SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Anaplastic lymphoma kinase assay
Device Trade Name:	VENTANA ALK (D5F3) CDx Assay
Device Procode:	PKW
Applicant's Name and Address:	Ventana Medical Systems, Inc. 1910 E. Innovation Park Drive Tucson, AZ 85755
Date of Panel Recommendation:	None

Premarket Approval Application (PMA) Number: P140025

Date of Notice of Approval:	June 12, 2015
Expedited:	Not Applicable

II. INDICATIONS FOR 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 automated staining instrument. It is indicated as an aid in identifying patients eligible for treatment with XALKORI® (crizotinib).

III. CONTRAINDICATIONS

There are no known contraindications for use for this test.

IV. WARNINGS AND PRECAUTIONS

Warnings and precautions can be found in the VENTANA ALK (D5F3) CDx Assay product labeling and in the BenchMark XT automated slide stainer labeling.

V. DEVICE DESCRIPTION

The VENTANA ALK (D5F3) CDx Assay consists of the following reagents and instruments:

- VENTANA ALK (D5F3) Antibody
- OptiView Amplification Kit
- OptiView DAB IHC Detection Kit
- BenchMark XT instrument (automated slide stainer)
- Rabbit Monoclonal Negative Control Ig

VENTANA ALK (D5F3) CDx Assay is a rabbit monoclonal primary antibody for use with the VENTANA OptiView DAB IHC Detection Kit and OptiView Amplification Kit on the automated BenchMark XT platform. The OptiView DAB IHC Detection Kit, OptiView Amplification Kit, Rabbit Monoclonal Negative Control Ig and the BenchMark XT instrument are all Ventana products and are procured by the end user commercially. The ALK (D5F3) antibody is a recombinant rabbit monoclonal antibody targeted for recognition of the C-terminal peptide region of the human ALK protein, and is purified from culture supernatant.

The ALK (D5F3) antibody binds to ALK in FFPE NSCLC tissue sections, which is followed by a sequential application of a secondary (linker) antibody that binds to the primary antibody, a tertiary antibody enzyme complex (multimer) that binds to the secondary antibody, and a chromogenic substrate. Interposed washing steps stop the reaction after appropriate incubation times between each of the steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site, which is DAB (3,3'-diaminobenzidine) with amplification of the signal via tyramide. The specimen is counterstained and cover slipped. Results are interpreted and visualized using light microscopy. The final slide staining results are evaluated by a pathologist. NSCLC cases that are positive with the VENTANA ALK (D5F3) CDx Assay demonstrate strong granular cytoplasmic staining in any percentage of tumor cells. NSCLC cases determined to be negative with the VENTANA ALK (D5F3) CDx Assay demonstrate an absence of strong granular staining. The VENTANA ALK (D5F3) CDx Assay scoring algorithm is specified in the product labeling, which includes the Interpretation Guide and Quick Reference Guide.

One 5 mL dispenser of VENTANA ALK (D5F3) CDx Assay contains approximately 70 µg of the rabbit monoclonal antibody targeted for the ALK protein, which is sufficient reagent for 50 tests. The antibody is diluted in 0.08 M PBS with 3% carrier protein and 0.05% ProClin 300, a preservative. Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 14 µg/mL. Ancillary reagents that are compatible with the BenchMark XT instrument system are required. The VENTANA ALK (D5F3) CDx Assay Interpretation Guide for NSCLC (containing color images of representative staining patterns and known artifacts) is available to the end user to assist in the interpretation of assay results.

Instruction in the product labeling states that “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.

Instrumentation and Software

VENTANA ALK (D5F3) CDx Assay was developed for use on a VENTANA BenchMark XT automated slide stainer in combination with Rabbit Monoclonal Negative Control Ig, OptiView DAB IHC Detection Kit, OptiView Amplification Kit and ancillary reagents. An assay specific staining procedure must be used with the VENTANA ALK (D5F3) CDx Assay.

The assay specific staining procedure software is named "XT VENTANA ALK(D5F3) CDx". The following steps in the staining procedure software are locked: deparaffinization, cell conditioning, pre-primary peroxidase inhibitor, antibody incubation, OptiView HRP Multimer, OptiView Amplification, OptiView AMP, OptiView H_2O_2 and OptiView AMP Multimer. The OptiView HQ Universal Linker, Counterstain and Post Counterstain steps remain selectable by users. For the OptiView HQ Universal Linker, the user can chose between only 2 options: 8 minutes or 12 minutes. The default option is 12 minutes, as specified in the product labeling. Similarly, for both the Counterstain and Post Counterstain options, users can select any time from 4 minutes to 32 minutes. The manufacturer's recommended counterstain times are 4 minutes for each counterstain step.

Test Controls and Calibrator

Ventana recommends the use of human NSCLC or appendix tissue as run controls to establish run validity of this assay. These tissue controls are not provided by Ventana and must be sourced by end users. Instructions are included in the product labeling on appropriate staining, qualification and interpretation of these end user provided tissue controls to assess run validity. Each run must have a system-level control and each case requires a corresponding slide stained with the negative reagent control.

Negative Reagent Control

The Rabbit Monoclonal Negative Control Ig is a pre-diluted, ready-to-use antibody product for use with Ventana detection chemistries on Ventana IHC instrument systems. The Rabbit Monoclonal Negative Control Ig should be run for each case to be assessed with the VENTANA ALK (D5F3) CDx Assay. The Rabbit Monoclonal Negative Control Ig is directed against DNP (Dinitrophenyl), which does not occur naturally, and should demonstrate appropriate negative staining results in human tissue. It is purified from culture supernatant.

System Level Controls

End user provided tissue controls (appendix or NSCLC) are required in each run with patient specimens. A major advantage of using end user provided tissue controls is that they monitor all aspects of pre-analytical variables from fixation to processing, to embedding and sectioning.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Currently, there is one FDA-approved alternative for detection of ALK status in FFPE NSCLC tissues, for the selection of patients who are eligible for XALKORI (crizotinib)

treatment. Namely, the Vysis ALK Break Apart FISH Probe Kit was approved under PMA P110012. This FDA-approved test is a fluorescence in situ hybridization (FISH)-based assay that detects rearrangements involving the ALK gene in human NSCLC tissue specimens. To date, there are no FDA-approved anti-ALK antibodies commercially available for use in immunohistochemistry applications.

