LCC-A Symposium: Original Article

A year of anaplastic large cell kinase testing for lung carcinoma: Pathological and technical perspectives

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Abstract

BACKGROUND: An in-frame fusion protein between echinoderm microtubule-associated protein-like 4 (EML4) and anaplastic large cell kinase (ALK) genes is seen in some non-small cell lung cancer (NSCLC). EML4-ALK demonstrates constitutive kinase activity. These ALK-positive lung carcinomas have been shown to respond to ALK kinase inhibitors. ALK gene rearrangement is commonly detected using fluorescent *in situ* hybridization (FISH). **AIMS:** To study the pathological features of ALK positive and negative NSCLC and evaluate the causes of uninterpretable FISH results. **MATERIALS AND METHODS:** This is a retrospective, observational study. The molecular pathology records of patients on whom test for ALK had been performed in a period of 1 year (February 2012 to February 2013) were accessioned. A total 224 cases were identified. Histological features were reviewed. The *in situ* hybridization was performed using Vysis ALK Dual Color Break Apart Rearrangement Probe (Abbott Molecular Inc.). Signal interpretation under the fluorescent microscope was performed in accordance with College of American Pathologists guidelines. **RESULTS:** Five patients showed ALK gene rearrangement, 182 were negative and 37 cases were uninterpretable. Five patients with ALK gene rearrangement had a mean age of 48 years and the male to female ratio was 2:3. In the ALK negative cases, the mean age was 54 years and male to female ratio was 3.2:1. Histologically, amongst the rearranged cases, three showed solid pattern, one showed acinar and one showed acinar with signet ring cells on histology. **CONCLUSION:** The percentage of ALK gene rearrangement was 2.7% (excluding the uninterpretable cases). These ALK positive patients were relatively younger than ALK negative patients. Solid pattern on histology was associated with ALK positivity. In a quarter of the uninterpretable results, the material submitted was fixed and processed outside.

Key Words: Adenocarcinoma, anaplastic large cell kinase, fluorescence *in situ* hybridization, India, lung cancer

Introduction

Non-small cell lung cancer (NSCLC) is a major cause of death world-wide, with most patients being diagnosed with disease in advanced stage, when palliation is the only available option.[1] Despite improvements in the detection and treatment of lung cancer, the overall 5-year survival rate remains at 15%.[2] Recent advances in the understanding of the molecular mechanisms of cancer pathogenesis demonstrate that malignancies can result from genetic alterations in a single gene. The cancer becomes dependent on signaling from the transcribed protein, often a receptor tyrosine kinase. Over the past 10 years, targeted therapies inhibiting such driver proteins have started a paradigm shift in treatment of cancer.

Transforming rearrangements and mutation of the anaplastic lymphoma kinase-now called anaplastic large cell kinase (ALK) gene have been recognized in some cancers viz. anaplastic large cell lymphoma,[3] neuroblastoma,[4] inflammatory myofibroblastic tumor,[5] and NSCLC.[6,7]

Access this article online

Quick Response Code:

Website: www.indianjcancer.com

DOI: 10.4103/0019-509X.117007

PMID: *******

These in-frame gene rearrangements place the ALK kinase domain under the promoter control of another gene with its intra-cellular localization and function potentially influenced by the specific N-terminal of the fusion partner.

The overall incidence of ALK gene rearrangements in NSCLC is estimated to be 3% with incidence up to 13% in East Asian patients.[7-14] A significantly higher incidence of ALK rearrangements is reported in the adenocarcinoma subset of NSCLC with further notable predilection for acinar and solid histologic subtypes with signet ring cells.[10,13,15,16] A higher rate of ALK rearrangement is encountered in NSCLC patients who never smoked or light smokers and younger age.[10,14] Coexistence of ALK rearrangement with (epidermal growth factor receptor [EGFR] or KRAS) mutations has been rarely described,[10,13] suggesting that ALK is a distinct oncogenic driver.

