ALK gene rearrangement testing in non-small cell lung carcinoma: correlation between ultrasensitive IHC and FISH.

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Abstract

Introduction / Background: Anaplastic lymphoma kinase (*ALK*) gene rearrangements in advanced non-small cell lung carcinomas (NSCLC) are an indication for targeted therapy with ALK-specific inhibitors, including crizotinib. *ALK* rearrangement status is commonly assessed by fluorescence in-situ hybridization (FISH) using the IVD class FISH system approved by the US FDA as a companion diagnostic tool for crizotinib-based treatment eligibility {Abbott Molecular Vysis (AMV)}. The utility of IHC, a more affordable and accessible method, has been challenged by low protein expression levels of ALK fusion transcripts in NSCLC. Here we assessed a modified ultrasensitive IHC method as an alternative to FISH for detecting *ALK* status in a NSCLC case series at our institution.

Materials and Methods: The study included 318 formalin-fixed paraffin-embedded samples (FFPE) from 296 patients with advanced NSCLC clinically referred for *ALK* testing. IHC was performed using a recently developed rabbit monoclonal antibody to the ALK protein c-terminus domain preserved in all known pathologic *ALK* fusions (D5F3, a generous gift from Cell Signaling Technology) linked to an ultrasensitive multimer-based detection system (OptiView DAB IHC Detection Kit, a generous gift from Ventana Medical Systems). To increase reproducibility, IHC staining was interpreted as positive or negative rather than scored on a semi-quantitative scale. FISH was performed using the AMV *ALK* Break Apart FISH Probe Kit (Abbott Molecular, USA). In a subset of cases, FISH was also performed on matched available ThinPrep material.

Results: Of 198 samples negative for *ALK* rearrangements by FFPE FISH, all were also negative for ALK expression by IHC. Of 33 samples positive by FFPE FISH, 31 were also positive by IHC. Both discordant cases approximated the FISH cut-off of 15% positive cells (15% and 18%) and were resolved by ThinPrep FISH as negative, in agreement with the initial IHC result. Overall, after discordance resolution, IHC demonstrated 100% sensitivity and specificity, and perfect agreement with FISH in detecting *ALK* status in NSCLC samples.

Conclusions: Ultrasensitive IHC can reliably detect *ALK*-encoded protein over-expression resulting from *ALK* gene rearrangements in NSCLC. The very high concordance between IHC and FISH warrants the routine use of IHC as the initial component of an algorithmic approach to clinical *ALK* molecular testing in NSCLC, followed by reflex FISH confirmation of IHC-positive cases.

Background

- Approximately 2-13% of NSLCL harbor *ALK* gene rearrangements, most commonly inv(2)(p21;p23), resulting in oncogenic ALK fusion kinase products, like ALK/EML4 (Figure 1).
- *ALK* rearrangements in advanced NSCLC are an indication for targeted therapy with ALK-specific inhibitors, including crizotinib.
- *ALK* rearrangement status is commonly assessed by FISH using the IVD class FISH system approved by the US FDA as a companion diagnostic tool for crizotinib-based treatment eligibility {Abbott Molecular Vysis (AMV)} (Figure 1).
- FISH for *ALK* rearrangements is often difficult to interpret due to probe binding design, requires a minimum number of tumor cells for informative results, allows limited morphologic evaluation and is expensive.
- The utility of IHC, a more affordable and accessible method, has been challenged by low protein expression levels of ALK fusion transcripts in NSCLC.
- We assessed a modified ultrasensitive IHC method as an alternative to FISH for detecting *ALK* rearrangements in a NSCLC case series at our institution (Cleveland Clinic Foundation).

Design

- We evaluated 318 FFPE samples from 296 patients with advanced NSCLC clinically referred for ALK testing.
- IHC for ALK overexpression was performed on all samples with the D5F3 rabbit monoclonal anti-human CD246 antibody recognizing the ALK c-terminus domain preserved in all known pathologic *ALK* fusions (Figure 1) (Cell Signaling Technology) linked to OptiView DAB IHC Detection Kit, an ultrasensitive multimer-based detection system (Ventana Medical Systems) (Figure 2a).
- To increase reproducibility, IHC staining was interpreted as positive or negative rather than scored on a semi-quantitative scale.
- FISH was performed using the AMV ALK Break Apart FISH Probe Kit (Abbott Molecular, USA) on all 318 FFPE samples (Figure 2b,c) and on 40 available matched ThinPrep preparations (Figure 2d).
- The cutoff of 15% positive cells was used for both FFPE FISH and ThinPrep FISH.

Figure 1. Schematic representation of inv(2)(p21;p23), and the detection of ALK/EML4 by FISH (red-green break-apart probes) and IHC (D5F3 antibody).

