

Client
Gurugram
 Pathkind Diagnostics Pvt. Ltd.
 Plot No. 55-56, Udhog Vihar Ph-IV, Gurugram - 122015

Processed By
Pathkind Diagnostics Pvt. Ltd.
 Plot No. 55-56, Udhog Vihar Ph-IV, Gurugram - 122015

Name : Mr. PL127	Billing Date : 07/07/2023 12:29:29
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. : P1000100012879	Report Released on : 18/07/2023 12:05:36
Accession No : 10002304935	Barcode No. : 10002304935
Referring Doctor : Self	
Referred By :	Ref no. :

Report Status - Final

Test Name	Result	Biological Ref. Interval	Unit
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MOLECULAR DIAGNOSTICS

Lung Cancer Panel- 3
(EGFR, ALK & ROS1)

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.

- 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 .
- 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.
- 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.
- 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.
- 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.
- 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.
- 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:

- 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
- Emily A. Barber and Karen L. Reckamp. Best Initial Treatment Strategies for *EGFR*-Mutant Lung Cancer. *AJHO*. 2016;12(12):4-7
- 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

Note: This Test has been validated and its performance evaluated at Pathkind Diagnostics Pvt. Ltd.
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CYTOGENETICS

ROS1 Gene Rearrangement By FISH

Sample: Paraffin Blocks
 Method: FISH

ROS1 Gene Rearrangement FISH Assay

Method: ROS1 by FISH
 Time of Fixation: Not provided
 Specimen: - P Blcks recd- -- Test was performed on --
 Probe Used: ZytoLight SPEC ROS1 Dual Color Breakapart Probe

Average Signals/ cell			No. of cells which exhibit this pattern	Analysis & Interpretation
ROS1 3' 6q22.1 Green	ROS1 5' 6q22.1 Orange	ROS1 fusion Yellow		
0	0	2	100	ROS1 gene NOT Rearranged
0	1	1	0	ROS1 gene NOT Rearranged
1	1	1	0	ROS1 gene REARRANGED
1	0	1	0	ROS1 gene REARRANGED

Nuclei Scored: 100

Result:
 nuc ish(5' ROS1,3' ROS1)x2(5' ROS1 con 3' ROS1)x2[100]
 The sample is NEGATIVE for ROS1 gene rearrangement

Clinical Information

1. ROS1 gene is located on 6q22.1 and encodes a receptor tyrosine kinase. Translocations affecting ROS1 have been detected in glioblastoma, cholangiocarcinoma and non-small cell lung cancer (NSCLC).
2. ROS1-rearranged NSCLC comprises 1-2% of all NSCLC adenocarcinoma cases. Patients who harbor ROS1-activated NSCLC tend to be

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- light or never smokers, younger and usually have tumors with solid growth and mucinous/cirbriform features.
- In NSCLC, several *ROS1* translocation partners have been detected all of which result in the fusion of variably truncated forms of eg. *TPM3*, *SDC4*, *SLC34A2*, *CD74*, *EZR* or *LRIG3* gene fused to the kinase domain of *ROS1*.
 - Patients that show *ROS1* gene rearrangement are candidates to be treated with anti- *ROS1* therapy with Crizotinib, Ceritinib or Entrectinib. etc. This treatment module has been shown to achieve significant improvement in clinical response and overall survival.

Interpretation and Scoring

The evaluation of *ROS1* 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 *ROS1* gene rearrangement usually shows one of the following patterns-

- One Yellow fusion signal, one Orange signal, and a separate Green signal indicates
- One Yellow fusion signal, and one or more isolated Green signals with loss of Orange signal (indicating deletion of *ROS1* 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 *ROS1* rearrangement. In such cases, a second reader should evaluate the slide. In the final analysis, if < 15% cells out of 100 show *ROS1* 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 *ROS1* 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:

- Ling Shan, Fang Lian, Lei Guo, Tian Qiu et al. Detection of *ROS1* Gene Rearrangement in Lung Adenocarcinoma: Comparison of IHC, FISH and Real-Time RT-PCR. PLOSOne.2015 Mar; 1-8.
- Toni-Maree Rogers, Prudence A. Russell, Gavin Wright, Zoe Wainer et al. Comparison of Methods in the Detection of *ALK* and *ROS1* Rearrangements in Lung Cancer. J Thorac Oncol. 2015;10: 611-618.
- Sehhoon Park, Beung-Chul Ahn, Sung Won Lim, Jong-Mu Sun et al. Characteristics and Outcome of *ROS1*-Positive Non-Small Cell Lung Cancer Patients in Routine Clinical Practice. J Thorac Oncol. 2018 Sep;13(9):1373-1382.