The US food and drug administration (FDA) has approved the use of ALK inhibitor, PF02341066 (crizotinib), in ALK-positive lung carcinoma. The FDA approved the Vysis ALK Break-Apart fluorescent *in situ* hybridization (FISH) Probe Kit (Abbott Molecular, Inc.) concurrently with the crizotinib approval. This companion diagnostic test is designed to detect ALK rearrangement in NSCLC. A phase I clinical trial of crizotinib given to 82 ALK positive patients with advanced NSCLC, the 1 year overall survival was 74% and 2 year overall survival was 54%; Survival in 30 ALK-positive patients who were given crizotinib in the second-line or third-line setting was significantly longer than in 23 ALK-positive controls given any second-line therapy, but not crizotinib (median overall survival not reached [14 months to not reached] versus 6 months, 1 year overall survival 70% versus 44%, and 2-year overall survival 55% versus 12%.[17]

The aim of this study was to retrospectively evaluate all cases where ALK by FISH was requested in one year. The incidence of ALK gene rearrangement and its association with any particular histological pattern would be analyzed. The reasons for uninterpretable result and the utility of repeat testing would be studied.

Materials and Methods

All cases of lung cancer where the testing for ALK was requisined over a period of 1 year were included. The demographic data, the histology, the type and the site of biopsy were analyzed.

The material obtained was either fresh tissue (biopsy and excision specimens) or formalin fixed paraffin embedded (FFPE) tissue blocks from outside. Pleural fluid was also received and a cell block was make out of it which was then routinely processed and embedded in paraffin.

The histology was reviewed and the pattern of adenocarcinoma was noted. All adenocarcinomas were classified according to The International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society, and the European Respiratory Society classification of 2011.[18]

The results of immunohistochemistry (IHC) were also reviewed and the reason for performing IHC was established.

FISH was carried out for all cases. The FISH assay was performed on FFPE tumour tissue using the ALK break-apart probe (Vysis, Abbott Molecular, Abbott Part, IL, USA). The reagent of this test comprised of a SpectrumOrange (red) labeled 250 kb probe to the 3' end of ALK with SpectrumGreen (green) labeled300 kb probe to the 5' end of ALK. Sections of 4-6 μm thickness of FFPE tissue were cut and taken onto these slides pre-coated with poly-L-lysine. The slides were deparaffinized, dehydrated and then put in 10 mM HCl. After drying the slides, they were rinsed with 2.5% Sodium Thiocyante solution and steamed in microwave for 2 min. Next, the slides were put in 10 mM Sodium Citrate buffer and boiled in the microwave for 4 min. The slides were immersed in Pepsin-HCl solution at 37°C for 25 min and then dehydrated. The probe mixture was added to the slide, cover slip applied, the edges sealed and placed in hybridizer at 80°C for 5 min to denature the probe. The hybridizer was sealed and the slides were incubated in it at 37°C for 16-20 h to allow hybridization. Post-hybridization washes with saline sodium citrate were given and 4,6-diaamidino-2-phenylindole (DAPI) counter-stain was applied. The signals for each probe were evaluated under a fluorescent microscope using an oil immersion objective.

There were two patterns of ALK rearrangement. One was break-apart where the red and green signals were split, and the distance between these two signals were physically separated by ≥2 signal diameters.[19] In the other pattern, the green signal was lost and only isolated or single red signals (3' ALK) were observed, and these denote deletion in the 5'ALK region in association with 2p inversion.[19] A minimum of 50 tumor cells were counted and cases were considered positive for ALK rearrangement when >15% (as defined by the company and used in crizotinib trials; Nearly, 15% falls within the non-overlapping area of

12% to 21% positivity that differentiates biology from assay variability) of the tumor cells showed split signals or single red signals; the rest of the cases were classified as ALK FISH negative.[13,15,19] While scoring, normal lung tissue, and lymphocyte nuclei were not counted. If no signals were detected, the test was repeated (if enough tissue was present). When the repeat test too showed no signals, the result of test uninterpretable was issued and the cause of uninterpretability was reported. The FISH results were evaluated and the reasons for uninterpretable results investigated.

