Client

Gurugram

Pathkind Diagnostics Pvt. Ltd.

Plot No. 55-56, Udhyog Vihar Ph-IV, Gurugram - 122015

Processed By

Pathkind Diagnostics Pvt. Ltd.

Plot No. 55-56, Udhyog Vihar Ph-IV, Gurugram - 122015

Name	:	**Mr. PL126**	Billing Date	:	07/07/2023 12:29:27
Age	:	45 Yrs	Sample Collected on	:	10/07/2023 10:01:31
Sex	:	Male	Sample Received on	:	10/07/2023 11:02:13
P. ID No.	:	P1000100012878	Report Released on	:	15/07/2023 16:26:50
Accession No	:	**10002304934**	Barcode No.	:	10002304934
Referring Doctor	:	Self			
Referred By	:		Ref no.	:	

Report Status - Final

Test Name	Result	Biological Ref. Interval	Unit

MOLECULAR DIAGNOSTICS

Lung Cancer Mutation Panel- 2 (EGFR, KRAS & ALK)

EGFR Mutation Detection By ARMS-PCR

Sample: Paraffin Blocks

EGFR Mutation Analysis by ARMS PCR

Specimen: 1 P Block- Test performed on: -
Methodology: ARMS-PCR
Mutations Screened in: Exons 18 to 21 of *EGFR* gene (Ref Seq NC_000007.14)

Exon	Mutation(s) Studied	Effect of Mutation/ Variant	Mutation Status
18	G719X	Activating/ Pathogenic	Not Detected
19	Deletion Mutations	Activating/ Pathogenic	Not Detected
20	S768I	Activating/ Pathogenic	Not Detected
	Insertion Mutations	De-sensitizing	Not Detected
	T790M	De-sensitizing	Not Detected
21	L858R	Activating/ Pathogenic	Not Detected
	L861Q	Activating/ Pathogenic	Not Detected

Result & Interpretation:
Wild type/ Normal gene sequences were observed in exons 18, 19,20 & 21 of *EGFR* gene. Absence of *EGFR* mutations denotes that the patient is unlikely to benefit from anti-EGFR TKI therapy.

Recommendation:
Further screening of *ALK, ROS1, PD-L1, BRAF, MET, RET and NTRK* mutations is recommended to identify the most appropriate first/second line treatment for the patient.

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Clinical Information:

Non-small-cell lung cancer (NSCLC) accounts for 75-85% of all lung cancers. In lung adenocarcinoma, a number of targetable major pathways have been identified, such as *EGFR, PI3K/AKT/mTOR, RAS-MAPK*, and *NTRK/ROS1* pathways. Many drugs targeting these pathways have been developed and show clinical benefits.

1. *EGFR* is a receptor tyrosine kinase glycoprotein found on the surface of epithelial cells and is a genetic driver in many carcinomas. Mutations in the *EGFR* gene that lead to activation/overexpression of the protein have been reported in a number of different cancers, including NSCLC, head and neck, esophagus, stomach, colon, liver, breast, ovary, cervix, endometrium, bladder and brain .
2. Pathogenic/ activating mutations of *EGFR* have been reported in 10-15% of Caucasians and 30-40% of Asian patients of NSCLC adenocarcinoma. The majority of mutations are located in the tyrosine kinase domain coded by exons 18- 21 of the gene.
3. The most common and best-characterized mutations of *EGFR* are small in-frame deletions in exon 19 , and L858R, a point mutation in exon 21. These two mutations account for > 80% of all *EGFR* alterations in lung cancer and are more frequent in never-smokers, Asian, and female lung cancer patients.
4. In-frame insertions within exon 20 of *EGFR* are the third most common type of mutation found in NSCLC, representing 4-10% of all *EGFR* mutations in NSCLC. These insertions affect amino acids 762-775 and are typically associated with resistance to radiation therapy or to *EGFR* TKIs.
5. T790M, a point mutation in exon 20, usually arises in response to and as a mechanism of resistance to first and second generation TKI therapy.
6. Many rare alterations in *EGFR* (exon 19 insertions, L861Q, S768I, G719X), which cumulatively account for 7-10% are also associated with responsiveness to *EGFR* TKI therapy.
7. Patients that test positive for the presence of activating mutations of *EGFR*, benefit from treatment with anti-EGFR TKIs such as Afatinib, Osimertinib, Gefitinib, Erlotinib.

