**ROS1 rearrangement and response to crizotinib in Stage IV non-small cell lung cancer**

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**ABSTRACT**

**Background:** The frequency of ROS1 rearrangement in non-small cell lung cancers has been reported from 1.6% to 2.3%. **Materials and Methods:** We examined 105 lung adenocarcinoma patients for ROS1 rearrangement which were negative for EGFR and anaplastic lymphoma kinase. Clinical characteristics of ROS1 rearranged patients and their responses to crizotinib therapy were studied. **Results:** Of the 105 patients, three cases were positive for ROS1 rearrangement by fluorescence in situ hybridization analysis. All of them showed heterogeneous pattern. All the 3 ROS1-positive patients were females in their forties and started on crizotinib. All of them responded to treatment. One of them developed resistance after 3 months. Another one showed marked systemic response but central nervous system lesions progressed. The third case is doing well till date with inactive lesions on positron emission tomography scan. **Conclusions:** The frequency of ROS1 rearrangement is low in non-small cell lung carcinoma, but their diagnosis offers patients an opportunity to receive highly effective targeted therapies.

**KEY WORDS:** Anaplastic lymphoma kinase, crizotinib, EGFR, ROS1

**INTRODUCTION**

The ROS1 rearrangement in non-small cell lung cancer (NSCLC) was discovered by Rikova et al.[1] The fusion partners include CD74, SLC34A2, SDC4, EZR, FIG, TPM3, LRRK3, KDELR2, LIMA1, MSN, CLTC, CCDC6, and TMEM106. Among these, CD74 is the most common fusion partner in NSCLC. The frequency of ROS1 rearrangement in NSCLC has been reported to range from 1.6% to 2.3%.[2]

The ROS1 oncogene encodes an orphan receptor tyrosine kinase related to anaplastic lymphoma kinase (ALK), along with members of the insulin receptor family.[3] ROS1 (ROS1 proto-oncogene receptor tyrosine kinase) is activated by chromosomal rearrangement in a variety of human cancers, including NSCLC, cholangiocarcinoma, gastric cancer, ovarian cancer, and glioblastoma multiforme.[1,4-7] Rearrangement leads to fusion of a portion of ROS1 that includes the entire tyrosine kinase domain with 1 of 13 different partner proteins.[8] The resulting ROS1 fusion kinases are constitutively activated and drive cellular transformation. Whether the various ROS1 fusion kinases may have different oncogenic properties is unknown. As ROS1 rearranged lung cancers are rare, they still remain poorly characterized aside from demographic features and fusion types. In particular, the histology of this type of cancers has not been widely investigated, and it is unclear whether this subset is associated with any characteristic.

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morphologic appearance. In addition, various modalities such as immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), RT-PCR, and NGS have been used in the diagnosis of these cancers.\(^9\) The present study determines the prevalence of ROS1 rearrangements in NSCLC through FISH in Indian patients. It also defines the clinicopathological characteristics, treatment response as well as prognostic impact of ROS1-positive NSCLC patients.

**MATERIALS AND METHODS**

ROS1 gene rearrangement was performed on 105, Stage IV-NSCLC patients. These were selected from 114 EGFR- and ALK-negative cases. Out of these 9 were rejected due to lack of tumor tissue. Their medical records were reviewed to extract data of clinicopathological characteristics, including age, sex, cancer stage, smoking history, histology, and treatment history. These cases were negative for EGFR and ALK mutations. EGFR gene mutation analysis in exon 18, 19, 20, 21 was done by real-time PCR using Qiagen Therascreen EGFR RGQ PCR kit and ALK protein expression was done on IHC using D5F3 antibody on Ventana Benchmark XT.

