

VENTANA ALK (D5F3) CDx Assay

# **ALK IHC Biomarker Testing** *for NSCLC treatment eligibility*



## Lung cancer is the leading cause of death

Lung cancer is the most prevalent form of cancer in the world. Each year, more than 1.8 million new cases are diagnosed. Lung cancer also has the highest mortality rate. Five year survival rates are as low as 16%. Adenocarcinoma, a subset of non-small cell lung carcinoma (NSCLC) is the most common, comprising approximately 40% of all lung disease.<sup>1,2</sup>

Figure 1. Five-year survival rate of patients after diagnosis with NSCLC is 16%



### ALK mutation in lung cancer

Genetic mutations are known to play critical roles in the progression to metastatic lung disease. The majority of these mutations are found in adenocarcinoma of young non-smokers. ALK is considered a key oncogenic driver in NSCLC. The ALK gene codes for a transmembrane glycoprotein with tyrosine kinase activity. In-frame rearrangements with the known fusion partners place the ALK kinase domain under the control of a different gene promoter. This fusion results in a chimeric protein (like EML4-ALK) with constitutive tyrosine kinase activity that has been demonstrated to play a key role in controlling cell proliferation. This unique protein is also a potential target for ALK-specific tyrosine kinase inhibitor (TKI) therapy.<sup>3,4,5</sup>

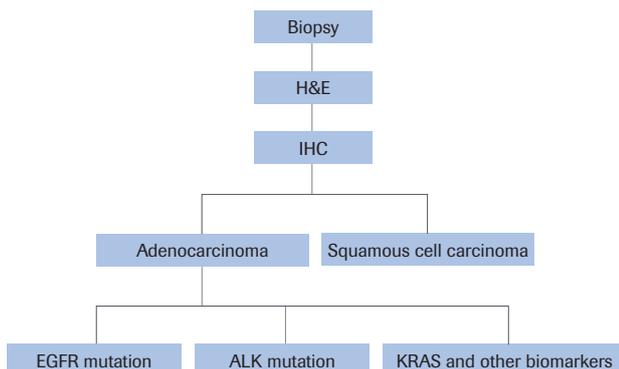
Figure 2. Breakdown of known gene mutations in NSCLC



### Testing for lung cancer

Clinical guidelines recommend routine testing for genetic mutations in all adenocarcinomas, including ALK EML4 gene rearrangement. Testing is recommended immediately after establishing histology and is required prior to initiating targeted therapy for a patient. The current approved methods for testing include IHC and FISH.<sup>6,7</sup>

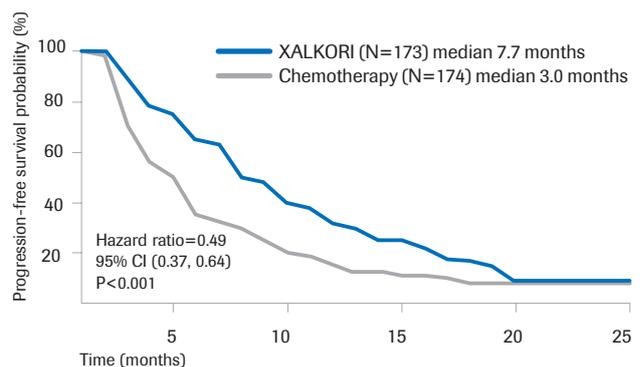
Figure 3. Common testing algorithm to determine indications for appropriate treatment in lung cancer



### Treatment for non-small cell lung cancer

XALKORI® (crizotinib) is a small molecular kinase inhibitor which inhibits ALK and other kinases. XALKORI is indicated for the treatment of patients with metastatic NSCLC whose tumors are ALK-positive as detected by an approved testing method for ALK.<sup>8</sup>

Figure 4. Kaplan Meier curves of progression-free survival of patients with NSCLC treated with XALKORI®

