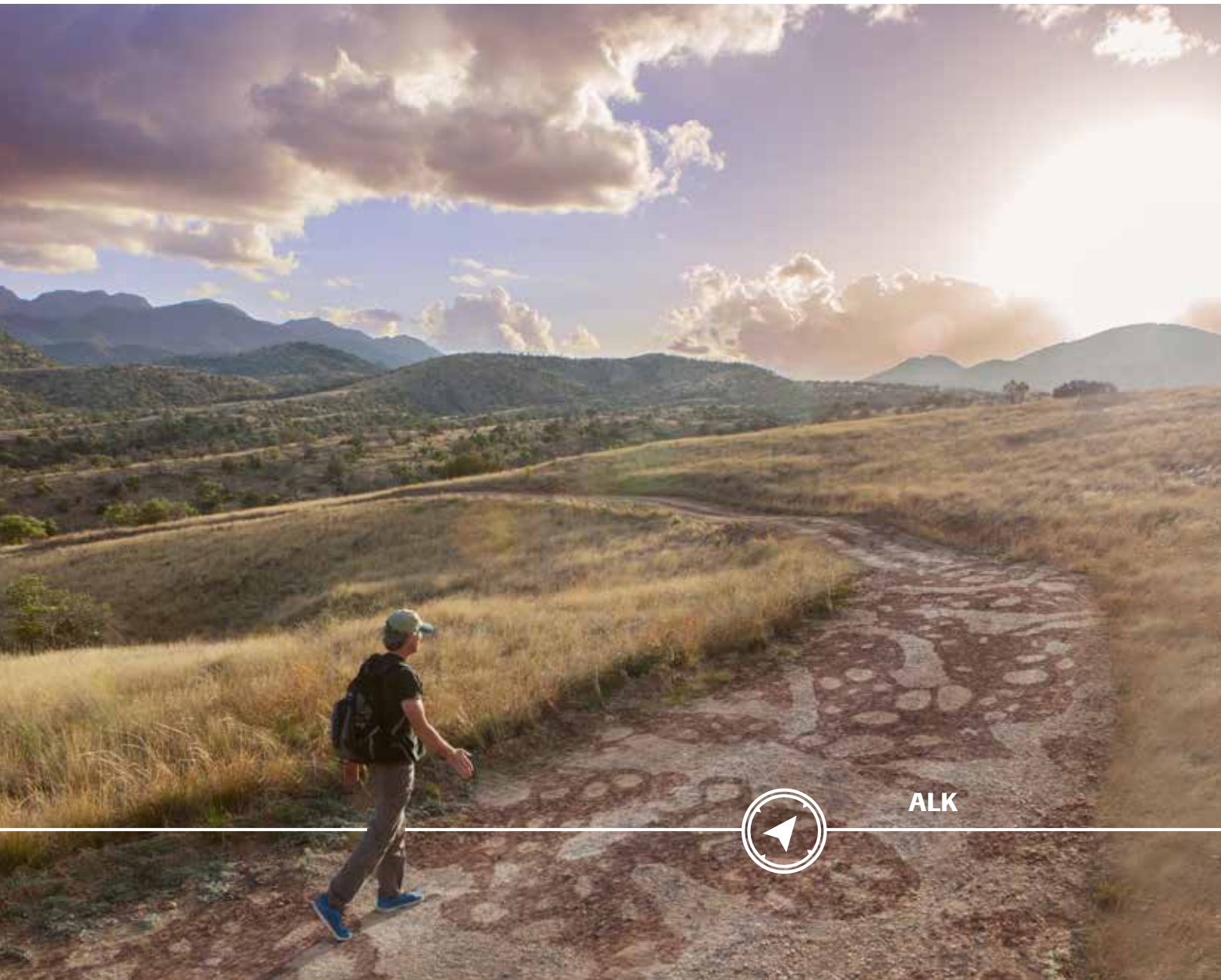
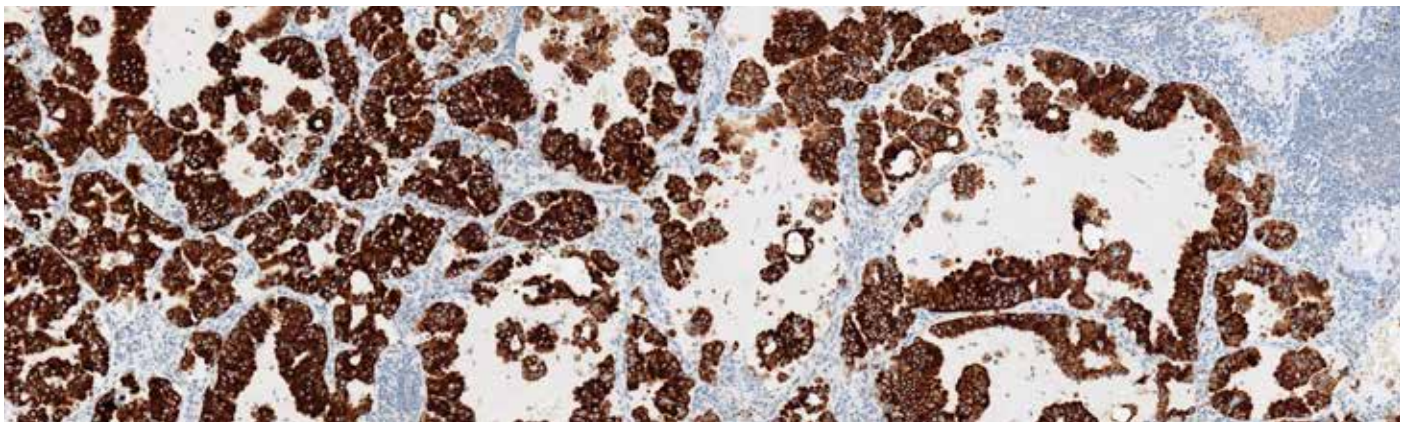
# VENTANA ALK (D5F3) CDx Assay

