Quantification of ALK from Non-Small Cell Lung Cancer (NSCLC) FFPE Tissue by Targeted Mass Spectrometry

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Overview

- ***ALK* break-apart FISH** is the FDA approved diagnostic test to detect *ALK* translocations, but, is laborious, expensive, and often challenging to interpret.
- **ALK IHC** is faster and more cost-efficient method to detect ALK protein expression; many reports have demonstrated excellent correlation between ALK IHC and *ALK* FISH results.
- **Mass Spectrometry-based SRM assay** simultaneously quantifies multiple biomarker proteins in FFPE tissues and avoids the triage of the specimens for FISH or IHC test to ensure all patients who may benefit from targeted therapy receive optimal treatment as early as possible.

Methods

Eighteen proteins were quantified in 18 FFPE NSCLC tissues

Figure 1. OncoPlex Diagnostics Liquid Tissue® technology platform

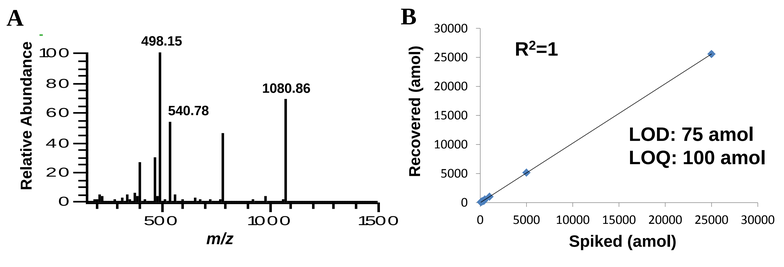


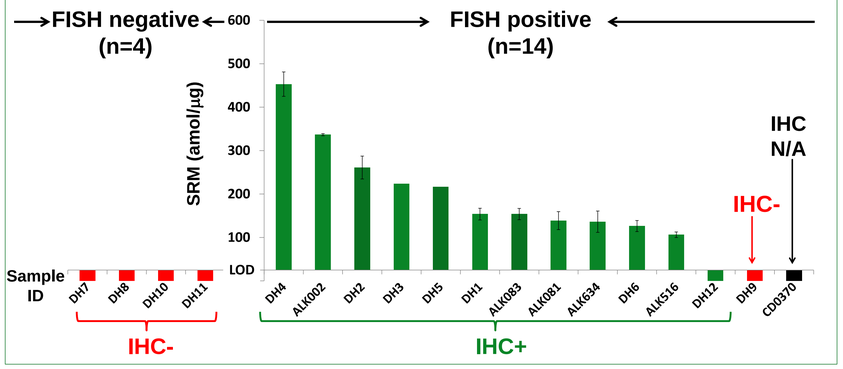
Figure 2. Development of ALK SRM assay. The fragmentation spectrum for ALK peptide (A) and the standard curve generated in *pyrococcus* complex matrix (B).

Currently Available Assays from OncoPlex Diagnostics

ALK	ROS1	RET	EGFR	HER2	HER3	MET	IGF1R	RON	PD-L1
RRM1	hENT1	ERCC1	XRCC1	TOPO1	TOPO2A	TS	TUBB3	SPARC	AR
FRalpha	DHFR	GARFT	FPGS	IDO1	MGMT	K5	K7	P63	TTF1
KRas-total	KRas-4B	AXL	MCL1	MDR1	MRP1	FGFR1	FGFR2	FGFR3	CD30
IRS-1	INSR	BRAF	Cav-1	Cbl	Paxillin	DR5	Ki67	E-Cad	Vimentin
MSLN	MUC1	Napsin	p16	PR	PSMA	PTEN	CAT	NQO1	Warburg

Results

Positive Correlation of ALK SRM with FISH and IHC in 18 Clinical FFPE Lung Tumors



SRM and IHC Confirm the Lack of ALK Protein in a FISH+ Crizotinib Non-Responder (DH9)

