ANAPLASTIC LYMPHOMA KINASE (ALK) GENE REARRANGEMENT DETECTION USING FLUORESCENE IN-SITU HYBRIDIZATION (FISH) IN LUNG CANCER PROGNOSTICATION

Alok Singh, A.N. Srivastava, Pooja Singh.
Department of Pathology
Era's Lucknow Medical College and Hospital, Lucknow, U.P., India.

Received : August 2016
Accepted : October 2016

Address for correspondence
Dr. Anand Narain Srivastava
Director Research & Consulting Faculty, Department of Pathology, Era's Lucknow Medical College and Hospital, Lucknow, U.P., India.
Email: ans4csmmu@gmail.com
Phone no:+91-9415409779

ABSTRACT

Lung cancer is a leading cause of cancer related death worldwide. It is increasing at a very fast rate in both men and women. Some significant mutations occurring at molecular level in lung adenocarcinoma, like ALK, EGFR, KRAS, MET, and, ALK (anaplastic lymphoma kinase) gene mutations for an ALK encoded transmembrane receptor tyrosine kinase domain and subsequently participating in the progression of Non-Small Cell Lung Adenocarcinoma (NSCLC). Some fusion partner genes involved in this process are EML-4, KLC1, *KIF5B* and *TFG*. The ALK-EML-4 rearrangement is the second most common oncogenic mutation in the non-small cell lung adenocarcinoma. There is 3-7% ALK mutation occurring in early or never-smokers in accompanying NSCLC. The NSCLC with ALK gene mutation generally do not have EGFR or KRAS gene mutation which are also molecular markers, which get mutated in cancer. For the detection of ALK mutation in NSCLC, different types of techniques like Fluorescence in situ Hybridization (FISH), Immunohistochemistry (IHC) and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) are being used. On the basis of sensitivity and specificity, FISH is gold standard in detecting the mutation when compared with other methodologies like IHC and RT- PCR. However in the Indian setting, FISH is more expensive and hence not available everywhere. In this review the efficacy of these different techniques in detecting ALK mutation and the detailed interpretation of results obtained with FISH has been discussed. For the treatment of ALK/MET mutated NSCLC patients an orally administered drug, crizotinib drug (tyrosine kinase inhibitor) has been approved by Food and Drug Administration (FDA) of United States. Highly sensitive and specific techniques are used for the detection of ALK gene mutation in NSCLC patients which have to be given for crizotinib treatment.

Keyword: Anaplastic Lymphoma Kinase; Non-Small Cell Lung Carcinoma; Polysomy.

INTRODUCTION

Lung Cancer

Nowadays lung cancer is one of the most common causes of cancer related deaths worldwide in both men and women, nearly more than one million deaths occur per year due to this dreaded disease.1 According to America cancer society (2016), death rate due to lung cancer is more than breast cancer, prostate cancer and colorectal cancer in the world.2 The 5 years survival in cases of lung cancer is only 15%. Hence necessity for searching more targeted and effective therapeutic drug for its treatment is the need of the hour.3 On the basis of cell shape and size the lung cancer is divided in two types, Small cell carcinoma (SCLC) and Non-small cell lung carcinoma (NSCLC).There are various causes of lung cancer viz tobacco smoking, (cigarette smoking)4, followed by exposure to asbestos, arsenic, chloromethyle, and radiation. These exposures cause genetic mutation in the patients leading to the development lung cancer.5,6 Some vegetables and fruits help in the protection from lung cancer risk, according to Sankaranarayanan *et al.(1994)* the green vegetable and banana reduces the risk of lung cancer. Onion and Pumpkin also reduce the risk of lung cancer. On the contrary, the animal fat and dietary cholesterol increase the lung cancer risk.7

Small Cell Lung Carcinoma (SCLC)

The metastasis rate is very high with this cancer and it contributes to about 15% of all types of lung cancer.2 According to *Sorensen M. (2010)* tobacco smoking is the main cause of SCLC.8 In SCLC, gene mutation has been found to occur in C-myc, L-myc, N-myc, C-raf (oncogene) Rb and p53 (tumour suppressor gene). Molecular studies have shown that the lung cancer is caused by genetic abnormalities due to deletion or translocation of the DNA sequences, which play the main role in cancer development. The genes where

mutation causes lung cancer may be proto-oncogene or tumour suppressor gene. After mutation, the proto-oncogene is converted into oncogene which causes carcinogenesis. The tumour suppressor gene suppresses the formation of tumour but after mutation it is converted into tumour promoting gene that may help in develop of the cancer.9,10