*Identifying ALK+ NSCLC patients for targeted treatment*



# VENTANA ALK (D5F3) CDx Assay

*Identify ALK+ NSCLC patients eligible for treatment with XALKORI, ZYKADIA or ALECENSA*



NSCLC tissue samples stained with the VENTANA ALK (D5F3) CDx Assay and OptiView DAB Detection and Amp

## Intended use

VENTANA ALK (D5F3) CDx Assay* is intended for the qualitative detection of the anaplastic lymphoma kinase (ALK) protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung carcinoma (NSCLC) tissue stained with a BenchMark XT or BenchMark ULTRA automated staining instrument. It is indicated as an aid in identifying patients eligible for treatment with XALKORI® (crizotinib), ZYKADIA® (ceritinib) or ALECENSA® (alectinib).

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This product is intended for in vitro diagnostic (IVD) use.

**ALK (D5F3) Assay*

## ALK value points

- ALK (D5F3) Assay stained with OptiView DAB IHC Detection and Amplification kits** detects the ALK protein that is the target of therapy
- Clinical guidelines recommend rapid turnaround for earlier targeted therapies
- ALK has comparable sensitivity and specificity relative to FISH
- More immediate treatment decisions can be made for advanced NSCLC patients by using the ALK (D5F3) Assay
- XALKORI (crizotinib), ZYKADIA (ceritinib) and ALECENSA (alectinib) are clinically effective and recommended for the treatment of ALK-positive patients[10, 17, 19]

