DNA Test Report

MGM2649 - Minimal Residual Disease (MRD) by NGS in Solid Tumours (Liquid Biopsy)

MEDGENOME | Prima Cancer Genetics

Patient: Mr J D J Roshan Perera/ 59 years **Sample ID/Order ID:** 8970666 / 1201918 **Tumor Type:** Colorectal neoplasm

Patient Details		Specimen Information		Ordering Clinician	
Name	Mr J D J Roshan Perera	**Sample ID**	8970666	**Clinician**	Dr. Mahendra Perera
		Order ID	1201918		
Gender/Age	M/59	**Specimen type***	Blood in Streck tube	**Affiliation**	Aegle Omics Private Limited
Patient ID	NA	**Date Received**	17.02.2025		
Tumor Type	Colorectal Cancer	**Date & Time of Report**	05.03.2025		
Test Code	MGM2649	**Test Name:**	**Minimal Residual Disease (MRD) by NGS in Solid Tumours (Liquid Biopsy)**		

CLINICAL DIAGNOSIS/INDICATIONS/HISTORY

Poorly differentiated adenocarcinoma of colon; pT4 pN1b pMx[as per the clinical details provided with the Test Requisition Form].

TEST RESULT SUMMARY

cfDNA mutations (MRD) - Detected

Date of Surgery	Date of Blood Collection (First follow-up)	Date of Blood Collection (second follow-up)	Treatment Details
03-02-2019	13-02-2025	NA	Chemotherapy

The date of surgery and date of blood collection is mentioned as per histopathology report and Test Requisition Form
MRD: Minimal Residual Disease; cfDNA: Circulating free DNA

Timeline

Time elapsed since surgery	6 Years	NA	NA	NA
Date of Blood Collection	13-02-2025	NA	NA	NA
ctDNA mutations	*ARID1B* variant detected	NA	NA	NA

Executive Summary

- The presence of baseline tumour mutations in liquid biopsy sample (cfDNA) after surgery has been associated with high risk of cancer recurrence.
- 6 years post-surgery, cfDNA was tested by NGS to track these mutations and to find any other mutations. The cfDNA NGS results were positive for clinically significant mutations in the *ARID1B* gene and variant of uncertain significance in *MSH6* gene (Please see report on Page number 2).
- Baseline tumor sample details are not available for comparison. *ARID1B and MSH6* variants are detected in the first follow up sample collected 6 years post-surgery.

Mr J D J Roshan Perera

AGE: 59 years | **Gender:** Male | **Coupon/UID:** NA / AOMG076

Diagnosis: Colorectal Neoplasms

MEDGENOME

MGM455 : Minimal Residual Disease (MRD) by NGS in Solid Tumours (Liquid Biopsy)

Report Details		Specimen Information		Ordering Clinician	
Sample ID / Order ID:	8970666 / 1201918	**Specimen Site:**	NA	**Clinician:**	Dr. Mahendra Perera
Collection Date:	13th February 2025	**Specimen Received:**	Blood in streck tube	**Affiliation:**	Aegle Omics Private Limited
Date Received:	17th February 2025	**Specimen Tested:**	Blood in streck tube	**Serviced By:**	18718
Report Date & Time:	5th Mar 2025 20:14 PM	**Tumor Content:**	NA	**Report Status:**	Final

Clinical Summary: Poorly differentiated adenocarcinoma of colon; pT4 pN1b pMx

TEST RESULT SUMMARY

Next Generation Sequencing (NGS) Test Result

Result - POSITIVE

CLINICALLY RELEVANT VARIANT/S DETECTED

AMP Classification ^	CDS variant details	Interpretation	Treatment Recommendations	$Treatment Response
***ARID1B* p.Pro1506GlnfsTer65 (FRAMESHIFT-DEL)** Variant Allele Frequency - 0.59%				
Tier II	c.4517del (ENST00000636930.2)	Oncogenic	NA	Diagnostic

^Refer to Glossary section for the classification criteria details.

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

NOTE: The patient tumor is wild type for KRAS, NRAS and BRAF mutation. Anti-EGFR monoclonal antibodies are indicated for the EGFR expressing colorectal cancer patients, having wild type KRAS, NRAS and BRAF.Kindly correlate clinically [NCCN Guidelines: Colon/Rectal Cancer, Version 4.2024].