ROS1 was tested using FISH assays with Break Apart Probe set (ZytoLight SPEC ROS1 Dual Break Apart Probe ZytoVision GmbH, Germany), according to the manufacturer’s instructions. FISH measurements were performed using fluorescent microscope Leica DM 6000 B (Leica, Japan) equipped with three filters (DAPI/green/red). The diagnostic criteria for ROS1 rearrangement were as follows: (1) A minimum of 50 tumor cells were evaluated; (2) fused, split signals or isolated green/orange signals were detected; (3) rearrangement positive cell rate (%) = ([number of cells with a split pattern + number of cells with isolated 3'] [green] pattern/total number of cells evaluated) × 100, (4) rearrangement-positive cells constituted no <15% of the enumerated tumor cells were considered positive. ROS1 tyrosine kinase domain is encoded by 3’ part of the gene, the unpaired 3’ signal indicates the oncogenic relevant fusion gene, whereas the unpaired 5’ signal represents a likely nonfunctional reciprocal fusion product. Hence, isolated 5’ signals were not included in the total count.

**RESULTS**

Out of 105 cases, three cases are ROS1 positive on FISH. The FISH pattern of all positive cases was similar. All cases showed heterogeneous positivity. All of them were women with median age 44 and nonsmoker status.

Case 1: A 47-year-old female came to our hospital with the complaint of chest pain, backache, hoarseness, cough with expectoration, and loss of weight and appetite for 2 months. Computed tomography (CT) thorax on December 15, 2014, showed hilar bronchogenic mass encasing and obliterating lumen of middle lobe bronchus with atelectasis. Positron emission tomography (PET)-CT showed metabolically active right lung lesion (possible primary) with lymph nodal, adrenal, liver, bony, and right adnexa involvement. Biopsy from left cervical lymph nodes revealed on IHC, the tumor cells expressing napsin and thyroid transcription factor-1 (TTF-1), with focal expression of p63 confirming of metastatic adenocarcinoma of lung origin. ALK protein expression and EGFR mutation were negative. She was treated with chemotherapy and showed partial response after 3 months on PET-CT. Four months later, the patient had a progressive disease. ROS1 gene rearrangement by FISH was positive on June 12, 2015, with rearrangement in 72% of cells. Predominant pattern was one fused and two separate green and orange signals. The split was easily discernible. She was started on crizotinib 250 mg BD. Within 3 months, there was marked symptomatic relief with a significant reduction in the size of the mass lesion [Figure 1]. She was continued on tablet crizotinib 250 mg BD. She complained of a severe headache, nausea, and blurring of vision, slurred speech in October 2015. Magnetic resonance imaging brain showed multiple supra and infratentorial brain lesion suggestive of central nervous system metastases along with occipital bony lesion. The patient was keen to get enrolled into a clinical trial with carbozantinib and went to Singapore where she was evaluated and also found to have granulomatous tubercular lesion in the lung. She was put on ATT and came back to India for further management. She received external beam radiotherapy to posterior fossa. The possibility of putting her on immune checkpoint inhibitors was explored. PDL1 studies were done using SP142 clone and were positive in 25% of the tumor cells. In December 2015, she succumbed after suffering cardiac arrest.

Case 2: A 44-year-old nonsmoker female presented with complaints of cough with mucoid expectoration for 4 months. PET-CT showed metabolically active right lung lesion with mediastinal lymph node involvement. There were subcentric brain and bone lesions. Biopsy from right lung showed TTF-1 positive lung adenocarcinoma.
Her ALK, EGFR, MET status were negative. She received 3 cycles of cisplatin-based chemotherapy and external beam radiotherapy with significant response to lymph nodal disease and persistent residual right lung mass. ROS1 gene rearrangement by FISH was performed on April 29, 2016, and showed rearrangement in 48% of the tumor cells. The predominant pattern was one fused and two separate green and orange signals. She was started on crizotinib 250 mg BD. After 5 months, she showed marked symptomatic relief with lung and nodal disease decreasing markedly [Figure 2] in size and lesions becoming inactive. She had stable disease for 9 months with persistence of subcentimeteric brain lesion. Another right temporal lobe lesion developed for which she underwent surgical intervention with radiation. The repeat FISH testing on the brain sample also showed ROS gene rearrangement. She is doing well and continuing on crizotinib.

