S A Shiranee M F Senevirathne

AGE: 76 years | **Gender:** Female | **Coupon/UID:** NA / AOMG008

Diagnosis: Endometrial Neoplasms

MEDGENOME | Prima Cancer Genetics

MGM2582 : Endometrial Cancer panel by NGS + dMMR by IHC

Report Details		Specimen Information		Ordering Clinician	
Sample ID / Order ID:	8690116 / 1042163	**Specimen Site:**	Endometrium	**Clinician:**	Dr. Mahendra Perera
Collection Date:	NA	**Specimen Received:**	FFPE Tissue Blocks [2]	**Affiliation:**	Aegle Omics Private Limited
Date Received:	4th September 2024	**Specimen Tested:**	XH1632 (Q, N) block	**Serviced By:**	18718
Report Date & Time:	25th Sep 2024 20:19 PM	**Tumor Content:**	50	**Report Status:**	Final

Clinical Summary: Endometrioid adenocarcinoma of endometrium

TEST RESULT SUMMARY

Immunohistochemistry (IHC) Results for Mismatch Repair (MMR) Proteins.

Marker	Expression
MLH1	Intact nuclear expression
MSH2	Intact nuclear expression
MSH6	Intact nuclear expression(Weak)
PMS2	Intact nuclear expression(Weak)

Impression: Intact DNA mismatch repair (MMR) function within the tumor (MMR proficient) .

Kindly refer to the complete MMR by Immunohistochemistry report below.

Next Generation Sequencing (NGS) Test Result

Result - POSITIVE

CLINICALLY RELEVANT VARIANT/S DETECTED

AMP Classification ^	CDS variant details	Interpretation	Treatment Recommendations	$Treatment Response
***PIK3CA* p.Glu545Asp (MISSENSE)** Variant Allele Frequency - 6.31%				
Tier II	c.1635G>T (ENST00000263967.4)	Likely oncogenic	NA	Prognostic
***PIK3CA* p.Arg88Gln (MISSENSE)** Variant Allele Frequency - 4.82%				
Tier II	c.263G>A (ENST00000263967.4)	Oncogenic	NA	Prognostic
***KRAS* p.Gly13Asp (MISSENSE)** Variant Allele Frequency - 14.11%				
Tier II	c.38G>A (ENST00000311936.8)	Oncogenic	NA	Diagnostic
***ARID1A* p.Ser711Ter (NONSENSE)** Variant Allele Frequency - 5.15%				

AMP Classification ^	CDS variant details	Interpretation	Treatment Recommendations	$Treatment Response
Tier II	c.2132C>G (ENST00000324856.13)	Oncogenic	NA	Prognostic
***PTEN* p.Arg130Gly (MISSENSE)** Variant Allele Frequency - 16.06%				
Tier II	c.388C>G (ENST00000371953.8)	Oncogenic	NA	Prognostic

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.

The *PIK3CA* p.Arg88Gln has been observed at Variant Allele Frequency below the limit of detection of this assay. Kindly correlate clinically.

NOTE: No clinically significant mutation has been detected in *TP53* and *POLE* genes in this sample.

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 (%)
PTEN	1	ENST00000371953 .8 c.44G>T chr10:g.87864513G>T	p.Arg15Ile	810x	6.91%	D(SIFT); D(LRT); BN(Polyphen2)	0 (1000G); 0 (gnomAD)

ACTIONABLE BIOMARKER DETAILS

PIK3CA (p.Glu545Asp) - MISSENSE

Gene: *PIK3CA*	**Exon:** 10	**Variant Allele Frequency:** 6.31%
Nucleotide change: chr3:g.179218305G>T	**Protein change:** p.Glu545Asp	**Population MAF:** 0 (1000G);0(gnomAD);
cDNA change: c.1635G>T	**Variant Type:** MISSENSE	**In-silico Predictions:** T(SIFT); D(LRT); PrD(Polyphen2)
Transcript ID: ENST00000263967.4	**Variant Allele Depth/Total depth:** 141/2235x	**Gene Function:** Oncogene

Gene Summary: Phosphatidylinositol 3-kinase is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The protein encoded by *PIK3CA* represents the catalytic subunit, which uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2. *PIK3CA* has been found to be oncogenic and has been implicated in cervical cancers. A pseudogene of *PIK3CA* has been defined on chromosome 22.

