VENTANA anti-ALK (D5F3) Rabbit Monoclonal Primary Antibody

REF 790-4794

06679072001

IVD Σ 50

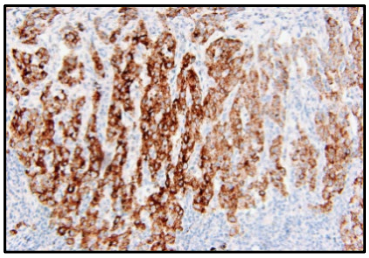

Figure 1. VENTANA anti-ALK (D5F3) assay expression on non-small cell lung carcinoma.

INTENDED USE

VENTANA anti-ALK (D5F3) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the qualitative detection of the anaplastic lymphoma kinase (ALK) protein in formalin-fixed, paraffin-embedded non-small cell lung carcinoma (NSCLC) tissue stained on a BenchMark IHC/ISH instrument. It is indicated as an aid in identifying patients eligible for treatment with XALKORI® (crizotinib), ZYKADIA® (ceritinib), ALECENSA® (alectinib), or lorlatinib.

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

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

SUMMARY AND EXPLANATION

The anaplastic lymphoma kinase (ALK) protein is a member of the insulin receptor superfamily of receptor tyrosine kinases.[1] ALK is a type I membrane glycoprotein that is normally expressed in the nervous system.[2] The ALK gene resides at chromosome 2p23 and is constructed of 2 large introns and 26 exons.[1] Molecular pathogenesis involving ALK begins with chromosomal rearrangements that partner the 3' coding sequences for the ALK intracellular signaling domain with the 5' promoter elements and coding sequences of other genes. The 5' promoter elements and coding sequences drive overexpression and ligand-independent oligomerization of the chimeric proteins, features common in fusion-type protein tyrosine kinase human neoplasms.

An inversion within chromosome 2p resulting in the formation of a fusion gene product comprising portions of the echinoderm microtubule associated protein-like 4 (EML4) gene and the ALK gene was discovered in 2007 in NSCLC cell lines and archived clinical specimens.[3] A subsequent series of published studies indicated that EML4-ALK inversion events produced at least 9 catalytically active kinase fusion protein variants, each containing the same portion of the ALK C-terminal kinase domain.[4-8] As with ALK gene fusions first identified in anaplastic large-cell lymphoma (ALCL), the EML4-ALK fusion protein was shown to have transforming activity. Consistent with this, EML4-ALK expression in lung alveolar epithelial cells in transgenic mice has been reported to be a potent oncogenic factor.[9]

CLINICAL SIGNIFICANCE

NSCLC is the most common type of lung cancer. ALK is now recognized as a key oncogenic driver in NSCLC, and although EML4 is the predominant fusion partner, other fusion partner genes have been identified.[10,11] The incidence of ALK gene rearrangements appears to range from 2-7%, translating to approximately 6000 ALK-positive patients/year in the United States (US) and 40 000 patients/year worldwide.[3,4,7]

The vast majority of ALK gene rearrangements in lung cancer have been in adenocarcinoma specimens rather than those with squamous or small cell histologies.[3-8] Some evidence suggests a correlation between ALK gene rearrangements and NSCLC in patients of “never or light” smoking status, although this may not be a statistically significant cofactor.[3,4,7,9] Importantly, ALK gene rearrangements are rarely coincident with EGFR, HER2, or KRAS mutations, demonstrating that ALK positivity is a distinct disease subtype.[9]

XALKORI® is a selective ATP-competitive small-molecule inhibitor of the ALK, ROS1 and c-Met/Hepatocyte Growth Factor Receptor (HGFR) tyrosine kinases and their oncogenic variants (e.g., ALK or ROS1 fusion proteins or c-Met/HGFR mutant variants). XALKORI® has demonstrated concentration-dependent inhibition of ALK and c-Met phosphorylation in cell-based assays using tumor cell lines. It has also demonstrated antitumor activity in mice bearing tumor xenografts expressing EML4- or NPM-ALK fusion proteins or Met.[12,13,14]

ZYKADIA® is a kinase inhibitor. Targets of ZYKADIA® inhibition identified in either biochemical or cellular assays at clinically relevant concentrations include ALK, insulin-like growth factor 1 receptor (IGF-1R), insulin receptor (InsR), and ROS1. Among these, ZYKADIA® is most active against ALK. ZYKADIA® inhibited autophosphorylation of ALK, demonstrated inhibition of ALK-mediated phosphorylation of the downstream signaling protein STAT3, and proliferation of ALK-dependent cancer cells in in vitro and in vivo assays.

ZYKADIA® inhibited the in vitro proliferation of cell lines expressing EML4-ALK and NPM-ALK fusion proteins and demonstrated dose-dependent inhibition of EML4-ALK-positive NSCLC xenograft growth in mice and rats.[15]

The clinical significance of ALK gene rearrangements has been demonstrated in randomized, active controlled, clinical trials of XALKORI®, conducted by Pfizer, and of ZYKADIA®, conducted by Novartis.[14,15]

ALECENSA® is a highly selective and potent ALK and RET tyrosine kinase inhibitor, which inhibits intracellular signaling pathways involved in tumor cell proliferation and survival and therefore, promotes cancer cell death and inhibits tumor cell growth and proliferation.[16] Based on preclinical data, ALECENSA® is not a substrate of the efflux transporters (PGP or BCRP) in the blood brain barrier and can therefore distribute into and be retained within the central nervous system. ALECENSA® induced tumor regression in preclinical mouse xenograft models, including antitumor activity in the brain, and prolonged survival in intracranial tumor animal models.[17] ALECENSA® is well-tolerated and provides a manageable safety profile.[18-20]

Lorlatinib is a third-generation, selective, adenosine triphosphate (ATP)-competitive, brain-penetrant, small molecule inhibitor of the ALK tyrosine kinase. It is designed to overcome or prevent major mechanisms of resistance that develop following previous ALK-inhibitor treatment and to penetrate the blood-brain-barrier. Lorlatinib is also a potent ROS1 tyrosine kinase inhibitor.[21] In vitro, lorlatinib potently inhibits the catalytic activity of non-mutated ALK and a broad range of clinically relevant mutant ALK kinases, including those conferring resistance to first and second-generation ALK tyrosine kinase inhibitors.[22] Lorlatinib demonstrated marked antitumor activity at low nanomolar free plasma concentrations in animal models harboring ALK mutations, including those that confer resistance to first and second-generation ALK inhibitors. Lorlatinib resulted in tumor shrinkage and prolonged survival by penetrating the blood-brain barrier and achieving efficacious brain exposure in orthotopic animal models.[23,24]

VENTANA anti-ALK (D5F3) Rabbit Monoclonal Primary Antibody (VENTANA anti-ALK (D5F3) assay) demonstrated concordance with the Abbott Vysis ALK Break Apart FISH Probe Kit (ALK FISH) in determining the ALK status of a patient’s NSCLC. ALK FISH can present technical challenges in evaluating the staining results. Intrachromosomal re-arrangements can yield subtle signal-splitting, leading to potential false negatives[25] Recent studies indicate that IHC is sensitive and specific for determining ALK status, and is a viable alternative to ALK FISH.[10,11,25,26,27] VENTANA anti-ALK (D5F3) assay and its associated scoring algorithm was developed to determine ALK status in NSCLC specimens.

Small tissue samples may be easily used in routine immunohistochemistry (IHC), making this technique, in combination with antibodies that detect antigens important for carcinoma interpretation, an effective tool for the pathologist in their diagnosis and prognosis of disease. One important marker in NSCLC is ALK.

Interpretation of the results of VENTANA anti-ALK (D5F3) assay staining of tissue samples should be made using the recommended scoring algorithm. Histological tissue preparations have the advantage of intact tissue morphology to aid in the interpretation of the ALK positivity of the sample. All histological tests should be interpreted by a pathologist, and the results should be complemented by morphological studies and proper controls and used in conjunction with other clinical and laboratory data. Target antigens of IHC assays are impacted by fixation time, type of fixative, and age of cut slides, therefore care must be taken to ensure compatibility of specimen preparation prior to staining (refer to the VENTANA anti-ALK (D5F3) Rabbit Monoclonal Primary Antibody Interpretation Guide for Non-Small Cell Lung Carcinoma (NSCLC) (P/N 1011879), and the Specific Limitations section below).

PRINCIPLE OF THE PROCEDURE

VENTANA anti-ALK (D5F3) assay is a rabbit monoclonal primary antibody that binds to ALK in paraffin-embedded tissue sections. The specific antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001) followed by the OptiView Amplification Kit (Cat. No. 760-099 / 06396518001 (50 test) or 860-099 / 06718663001 (250 test)). Refer to the appropriate OptiView DAB IHC Detection Kit and OptiView Amplification Kit method sheets for further information.

MATERIAL PROVIDED

VENTANA anti-ALK (D5F3) assay contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA anti-ALK (D5F3) assay contains approximately 70 µg of the rabbit monoclonal (D5F3) antibody.

The antibody is diluted in PBS with carrier protein and 0.05% ProClin 300, a preservative.

Specific antibody concentration is approximately 14 µg/mL.