Methodology, Test Attributes and Limitations:

The Kit used is an in vitro diagnostic test for the detection of 29 somatic mutations in the *EGFR* gene and provides a qualitative assessment of the mutation status. This assay is based upon a combination of ARMS and Scorpion technology to detect mutations on a Real Time platform. The analytical sensitivity of the test allows detection of the mutation when the mutant clone comprises at least 1-5% of the total genomic DNA.

The kit detects 19 deletions in exon 19 but does not distinguish between them. G719X positive denotes the presence of G719S, G719A, or G719C, but does not distinguish between them. Similarly, though the kit detects 3 insertions in exon 20 but it does not distinguish between them.

ASCO guidelines recommend that the tissue sample should be fixed in 10% neutral buffered formalin for 6-72hrs, depending upon the size of the tissue. The volume of formalin used should be 10 times the volume of the specimen. Decalcification solutions with strong acids should not be used, as these lead to degradation of the DNA and result in failure of PCR assay. PCR is a highly sensitive technique; reasons for apparently contradictory results may be due to improper quality control during sample collection and fixation, high degree of necrosis, mucin content and/or presence of PCR inhibitors.

References:

1. Min Yuan, Li-Li Huang1, Jian-Hua Chen1, Jie Wu et al. The emerging treatment landscape of targeted therapy in nonsmall-cell lung cancer. Signal Transduction and Targeted Therapy (2019) 4:61
2. Emily A. Barber and Karen L. Reckamp. Best Initial Treatment Strategies for *EGFR*-Mutant Lung Cancer. AJHO. 2016;12(12):4-7
3. Gristina V, Malapelle U, Galvano A, Pisapia P, et al. The significance of epidermal growth factor receptor uncommon mutations in non-small cell lung cancer: A systematic review and critical appraisal. Cancer Treatment Reviews, 21 Feb 2020, 85

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KRAS Mutation Detection

Sample: Paraffin Blocks
Method: ARMS-PCR

KRAS Mutation Analysis (Qualitative)

Specimen: 1 P Block- Test performed on: -
Methodology: EGFR & KRAS by ARMS-PCR
Mutations Screened in: Exon 2, 3 & 4 of *KRAS* gene (NCBI Refseq NM_033360)

Exon	Mutations Screened	Mutation Status	Variant Effect
2	G12A, G12D, G12R, G12V, G13D	No Mutation Detected	###
	G12C, G12S	No Mutation Detected	###
3	Q61H, Q61L, Q61R, A59E, A59G, A59T	No Mutation Detected	###
4	K117N, K117R, K117E	No Mutation Detected	###
	A146T, A146P, A146V	No Mutation Detected	###

Result & Interpretation:
No Pathogenic mutation was observed in *KRAS* gene, in the sample provided
Further screening of *NRAS* and *BRAF* mutations is recommended to evaluate whether the patient would derive optimal benefit from anti-EGFR therapy

Clinical Information:

1. *KRAS* gene encodes a small GTPase that plays a key role in transducing signals from the epidermal growth factor receptor (*EGFR*) to downstream effectors. *KRAS* mutations have been commonly found in several types of human malignancies, such as metastatic colorectal cancer (mCRC), lung adenocarcinoma and thyroid cancer.
2. The most common mutations are found in codons 12, 13 and 61. Several studies have demonstrated that tumors carrying any of these mutant forms of the *KRAS* gene are less likely to respond to anti-EGFR antibody therapy such as Cetuximab or Panitumumab.
3. As per NCCN guidelines, all patients of metastatic colorectal carcinoma should have tumor tissue genotyped for *KRAS, NRAS* and *BRAF* mutations

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Colorectal Cancer:
Acquiring *KRAS* mutation after *APC* gene mutation in colon epithelial cells contributes to progression of adenoma to carcinoma. 40-50% of CRC patients have constitutively activating *KRAS* mutations, located mainly in codons 12, 13, and 61, that are resistant to EGFR therapies. *KRAS* mutations are associated with overall poor patient prognosis in colorectal cancer. Patients with *KRAS* mutation are poor responders to anti-EGFR therapy (Panitumumab and Cetuximab) in metastatic colon cancer. Hence, testing for *KRAS* gene mutations prior to using anti-EGFR antibodies for treatment of CRC is recommended.

