The use of Fluorescence in Situ Hybridization (FISH) in detecting ROS1 gene rearrangements in Non Small Cell Lung Cancer.

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INTRODUCTION

Lung cancer is the second most common cancer with an estimated 220,000 new cases in 2011. CDC statistics also suggest that approxi-
mately 157,000 deaths will be caused by lung cancer which makes it more deadly than colon, breast and prostate cancers combined. About 85% of all lung cancers are categorized as non-small cell lung cancer (NSCLC) including adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. There are tests available for a number of genes known to be involved in NSCLC tumorigenesis including EGFR, KRAS, BRAF, HER2, and ALK. Recently, ROS1 (c-ros oncogene 1, receptor tyrosine kinase) gene rearrangements have been identified in patients with NSCLC. Located on chromosome 6, ROS1 gene rearrangements have been found in 1-2% of all NSCLC patients. Rearrangements of the ROS1 gene leads to kinase activation and subsequent up-regulation in the cell proliferation pathway and reduced apoptosis. Recent literature sug-
gests that Xalkori (crizotinib) a kinase inhibitor could be considered as a treatment for NSCLC patients that harbor ROS1 gene rearrangements. In January, 2013 ROS1 was mentioned in the NCCN guidelines under di-
agnostic and treatment options for patients with NSCLC. ROS1 has multi-
pie rearrangement partners making a ROS1 break apart FISH probe set the most cost-effective strategy for identifying gene rearrangements in paraffin embedded tissues. We present our data on ROS1 gene rear-
rangements in NSCLC tumor samples. Of note is the observation that ROS1 shows similar FISH signal patterns, both normal and abnormal, to what has been reported in ALK-rearranged NSCLC tumors.

RESULTS

Probe Validation for the ROS1 probe from MetaSystems, Altlussheim, Ger-
many, was conducted on at least 13 normal specimens relabeled for ade-
nocarcinoma or Non-Small Cell Lung Cancer to establish normal cutoff values. One abnormal specimen was run to confirm 100% sensitivity. Five metaphase spreads were probed and karyotyped with G- banding to confirm probe localization to 6q22 (Figure 3), a 370kb 3’ segment distal to the ROS1 gene (GREEN signal) and a 370kb 3’ segment proximal to the ROS1 gene (RED signal). Cutoff values for (3F or 1F) are calculated at 3 standard deviations from the average for each signal pattern observed. The germline (non-rearranged) normal signal pattern is two orange-green fusion signals (2F). The typical abnormal signal pattern includes one green, one orange and one green-orange fusion signal (1R1G1F) indicating a chromosome break in the ROS1 locus. Some variant or atypical abnormal signal patterns include 1R1F, 2R1G1F, 2R1G2F, and 1R2G1F. We present our data collected from 100 different patients that ROS1 probe set and ROS1 probe set were performed on paraffin embedded lung tissues. This data shows how many cells were counts as normal (0F) or abnor-
mal (1R1G1F, 1R1G1F, 1G1F, nF or nF) in each probe set (see Table 1). For ROS1, the average number of normal signal patterns were 82.7 and 13.8, respectively. For ALK, the average number of 1F pattern was 3.0 and the 3F signal pattern was 20.6. The number of samples that showed a hyperdiplo-
dy pattern for both probe sets was 36%. Only 2 samples were confirmed positive for a ROS1 gene rearrangement and 3 were positive for ALK.

DISCUSSION

Lung cancer is estimated to cause over 150,000 deaths and is the second most common cause of cancer. NSCLC represents about 85% of lung cancer cases and finding treatment for this condition is a medical necessity. Literature suggests patients with ALK or ROS1 rearrangements have an increased survival if treated with Xalkori (crizotinib). Herein, using FISH to identify this molecular abnormality is critical.

In this study, we report our ALK and ROS1 FISH analysis on tumor biopsies from 100 patients relabeled for NSCLC. Tissues were sectioned and hybridized using on an FDA protocol for ALK and a laboratory developed test for ROS1. We found a single fusion signal pattern in approximately 3% for ALK probe set and 8% for ROS1 in our sample population. Approximately 20% of sam-
ples showed hyperdiploidy (n>3F) for ALK and 14% had hyperdiploidy for ROS1. Of the 71 samples we tested for both ALK and ROS1, approximately 26% had hyperdiploidy of at least 1XALK or 1XROS1. This would suggest that copy number gains in NSCLC is common. For ALK, we found that 3 of 100 cases were positive for ALK gene rear-
rangements of greater than 1%, however we found seven (7) cases that had positive ALK rearrangements in 14% of nuclei analyzed. Because re-
duction in the treatment using Xalkori (crizotinib) was only predicted to show response in patients with 15% rearrangements, patients within the 8-14 range were not eligible. For ROS1, we found 2% of cases had greater than 1% gene rearrange-
ments, another 2 of 100 samples showed greater than 8% abnormality which would also prevent them from receiving Xalkori (crizotinib) treat-
ment. None of the patients were positive for both ALK and ROS1, gene rear-
rangements, confirming that the rearrangements are mutually exclusive.

CONCLUSIONS

In this study of 100 cases of NSCLC cases relabeled for ALK and ROS1 testing, we found an abnormality rate of 3% and 2%, respectively. However, we found that rearrangements of 8% or greater was found in 7 samples for ALK and 2 for ROS1 which would exclude them from being eligible for Xalkori (crizotinib) treat-
ment. None of the patients were positive for both ALK and ROS1, gene rear-
rangements, confirming that the rearrangements are mutually exclusive.

REFERENCES


ABSTRACT

Lung cancer is one of the leading causes of cancer deaths worldwide. Approximately 200,000 new cases were identified in 2011. The Centers for Disease Control and Prevention suggest that over 157,000 lung cancer deaths will occur making lung cancers frequency higher than that of colon, breast and prostate cancer combined. Non small cell lung cancer (NSCLC) represents approximately 85% of all cases of lung cancer. NSCLC is represented by adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. A number of genes are known to be involved in lung cancer tumorigenesis including EGFR, KRAS, BRAF, HER2, and ALK. New research suggests that ROS1, formally known as c-ros oncogene 1, is a rece-
tor tyrosine kinase located on chromosome 6 that is an oncogene when mutated/rearranged causes lung cancer. ROS1 gene rearrangements can be detected by FISH analysis and are reported at a frequency of 1-2% of known NSCLC cases. The method to detect ROS1 gene rearrangements involves hybridizing FISH probes that flank the ROS1 gene. This FISH test is performed on formalin fixed paraffin embedded FFPE lung tissue and allows the detection of the majority of ROS1 gene rearrange-
ments reported in NSCLC. The ROS1 gene shares similar biologic activity with ALK, including predominant histology and patient demographics (young, never-smokers, increased frequency of Asians). Pre-clinical studies and early clinical reports show ROS1-rearranged tumors are sensitive to crizotinib, a kinase inhibitor and demonstrates response rates comparable to ALK rearranged tumors. FISH testing has become crucial in the diagnosis of ROS1 positive NSCLC cases.