

Test Description

The MolQ Precision Panel includes genes, involving hotspot regions and unique variants, applicable to a wide range of tumor types for detection of SNV (single and multiple nucleotide variation) and short Insertion-Deletion.

Patient Demographic

Name: Mr. M.K. Khungar
Sex: Male
Date of Birth/Age: 81 years
Disease: Liposarcoma

Clinician

Clinician Name: Dr Amit Verma
Medical Facility: Dr AV Institute of Personalized Cancer Therapy and Research
Pathologist: Not Provided

Specimen

Booking ID: 012412110027
Sample Type: FFPE Block No. 2Hx-8257-A2
Tumor Content Percentage: 80%
Date of Collection: 10-12-2024
Date of Booking: 10-12-2024

CLINICAL SYNOPSIS

M. K. Khungar, is a known case of liposarcoma. He has been evaluated for pathogenic variations in the genes listed in Appendix 2.

RESULT SUMMARY

No clinically significant alterations were identified in the analyzed gene panel for this specimen.

Microsatellite Instability (MSI) Status: High.

RESULTS

No pathogenic variants causative of the reported phenotype was detected.

PRIMARY FINDINGS

Gene (Transcript)	Variant	Variant Effect	Relevant Therapies	Evidence Tier
	NA			

UNKNOWN CLINICAL SIGNIFICANCE

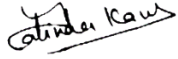
Gene Fusion	Unique Molecule Count (UMI VAF)	Relevant Therapies	Evidence Tier
<i>NTRK1 Fusion</i> RAB11FIP1(NM_025151:3)::NTRK1(NM_002529:11) 5'-chr8:37732033-chr1:156844698-3'	21 (5.34%)	Entrectinib Larotrectinib	Tier III

OTHER RELATED TEST FINDINGS

None.

Quality Metrics

DNA QC metrics: Total Reads: 65,460,872; Total mapped reads: 62,753,830; Percent mapped reads: 95.86%

A handwritten signature in black ink that reads 'Jatinder Kaur'.

Jatinder Kaur, PhD
Head, Molecular Biology & Genomics

A handwritten signature in black ink that reads 'Gulshan'.

Dr. Gulshan Yadav, MD
Head, Pathology

APPENDIX 1: TEST METHODOLOGY

Method

Pathology assessment

For FFPE/fresh tissue/frozen/cytology tissues, histopathological review on the H&E-stained section is done initially to assess the tumor type and location, pathological stage, the content of tumor cells, stromal changes such as necrosis, fibrosis/gliosis, inflammatory infiltrate, processing, fixation and embedding changes.

Assay Method

Genomic DNA and RNA was extracted from FFPE tissue using the Qiagen AllPrep DNA/ RNA FFPE Kit. The protocol prescribed by the manufacturer was meticulously followed to ensure the isolation of high-quality DNA and RNA from the FFPE samples. Following the pre-library quality assessment, the genomic DNA samples with minimum 100 ng of concentration and RNA samples with 500 ng input concentration were considered for the sequencing library preparation. The sequencing ready libraries were prepared using the SOPHiA GENETICS™ Universal Library Preparation kit and BS - SOPHiA DDM™ Solid Tumor Plus Solution v1 - ILL - R - SM - Y10 - 16rx kit.

Quality Assessment

Following the extensive qualitative and quantitative assessment (Tapestation and Qubit), the sequencing ready libraries underwent sequencing on Illumina X-plus platform, generating 150 base-paired end reads.

Variant Calling and Annotation

Alignment, post-processing, and default quality-filtered variant calling were executed on SOPHiA DDM™ 6.5.0. OncoPortal™ Plus was used to support decisions based on the JAX-CKB, database and CAP, ASCO, AMP as well as other guidelines. Variants were also annotated using GoldenHelix VSclinical pipeline utilizing a vast collection of databases. The variants were manually evaluated and reported. For each putative fusion, the number of unique molecules (unique molecule count) is determined based on Unique Molecular Identifiers (UMIs). Additionally, the percentage of unique molecules mapped to a specific fusion, relative to the total mapped molecules (UMI VAF), is provided.

Variant Classification and Evidence Tiers

The variant classification system used in this report is based on joint consensus recommendations of the Association for Molecular Pathology, American Society of Clinical Oncology, and the College of American Pathologists (PMID: 27993330). Tiers IA, IB, IIC, IID, III and IV describe variant categories of descending clinical significance in the patient. Variants in Tier IV are not reported in accordance with the consensus recommendations.