***OptiView DAB Detection and Amp*

**Full automation**

Reproducible staining

**Standardization**

**In-house testing**

Efficient workflow

**Rapid results**

**Training**

Precision scoring

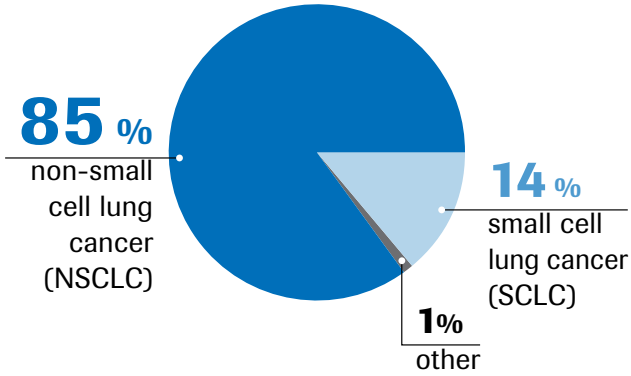
**Confidence**

Lung cancer is the most common cancer worldwide with more than

**1.8 million new cases**

**each year**[2]

## Main types of lung cancer




Lung cancer is the leading cause of cancer death for men and women globally with more than

**4,000 people**

**dying every day**

## Poor 5-year survival rate

when compared to other cancers

**91 %** breast cancer

**18 %** lung cancer

## About non-small cell lung cancer

Lung cancer has been the most common cancer in the world for several decades and remains the leading cause of cancer deaths worldwide. It is estimated to account for 12.9% of all new cancer cases and is responsible for nearly 1.59 million deaths annually worldwide, or approximately one-in-five cancer-related deaths.[5] Although improvements have been made in diagnosis and therapy options, prognosis remains poor with low long-term survival rates for all stages. Over the past three decades, lung cancer has shown among the least improvement in survival rates when compared with other cancers.[4]

Non-small cell lung cancer (NSCLC), one of the two major types of lung cancer, accounts for approximately 85% of all lung cancer cases.[4] In more than half of patients newly diagnosed with NSCLC, the disease has already metastasized, greatly decreasing the likelihood of survival. The five-year relative survival rate for NSCLC diagnosed as distant disease is 4.7%.[6] The majority of patients with NSCLC present with inoperable, locally advanced disease (Stage IIIB) or metastatic disease (Stage IV), neither of which currently has any curative treatment options. On average, these patients die within a year of diagnosis. Improvement in the clinical outcome of lung cancer is likely to be achieved through better understanding of the molecular events that underlie its pathogenesis, identifying new biomarker targets and developing new treatment options.

## 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 practice for testing include IHC and FISH.[4]

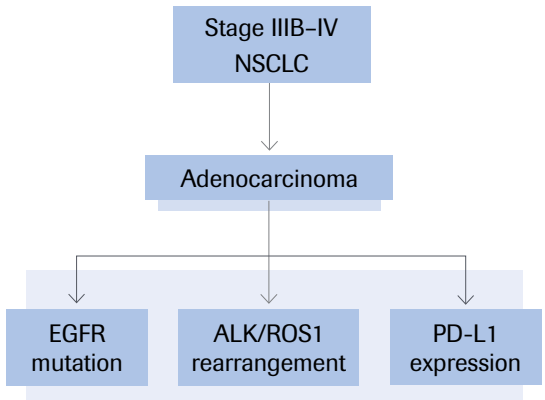



Figure 1. Common testing algorithm to determine indications for appropriate treatment in lung cancer

## 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 18%. Adenocarcinoma, a subset of NSCLC, is the most common, comprising approximately 40% of all lung disease.[7, 8]