Results

The Molecular Pathology Division of the Department of Pathology received a total of 224 cases of lung cancer for ALK gene rearrangement by FISH.

Patients included 165 men and 59 women (male to female ratio of 2.8:1). The age of patients ranged from 21 years to 81 years with a mean age of 54 years.

The sites from which the tissue for ALK FISH was obtained were: (1) Lung, which included lobectomies – 8 cases, computed tomography (CT) guided biopsies – 134 cases and transbronchial biopsies – 11 cases (overall 68%); (2) Metastatic lymph nodes (mainly supraclavicular, but also mediastinal, paratracheal and axillary) – 52 cases (23%); (3) Pleural biopsy– 7 cases; (4) Bone metastasis – 10 cases; (5) Liver metastasis – 5 cases; (6) Paravertebral metastatic mass – 1 case; (7) Brain metastasis – 1 case; (8) Mediastinal mass – 1 case and (9) Pleural fluid cell block – 2 cases.

The biopsies and excision were performed in house in 170 cases and we received FFPE from outside as consultation in 42 cases. We had two cases where a cell block was made from the pleural fluid, which was embedded in agar and subsequently fixed and processed as a biopsy sample.

The histopathological diagnosis included: (1) Adenocarcinoma or NSCLC, favor adenocarcinoma – 217 cases (97%), (2) Adenosquamous Carcinoma – 3 cases, (3) Sarcomatoid/Anaplastic Carcinoma (no focal squamous or adenocarcinoma features) – 3 cases, and NSCLC – 1 case. More than 60% of the adenocarcinomas showed acinar pattern and about a quarter showed solid patterns. The remainder of the cases showed papillary, invasive mucinous, lepidic, cribriform, micropapillary, and fetal patterns.

Immunohistochemical analysis (IHC) was carried out in 84 cases (38%). The commonly used IHC markers were TTF1, CK7, p63, CK5/6, Napsin A and surfactant protein B (SPB). TTF1 was positive in 55 of 77 cases on which it was performed (71%), CK7 in 33 of 37 cases (89%), Napsin A in 1 of 2, SPB in 9 of 19, p63 was focally positive in 9 of 37 cases (24%) of which two positive cases were adenosquamous carcinomas and CK5/6 was only focally positive in 2 of 8 cases. Both CK7 and TTF1 were positive in 50 of 63 cases (79%).

Different signal patterns were observed. They were (i) single fused signals, (ii) double fused signals, (iii) multiple fused signals, (iv) one fused and one green, (v) narrow split (<2 probe diameter), (vi) one fused and one split, (vii) two splits, (viii) one fused and one red, (ix) one fused and multiple red, (x) Single red, and (xi) Single green. The presence of one or more fused signals and one fused with one green signal was considered as negative. One fused and one split, both split, one fused with isolated red signal and one fused with multiple red signals were all considered as positive for ALK gene rearrangement. The abnormal patterns, where present, were noted in more than 15% of the tumor nuclei. A minimum of 50 cells were counted in each case.

The analysis of the FISH results for ALK gene rearrangement revealed 5 cases with gene rearrangement, 37 uninterpretable results and 182 negative.

Cases reported as ALK gene rearrangement not detected or negative (182 cases)

One hundred and eight two cases were in this group. Patients included 139 men and 43 women (M:F = 3.2:1), with age range of 21-79 years (mean – 54 years). On FISH analysis, they all showed fused red and green signals [Figure 1].