Tier I Strong Clinical Significance	Level A Evidence (approved therapy or practice guideline in patient's tumor type) Level B Evidence (consensus in the field based on well-powered studies in patient's tumor type)
Tier II Potential Clinical Significance	Level C Evidence (approved therapy or practice guideline in other tumor type(s), evidence from multiple small, published studies, or based on availability of investigational therapies) Level D evidence (case reports or preclinical studies).
Tier III Variants of uncertain significance	
Tier IV Benign or likely benign variant	

DISCLAIMER

- As a standard of care, our case selection criteria for NGS run is ≥20% tumor content. The run was performed in this case after receiving informed consent from the clinician.
- Interpretation of variants in this report is performed to the best knowledge of the laboratory based on the information available at the time of reporting. The classification of variants can change over time and MolQ cannot be held responsible for this. Please feel free to contact MolQ Laboratory (contact@molq.in) in the future to determine if there have been any changes in the classification of any variations. Re-analysis of variants in previously issued reports in light of new evidence

- is not routinely performed, but may be considered upon request.
- The tests are carried out in the lab with the presumption that the specimen belongs to the patient named or identified in the bill/test request form.
 - The test results relate specifically to the sample received in the lab and are presumed to have been generated and transported per specific instructions given by the physicians/laboratory.
 - Some tests are referred to other laboratories to provide a wider test menu to the customer.
 - The report shall be generated within turnaround time (TAT), however, such TAT may vary depending upon the complexity of test(s) requested. MolQ Laboratory under no circumstances will be liable for any delay beyond afore mentioned TAT.
 - It is hereby clarified that the report(s) generated from the test(s) do not provide any diagnosis or opinion or recommends any cure in any manner. MolQ Laboratory hereby recommends the patient and/or the guardians of the patients, as the case may be, to take assistance of the clinician or a certified physician or doctor, to interpret the report(s) thus generated. MolQ Laboratory hereby disclaims all liability arising in connection with the report(s).
 - In a very few cases genetic test may not show the correct results, e.g. because of the quality of the material provided to MolQ Laboratory. In case where any test provided by MolQ Laboratory fails for unforeseeable or unknown reasons that cannot be influenced by MolQ Laboratory in advance, MolQ Laboratory shall not be responsible for the incomplete, potentially misleading or even wrong result of any testing if such could not be recognized by MolQ Laboratory in advance. MolQ shall in no event be liable for accidental damage, loss, or destruction of specimen, which is not attributable to any direct and mala fide act or omission

LIMITATIONS

- This Report was generated using the materials and methods described above, which required the use of various reagents, protocols, instruments, software, databases, and other items, some of which were provided or made accessible by third parties. A defect or malfunction in any such reagents, protocols, instruments, software, databases, and/or other items may compromise the quality or accuracy of the Report.
- The Report has been created based on, or incorporates references to, various scientific manuscripts, references, and other sources of information, including without limitation manuscripts, references, and other sources of information that were prepared by third parties that describe correlations between certain genetic mutations and particular diseases (and/or certain therapeutics that may be useful in ameliorating the effects of such diseases). Such information and correlations are subject to change over time in response to future scientific and medical findings. MolQ Laboratory makes no representation or warranty of any kind, expressed or implied, regarding the accuracy of the information provided by or contained in such manuscripts, references, and other sources of information. If any of the information provided by or contained in such manuscripts, references, and other sources is later determined to be inaccurate, the accuracy and quality of the Report may be adversely impacted. MolQ Laboratory is not obligated to notify you of any impact that future scientific or medical research findings may have on the Report.
- The Report must always be interpreted and considered within the clinical context, and a physician should always consider the Report along with all other pertinent information and data that a physician would prudently consider prior to providing a diagnosis to a patient or developing and implementing a plan of care for a patient. The Report should never be considered or relied upon alone in making any diagnosis or prognosis. The manifestation of many diseases is caused by more than one gene variant, a single gene variant may be relevant to more than one disease, and certain relevant gene variants may not have been considered in the Report. In addition, many diseases are caused or influenced by modifier genes, epigenetic factors, environmental factors, and other variables that are not addressed by the Report (or that are otherwise unknown). This Report is based on a next generation sequencing assay which does not distinguish between somatic and germline variants. If a germline variant is in question, further testing may be recommended. As such, the relevance of the Report should be interpreted in the context of a patient's clinical manifestations. The Report provided by MolQ Laboratory is provided on an "AS IS" basis. MolQ Laboratory makes no representation or warranty of any kind, expressed or implied, regarding the Report. In no event shall MolQ Laboratory be liable for any actual damages, indirect damages, and/or special or consequential damages arising out of or in any way connected with the Report, your use of the Report, your reliance on the Report, or any defect or inaccurate information included within the Report. Medical knowledge annotation is constantly updated and reflects the current knowledge at the time.
- The test performance characteristics were determined and report generated by the reference laboratory as required by the CLIA 1988 regulations. The Report, and the tests used to generate the Report, have not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. The test results have been scientifically shown to be clinically useful. The reference laboratory is under the process of CAP/CLIA as well as ISO-15189 certifications.
- Negative findings can be due either to low tumor cellularity (TC < 20%) or the region not being targeted by this assay. The

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optimal TC cutoffs for the assay for the detection of different variant types were concluded from the validation to be 20% for single nucleotide variants (SNV) and insertions/deletions (INDEL), 20% for microsatellite analysis (MSI). Low TC may negatively impact the technical performance of this assay and could result in false- negative findings. Submission of another specimen with higher tumor cellularity is recommended.

- This test is designed currently to identify exclusively the single nucleotide variants (SNVs), multi-nucleotide variants (MNVs), and short insertions and deletions (Indels). The test will not be able to identify copy number variations/gene amplifications, alternately spliced variants, intronic variants, gene fusions, or genomic-level mutations such as microsatellite instability or tumor mutation burden.