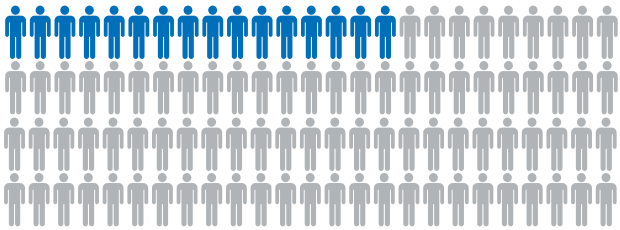


Figure 2. Five-year survival rate of patients after diagnosis with NSCLC is 18%

## 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. [3, 5, 9]

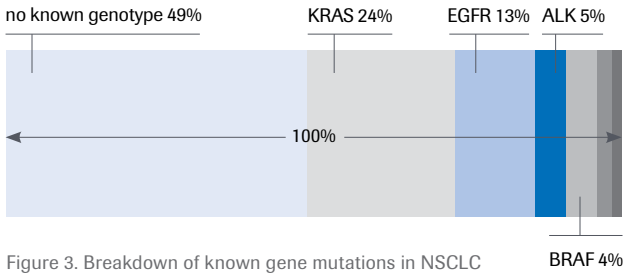



Figure 3. Breakdown of known gene mutations in NSCLC

| | PROFILE 1014 | | ASCEND 4 TRIAL | | ALEX STUDY | |
|---|---|---|---|---|---|---|
| **Progression-Free Survival** | **XALKORI (n=172)** | **Chemotherapy (n=171)** | **ZYKADIA (n=189)** | **Chemotherapy (n=187)** | **ALECENSA (n=152)** | **XALKORI (n=151)** |
| Median, months (95% CI) [a] | 10.9 (8.3, 13.9) | 7.0 (6.8, 8.2) | 16.6 (12.6, 27.2) | 8.1 (5.8, 11.1) | (17.7-not reached)* | 11.1 (9.1,13.1) |
| HR (95% CI) [b] | 0.45 (0.35, 0.60) | | 0.55 (0.42, 0.73) | | 0.47 (0.34, 0.65) | |
| p-valued [c] | <0.001 | | <0.0001 | | <0.0001 | |

Figure 4. Clinical benefit of XALKORI, ZYKADIA and ALECENSA (progression free survival).

HR=hazard ratio; CI=confidence interval; BIRC=Blinded Independent Review Committee; NR=not reached; NE=not estimable

[a] Estimated using the Kaplan-Meier method.

[b] A Cox regression model stratified by randomization stratification factors (WHO performance status: 0 vs. 1-2; presence or absence of BM, presence or absence of previous neo-/adjuvant chemotherapy)) was used to estimate the hazard ratio of PFS, along with 95% CI based on the Wald test.

[c] Based on the stratified log-rank test (same stratification as [b]).

*Median PFS not estimable due to insufficient patients demonstrating disease progression while on alectinib therapy.

## Treatment options for non-small cell lung cancer

XALKORI, ZYKADIA and ALECENSA are indicated for the treatment of patients with metastatic NSCLC whose tumors are ALK-positive as detected by an approved testing method for ALK.[10, 17, 19]

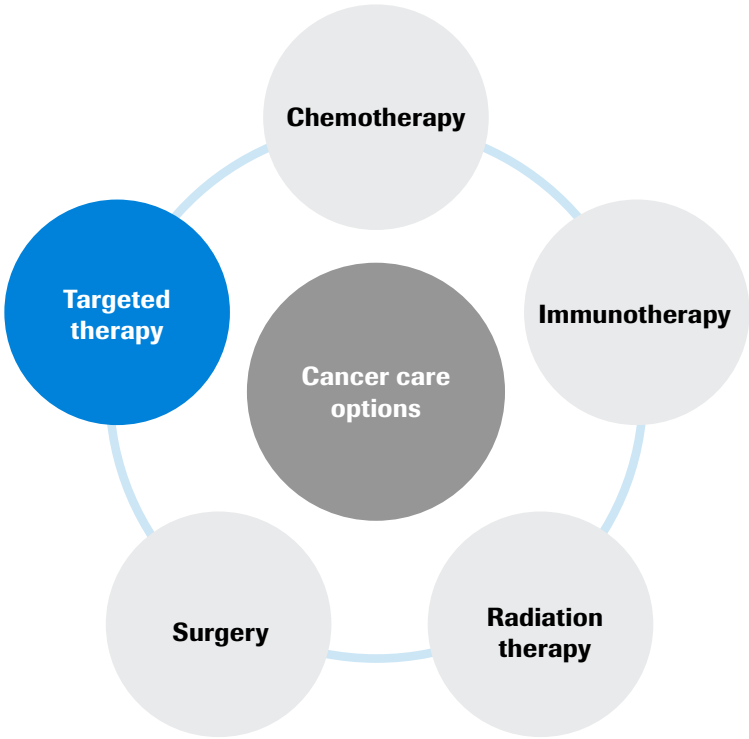
XALKORI (crizotinib) is indicated for the treatment of patients with ALK positive metastatic NSCLC and other kinases.[10]

