

MGM2529 : Colorectal advanced panel by NGS & Microsatellite Instability (MSI) by fragment analysis

Report Details

Sample ID / Order ID: 8758711 / 1082072
 Collection Date: 27th July 2024
 Date Received: 16th October 2024
 Report Date & Time: 2nd Nov 2024 21:12 PM

Specimen Information

Specimen Site: Lung
 Specimen Received: FFPE Tissue Blocks [2]
 Specimen Tested: RP3219 (B1, B2) block
 Tumor Content: 40

Ordering Clinician

Clinician: Dr. Senaka Kandegedara
 Affiliation: Aegle Omics Private Limited
 Serviced By: 18718
 Report Status: Final

Clinical Summary: Metastatic colonic carcinoma

TEST RESULT SUMMARY

Microsatellite Instability (MSI) Test

Status - Stable

Kindly refer to the complete MSI reports below.

Next Generation Sequencing (NGS) Results

POSITIVE

Gene	Findings	Gene	Findings
AKT1	Not Detected	BRAF	Not Detected
ERBB2	CNV	HRAS	Not Detected
KRAS	Not Detected	NRAS	Not Detected
NTRK1	Not Detected	NTRK2	Not Detected
NTRK3	Not Detected	PIK3CA	Not Detected
POLD1	Not Detected	POLE	Not Detected
PTEN	Not Detected	RET	Not Detected
SMAD4	Not Detected		

Please refer to the complete variant details in the result table in page 2.

Next Generation Sequencing (NGS) Test Result

Result - POSITIVE

CLINICALLY RELEVANT VARIANT/S DETECTED

AMP Classification [^]	CDS variant details	Interpretation	Treatment Recommendations	[§] Treatment Response
ERBB2 Copy Number Gain (CNV)				
Tier I	NA	Oncogenic	Tucatinib + Trastuzumab	Effective

No clinically significant fusion has been detected in this sample

[^]Refer to Glossary section for the classification criteria details.

[§]Drug Approvals are based on US-FDA Guidelines. Kindly refer to local guidelines if required.

ADDITIONAL BIOMARKERS DETECTED

This section provides information about variants that do not have any therapeutic value. However, these variants may or may not have a likely oncogenic effect.

No other biomarkers that warrants to be reported was detected

ACTIONABLE BIOMARKER DETAILS

ERBB2 (CNV)Gene: *ERBB2*

CNV type: Copy Number Gain

Gene Summary: *ERBB2* encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. This protein has no ligand binding domain of its own and therefore cannot bind growth factors. However, it does bind tightly to other ligand-bound EGF receptor family members to form a heterodimer, stabilizing ligand binding and enhancing kinase-mediated activation of downstream signalling pathways, such as those involving mitogen-activated protein kinase and phosphatidylinositol-3 kinase. Allelic variations at amino acid positions 654 and 655 of isoform a (positions 624 and 625 of isoform b) have been reported, with the most common allele, Ile654/Ile655, shown here. Amplification and/or overexpression of *ERBB2* has been reported in numerous cancers, including breast and ovarian tumors. Alternative splicing results in several additional transcript variants, some encoding different isoforms and others that have not been fully characterized.

Clinical and Therapeutic Relevance: *ERBB2* Amplification present in 2.22% of all colorectal carcinoma patients [www.mycancergenome.org]. However, the *ERBB2* amplification detected in this subject is recommended to be confirmed by alternative tests (IHC, FISH). The Food and Drug Administration (FDA) granted accelerated approval to tucatinib in combination with trastuzumab for RAS wild-type HER2-positive unresectable or metastatic colorectal cancer that has progressed following fluoropyrimidine-, oxaliplatin-, and irinotecan-based chemotherapy. Efficacy was evaluated in 84 patients in MOUNTAINEER (NCT03043313), an open-label, multicenter trial. Patients were required to have HER2-positive, RAS wild-type, unresectable or metastatic colorectal cancer and prior treatment with fluoropyrimidine, oxaliplatin, irinotecan, and an anti-vascular endothelial growth factor (VEGF) monoclonal antibody (mAb) [<https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-tucatinib-trastuzumab-colorectal-cancer>].

