGNIFICANT VARIANT(S) WAS FOUND						
PTEN	NO CLINICALLY SIGNIFICANT VARIANT(S) WAS FOUND						

EVIDENCE BASED VARIANT CATEGORIZATION: Variant classification (Based on AMP recommendations)

Tier 1 – Variants with strong clinical significance for therapeutic, prognostic and diagnostic for the same tumor type

Tier 2 – Variants with potential clinical significance for therapeutic, prognostic and diagnostic for the different tumor type

Tier 3 – Variants of unknown clinical significance

Tier 4 – Variants deemed benign or likely benign

* Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer (PMID:

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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

This panel detects SNPs, MNVs and InDels in seven genes using methodologies of next generation sequencing. Sensitivity of the tests depends on the quality of the block, and tumor content. After next generation 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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