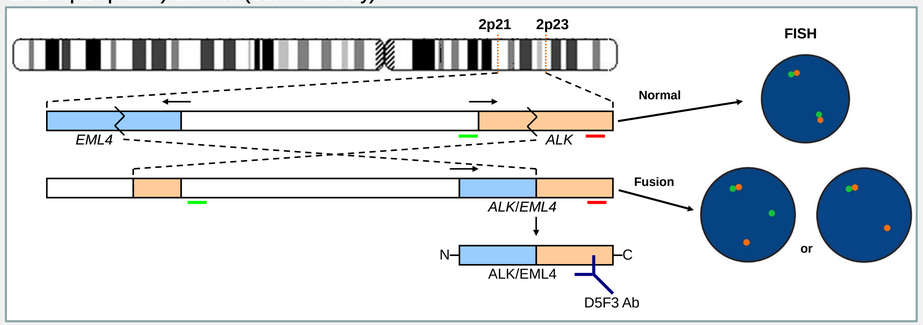

Figure 2. Representative images of NSCLC samples positive for ALK overexpression by D5F3-IHC (a), and for *ALK* rearrangements by FFPE-FISH (b, c) and ThinPrep-FISH (d).

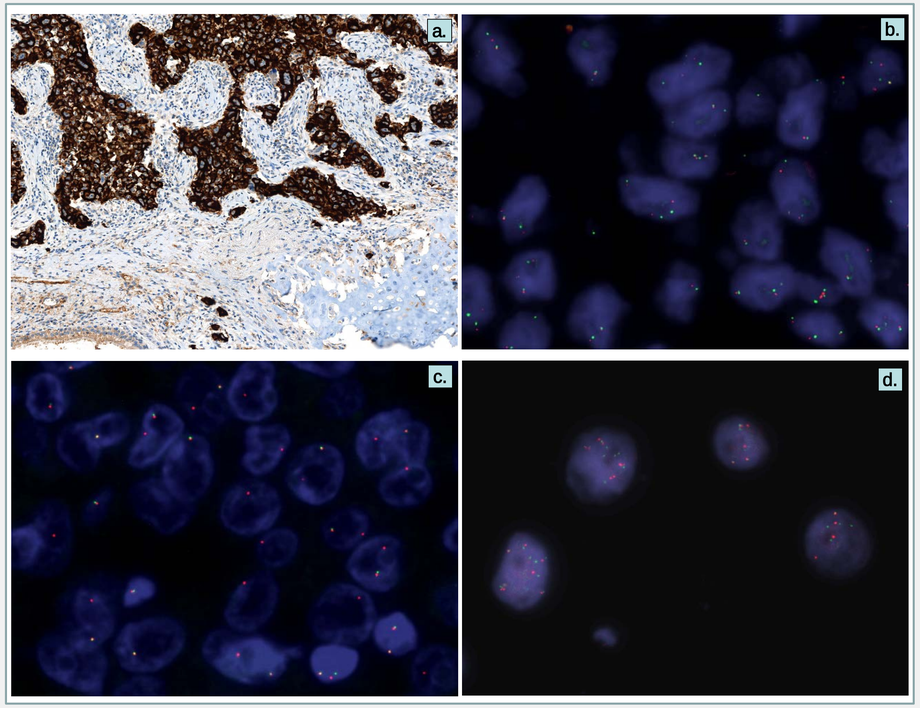

Figure 3. Concordance between IHC and FISH in detecting ALK status:
- with FFPE-FISH after discordant resolution by ThinPrep-FISH (a);
- with ThinPrep-FISH on cases with uninformative FFPE-FISH (b).

a.

ALK D5F3 IHC	FISH Positive	FISH Negative	Total
Positive	31	0	
Negative	0	200	
			231
Sensitivity	100.0%		
Specificity	100.0%		

b.

ALK D5F3 IHC	ThinPrep-FISH Positive	ThinPrep-FISH Negative	Total
Positive	1	0	
Negative	0	17	
			18
Agreement	100.0%		

Results

- 235/318 (73%) FFPE-FISH results and 304/318 (95%) IHC results were informative;; 231/318 (72%) samples had paired informative results.
- Of 198 samples negative for *ALK* rearrangements by FFPE-FISH, all were also negative by ALK IHC.
- Of 33 samples positive by FFPE FISH, 31 were also positive by IHC. Both discordant samples were FFPE cytopathology cell block specimens, and approximated the FISH threshold of 15% (15% and 18%). Confirmatory FISH on matched available ThinPrep material was negative, in agreement with the IHC result.
- Overall, after discordance resolution, IHC demonstrated 100% sensitivity and specificity, and perfect agreement with FISH in detecting ALK status in NSCLC samples (Figure 3a).
- Within the FFPE-FISH uninformative cases, ALK IHC was 100% concordant with available ThinPrep FISH on 18 matched samples (17 negative, 1 positive) (Figure 3b), and was positive on additional 3/55 cases with uninformative FFPE or ThinPrep FISH.

Conclusions

- Ultrasensitive IHC is highly concordant with FISH in detecting ALK-status in NSCLC.
- IHC can detect ALK-overexpression in NSCLC samples not qualitatively adequate for FISH.
- The high concordance between IHC and FISH warrants the routine use of IHC as the initial component of an algorithmic approach to clinical ALK molecular testing in NSCLC, followed by reflex FISH confirmation of IHC-positive cases.

References

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