VENTANA anti-ALK (D5F3) assay is a recombinant rabbit monoclonal antibody produced as purified cell culture supernatant.

VENTANA anti-ALK (D5F3) Rabbit Monoclonal Primary Antibody Interpretation Guide for Non-Small Cell Lung Carcinoma (NSCLC) (P/N 1011879).

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Human appendix or ALK-positive and ALK-negative non-small cell lung carcinoma specimens for use as control tissue
2. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
3. Microscope slides, positively charged
4. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. OptiView Amplification Kit (Cat. No. 760-099 / 06396518001 (50 test) or 860-099 / 06718663001 (250 test))
6. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
7. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
8. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
9. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
10. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
11. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
12. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
13. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
14. Permanent mounting medium
15. Cover glass
16. Automated coverslipper
17. General purpose laboratory equipment
18. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin-embedded (FFPE) tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments.

On the basis of xenograft models generated from the NCI-H2228 human NSCLC cell-line, which is positive for ALK, the recommended tissue fixation is 10% neutral buffered formalin (NBF) for at least 6 hours. Fixation times of less than 6 hours result in a significant loss of staining intensity for ALK. Zinc formalin fixative also is acceptable for a fixation time of at least 6 hours. The amount used should be 15 to 20 times the volume of tissue. No fixative will penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24-hour period. Fixation can be performed at room temperature (15-25°C).[28,29]

Fixatives such as alcohol formalin acetic acid (AFA), PREFER fixative, B5, and other acid and/or alcohol-containing fixatives have demonstrated a loss of staining intensity for ALK at all fixation times tested (1 to 72 hours). These fixatives are not recommended for use with this assay. Delay to fixation studies also revealed a loss of staining intensity for ALK when xenograft specimens were not fixed within 6 hours of excision. Refer to the interpretation guide for further discussion of the impact of specimen preparation on ALK staining intensity.

Sections should be cut approximately 4 µm thick and mounted on positively-charged glass slides. Slides should be stained promptly, as antigenicity of cut tissue sections may diminish over time and is compromised within 3 months after cutting from the paraffin block (refer to the interpretation guide, and the Performance Characteristics section below).

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. Do not use beyond the specified number of tests.
4. CAUTION: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
5. ProClin 300 solution is used as a preservative in this reagent. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
6. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
7. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[30,31]
8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at dialog.roche.com.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Warning 	H317	May cause an allergic skin reaction.
	P261	Avoid breathing mist or vapours.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.

Hazard	Code	Statement
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, reaction mass of: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

STAINING PROCEDURE

VENTANA anti-ALK (D5F3) assay has been developed for use on BenchMark IHC/ISH instruments in combination with Rabbit Monoclonal Negative Control Ig, OptiView DAB IHC Detection Kit, OptiView Amplification Kit, and ancillary reagents. Refer to the tables below for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent. Deviating from the staining protocol in Table 2, Table 3, or Table 4 can result in false positive or false negative results. For the BenchMark ULTRA instrument an ALK-specific staining procedure has been developed, U VENTANA ALK (D5F3). Prior to selecting protocol conditions from the ALK-specific staining procedure the BenchMark ULTRA instrument must have VSS 12.3 software with the OptiView v5 staining procedure or higher.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-4794.

Table 2. Recommended staining protocol for VENTANA anti-ALK (D5F3) assay and Rabbit Monoclonal Negative Control Ig with OptiView DAB IHC Detection Kit and OptiView Amplification Kit on a BenchMark ULTRA or BenchMark ULTRA PLUS instrument.

Staining Procedure	U VENTANA ALK (D5F3)
Protocol Step	Parameter Input
Antibody (Primary)	VENTANA ALK AB – 16 Min (If no reagent is selected, the default will be 4794) -4794 -4796 -7155 -7157 (Select reagent above based on region of distribution.) Or Negative Control
Counterstain	Hematoxylin II, 4 Minutes
Post Counterstain	Bluing, 4 Minutes

Table 3. Recommended staining protocol for VENTANA anti-ALK (D5F3) assay and Rabbit Monoclonal Negative Control Ig with OptiView DAB IHC Detection Kit and OptiView Amplification Kit on a BenchMark XT instrument.

Procedure Type	Method
IHC Synchronization Option	Selected [a]
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 92 Minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected
Antibody (Primary)	Ventana anti-ALK (D5F3) [-4794] or Rabbit Mono Neg [-4795] 16 Minutes, 37°C
OptiView HQ Univ Linker	12 Minutes
OptiView HRP Multimer	12 Minutes
OptiView Amplification	Selected
OV AMP H2O2, OV Amplifier	8 Minutes
OV AMP Multimer	8 Minutes
Counterstain	Hematoxylin II, 4 Minutes
Post Counterstain	Bluing, 4 Minutes

[a] This selectable step is only applicable when running XT OptiView DAB v4 and is not available with previous software versions.

Table 4. Recommended staining protocol for VENTANA anti-ALK (D5F3) assay and Rabbit Monoclonal Negative Control Ig with OptiView DAB IHC Detection Kit and OptiView Amplification Kit on a BenchMark GX instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 92 Minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected
Antibody (Primary)	Ventana anti-ALK (D5F3) [-4794] or Rabbit Mono Neg [-4795] 16 Minutes, 37°C
OptiView HQ Univ Linker	12 Minutes
OptiView HRP Multimer	12 Minutes
OptiView Amplification	Selected
OV AMP H2O2, OV Amplifier	8 Minutes
OV AMP Multimer	8 Minutes
Counterstain	Hematoxylin II, 4 Minutes
Post Counterstain	Bluing, 4 Minutes

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to “Immunohistochemistry Principles and Advances”.[32]

QUALITY CONTROL PROCEDURES

Rabbit Monoclonal Negative Control Ig

A matched negative reagent control slide must be run for every specimen to aid in the interpretation of results. Rabbit Monoclonal Negative Control Ig, a negative reagent control antibody, is specifically matched for this assay and is used in place of the primary antibody to evaluate nonspecific staining. The staining procedure for the negative reagent control should equal the primary antibody incubation period. Use of a different negative control reagent, or failure to use the recommended negative control reagent, may cause false results.

System-Level Controls

System-level controls must be run with patient samples. They can be either human appendix[33] or known ALK-positive/negative NSCLC tissue samples.

Control tissue should be autopsy, biopsy, or surgical specimens prepared and fixed as soon as possible in a manner identical to test sections. Such tissue may monitor all steps of the analysis, from tissue preparation through staining. Use of a tissue section fixed or

processed differently from the test specimen provides control for all reagents and method steps except fixation and tissue preparation.

Appendix or ALK Positive / Negative NSCLC Tissue Controls

An ALK-positive and ALK-negative control tissue must be run with every VENTANA anti-ALK (D5F3) assay staining procedure performed.

NSCLC cases with staining representative of clinically ALK-positive and clinically ALK-negative results are suitable for optimal quality control, including detection of minor levels of reagent degradation or instrument out-of-specification issues.

Human appendix tissue contains positive and negative staining elements for the ALK protein and is also suitable for use as a system-level control. The positive staining tissue components are used to confirm that the antibody was applied and the instrument functioned properly; the negative staining elements are used to detect minor levels of reagent degradation or instrument out-of-specification issues

Appropriate staining of ALK-positive and negative NSCLC and appendix tissue components are described in Table 5 and Table 6 and in the interpretation guide.

Known positive and known negative tissue controls utilized for monitoring the performance of the ALK assay on patient specimens must be fixed and processed in a similar manner as the patient specimen. Otherwise, the tissue controls can only be used to monitor for the correct performance of the test reagents. The tissue controls should not be used to aid in diagnosing patient samples.

Assay Verification

Prior to initial use of an antibody or staining system in a diagnostic procedure, the specificity of the antibody should be verified by testing it on a series of tissues with known IHC performance characteristics representing ALK-positive and -negative tissues (refer to the Quality Control Procedures previously outlined in this section of the product insert and to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist[34] or the CLSI Approved Guideline[35]). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. NSCLC tissues with known ALK status, or human appendix samples, are suitable for assay verification.

STAINING INTERPRETATION / EXPECTEDRESULTS

The VENTANA automated immunostaining procedure causes a brown colored DAB reaction product to precipitate at the antigen sites localized by VENTANA anti-ALK (D5F3) assay. A qualified pathologist experienced in IHC procedures must evaluate negative reagent controls, system-level controls and qualify the stained product before interpreting results.

Positive / Negative System-Level Tissue Controls

The stained positive and negative tissue controls should be examined to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction product on the positive control tissue within the cytoplasm of the target cells is indicative of positive reactivity.

If the positive or negative tissue controls fail to demonstrate appropriate staining or demonstrate a change in clinical diagnostic interpretation, any results with the test specimens should be considered invalid.

Table 5. Appendix tissue control evaluation criteria. Representative images are provided in the interpretation guide.

Acceptable	Unacceptable
Presence of strong granular cytoplasmic staining in ganglion cells. (See note)	Absence of strong granular cytoplasmic staining in ganglion cells.
Absence of strong granular cytoplasmic staining in glandular epithelial cells, muscle, and lymphoid tissue (scant or rare staining of lymphoreticular cells may be observed in lymphoid aggregates).	Excessive non-specific background staining of glandular epithelial cells, muscle, or lymphoid tissue that interferes with scoring.

