Overview

• While FISH is the standard diagnostic test to detect ALK, ROS1 or RET translocation, it is low-throughput and performing FISH on multiple targets is tissue-consuming. Therefore, a higher-throughput multiplex method is necessary especially for the detection of oncogenic drivers of low frequencies (ALK rearrangement incidence rate: 2-5%; ROS1 and RET: 1-2%).

• Quantitation of protein may provide a more relevant measure of the ALK pathway. Therefore, a specific, objective, sensitive, and accurate proteomics-based quantitative assay would be ideal.

• In this report, we developed a clinically-validated multiplex MS assay to quantify ALK, ROS1, and RET protein levels from formalin-fixed paraffin-embedded (FFPE) NSCLC tissues.

• We are running the assay in a CLIA-certified-CAP-accredited laboratory to concurrently assess protein expression levels for translocation markers and several diagnostic and potentially targetable biomarkers, e.g. TTF1, K7, p63, K5, EGFR, HER2, HER3, MET, KRAS and IGF1R, from NSCLC biopsies.

ALK Fusion Proteins Identified in NSCLC

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breakpoint: exon 20 of ALK (ex 1505-1119)

ALK tyrosine kinase domain


Results

Translocation Markers- SRM Assay Development

Quantitation of ALK and ROS1 in Rearrangement Positive and Negative Tissues

Comparison of SRM with ALK DNA Sequence, FISH, and IHC in DH9

Analysis of Lung OncoPlex in FFPE NSCLC Tissues

Figure 1: Liquid Tissue®-SRM workflow for analysis of proteins from FFPE tissue.

Figure 2: Calibration curve of translocation markers. The calibration curve was built using various concentrations of an unlabeled synthetic peptide (light) into a matrix containing 5 fold of isotope-labeled peptide. Assay parameters are summarized in the table.

Figure 3: Summary of the expression of translocation markers in eighteen FFPE NSCLC tissues and H3122 cells. ALK or ROS1 rearrangement status is listed and samples were analyzed by mass spectrometry to quantify the expression of ALK and ROS1 protein. Analytes were quantitated in triplicate 1 μg injections. Pinpoint spectra on the right represented highlighted rows in the table.

Figure 4: Comparison of SRM with ALK FISH, IHC and DNA sequencing in 11 samples. The table summarizes SRM data, sequencing results for ALK peptide-encoding region, and FISH/ IHC status. The upper figures represent FISH and paired IHC for DH4 and DH9. In both cases, ALK FISH testing shows deletion of the 5' (green) signal with retained 3' (orange) signal consistent with ALK rearrangement. Arrows indicate the re-arranged red signal. ALK IHC, however, is negative in DH9.

Figure 5: NSCLC tissue expression for each of the targets within the Lung OncoPlex as a multiplex analysis, sorted by K7 expression from low to high, left to right. The 87 samples represent a mixture of 12 ALK rearrangement positive controls and a cohort of 75 ALK negative NSCLC. The Lung OncoPlex assay not only confirmed pathologist’s subtyping but also quantified the other potentially targetable biomarkers.

Conclusions

• We have developed a quantitative mass-spectrometry-based assay for ALK and ROS1 to evaluate protein expression level in FFPE samples.

• Including these markers within the Lung OncoPlex assay allows simultaneous assessment of multiple clinically actionable gene rearrangements and biomarker targets.

• The multiplexed proteomic screening of patient tissue could be performed at the time of initial biopsy to maximize information in limited tissue. Clinicians could use the information to strategically order appropriate tests, leading to the best patient care.