Case 3: A 43-years-old nonsmoker female came to our hospital on May 26, 2016, with complaints of chest pain, back pain, and vomiting for 15–20 days. PET-CT scan on May 26, 2016, showed metabolically active right lung lesion (possible primary) with lymph nodal and bony involvements along with metabolically active pericardial deposits. Forceps biopsy from left lung nodule revealed malignant cells weakly expressing both TTF-1 and P40. EGFR gene mutation, ALK protein expression, and MET amplification were negative. The patient received one cycle of cisplatin-based chemotherapy. In the meantime, ROS1 gene rearrangement was detected. It showed rearranged signals in 62% of the tumor cells with predominant one fused and two separate green and orange patterns [Figure 3]. The patient was started on crizotinib after the first cycle. She showed dramatic response after 5 months of treatment with the loss of PET avidity and shrunken tumor size. She is doing well till date (8 months) with no progression.

DISCUSSION

This study is the earliest study on ROS1 rearrangement from India. All tumors presented with a solid pattern on histology with TTF-1 positivity on IHC. Most ROS1 rearranged tumors are TTF-1 immunoreactive, but it is far from specific. Many other molecular subtypes of lung adenocarcinoma, such as EGFR mutant, also label for this marker.[10,11] All cases were Stage IV relatively young females in their forties, nonsmokers and negative for other molecular markers, namely, EGFR and ALK.[12]

FISH is a robust technique of identifying ROS-1 rearrangement. All positive cases showed diffuse positivity. This finding along with mutual exclusivity with other markers points toward the driver status of ROS1. This gene maps at 6q22.1 and known to be activated by multiple partners. Most of these partners are mapped in chromosomes other than 6; thus, the molecular fusion is either translocation or insertion. In contrast, five partner genes (HLA-A, GOPC, CEP85 L, TPD52 L1, and EZR) are located in chromosome 6 where deletions, duplications and inversions lead to fusions. Some of these may be challenging on FISH and may lead to false-negative results.[13,14] Next generation sequencing/RT-PCR appear to be promising in these scenarios.[15]

Based on a median duration of response of 17.6 months and median progression-free survival of 19.2 months in PROFILE 1001 trail (NCT00585195), US Food and Drug Administration rapidly approved the use of crizotinib for ROS1-positive cases on March 11, 2016. This overwhelming response rate of 72% among lung cancer was identified by break-apart FISH assay.[16] ROS1 rearrangement defines a second molecular subgroup of NSCLC besides ALK rearrangement for which crizotinib is
highly active. Most of the amino acid differences between ALK and ROS1 are in conservative regions or do not contact crizotinib. Only a valine to leucine difference at codon 1180 of ALK and codon 2010 of ROS1 is predicted to have an effect on binding. A larger leucine in ROS1 extends closer to and makes more direct contact with crizotinib. This probably might explain increased efficacy and longer survival of ROS1-positive patients as compared to ALK in larger cohorts.\textsuperscript{[17]}

All three of our positive cases received crizotinib. Case 2 and 3 showed significant response with stable disease till date. Case 2 had ROS1-positive brain metastasis, which did not respond to treatment, owing to lack of crizotinib to cross blood brain barrier. This opens the need for TKIs that do not respond to treatment, owing to lack of crizotinib.

This paves the way for the next generation of more selective tyrosine kinase inhibitors, such as entrectinib, ceritinib, and PF-06463922, which have been shown to be effective in preclinical models.\textsuperscript{[10-21]}

**CONCLUSIONS**

Albeit a small number, this study is indicating an incidence of up to 2.8% (3 out of 105) in ROS1 rearrangement of lung adenocarcinoma in Indian population. Although the frequency of these genomic aberrations is low, their diagnosis offers patients with lung cancer an opportunity to receive highly effective targeted therapies.

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**Conflicts of interest**

There are no conflicts of interest.

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