Note: The nerve in appendix muscular layers shows positive staining.

Negative Reagent Control

Nonspecific staining, if present, will have a diffuse appearance and can be evaluated using the negative reagent control slide stained with Rabbit Monoclonal Negative Control Ig. Intact cells should be used for interpretation of staining results, as necrotic or degenerated cells often stain nonspecifically. If background staining is excessive, results from the test specimen should be considered invalid. Examples of acceptable levels of background staining for this assay can be found in the interpretation guide.

Patient Tissue

Patient tissue must be evaluated according to VENTANA anti-ALK (D5F3) assay scoring algorithm provided in Table 6. Refer to the interpretation guide.

Table 6. Scoring algorithm for VENTANA anti-ALK (D5F3) assay. Representative images are provided in the interpretation guide.

Clinical Interpretation	Staining Description
Positive for ALK	Presence of strong granular cytoplasmic staining in tumor cells (any percentage of positive tumor cells). Certain staining elements should be excluded, including: · light cytoplasmic stippling in alveolar macrophages, · cells of neural origin (nerve and ganglion cells), · glandular epithelial staining, and · scattered lymphoreticular cells within lymphocytic infiltrates. Some background staining also may be observed within normal mucosa in NSCLC (including mucin) and in necrotic tumor areas, which should be excluded from the clinical evaluation.
Negative for ALK	Absence of strong granular cytoplasmic staining in tumor cells.

SPECIFIC LIMITATIONS

1. This assay has not been validated for use with cytology smears or decalcified specimens.
2. Patient tissue must be stained within 3 months of sectioning from the tissue block. Loss of staining performance has been observed with VENTANA anti-ALK (D5F3) assay on sections that have been stored at room temperature for longer than 3 months.
3. Some staining artifacts have been noted with VENTANA anti-ALK (D5F3) assay. Light granular cytoplasmic stippling in alveolar macrophages may be present on both the anti-ALK and negative reagent control stained slides, indicating that it is an artifact of the detection system and should not be evaluated as anti-ALK positive staining. In addition, punctate staining has been observed on necrotic tumor areas; such staining should also be disregarded during patient sample evaluation. Staining of neural tissue, including nerve, and occasional lymphoreticular cells within lymphocytic infiltrate has been observed with VENTANA anti-ALK (D5F3) assay. Refer to the interpretation guide for further discussion.
4. Slight variability in overall staining intensity may be observed on the tissue controls due to the OptiView Amplification Kit. Refer to the interpretation guide for examples of acceptable staining performance.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, precision, and method comparison were conducted and the results are listed below.

Sensitivity and Specificity

Table 7. Sensitivity/specificity of VENTANA anti-ALK (D5F3) assay was determined by testing FFPE normal tissues.

Tissue	# Positive / Total Cases	Tissue	# Positive / Total Cases
Cerebrum [a]	0/3	Thymus	0/3
Cerebellum	0/3	Myeloid (bone marrow)	0/3
Adrenal gland	0/3	Lung	0/3

Tissue	# Positive / Total Cases	Tissue	# Positive / Total Cases
Ovary	0/3	Heart	0/3
Pancreas	0/3	Esophagus	0/3
Parathyroid gland	0/3	Stomach	0/3
Pituitary gland [b]	0/3	Small intestine [c]	0/3
Testis	0/3	Colon [c]	0/3
Thyroid	0/3	Liver	0/3
Breast	0/3	Salivary gland	0/3
Spleen	0/3	Kidney	0/3
Tonsil	0/3	Prostate	0/3
Endometrium	0/3	Cervix	0/3
Skeletal muscle	0/4	Skin	0/3
Nerve (sparse)	0/3	Mesothelium	0/3

[a] 2/3 A few glial cells in the cerebrum showed weak to moderate positivity.

[b] 3/3 Pituitary gland stained weakly.

[c] Ganglion cells within 4/6 intestinal tissues stained positive for ALK at varying intensities.

Table 8. Sensitivity/specificity of VENTANA anti-ALK (D5F3) assay was determined by testing a variety of FFPE tissues.

Pathology	# Positive / Total Cases
Glioblastoma (Cerebrum)	0/1
Meningioma (Cerebrum)	0/1
Ependymoma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Serous adenocarcinoma (Ovary)	1/1
Adenocarcinoma (Ovary)	0/1
Neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/2
B-cell lymphoma; NOS (Spleen)	0/3
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Mucinous adenocarcinoma (Stomach)	0/1
Adenocarcinoma (Gastrointestinal)	0/1
Gastrointestinal stromal tumor (GIST) (Colon)	0/1
Adenocarcinoma (Rectum)	0/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	1/1
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/2
Leiomyoma (Uterus)	0/1
Adenocarcinoma (Uterus)	0/1
Clear cell carcinoma (Uterus)	0/1
Squamous cell carcinoma (Uterus)	0/2
Embryonal rhabdomyosarcoma	0/1
Melanoma (Anus)	0/1
Basal cell carcinoma (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Neurofibroma (Lumbar)	0/1
Neuroblastoma (Retroperitoneum)	1/1
Mesothelioma	0/1
Hodgkin lymphoma (Lymph node)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/1
Urothelial carcinoma (Bladder)	0/1
Leiomyosarcoma	0/2
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma	0/1

Platform Concordance (Bridging Study)

VENTANA anti-ALK (D5F3) assay was initially released on the BenchMark XT and GX instruments. To demonstrate equivalent performance of the assay between the BenchMark ULTRA and BenchMark XT instruments, a bridging study was performed. This study evaluated ALK clinical status (based on the ALK scoring algorithm found in Table 6) in 184 unique NSCLC specimens stained with VENTANA anti-ALK (D5F3) assay across both platforms. The resulting stained slides were blinded and randomized then evaluated by three pathologists. Results of platform concordance for this study can be found in Table 9 and Table 10.

Table 9. Concordance results between BenchMark XT and BenchMark ULTRA instruments.

VENTANA anti-ALK (D5F3) assay concordance between the BenchMark ULTRA and BenchMark XT instruments			
BenchMark ULTRA	BenchMark XT		
	Positive	Negative	Total
Positive	85	1	86
Negative	1	97	98
Total	86	98	184

Table 10. Concordance of ALK status between BenchMark XT and BenchMark ULTRA instruments.

Platform Concordance Agreement Rates	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)	Overall Percent Agreement (95% CI)
Concordance between BenchMark XT instrument and BenchMark ULTRA instrument	98.8% (93.7-99.8%)	99.0% (94.4-99.8%)	98.9% (96.1-99.7%)

Tissue Thickness

Tissue thickness was evaluated using 4 unique cases of human NSCLC (3 ALK-positive and 1-ALK negative) and 4 unique cases of human appendix. Tissues were sectioned and tested in duplicate at 3, 4, 5, 6, and 7 microns. All tissue thicknesses demonstrated appropriate specific staining for ALK and appropriate background levels with VENTANA anti-ALK (D5F3) assay. No specimens exhibited a change in clinical ALK status within this range of thickness. Specimens should be cut at 4-6 microns for the assay.

Repeatability and Intermediate Precision Studies

Repeatability and intermediate precision of VENTANA anti-ALK (D5F3) assay were evaluated on the BenchMark ULTRA, XT, and GX instruments in combination with the OptiView DAB IHC Detection and OptiView Amplification kits.

Ten unique NSCLC tissue specimens (5 ALK-positive and 5 ALK-negative) were evaluated on both the BenchMark XT and BenchMark ULTRA instruments. For Intra-Day precision, 5 replicate slides from each of the NSCLC specimens were stained across a single BenchMark XT or a single BenchMark ULTRA instrument. For intra-instrument precision testing, 3 replicate slides from each of the NSCLC specimens were stained with VENTANA anti-ALK (D5F3) assay across three BenchMark XT instruments while 2 replicate slides from each of the NSCLC specimens were stained across three BenchMark ULTRA instruments. For Inter-Day precision, 2 replicate slides from each of the NSCLC specimens were stained with VENTANA anti-ALK (D5F3) assay on a single BenchMark XT or BenchMark ULTRA instrument across 5 non-consecutive days. All slides were blinded, randomized within each instrument tissue cohort. Each cohort was evaluated individually by a pathologist using the VENTANA anti-ALK (D5F3) assay scoring algorithm (provided in Table 6). Each replicate NSCLC specimen produced equivalent ALK IHC staining results. A summary of the results for repeatability and intermediate precision for the BenchMark XT and BenchMark ULTRA instruments can be found in Table 11 and Table 12, respectively.

An inter-platform study was performed comparing the performance of VENTANA anti-ALK (D5F3) assay on the BenchMark XT and BenchMark GX instruments. In this study two multi-tissue blocks each containing 8 NSCLC specimens (3 ALK-positive, 1 ALK-negative per block) were evaluated. For this comparison 5 replicate slides were stained on three BenchMark XT instruments and three BenchMark GX instruments. These slides were evaluated for appropriate staining based on the VENTANA anti-ALK (D5F3) IHC Scoring Algorithm found in Table 6. Each replicate NSCLC specimen produced equivalent ALK IHC staining results between the two platforms. A summary of the results can be found in Table 13.