Non-Small Cell Lung Carcinoma (NSCLC)

NSCLC is a well-known type of lung cancer and constitute nearly 85% in all type of lung carcinoma. NSCLC metastasizes slower than small cell lung carcinoma and its diagnosis is very difficult in comparison to SCLC in early stage. Approximately 70% of cases of NSCLC are diagnosed at advanced stage.2 The gene which are associated with NSCLC are EGFR, ALK, KRAS, HER2, PI3KCA, MAP2K1, BRAF, MET3 N-ras, H-ras, C-raf, p16, Rb.9,10

Lung Cancer in Indian Scenario

Ito. Y *et al*. (2007), reported that the 5 year survival of NSCLC in India was 12-14% and in case of SCLC was only 2-3%.11 Ferguson MK. et al. (2000) have found better survival with squamous cell carcinoma in comparison to other types of lung carcinoma.12 Radzikowska E. et al. in 2003 reported that women have better survival rate in comparison to men. However, some workers have found an opposite trend.13

Anaplastic Lymphoma Kinase Gene (ALK)

The ALK gene occurs on short arm (p) of chromosome number two (2p23),14 which codes a transmembrane signaling receptor protein (receptor tyrosine kinase), a member of the insulin like tyrosine receptor kinase superfamily.15 Generally the expression of receptor tyrosine kinase is not noticed in lung cancer.16 In 2007, Soda *et al.* revealed a mutation causing the fusion between two different genes- ALK and EML-4 (Echinoderm microtubule protein like four). EML-4 is another mutated gene, also present on short arm (p) of chromosome number two (2p21), present upstream of ALK gene and its mutation culminates into different types of Non-Small Cell Lung Carcinoma.17 EML-4 encoded protein may aid in assembly of microtubules.19 Fusion between ALK and EML-4 results in the constitutive kinase activity of ALK receptor tyrosine kinase that induces the abnormal proliferation of cells which cause cancer like lung carcinoma.18 According to Soda *et al. (2007)* signaling part of ALK receptor tyrosine kinase is fused with the N- terminal end of EML-4 protein due to inversion between ALK and EML-4 gene.17 There are several types of genes involved in the fusion with ALK gene which participates in the NSCLC development such as KLC1, *KIF5B* and *TFG* gene 20 but EML-4 is predominant fusion partner. The fusion of ALK and EML-4 on chromosome '2p' has also been observed in non-smokers, light smoker and young age patients.22 EML4-ALK fusion is found with wild type EGFR and KRAS gene.23

Targeted Therapy

United States, FDA approved ALK/MET and ROS1 tyrosine kinase inhibitor Crizotinib in August 2011, as a therapeutic drug for NSCLC.24 This drug is taken up orally and it interferes with the constitutive phosphorylating activity of the tyrosine kinase domain of ALK receptor.25 EGFR tyrosine kinase inhibitor does not have affinity to inhibit the ALK mutated tyrosine kinase activity of the receptor.26 The copy number of ALK gene with its fusion partner increases in cases of crizotinib resistance. ALK gene fusion copy number is detected in each cell before and after crizotinib treatment using fluorescence *in situ* hybridization method. The copy number increases nearly more than 2 time of ALK gene rearrangement in every cell after the treatment than before treatment with crizotinib in the samples and sometimes it may be >4 times.27 An increased copy number of non-rearranged ALK gene signals correspond to polysomy (≥ 4 ALK copies in ≥ 10% cell nuclei in figure 1) or ALK amplification (≥10 ALK copies in ≥10% cell nuclei). The ALK gene copy number observed in 10% to 39% of nuclei is considered as low grade polysomy, whereas >40% of nuclei is taken as high grade polysomy (Cappuzzo et al. 2005).28