Clinical and Therapeutic Relevance: *PIK3CA* is the catalytic subunit of the lipid phosphoinositide-3-kinase (PI3K) that activates the PI3K/AKT signaling pathway to promote cell proliferation and survival. This variant tis located in exon 10, a known hotspot region, and has been shown to promote cell transformation and confer ligand-independent proliferation in cell lines. The PI3K inhibitor alpelisib plus fulvestrant or the AKT inhibitor capivasertib plus fulvestrant are indicated for the treatment of hormone receptor (HR)-positive, HER2-negative locally advanced or metastatic breast cancer with this variant. An exploratory analysis of two phase 3 trials suggests that HER2-positive patients with *PIK3CA* mutations show shorter progression-free survival (PFS) to trastuzumab compared to *PIK3CA* wild-type tumors. The combination with the mTOR inhibitor everolimus results in improvement of PFS. The clinical and genomic data of 307 patients with endometrioid endometrial adenocarcinoma (EEC) were obtained from TCGA project that includes 90 patients in the copy-number low-EEC (CNL-EEC) subgroup. In CNL-EEC subgroup patients, somatic *PIK3CA* mutations (48/90 cases) were associated with significantly improved overall survival compared with that of wild-type *PIK3CA* (P=0.018). Furthermore, this improved survival was specific to the CNL-EEC subgroup and was not observed in other TCGA molecular subgroups. The majority of CNL-EEC cases were low-stage (stage I) and low-to-intermediate grade (grades 1– 2) endometrioid tumors. Overall, in the TCGA cohort, *PIK3CA* mutations had a favourable effect on the survival of patients with EEC, and this effect was dependent on tumoral molecular sub-stratification [PMID: 26722235].

PubMed References: 37256976, 35759724, 31091374, 27091708, 26722235

PIK3CA (p.Arg88Gln) - MISSENSE

Gene: *PIK3CA*	**Exon:** 2	**Variant Allele Frequency:** 4.82%
Nucleotide change: chr3:g.179199088G>A	**Protein change:** p.Arg88Gln	**Population MAF:** 0 (1000G);0(gnomAD);
cDNA change: c.263G>A	**Variant Type:** MISSENSE	**In-silico Predictions:** D(SIFT); D(LRT); PrD(Polyphen2)
Transcript ID: ENST00000263967.4	**Variant Allele Depth/Total depth:** 119/2469x	**Gene Function:** Oncogene

Gene Summary: Phosphatidylinositol 3-kinase is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The protein encoded by *PIK3CA* represents the catalytic subunit, which uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2. *PIK3CA* has been found to be oncogenic and has been implicated in cervical cancers. A pseudogene of *PIK3CA* has been defined on chromosome 22.

PIK3CA (p.Arg88Gln) - MISSENSE

Clinical and Therapeutic Relevance: *PIK3CA* is the catalytic subunit of the lipid phosphoinositide-3-kinase (PI3K) that activates the PI3K/AKT signaling pathway to promote cell proliferation and survival. This variant strongly activates the downstream pathway in preclinical settings and lies outside the hotspot regions, exon 8, 10, and 21 (sometimes reported as exon 7, 9, or 20). The AKT inhibitor capivasertib in combination with fulvestrant is indicated for adult patients with hormone receptor (HR)-positive, HER2-negative locally advanced or metastatic breast cancer with this variant. The PI3K inhibitor alpelisib in combination with fulvestrant is indicated as second-line therapy in HR-positive/HER2-negative locally advanced or metastatic breast cancer with *PIK3CA* mutations (EMA) and with mutations in exon 8, 10, or 21 (ESMO guidelines). The HER2-positive tumor of a patient with this variant was resistant to trastuzumab. Although not specifically tested for this mutation, preclinical models with activating variants are sensitive to alpelisib, to the mTOR inhibitors everolimus and sirolimus, and have reduced sensitivity to trastuzumab. An exploratory analysis of two phase 3 trials suggests that HER2-positive patients with *PIK3CA* mutations show shorter progression-free survival (PFS) to trastuzumab compared to *PIK3CA* wild-type tumors. The combination with everolimus results in improvement of PFS. The clinical and genomic data of 307 patients with endometrioid endometrial adenocarcinoma (EEC) were obtained from TCGA project that includes 90 patients in the copy-number low-EEC (CNL-EEC) subgroup. In CNL-EEC subgroup patients, somatic *PIK3CA* mutations (48/90 cases) were associated with significantly improved overall survival compared with that of wild-type *PIK3CA* (P=0.018). Furthermore, this improved survival was specific to the CNL-EEC subgroup and was not observed in other TCGA molecular subgroups. The majority of CNL-EEC cases were low-stage (stage I) and low-to-intermediate grade (grades 1– 2) endometrioid tumors. Overall, in the TCGA cohort, *PIK3CA* mutations had a favourable effect on the survival of patients with EEC, and this effect was dependent on tumoral molecular sub-stratification [PMID: 26722235].