Cases showing ALK gene rearrangement (5 cases)

The cases with ALK gene rearrangement comprised 2% of all cases tested and 2.7% of all interpretable results. Patients included three females and two males with an age range of 35-61 years with a mean of age of 48 years [Table 1]. All these cases had in house biopsies – four supraclavicular lymph nodes and one CT guided lung biopsy. All these were adenocarcinoma with three showing solid pattern, one acinar pattern and one acinar pattern with signet ring cells. The results were also reviewed by another Pathologist. Three cases had split signals – accounting for 22%, 38%, and 64% of all tumor cell nuclei counted [Figure 2]. One case showed isolated red signals or loss of green signals in 39% of the nuclei [Figure 3]. The final case (case 2) showed multiple red signals (polysomy) with one fused signal [Figure 4]. This case was reported as showing polysomy with the loss of green signals.

Table 1: Cases showing ALK gene rearrangement

Case	Age (year)	Sex	Site of biopsy	Histology	FISH result (in tumor nuclei) %
1	61	M	SCLN	Solid	Split signals in 22
2	42	F	Lung (guided)	Acinar	Polysomy and loss of green in 22
3	35	F	SCLN	Solid	Split signals in 38
4	46	M	SCLN	Solid with mucin	Isolated red signals in 39
5	55	F	PCLN	Acinar with signet ring cells	Split signals in 64

M=Male; F=Female; SCLN=Supraclavicular lymph node; PCLN=Posterior cervical lymph node; ALK=Anaplastic large cell kinase; FISH=Fluorescent *in situ* hybridization

Cases reported as uninterpretable (37 cases)

A total of 37 cases were deemed uninterpretable due to the absence of signals [Figure 5]. The test was repeated in 28 cases and in 9 cases it was not repeated as the material remaining in the paraffin block was extremely scanty. Of the 42 cases where we received outside FFPE tissue, 11 were uninterpretable (26%). In comparison, 26 of 182 cases with in-house biopsies were uninterpretable. Many of the referral cases where the paraffin block was submitted, the fixation and processing were suboptimal.

Discussion

In 2007, two groups independently described some NSCLC harboring ALK gene rearrangement creating an in-frame fusion protein between echinoderm microtubule-associated protein-like 4 (EML4) and ALK genes.[6,7] The novel fusion gene arose from an inversion on the short arm of chromosome 2 (inv (2)(p21p23)) that joined exons 1-13 of EML4 to exons 20-29 of ALK. In consequence, the ALK tyrosine kinase domain is constitutively activated as well as the downstream signaling pathways of MAPK, PI3K/AKT, and STAT3.[7,8,20] EML4-ALK demonstrates constitutive kinase activity, and ALK-rearranged lung cancer cell lines are dependent upon ALK kinase activity for growth and survival.[6,8,21] Since, the ALK tyrosine kinase activity is necessary for its transforming activity and oncogenicity, several ALK kinase inhibitors have been identified.[8,21] ALK inhibitors lead to apoptosis *in vitro* and tumor shrinkage *in vivo* thus demonstrating the phenomenon of "oncogene addiction."[8] Therefore, a need exists for molecular testing to correctly identify the correct target patient population.

In the present study, it was seen that patients with ALK rearrangement were comparatively younger with a median age of 48 years as opposed to 54 years in the ALK negative subgroup. The male to female ratio was 0.7:1 compared to 3.2:1 in the ALK negative subgroup. Studies have shown that the patients harboring this translocation are significantly younger than patients who did not have the ALK translocation.[13,14] Female predominance in ALK rearranged tumors is also documented.[16]

We received tissue from a variety of sites which included the lung (68%), metastatic nodes (23%), and other metastatic sites (9%).

On reviewing the histology, almost all the cases were adenocarcinoma, NSCLC – favor adenocarcinoma or adenosquamous carcinoma (98%). The major histological pattern noted was acinar (in more than 60% of the cases). A variety of histological features have been shown to be associated with ALK-rearranged lung adenocarcinomas. In the Asian population, acinar and lepidic pattern[9] and in the Western patients the solid pattern and signet-ring cell histology[13,15] were the most common histologic subtypes associated with EML4-ALK translocations. We had three cases with a solid pattern and two with acinar.