In addition, repeatability of VENTANA anti-ALK (D5F3) assay staining on human appendix (system-level control) was also evaluated. Eight unique human appendix tissues were used for this study. For Intra-Day precision, 13 replicate slides from two multi-tissue blocks containing 4 appendix specimens were stained on a single BenchMark XT instrument. For Inter-Instrument precision, 5 replicate slides from two multi-tissue blocks containing 4 appendix specimens each were stained with VENTANA anti-ALK (D5F3) assay across three BenchMark XT instruments. For Inter-Day precision, 5 replicate slides from each of two multi-tissue blocks containing 4 appendix specimens were stained with VENTANA anti-ALK (D5F3) assay on a single BenchMark XT instrument across 5 non-consecutive days. All slides were evaluated by a pathologist using the VENTANA anti-ALK (D5F3) assay scoring guide for appendix control tissue (provided in Table 5). Each replicate appendix specimen produced equivalent ALK IHC staining results. The overall percent agreement for intra-day and inter-instrument (across 3 instruments) repeatability was 100%, while the inter-day repeatability (across 5 non-consecutive days) was 98%.

Table 11. Repeatability and intermediate precision of VENTANA anti-ALK (D5F3) assay on individual NSCLC specimens stained on the BenchMark XT instrument.

NSCLC Tissue Repeatability / Precision	N = Total Slides Evaluated in the Cohort	Overall Percent Agreement for ALK Status (95% CI)
Intra-Day Repeatability	50	100% (97.5-100%)
Intra-Platform Precision (across 3 BenchMark XT instruments)	60	100% (97.9-100%)
Inter-Day Precision (5 non-consecutive days)	100	100% (98.7-100%)

Table 12. Repeatability and intermediate precision of VENTANA anti-ALK (D5F3) assay on individual NSCLC specimens on the BenchMark ULTRA instrument.

NSCLC Tissue Repeatability / Precision	N = Total Slides Evaluated in the Cohort	Overall Percent Agreement for ALK Status (95% CI)
Intra-Day Repeatability	50	100% (92.9-100.0%)
Intra-Platform Precision (across 3 BenchMark ULTRA instruments)	60	100% (94.0-100.0%)
Inter-Day Precision (5 non-consecutive days)	100	100% (96.3-100.0%)

Table 13. Inter-Platform Precision of VENTANA anti-ALK (D5F3) assay on multi-tissue block NSCLC specimens using the BenchMark XT and BenchMark GX instruments.

NSCLC Tissue Precision	N = Total Slides Evaluated in the Cohort	Overall Percent Agreement for ALK Status
Inter-Platform Precision BenchMark XT to BenchMark GX instrument (across 3 instruments)	30	100%

Lot-to-Lot Reproducibility

Lot-to-lot reproducibility of VENTANA anti-ALK (D5F3) assay was determined by testing three lots of VENTANA anti-ALK (D5F3) assay across 38 unique NSCLC cases (21 ALK-positive specimens (from 18 unique cases) and 20 ALK-negative NSCLC tissue specimens) on the BenchMark XT instrument using the OptiView DAB IHC Detection and OptiView Amplification kits. All cases were stained in duplicate with each of the three lots of primary antibody. Slides were blinded and randomized prior to evaluation for clinical status as determined by the VENTANA anti-ALK (D5F3) assay scoring algorithm (provided in Table 6). All three lots of antibody exhibited greater than 90% concordant staining results for ALK status across the 41 NSCLC tissue specimens evaluated. Results are reported as overall percent agreement, average positive agreement, and average negative agreement rates. The overall percent agreement rate between lots was 99.2%; therefore,

VENTANA anti-ALK (D5F3) assay is reproducible in its staining results across antibody lots. Results can be found in Table 14.

Lot-to-lot reproducibility of VENTANA anti-ALK (D5F3) assay using the BenchMark ULTRA instrument was determined by testing three lots of VENTANA anti-ALK (D5F3) assay across 30 unique NSCLC cases (15 ALK-positive specimens and 15 ALK-negative NSCLC tissue specimens) using the OptiView DAB IHC Detection and OptiView Amplification kits. All cases were stained in duplicate with each of the three lots of primary antibody. Slides were blinded and randomized prior to evaluation for clinical status as determined by VENTANA anti-ALK (D5F3) assay scoring algorithm (provided in Table 6) by a pathologist. All three lots of antibody exhibited greater than 90% concordant staining results for ALK status across the 30 NSCLC tissue specimens evaluated. Results are reported as overall percent agreement, average positive agreement, and average negative agreement rates. The overall percent agreement rate between lots was 99.1%; therefore, VENTANA anti-ALK (D5F3) assay is reproducible in its staining results across antibody lots. Results can be found in Table 15.

Lot-to-lot reproducibility of VENTANA anti-ALK (D5F3) assay was also evaluated using 12 unique human appendix tissue specimens. Reproducibility was determined by testing three lots of antibody in combination with three lots of OptiView DAB IHC Detection and OptiView Amplification Kits across three BenchMark XT instruments. The overall agreement rate for appropriate positive and negative staining elements of the appendix using VENTANA anti-ALK (D5F3) assay was 100%.

Table 14. Lot-to-lot reproducibility agreement rates across 41 NSCLC tissue specimens on the BenchMark XT instrument. Twenty-one ALK-positive specimens (from 18 unique cases) and 20 ALK-negative specimens were tested.

Lot –to-Lot Reproducibility Agreement Rates	Average Positive Agreement (95% CI)	Average Negative Agreement (95% CI)	Overall Percent Agreement (95% CI)
Average of all three lot to lot comparisons	99.2% (97.4-100%)	99.1% (96.8-100.0%)	99.2% (97.5-100%)

Table 15. Lot-to-lot reproducibility agreement rates across 30 NSCLC tissue specimens on the BenchMark ULTRA instrument. Fifteen ALK-positive specimens and 15 ALK-negative specimens were tested.

Lot –to-Lot Reproducibility Agreement Rates	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)	Overall Percent Agreement (95% CI)
Average of all three lot to lot comparisons	98.9% (96.8-99.6%)	99.3% (97.3-99.8%)	99.1% (97.9-99.6%)

Inter-Reader Precision Studies

Several inter-reader precision studies were performed: two studies on the BenchMark XT instrument, and one on the BenchMark ULTRA instrument.

In BenchMark XT Inter Reader Precision Study, three pathologists evaluated a total of 185 unique cases. The 185 cases correlated with 100 ALK-positive and 100 ALK-negative blocks that were stained with VENTANA anti-ALK (D5F3) assay. The cases were blinded and randomized prior to evaluation for ALK IHC staining results per VENTANA anti-ALK (D5F3) assay scoring algorithm provided in Table 6. The results provided in Table 16 below reflect the inter-reader precision rates for unique cases from the study cohort.

Table 16. Inter-Reader Precision Study 1 on the BenchMark XT instrument

Inter-Reader Precision	Average Positive Agreement (95% CI)	Average Negative Agreement (95% CI)	Overall Percent Agreement (95% CI)
Average of all three readers comparisons	98.8% (97.3-100%)	99.0% (97.7-100%)	98.9% (97.4-100%)

BenchMark XT Inter-Reader Precision Study 2 was performed on a cohort of cases from a randomized clinical study of ALK-positive NSCLC patient specimens enrolled with the Abbott Vysis ALK Break Apart FISH Probe Kit. Approximately 300 cases were stained with VENTANA anti-ALK (D5F3) assay on the BenchMark XT instrument. The cases were blinded for ALK FISH status, randomized, and provided to three readers, who evaluated the ALK IHC staining results per the VENTANA anti-ALK (D5F3) assay scoring algorithm provided in Table 6. The results provided in Table 17 reflect the inter-reader precision rates from this clinical trial cohort.

For the BenchMark ULTRA instrument Inter-Reader Precision Study, a cohort of 184 unique NSCLC cases was evaluated. The cohort consisted of 90 ALK positive and 94 ALK negative cases which were stained with VENTANA anti-ALK (D5F3) assay on the BenchMark ULTRA instrument. The cases were blinded, randomized and provided to three readers, who evaluated the ALK IHC staining results per the VENTANA anti-ALK (D5F3) assay scoring algorithm provided in Table 6. Table 18 reflects the inter-reader precision rates from this study.

Table 17. Inter-Reader Precision Study 2 for ALK status in NSCLC specimens obtained from clinical method comparison Cohort # 1 stained with VENTANA anti-ALK (D5F3) assay on the BenchMark XT instrument.

Inter-Reader Precision	Average Positive Agreement (95% CI)	Average Negative Agreement (95% CI)	Overall Percent Agreement (95% CI)
Average of all three reader comparisons	97.6% (95.0-99.5%)	99.5% (98.9-99.9%)	99.1% (98.2-99.8%)
Reader 1 vs Reader 2	99.1% (97.1-100%)	99.8% (99.4-100%)	99.7% (98.2-99.9%)
Reader 1 vs Reader 3	96.3% (92.3-99.2%)	99.2% (98.3-99.8%)	98.6% (96.6-99.5%)
Reader 2 vs Reader 3	97.2% (93.5-100%)	99.4% (98.6-100%)	99.0% (97.1-99.7)

Table 18. Inter-Reader Precision for ALK status in NSCLC specimens stained with VENTANA anti-ALK (D5F3) assay on the BenchMark ULTRA instrument.

