Frequencies of ALK and ROS in NSCLC FFPE tumor samples utilizing a highly specific and sensitive immunohistochemistry-based assay and FISH analysis.

Receptor tyrosine kinase ALK and ROS are expressed as oncogenic fusion proteins in approximately 2-5% of all NSCLC patients. ALK and ROS targeted therapeutics are currently in development thus identifying patients with pathologic ALK and ROS expression is necessary for selecting patients in clinical trials. Currently, molecular assays such as fluorescence in situ hybridization (FISH) and RT-PCR are used to identify these patients. We reasoned that an immunohistochemistry-based assay could provide a fast, inexpensive alternative for diagnosing patients with ALK and ROS abnormalities. We have developed a reliable immunohistochemistry-based assay for the detection of low level ALK and ROS in NSCLC patients with high specificity and sensitivity.

Study Goals:
- Determine ALK and ROS frequencies in a Chinese tumor set
- Develop an ALK and ROS IHC based assay and validate by FISH analysis

Materials and Methods:

Human NSCLC Tumor Tissues: A Tissue bank was provided by the Second Xiangya Hospital, Central South University, Changsha, Hunan, P.R. China. Human samples of NSCLC paraffin blocks were provided by the Second Xiangya Hospital.

Immunohistochemistry: 4 µm tissue sections were deparaffinized and rehydrated through xylene and graded ethanol, respectively. Slides were blocked in 3% hydrogen peroxide in Tris-buffered saline (pH 7.6) for 30 minutes, then washed in phosphate-buffered saline (PBS). The sections were incubated with 0.1% trypsin in PBS at 37°C for 10 minutes. After blocking in Tris buffered saline (pH 7.6), the samples were incubated overnight at 4°C with ROS (D4D6) XP mAb at 0.19 µg/ml diluted in SignalStain® + 0.5% Tween-20 (TBST)/5% goat serum in a humidified chamber. Slides were developed for 1 minute then rinsed in diH2O. Slides were dehydrated and cleared. Then coverslipped.

Immunohistochemistry using ALK D5F3 monoclonal antibody:

- ALK IHC (+) ALK FISH (+)
- ALK IHC (-) ALK FISH (-)

Results:

<table>
<thead>
<tr>
<th>ALK Antibody</th>
<th>Comparison of IHC and FISH results:</th>
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<tbody>
<tr>
<td>ALK IHC (+)</td>
<td>False Positive</td>
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<tr>
<td>ALK IHC (-)</td>
<td>Not scoreable by FISH</td>
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</tbody>
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Conclusions:

- In NSCLC, ALK and ROS are expressed as oncogenic fusion proteins in approximately 2-5% of all NSCLC patients. ALK and ROS targeted therapies are currently in development thus identifying patients with pathologic ALK and ROS expression is necessary for selecting patients in clinical trials.
- We reasoned that an immunohistochemistry-based assay could provide a fast, inexpensive alternative for diagnosing patients with ALK and ROS abnormalities.
- We have developed a reliable immunohistochemistry-based assay for the detection of low level ALK and ROS in NSCLC patients with high specificity and sensitivity.