IHC was performed in a third of the cases (84 cases, 38%). About half of the IHC was done on the lung biopsies (52%) and the remainder on the tissue from the metastatic sites (including pleura and lymph nodes). The reasons for IHC were to either establish the primary site of the adenocarcinoma as lung or to differentiate adenocarcinoma from squamous carcinoma. A combination of TTF1 or CK7 with p63 was very useful in deciding whether a poorly differentiated NSCLC was adenocarcinoma or squamous carcinoma. IHC staining with p63, when present, was always focal in adenocarcinoma as opposed to the diffuse pattern in squamous carcinoma [ref]. When some cells in a tumor showed immunoreactivity for TTF1/CK7 and were negative for p63 and the other tumor cells showed the opposite (strong and diffuse staining with p63 and immunonegative for TTF1 and CK7), the tumor was called adenosquamous carcinoma. It must be emphasized that special stains and IHC should be used sparingly as the tissue is required for molecular analysis (EGFR, ALK, etc.). It might be prudent to divide the biopsy cores and put them into different paraffin block so that the tissue does not get depleted. Performing IHC on the cell block obtained from pleural fluid (TTF1 or Napsin A), to identify and roughly quantify the adenocarcinoma cells would be useful as too many reactive mesothelial cells may make it difficult to identify the carcinoma cells on fluorescent microscopy.

Formalin fixed and paraffin embedded tissue is good for FISH analysis. The slide should be scanned to assess the distribution of the cancer cells and the

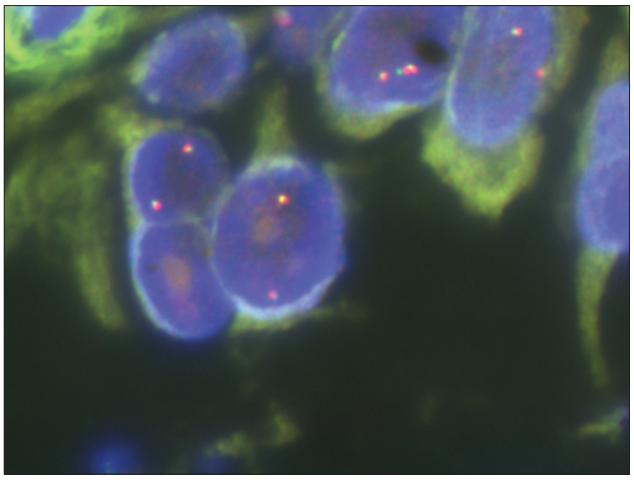

Figure 1: Fluorescent *in situ* hybridization for anaplastic large cell kinase showing fused red and green signals (yellow color). Oil ×100

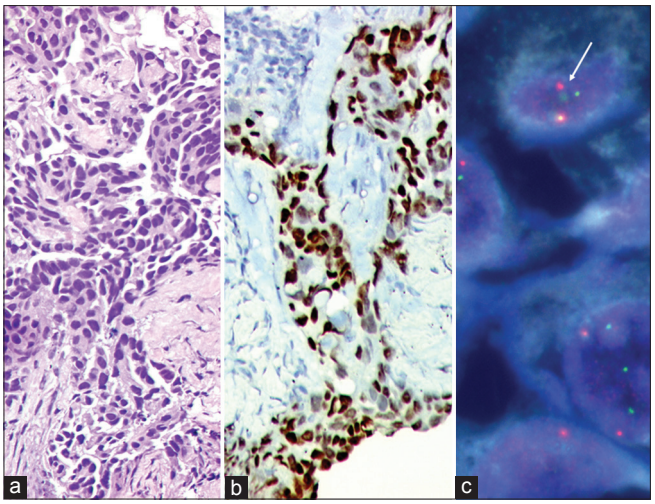

Figure 2: (a) Adenocarcinoma with acinar pattern (H and E, ×40). (b) The tumor cells show nuclear TTF1 expression (×40). (c) Fluorescent *in situ* hybridization result showing split red and green signals (arrow) and one fused yellow signal (Oil, ×100)