Screening Techniques

IHC, FISH, and RT-PCR are in use for the detection of ALK-EML4 fusion in NSCLC on formalin fixed paraffin embedded tissue (FFPE) sample but some problems occur during these experiments. For IHC and FISH the cancer tissue should be enough because it is not always possible to obtain large tissue from the NSCLC patients. The tissue size is a major issue with the IHC and FISH. FISH is a more expensive test and may not be suitable for mass screening.29 RT-PCR for detecting ALK-EML-4 mutation is not easy like FISH and IHC because it requires sufficient quantity of RNA for the experiment and the RNA extraction should be done instantly after the sample collection from the NSCLC patients for the feasibility of the result. More researchers have studied ALK mutation in NSCLC, by Reverse Transcriptase Polymerase Chain Reaction

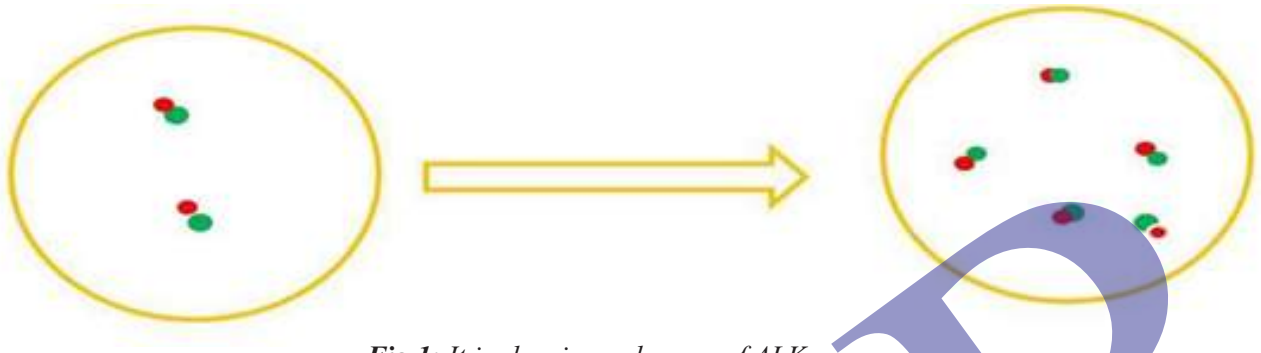

Fig.1: *It is showing polysomy of ALK gene*

(RT-PCR),Immunohistochemistry(IHC)and Fluorescence In-Situ Hybridization (FISH). To determine the ALK mutation in NSCLC patients,IHC is cheap as compared to other techniques but it does not give confirmatory results. FISH and RT-PCR provide confirmatory results. On the basis of sensitivity and specificity, the FISH is a gold standard method for the analysis of the ALK gene rearrangement in NSCLC patients (Table 1).30

Immunohistochemistry

There are different types of monoclonal antibody clones (ALK1, 5A4 and D5F3) showing variation in sensitivity and specificity (Table-2), are available in the market for the analysis of ALK gene mutation detection in NSCLC cases using immunohistochemical techniques.31 Thunnissen et,al. (2012) described the recent challenges in the standardization of IHC for ALK mutation detection in NSCLC patients like tissue preparation, antibody alternatives, signal intensity improvement and exact scoring. The benefits of IHC are that it is less expensive technique, easy to interpret the result by general pathologists, less time taking technique.

TECHNIQUES	ADVANTAGES	DRAWBACKS
Fluorescent in-situ Hybridization	1. On FFPE tissue 2. It can distinguish any rearrangement	1. Costly 2. Require expert for result interpretation 3. Difficult in result interpretation
Immunohistochemistry	1. It is cheap in comparison to other techniques 2. On FFPE sample	1. Unique antibodies are not present in the market for best expression.
RT-PCR	1. Require a smaller amount material	1. It cannot identify different fusion. 2. Chances of RNA degradation Contamination problem

Table 1. *Advantages and disadvantages of different types of techniques for the detection of ALK-EML-4 rearrangement in Lung Adenocarcinoma.36*

However, it has been recently seen that ALK mutation by IHC in NSCLC require further scrutiny and validation using FISH because sometimes IHC positive cases become negative after the FISH interpretation (Figure-2).32