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.

Gene	Exon	Nucleotide change	Protein change	Alternate allele Depth (x)	Allele Burden (%)	Functional predictions	Population MAF (%)
MSH6	1	ENST00000234420 .11 c.220G>A chr2:g.47783453G>A	p.Gly74Arg	875x	51.08%	T(SIFT); U(LRT); NA(Polyphen2)	0 (1000G); 0 (gnomAD)

ACTIONABLE BIOMARKER DETAILS

ARID1B (p.Pro1506GlnfsTer65) - FRAMESHIFT-DEL

Gene: *ARID1B*	**Exon:** 18	**Variant Allele Frequency:** 0.59%
Nucleotide change: chr6:g.157200742del	**Protein change:** p.Pro1506GlnfsTer65	**Population MAF:** 0 (1000G);0(gnomAD);
cDNA change: c.4517del	**Variant Type:** FRAMESHIFT-DEL	**In-silico Predictions:** NA(SIFT); NA(LRT); NA(Polyphen2)
Transcript ID: ENST00000636930.2	**Variant Allele Depth/Total depth:** 15/2547x	**Gene Function:** Tumor Suppressor Gene

Gene Summary: *ARID1B* (AT-rich interactive domain-containing protein 1B), also known as BAF250B, is a member of the SWI/SNF chromatin-remodeling complex, and plays a role in altering chromatin structure for various cellular functions, including transcription, DNA synthesis and DNA repair [PMID: 25387058]. ARID1B binds to AT-rich regions of DNA and helps recruit other members of the SWI/SWF complex, such as SMARCA and BAF complexes. Together, these complexes are involved in ATP-dependent chromatin

Clinical and Therapeutic Relevance: *ARID1B*, a tumor suppressor involved in transcriptional regulation, is inactivated by mutation or deletion in various cancer types. *ARID1B* can substitute for *ARID1A* in BAF complexes despite ARID1A being more commonly present [PMID: 15170388]. Like ARID1A, germline mutations in *ARID1B*result in Coffin-Siris syndrome, which is characterized by developmental delay and coarse facial features [PMID: 22426309]. Inactivating ARID1B mutations have been identified in breast cancer [PMID: 22722201], gynecologic carcinosarcoma [PMID: 25233892], pancreatic cancer [PMID: 22233809] and neuroblastoma [PMID: 23202128]. ARID1B is also lost in certain cancers, such as colorectal, pancreatic, hepatocellular carcinoma, malignant melanoma, breast cancer, and neuroblastoma. **However, the mutation identified in this patient is not well documented in the prognostic and therapeutic relevance in medical literature for the tumor type in the patient. Kindly correlate clinically.**

PubMed References: 15170388, 22426309, 25233892, 22722201, 22233809, 23202128

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 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 till date. 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 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 cfDNA 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.
- Detection of large insertions, deletions, copy number variations, gene rearrangements and deep intronic variations are beyond the scope of this test.
- This test has been validated at MedGenome Labs and the limit of detection (LOD) of allele fraction for SNVs and short InDels is 0.25% VAF. However, the report may include, at the discretion of laboratory director, the variants with lower allele burden having strong or potential clinical significance or those have been reported earlier in the patient.
- Variants with <0.1% allele fraction and variants of uncertain significance with <0.25% 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.

- ***Additional case specific disclaimer*:** None

TEST DESCRIPTION

The MedGenome's Oncotrack Ultima is a liquid biopsy based high throughput next-generation sequencing assay to detect cancer causing genomic alterations (SNVs, Indels) in 118 tumour agnostic genes. Complete coding regions of all guideline recommended actionable genes are covered.
The test is performed on circulating free DNA (cfDNA) isolated from blood plasma (liquid biopsy). cfDNA comprises circulating tumor DNA (ctDNA) present in blood plasma that is shed from tumor tissue and is the source of tumor genetic material. Unlike traditional biopsy, liquid biopsy is non-invasive as it requires only a peripheral blood draw in Streck tube from the cancer patient. Liquid Biopsy NGS testing is very powerful clinically as it provides- (a) real-time treatment monitoring to evaluate the drug response in cancer patients, (b) early detection of acquired resistance mutations to targeted therapy, (c) detection of recurrence at early stages before significant accumulation of tumor cell mass, (d) identification of tumor heterogeneity arising due to multiple clones and hence the disease progression

TEST METHODOLOGY

Sample Type: Peripheral blood in Streck tube

Extraction and Library Preparation: cfDNA isolated from blood plasma is used to perform UMI-based target enrichment and sequencing using a custom capture kit.