PubMed References:

AMP-ASCO-CAP CLASSIFICATION CRITERIA

Genetic test results are reported based on the somatic variant classification recommendations of College of American Pathologists (CAP) /American society for Clinical Oncology (ASCO)/Association of Molecular Pathologists (AMP) [PMID: 27993330] as described in the table below:

Tier	Criteria
Tier I	Variants of strong clinical significance.
Tier II	Variants of potential clinical significance.
Tier III	Variants of unknown clinical significance
Tier IV	Benign or likely benign variants

DISCLAIMER

- **Decisions regarding treatment action plans should not be solely based on these test results. These findings are highly recommended to be correlated with the patient's clinical, pathological, radiological and family history for decisions on diagnosis, prognosis, or therapeutics.**
- The therapy information provided in this report is based on FDA approved drugs data, NCCN guidelines, peer reviewed published literature, standard clinical databases, and strength of biomarker results till date. These therapies may or may not be suitable/beneficial to a particular patient. This clinical report summarises potentially effective medications, potentially ineffective medications, and medications that may pose a higher risk of adverse reactions by mapping the patient's genetic alterations to the biomedical reference information. The report may also provide prognostic and diagnostic biomarkers detected or shown for the given disease context. The treatment recommendations for the variants classified in Tier II are not provided.
- The clinical trials information provided in this report is compiled from www.clinicaltrials.gov as per currently available data, however completeness of information provided herein cannot be guaranteed. This information should only be used as a guide and specific eligibility criteria should be reviewed thoroughly for the concerned patient. MedGenome Labs does not guarantee or promise an enrolment in any clinical trials.
- The identification of a genomic biomarker does not necessarily imply pharmacological effectiveness or ineffectiveness. The medications identified by the treating physician may or may not be suitable for use on a particular patient. Thus, the clinical report does not guarantee that any particular agent will be effective in the treatment of any particular condition. Also, the absence of a treatment option does not determine the effectiveness or predict an ineffective or safety-relevant effect of a medication selected by the treating physician.
- The classification and clinically relevant information for the reported variants is based on peer-reviewed publications, public clinical databases, medical guidelines (NCCN, ASCO, AMP) or other publicly available information and it has been ensured that the information provided is up to date at the time of report generated, however continuous updates may happen in public domains. Also, the classification of variants can change based on the updated literature evidence. Re-analysis of the results can be requested at additional cost.
- This test is performed on the patient's tumor sample without a paired blood sample; therefore, it may include variations which may be of germline origin. However, this test is designed and validated for the detection and reporting of somatic genomic variants only and does not discriminate between germline and somatic variants. If clinically warranted, appropriate germline testing and genetic counselling for the patient should be considered for further evaluation.
- Due to poor quality of FFPE tissue blocks, the QC parameters for extracted RNA may not pass to proceed further with the testing, therefore there is a possibility of assay failure at various steps (RNA QC, Library QC, Bioinformatics QC) or compromised results that include low gene coverage and low variant depth. However, sample status in such scenarios shall be sent through mail to the ordering clinician.
- This test has been validated at MedGenome Labs and the limit of detection (LOD) of allele fraction for SNVs and InDels is $\geq 5\%$ and for fusions is ≥ 10 spanning reads. However, the report may include, at the discretion of laboratory director, the variants with lower allele burden (3-5%) having strong or potential clinical significance or those have been reported earlier in the patient. Variants with $< 1\%$ allele

fraction and variants of uncertain significance with <5% allele fraction are not routinely reported. However, possibility of false negative or false positive below the limit of detection of this assay cannot be ruled out.

- Large deletions and deep intronic variations are not detected in this assay.
- **Copy Number Variations (CNVs) are based on the RNA expression data using a CNV prediction model developed with control samples. Hence, the chromosome coordinates and size of the CNV can not be determined. It is recommended to confirm the CNVs by alternate methods, such as FISH as the sensitivity of NGS for detecting CNVs is not 100%.**
- **Additional case specific disclaimer : None**

TEST DESCRIPTION

The MedGenome's Colorectal panel is a high throughput next-generation sequencing assay covering key genes to detect SNVs, InDels, CNVs and fusions and aids in diagnosis, prognosis and therapeutics of the colorectal cancer patients.

TEST METHODOLOGY

Sample type: FFPE Specimen; A histopathologic review is performed to determine the tumor content in the FFPE block/curls.

Extraction and Library Preparation: Tumor nucleic acid is extracted from FFPE (Formalin fixed) tissue block and used to perform targeted gene capture using a custom hybrid capture kit.

Sequencing: The QC passed libraries are sequenced to a minimum depth of 250X on validated Illumina sequencing platform.

Data Analysis: The sequences are processed using a customized and validated analysis pipeline designed to accurately detect all classes of genomic alterations (SNVs, InDels, CNVs and Fusions).

Variant Annotation and Reporting: The variants are annotated using our in-house annotation pipeline. Reportable genomic alterations and fusions are prioritized, classified, and reported based on AMP-ASCO-CAP guidelines [PMID: [27993330](#)] and NCCN guidelines.

Limit of Detection (LOD): The LOD for SNVs and InDels is 5% Variant allele Frequency (VAF) and for fusions is >10 spanning reads.