VII. MARKETING HISTORY

The VENTANA ALK (D5F3) CDx Assay has not been marketed in the United States. It is marketed in the following countries under the VENTANA anti-ALK (D5F3) Rabbit Monoclonal Primary Antibody product name: Austria, France, Germany, Italy, Middle East, Belgium, Denmark, Finland, Netherlands, Norway, Portugal, Sweden, Slovenia, Baltics, Croatia, Czech Republic, Estonia, Greece, Hungary, Poland, Romania, Russia, Slovakia, Spain, Switzerland, Turkey, United Kingdom, Argentina, Brazil, Chile, Colombia, Ecuador, Mexico, Peru, Venezuela, Uruguay, New Zealand, India, Indonesia, Malaysia, Singapore, Taiwan, Vietnam, China, Hong Kong, Thailand, Israel, and South Africa.. The device has not been withdrawn to date from the market in any country for reasons relating to safety and effectiveness of the device.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect ALK test results, and consequently improper patient management decisions in NSCLC treatment.

A false negative test result may lead to XALKORI (crizotinib) treatment being withheld from a patient who might have benefited. A false positive test result may lead to administration of XALKORI (crizotinib) treatment to a patient who may experience adverse side effects associated with treatment without clinical benefit.

The most common adverse reactions ($\geq$ 25%) that occurred in clinical trials of XALKORI (crizotinib) are vision disorders, nausea, diarrhea, vomiting, constipation, edema, elevated transaminases, and fatigue.

IX. SUMMARY OF PRECLINICAL STUDIES

All preclinical studies were performed at Ventana using the VENTANA ALK (D5F3) CDx Assay, which is also referred to as ALK IHC Assay below. These studies were conducted to characterize the assay, demonstrate the impact of pre-analytical variables on assay performance, verify precision and robustness of the assay, and establish assay stability.

The scoring algorithm used in these studies included a clinical score (i.e., ALK positive or negative) and/or an analytical score (i.e., 0-3 scale for staining signal intensity and distribution). Clinical scores were recorded for all studies with the scoring algorithm developed for clinical interpretation of the ALK IHC Assay. Analytical scores were

assessed for some studies to ensure assay performance in borderline cases. The analytical score is not part of the interpretation of ALK IHC staining status in the device labeling.

A. Laboratory Studies

1. Antibody Specificity

a. Western Blot

The D5F3 clone has been licensed from CST (Cell Signaling Technology). Western blot studies were conducted to characterize the VENTANA ALK (D5F3) antibody. Cell lysates were prepared from the following four different cell lines, which have been characterized in the literature for ALK status: the NCI-H2228 cell line (a lung adenocarcinoma NSCLC cell line) expresses the ALK fusion protein EML4-ALK variant 3; the Karpas 299 cell line (a CD30+ anaplastic large cell lymphoma [T-cell Non-Hodgkin's lymphoma] cell line) expresses the NPM-ALK fusion protein; the Rh30 cell line (origin is rhabdomyosarcoma) expresses the ALK wild-type protein; and the Calu-3 cell line (origin is NSCLC) does not express the ALK protein and therefore was used as a negative control.

The Western blot results on the four cell lysates show that the anti-ALK (D5F3) antibody recognizes the EML4-ALK fusion protein present in NSCLC, the NPM-ALK fusion protein, and the wild-type ALK protein that does not have a fusion partner.

b. Immunoreactivity in Human Tissues

Studies were conducted to demonstrate specificity of the VENTANA ALK (D5F3) CDx Assay for use with NSCLC tissues. One lot of anti-ALK (D5F3) antibody was stained on commercially available tour of body (TOB) and tour of tumor (TOT) tissue microarrays (TMAs). Slides were evaluated by one reader for presence or absence of specific and background staining on each tissue type.

Normal tissue screened are shown in **Table 1** below, which included central nervous system, endocrine, breast, hematopoietic, respiratory, cardiovascular, gastrointestinal, genitourinary, musculoskeletal, skin, peripheral nerve, and mesothelial cells. Three unique samples for each anatomic site were assessed.

Table 1: Normal Tissue Screened for ALK Status (Tour of Body)

Tissue	# Positive / total cases	Tissue	# Positive / total cases
Cerebrum	0/3*	Thymus	0/3
Cerebellum	0/3	Myeloid (bone marrow)	0/3
Adrenal gland	0/3	Lung	0/3
Ovary	0/3	Heart	0/3
Pancreas	0/3	Esophagus	0/3
Parathyroid gland	0/3	Stomach	0/3
Hypophysis	0/3**	Small intestine	0/3***
Testis	0/3	Colon	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 and lung	0/3

* 2/3 cases showed weak to moderate positivity in a few glial cells in the cerebrum.

** 3/3 cases of hypophysis stained weakly.

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

Neoplastic tissues screened included a sampling of several neoplastic tissue types, as shown in **Table 2** below. One to three cases were included per evaluated tissue type. Appropriate background staining was observed in 100% of cases (143 of 143) with the VENTANA ALK (D5F3) CDx Assay. No unexpected cross reactivity was observed. Of the 54 neoplastic tissues stained with the VENTANA ALK (D5F3) CDx Assay, some cases demonstrated positive staining. These included an ovarian serous adenocarcinoma, two blastomas, one hepatoblastoma and one neuroblastoma. Literature references support this observation, as all these tumor types have been shown to express ALK.