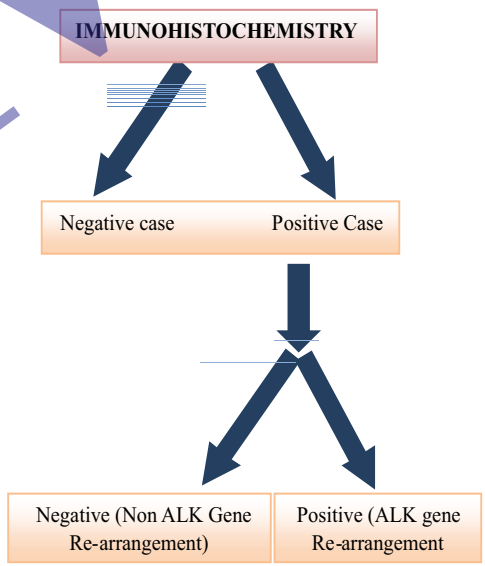

Figure 2. *Difference between Immunohistochemistry and FISH Interpretation of ALK Mutation in NSCLC.32*

Fluorescence in- situ Hybridization for ALK Mutation Analysis

For the interpretation of ALK mutation of 2p23 locus in NSCLC patients a FDA approved diagnostic probe kit is available in the market known as Vysis LSI ALK dual colour Break Apart Rearrangement Probe (United

State). The probes are of two types for the same gene which are labeled with two different colour green and orange (Red) fluorochromes which bind at a specific DNA target sequence (fig.-3).33 In non ALK mutated cells the green and orange signals are fused or less than two diameters distance. In case of gene rearrangement of ALK gene with EML-4 gene, the orange and green signals appears separately (>2 diameter) and one fused signals, sometimes due to deletion, only one orange and one fused signal appear (fig.-4).

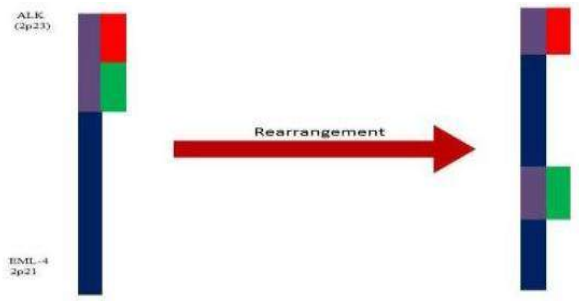

Figure 3. *Rearrangement pattern of ALK gene with EML-4 gene by the help of dual colour ALK break apart probe.*

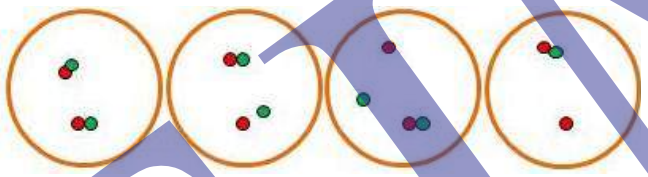

Figure 4. *Showing FISH result interpretation in various type (a) Showing two fused (red and green) signals (Negative) (b) Show one fused and one break apart signal but less than two diameter (Negative) (c) One fused and one break apart signal of more than two diameter (Positive) (d) One fused and one separate red signal which is presenting green signal deletion but it will be considered as positive.*

Immunohistochemistry Comparison	ALK1	5A4	D5F3
Sensitivity (%)	100	100	100
Specificity (%)	99	98	99
Positive Predictive Value	54	39	54
Negative Predictive Value	100	100	100

Table 2. Showing comparison of different types of antibodies (Selinger et al. (2013).31

