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

Receptor tyrosine kinases 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. 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
- Develope an ALK and ROS IHC based assay and validate by FISH analysis

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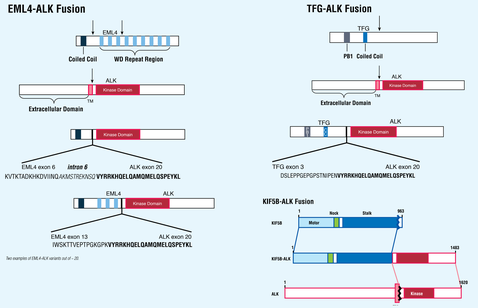
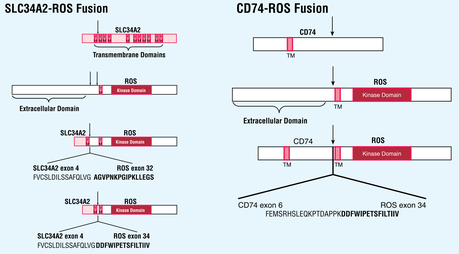
ALK and NSCLC:

- EML4-ALK fusion present in ~ 5% lung adenocarcinoma cases (1,2)
- ALK-KIF5B and ALK-TFG also described (1,3)
- ALK inhibitors show clinical response in patients with ALK-rearranged tumors (4)
- ALK FISH is difficult to interpret leading to false negative results
- IHC using monoclonal antibody D5F3 reliably detects low level ALK in lung adenocarcinomas (5)



ROS in NSCLC:

- SLC34A-ROS and CD74-ROS fusions first identified in large scale survey of tyrosine kinases in lung cancer (1)
- Ros fusions are generated by chromosomal translocations
- ROS kinase inhibitors in development (6)
- Patient response to ROS inhibitors still to be determined

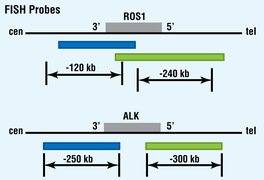


Materials and Methods:

Human NSCLC Tumor Tissues: IRB approval was granted by the Second Xiangya Hospital, Central South University (Chansha, Hunan, P.R. China). Human samples of NSCLC paraffin blocks were provided by Second Xiangya hospital.

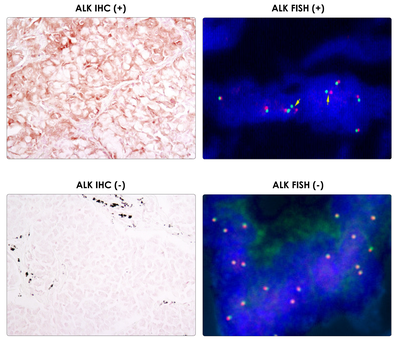
Immunohistochemistry: 4-6 µm tissue sections were deparaffinized and rehydrated through xylene and graded ethanol, respectively. Slides were rinsed in diH2O, then were subjected to antigen retrieval in a Decloaking Chamber (Biocare Medical, Concord, CA) using 1.0 mM EDTA, pH 8.0. Slides were quenched in 3% H2O2 for 10 minutes, then washed in diH2O. After blocking in Tris buffered saline + 0.5% Tween-20 (TBST)/5% goat serum in a humidified chamber, slides were incubated overnight at 4°C with Ros (D4D6) XP™ Rabbit mAb at 0.19 µg/ml diluted in SignalStain® Antibody Diluent (#8112 Cell Signaling Technology, Danvers, MA). After washing with TBST, detection was performed with Envision+ (Dako, Carpinteria, CA). After washing slides were exposed to NovaRed (Vector Laboratories, Burlingame, CA) prepared per the manufacturer's instructions. Slides were developed for 1 minute then rinsed in diH2O. Slides were dehydrated and cleared. Then coverslipped.

Fluorescence in situ hybridization: FISH was done on 4-µm thick FFPE tissues sections. ALK was analyzed with the use of break-apart probe specific to the ALK locus (Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe; Abbott Molecular). ROS break-apart probe was developed in house utilizing bacterial artifical chromosomes: RP1-179P9, RP11-323017 and RP1-94G16 purchased from Invitrogen. BACS were labeled with orange and green dUTPS with the nick end translation kit (Enzo). FISH-positive cases were defined as >15% split signals in tumor cells.

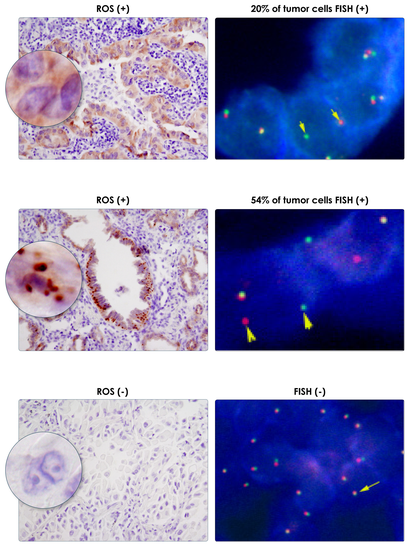


Results:

FISH Validation of IHC using ALK D5F3 monoclonal antibody:



FISH Validation of IHC using ROS D4D6 monoclonal antibody:



Comparison of IHC and FISH results:

	FISH +	FISH -	IHC +	IHC -
ROS (# of Lungs)	7	136	10	646
ALK (# of Lungs)	24	266	27	629

All IHC positive tissues were analyzed by FISH.
Additional arrays were scored to generate negative data.

ROS Antibody	# of Lungs
False Positive	1
Not scoreable by FISH	1
TBA	1

ALK Antibody	# of Lungs
False Positive	0
Not scoreable by FISH	3

Comparison of IHC Results and FISH Analysis	ROS	ALK
Sensitivity	100%	100%
Specificity	99.3%	100%

Frequencies of ROS and ALK	ROS	ALK
Positive Cases	7/656	24/656
Frequency	1.1 – 1.4%*	3.8%

*Frequency was calculated as a ratio of FISH (+) and IHC (+) tumors out of the total number of samples. *Pending FISH follow up work.*

References:

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3. Takeuchi, K. (2009) *Clin. Cancer Res.* 15, 3143–3149.
4. Kwak, E.L. (2009) *J. Clin. Oncol.* 27:8 abstr 3509.
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Conclusions:

ALK is positive in 3.8% of NSCLC Chinese tumor set

ROS is positive in 1.1-1.4% of NSCLC Chinese tumor set

IHC has been proven to be a sensitive and specific assay for identifying ROS and ALK positive patients