Table 2: Neoplastic Tissue Screened for ALK Status (Tour of Tumor)

Tissue	# Positive / total cases	Tissue	# Positive / total cases
Glioblastoma	0/1	Rectal adenocarcinoma	0/1
Atypical meningioma	0/1	Rectal malignant interstitialoma	0/1
Malignant ependymoma	0/1	Hepatocellular carcinoma	0/1
Malignant oligodendroglioma	0/1	Hepatoblastoma	1/1

Tissue	# Positive / total cases	Tissue	# Positive / total cases
Serous ovarian adenocarcinoma	1/1	Renal clear cell carcinoma	0/1
Ovarian adenocarcinoma	0/1	Prostatic adenocarcinoma	0/2
Islet cell carcinoma	0/1	Leiomyoma	0/1
Pancreatic adenocarcinoma	0/1	Endometrial adenocarcinoma	0/1
Seminoma	0/1	Endometrial clear cell carcinoma	0/1
Embryonal carcinoma	0/1	Uterine squamous cell carcinoma	0/2
Medullary carcinoma	0/1	Embryonal rhabdomyosarcoma	0/1
Papillary carcinoma	0/1	Anal malignant melanoma	0/1
Breast intraductal carcinoma	0/1	Basal cell carcinoma	0/1
Breast invasive ductal carcinoma	0/2	Squamous cell carcinoma	0/1
Diffuse B-cell lymphoma	0/3	Neurofibroma	0/1
Lung small cell undifferentiated carcinoma	0/1	Retroperitoneal neuroblastoma	1/1
Lung squamous cell carcinoma	0/1	Malignant mesothelioma	0/1
Lung adenocarcinoma	0/1	Hodgkin lymphoma	0/1
Esophageal squamous cell carcinoma	0/1	Anaplastic large cell lymphoma	0/1
Esophageal adenocarcinoma	0/1	Bladder transitional cell carcinoma	0/1
Gastric mucinous adenocarcinoma	0/1	Low grade leiomyosarcoma	0/1
Gastrointestinal adenocarcinoma	0/1	Osteosarcoma	0/1
Malignant interstitialoma	0/1	Spindle cell rhabdomyosarcoma	0/1
		Intermediate grade leiomyosarcoma	0/1

2. Pre-Analytical Variables

Several studies were conducted to demonstrate the impact of pre-analytic variables on the performance of the assay. These studies included assessment of the stability of the ALK antigen in sectioned (cut) FFPE tissue (after being cut from the block), and the effect of tissue thickness on reproducibility of the assay. In addition, the impact of a delay-to fixation in neutral buffered formalin (NBF), and the impact of various fixation types and times were evaluated.

a. Cut Slide Stability

The stability of the ALK antigen in NSCLC specimens after sectioning onto glass slides and storing at room temperature was evaluated. Four cases across the assay dynamic range, representing positive and negative ALK clinical status, were included in the study. Three cases were positive for ALK and one case was negative for ALK. Of the three ALK positive cases, one was homogeneously positive, one was heterogeneously positive and one was focal positive. All sections were compared to a baseline slide (Day 0) at each pre-defined interval (1 month apart). Wildcard slides were stained and randomized with study slide cohorts. The wildcard tissues were assessed, but not included in the final data analysis. A decrease in overall staining intensity was observed at 7 months after the positive ALK cases were sectioned from the blocks and stored at room temperature. Specifically, for the weak positive NSCLC tissue, the clinical score changed from ALK positive to ALK negative because the analytical score changed from 1.0 to 0.5. For the strong positive NSCLC tissue, the clinical score remained ALK positive but the analytical score changed from 3.0 to 2.0 (i.e., 1.0 staining intensity difference). Therefore, the NSCLC cut slide sections should be stained with the ALK IHC Assay no longer than 3 months after being cut from the blocks.

b. Tissue Thickness

The effect of tissue thickness on the performance of the VENTANA ALK (D5F3) CDx Assay was studied using cases representing the dynamic range of the ALK assay. Four cases were included in the study with three cases demonstrating positive ALK clinical status and one case demonstrating negative ALK clinical status. Of the three ALK positive cases, one was homogeneously positive, one was heterogeneously positive and one was focal positive. Each case was sectioned at 3-, 4-, 5-, 6- and 7-micron thicknesses and stained in duplicate with the VENTANA ALK (D5F3) CDx Assay. Wildcard slides were stained and randomized with study slide cohorts. The wildcard tissues were assessed, but not included in the final data analysis. All slides stained at the various thicknesses resulted in staining intensities within 1.0 point across all tissues tested (i.e., analytical score in terms of staining intensity), and all tissue thicknesses for all specimens maintained their clinical ALK status (i.e., clinical score,

positive or negative for ALK). Ventana recommends using sections from 4-6 um in thickness for the assay.

c. **Delay-to-Fixation (Ischemia)**

The impact of delaying the fixation of NSCLC specimens (in 10% Neutral Buffered Formalin: NBF) on the staining performance of the VENTANA ALK (D5F3) CDx Assay was evaluated. Xenograft tumors generated from a human ALK positive NSCLC cell line NCI-H2228 were used for this study. The NCI-H2228 xenograft tumors were harvested and left at room temperature conditions for 30-min, 1-hr, 2-hr, 6-hr, and 24-hr time delays after harvesting and prior to fixation in 10% NBF. The staining intensities were compared to those from reference xenograft tumors that were excised and fixed immediately in 10% NBF. For VENTANA ALK (D5F3) antibody, there was little-to-no difference in staining intensity for ischemic samples for the time points of 30 min, 1-hr, 2-hrs, and 6-hrs. A drop in staining intensity was observed 24 hours post-excision prior to fixation in 10% NBF.

A loss of staining performance was noted on slides with xenograft tumors with a delay-in-fixation greater than six hours in 10% NBF that were stained with the VENTANA ALK (D5F3) CDx Assay. Therefore, the recommendation for ALK antigen preservation is to fix samples in 10% NBF immediately if possible or within six hours of excision.

d. **Fixation Time and Fixative Types**

The impact of fixative type and fixation time on staining performance of the VENTANA ALK (D5F3) CDx Assay was evaluated. Xenograft tumors generated from the human ALK positive cell line NCI-H2228 were used for this study. Xenograft tumors were excised and then fixed in various fixative types including 10% NBF, Zinc formalin, Prefer fixative, AFA (alcohol-formalin-acetic acid), B5, and 95% alcohol for a range of times prior to processing (**Table 3**). Xenograft tumors excised and fixed immediately in 10% NBF for 6-hr, 12-hr and 24-hrs served as reference for the other fixation conditions evaluated. The xenograft tumors were stained with the VENTANA ALK (D5F3) CDx Assay and the stained slides were evaluated for staining intensity using the analytical score, i.e., 0-3 staining intensity range.