PubMed References: 37256976, 35759724, 34678411, 33246021, 28382169, 27091708, 23092874, 21358673, 26722235

PTEN (p.Arg130Gly) - MISSENSE

Gene: *PTEN*	**Exon:** 5	**Variant Allele Frequency:** 16.06%
Nucleotide change: chr10:g.87933147C>G	**Protein change:** p.Arg130Gly	**Population MAF:** 0 (1000G);0(gnomAD);
cDNA change: c.388C>G	**Variant Type:** MISSENSE	**In-silico Predictions:** D(SIFT); D(LRT); PrD(Polyphen2)
Transcript ID: ENST00000371953.8	**Variant Allele Depth/Total depth:** 2263/14094x	**Gene Function:** Tumor Suppressor Gene

Gene Summary: *PTEN* was identified as a tumor suppressor that is mutated in a large number of cancers at high frequency. The protein encoded by *PTEN* is a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase. It contains a tensin like domain as well as a catalytic domain similar to that of the dual specificity protein tyrosine phosphatases. Unlike most of the protein tyrosine phosphatases, this protein preferentially dephosphorylates phosphoinositide substrates. It negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate in cells and functions as a tumor suppressor by negatively regulating AKT/PKB signaling pathway. The use of a non-canonical (CUG) upstream initiation site produces a longer isoform that initiates translation with a leucine, and is thought to be preferentially associated with the mitochondrial inner membrane. This longer isoform may help regulate energy metabolism in the mitochondria. A pseudogene of *PTEN* is found on chromosome 9. Alternative splicing and the use of multiple translation start codons results in multiple transcript variants encoding different isoforms.

Clinical and Therapeutic Relevance: The phosphatase and tensin homolog (PTEN) is a dual-specificity protein phosphatase that inactivates the PI3K/AKT signaling pathway to inhibit cell proliferation and survival. This inactivating mutation leads to constitutive activation of this pathway and was observed in many types of cancer, including endometrial, breast, prostate, and colorectal carcinomas. Capivasertib plus fulvestrant is indicated for the treatment of HR-positive, HER2-negative locally advanced or metastatic breast cancer with *PTEN* inactivating alterations (phase III trial, CAPItello-291). *PTEN* loss is observed in more aggressive stages of the disease, including metastatic castration-resistant prostate cancers (mCRPC). A phase II study combining ipatasertib and abiraterone has reported that radiographic progression-free survival (rPFS) was prolonged in the ipatasertib cohort vs placebo in mCRPC patients, with a superior antitumor activity observed in those with *PTEN* loss. Consistently, tumors in prostate cancer xenograft models were sensitive to ipatasertib treatment. In a phase 1b study, the combination of CC-115 and enzalutamide in mCRPC patients resulted in a non-statistically significant trend towards improved PSA response in patients harboring PI3K pathway alterations (n=41), including 11 patients with *PTEN* mutations or deletion. The growth of the *PTEN*-negative PC346C xenograft model was inhibited by the combination of capivasertib (AZD53630) with castration. Although *PTEN*-deficient cells showed some sensitivity to everolimus, in a phase II trial, treating advanced solid tumors with PTEN loss with everolimus did not show any clinical benefit. In a clinical study involving 221 primary endometrial carcinoma patients, mutational analysis of *PTEN* gene was done along with immuno-histochemical analysis. Expression of *PTEN* was lost in 56 patients (25%), and *PIK3CA* was overexpressed in 159 patients (72%). Overexpression of *PIK3CA* was associated with p-Akt overexpression (P<0.001),which was in turn associated with loss of nuclear p27 expression (P=0.028). Loss of *PTEN* expression was found to be associated with endometrioid histology (P=0.03) and was inversely associated with the presence of lymphovascular space invasion (P=0.03). Univariate and multivariate survival analyses revealed that factors of *PTEN* loss, age <70, histological grade 1,early International Federation of Gynecology and Obstetrics (FIGO) stage, and absence of lymphovascular invasion were independent prognostic indicators for better overall survival (P=0.03, 0.04, 0.01, <0.001, and 0.03,respectively). The subset analysis showed a stronger tendency of *PTEN* loss towards favourable survival in advanced-stage (III and IV) disease than in early-stage (I and II) disease (P=0.05 vs 0.14). Moreover, this mutational analysis demonstrated that *PTEN* expression loss was associated with *PTEN*-truncating mutations (P=0.03). Thus, loss of PTEN expression is a significant and independent prognostic factor for favourable survival in the disease [PMID: 23949151].