The transcript used for clinical reporting generally represents the canonical transcript (according to Ensembl release 99 human gene model), which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported. Variants annotated on incomplete, and nonsense mediated decay transcripts are not reported.

§This test is developed, and its performance characteristics is determined by MedGenome Labs Ltd.

GENES ANALYSED

SNVs/InDels							
AKT1	BRAF	ERBB2	HRAS	NRAS	POLE	POLD1	PIK3CA
PTEN	SMAD4	KRAS					

CNVs
ERBB2

FUSIONS			
NTRK1	NTRK2	NTRK3	RET

CLINICAL TRIALS

The following trials are potentially best suited for your patient's indication, considering all reported treatment recommendations. See <https://clinicaltrials.gov> (clinical trials from NCT) or <https://trialsearch.who.int> (clinical trials from other registries) for more information.

Clinical trials in total : 0 **Trial countries :** IN-India, US-United States

S.No	Title	Phase and ID	Intervention	Disease	Age & Sex
No Clinical Trials.					

Aparna Natarajan, Ph.D
Lead - Genome Analyst (Oncology)

Dr. Syed Muqlisur Rehman, MD Path
Molecular Pathologist
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END OF REPORT

MGM527: Microsatellite Instability (MSI) by fragment analysis

Patient: DR. D.R.P.A WICKRAMASINGHE / 64 years

SID/OID: 8758711/ 1082072

Patient Details		Specimen Information		Ordering Clinician	
Name	DR. D.R.P.A WICKRAMASINGHE	Sample ID	8758711	Clinician	Dr. Senaka Kandegedara
		Order ID	1082072		
Gender/Age	Male / 64 Years	Specimen Type	FFPE Tumor	Affiliation	Aegle Omics Private Limited (Colombo)
Patient ID	NA	Date Received	16 th October 2024		
Tumor Type	Colorectal neoplasm	Date and Time of Report	23 rd October 2024		
Test Code	MGM527	Test Name	Microsatellite Instability (MSI) by fragment analysis		

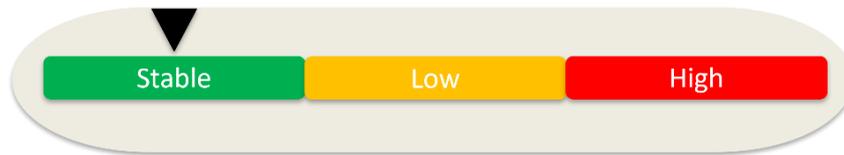
CLINICAL DIAGNOSIS/INDICATIONS/HISTORY

Metastatic carcinoma sigmoid colon; T3N2bM1b. The tumor content estimated for the tissue available in the FFPE block [RP3219 (B1, B2)] was adequate for further analysis.

Kindly note that this is the MSI report. The final NGS report, including the status of SNVs & Indels, Fusions and CNVs, will be released on or before 02-11-2024 based on the QC status.

TEST RESULT SUMMARY

Microsatellite Instability (MSI) Status - MSS



Summary of Markers	
Count of markers reported Unstable	0
Count of markers reported Stable	12
Reported Unstable Rate	0%
Unstable Markers	NA

CLINICAL SIGNIFICANCE

- MSI screening has long been recognized as important in the care of patients with colorectal cancer (CRC) or endometrial cancer (EC).
- High-frequency MSI (MSI-H) is also recognized as a potential marker for germline mutations in certain DNA mismatch repair (MMR) genes associated with Lynch syndrome [PMID: 15872200].
- MSI has been found in several cancer types, including non-small cell lung cancer, melanoma, breast cancer, urothelial cancer, pancreatic ductal adenocarcinoma and brain cancer. The expansion of MSI clinical trials into other cancers may elucidate the prognostic and predictive value of MSI for non-colorectal [PMID: 35955855].
- NCCN® guidelines recommend universal screening for 15+ different cancer types by MSI and/or IHC analysis [www.nccn.org]
- MSI-H status is predictive of a positive response to immunotherapies such as immune checkpoint blockade inhibitors [PMID: 26028255]
- The 2015 paper by Le et al. reported the extended analysis on the efficacy of PD-1 blockade in patients with advanced mismatch repair-deficient cancers of both colorectal cancer and non-colorectal origins. Following 41 patients, the study found that patients with mismatch repair deficient

tumors, experienced an objective response rate of 40% and a progression-free survival rate of 78%. In contrast, the objective response rate was 0% and the progression-free survival rate was 11% for mismatch repair-proficient patients [[PMID: 26028255](#)].