Inter-Reader Precision	Average Positive Agreement (95% CI)	Average Negative Agreement (95% CI)	Overall Percent Agreement (95% CI)
Average of all three Readers comparisons	98.4% (96.5-99.6%)	98.6% (96.9-99.7%)	98.5% (96.7-99.6%)
Reader 1 vs Reader 2	98.9% (96.8-100%)	98.9% (97.0-100%)	98.9% (96.0-99.7%)
Reader 1 vs Reader 3	98.8% (96.7-100%)	99.0% (97.2-100%)	98.9% (96.0-99.7%)
Reader 2 vs Reader 3	97.6% (94.7-99.4%)	97.9% (95.4-99.5%)	97.8% (94.4-99.1%)

Concordance with ALK FISH

Three cohorts were used to compare the staining results from VENTANA anti-ALK (D5F3) assay with ALK FISH in terms of ALK clinical status. The cohorts included a range of human NSCLC tissue samples from primary and metastatic tumors, including resections, needle biopsies, bronchial biopsies, and FFPE cell blocks from FNAs. All studies were scored using the scoring algorithm (described in Table 6).

Concordance Study 1

A study was conducted in an external laboratory comparing VENTANA anti-ALK (D5F3) assay with retrospective Abbott Vysis ALK Break Apart FISH Probe Kit data. The external site stained approximately 100 NSCLC cases using VENTANA anti-ALK (D5F3) assay on a BenchMark XT instrument. VENTANA anti-ALK (D5F3) assay demonstrated > 98% overall percent agreement with the retrospective Abbott Vysis ALK Break Apart FISH Probe Kit data on this NSCLC sample cohort. The results are detailed in Table 19 and Table 20. Note that 86 of the 100 cases had available FISH data and sufficient tumor present for comparison with the ALK IHC result.

Table 19. VENTANA anti-ALK (D5F3) assay compared to Abbott's Vysis ALK Break Apart FISH Probe Kit.

VENTANA anti-ALK (D5F3) assay Comparison to Abbott Vysis ALK Break Apart FISH Probe Kit			
VENTANA anti-ALK (D5F3) assay	Abbott Vysis ALK Break Apart FISH Probe Kit		Total
	Positive	Negative	
Positive	10	0	10
Negative	1	75	76
Total	11	75	86

Table 20. Percent overall, positive, and negative agreement rates for VENTANA anti-ALK (D5F3) assay compared to Abbott Vysis Break Apart FISH Probe Kit.

Percent Overall, Positive, and Negative Agreement Rates			
Rate	n/N	%	95% CI [a]
Overall Percent Agreement	85/86	98.8	93.7, 99.8
Positive Percent Agreement	10/11	90.9	62.3, 98.4
Negative Percent Agreement	75/75	100.0	95.1, 100.0

[a] Two-sided 95% confidence interval calculated using the score method.

Note that preparation of tissue specimen from this study was not verified as having followed the specimen preparation procedures recommended for this assay.

Concordance Study 2

A study was conducted in a second external laboratory comparing VENTANA anti-ALK (D5F3) assay with Abbott Vysis ALK Break Apart FISH Probe Kit data on 73 NSCLC cases (cut within one week of staining). The external site stained the cases using VENTANA anti-ALK (D5F3) assay on a BenchMark XT instrument. VENTANA anti-ALK (D5F3) assay demonstrated > 93% overall percent agreement with the retrospective Abbott Vysis ALK Break Apart FISH Probe Kit data on this NSCLC sample cohort. The results are detailed in Table 21 and Table 22.

Table 21. VENTANA anti-ALK (D5F3) assay compared to Abbott Vysis ALK Break Apart FISH Probe Kit.

VENTANA anti-ALK (D5F3) assay Comparison to Abbott Vysis ALK Break Apart FISH Probe Kit			
VENTANA anti-ALK (D5F3) assay	Abbott Vysis ALK Break Apart FISH Probe Kit		Total
	Positive	Negative	
Positive	2	4	6
Negative	0	56	56
Total	2	60	62

Table 22. Percent overall, positive, and negative agreement rates for VENTANA anti-ALK (D5F3) assay compared to Abbott Vysis Break Apart FISH Probe Kit.

Percent Overall, Positive, and Negative Agreement Rates			
Rate	n/N	%	95% CI [a]
Overall Percent Agreement	58/62	93.5%	84.6-97.5
Positive Percent Agreement	2/2	100%	34.2-100.0
Negative Percent Agreement	56/60	93%	84.1-97.4

[a] Two-sided 95% confidence interval calculated using the score method.

Of the four discordant (FISH negative, ALK IHC positive) cases, additional unstained slides were tested with another ALK (different clone and detection system). Three of the four cases agreed with VENTANA anti-ALK (D5F3) assay in terms of ALK IHC staining detected.

There were also 10 cases where FISH results were undetermined or was not performed. Four of these cases were positive by VENTANA anti-ALK (D5F3) assay and the other ALK clone, and six were negative by ALK IHC. There was one case that was positive by FISH but not enough sample was available to stain with IHC.

Concordance Study 3

In this study, approximately 300 cases from an on-going, global clinical study of ALK positive NSCLC patients enrolled with the Abbott Vysis ALK Break Apart FISH Probe Kit were stained with VENTANA anti-ALK (D5F3) assay. This is the same cohort that was previously discussed for the Inter-Reader Precision Study. Of the approximately 300 cases, some were categorized as "uninformative" by FISH or "FISH assay not performed" and were stained and evaluated for informational purposes only.

The cases were blinded for FISH status, randomized, and provided to two readers, who evaluated the staining results. Results were compared with the FISH status obtained from the global clinical study.

The results of the comparison of ALK IHC with ALK FISH are shown in Table 23.

Table 23. Agreement of VENTANA anti-ALK (D5F3) assay with Abbott Vysis ALK Break Apart FISH Probe Kit as evaluated by 2 pathologists.

VENTANA anti-ALK (D5F3) assay Comparison to Abbott Vysis ALK Break Apart FISH Probe Kit				
VENTANA anti-ALK (D5F3) assay		Abbott Vysis ALK Break Apart FISH Probe Kit		Total
Reader		Positive	Negative	
Reader 1	Positive	37	13	50
	Negative	11	223	234
	Total	48	236	284
Reader 2	Positive	37	12	49
	Negative	11	225	236
	Total	48	237	285

Note that preparation of tissue specimens from this study was not verified as having followed the specimen preparation procedures recommended for this assay.

Discrepant cases that were VENTANA anti-ALK (D5F3) assay ALK positive, ALK FISH negative:

- There were 4 cases evaluated by at least one reader as ALK IHC positive, FISH negative. Upon consensus review, it was determined that they should be evaluated as IHC negative. These cases had focal cytoplasmic/membrane staining and are explained in the interpretation guide.
- There were 9 ALK IHC positive, ALK FISH negative cases that were considered true discrepant cases.

Of the 9 discordant cases, 7 had unstained slides that were available for additional ALK diagnostic testing (molecular testing and IHC testing using a different clone and detection system). These additional testing results indicated that the majority of discrepant cases favored the positive IHC evaluation for ALK status when ALK FISH was negative. (Note that cut slides from these cases exceeded the recommended 3 months).

Discrepant cases that were VENTANA anti-ALK (D5F3) assay negative, ALK FISH positive:

- There were 11 cases that were positive by FISH but negative by VENTANA anti-ALK (D5F3) assay. 10 cases had unstained slides that were available for the additional ALK diagnostic testing with molecular techniques and IHC. These additional testing results indicated that the majority of cases that were negative by VENTANA anti-ALK (D5F3) assay were also negative by another ALK IHC system, but were positive by one or more molecular assays. Note that cut slides from these cases exceeded the recommended 3 months for ALK IHC.
- Finally, there were 14 cases in the cohort that were uninformative by FISH (no result was obtained). Of these, 3 were evaluated as positive by both readers by VENTANA anti-ALK (D5F3) assay. In addition, there were 19 cases where the FISH assay could not be performed, based on the H&E slide (usually due to the tumor content being insufficient). Of these, both readers evaluated the ALK IHC staining results as positive in 4 cases. Therefore, on average, 21% of the cases where FISH results were not obtained had a positive ALK status by VENTANA anti-ALK (D5F3) assay.

Inter-Laboratory Reproducibility Study on the BenchMark XT Instrument

An Inter-Laboratory Reproducibility Study for VENTANA anti-ALK (D5F3) assay was completed to demonstrate reproducibility of the assay in determining ALK clinical status on the BenchMark XT instrument, using NSCLC (6 ALK-positive and 6 ALK-negative) tissue specimens run across 1 reagent lot, 3 instruments and 5 non-consecutive days at three external laboratories. The specimens were randomized and evaluated by a total of 6 readers (2 readers/site) who were blinded to the ALK clinical status of the cohort. This cohort contained 180 slides generated from 12 NSCLC cases positive and negative for ALK expression by IHC and FISH. These cases were stained in replicate over 21 days at the 3 laboratories. See Table 24 for results. The acceptability rate for morphology and background in these studies was 100%. The Inter-Laboratory Reproducibility (ILR) study for VENTANA anti-ALK (D5F3) assay on the Benchmark XT instrument provided average positive agreement (APA) and average negative agreement (ANA) for between-site, between-reader, and between-run (day) comparisons performed pairwise utilizing evaluable observations.