Lung Cancer:
In NSCLC, one of the most frequently reported alterations in the EGFR-signaling pathway is the presence of a mutation in the proto-oncogene *KRAS*. *KRAS* is recruited by ligand-bound (active) EGFR to initiate the signaling cascade induced by the RAS/MAPK pathway. Because mutant *KRAS* constitutively activates the RAS/MAPK pathway downstream of *EGFR*, agents that prevent ligand-binding to EGFR do not appear to have any meaningful inhibitor activity on cell proliferation in the presence of mutant *KRAS*. Current data suggest that the efficacy of EGFR-targeted therapies in NSCLC is confined to patients with tumors lacking *KRAS* mutations. As a result, the mutation status of *KRAS* can be a useful marker by which patients are selected for EGFR-targeted therapy.

Methodology, Test Attributes and Limitations:
The Mutation Screening Panel is a polymerase chain reaction (PCR)-based assay that uses allele-specific primers in a multiplex reaction to identify the presence of the mutations in a total of 5 reactions per sample. Exons 2, 3 and 4 are screened for the presence of Mutations as shown in the Table. The test identifies but does not distinguish between mutations within each group. The analytical sensitivity of the test allows detection of the mutation when the mutant clone comprises at least 5% of the total genomic DNA.
Tumor sample must be fixed under appropriate conditions (10% Neutral buffered formalin for 24-72hrs) to ensure preservation of amplifiable quality DNA. PCR is a highly sensitive technique; reasons for apparently contradictory results may be due to improper quality control during sample collection and fixation, high degree of necrosis, mucin content and/or presence of PCR inhibitors.

References:

1. Eberhard DA, Johnson BE, Amler LC, et al: Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol 2005;23(25):5900-5909
2. Ladanyi M, Pao W: Lung adenocarcinoma: guiding EGFR-targeted therapy and beyond. Mod Pathol 2008;21 Suppl 2:S16-S22
3. Lam DC: Clinical testing for Molecular targets for personalized treatment in lung cancer. Respirology 2013 Feb;18(2):233-237
4. Tormod Kyrre Guren, Maria Thomsen, Elin H Kure, Halfdan Sorbye, Bengt Glimelius et al. Cetuximab in treatment of metastatic colorectal cancer: final survival analyses and extended RAS data from the NORDIC-VII study. Br J Cancer. 2017 May 9; 116(10): 1271-1278.

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CYTOGENETICS

ALK Gene Rearrangement By FISH

Sample: Paraffin Blocks
Method: FISH

ALK Gene Rearrangement FISH Assay

Method: ALK by FISH
Time of Fixation: Not provided
Specimen: - P Blcks recd- --. Test was performed on --
Probe Used: ZytoLight SPEC *ALK* Dual Color Breakapart Probe

Average Signals / Cell				
ALK 3' 2p23 Orange	*ALK* 5' 2p23 Green	*ALK* fusion Yellow	No. of cells which exhibit this pattern	Analysis & Interpretation
0	0	2	100	*ALK* gene NOT Rearranged
0	1	1	0	*ALK* gene NOT Rearranged
1	1	1	0	*ALK* gene REARRANGED
1	0	1	0	*ALK* gene REARRANGED

Nuclei Scored: 100

Result:
nuc ish(5'*ALK*,3'*ALK*)×2(5'*ALK* con 3'*ALK*×2)[50]
The sample is NEGATIVE for *ALK* gene rearrangement

Clinical Information

1. *ALK* gene encodes a transmembrane receptor tyrosine kinase. Molecular alterations leading to heightened *ALK* activation have been implicated in several cancers including non-Hodgkin's lymphoma, rhabdomyosarcomas, renal cell carcinoma, thyroid cancer, neuroblastoma, and NSCLC.

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2. *ALK*-rearranged NSCLC comprises 2% to 5% of all NSCLC cases. Patients who harbor *ALK*-activated NSCLC tend to be light or never smokers, younger, of male gender and usually have tumors with adenocarcinoma signet-ring cell type histology.
3. In *ALK*-activated NSCLC, a gene inversion leads to fusion of N-terminal portion of *EML4* gene with the intracellular domain of the *ALK* tyrosine kinase and causes constitutive activation of downstream pathways. Less commonly, fusion with other partners, including *KIF5B*, *KLC1* and *TPR* have been reported.
4. Patients that show *ALK* gene rearrangement are candidates to be treated with anti-ALK therapy with Alectinib, Crizotinib, Ceritinib etc. This treatment module has been shown to achieve significant improvement in clinical response and overall survival.