Based on the results in this study (**Table 3**), Ventana recommends fixing samples in 10% NBF or zinc formalin for a minimum of 6 hours; fixation times less than 6 hours resulted in suboptimal staining. Fixatives such as AFA, Prefer fixative, B5, and other alcohol fixatives demonstrated a loss of specific ALK staining intensity at all fixation times tested (1-72 hours), and are not recommended for use with this assay.

Table 3: Fixation Parameters and Study Results

Fixation Time	ALK Staining Intensity					
	10% NBF	Zinc Formalin	AFA	95% Alcohol	B5	Prefer
1 hr	1+	1	1	0.5	1	0.5-1
*6 hr	2.5	2.5	1	No tumor	1.5-2	1
*12 hr	3	3	1.5	1	1.5-2	1.5-2
*24 hr	2.5	2.5	1	0.5	1	1
72 hr	3	2.5	1.5-2	0.5-1	1.5-2	1.5-2

*The 6, 12, and 24 hour fixation times in 10% NBF served as the gold standard and reference staining against which the remainder of the fixation types and times were compared.

3. Precision Studies

Several studies were conducted to demonstrate the precision of the VENTANA ALK (D5F3) CDx Assay. These studies included intra-day, inter-day, and inter-platform intermediate precision studies, a lot-to-lot precision study, as well as an inter-reader precision study.

a. **Intermediate Precision – Intra-Day, Inter-Day, Inter-Platform**

The repeatability study protocol was conducted to verify repeatability of the ALK IHC (D5F3) Assay. This study was performed using human NSCLC tissue to evaluate variability from intra-day (performance within multiple slides of a single run), inter-platform (performance between three BenchMark XT instruments), and inter-day (performance between several days on a single BenchMark XT).

Ten (10) NSCLC (5 ALK positive and 5 ALK negative) whole tissue blocks (unique cases) and one appendix tissue block were pre-qualified and stained in the repeatability testing. To allow for the maximum number of unique cases of NSCLC to be stained in a given run, a system level control, appendix tissue, was sectioned onto the slides with the NSCLC test samples. One such slide, containing both a control appendix tissue and an ALK positive NSCLC test sample, was included in every instrument run. The appendix tissue was evaluated for both positive and negative ALK staining elements in accordance with the appendix scoring criteria. One slide from each of the 10 NSCLC cases was also stained with Rabbit Monoclonal Negative Control Ig Primary Antibody in each study.

The stained and randomized NSCLC slides were evaluated by three pathologists for ALK clinical score. No wildcard cases were included in the study. All negative reagent control stained slides were acceptable across all three groups (Intra-Day, Inter-Platform, and Inter-Day testing).

Intra-Day (repeatability within a run) Testing:
Five slides from each of the 10 NSCLC cases were stained on a single BenchMark XT instrument with the VENTANA ALK (D5F3) CDx Assay.

All (100%) of the 10 NSCLC stained cases demonstrated reproducible ALK clinical scores as determined by three pathologists. All 5 replicates of the 5 ALK negative NSCLC cases were evaluated as negative, and all 5 replicates of the 5 ALK positive NSCLC cases were evaluated as positive.

Inter-Day (Day-to-Day) Testing:
Two slides from each of the 10 NSCLC cases were stained on two BenchMark XT instruments with the VENTANA ALK (D5F3) CDx Assay for 5 non-consecutive days spanning no less than 20 total days. The first two slides of each NSCLC case stained from the Intra-Day testing served as day 1 for the Inter-Day testing. All (100%) of the 10 NSCLC cases evaluated by the three pathologists demonstrated reproducible ALK clinical scores. All duplicate slides of the 5 ALK negative NSCLC cases were evaluated as negative for ALK across all five non-consecutive runs. All duplicate slides of the 5 ALK positive NSCLC cases were evaluated as positive for ALK across all five non-consecutive runs.

Inter-Platform (Instrument-to-Instrument) Testing:
Two slides from each of the 10 NSCLC cases were stained on three BenchMark XT instruments with the VENTANA ALK (D5F3) CDx Assay. All (100%) of the 10 NSCLC cases evaluated by three pathologists demonstrated reproducible ALK clinical scores. All duplicate slides of the 5 ALK negative NSCLC cases were evaluated as negative for ALK across all three BenchMark XTs. All duplicate slides of the 5 ALK positive NSCLC cases were evaluated as positive for ALK across all three BenchMark XTs.

In all three repeatability studies, one of the three pathologists evaluated the cohort for semi-quantitative signal intensity, i.e., analytical score. The semi-quantitative signal intensity did not fluctuate more than a point across all evaluated slides. Thus, staining of NSCLC tissues with the ALK IHC Assay demonstrated acceptable Intra-Day, Inter-Day, and Inter-Platform repeatability based on clinical scores. The study designs and results are summarized in **Table 4** below.

Table 4: Summary of Intermediate Precision Studies

Repeatability Study – NSCLC Tissues		Intra-Day Repeatability	Inter-Platform Repeatability	Inter-Day Repeatability
Total slides stained and evaluated		50	90	100
ALK (D5F3) stained replicates/case		5	2	2
Blinded and Randomized		Yes	Yes	Yes
Distribution of NSCLC Cases in the Study Cohort	**ALK Positive**	5	5	5
	ALK Negative	5	5	5
Distribution of ALK Positive NSCLC Cases	**Focal Positive**	1	1	1
	Heterogeneous Positive	2	2	2
	Homogenous Positive	2	2	2
Result (with 95% CI)		100% (97.5-100%)	100% (97.9-100%)	100% (98.7-100%)

b. **Lot-to-Lot Precision**

Lot-to-lot testing was conducted to demonstrate the reproducibility of the VENTANA ALK (D5F3) CDx Assay across multiple manufactured lots of antibody. Three lots of the VENTANA ALK (D5F3) antibody were tested. A total of 41 NSCLC tissue samples were included in the study with 21 ALK positive tissue samples (18 unique cases) and 20 ALK negative cases. Slides were blinded and randomized prior to evaluation across multiple days by a single pathologist.