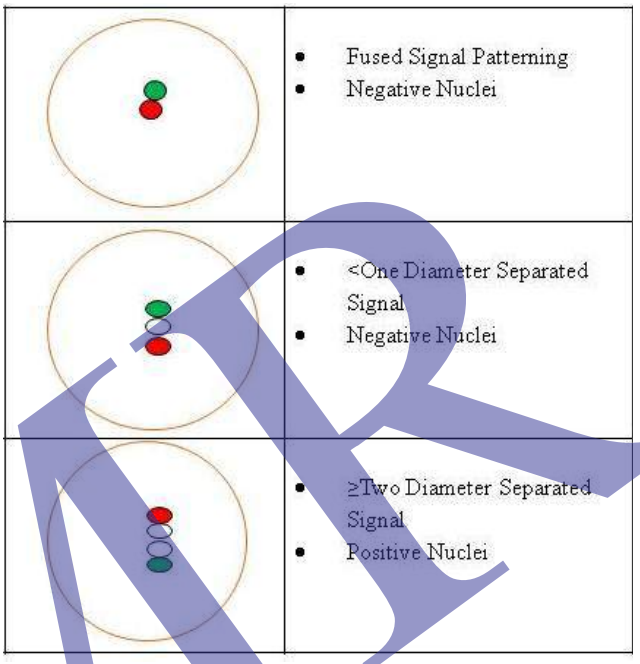

Figure 5. *Signal patterning of ALK FISH result analysis 35*

The interpretation criteria are 50% cells should be positive out of 50 cells.34 Signal patterning for positive and negative nucle i is given bellow (fig.-5). Thunnissen E *et al.* (2012) reviewed that if the tissue sample has less than five nuclei (10%) are positive, then the sample will be considered as FISH negative or if the number of positive nuclei are 10-50 % in this case, a second expert should evaluate the result after that if average of two evaluator is at least 15% then the sample will be considered as FISH positive otherwise equivocal (fig.-6).

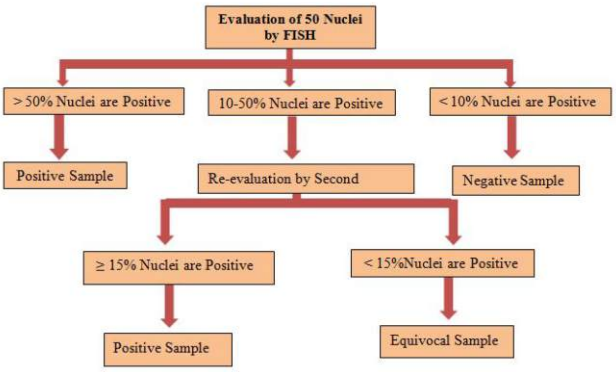

Figure 6. *This diagram is showing interpretation criteria*

Table 3. *ALK deregulated protein in different types of cancer (Taken from the review of Shackelford et al.2014)*

Tumor Type	ALK Aberration	ALK Mutation Percentage
Non-Small cell lung carcinoma	Fusion between EML-4 and ALK on 2p arm of the chromosome	3-7% of NSCLC are ALK positive 37
Anaplastic Non-Hodgkin Lymphoma's (ALCL)	ALK-Nucleophosmin chromosomal translocation	ALCL are 60-85% +ve, rare fusion with ATIC, MYH9, ALO17, TPM3, TPM4, TFG, MSN and ALTC 38
Basal cell cancer	Over Expression of ALK	250 fold increased phosphor-ALK expression in near about 100% of BCCs 39
Glioblastoma	Over Expression of ALK	ALK is over expressed , lowering ALK expression, decreases glioblastoma tumor growth 40
Breast Carcinoma	ALK-EML-4 fusion	2.4% positive rearrangement 80% inflammatory breast carcinoma show increased ALK protein expression 41
Neuroblastoma	Over expression of ALK	ALK shows over expression in over 50% of cancer. Nearly 12.4% of cancer carry ALK point mutation which are common in familial neuroblastoma 42
Ovarian carcinoma	Over expression of ALK	ALK over expressed in 2-4% of ovarian cancers, one stromal carried a FNI-ALK fusion protein 43
Melanoma	Over expression of ALK	6.9% of acral melanomas were ALK positive, ALK breakpoints suggest that translocations are present 44
Anaplastic thyroid carcinoma	ALK activating point mutation	L1198F and G1201E amino acid changes result in constitutive ALK kinase activation 45
Colorectal carcinoma	ALK-EML4 fusion	2.4% fusion positive by exon array profiling 46
Extramedullary plasmacytoma	ALK over-expression	1 case in 46 extramedullary plasmacytomas was ALK positive by immunohistochemistry and FISH analysis 47
Esophageal squamous cell carcinoma	TPM4–ALK fusion	TPM4–ALK fusion oncoprotein type 2 found in ~20% of cases 48
Renal carcinomas	Several translocations	ALK translocations with EML4, TPM3, and VCL fusion partners, the translocations appear to occur at a low frequency 49

Table 4. *Mechanism of crizotinib resistance (Taken from the review of Shackelford et al.2014)*

