

Association for Molecular Pathology: Solid Tumors Reviews

ALK Rearrangement in Non Small Cell Lung Carcinoma

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Indication

Detection of *ALK* gene fusions is currently indicated as a predictive marker of treatment response to small molecule inhibitors of *ALK* in non-small cell lung carcinomas (NSCLC). A recent Phase I study evaluating the role of crizotinib (PF-02341066 or PF-1066), a potent dual inhibitor of *ALK* and *MET*, in NSCLC found a complete or partial response in the majority of *ALK*-rearranged tumors(1). An international randomized Phase III study comparing the efficacy of crizotinib therapy to standard second-line chemotherapy in *ALK* FISH positive advanced NSCLC patients is underway.

Background

Transforming rearrangements of the anaplastic lymphoma kinase (*ALK*) gene have been recognized in a subset of human hematological and solid malignancies (2). These in-frame gene rearrangements place the *ALK* kinase domain under the promoter control of another gene with its intra-cellular localization and function potentially influenced by the specific N-terminal of the fusion partner. In 2007, an *ALK* gene rearrangement creating an in-frame fusion protein between echinoderm microtubule-associated protein-like 4 (*EML4*) and *ALK* was described in NSCLC (4). The novel fusion gene arose from an inversion on the short arm of chromosome 2 [inv(2)(p21p23)] that joined exons 1–13 of *EML4* to exons 20–29 of *ALK*. In consequence, the *ALK* tyrosine kinase domain is constitutively activated as well as the downstream signaling pathways of MAPK, PI3K/AKT, and STAT3 (4-6). Since then, numerous transforming in-frame fusion variants involving different breakpoints in *EML4* and *ALK* have been described in NSCLC. Other rarer non-*EML4* fusion partners, including *TFG* and *KIF5B*, have also been identified (4-11). Rearrangements involving *EML4* and *ALK* have also been reported as rare events in breast and colorectal carcinomas (12).

The overall incidence of *ALK* gene rearrangements in NSCLC is estimated to be lower than 3%. A significantly higher incidence of *ALK* rearrangements is reported in the adenocarcinoma subset of NSCLC with further notable predilection for acinar and solid histologic subtypes with signet-ring features (13-15). A higher rate of *ALK* rearrangement is encountered in NSCLC patients who never smoked or of light smoking status while discordant findings reported in relation to age, gender, and stage (12). Comparable overall incidence of *ALK* fusion is found in series conducted in Asian and Western NSCLC patients.

Coexistence of *ALK* rearrangement with *EGFR* or *KRAS* mutations has been rarely described, suggesting that *ALK* is a distinct oncogenic driver. The scarce number of *ALK*-positive patients available as of now (<200) has prevented solid conclusions on clinical outcomes, but there are suggestions that these patients have a good prognosis, present similar response rates to chemotherapy as patients who have *EGFR* wild-type tumors, and do not benefit from treatment with *EGFR* inhibitors (1, 16,17).

Methodology

Currently, RT-PCR of cDNA and fluorescence in situ hybridization (FISH) assays are the most commonly used platforms for detection of *ALK* gene rearrangements in NSCLC (see Table). Immunohistochemical detection of *ALK* fusion protein has also been pursued. Early on, the mouse monoclonal anti-human CD246 (clone *ALK1*; Dako USA) antibody, utilized for evaluation of *ALK* expression in anaplastic large cell lymphoma (ALCL) and inflammatory myofibroblastic tumors, was found to be unreliable in NSCLC (18). Recently, the rabbit monoclonal anti-human CD246 (clone D5F3; Cell Signaling Technology, Danvers, MA) has been shown to detect *ALK* fusion protein in lung adenocarcinomas with very high sensitivity and specificity (18). Additionally, complex strategies to improve the accuracy of *ALK* protein detection in lung cancer including amplification of the signal with a tyramide cascade (14) or intercalation of an antibody-enhanced polymer (10) seems to be advantageous.