ZYKADIA (ceritinib) is indicated for the treatment of patients with ALK-positive metastatic NSCLC who have had no previous treatment, or who have progressed on, or who are intolerant to, crizotinib.[17]

ALECENSA (alectinib) is indicated for the treatment of patients with ALK-positive metastatic NSCLC who have had no previous treatment, or who have progressed on, or who are intolerant to, crizotinib.[19]

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

Patients with late-stage lung cancer need a fast, reliable and standardized way to assess treatment options. Roche developed the ALK (D5F3) Assay to be used with OptiView DAB IHC 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.




## Best in quality

The ALK (D5F3) Assay stained with OptiView DAB Detection and Amp scored high in External Quality Assurance testing vs. all other ready-to-use antibodies for demonstration of ALK rearrangement.[11]

## Fast turnaround time

The approved ALK (D5F3) Assay stained with OptiView DAB Detection and Amp is a 4½-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.[4]

## Easy to score

The sensitivity of the approved ALK (D5F3) 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.[1]

## Reagents required

The ALK (D5F3) Assay is fully optimized for use on the BenchMark IHC/ISH staining instrument.

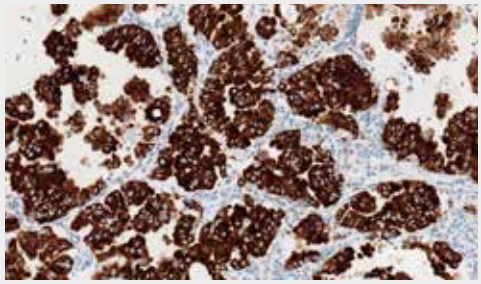
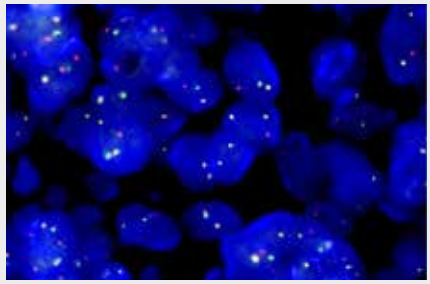
| | |
|---|---|
| ALK (D5F3) Assay | Ref: 06687199001 |
| Rabbit Monoclonal Negative Control Ig | Ref: 06683380001 |
| OptiView DAB IHC Detection Kit | Ref: 06396500001 |
| OptiView Amplification Kit | Ref: 06396518001 |

# ALK (D5F3) Assay and Detection with 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 ALK (D5F3) 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.[12, 13, 14]

Figure 4. Comparison of ALK (D5F3) Assay stained with OptiView DAB Detection and Amp vs. FISH testing for ALK mutation

| | ALK (D5F3) Assay with OptiView DAB Detection and Amp | ALK FISH |
|---|---|---|
| **Easy to score** | ▪ Binary (+/-) scoring<br>▪ Any strong positive staining in any number of cells is positive for ALK | ▪ Requires a dual-color scoring algorithm<br>▪ Requires 50 enumerable cells and specific cutoff ratios to be calculated |
| **Faster turnaround times** | ▪ 4½ hours, fully automated<br>▪ Routine IHC testing | ▪ 12+ hours, semi-automated<br>▪ Typically batch or send-out testing |
| **Brightfield vs. fluorescent staining** | ▪ Standard brightfield microscope<br>▪ Fully archivable results<br>▪ Full visibility of tumor morphology | ▪ Requires a fluorescent microscope<br>▪ Staining and signal fade over time<br>▪ Loss of tissue morphology |
| |  |  |