PubMed References: 38478799, 37980367, 35671774, 34246347, 32451180, 30037818, 27872130, 26910118, 25220373, 24141624, 23949151

ARID1A (p.Ser711Ter) - NONSENSE

Gene: *ARID1A*	**Exon:** 5	**Variant Allele Frequency:** 5.15%
Nucleotide change: chr1:g.26761067C>G	**Protein change:** p.Ser711Ter	**Population MAF:** 0 (1000G);0(gnomAD);
cDNA change: c.2132C>G	**Variant Type:** NONSENSE	**In-silico Predictions:** NA(SIFT); NA(LRT); NA(Polyphen2)
Transcript ID: ENST00000324856.13	**Variant Allele Depth/Total depth:** 158/3068x	**Gene Function:** Tumor Suppressor Gene

Gene Summary: *ARID1A* encodes a member of the SWI/SNF family, whose members have helicase and ATPase activities and are thought to regulate transcription of certain genes by altering the chromatin structure around those genes. The encoded protein is part of the large ATP-dependent chromatin remodeling complex SNF/SWI, which is required for transcriptional activation of genes normally repressed by chromatin. It possesses at least two conserved domains that could be important for its function. First, it has a DNA-binding domain that can specifically bind an AT-rich DNA sequence known to be recognized by a SNF/SWI complex at the beta-globin locus. Second, the C-terminus of the protein can stimulate glucocorticoid receptor-dependent transcriptional activation. It is thought that the protein encoded by *ARID1A* confers specificity to the SNF/SWI complex and may recruit the complex to its targets through either protein-DNA or protein-protein interactions. Two transcript variants encoding different isoforms have been found for *ARID1A*.