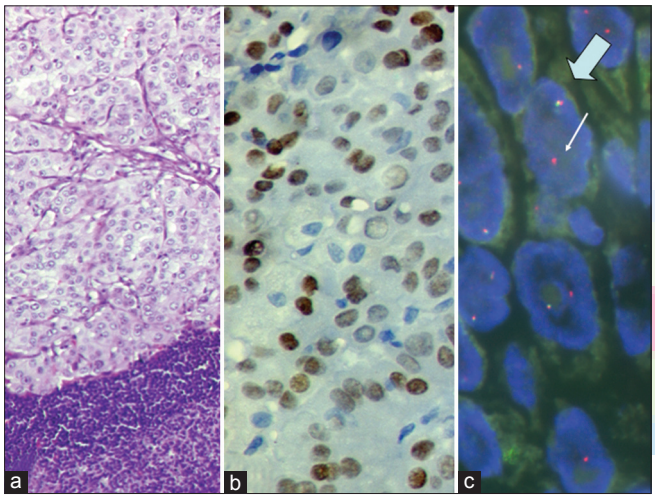

Figure 3: (a) Nodal metastasis of adenocarcioma with solid pattern (H and E, ×20). (b) The tumor cells express TTF1 (×40). (c) fluorescent *in situ* hybridization reveals fused signal (thick arrow) and isolated red signal (thin arrow) in the same nucleus (Oil, ×100)

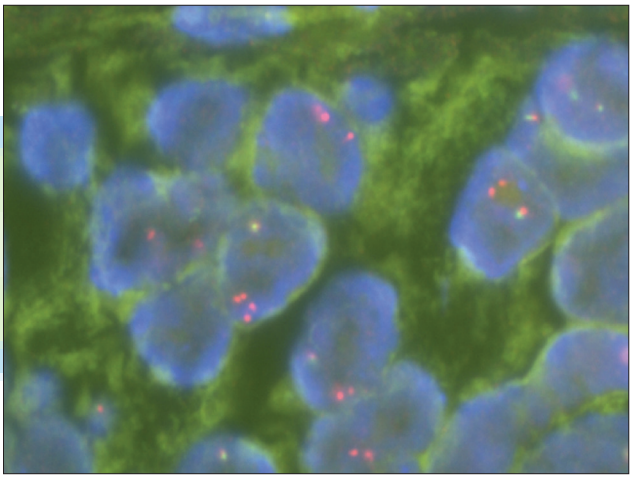

Figure 4: Fluorescent *in situ* hybridization result showing one fused signal and multiple red signals suggesting polysomy (Oil, ×100)

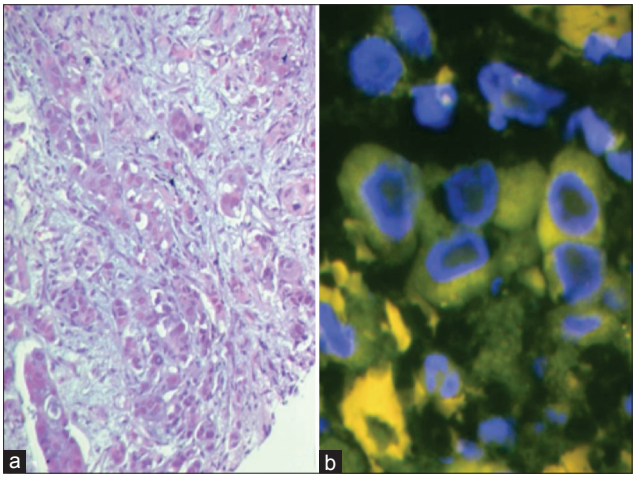

Figure 5: (a) Poor tissue morphology due to suboptimal fixation and processing (H and E, ×20). (b) The fluorescent *in situ* hybridization test revealed nuclear distortion and no appreciable signals

interpretation must be performed in the area with minimal overlapping of nuclei and good signal quality. The IASLC, in association with the College of American Pathologists and the Association for Molecular Pathology has come up with Lung Cancer Biomarkers Guidelines.[22] Only signals with a split of more than two probe diameters should be considered as positive. Though the cut-off of 15% for calling a tumor positive or negative for rearrangement might seem arbitrary, this cut-off falls within the non-overlapping area of 12% to 21% positivity that seems to accurately differentiate biology from assay variability.[23]