RT-PCR of cDNA has been a commonly applied screening strategy for *ALK* gene rearrangements. Initially, only a few primer sets were tested, but currently, a number of multiplex assays have been designed to simultaneously capture all possible in-frame fusions between *EML4* and *ALK* including the kinase domain of *ALK* (4, 6, 9, 10). However, although different breakpoints in *EML4* or *ALK* may be identified, novel *ALK* fusion partners will not be detected with such techniques. Furthermore, to date, RT-PCR based detection of *ALK* rearrangement has been largely constrained to fresh or frozen tissue. The primers most commonly utilized for *ALK* rearrangement testing by RT-PCR are generally not suitable for use in formalin-fixed paraffin-embedded (FFPE) specimens due to somewhat large amplicon size (9). One study in which RT-PCR was performed on FFPE specimens demonstrated a significant false-negative rate in the RT-PCR analysis compared to FISH analysis (21). While it is possible to design primers which would result in amplicons which are small enough for routine use in FFPE, there is, to date, no published study which demonstrates the performance characteristics of this approach in a clinical setting.

FISH assays for detection of *ALK* rearrangements using commercial break apart *ALK* probe has been the method of choice for selection of patients in the current clinical trials. The break apart FISH assays are valid surrogate for detecting *EML4-ALK* rearrangement using the *ALK* Dual Color Break-Apart probe (Abbott Molecular, Des Plaines, IL) (14, 16, 19, 20) or “homebrew” reagents (16, 21). The commercial reagent is comprised of a SpectrumOrange (red)-labeled 250 kb probe to the 3’ end of *ALK* with a SpectrumGreen (green)-labeled 300 kb probe to the 5’ end of *ALK*. Using the *ALK* break-apart probe, red and green signals physically separated by ≥ 2 signal diameters are considered split (16). Frequently, there is a deletion in the 5’*ALK* region in association with the 2p inversion (16). In these cases, the green signal is lost and single red signals (3’*ALK*) are observed. A minimum of 50 tumor cells must be scored and a specimen is considered positive for *ALK* rearrangement when >15% of the cells show split signals or single red (3’ *ALK*) signals; otherwise the specimen is classified as *ALK* FISH negative (14, 16, 19). FISH assays can be performed on relatively small specimens provided the minimum number of tumor cells is

present (requirements vary by laboratory but can be as low as 100 cells). These include routine anatomic pathology pulmonary specimens such as cytology cell blocks, touch preps, and FFPE transbronchial biopsies.

Because the *EML4* and *ALK* loci are mapped relatively close on 2p (~12.5 MB apart), the interpretation of a positive rearrangement through the introduction of a gap between the red and green probes in formalin-fixed, paraffin-embedded tissue sections can be challenging. This distance is estimated using the signal size as a reference, therefore analyses must be performed by experienced laboratory personnel. Moreover, it is recommended that a two-person scoring approach be adopted when the percentage of positive cells is close to the cut-off (between 10% and 30%) due to the subtle nature of some positive cases. It is also critical to use adequate positive and negative control specimens in each assay.

More sophisticated strategies such as a three color probe set with an extra fluorescent probe targeting the deleted portion of chromosome 2 in *ALK*-rearranged NSCLC (19) or dual-fusion color probe directed against both fusion derivatives have also been proposed (16). Although none of these FISH probe sets can comprehensively identify all novel rearrangements, they are able to detect atypical cytogenetic patterns which in turn open the way for further molecular exploration.

TABLE

Diagnostic method	Advantages	Limitations
ALK immunohistochemistry	Allows simultaneous evaluation of morphology	Low sensitivity (varies between antibodies)
ALK FISH	Can detect unusual variants; methodology used in clinical trials	Limited probe separation in some positives reduces sensitivity
RT-PCR	High sensitivity; specific fusion variants identified	Does not detect rare fusion variants; sensitivity may be reduced by tissue fixation; careful primer design required

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