Clinical and Therapeutic Relevance: The AT-rich interactive domain-containing protein 1A (ARID1A) is part of the large ATP-dependent chromatin remodeling complex SWI/SNF that epigenetically controls gene expression to regulate cell proliferation, differentiation, and apoptosis. *ARID1A* is frequently mutated in different types of cancer, with mutations in up to 57% of ovarian clear cell carcinomas and 30% of ovarian endometrioid carcinomas, in 27% of gastric and 10% of esophageal cancers, in 5-10% of colorectal cancer and 13% in pancreatic cancer, in 15% of metastatic and 7% of primary breast cancer, in 17% of hepatocellular carcinoma and up to 36% in cholangiocarcinoma, among others. Nonsense and frameshift mutations lead to premature truncation thus they likely result in loss of protein expression and/or function. *ARID1A* mutations were found in 57% of ovarian clear-cell carcinomas, 40% of uterine endometrioid carcinomas, and between 20% and 36% of uterine carcinosarcomas. A study investigating the *ARID1A* loss in patients with endometrioid carcinoma reported that no *ARID1A* loss was seen in complex atypical hyperplasia, with loss increasing to 25% and 44% of patients with low-grade and high-grade endometrioid carcinomas, respectively. *ARID1A* has also been used as a prognostic marker in endometrial cancer. A significant association of reduced *ARID1A* expression has been found with shorter progression-free survival in patients with endometrium-related cancer and ovarian clear-cell carcinoma, as well as with higher FIGO stage of both endometrial and ovarian cancer. A total of 67 patients with pathologically confirmed grade 3 endometrioid endometrial carcinoma (G3EEC) were included in the study. The recurrence-free survival (RFS) and overall survival (OS) were estimated using the Kaplan-Meier method and compared with a log-rank test. A recurrence was observed in 9 (13%) of the 67 patients with early stage G3EEC. The respective 5-years RFS and OS rates were 87.7% and 93.7%, and 68.6% and 85.7%, respectively for stages I and II. Multivariate analysis showed significantly longer RFS among patients with *ARID1A* loss (hazard ratio = 8.7; 95% CI, 1.09–69.6, p = 0.04). No significant differences were observed in RFS and OS of patients according to p53 and MMR expression status. *ARID1A* expression status was a prognosticator for patients with early stage G3EEC without adjuvant therapy, whereas p53 and MMR expression status showed no impact on survival outcomes. *ARID1A* may become a useful biomarker for stratification of adjuvant treatment for early stage G3EEC patients.

PubMed References: 36910637, 36882165, 36811690, 33785559, 32111729, 31949479, 31932695, 30686770, 30097580, 29767248, 28737768, 27958275, 27364904, 26069190, 24979463, 28055103, 16495918, 19592079, 24076775, 28466574, 34257552

KRAS (p.Gly13Asp) - MISSENSE

Gene: *KRAS*	**Exon:** 2	**Variant Allele Frequency:** 14.11%

KRAS (p.Gly13Asp) - MISSENSE

Nucleotide change: chr12:g.25245347C>T	**Protein change:** p.Gly13Asp	**Population MAF:** 0 (1000G);0.001971(gnomAD);
cDNA change: c.38G>A	**Variant Type:** MISSENSE	**In-silico Predictions:** D_lc(SIFT); D(LRT); NA(Polyphen2)
Transcript ID: ENST00000311936.8	**Variant Allele Depth/Total depth:** 147/1042x	**Gene Function:** Oncogene

Gene Summary: *KRAS*, a Kirsten ras oncogene homolog from the mammalian ras gene family, encodes a protein that is a member of the small GTPase superfamily. A single amino acid substitution is responsible for an activating mutation. The transforming protein that results is implicated in various malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas and colorectal carcinoma. Alternative splicing leads to variants encoding two isoforms that differ in the C-terminal region.

Clinical and Therapeutic Relevance: The small GTPase KRAS activates the RAS/MAPK signaling pathway to promote cell proliferation and survival. In preclinical studies, this variant promotes transformation due to enhanced downstream signaling. Clinical studies have established that metastatic colorectal cancers (CRC) with mutations at this codon show resistance to the anti-EGFR antibodies cetuximab and panitumumab. In clinical studies, patients with lung cancer demonstrated no clinical benefit of monotherapy with MEK inhibitor selumetinib or trametinib compared to standard therapy. In a few clinical cases, resistance to crizotinib was reported in ALK-rearranged lung tumors. A CRC patient treated with PLK1 inhibitor onvansertib (nms-1286937) combined with FOLIFRI/bevacizumab achieved a partial response (PR). Onvansertib antitumor activity was confirmed in a mouse model. CRC cell lines with this variant were resistant to EGFR inhibitor neratinib but sensitive to MEK inhibitors trametinib or selumetinib and moderately sensitive to cobimetinib. Dual node inhibition with MEK inhibitor cobimetinib and ERK inhibitor ulixertinib had a synergistic effect. In a CRC cell line harboring the BRAF.V600E mutation, this variant conferred resistance to the combination treatment of cetuximab with BRAF inhibitors vemurafenib, dabrafenib, or encorafenib, or the triple combination of encorafenib, cetuximab, and PI3K inhibitor alpelisib. In addition, this cell model showed resistance to combination treatment with MEK inhibitor selumetinib and cetuximab or MEK inhibitor trametinib and dabrafenib but moderate resistance to the triple combination of trametinib, dabrafenib, and cetuximab. In Ba/F3 cells with the KRAS.G12C variant, this variant was acquired upon resistance to sotorasib. *KRAS* seems to be directly associated with type I Endometrial cancer, and most studies support its early involvement in carcinogenesis. Current evidence correlates *KRAS* mutations with increased cell proliferation and apoptosis, as well as up-regulation of endometrial cell oestrogen receptors. *KRAS* mutations in patients with hyperplastic endometrium or early-stage type I Endometrial cancer, may provide important information for prognosis stratification, and further provision of personalised treatment options [PMID: 30711927]. MEK inhibition in *KRAS* mutant cells results in activation of ER signaling and prevents the abrogation of signaling through ERK1/2 and p90RSK that is achieved in *KRAS* wild-type EC cells. Combination therapy with MEK inhibition plus anti-estrogen therapy may be necessary to improve response rates in patients with *KRAS* mutant Endometrial cancer [PMID: 28498246].

