FISH for *ALK* ThinPrep NSCLC

Background

The Food and Drug Administration's August 2011 approval of the targeted therapy crizotinib (Xalkori®, Pfizer, New York, N.Y.) and the companion diagnostic assay Vysis *ALK* Break Apart FISH Probe Kit (Abbott Molecular, Inc., Chicago, Ill.) opened the door to a new era in the treatment of late-stage non-small cell lung cancer (NSCLC). The FDA-approved assay is performed on a formalin-fixed, paraffin-embedded (FFPE) NSCLC tissue specimen. An alternative approach is to perform the *ALK* FISH procedure on ThinPrep cytology slides (Cytyc, Boxborough, Massachussetts) rather than FFPE tissue sections targeting cytologically abnormal cells.

Lung cancer is the leading cause of cancer death in men and women worldwide. Approximately 85% of all lung cancers are of non-small cell type. Advances in surgical treatment and combination therapies have marginally improved the average one-year survival rate (38% for stage IA-IIB, Source: National Cancer Institute Surveillance, Epidemiology, and End Results [SEER] database) from the period 1975-1979 to the present; but the five-year survival rate for all stages of lung cancer combined remains at 15%. The primary reason is that nearly 85% of cases present at an advanced stage.

Crizotinib is highly effective in treating patients whose NSCLC tumors harbor a rearrangement of the anaplastic lymphoma kinase (*ALK*) gene. The *ALK*-FISH assay allows for selection of the subgroup of NSCLC patients who are potentially therapy responsive based on identification of *ALK* rearrangements at the 2p23 chromosome in the tumor genome. This genetic alteration occurs in 2 to 7% of NSCLC patients.

The *ALK* gene encodes for a transmembrane glycoprotein with tyrosine kinase activity that normally is expressed only in select neuronal cell types. The constitutive kinase activity of ALK is essential for cellular proliferation in this subset of NSCLC. Gene rearrangements in the presence of known fusion partners, including *EML4*, *TFG* and *KIF5B*, result in a chimeric protein with tyrosine kinase activity. In particular the *EML4/ALK* fusion appears to be a key driver of tumorigenesis in NSCLC. The *EML4/ALK* fusion also has been identified in about 2.5% of breast and colon carcinomas using exonic sequencing.

ALK rearrangements are typically mutually exclusive from *EGFR* and *KRAS* mutations, and the majority are found in adenocarcinomas. Patients with tumors exhibiting *ALK* rearrangements are usually younger, male and light or never-smokers. Lung adenocarcinomas with *ALK* rearrangement occur in 15% of this population with advanced stage NCSLC.

Crizotinib works by blocking certain kinases, including those produced by the abnormal *ALK* gene. Studies demonstrate crizotinib treatment of patients with tumors exhibiting *ALK* rearrangements can halt tumor progression or result in tumor regression.

Methodology

The FISH for *ALK* ThinPrep NSCLC assay is a qualitative fluorescence *in situ* hybridization (FISH) test that detects *ALK* gene rearrangements with all potential fusion partners, including *EML4*, *TFG* and *KIF5B*. Other available molecular testing methodologies such as RT-PCR detect only the fusion target for which they have been constructed (e.g. *EML4/ALK* fusion product) and, of course, other potentially clinically significant fusion targets will not be detected.

The Abbott Molecular Vysis (AMV) *ALK* FISH kit was used in one of the two single-arm trials leading to the FDA-approval of crizotinib and has become the gold standard for detecting *ALK* rearrangement in NSCLC. The AMV *ALK* Break Apart FISH Probe mixture consists of two fluorophore-labeled DNA probes in hybridization buffer containing dextran sulfate, formamide, and SSC with blocking DNA. The two probes used are Vysis LSI 3 *′-ALK* SpectrumOrange and LSI 5 *′-ALK* SpectrumGreen.