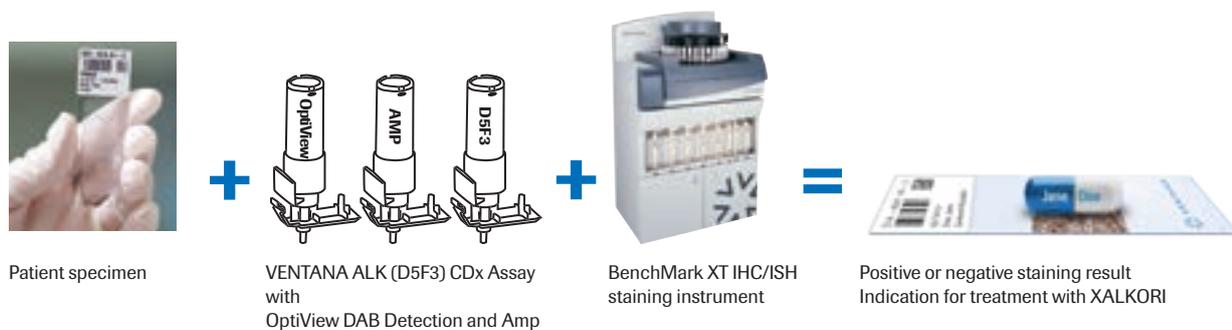


## VENTANA ALK (D5F3) CDx Assay with OptiView DAB Detection and Amp

The VENTANA ALK (D5F3) Rabbit Monoclonal Primary Antibody (VENTANA ALK (D5F3) CDx Assay) is intended for qualitative detection of the ALK protein in formalin-fixed, paraffin-embedded NSCLC tissue stained on the VENTANA BenchMark IHC/ISH XT staining instrument. It is indicated as an aid in identifying patients eligible for treatment with XALKORI®.<sup>9</sup>

### Standardization of ALK IHC testing: VENTANA ALK (D5F3) CDx Assay and OptiView DAB Detection and Amp

Patients with late stage lung cancer need a fast, reliable and standardized diagnostic test to assess treatment options. Ventana developed the ALK (D5F3) CDx Assay to be used with OptiView DAB IHC Detection and OptiView Amplification (OptiView DAB Detection and Amp) to identify these patients who are eligible for ALK targeted therapy. A full range of human NSCLC tissue specimen types can be tested; including resections, needle biopsies, bronchial biopsies and formalin-fixed, paraffin-embedded cell blocks.



### Clinical Confidence

The approved VENTANA ALK (D5F3) CDx Assay stained with OptiView DAB Detection and Amp scores the highest in External Quality Assurance testing vs. all other clones and antibody vendors for demonstration of ALK rearrangement.<sup>10</sup>

### Fast turnaround time

The approved VENTANA ALK (D5F3) CDx Assay stained with OptiView DAB Detection and Amp is a 4-hour, fully automated test to be stained with all other routine IHC testing for same-day results and to meet current CAP/IASLC/AMP guidelines for testing patients with lung cancer.<sup>6</sup>

### Easy to score

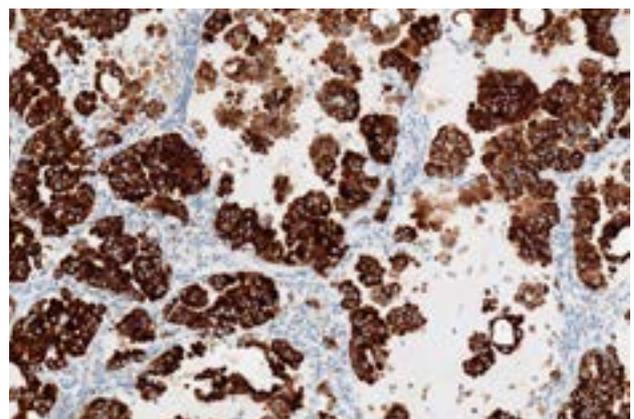
The sensitivity of the approved VENTANA ALK (D5F3) CDx Assay stained with OptiView DAB Detection and Amp enables a reproducible, binary scoring system for evaluating staining results without the need for quantification of cells or staining.<sup>9</sup>

### Reagents required

The VENTANA ALK (D5F3) CDx Assay is fully optimized for use on the Benchmark XT IHC/ISH staining instrument.