Table 24. ILR: Agreement rates for VENTANA anti-ALK (D5F3) assay on the BenchMark XT instrument (n = 180 slides evaluated).

Agreement Rates for Inter-Laboratory Reproducibility (ALK Clinical Status)	Average Positive Agreement (95% CI)	Average Negative Agreement (95% CI)	Overall Percent Agreement (95% CI)
Between-site (3 sites)	93.8% (76.2-100%)	94.9% (79.2-100%)	94.4% (83.3-100%)
Between-day (5 non-consecutive days)	99.1% (96.4-100%)	99.2% (96.9-100%)	99.2% (97.5-100%)
Between-reader (2 readers/site)	98.8% (95.2-100%)	99.0% (95.8-100%)	98.9% (96.7-100%)

Inter-Laboratory Reproducibility Study on BenchMark ULTRA Instrument

An additional Inter-Laboratory Reproducibility Study for VENTANA anti-ALK (D5F3) assay was completed to demonstrate reproducibility of the assay in determining ALK clinical status on the BenchMark ULTRA instrument, using NSCLC (7 ALK-positive and 7 ALK-negative) tissue specimens run across 1 reagent lot, 3 instruments and 5 non-consecutive days at three external laboratories on the BenchMark ULTRA instrument. The specimens were randomized and evaluated by a total of 6 readers (2 readers/site) who were blinded to the ALK clinical status of the cohort. This cohort contained 210 slides generated from 14 NSCLC cases positive and negative for ALK expression by IHC and FISH. These cases were stained in replicate over 21 days at the 3 laboratories. See Table 25 for results. The overall final staining acceptability rate for all data pooled was 99%. The acceptability rate for morphology and background in these studies was 100%. See Table 26 for results.

The ILR study for VENTANA anti-ALK (D5F3) assay on the BenchMark ULTRA instrument provided positive percent agreement (PPA) and negative percent agreement (NPA) across all evaluable observations obtained from the study by pooling all sites, readers, and days, when using the consensus score as a reference standard.

Table 25. ILR: Agreement rates for VENTANA anti-ALK (D5F3) assay on BenchMark ULTRA instrument (n = 210 slides evaluated).

Agreement Rates for Inter-Laboratory Reproducibility (ALK Clinical Status)	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)	Overall Percent Agreement (95% CI)
Across all evaluable observations	92.8% (88.4, 95.6%)	100.0% (98.2, 100.0%)	96.4% (94.1, 97.8%)

Table 26. Inter-Laboratory Reproducibility: Inter-reader agreement rates for VENTANA anti-ALK (D5F3) assay on BenchMark ULTRA instrument (n = 210 slides evaluated).

Agreement Rates for Inter-Laboratory Reproducibility (ALK Clinical Status) between reader agreement rates	Average Positive Agreement (95% CI)	Average Negative Agreement (95% CI)	Overall Percent Agreement (95% CI)
Readers A1 vs A2	97.0% (87.5-100%)	97.3% (89.5-100%)	97.1% (90.2-99.2%)
Readers B1 vs B2	93.5% (71.4-100%)	94.7% (81.0-100%)	94.2% (86.0-97.7%)
Readers C1 vs C2	92.3% (66.7-100%)	93.2% (74.7-100%)	92.8% (84.1-96.9%)
Overall	94.3% (75.6-100%)	95.1% (81.6-100%)	94.7% (84.1-100%)

Inter-Laboratory and Inter-Reader Reproducibility Study on BenchMark ULTRA PLUS Instrument Using OptiView DAB IHC Detection and OptiView Amplification Kits.

The BenchMark ULTRA PLUS Instrument Inter-Laboratory Reproducibility Study for VENTANA anti-ALK (D5F3) Assay was completed to demonstrate reproducibility of the assay in determining ALK clinical status on the BenchMark ULTRA PLUS instrument using NSCLC (14 ALK-positive and 14 ALK-negative) tissue specimens run across a reagent lot, and instrument at each of three external laboratories for 5 non-consecutive days. Specimens were randomized and evaluated by a total of 6 readers (2 readers/site). For each case, all available reader observations were compared against the modal status for the case. Negative reagent control slide and ALK-stained slide acceptability was 100% for all 420 slides (140 per site) evaluated. See Table 27, Table 28, and Table 29 for results

The VENTANA anti-ALK (D5F3) BenchMark ULTRA PLUS ILR analysis provided positive percent agreement (PPA) and negative percent agreement (NPA) across all evaluable observations obtained from the study by pooling all sites, readers, and days, when using the case-level modal ALK status as a reference standard.

Table 27. Inter-Laboratory Reproducibility: Agreement rates for VENTANA anti-ALK (D5F3) Assay on BenchMark ULTRA PLUS instrument (n=420 slides evaluated).

Inter-Laboratory Reproducibility of ALK Clinical Status	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)	Overall Percent Agreement (95% CI)
Across all evaluable observations	98.6% (97.3, 99.7)	99.5% (98.7-100%)	99.0% (98.3-99.7%)

In addition to the case-level modal status approach analysis, pair-wise approach analyses were also performed and average positive agreement (APA), average negative agreement (ANA) results are presented below.

Table 28. Inter-Laboratory Reproducibility: Agreement rates for VENTANA anti-ALK (D5F3) Assay on BenchMark ULTRA PLUS instrument (n=420 slides evaluated).

Agreement Rates for Inter-Laboratory Reproducibility (ALK Clinical Status)	Average Positive Agreement (95% CI)	Average Negative Agreement (95% CI)	Overall Percent Agreement (95% CI)
Between-site (3 sites)	98.1% (96.6-99.4%)	98.1% (96.8-99.4%)	98.1% (96.7-99.4%)
Between-day (5 non-consecutive days)	98.3% (96.9-99.4%)	98.3% (97.2-99.5%)	98.3% (97.1-99.5%)

Table 29. Inter-Laboratory Reproducibility: Inter-reader agreement rates for VENTANA anti-ALK (D5F3) Assay on the BenchMark ULTRA PLUS instrument (n=420 slides evaluated).

Inter-Laboratory Reproducibility of ALK Clinical Status between Readers	Average Positive Agreement (95% CI)	Average Negative Agreement (95% CI)	Overall Percent Agreement (95% CI)
Readers A1 vs A2	99.3% (97.7-100%)	99.3% (98.0-100%)	99.3% (97.9-100%)
Readers B1 vs B2	94.8% (90.5-98.6%)	95.2% (91.4-98.6%)	95.0% (91.1-98.6%)
Readers C1 vs C2	100.0% (97.3-100%)	100.0% (97.3-100%)	100.0% (97.3-100%)
Overall	98.1% (96.6-99.4%)	98.1% (96.7-99.4%)	98.1% (96.7-99.4%)

Concordance between BenchMark ULTRA PLUS and BenchMark ULTRA Instruments

Three laboratories, from separate institutions in the United States, participated in a concordance study between the BenchMark ULTRA PLUS instrument and the BenchMark ULTRA instrument. One hundred and forty-four cases representing the staining range of the intended use population of the VENTANA anti-ALK (D5F3) assay were selected for use in this study. All 144 cases were stained at Ventana on a BenchMark ULTRA using the OptiView DAB IHC Detection kit paired with the OptiView Amplification Kit. Cut slides from the same cases were randomized and equally distributed (48 cases/per site) across study sites for staining on a BenchMark ULTRA PLUS instrument using the recommended staining protocol with one lot of OptiView DAB IHC Detection and OptiView Amplification Kits. Two pathologists per site, blinded to case status, evaluated the slides stained on the corresponding BenchMark ULTRA PLUS instrument and provided a clinical status. After a 2 week washout period, corresponding case slides previously stained at Ventana on the BenchMark ULTRA instrument were distributed to the appropriate sites for clinical evaluation. Additionally, one internal pathologist reviewed all study slides and was included as a third pathologist for each of the sites. The results were analyzed by Ventana for an ALK-positive or negative clinical status. The OPA, PPA and NPA rates were 95.4% (412/432), 93.5% (202/216), and 97.2% (210/216), respectively. The two-sided 95% CIs were calculated using the percentile bootstrap method. Background and morphology acceptability rates for all cases were 100% for both instruments. Results can be found in Table 30.

Table 30. Pooled Agreement of ALK NSCLC Cases Stained with VENTANA anti-ALK (D5F3) Assay on BenchMark ULTRA PLUS vs BenchMark ULTRA Instruments.

BenchMark ULTRA PLUS ALK Status	BenchMark ULTRA ALK Status		
	Positive	Negative	Total
Positive	202	6	208
Negative	14	210	224
Total	216	216	432
	n/N	% (95% CI[a])	
Positive Percent Agreement	202/216	93.5 (89.5, 97.2)	
Negative Percent Agreement	210/216	97.2 (94.5, 99.5)	
Overall percent agreement	412/432	95.4 (92.8, 97.5)	

[a] Two-sided 95% CIs were calculated using the percentile bootstrap method with 2000 replicates selected with stratification by qualification bin [ALK-positive, ALK-negative, challenging ALK-positive, challenging ALK-negative].