The ThinPrep version of the test uses cytopathology ThinPrep slides for probe hybridization rather than FFPE tissue sections, with morphologic correlation. Following appropriate specimen preparation, the specimen is hybridized using the probe mixture described above at 37°C for 14 to 24 hours. After washing and counterstaining, slides are evaluated for adequate hybridization. Slides are then assessed for the quality of the *ALK* signal. Signals from 50 tumor cells from representative areas of the slide are recorded.

Depending on the monoclonal antibody and concentration used in IHC, overall sensitivity of IHC for *ALK* rearrangements ranges from 80 to 95% with specificity of 100%. A testing algorithm using IHC as an initial screening in all cases of NSCLC has been proposed in which *ALK* IHC 2+ tumor cells would undergo *ALK*-FISH; 3+ cells would be reported as *ALK*-positive; 0 and 1+ cells would be reported as *ALK*-negative.

Clinical Indications

Patients with late-stage, non-small cell lung cancers may benefit from treatment with crizotinib. The National Comprehensive Cancer Network (NCCN Guidelines™) recognizes FISH as a specifically designed method for diagnosing *ALK*-rearranged adenocarcinomas. These guidelines recommend *ALK* testing concurrent with *EGFR* mutation testing in the diagnosis of adenocarcinoma, large cell and other nonspecified histologic subsets of NSCLC.

Interpretation

When hybridized with the *ALK* FISH probes, the 2p23 *ALK* region in its native state will be observed as two immediately adjacent or fused (overlapping) orange/green (yellow) signals. However, if a chromosome rearrangement at the 2p23 *ALK* breakpoint region has occurred, one orange and one green signal separated by at least three signal diameters will be identified. Alternatively, a single orange signal (deletion of green signal) in addition to a fused or broken apart signal may be observed.

Cells are considered negative (non-rearranged) when orange and green signals are adjacent or fused (appear yellow under the Orange/Green V2 filter). A single green signal without a corresponding orange signal is also abnormal, but represents a rearrangement at the 2p23 locus that is not predictive of over-expressed ALK protein. Orange and green signals that are less than three signal diameters apart are classified as a single fused signal.

Cells are considered positive for a rearrangement when at least one set of orange and green signals are three or more signal diameters apart, or a single orange signal occurs without a corresponding green signal in addition to fused and/or broken apart signals. Multiple 2p23 signals are often observed as well, indicative of either genomic gain at 2p23 or an aneusomic state for chromosome 2.

A sample is classified as positive for *ALK* rearrangement if 15% or more of tumor cells are positive for the presence of a rearrangement. When the initial signal count identifies >10% but less than 50% of tumor cells positive, another molecular technologist recounts the specimen, and the two counts are averaged. The molecular pathologist reviews the FISH slides and provides the final interpretation.

Patients who are *ALK*-positive are candidates for crizotinib therapy. For patients who are responsive to treatment, crizotinib yields significant clinical benefits. However, not all *ALK*-positive patients will respond, and those who do will eventually develop resistance to crizotinib. Time-to-drug resistance is unknown as yet; a variety of underlying molecular mechanisms are responsible for development of resistance.

Limitations of the Assay

FISH for *ALK* ThinPrep NSCLC is intended to be used for therapeutic purposes in lung cancer. It does not rule out other chromosome abnormalities. Results may indicate likely response to *ALK* inhibitor therapy, however, selection of treatment remains a clinical decision.

References

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Test Overview

Test Name	FISH for *ALK* ThinPrep NSCLC
Methodology	Multicolor interphase FISH using ThinPrep slides (Cytyc, Boxborough, Massachussetts) rather than FFPE tissue sections; the multicolor FISH probe targets cytologically abnormal cells.
Specimen Requirements	ThinPrep cytology slide or FNA or other aspirated cytology specimen in CytoLyt solution.
Clinical Information	The presence of an *ALK* rearrangement detected by FISH may qualify the patient for treatment with crizotinib.
Reference Range	Negative for *ALK* rearrangement, Positive for *ALK* rearrangement, Atypical *ALK* rearrangement identified, Indeterminate, Technically Unsatisfactory. The percentage of positive cells is specified.
Billing Code	88843
CPT Code	88368 x2

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