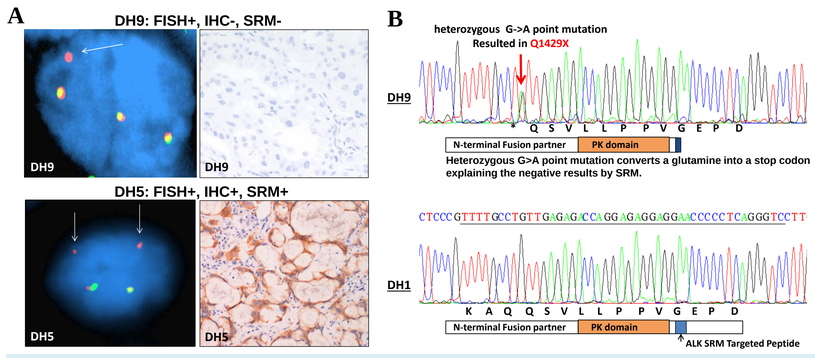
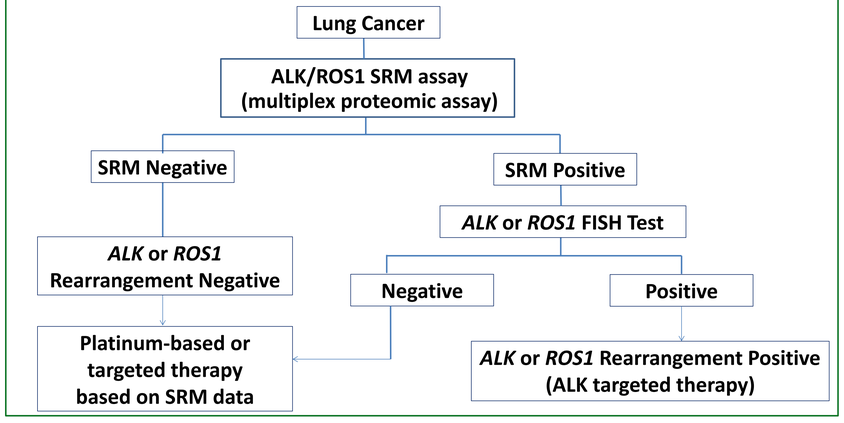


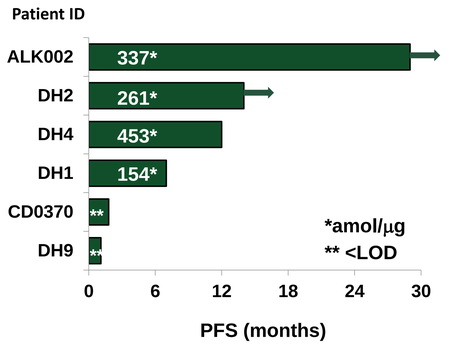
Figure 3. Comparison of SRM with *ALK* FISH, IHC, and DNA sequencing. FISH and paired IHC for patients DH9 and DH5. Arrows indicate the re-arranged red signal (A). The FISH positive, SRM and IHC negative case (DH9) showed a point mutation that resulted in a stop codon; therefore, non-functional protein would be produced (B).

Potential ALK (and ROS1) Testing Algorithm in NSCLC



Results continued

Patient Responses to Crizotinib in *ALK*-Rearranged NSCLC



Crizotinib-treated patients:

Patient ID#	Crizotinib Treatment	Overall Response
ALK002	Yes	PFS=29mos; initial PR, then CR, and currently NED
DH2	Yes	PFS=14mos; then NED
DH4	Yes	PFS=12mos; then AWD
DH1	Yes	PFS=7mos; loss to follow-up
CD0370	Yes	PFS<2mos
DH9	Yes	PD; DOD
DH3	Yes (5mos)	PFS=22mos; then NED. Independent of crizo because pt. was on crizo for only 5 mos
DH12	Yes	PR for 10mos; DOD

Figure 4: Duration of response among patients treated with crizotinib. Arrows indicate patients who are recently assessed to be NED (CR). Numbers in the bar indicate ALK protein expression by SRM. Highlighted rows in the table represent samples in figure above.

PFS: progression free survival; CR: complete response; NED: no evidence of disease ; AWD: alive with disease; PD: progressive disease; DOD: dead of disease; PR: partial response.

Multiplex SRM Analysis of Biomarkers in FFPE NSCLC Tissue Identifies MET as a potential target for crizotinib in DH12

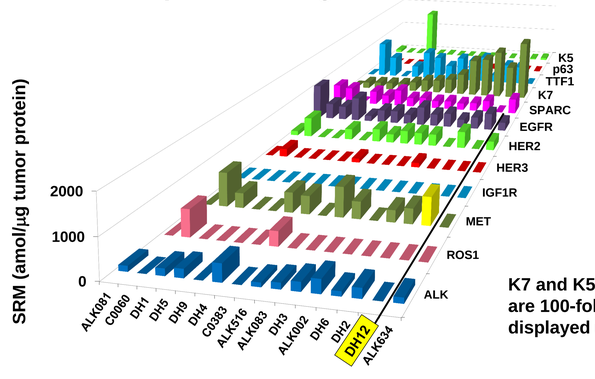


Figure 5: NSCLC tissue expression for each of the targets as a multiplex analysis, sorted by K7 expression from low to high, left to right. The 15 samples represent a mixture of 13 *ALK* rearrangement positive controls and 2 *ROS1* rearrangement positive controls.

Conclusions

- The OncoPlex Diagnostics SRM assay identifies ALK positive patients who responded to crizotinib treatment; SRM positive cases would be reflexed to FISH for confirmation.
- Multiplex SRM assay identifies other proteins (e.g. ROS1 and MET) that are known targets to crizotinib therapy and may be responsible for patient's response.
- Multiplex screening of patient tissue at the time of initial biopsy maximizes information in limited tissue and provides the clinician with valuable, actionable diagnostic information.