VENTANA anti-ALK (D5F3) CDx Assay	06687199001
Rabbit Monoclonal Negative Control Ig	06683380001
OptiView DAB IHC Detection Kit	06396500001

Figure 5. NSCLC tissue samples stained with the VENTANA ALK (D5F3) Assay and OptiView DAB Detection + Amp

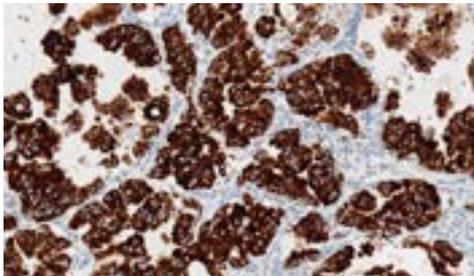
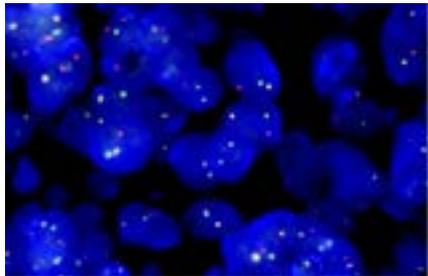


## VENTANA ALK (D5F3) CDx Assay and OptiView DAB Detection and Amp vs. FISH

### Technical benefits of IHC testing

ALK FISH can present technical challenges in evaluating patient results and offers the potential for false negatives. Recent studies indicate that the VENTANA ALK (D5F3) CDx Assay stained with OptiView DAB Detection and Amp is sensitive and specific for determination of ALK status, and a better alternative to ALK FISH. There are reports of ALK IHC-positive, FISH-negative patients benefitting from treatment with XALKORI.<sup>11,12,13</sup>

Figure 6. Comparison of VENTANA ALK (D5F3) CDx Assay stained with OptiView DAB Detection and Amp vs. FISH testing for ALK mutation

	VENTANA ALK (D5F3) CDx Assay with OptiView DAB Detection and Amp	ALK FISH
<b>Easy to score</b>	<ul style="list-style-type: none"> <li>Binary (+/-) scoring</li> <li>Any strong positive staining in any number of cells is positive for ALK</li> </ul>	<ul style="list-style-type: none"> <li>Requires a dual color scoring algorithm</li> <li>Requires 50 enumerable cells and specific cutoff ratios to be calculated</li> </ul>
<b>Faster turnaround times</b>	<ul style="list-style-type: none"> <li>4 hours, fully automated</li> <li>Routine IHC testing</li> </ul>	<ul style="list-style-type: none"> <li>12+ hours, semi-automated</li> <li>Typically batch or send-out testing</li> </ul>
<b>Bright field vs. fluorescent staining</b>	<ul style="list-style-type: none"> <li>Standard brightfield microscope</li> <li>Fully archivable results</li> <li>Full visibility of tumor morphology</li> </ul> 	<ul style="list-style-type: none"> <li>Requires a fluorescent microscope</li> <li>Staining and signal fade over time</li> <li>Loss of tissue morphology</li> </ul> 

### Economic benefits of IHC testing

The VENTANA ALK (D5F3) CDx Assay stained with OptiView DAB Detection and Amp is approved to identify patients eligible for treatment with XALKORI. IHC is a routine testing method in a majority of pathology laboratories and an economical option to more expensive and labor-intensive molecular testing techniques.<sup>14</sup>



Automation with VENTANA Benchmark XT eliminates up to 80% of the labor required for manual staining.