APPENDIX 2: GENE LIST

SNVs and InDels

Gene	Gene	Gene	Gene	Gene	Gene	Gene
<i>AKT1</i>	<i>ALK</i>	<i>BRAF</i>	<i>CDK4</i>	<i>CDKN2A</i>	<i>CTNNB1</i>	<i>DDR2</i>
<i>DICER1</i>	<i>EGFR</i>	<i>ERBB2</i>	<i>ERBB4</i>	<i>FBXW7</i>	<i>FGFR1</i>	<i>FGFR2</i>
<i>FGFR3</i>	<i>FOXL2</i>	<i>GNA11</i>	<i>GNAQ</i>	<i>GNAS</i>	<i>H3F3A</i>	<i>H3F3B</i>
<i>IST1H3B</i>	<i>HRAS</i>	<i>IDH1</i>	<i>IDH2</i>	<i>KIT</i>	<i>KRAS</i>	<i>MAP2K1</i>
<i>MET</i>	<i>MYOD1</i>	<i>NRAS</i>	<i>PDGFRA</i>	<i>PIK3CA</i>	<i>PTPN11</i>	<i>RAC1</i>
<i>RAF1</i>	<i>RET</i>	<i>ROS1</i>	<i>SF3B1</i>	<i>SMAD4</i>	<i>TERT</i>	<i>TP53</i>

FUSION

Gene	Gene	Gene	Gene
<i>ACPP-FGFR1</i>	<i>AGAP3-BRAF</i>	<i>AGK-BRAF</i>	<i>AHCYL1-FGFR2</i>
<i>ATIC-ALK</i>	<i>C11orf95-RELA</i>	<i>C19orf12-AKT2</i>	<i>CCDC6-BRAF</i>
<i>CCDC6-RET</i>	<i>CD74-ROS1</i>	<i>CDK5RAP2-BRAF</i>	<i>CEP112-FGFR2</i>
<i>CEP85L-ROS1</i>	<i>CLTC-ALK</i>	<i>CTNNA3-FGFR2</i>	<i>CUL1-BRAF</i>
<i>DCTN1-ALK</i>	<i>EGFR-RAD51</i>	<i>EML4-ALK</i>	<i>EML4-NTRK3</i>
<i>ERBB2-Clorf87</i>	<i>ERBB2-GRB7</i>	<i>ETV6-NTRK3</i>	<i>EZR-ROS1</i>
<i>FAM131B-BRAF</i>	<i>FGFR2-BICC1</i>	<i>FGFR2-CCSER1</i>	<i>FGFR2-KCNH7</i>
<i>FGFR2-KIAA1217</i>	<i>FGFR2-KIF14</i>	<i>FGFR2-LAMC1</i>	<i>FGFR2-NOL4</i>
<i>FGFR2-NRAP</i>	<i>FGFR2-NRBF2</i>	<i>FGFR2-PPP1R21</i>	<i>FGFR2-RABGAP1L</i>
<i>FGFR2-RASAL2</i>	<i>FGFR2-ROCK1</i>	<i>FGFR2-SHC2</i>	<i>FGFR2-TFEC</i>
<i>FGFR2-VCL</i>	<i>FGFR3-JAKMIP1</i>	<i>FGFR3-TACC3</i>	<i>FGFR3-TNIP2</i>
<i>FN1-ALK</i>	<i>GCC2-ALK</i>	<i>GIPC2-BRAF</i>	<i>GOLGB1-ROS1</i>
<i>GON4L-NTRK1</i>	<i>GOPC-ROS1</i>	<i>KHDRBS1-NTRK3</i>	<i>KIAA1549-BRAF</i>
<i>KIF5B-ALK</i>	<i>KIF5B-RET</i>	<i>LMNA-NTRK1</i>	<i>MKRN1-BRAF</i>
<i>MYH14-BRAF</i>	<i>MYOSA-NTRK3</i>	<i>NCOA4-RET</i>	<i>OSBPL9-BRAF</i>
<i>PAX8-PPARG</i>	<i>PDCD10-RET</i>	<i>PHTF2-BRAF</i>	<i>PJA2BRAF</i>
<i>PLAGL1-ROS1</i>	<i>PRKAR1A-RET</i>	<i>PRKAR1B-BRAF</i>	<i>PRKAR2B-BRAF</i>
<i>RAB11FIP1-FGFR1</i>	<i>RANBP2-ALK</i>	<i>RASSF4-RET</i>	<i>RBM33-BRAF</i>
<i>SCRIB-BRAF</i>	<i>SDC4-ROS1</i>	<i>SLC16A10-ROS1</i>	<i>SLC34A2-ROS1</i>
<i>SLC4A4-ROS1</i>	<i>SND1-BRAF</i>	<i>TFG-RET</i>	<i>TPM3-ALK</i>
<i>TPM3-NTRK1</i>	<i>TPM3-ROS1</i>	<i>TSHZ3-AKT2</i>	<i>VCL-ALK</i>
<i>ZCCHC8-ROS1</i>	<i>ZNF207-BRAF</i>		