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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			No. of cells which exhibit this pattern	Analysis & Interpretation
ALK 3' 2p23 Orange	ALK 5' 2p23 Green	ALK fusion Yellow		
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.
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

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ALK tyrosine kinase and causes constitutive activation of downstream pathways. Less commonly, fusion with other partners, including *KIF5B*, *KLC1* and *TPR* have been reported.

- 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-

- One Yellow fusion signal, one Orange signal, and a separate Green signal indicates
- 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:

- Tri Le and David E. Gerber. *ALK* mutation and inhibition in lung cancer. Semin Cancer Biol. 2017 Feb; 42: 81-88.
- Grande E, Bolos MV, Arriola E. Targeting oncogenic *ALK*: a promising strategy for cancer treatment. Mol Cancer Ther. 2011;10(4):569-79
- 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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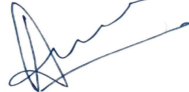
Test Name	Result	Biological Ref. Interval	Unit
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**** End of Report****



Dr. Sarjana Dutt

PhD
Director Molecular Biology & Cytogenetics



Dr. Avijit Guha

Scientist (Molecular)
PhD (Molecular)

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PATHKIND DIAGNOSTICS PVT. LTD.

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Customer Care: 75000-75111

10002304935 Mr. PL127

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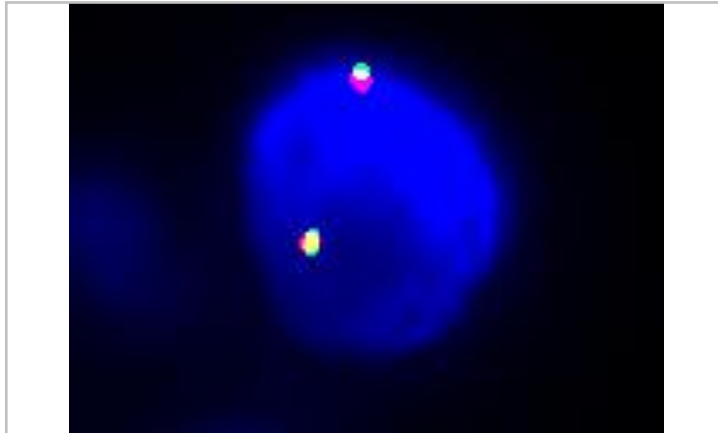
जांच सही तो इलाज सही



Patient ID: CG23-SF-91
Patient Name: Dummy
Gender: Male
Specimen: Tissue, Solid
Clinical Indication: ? ROS1
Method: Fluorescence in situ hybridization (FISH) was performed on the nuclei for rearrangement of ROS1 gene.
Probe details: ZytoLight SPEC ROS1 Dual Color, Break Apart Rearrangement Probe

FISH REPORT

Slide Label : ROS1



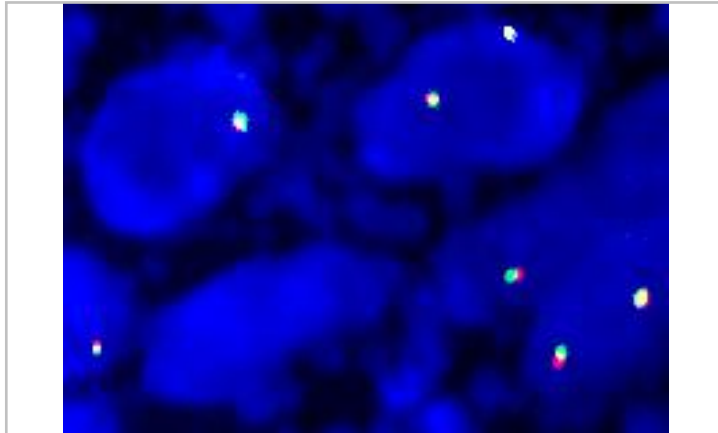
Block No. : --

Results : nuc ish(5'ROS1,3'ROS1)x2(5'ROS1 con 3'ROS1)x2[100]

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]