## VENTANA ALK (D5F3) CDx Assay with OptiView DAB Detection and Amp vs. other ALK testing methods

### ALK antibody clones

There are many available ALK antibody clones and detection kits. Only VENTANA ALK (D5F3) CDx Assay stained with OptiView DAB Detection and Amp is approved to identify patients eligible for treatment with XALKORI.<sup>9</sup>

Figure 7. NSCLC tissue samples stained with different ALK IHC methods



VENTANA ALK (D5F3) CDx Assay with OptiView DAB Detection and Amp



ALK antibody, clone 5A4 with Polymer Detection

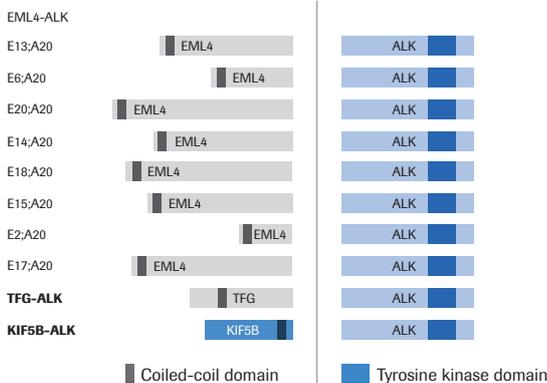
### PCR testing and DNA sequencing

Over ten genetic variants of EML4-ALK mutations have been identified. VENTANA ALK (D5F3) CDx Assay stained with OptiView DAB Detection and Amp identifies a conserved protein sequence common to known variants of the ALK mutation. Current PCR and sequencing methods do not identify all known ALK genetic variants and are not recommended as an alternative testing method to select patients for ALK inhibitor therapy.<sup>6</sup>

Figure 8. Different variants of EML4-ALK and non-EML4 fusion partners<sup>20</sup>

The EML4 genetic sequence is diverse and creates multiple targets for PCR

A conserved protein sequence common to all known ALK mutations is detected by VENTANA ALK (D5F3) CDx Assay



- There are multiple genetic variants of the ALK mutation that lead to NSCLC
- PCR and genetic sequencing do not identify all ALK gene variants and can miss positive cases
- D5F3 clone is specific to the common kinase domain of all ALK mutations and identifies all genetic variants

PCR and DNA sequencing techniques rely upon good sample integrity and require sophisticated computational analysis to interpret results. Formalin-fixed, paraffin-embedded tissues provide a significant challenge as genetic material is known to degrade in sample preparation. Even when properly performed, interpreting the results of these techniques is not standardized.<sup>6</sup>

### ALK testing with VENTANA ALK (D5F3) CDx Assay with OptiView DAB Detection and Amp offers many benefits:

- An approved, fully automated testing method to indicate treatment with XALKORI
- Fastest turnaround time to meet the current CAP/IASLC/AMP guidelines for testing lung patients
- Can be integrated into a routine IHC panel of antibodies to classify NSCLC