All three lots of anti-ALK antibody exhibited equivalent staining results across the 41 tissues evaluated, as assessed by average positive agreement (APA), average negative agreement (ANA) and overall percent agreement (OPA) for ALK clinical status. The study analysis also included evaluation of analytical scores, which were captured on a 0-3 scale for informational purposes only. All lots performed concordantly, and fluctuations in staining intensity for each tissue were within the acceptable ± 0.5 points on a 0-3 scale. Agreement rates were calculated for each lot-to-lot comparison as a weighted average of all comparisons (**Table 5**). Overall percent agreement between the three lots of anti-ALK (D5F3) antibody was 99.2% agreement for all 41 cases.

Table 5: VENTANA ALK (D5F3) Antibody Lot-to-Lot Reproducibility

Comparison	Rate	n/N	%	95% Confidence Interval
Average of all three lot-to-lot comparisons	APA	488/492	99.2	97.4-100%
	ANA	456/460	99.1	96.8-100%
	OPA	472/476	99.2	97.5-100%

c. **Inter-Reader Precision**

Study 1

Reader precision testing was conducted to demonstrate reader agreement rates using the ALK scoring algorithm for slides stained with the VENTANA ALK (D5F3) CDx Assay. **Table 6** below lists the case distribution for the ALK positive NSCLC cases in this study, including 10-15% of focal positive cases (i.e., 11 cases with analytical scores of 1). A total of 185 unique tissue cases were stained, which correlated to 100 ALK positive and 100 ALK negative blocks due to the use of sibling blocks (i.e., different blocks from the same patient case). The study slides were blinded and randomized and then evaluated by three readers.

Table 6: Dynamic Range of ALK Positive NSCLC Used in Inter-Reader Precision

Classification for ALK Positive NSCLC	Total number of blocks used
Homogeneous	21
Heterogeneous	56
Focal	11
Not classified	12

Overall percent agreement (OPA), average positive agreement (APA), and average negative agreement (ANA) were calculated. The weighted average of the agreement rates (APA, ANA, and OPA) for all three reader pairs are reported in **Table 7** below. Results showed 98.8% APA, 99.0% ANA, and 98.9% OPA based on clinical scores (ALK positive vs. ALK negative).

Table 7: Inter-Reader Precision Study Results – Study 1

Comparison	Rate	n/N	%	95% Confidence Interval
Average of all three reader-to-reader comparisons	APA	496/502	98.8	97.3-100%
	ANA	584/590	99.0	97.7-100%
	OPA	540/546	98.9	97.4-100%

Study 2

The inter-reader precision was also evaluated on a cohort of cases from a clinical study of ALK positive NSCLC patients enrolled based on the results from the Abbott Vysis ALK Break Apart FISH Probe Kit. Approximately 300 cases were stained with the VENTANA ALK (D5F3) CDx Assay. The cases were blinded for ALK FISH status, randomized, and provided to three readers, who evaluated the ALK IHC staining results per the VENTANA ALK (D5F3) CDx Assay scoring algorithm. The results are provided in **Table 8** below.

Table 8: Inter-Reader Precision Study Results – Study 2

Comparison	Rate	n/N	%	95% Confidence Interval
Average of all three reader-to-reader comparisons	APA	320/328	97.6	95.0-99.5%
	ANA	1466/1474	99.5	98.9-99.0%
	OPA	893/901	99.1	98.2-99.8%

4. Inter-Laboratory (Site-to-Site) Reproducibility

The inter-laboratory reproducibility (ILR) study for the VENTANA ALK (D5F3) CDx Assay was conducted at 3 external sites in the United States, sites A, B, and C. A total of 6 ALK positive and 6 ALK negative NSCLC specimens were procured from vendors and screened by Ventana pathologists to select 12 whole tissue blocks that together represented the dynamic range of the ALK IHC Assay in the intended use population. This study used 180 slides from 12 NSCLC cases (5 triplets per case). Sample characteristics of the 12 NSCLC cases are tabulated in **Table 9** below.

Table 9: ILR: Sample Characteristics

Qualification Status with VENTANA D5F3			FISH Status[b]	
ALK Status[a]	**Overall Specific Intensity (0-3)**	**Overall Background Intensity (0-3)**	**% Abnormal Cells ALK[c]**	**ALK Gene Rearrangement Status[d]**
Positive	1	0	56	Positive
Positive	3	0.5	49	Positive
Positive	3	0	33	Positive
Positive	3	0	52	Positive
Positive	2.5	0	20	Positive
Positive	3	0	60	Positive

Qualification Status with VENTANA D5F3			FISH Status[b]	
ALK Status[a]	Overall Specific Intensity (0-3)	Overall Background Intensity (0-3)	% Abnormal Cells ALK[c]	ALK Gene Rearrangement Status[d]
Negative	0	0	0	Negative
Negative	0	0	0	Negative
Negative	0	0	0	Negative
Negative	0	0.5	0	Negative
Negative	0	0	0	Negative
Negative	0	0	0	Negative

[a] Results of the VENTANA ALK (D5F3) CDx Assay performed during case qualification.
[b] The Vysis ALK Break Apart FISH Probe Kit assay (FISH assay) was performed for informational purposes only.
[c] Percent of tumor cells staining positive for ALK gene rearrangement in the FISH assay.
[d] Cases scoring ≥15% positive in the FISH assay are considered ALK positive.