A quarter of the cases where we received FFPE from outside were uninterpretable. This might have to do with poor primary fixation, suboptimal processing or both. The in house cases, which were uninterpretable

were old excision specimens and smaller biopsies. In many cases (which were promptly and adequately fixed and optimally processed), the cause of uninterpretable result or the absence of signals was unexplainable. There were 10 cases where the bone was the site of the biopsy. As decalcification is thought to damage the cells and the deoxyribonucleic acid, the material is traditionally not thought to be good for FISH analysis.

In a total of 39 cases, the test was repeated as the first result was uninterpretable. Of these, 28 were again uninterpretable, but 11 (28% of repeats) showed assessable signals on repeat test, and were negative for ALK gene rearrangement. Hence, it might be worthwhile repeating the test if the signals have been absent as sometimes technical issues (though not readily identifiable) can result in lack of signals.

The advantages of FISH technique include commercial availability of the probe and detection of cases with non-EML4 fusion partners. The disadvantages include the cost, the subtlety of the split signals and non-tumor portions showing split signals![23]

The other modes of detecting ALK gene rearrangement include (i) reverse transcriptase – polymerase chain reaction (RT-PCR), (ii) IHC and (iii) chromogen *in situ* hybridization (CISH). RT-PCR is a rapid and extremely sensitive method for identifying ALK translocated NSCLC. It is capable of definitely defining both ALK fusion partners and the precise fusion variant. However, as there are 11 variant EML4-ALK fusions and non-EML4 translocation partners, all these primers must be incorporated in the assay and novel fusions may be missed. IHC is easy to apply on FFPE and the pathologist can readily relate with it. However, interpretation might be difficult and some type of signal enhancement would be required.[24] In CISH, chromogenic signals instead of fluorescent signals are used. The advantages include the use of bright-field microscopy, visualization of morphology and the stains do not fade over time, unlike the fluorescent dyes. The results are comparable to FISH.[25] All these methods are complimentary, but FISH is considered to be the gold standard.

Acknowledgment

We would like to thank Dr. Sangeeta Desai, Officer-in-charge of the Molecular Pathology Division for her unflinching support and encouragement. We thank the technical staff – Rajani Bagal, Jyoti Bodake, and Sandeep Dhanavade for doing the fluorescent *in situ* hybridization tests with finesse and élan.