Crizotinib resistance mechanism	Explanation
ALK Copy Number Gain	Two cases; one with and one without an ALK mutation, 4-5-fold increased expression 50
c-Kit	5 fold –KIT amplification 51
C1156Y EML-ALKpm*	Alters ALK crizotinib binding cavity, reducing crizotinib-protein interactions 52
G1202R EML-ALKpm	A mutation-specific strong H-bond pulls crizotinib out of the position found in the non-crizotinib resistant EML-ALK fusion gene 53
L1152R EML-ALKpm	Mutation resistant to crizotinib and the structurally unrelated compound TAE684 54
S1206Y EML-ALKpm	Lowers crizotinib-protein affinity by eliminating two H-bonds between crizotinib and the ALK binding site 53
1151Tins	Thr insertion is predicted to alter ATP binding to ALK 51
G1269A EML-ALKpm	Gly→Ala reduces crizotinib binding ATP-binding pocket by steric hindrance 50
EGFR Alterations	L585R mutation in one case, other cases often show in EGFR and EGFR amplification 50
KRAS Mutations	G12C and G12V activating KRAS mutations 50
L1196M EML-ALKpm	Gatekeeper residue mutation blocks binding 51

****pm=Point Mutation***

CONCLUSION

Immunohistochemistry seems to be the most reliable screening technique for large-scale clinical practice, when performed with different types of sensitive antibody clones. For initial routine workup molecular technique in NSCLC should be IHC followed by ALK FISH as a confirmatory test of IHC positive cases. Using ALK FISH break-apart probe for ALK gene rearrangement analysis in NSCLC is documented as gold standard diagnostic criteria for ALK tyrosine kinase inhibitor therapy, the screening algorithm for detection of ALK-rearranged lung cancer is still under development. Many studies are being conducted for the standardization of IHC and FISH technique as well as assessment of the sensitivity and specificity of IHC as a screening method. After FISH, RT-PCR can be used as a second confirmatory tool whenever FISH is not available.

References:

- American Cancer Society. Cancer Facts and Figures 2007. Atlanta, Ga: American Cancer Society, 2007;(1);18-28.
- American cancer society. Cancer Facts and Figures 2016. Atlanta, Ga: American Cancer Society; 2016;(4);69-89.
- Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med. 2002; (346); 92–98.