*In one study, van der Wekken* et al. *found that "Dichotomous ALK-IHC is superior to ALK-FISH on small biopsies and FNA to predict tumor response and survival to crizotinib for advanced NSCLC patients."*[18]

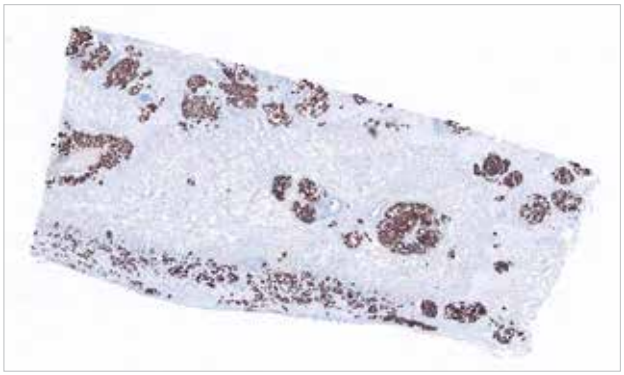
Visit ALKIHC.com and contact your local Roche representative to learn more.

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

## ALK antibody clones

There are a many available ALK antibody clones and detection kits. Only ALK (D5F3) Assay stained with OptiView DAB Detection and Amp is approved as an aid to identify patients eligible for treatment with XALKORI, ZYKADIA, or ALECENSA.[1]

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



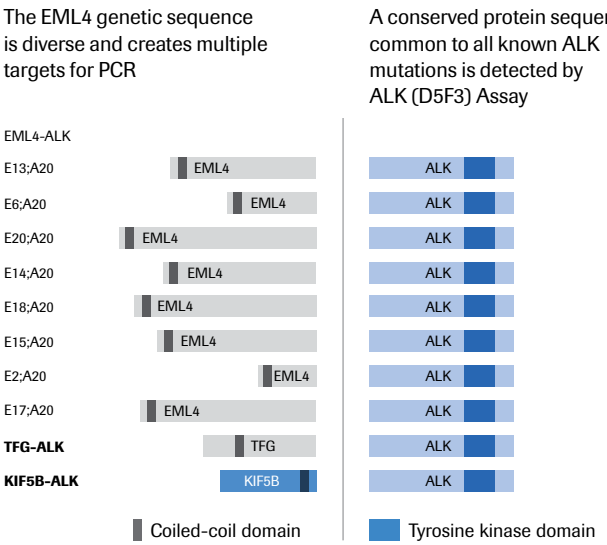
VENTANA ALK (D5F3) Assay with OptiView DAB Detection and Amp

ALK antibody, clone 5A4 with Polymer Detection

## Molecular testing

Over ten genetic variants of EML4-ALK mutations have been identified. ALK (D5F3) Assay stained with OptiView DAB Detection and Amp identifies a conserved protein sequence common to known variants of the ALK mutation. Current molecular testing does not identify all known ALK genetic variants and is not recommended as an alternative testing method to select patients for ALK inhibitor therapy.[4]

Figure 8. Different variants of EML4-ALK and non-EML4 fusion partners[19]




- There are multiple genetic variants of the ALK mutation that lead to NSCLC
- Molecular testing does 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 should identify all genetic variants

Molecular testing 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.[4]

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

- A fully automated test to select patients for treatment with XALKORI, ZYKADIA or ALECENSA
- 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 stratify NSCLC patients