References

1. Thun MJ, DeLancey JO, Center MM, Jemal A, Ward EM. The global burden of cancer: Priorities for prevention. Carcinogenesis 2010;31:100-10.
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, *et al*. Cancer statistics, 2008. CA Cancer J Clin 2008;58:71-96.
3. Morris SW, Xue L, Ma Z, Kinney MC. Alk+CD30+lymphomas: A distinct molecular genetic subtype of non-Hodgkin's lymphoma. Br J Haematol 2001;113:275-95.
4. Janoueix-Lerosey I, Lequin D, Brugières L, Ribeiro A, de Pontual L, Combaret V, *et al*. Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. Nature 2008;455:967-70.
5. Griffin CA, Hawkins AL, Dvorak C, Henkle C, Ellingham T, Perlman EJ. Recurrent involvement of 2p23 in inflammatory myofibroblastic tumors. Cancer Res 1999;59:2776-80.
6. Rikova K, Guo A, Zeng Q, Possemato A, Yu J, Haack H, *et al*. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 2007;131:1190-203.
7. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, *et al*. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561-6.
8. Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, Holmes AJ, *et al*. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin Cancer Res 2008;14:4275-83.
9. Inamura K, Takeuchi K, Togashi Y, Nomura K, Ninomiya H, Okui M, *et al*. EML4-ALK fusion is linked to histological characteristics in a subset of lung cancers. J Thorac Oncol 2008;3:13-7.
10. Inamura K, Takeuchi K, Togashi Y, Hatano S, Ninomiya H, Motoi N, *et al*. EML4-ALK lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. Mod Pathol 2009;22:508-15.
11. Shinmura K, Kageyama S, Tao H, Bunai T, Suzuki M, Kamo T, *et al*. EML4-ALK fusion transcripts, but no NPM-, TPM3-, CLTC-, ATIC-, or TFG-ALK fusion transcripts, in non-small cell lung carcinomas. Lung Cancer 2008;61:163-9.
12. Martelli MP, Sozzi G, Hernandez L, Pettirossi V, Navarro A, Conte D, *et al*. EML4-ALK rearrangement in non-small cell lung cancer and non-tumor lung tissues. Am J Pathol 2009;174:661-70.
13. Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, *et al*. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol 2009;27:4247-53.
14. Wong DW, Leung EL, So KK, Tam IY, Sihoe AD, Cheng LC, *et al*. The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS. Cancer 2009;115:1723-33.
15. Rodig SJ, Mino-Kenudson M, Dacic S, Yeap BY, Shaw A, Barletta JA, *et al*. Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the western population. Clin Cancer Res 2009;15:5216-23.
16. Takahashi T, Sonobe M, Kobayashi M, Yoshizawa A, Menju T, Nakayama E, *et al*. Clinicopathologic features of non-small-cell lung cancer with EML4-ALK fusion gene. Ann Surg Oncol 2010;17:889-97.
17. Shaw AT, Yeap BY, Solomon BJ, Riely GJ, Gainor J, Engelman JA, *et al*. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: A retrospective analysis. Lancet Oncol 2011;12:1004-12.
18. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, *et al*. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 2011;6:244-85.
19. Varella Garcia M, Cho Y, Lu X, Baron AE, Terraciano L, Camidge DR *et al*. ALK gene rearrangements in unselected Caucasians with non-small cell lung carcinoma (NSCLC). J Clin Oncol 2010;suppl 28:18s. abstr 10533.
20. McDermott U, Iafrate AJ, Gray NS, Shioda T, Classon M, Maheswaran S, *et al*. Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase

inhibitors. Cancer Res 2008;68:3389-95.
21. Soda M, Takada S, Takeuchi K, Choi YL, Enomoto M, Ueno T, *et al*. A mouse model for EML4-ALK-positive lung cancer. Proc Natl Acad Sci U S A 2008;105:19893-7.
22. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, *et al*. Molecular Testing Guidelines for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors: Guidelines from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. Arch Pathol Lab Med 2013;137:828-860.
23. Camidge DR, Kono SA, Flacco A, Tan AC, Doebele RC, Zhou Q, *et al*. Optimizing the detection of lung cancer patients harboring anaplastic lymphoma kinase (ALK) gene rearrangements potentially suitable for ALK inhibitor treatment. Clin Cancer Res 2010;16:5581-90.
24. Mano H, Takeuchi K. EML4-ALK fusion in lung. Am J Pathol 2010;176:1552-3.
25. Kim H, Yoo SB, Choe JY, Paik JH, Xu X, Nitta H, *et al*. Detection of ALK gene rearrangement in non-small cell lung cancer: A comparison of fluorescence *in situ* hybridization and chromogenic *in situ* hybridization with correlation of ALK protein expression. J Thorac Oncol 2011;6:1359-66.

How to site this article: Desai SS, Shah AS, Prabhash K, Jambhekar NA. A year of anaplastic large cell kinase testing for lung carcinoma: Pathological and technical perspectives. Indian J Cancer 2013;50:80-6.
Source of Support: Nil. **Conflict of Interest:** None declared.