- Behera D, Balamugesh T. Lung Cancer in India. Indian J Chest Dis Allied Sci. 2004;(46);269-281.
- Gupta D, Boffetta P, Gaborieau V, Jindal SK. Risk factors of lung cancer in Chandigarh, India. Indian J Med Res. 2001;(113);142-50.
- Coultas DB, Samet JM. Occupational lung cancer. Clin Chest Med. 1992;(13);341-354.
- Sankarnarayanan R, Varghese C, Dugffy SW, Psdmakumary G, Day NE, Nair MK. A case control study of diet and lung cancer in Kerala, South India. Int J Cancer 1994;(58);644-649.
- Sørensen M, Pijls-Johannesma M, Felip E. Small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology. 2010;(21); 120–125.
- Jockel KH, Ahrens W, Wichmann HE. Occupational and environmental hazards associated with lung cancer. Int J Epidemiol. 1992;21(2);202-13.
- Minna JD. Genetic events in the pathogenesis of lung cancer. Chest. 1989;(96);17S.
- Ito Y, Ohno Y, Rachet B, et al: Cancer survival trends in Osaka, Japan: the influence of age and stage at diagnosis. Jpn J Clin Oncol. 2007; 37(37);452-8.
- Ferguson MK, Wang J, Hoffman PC, et al: Sex-associated differences in survival of patients undergoing resection for lung cancer. Ann Thoracic Sur. 2000;69(1);245-9.
- Radzikowska E, Gtaz P, Roszkowski K: Lung cancer in women: age, smoking, histology, performance status, stage, initial treatment and survival. Population-based study of 20 561 cases. Ann Oncol. 2002;13(7);1087-93.
- Morris SW, Kirstein MN, Valentine MB: Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science.1994;263(5151);1281-4.
- Stoica GE, Kuo A, Power C, et al. Bowden ET, Sale EB, Riegel AT, Wellstein A Midkine binds to anaplastic lymphoma kinase (ALK) and acts as a growth factor for different cell types.J BiplChem. 2002;277(39);35990-8.
- Morris SW, Naeve C, Mathew P, et al. ALK, the chromosome 2 gene locus altered by the t(2;5) in non-Hodgkin's lymphoma, encodes a novel neural receptor tyrosine kinase that is highly related to leukocyte tyrosine kinase (LTK). Oncogene. 1997;14(18);2175-88.
- Soda M, Choi YL, Enomoto M, et al. Takada S, Yamashita Y, Ishikawa S, Fujiwara S, Watanabe H, Kurashina K, Hatanaka H, Bando M, Ohno S, Ishikawa Y, Aburatani H, Niki T, Sohara Y, Sugiyama Y, Mano H. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature. 2007;(448);561–566.
- Ma Z, Cools J, Marynen P, et al. Inv(2) (p23q35) in anaplastic large-cell lymphoma induces constitutive anaplastic lymphoma kinase (ALK) tyrosine kinase activation by fusion to ATIC, an enzyme involved in purine nucleotide biosynthesis. *Blood*. 2000;95(6):2144–9.
- Pollmann M, Parwaresch R, Adam-Klages S, et al. Human EML4, a novel member of the EMAP family, is essential for microtubule formation. Exp Cell Res. 2006;312(17):3241–51.
- Togashi Y, Soda M, Sakata S, Hatano S, et al. KLC1-ALK: a novel fusion in lung cancer identified using a formalinfixed paraffin-embedded tissue only. PLoS One. 2012;7(2);31323.
- Wu Y-C, Chang I-C, Wang C-L, et al. Comparison of IHC, FISH and RT-PCR Methods for Detection of *ALK* Rearrangements in 312 Non-Small Cell Lung Cancer Patients in Taiwan. PLoS One. 2013;8(8);70839.
- Horn L, Pao W. *EML4-ALK*: honing in on a new target in non-small cell lung cancer. *J Clin Oncol*. 2009; (27); 4247–53.
- Shaw AT, Yeap BY, Mino-Kenudson M, et al. clinical feature and outcome of patients with non-small cell lung cancer harboring EML4-ALK. J Clin Oncol. 2009;(27);4247-4253.
- Ou SH, Kwak EL, Siwak-Tapp C, et al. Activity of crizotinib (PF02341066), a dual mesenchymal-epithelial transition (MET) and anaplastic lymphoma kinase (ALK) inhibitor, in non small cell lung cancer patient with de novo MET amplification, J Thorac Oncol. 2011; (6);942-6.