PubMed References: 35797463, 33579957, 31088841, 30194935, 30711927, 28498246

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. 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 (WHO, 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 ≥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.
- **Additional case specific disclaimer : Although the panel coverage is >95%, the average depth of *NTRK1* and *RET* genes is below the threshold criteria for routine reporting. Hence, the possibility of false negative result with respect to these genes cannot be ruled out. Kindly correlate clinically.**

TEST DESCRIPTION

The MedGenome's Endometrial cancer panel is a high throughput next-generation sequencing based single assay that may provide treatment benefit to the patients. This panel covers a total of 32 key endometrial cancer genes for the assessment of various SNVs, InDels, and Fusions (in RNA).

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 and Fusions).

Variant Annotation and Reporting: The variants are annotated using our in-house annotation pipeline. Reportable genomic alterations 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	*ARID1A*	*BRCA1*	*BRCA2*	*BRAF*	*CHEK2*	*CTNNB1*	*EPCAM*
ERBB2	*FBXW7*	*FGFR1*	*FGFR2*	*FGFR3*	*KRAS*	*MLH1*	*MSH2*
MSH3	*MSH6*	*MUTYH*	*NTRK1*	*NTRK2*	*NTRK3*	*PIK3CA*	*PIK3R1*
PMS2	*POLD1*	*POLE*	*PTEN*	*RET*	*SMARCA4*	*STK11*	*TP53*

FUSIONS						
FGFR1	*FGFR2*	*FGFR3*	*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
KMC Registration No. 71468

END OF REPORT

Medgenome Labs Ltd
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Karnataka, 560100.

MMR by Immunohistochemistry - Test Report

Patient Name	S A Shiranee M F Senevirathne	Order ID	1042163
Age / Gender	76 Years / Female	Sample ID	8690116
Physician	Dr. Mahendra Perera	Collection Date	NA
Customer	MCC18718-Aegle Omics Private Limited	Sample Received Date	04-09-2024 03:30 PM
Report Date	06-09-2024 09:45 PM	Report Status	Final

Lab/Biopsy No : MBH-4307/24

Clinical Details : Ca. Endometrium.

Specimen received : 2 blocks. Hysterectomy specimen blocks.

Gross examination : Received two paraffin blocks No XH1632 - N, Q. From Lanka Hospitals. For IHC.
IHC done on N and Q blocks.

Immunohistochemistry (IHC) Testing for Mismatch Repair (MMR) Proteins.

Immunohistochemistry Microscopy :

IHC Markers	Pattern of expression
1. MLH1	Intact nuclear expression
2. MSH2	Intact nuclear expression
3. MSH6	Intact nuclear expression (Weak)
4. PMS2	Intact nuclear expression (Weak)

Background nonneoplastic tissue / internal control with intact nuclear expression : Yes

Impression:

Intact DNA mismatch repair (MMR) function within tumor (MMR proficient).

NOTE : Tumor is poorly preserved.

Medgenome Labs Ltd
Sy No 94/1C and 94/2, Tower-1, Veerasandra Village,
Attibele Hobli, Electronic City, Phase-1, Bangalore,
Karnataka, 560100.