Each site conducted staining runs with slides from all 12 cases (together with control slides) on each of 5 non-consecutive days. The first and last staining days at each site were at least 20 days apart. Sites used one randomized slide group (containing two slides for each of the 12 cases) and two pairs of control slides on each staining day. The order of cases was randomized for each staining day. At each site, one run was conducted using a single BenchMark XT instrument per day. Specimens were randomized before and after staining. Slides stained at a study site were independently evaluated by two readers at that site. Readers were blinded to run positions, staining days, previously determined ALK statuses, and case IDs. Each reader evaluated his or her reading sets without consulting any other pathologist and without knowledge of the other study pathologists' findings. Their determinations of ALK protein expression status (positive or negative) for each anti-ALK case slide were used to determine between-site, between-day, and between-reader agreement rates. Of the 180 ALK (D5F3 stained) case slides stained at sites A, B, and C, all were considered evaluable by both readers. Results for between-site, between-day, and between-readers agreements are outlined in **Table 10, Table 11**, and **Table 12** below, respectively.

Table 10: ILR: Between-Site Reproducibility

Comparison	Rate	n/N	%	95% Confidence Interval
Average of all three Sites comparisons	APA	3040/3240	93.8	76.2-100.0%
	ANA	3760/3960	94.9	79.2-100.0%
	OPA	3400/3600	94.4	83.3-100.0%

Table 11: ILR: Between-Day Reproducibility

Comparison	Rate	n/N	%	95% Confidence Interval
Average of all Days comparisons	APA	642/648	99.1	96.4-100.0%
	ANA	786/792	99.2	96.9-100.0%
	OPA	714/720	99.2	97.5-100.0%

Table 12: ILR: Between-Reader Reproducibility

Comparison	Rate	n/N	%	95% Confidence Interval
Average of all six Readers comparisons	APA	160/162	98.8	95.2-100.0%
	ANA	196/198	99.0	95.8-100.0%
	OPA	178/180	98.9	96.7-100.0%

5. <u>Reagent Lot-to-Lot Interchangeability</u>

This study included testing of three lots of VENTANA ALK (D5F3) antibody in combination with three lots of the OptiView Detection Kit and OptiView Amplification Kit, to assess ALK IHC Assay performance across a cohort of NSCLC cases which represent the dynamic range of the intended use population. A total of 9 different lot combinations were tested. Testing was performed using two prequalified multi-tissue blocks (MTBs) containing four NSCLC and control slides. Each MTB consisted of 2 ALK strong-positive tissues, a third tissue which is considered a borderline positive, and a fourth tissue which is negative for ALK. Two slides from each multi-tissue block and two control slides were stained. To reduce the possibility of reader bias during the evaluation of the data set, additional wildcard slides were stained and incorporated into the randomization scheme but were not included in the analysis of the data.

A total of 81 slides (27 per run) was stained over three runs. Each run tested three lots of VENTANA ALK (D5F3) antibody with a different lot of OptiView DAB IHC Detection Kit and OptiView Amplification Kit. The positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) are outlined in **Table 13** below.

Table 13: Reagent Lot-to-Lot Interchangeability

Comparison	Rate	n/N	%	95% Confidence Interval
Average of all Lot-to-Lot comparisons	PPA	102/108	94.4	88.4-97.4%
	NPA	36/36	100	90.4-100.0%
	OPA	138/144	95.8	91.2-98.1%

6. Staining Procedure Robustness

The staining protocol for the ALK IHC Assay allows users to select either 8 or 12 minutes at the OptiView HQ Universal Linker step, and 4 to 32 minutes at the counterstain and post counterstain steps. This study was conduct to test the robustness of the staining procedure within these variable parameters. Two positive and one negative NSCLC cases were utilized and tested with each of the 32 conditions (**Table 14**). The stained slides were blinded, randomized, and evaluated by a single pathologist. Slides were given an ALK positive or negative result based on the ALK scoring algorithm. Qualitative scoring using a 0-3+ staining intensity categorization were captured for each case for information purposes only.

Table 14: Staining Procedure Robustness – Variable Parameters Tested

Linker Incubation	Hematoxylin Incubation	Bluing Incubation
8 min or 12 min	4 min	4 min
		12 min
		24 min
		32 min
	12 min	4 min
		12 min
		24 min
		32 min
	24 min	4 min
		12 min
		24 min
		32 min
	32 min	4 min
		12 min
		24 min
		32 min

The ALK staining intensity ranged from 2.0 – 2.75 when the Linker incubation time was 8 minutes, and ranged from 2.25 – 3.0 when the Link incubation time was 12 minutes. An agreement rate based on clinical scores of the ALK IHC Assay was calculated by comparing results from the test condition to the nominal recommended protocol (OptiView Linker at 12 min,

Hematoxylin at 4 min, and Bluing Reagent at 4 min), and determined to be 100% (n=93/93 for ALK positive cases, n=31/31 ALK negative cases).

7. Stability Studies

a. VENTANA ALK (D5F3) CDx Assay

This study was designed to evaluate the stability of three production lots of the VENTANA ALK (D5F3) antibody and assay kits under different storage conditions. Stability testing was conducted using three Stability Master Lots (SMLs). Each SML was comprised of a single lot of the VENTANA anti-ALK (D5F3) Rabbit Monoclonal Primary Antibody, the OptiView DAB IHC Detection Kit and the OptiView Amplification Kit. The time points planned tested were: Day 0, month 3, 6, 8, 9, 12, 13, 14, 18, 20, 24 and 26.

The following stability categories were tested:

1. Intended Storage (2-8 °C);
2. Hot Ship Stress – Category A (30 °C ± 5 ºC initial 192 hours, then replaced in 2-8 °C storage for remainder of study);
3. Hot Ship Stress – Category B (15 °C ± 5 ºC initial 192 hours, then replaced in 2-8 °C storage for remainder of study);
4. Cold Ship Stress – Freeze/Thaw (-20°C± 5 ºC initial 188 hours, then replaced in 2-8 °C storage for remainder of study).