- Christensen JG, Zou HY, Arango ME, et al. Cytoreductive antitumor activity of PF-2341066, A novel inhibitor of anaplastic limphoma kinase and c-Met, in experimental models of anaplastic large-cell lymphoma, Mol cancer Ther. 2007;6(12); 3314-3322.
- Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibitor in non-small cell lung cancer. Nengl J Med. 2010; (363);1693-1703.
- Doebele RC, Pilling AB, Aisner DL, et al.Mechanism of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clin Cancer Res. 2012; (18);1472-82.
- Cappuzzo F,Hirsch FR,RossiE, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small cell lung cancer. J Natl Cancer Inst. 2005; (97);643-55.
- Mitsudomi T, Yatabe Y, Akita H, et al. Guidance for ALK gene testing in lung cancer patients, Biomarker Committee, the Lung Cancer Society. 2011;(14);48-69.
- Zhang YG, Jin ML, Li L et al. Evaluation of ALK rearrangement in Chinese non small cell lung cancer using FISH, Immunohistochemistry, and real time quantitative RT-PCT on paraffin-embedded tissue. PLoS One. 2013;31(8(5)); 64-82.
- Selinger CI, Rogers TM, Russel PA, et al. Testing for ALK rearrangement in lung adenocarcinoma: amulticenter comparision of immunohistochemistry and fluorescent in situ hybridization. Modern Pathology. 2013; (26);1545-1553.
- Thunnissen E, Bubendorf L, Dietel M. et al. EML4-ALK testing in non small cell carcinomas of the lung: a review with recommendations. Virchows Arch. 2012;(461); 245-257.
- Marchetti A, Ardizzoni A, Papotti M et al. Recommendation for the analysis of ALK gene rearrangements in non –small cell lung cancer: a consensus of Italian Association of Medical Oncology and the Italian Society of Pathology and Cytopathology. J Thorac Oncol. 2013; 8(3);352-8.
- Vysis ALK Break Apart FISH Probe Kit datasheet.Availableat:http//www.abbottmolecular.com/static/cms. Accessed August 2011;(47);78-169.
- Wojas-Krawczyk K, Krawczyk PA, RamlauRA et al. The analysis of ALK gene rearrangement by fluorescence in situ hybridization in non small cell lung cancer patients. ContempOncol (Pozn). 2013;17 (16);484-92.
- Gandhi L, Janne PA. Crizotinib for ALK-rearranged non-small cell lung cancer: anew targeted therapy for a new target. Clin Cancer Res. 2012;18(14);3737-42.
- Hallberg B,Palmer RH. Mechanistic insight into ALK receptor tyrosin kinase in human cancer biology. Nat Rev Cancer. 2013;(13);685-700.
- Pulford K, Lamant L, Espinos E, et al. The emerging normal and disease-related related roles of anaplastic lymphoma kinase. Cell Mol Life Sci. 2004;(61);2939-53.
- Ning H, Mitsui H, Wang CQ, et al. Identification of anaplastic lymphoma kinase as a potential therapeutic target in Basal Cell Carcinoma. Oncotarget. 2013; 4(12);2237-48.
- Bilsland JG. Wheeldon A, Mead A, et al. Behavioral and neurochemical alterations in mice deficient in anaplastic lymphoma kinase suggest therapeutic potential for psychiatric indications. Neuropsychopharmacology. 2008;(33);685-700.
- Robert FM, Ill PEF, Laere SJV, et al. Presence of anaplastic lymphoma kinase in inflammatory breast cancer. SpringerPlus. 2013;(2);497.
- Wang M, Zhou C, Sun Q, et al. ALK amplification and protein expression predict inferior prognosis in neuroblastomas. Exp Mol Pathol. 2013;(95);124-30.
- Ren H, Tan ZP, Zhu X, et al. Identification of anaplastic lymphoma kinase as a potential therapeutic target in ovarian cancer. Cancer Res. 2012;(72);3312-23.
- Niu HT, Zhou QM, Wang F, et al. Identification of anaplastic lymphoma kinase break points and oncogenic mutation profiles in acral mucosal melanomas. Pigment Cell Melanoma Res.2013;26(5);646-53.

- Murugan AK,Xing M. Anaplastic thyroid cancer harbor novel oncogenic mutations of the ALK gene. Cancer Res. 2011;71(13);4403-11.
- Lin E, Li I, Guan Y et al. Exon array profiling detects EML4-ALK fusion in breast, colorectal, and non small cell lung cancers. Mol Cancer Res. 2009;7(9);1466-7
- Wang WY, Gu L, Liu WP, et al. ALK positive extramedullary plasmacytoma with expression of the CLTC_ALK fusion transcript. Pathol Res Pract. 2011;(207);587-91.
- Du XL, Hu H, Lin DC et al. Proteomic profiling of proteins dysregulated in Chinese esophageal squamous cell carcinoma. J Mol Med. 2007;85(8);863-75.
- Sugawara E, Togashi Y, Kuroda N, et al. Identification of anaplastic lymphoma kinase fusion in renal cancer: large scale immunohistochemical screening by the intercalated antibody- enhanced by polymer method. Cancer. 2012;118(18);4427-36.
- Doebele RC, Pilling AB, Aisner DL, et al. Mechanism of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clin Cancer Res. 2012;18(5):1472-82.
- Katayama R, Shaw AT, Khan TM, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung Cancers. Sci Transl Med. 2012;(4);120-17.
- Choi YL, Soda M, Yamashita Y, et al. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. N Engl J Med. 2010;(363);1734-9.
- Sun HY, Ji FQ. A molecular dynamics investigation on the crizotinib resistance mechanism of C1156Y mutation in ALK. Biochem Biophys Res Commun. 2012;(423);319-24.
- Sasaki T, Koivunen J, Ogino A, et al. A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. Cancer Res. 2011;(71);6051-60.
- Thunnissen E, Bubendorf L, Dietel M et al. EML4-ALK testing in non-small cell carcinomas of the lung: a review with recommendations. Virchows Arch. 2012 ;(461);245–257.