CLINICAL PERFORMANCE

Method Comparison Study on BenchMark XT Instrument

The Method Comparison Study cohorts were generated from two independent, randomized clinical trials of crizotinib (designated Trial #1 and Trial #2) that enrolled patients with ALK-positive NSCLC. ALK status for these patients was determined using the Vysis ALK Break Apart FISH Probe Kit clinical trial assay at multiple central laboratories. Valid Vysis ALK FISH results were obtained for a total of 1644 NSCLC tissue specimens (1018 and 626 specimens for Trials #1 and #2, respectively).

In VENTANA anti-ALK (D5F3) assay Method Comparison Study, specimens from patients screened for Trials #1 and #2 were sent to a central laboratory for staining with VENTANA anti-ALK (D5F3) assay and evaluation for ALK IHC status based on VENTANA anti-ALK (D5F3) assay scoring algorithm criteria (Table 6). Of the specimens yielding valid Vysis ALK FISH results in clinical trial screening, 933 specimens from Trial #1 (Table 31) and 598 specimens from Trial #2 (Table 33) also yielded valid results for VENTANA anti-ALK (D5F3) assay.

The numbers of specimens yielding ALK-positive and ALK-negative results for each assay are shown in Tables 31 and 33 for the Trial #1 and #2 cohorts, respectively. The agreement rates between the two assays are shown in Tables 32 and 34 for the Trial #1 and #2 cohorts, respectively. The reported positive and negative percent agreement rates were 86.0% and 96.3%, respectively, for Trial #1 (Table 32) and 92.7% and 94.8%, respectively, for Trial #2 (Table 34).

Table 31. ALK status comparison in NSCLC specimens (cohort from Trial #1) determined using VENTANA anti-ALK (D5F3) assay vs. Vysis ALK Break Apart FISH Probe Kit.

ALK Status		Vysis ALK Break Apart FISH Probe Kit		
		Positive	Negative	Total
VENTANA anti-ALK (D5F3) assay	Positive	154	28	182
	Negative	25	726	751
	Total	179	754	933

Table 32. Agreement rates between VENTANA anti-ALK (D5F3) assay and Vysis ALK Break Apart FISH Probe Kit in Trial #1.

Agreement Rates between ALK Assays	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)	Overall Percent Agreement (95% CI)
VENTANA anti-ALK (D5F3) antibody and Vysis ALK Break-Apart FISH Probe Kit	86.0% (80.2-90.4%)	96.3% (94.7-97.4%)	94.3% (92.6-95.6%)

Table 33. ALK status comparison cohort from Trial #2 in NSCLC specimens determined using VENTANA anti-ALK (D5F3) assay vs. Vysis ALK Break Apart FISH Probe Kit.

ALK Status		Vysis ALK Break Apart FISH Probe Kit		
		Positive	Negative	Total
VENTANA anti-ALK (D5F3) antibody	Positive	179	21	200
	Negative	14	384	398
	Total	193	405	598

Table 34. Agreement rates between VENTANA anti-ALK (D5F3) assay and Vysis ALK Break Apart FISH Probe Kit in Trial #2.

Agreement Rates between ALK Assays	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)	Overall Percent Agreement (95% CI)
VENTANA anti-ALK (D5F3) assay and Vysis ALK Break Apart FISH Probe Kit	92.7% (88.2-95.6%)	94.8% (92.2-96.6%)	94.1% (92.0-95.8%)

Note that tissue specimens used in Trial #1 and Trial #2 were not verified as having been prepared according to the specimen preparation procedures recommended for VENTANA anti-ALK (D5F3) assay.

Crizotinib Clinical Outcome Study

The clinical efficacy analysis for VENTANA anti-ALK (D5F3) assay as a diagnostic device for selection of patients who might benefit from treatment with crizotinib, an ALK-targeted agent, was based on Trial #1. These patients were tested with VENTANA anti-ALK (D5F3) assay under the Method Comparison Study as well as an additional study. Trial #1 was a multicenter, multinational, randomized, open-label, Phase 3 efficacy and safety study of crizotinib vs. first-line chemotherapy (pemetrexed/cisplatin or pemetrexed/carboplatin) in previously untreated patients with ALK-positive advanced non-squamous NSCLC. The Vysis ALK Break Apart FISH Probe Kit (ALK FISH) was used to determine ALK positivity and trial eligibility for Trial #1. Based on the Vysis ALK FISH assay results, 343 patients were in the randomized set (172 in the crizotinib arm and 171 in the chemotherapy arm). In VENTANA anti-ALK (D5F3) assay Clinical Outcome Study, tissue specimens from Trial #1 were retrospectively tested with VENTANA anti-ALK (D5F3) assay. Of the 343 patients enrolled in Trial #1, 133 had been tested with VENTANA anti-ALK (D5F3) assay under the Method Comparison Study protocol, and an additional 39 patients had been tested under a separate study protocol, for a total of 172 patients tested with VENTANA anti-ALK (D5F3) assay. Of these patients, 166 were diagnosed as ALK-positive or ALK-negative by ALK (D5F3) IHC. The overall efficacy results for these patients are summarized according to VENTANA anti-ALK (D5F3) assay results in Table 35.

Table 35. Clinical benefit of crizotinib (progression-free survival) for patients enrolled in Trial #1.

ALK Status		HR [a]	SE [a]	95% CI [a]	Sample Size	
					Chemotherapy Arm	Crizotinib Arm
Total Enrolled	FISH+	0.454	0.139	(0.346, 0.596)	171	172
ALK IHC Tested	FISH+ [b]	0.407	0.214	(0.267, 0.618)	82	90
	FISH+/IHC+	0.401	0.237	(0.252, 0.639)	63	78
	FISH+/IHC-	1.711	0.703	(0.431, 6.789)	17	8

[a] Observed hazard ratio (HR) for progression-free survival (PFS) of crizotinib versus chemotherapy; standard error (SE); and 2-sided 95% confidence interval (CI). Results were estimated using a stratified Cox model with the following strata: race, brain metastasis, and ECOG score.

[b] For two ALK FISH+ patients in the chemotherapy arm and 4 patients in the crizotinib arm, no positive or negative ALK IHC result was obtained.

Additional imputation analyses were performed to include patients with missing or invalid VENTANA anti-ALK (D5F3) assay test results and to evaluate the robustness of study conclusions. Statistical analysis of discordant patients not enrolled in Trial #1 involved simulation of a range of possible outcomes for these patients. Results from all of the hypothetical analyses were generally similar to those from the primary efficacy analysis.

FISH+ / IHC- Discordant Cases from Trial #1:

Method Comparison Study

In the Method Comparison Study (Table 35), 25 patients from Trial #1 were evaluated as FISH+/IHC-. The median Vysis ALK FISH score (% tumor cells positive for ALK gene rearrangement) for these cases was 20% (mean 31.6%, SD 21.58%), and for 14 of these cases, the FISH score was 25% or less. While all of these cases had Vysis ALK FISH scores above the 15% cut-off for ALK positivity, their scores were in the FISH equivocal zone (10%–50%). In contrast, the median FISH score observed for all enrolled patients tested with IHC was 58% (mean 56.9%, SD 21.97%).

Clinical Outcome Study

In the Clinical Outcome Study, 25 patients enrolled into Trial #1 were evaluated as FISH+/IHC- (see last row of Table 35). Eight of these cases were randomized to the crizotinib arm of the clinical study. Of these patients, five had FISH scores very close to the FISH cut-off (15%–18% of tumor cells positive for ALK gene rearrangement) and also exhibited objective progression or stable disease/no response. Two of the 8 patients had FISH scores outside the FISH equivocal zone (66% and 72% of tumor cells positive for ALK gene rearrangement) and exhibited a partial objective tumor response. The eighth IHC- patient was FISH- and was enrolled erroneously; this patient exhibited an indeterminate response.

FISH- / IHC+ Discordant Cases from Trial #1

In the VENTANA anti-ALK (D5F3) assay Method Comparison Study, 28 cases screened for Trial #1 were evaluated as FISH-/IHC+. Since FISH was the clinical trial assay, and only FISH+ cases were enrolled into Trial #1, no outcome data are available on the FISH-/IHC+ discordant cases.

Ceritinib Clinical Outcome Study

The clinical efficacy analysis of VENTANA anti-ALK (D5F3) assay as a diagnostic device for selection of patients who might benefit from treatment with ceritinib was based on an open-label, randomized, active-control multi-center, Phase 3 study (Trial #3) of oral ceritinib. This study compared the clinical efficacy and safety of ceritinib treatment to that of chemotherapy (platinum-based doublet with pemetrexed followed by pemetrexed maintenance in patients without progressive disease after 4 cycles) in previously untreated adult patients with ALK-positive, locally advanced or metastatic, non-squamous NSCLC. VENTANA anti-ALK (D5F3) assay was used on the BenchMark XT instrument to test a total of 1778 patients for Trial #3 eligibility, which required a positive ALK status. A total of 376 patients whose tumors yielded ALK-positive results from the assay were in the

randomized set (189 in the ceritinib arm and 187 in the chemotherapy arm). The overall efficacy results for the ceritinib-treated patients are summarized in Table 36. Ceritinib demonstrated a statistically significant and clinically meaningful benefit over chemotherapy, with a 45% risk reduction in PFS per BIRC (HR = 0.55; 95% CI: 0.42, 0.73; p < .001), for patients selected using VENTANA anti-ALK (D5F3) assay. The median PFS per BIRC assessment was 16.6 months (95% CI: 12.6, 27.2) and 8.1 months (95% CI: 5.8, 11.1) for the ceritinib and chemotherapy arms, respectively.