## References


1. VENTANA ALK (D5F3) Rabbit Monoclonal Primary Antibody [package insert] Tucson, AZ: Ventana Medical Systems, Inc.; 2014.
2. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. (2012). GLOBOCAN v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 Lyon, France: International Agency for Research on Cancer; 2013. Available at: http://globocan.iarc.fr (last accessed September 2017).
3. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. *Lancet Oncol.* 2011 Feb1; 12 (2):175-180. doi: 10.1016/ S1470-2045(10)70087-5.
4. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *J Thorac Oncol.* 2013 Jul; 8(7): 823–859. doi: 10.1097/ JTO.0b013e318290868f.
5. Shaw AT, Yeap BY, Mino-Kenudson M, et al. Clinical features and outcome of patients with non-small–cell lung cancer who harbor EML4-ALK. *J Clin Oncol.* 2009; 27:4247- 4253.
6. NCCN Clinical Practice Guidelines in Oncology™. Non-small cell lung cancer. v 2.2010. Available at: http://www.nccn.org/professionals/ physician_gls/PDF/nscl.pdf. Accessed August 1, 2014.
7. World Health Organization. International Agency for Research on Cancer. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. Lyon, France http://globocan.iarc.fr/ Default.aspx. Accessed September 2017.
8. Lung Cancer Survival Rates and Prognosis. National Cancer Institute at the National Institutes of Health. Bethesda, MD. http://www.cancer.gov/ cancertopics/types/lung/cancer-survival-prognosis. Accessed August 1, 2014.
9. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature.* 2007 Aug 2; 448(7153):561-566.
10. XALKORI® (crizotinib) [package insert]. New York, NY: Pfizer; 2012.
11. http://www.nordiqc.org/downloads/assessments/45_14.pdf.
12. Zhou J, Zhao J, Sun K, Wang B, Wang L, et al. Accurate and Economical Detection of ALK Positive Lung Adenocarcinoma with Semiquantitative Immunohistochemical Screening. *PLoS ONE.* 2014; 9(3): e92828. doi:10.1371/journal.pone.0092828.
13. Shan L, Lian F, Guo L, Yang X, Ying J and Lin D. Combination of conventional immunohistochemistry and qRT-PCR to detect ALK rearrangement. *Diagn Pathol.* 2014; 9:3. doi:10.1186/1746-1596-9-3.
14. Ying J, Guo L, Qiu T, Shan L, LingY, Liu X, Lu N. Diagnostic value of a novel fully automated immunochemistry assay for detection of ALK rearrangement in primary lung adenocarcinoma. *Ann Oncol.* 2013 Oct 2; 24(10):2589-2593.
15. Selinger CI, Rogers TM, Russell PA, et al. Testing for ALK rearrangement in lung adenocarcinoma: a multicenter comparison of immunohistochemistry and fluorescent in situ hybridization. *Mod Pathol.* 2013; 26(12):1545–1553. doi:10.1038/modpathol.2013.87; published online 7 June 2013.
16. Alì G, Proietti A, Pelliccioni S, et al. ALK Rearrangement in a Large Series of Consecutive Non–Small Cell Lung Cancers:Comparison Between a New Immunohistochemical Approach and Fluorescent In Situ Hybridization for the Screening of Patients Eligible for Crizotinib Treatment. *Arch Pathol Lab Med.* 2014 June 2.
17. ZYKADIA® (ceritinib) [package insert]. Whippany, NJ: Novartis Pharmaceuticals Corporation; 2016.
18. van der Wekken, et al. Dichotomous ALK-IHC is a better predictor for ALK inhibition outcome than traditional ALK-FISH in advanced non-small cell lung cancer, Clin Cancer Res. 2017. Available at http:// clincancerres.aacrjournals.org/content/early/2017/02/09/1078-0432. CCR-16-1631.
19. ALECENSA® (alectinib) [package insert]. San Francisco, CA: Genentech; 2015.





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