MMR by Immunohistochemistry - Test Report

Patient Name	S A Shiranee M F Senevirathne	Order ID	1042163
Age / Gender	76 Years / Female	Sample ID	8690116
Physician	Dr. Mahendra Perera	Collection Date	NA
Customer	MCC18718-Aegle Omics Private Limited	Sample Received Date	04-09-2024 03:30 PM
Report Date	06-09-2024 09:45 PM	Report Status	Final

Note:

Appropriate dual control tissue run with the test is satisfactory. Reagents used are as follows **Anti MLH1(GM011 clone)** mouse monoclonal antibody , **Anti MSH2 (RED2 clone)** rabbit monoclonal antibody , **Anti-MSH6 (EP49 clone)** rabbit monoclonal antibody , **Anti PMS2 (EP51 clone)** Rabbit monoclonal antibody .

This assay has not been validated on decalcified tissue and result should be interpreted with caution given the likelihood of false negativity of decalcified specimen. Specimen should be processed by routine tissue processing method. Inappropriate fixation (nonformalin) and processing may give erroneous result. The performance characteristics of these assays have been determined by MedGenome. Performance characteristics refer to the analytical performance of the test.

Interpretation of IHC MMR:

No loss of nuclear expression of mismatch repair (MMR) proteins : Low probability of microsatellite instability-high (MSI-H).

Loss of nuclear expression of MLH1 and PMS2: testing for methylation of the MLH1 promoter and/or mutation of BRAF is indicated (the presence of a BRAF V600E mutation and/or MLH1 methylation suggests that the tumor is sporadic and germline evaluation is probably not indicated; absence of both MLH1 methylation and of BRAF V600E mutation suggests the possibility of Lynch syndrome, and sequencing and/or large deletion/duplication testing of germline MLH1 may be indicated)*

Loss of nuclear expression of MSH2 and MSH6: high probability of Lynch syndrome (sequencing and/or large deletion/duplication testing of germline MSH2 may be indicated, and, if negative, sequencing and/or large deletion/duplication testing of germline MSH6 may be indicated)*

Loss of nuclear expression of MSH6 only: high probability of Lynch syndrome (sequencing and/or large deletion/duplication testing of germline MSH6 may be indicated)*

Loss of nuclear expression of PMS2 only: high probability of Lynch syndrome (sequencing and/or large deletion/duplication testing of germline PMS2 may be indicated)*

* There are exceptions to the above IHC interpretations. These results should not be considered in isolation, and clinicalcorrelation with genetic counseling is recommended to assess the need for germline testing.

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MMR by Immunohistochemistry - Test Report

Patient Name	S A Shiranee M F Senevirathne	Order ID	1042163
Age / Gender	76 Years / Female	Sample ID	8690116
Physician	Dr. Mahendra Perera	Collection Date	NA
Customer	MCC18718-Aegle Omics Private Limited	Sample Received Date	04-09-2024 03:30 PM
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Explanatory Notes

Mismatch Repair Testing: Microsatellite instability and Immunohistochemistry

Detection of defective mismatch repair in colorectal carcinomas is important for detection of Lynchsyndrome (hereditary nonpolyposis colorectal cancer syndrome [HNPCC]), which accounts for approximately 2% to 3% of all colorectal carcinomas and has clinical implications for treatment of the affected patient and family members. Microsatellite instability (MSI) testing can be used to cost-effectively screen colorectal cancer patients for possible Lynch syndrome. Patients with a microsatellite instability-high (MSI-H) phenotype that indicates mismatch repair deficiency in their cancer may have a germline mutation in one of several DNA mismatch repair (MMR) genes (eg, MLH1, MSH2, MSH6, or PMS2) or an altered EPCAM (TACSTD1) gene. After appropriate genetic counseling, patients may want to consider testing to identify the causative heritable abnormality.

An MSI-H phenotype is more frequently observed in sporadic colorectal cancer (about 15% of cases) due to somatic abnormalities, usually hypermethylation of the MLH1 gene promoter. The specificity of MSI testing can be increased by using it primarily on at-risk populations, such as colorectal cancer patients younger than 50 years, or patients with a strong family history of Lynch associated tumors (eg, colorectal, endometrial, gastric, or upper urinary tract urothelial carcinoma), but with sacrifice of sensitivity, since a sizeable minority of cases lacks these clinical characteristics.