Interpretation and Scoring
The evaluation of *ALK* gene rearrangement is based on counting of the Breakapart signals in the nuclei of tumor cells. In a normal interphase nucleus, two or more Yellow fusion signals are expected. A tumor cell affected by *ALK* gene rearrangement usually shows one of the following patterns-

1. One Yellow fusion signal, one Orange signal, and a separate Green signal indicates
2. One Yellow fusion signal, and one or more isolated Orange signals with loss of Green signal (indicating deletion of *ALK* 5' region).

The interpretation and scoring are done as per recommended guidelines -
A sample is considered Negative if <5 cells out of 50 show a rearranged pattern; and considered positive if > 25 cells out of 50 exhibit a rearranged pattern. A sample is considered equivocal if 5- 25 cells out of 50 show *ALK* rearrangement. In such cases, a second reader should evaluate the slide. In the final analysis, if < 15% cells out of 100 show *ALK* gene rearrangement then the sample is considered Negative and it is considered Positive if > 15% show the Rearrangement.

Methodology, Test attributes and Limitations:
The evaluation of *ALK* gene rearrangement is based on counting of the Breakapart signals in the nuclei of tumor cells. The interpretation and scoring are done as described above.
ASCO guidelines recommend that the tissue sample should be fixed in 10% neutral buffered formalin for 6-72hrs, depending upon the size of the tissue. The volume of formalin used should be 10 times the volume of the specimen. Decalcification solutions with strong acids should not be used, as these lead to degradation of the DNA and result in failure of FISH assay. Kindly note that eosin stain has a strong auto-fluorescence and interferes with reading of the FISH signals; hence the biopsy samples should not be stained with eosin during processing. It is suggested that small tissue biopsies may be stained with Tomato Red dye instead of Eosin to allow sample tracking during tissue processing.
The performance characteristics of this kit have been standardized for FFPE tissues. Other types of specimen or fixatives should not be used.

References:

1. Tri Le and David E. Gerber. *ALK* mutation and inhibition in lung cancer. Semin Cancer Biol. 2017 Feb; 42: 81-88.
2. Grande E, Bolos MV, Arriola E. Targeting oncogenic *ALK*: a promising strategy for cancer treatment. Mol Cancer Ther. 2011;10(4):569-79
3. Vittoria Martin, Barbara Bernasconi, Elisabetta Merlo, Piera Balzarini et al. *ALK* testing in lung adenocarcinoma: technical aspects to improve FISH evaluation in daily practice. J Thorac Oncol. 2015 Apr;10(4):595-602.

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**** End of Report****

Dr. Sarjana Dutt
PhD
Director Molecular Biology & Cytogenetics

Dr. Avijit Guha
Scientist (Molecular)
PhD (Molecular)

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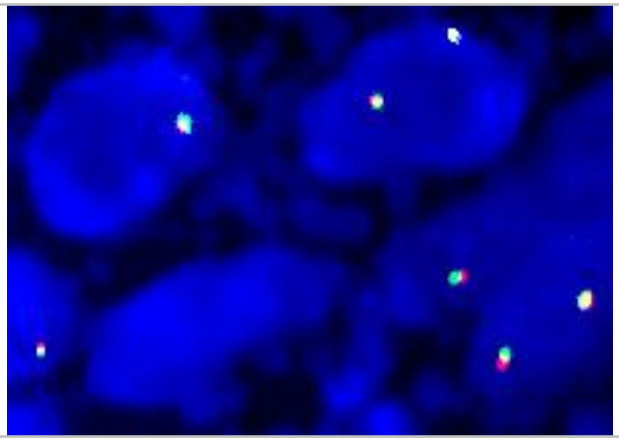
Patient ID:	**CG23-SF-53**
Patient Name:	**Mr. Dummy**
Gender:	**Male**
Specimen:	**Tissue, Solid**
Clinical Indication:	**? ALK**
Method:	**Fluorescence in situ hybridization (FISH) was performed on the nuclei for rearrangement of ALK gene.**
Probe details:	**ZytoLight SPEC ALK Dual Color, Break Apart Probe**

FISH REPORT

Slide Label : **ALK**



Block No. : **--**

Results **:** nuc ish(5'ALK,3'ALK)x2(5'ALK con 3'ALK)x2[100]