Table 36. Clinical benefit of ceritinib (progression-free survival) for patients enrolled in Trial #3.

Progression-Free Survival	Ceritinib (N = 189)	Chemotherapy (N = 187)
Median, months (95% CI) [a]	16.6 (12.6, 27.2)	8.1 (5.8, 11.1)
HR (95% CI) [b]	0.55 (0.42, 0.73)	
p-valued [c]	< 0.001	

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 Note b).

Staining acceptability rates for VENTANA anti-ALK (D5F3) assay in the intent-to-diagnose population (the 1778 patients tested with the assay) are reported in Table 37. The rates of acceptable morphology and acceptable background for VENTANA anti-ALK (D5F3) assay-stained slides are also reported. For 122 specimens, the initial VENTANA anti-ALK (D5F3) assay staining attempt failed, and another staining attempt was performed. On the final staining attempt, 48 of the 122 specimens remained unacceptable (1 due to invalid run control, 30 due to unacceptable H&E, and 12 due to unacceptable negative reagent control, 1 due to unacceptable background, 2 due to unacceptable background and morphology, and 2 due to unevaluable IHC slide). VENTANA anti-ALK (D5F3) assay demonstrated high initial and final overall staining acceptability rates; 93.1% and 97.3% respectively. Final morphology and background acceptability rates were 99% or greater.

Table 37. Initial and final VENTANA anti-ALK (D5F3) assay staining performance characteristics for NSCLC study specimens screened for enrollment into Trial #3.

Evaluated Staining Attributes	Acceptability Rate % (n/N) (95% CI)	
	Initial Staining Attempt	Final Staining Attempt
Overall ALK IHC staining acceptability rate	93.1% (1656/1778) (91.9-94.2%)	97.3% (1730/1778) (96.4-98.0%)
Background staining	99.0% (1655/1672) (98.4-99.4%)	99.8% (1727/1730) (99.5-99.9%)
Morphology	99.0% (1657/1674) (98.4-99.4%)	99.9% (1728/1730) (99.6-100%)

Alectinib Clinical Outcome Study

The clinical efficacy analysis of VENTANA anti-ALK (D5F3) assay as a diagnostic device for selection of patients who might benefit from treatment with alectinib was based on an open-label, randomized active-control multi-center Phase 3 study (Trial #BO28984) of oral alectinib. This study compared the clinical efficacy and safety of alectinib treatment to that of crizotinib in previously untreated adult patients with ALK-positive, locally advanced or metastatic, NSCLC. VENTANA anti-ALK (D5F3) assay was used on the BenchMark XT instrument to test a total of 1239 patients for Trial #BO28984 eligibility, which required a positive ALK status by central testing. A total of 303 patients whose tumors yielded ALK-positive results from the assay were randomized and analyzed for efficacy (152 in the alectinib arm and 151 in the crizotinib arm). The overall efficacy results are summarized in Table 38. Alectinib demonstrated a statistically significant and clinically meaningful benefit over crizotinib with a 53% risk reduction in PFS per investigator assessment (HR = 0.47, 95% CI: 0.34, 0.65; p < 0.0001) and a 50% risk reduction in PFS per IRC (HR = 0.50, 95% CI: 0.36, 0.7; p < 0.0001), for patients selected using the VENTANA anti-ALK (D5F3) assay. The median PFS per investigator assessment has not been reached in the alectinib arm and per IRC assessment was 25.7 months (95% CI: 19.9, NE) and 10.4 months (95% CI: 7.7, 14.6) for the alectinib and crizotinib arms, respectively.

Table 38. Clinical benefit of alectinib or crizotinib (progression-free survival) for patients enrolled in Trial #BO28984.

Progression-Free Survival Investigator-assessed	Alectinib (N = 152)	Crizotinib (N = 151)
Median, months (95% CI) [a]	NE (17.7, NE%)	11.1 (9.1,13.1%)
HR (95% CI) [b]	0.47 (0.34, 0.65%)	
p-valued [c]	< 0.0001	
Progression-Free Survival IRC-assessed	**Alectinib (N = 152)**	**Crizotinib (N = 151)**
Median, months (95% CI) [a]	25.7 (19.9, NE%)	10.4 (7.7, 14.6%)
HR (95% CI) [b]	0.50 (0.36, 0.70%)	
p-valued [c]	< 0.0001	

HR = hazard ratio; CI = confidence interval; IRC = Independent Review Committee; NE = not estimable

[a] Estimated using the Kaplan-Meier method.

[b] Hazard ratio was estimated by Cox regression, stratified for covariates Race (Asian vs Non-Asian) and CNS metastases at baseline (presence/absence) by IRC

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

Staining acceptability rates for VENTANA anti-ALK (D5F3) assay in the intent-to-diagnose population (the 1239 patients tested with the assay) were comparable to results in trial A2301.

Lorlatinib Clinical Study Outcome

The efficacy of lorlatinib for the treatment of patients with ALK-positive NSCLC who had not received prior systemic therapy for metastatic disease was established in an open-label, randomized, active-controlled, multicenter study (Study B7461006; NCT03052608; CROWN). Patients were required to have an ECOG performance status of 0-2 and ALK-positive NSCLC as identified by VENTANA anti-ALK (D5F3) assay. Neurologically stable patients with treated or untreated asymptomatic central nervous system (CNS) metastases, including leptomeningeal metastases, were eligible.

A total of 296 patients were randomized 1:1 to receive lorlatinib 100 mg orally once daily (n = 149) or crizotinib 250 mg orally twice daily (n = 147). Randomization was stratified by ethnic origin (Asian vs. non-Asian) and the presence or absence of CNS metastases at baseline. Treatment on both arms was continued until disease progression or unacceptable toxicity. The major efficacy outcome measure was progression-free survival (PFS) as determined by Blinded Independent Central Review (BICR) according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1).

The study results demonstrated a substantial and statistically significant improvement in PFS for the lorlatinib arm over the crizotinib arm, with a 72% reduction in the risk of disease progression or death per BICR (HR = 0.28; 95% CI: 0.19, 0.41; p < 0.0001; Table 39). OS data were immature at the time of the clinical data cutoff.

Subgroup analyses in participants with or without brain metastasis at baseline yielded results consistent with the primary PFS outcome. The probability that the CNS would be the first site of disease progression, alone or with concurrent systemic progression, was markedly lower in the lorlatinib arm (2.7%) than in the crizotinib arm (23.8%), with a cause-specific stratified HR of 0.06 (95% CI: 0.02, 0.18).[36]

Table 39. Overall Efficacy Results in Study B7461006 (CROWN).

Progression-Free Survival (BICR-Assessed)	Lorlatinib (N = 149)	Crizotinib (N = 147)
Number of events, n (%)	41 (27.5%)	86 (58.5%)
Progressive disease, n (%)	32 (21.5%)	82 (55.8%)
Death, n (%)	9 (6.0%)	4 (2.7%)
Probability of PFS at 12 months (95% CI) [a]	0.78 (0.70, 0.84)	0.39 (0.30, 0.48)
Hazard ratio (95% CI) [b]	0.28 (0.19, 0.41)	
p-value [c]	< 0.0001	

Abbreviations: CI = confidence interval; NE = not estimable; PFS = progression free survival.

[a] CIs were derived using the log-log transformation with back transformation to original scale.

[b] Stratified hazard ratio based on Cox proportional hazards model.

[c] One-sided p-value based on stratified log-rank test.

VENTANA anti-ALK (D5F3) assay testing of 232 Study B7461006 screening specimens at central laboratories yielded staining acceptability rates that were nearly identical to those observed in Trial A2301.

CONCLUSION

VENTANA anti-ALK (D5F3) assay is reproducible in its staining results for clinical ALK status on BenchMark IHC/ISH instruments. The binary scoring algorithm is highly reproducible across readers. The assay is concordant with Vysis ALK Break Apart FISH Probe Kit for ALK status. VENTANA anti-ALK (D5F3) assay may be used in identifying patients eligible for treatment with XALKORI® (crizotinib), ZYKADIA® (ceritinib), ALECENSA® (alectinib) or lorlatinib.

TROUBLESHOOTING

If inappropriate staining is observed on either the system-level human NSCLC or appendix tissue controls, or patient specimens, ensure that instrument maintenance procedures have been followed for the BenchMark IHC/ISH instrument. If no technical issues or deviations are noted then, prior to conducting a repeat run, please contact your local support representative.

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:
https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
B	Updates to Clinical Significance, Warnings and Precautions, Staining Procedure, Staining Interpretation / Expected Results, Analytical Performance, References and Intellectual Property sections. Added BenchMark ULTRA PLUS instrument.

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