At the 18 month time point, invalid results were recorded and investigated. Additional testing conducted during the investigations concluded that this specific lot of OptiView Amplification Kit in the SML was responsible for the lighter staining intensity and the invalid results. Repeat runs with all lots of the ALK antibody (i.e., SML antibody lots) under test conditions using other lots of the OptiView Amplification Kit (i.e., older lots: 24, 35, and 36 months) passed the acceptance criteria, confirming that the ALK antibody is functioning as intended at Month 18. From Month 20 onward, the stability testing protocol was modified to include new OptiView DAB IHC Detection and OptiView Amplification Kits, rather than the original SML kits; the same lots of the VENTANA anti-ALK (D5F3) Rabbit Monoclonal Primary Antibody (part of the original SML configuration) continued to be tested to complete stability study. Due to the substitution of different lots of the OptiView DAB IHC Detection and OptiView Amplification Kits within real time and ship stress stability testing, the results support expiry dating of 18 months at 2 – 8°C for the VENTANA ALK (D5F3) CDx Assay.

In addition, independent accelerated stability testing of three lots of the VENTANA ALK (D5F3) antibody was conducted after storage at 45ºC

over 236 hours (approximately 9.5 days). Results showed no alterations in staining pattern when compared to time 0 staining (i.e., analytical scores were recorded and compared in the study).

B. Animal Studies

None.

C. Additional Studies

1. Appendix Tissue Control Slides

Repeatability
The VENTANA ALK (D5F3) CDx Assay was evaluated for repeatability using eight unique human appendix tissues. For intra-day precision, 13 replicate slides from two multi-tissue blocks containing 4 appendix specimens were stained across a single BenchMark XT instrument. For inter-instrument precision testing, 5 replicate slides from two multi-tissue blocks containing 4 appendix specimens each were stained with the VENTANA ALK (D5F3) CDx Assay across three BenchMark XT automated staining platforms. For inter-day precision, 5 replicate slides from each of two multi-tissue blocks containing 4 appendix specimens were stained with the VENTANA ALK (D5F3) CDx Assay on a single BenchMark XT instrument across 5 non-consecutive days. All slides were evaluated by a pathologist using the VENTANA ALK (D5F3) CDx Assay scoring guide for appendix control tissue. 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%.

Lot-to-lot Reproducibility
The VENTANA ALK (D5F3) CDx Assay was also evaluated for reproducibility 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 automated slide stainers. The overall agreement rate for appropriate positive and negative staining elements in the appendix tissues using the VENTANA ALK (D5F3) CDx Assay was 100%.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The safety and effectiveness of the VENTANA ALK (D5F3) CDx Assay was evaluated in a study using clinical outcome results and retrospective samples from the randomized Phase 3 Pfizer Study A8081014 (Study 1014). Study 1014 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 locally advanced or metastatic nonsquamous NSCLC. The Vysis ALK Break Apart FISH Probe Kit from Abbott Molecular, Inc. was used to determine ALK positivity and trial enrollment for Study 1014. Please see section "A. Study Design (for Therapeutic Clinical Trial)" below for more details regarding Study 1014.

Ventana conducted retrospective testing of a proportion of the screened patient samples from Study 1014 under the diagnostic study protocols. Please see below section on "B. Study Design (for Bridging Study)" and "C. Accountability of PMA Cohort" for more details.

A. Study Design (for Therapeutic Clinical trial)

Study 1014 was a multicenter study conducted at 169 centers in 31 countries. A total of 343 patients were randomized and included in the therapeutic Full Analysis (FA) population, with 172 patients enrolled in crizotinib arm, and 171 patients enrolled in chemotherapy arm. The choice of platinum-doublet chemotherapy (pemetrexed/cisplatin or pemetrexed/carboplatin) in the chemotherapy arm was made by the investigator.

The presence of an ALK fusion event in patients with NSCLC, determined by the central laboratory, was required for eligibility as these patients represent the target population for crizotinib. Samples provided to the central laboratory were paraffin block(s) of adequate size to allow, if possible, for at least 10 slides with cuts that are 5-microns thick or if a paraffin block was not available, at least 10 slides with cuts that are 5-microns thick were acceptable. Archived or fresh tumor samples were accepted according to the institutional practice for biopsy. The mandatory tumor tissue could be obtained outside the 28-day screening window.

1. Key Clinical Inclusion and Exclusion Criteria

   Male or female patients were to be 18 years of age or older; have histologically or cytologically proven diagnosis of locally advanced, not suitable for local treatment, recurrent, or metastatic non-squamous NSCLC; positive for translocation or inversion events involving the ALK gene locus as determined by the Vysis ALK Break Apart FISH Probe Kit assay; have had no prior systemic treatment for locally advanced or metastatic disease (except prior adjuvant chemotherapy for Stage I-III or combined modality chemotherapy radiation for locally advanced disease if completed >12 months prior to documented PD); measurable disease as per RECIST (Version 1.1); and ECOG PS 0-2.

2. Follow-up Schedule

   Post-treatment Follow-Up: Disease assessment and other clinical assessment were conducted post-treatment accordingly. Clinical assessments include adverse events, clinical laboratory tests, ECGs, vital signs, and hospitalization, as well as concomitant medications/treatments.

Survival Follow-Up: After discontinuation of study treatment and /or confirmed progression of disease, post-study survival status will be collected every 2 months until death or until 18 months after the randomization of the last patient (End of Trial definition). This includes collection of information on subsequent anticancer therapies.

3. Clinical Endpoints
   The primary clinical endpoint of Study 1014 was progression-free survival (PFS) in patients with advanced NSCLC whose tumors harbor a translocation or inversion event involving the ALK gene locus.

B. Study Design (for Bridging Study)

The objectives of the bridging study were to determine the concordance between the VENTANA ALK (D5F3) CDx Assay (ALK IHC) and the Vysis ALK Break Apart FISH Probe Kit assay (ALK FISH) using FFPE patient samples from Study 1014. In addition, this study evaluated the hazard ratio of progression-free survival (PFS) for the comparison between the crizotinib-treated arm and the chemotherapy-treated arm of Study 1014, for those cases designated ALK positive by the ALK IHC Assay.