- The College of American Pathologists (CAP), in collaboration with the Association of Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO), and patient advocacy group Fight Colorectal Cancer (Fight CRC) convened a multidisciplinary expert and advisory panel to develop evidence-based guidelines to identify the optimal clinical laboratory test to identify defects in DNA mismatch repair (dMMR) in patients with solid tumor malignancies who are being considered for immune checkpoint inhibitor (ICI) therapy. MSI by PCR was recommended for colorectal cancer, patients with gastroesophageal and small bowel cancer and other solid malignancies [[PMID: 35920830](#)].
- On June 29, 2020, the Food and Drug Administration approved pembrolizumab (KEYTRUDA, Merck & Co.) for the first-line treatment of patients with unresectable or metastatic microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) colorectal cancer [www.fda.gov]
- The FDA approved pembrolizumab on May 23, 2017, for the treatment of adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H), or mismatch repair deficient (dMMR) solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options and for the treatment of unresectable or metastatic MSI-H or dMMR colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan [www.fda.gov].

DISCLAIMER

- **Decisions regarding treatment action plan should not be solely based on these test results. These findings are highly recommended to be correlated with the patient's clinical, pathological, radiological and family history for decisions on diagnosis, prognosis, or therapeutics.**
- The therapy information provided in this report is based on FDA approved drugs data, NCCN guidelines, peer-reviewed published literature, standard clinical databases, and strength of biomarker results. These therapies may or may not be suitable/beneficial to a particular patient. This clinical report summarizes potentially effective medications, potentially ineffective medications, and medications that may pose a higher risk of adverse reactions by mapping the patient's genetic alterations to the biomedical reference information. The report may also provide prognostic and diagnostic biomarkers detected or shown for the given disease context.
- The identification of a genomic biomarker does not necessarily imply pharmacological effectiveness or ineffectiveness. The medications identified by the treating physician may or may not be suitable for use on a particular patient. Thus, the clinical report does not guarantee that any particular agent will be effective in the treatment of any particular condition. Also, the absence of a treatment option does not determine the effectiveness or predict an ineffective or safety-relevant effect of a medication selected by the treating physician.
- Due to poor quality of FFPE tissue blocks, the QC parameters for extracted DNA may not pass to proceed further with the testing, therefore there is a possibility of assay failure or compromised. However, sample status in such scenarios shall be sent through mail to the ordering clinician.
- This test has been validated at MedGenome Labs as per the CAP guidelines with 100% sensitivity and specificity.
- The results of this test are dependent on the tumor content in the tissue sample provided. A minimum of >10% tumour content is required for a successful testing.
- In case of MSI negative or MSS patients, if there is a co-existing strong personal or family history of HNPCC related cancers for this patient, consider microsatellite instability and IHC testing on a different tumor block to further evaluate the possible role of defective DNA mismatch repair.
- **Additional case specific disclaimer: In this case, the markers "ABI-20A" had failed in amplification. Hence, the MSI status of this subject has been interpreted based on the status of 12 out of 13 markers. Kindly correlate clinically.**

TEST METHODOLOGY

This assay detects the presence of microsatellite instability (MSI) in DNA samples through multiplex PCR [1] and fragment analysis and screens for 13 mononucleotide markers listed in table below. Mononucleotide markers like BAT-25, BAT-26 and BAT-40 markers are selected as per the NCI guidelines. A revised guidelines suggests mononucleotide marker panel is more sensitive for MSI-H tumors than other microsatellite markers. Dinucleotide markers are less sensitive, and if only dinucleotide markers are positive, it is mandatory to test additional mononucleotide markers to rule out MSI-L [PMID: 14970275]. This kit contains 13 mononucleotide markers for higher resolution and two STR sequences that can be used to track sample identity [PMID: 35884597, PMID: 35982978].

BAT-25	NR-21	ABI-16	ABI-20B
BAT-26	NR-22	ABI-17	
BAT-40	NR-24	ABI-19	
CAT-25	NR-27	ABI-20A	

The primers are fluorophore tagged at the 5' end and the end-point PCR product is analyzed by Fluorophore Capillary Electrophoresis. The tumor tissue is classified as MSS/MSI-L/MSI-H as mentioned in the table below.

MSI Result	Interpretation [PMID: 35884597]
MSI-High	Unstable marker rate:- 30% - 100%
MSI-Low	Unstable marker rate:- 5% - 30%
MSS (Microsatellite Stable)	Unstable marker rate:- 0%

Recommendation: Test results should be interpreted in context of clinical findings, family history, and other laboratory data. If results obtained do not match other clinical or laboratory findings, please contact the laboratory for possible interpretation. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

Reference:

1. Application note: TrueMark MSI Assay—a simplified solution for analyzing microsatellite instability in FFPE tumor samples, 2020.



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End of Report