## Publications for VENTANA ALK (D5F3) CDx Assay stained with OptiView DAB Detection and Amp

Title	Findings and recommendations
Diagnostic value of a novel fully automated immunochemistry assay for detection of ALK rearrangement in primary lung adenocarcinoma <sup>13</sup>	<p>“Using FISH as the standard procedure, we demonstrated that the novel fully automated IHC assay using pre-diluted Ventana anti-ALK (D5F3) Rabbit monoclonal primary antibody, together with the Optiview DAB detection and Amplification kit, is a highly sensitive (100%) and specific (98%) method for detection of the ALK rearrangement in primary lung adenocarcinoma.”</p> <p>“In summary, we report that the fully automated IHC is a sensitive and specific screening method to detect ALK rearrangement in lung cancer. IHC would be served as an effective and rapid detection method in routine pathologic laboratories for the identification of suitable candidates for ALK-targeted therapy.”</p>
ALK Rearrangement in a Large Series of Consecutive Non-Small Cell Lung Cancers: Comparison Between a New Immunohistochemical Approach and Fluorescent <i>In Situ</i> Hybridization for the Screening of Patients Eligible for Crizotinib Treatment <sup>15</sup>	<p>“We used a new, high-sensitivity, and high-specificity monoclonal antibody (D5F3) and a highly sensitive detection system (OptiView DAB IHC detection kit and OptiView amplification kit) to enhance the assay sensitivity. The advantage of this anti-ALK IHC assay is that the use of these kits enabled most NSCLC cases to easily be interpreted as “positive” or “negative,” providing more objective evaluation of the results by pathologists.”</p> <p>“After further confirmatory studies in large subsets of FISH positive cases, IHC could represent the best method for selecting patients for ALK inhibitor therapy in NSCLC.”</p>
Immunohistochemistry as a screening tool for ALK rearrangement in NSCLC: evaluation of five different ALK antibody clones and ALK FISH <sup>16</sup>	<p>“Ventana D5F3 exclusively stained cases that harboured ALK rearrangements with strong intensity (3+, positive) at any percentage of tumour cells.”</p> <p>“Only D5F3 combined with OptiView stained rearranged cases exclusively at strong intensity (3+) and reached an NPA and a PPA of 100%. Therefore, the number of required ALK FISH analyses was reduced substantially, reducing the time, work and costs without any loss of diagnostic quality and accuracy.”</p>
A Comparison of Immunohistochemical Assays and ISH in Detecting the ALK Translocation in Diagnostic Histological and Cytological Lung Tumor Material <sup>17</sup>	<p>“IHC with all three antibodies is especially highly specific (100%) although variably sensitive (71%-86%), specifically in cases with scanty material. D5F3 assay was most sensitive in these latter cases. Occasional cases are IHC-positive but FISH-negative, suggesting either inaccuracy of one assay or occasional tumors with ALK rearrangement that do not express high levels of ALK protein.”</p> <p>“In summary, we find IHC to be a highly sensitive (86%) and specific (100%) test for ALK rearrangement in lung adenocarcinoma. We find a slight advantage of a proprietary amplified assay (D5F3 Ventana) over two other antibodies with conventional DAB staining (ALK1 Dako and 5A4 Abcam), but only in scanty samples. Intensity of staining was the most discriminating measure, and the proportion of cells staining did not contribute. We identified two cases that were positive for the ALK rearrangement by FISH but negative by all immunohistochemical assays and suggest that in discordant cases the IHC test result may be more predictive of treatment response than FISH.”</p>
ALK Status Testing in Non Small Cell Lung Carcinoma: Correlation Between Ultrasensitive IHC and FISH <sup>18</sup>	<p>“The ultrasensitive D5F3-IHC method revealed a very high correlation with FISH in assessing ALK status. The 100% sensitivity and specificity (95% CI, 0.86 to 1.00 and 0.97 to 1.00, respectively) observed in our study surpass those reported for IHC in other studies using the same or different antibodies.”</p> <p>“Taken together, our data demonstrate that ultrasensitive automated IHC represents a reliable alternative to FISH for initial ALK screening in NSCLC.”</p>
An International Interpretation Study Using the ALK IHC Antibody D5F3 and a Sensitive Detection Kit Demonstrates High Concordance between ALK IHC and ALK FISH and between Evaluators <sup>19</sup>	<p>“IHC correlates well with FISH with a very few cases showing discrepancy, but the biggest limitation is that the antibody clones, antigen retrieval, detection systems, and scoring methodology were not standardized.”</p> <p>“The most favorable IHC assay for this screening should be one that has been thoroughly standardized and has exquisitely sensitive detection/amplification systems with limited background to consistently identify all levels of ALK protein expression.”</p>

**Contact your local Account Manager for more information on the VENTANA ALK (D5F3) CDx Assay.**

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