Sequencing: The QC passed libraries are sequenced to a minimum depth >20000X (pre-UMI) on validated Illumina sequencing platform and compressed to >2000X (post-UMI) for variant analysis

Data Analysis: The sequences obtained are aligned to human reference genome (GRCh38/hg38) using BWA program [PMID:19451168, PMID: 23155063]. Somatic mutations are identified using UMI corrected Sention pipeline [PMID: 31481971]. Only non-synonymous and splice site variants found in the coding regions are used for clinical interpretation. The mutations are annotated using our in-house annotation pipeline (VariMAT).

Reporting: Reportable alterations are prioritized, classified, and reported based on AMP-ASCO-CAP guidelines [PMID: 27993330].

Analytical performance: A minimum of 30ng cfDNA isolated from plasma is considered as an acceptable criterion for proceeding with this testing. Analytical validation of this test in our laboratory has shown sensitivity, and specificity of 100% at Limit of Detection at 0.25% VAF.

Limit of Detection (LOD): The Limit of detection of the assay for somatic mutations is 0.25% for SNVs and short INDELs [PMID: 29379323].

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

Complete coding regions of 73 genes are covered (Black font) and hotspot regions of 45 genes (Blue font) are covered in this panel. All genes that are diagnostically, prognostically and therapeutically significant according to the NCCN guidelines in multiple cancer types are completely covered in this test.

SNVs/InDels							
ABL1	*ABL2*	*AKT1*	*ALK*	*APC*	*AR*	*ARAF*	*ARID1A*
ARID1B	*ATM*	*ATR*	*ATRX*	*BAP1*	*BARD1*	*BRAF*	*BRCA1*
BRCA2	*BRIP1*	*C11orf65*	*CCND1*	*CDH1*	*CDK12*	*CDK4*	*CDKN2A*
CDX2	*CHEK1*	*CHEK2*	*CSF1R*	*CTNNB1*	*DDR2*	*EGFR*	*ERBB2*
ERBB3	*ERBB4*	*ERCC2*	*ESR1*	*EZH2*	*FANCL*	*FBXW7*	*FGFR1*
FGFR2	*FGFR3*	*FGFR4*	*FLT3*	*FOXA1*	*FOXL2*	*GATA3*	*GNA11*
GNAQ	*GNAS*	*HNF1A*	*HRAS*	*IDH1*	*IDH2*	*INPP4B*	*JAK1*
JAK2	*JAK3*	*KDM5C*	*KDM6A*	*KEAP1*	*KIT*	*KRAS*	*MAP2K1*
MAP2K2	*MAPK1*	*MET*	*MLH1*	*MPL*	*MSH2*	*MSH6*	*MTOR*
MUTYH	*MYC*	*MYCN*	*MYD88*	*NF1*	*NF2*	*NOTCH1*	*NPM1*
NRAS	*NTRK1*	*NTRK3*	*PALB2*	*PBRM1*	*PDGFRA*	*PIK3CA*	*PMS2*
POLD1	*POLE*	*PPP2R2A*	*PTCH1*	*PTEN*	*PTPN11*	*RAD51B*	*RAD51C*
RAD51D	*RAD54L*	*RAF1*	*RB1*	*RET*	*RHEB*	*RHOA*	*RIT1*
ROS1	*SETD2*	*SF3B1*	*SMAD4*	*SMARCB1*	*SMO*	*SPOP*	*SRC*
STK11	*TERT*	*TP53*	*TSC1*	*TSC2*	*VHL*		

Note: *MET* exon 14 skipping mutations included.

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
KMC Registration No. 71468

END OF REPORT