MSI testing of tumor DNA is generally performed with at least 5 microsatellite markers, generally mononucleotide or dinucleotide repeat markers. In 1998, a National Institutes of Health consensus panel proposed that laboratories use a 5-marker panel consisting of 3 dinucleotide and 2 mononucleotide repeats for MSI testing. Recent data suggests that dinucleotide repeats may have lower sensitivity and specificity for identifying tumors with an MSI-H phenotype. As a consequence, there has been a move towards including more mononucleotides and fewer dinucleotides in MSI testing panels. Many laboratories now use a commercially available kit for MSI testing that utilizes 5 mononucleotide markers.

MSI testing is frequently done in conjunction with immunohistochemical (IHC) testing for DNA MMR protein expression (ie, MLH1, MSH2, MSH6, and PMS expression). If DNA MMR IHC has not been performed, this testing should be recommended for any case that shows an MSI-H phenotype, because this information will help identify the gene that is most likely to have a germline mutation (eg, a patient whose tumor shows loss of MSH2 and MSH6 expression, but retention of MLH1 and PMS2 expression, is likely to have an MSH2 germline mutation). If the results of DNA MMR IHC and MSI testing are discordant (eg, MSI-H phenotype with normal IHC or abnormal IHC with MSS phenotype), then the laboratory should make sure that the same sample was used for MSI and IHC testing and that there was no sample mix-up. However, MSI-H may not occur in colorectal cancers of patients with germline MSH6 mutation.

Intact expression of all 4 proteins indicates that MMR enzymes tested are intact but does not entirely exclude Lynch syndrome, as approximately 5% of families may have a missense mutation (especially in MLH1) that can lead to a nonfunctional protein with retained antigenicity. Defects in lesser-known MMR enzymes may also lead to a similar result, but this situation is rare.

Any positive reaction in the nuclei of tumor cells is considered as intact expression (normal), and it is common for intact staining to be somewhat patchy. An interpretation of expression loss in tumor cells should be made only if a positive reaction is seen in internal control cells, such as the nuclei of stromal, inflammatory, or nonneoplastic epithelial cells. Loss of expression of MLH1 may be due to Lynch syndrome or methylation of the MLH1 promoter region (as occurs in sporadic MSI colorectal carcinoma). Genetic testing is ultimately required for this distinction, although a specific BRAF gene mutation (V600E) is present in many sporadic cases, but not familial cancers. Loss of MSH2 expression strongly suggests Lynch syndrome. PMS2 loss is often associated with loss of MLH1 and is only independently meaningful if MLH1 is intact. MSH6 is similarly related to MSH2. One should also keep in mind that nucleolar staining or complete loss of MSH6 staining has been described in colorectal cancer cases with prior radiation or chemotherapy, and a significant reduction of MSH6 staining has been described in a small percentage of colorectal carcinomas with somatic mutations of the coding region microsatellites of the MSH6 gene in MLH1/PMS2-deficient carcinomas.

Enclosed: 2 blocks

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Approved By

Dr. Rakshith V
Pathologist
KMC95334

*****End of Report*****

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CONDITIONS OF LABORATORY TESTING AND REPORTING

Medgenome Labs Ltd, Bangalore, Karnataka, India

- Laboratory results should be used with other clinical information to determine a final diagnosis.
- In case of unexpected test results please contact the laboratory. We will investigate and repeat analysis if possible.
- The medical report must be viewed and reproduced as a whole
- This medical report is not intended for medico-legal purposes.
- The medical report is to be interpreted and used by medical personnel only
- Assays are performed and reported in accordance with the stated schedule.
- There may be circumstances beyond our control that delay results, e.g., invalid assay run.
- The results of a laboratory test are dependent on the quality of the sample as well as the assay procedure.
- A requested test may not be carried out if:
- Sample is insufficient or inappropriate
- Sample quality is unsatisfactory
- Request for testing is withdrawn by the ordering doctor or patient
- There is discord between the labelling of the sample container and the name on the test requisition.
- For any query contact customer support : +91(0)8067154932/33