An independent central laboratory served as the sole investigative site for this study. The laboratory received unstained tissue sections from all available specimens screened for Study 1014 and tested for ALK IHC status using the VENTANA ALK (D5F3) CDx Assay. All available NSCLC specimens from Study 1014, whether ALK positive, ALK negative, or ALK uninformative by the ALK FISH assay (i.e., whether "ALK FISH+", "ALK FISH–," or "FISHu"), were eligible for inclusion in this study. This diagnostic study was conducted under protocol D032361.

1. Diagnostic Inclusion and Exclusion Criteria
   Inclusion Criteria
   To be enrolled in the study, a case specimen had to meet all of the following criteria:
   - It had to be an FFPE specimen of NSCLC tissue;
   - It had to have been submitted to a central clinical laboratory for ALK FISH assessment of ALK gene fusion under a Pfizer protocol; and
   - It had to have least three unstained slide sections available for staining with the VENTANA ALK (D5F3) CDx Assay.

   Exclusion criteria
   A case was excluded from the study if:
   - There was no informed consent for research use of the specimen under a Pfizer protocol; or
   - There was insufficient tumor for evaluation, as determined by H&E staining of the specimen.

2. Criteria for Evaluation

VENTANA ALK (D5F3) antibody-stained case slides had to satisfy the following criteria to be deemed adequate for scoring:

- No tissue loss that interfered with the qualified reader's ability to score the slide.
- For cases from Study 1014, appropriate staining of both slides used to validate the IHC staining run (human NSCLC tissue controls).
- Appropriate staining of the case slide stained with Negative Control Ig.
- Acceptable background and morphology on the case slide stained with VENTANA ALK (D5F3) antibody.

If the above criteria were not met, the case could not be evaluated.

C. Accountability of PMA Cohort

Specimens were obtained from clinical sites and submitted to one of four central laboratories participating in enrollment screening in Study 1014. These laboratories screened NSCLC cases (tissue blocks or unstained tissue sections) for potential inclusion in the clinical study using the ALK FISH assay; only those cases found to be ALK FISH+ were eligible for enrollment. Subjects enrolled in the drug study were randomized to treatment with crizotinib or standard chemotherapy (pemetrexed/cisplatin or pemetrexed/carboplatin).

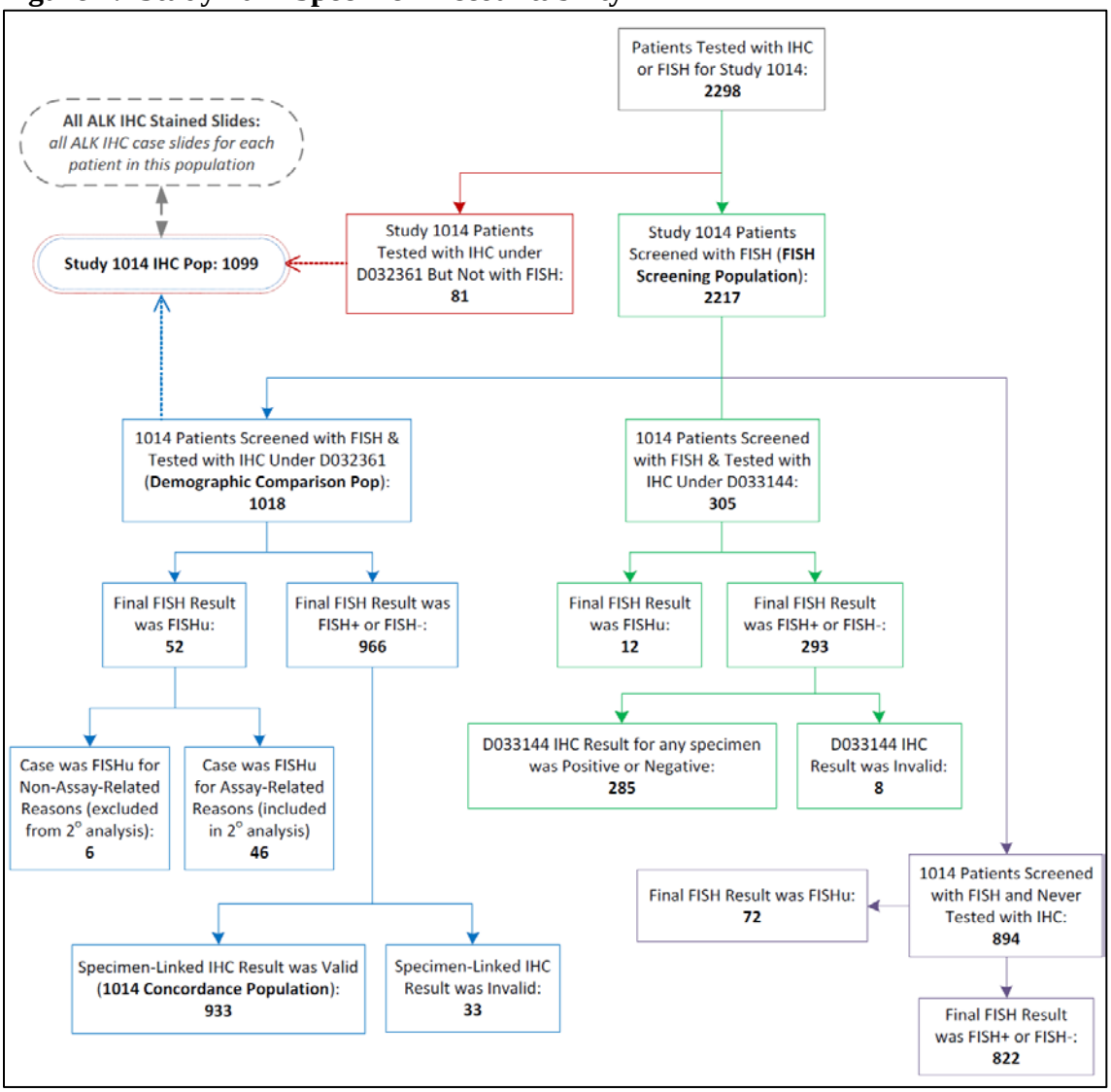
All available specimens screened for Study 1014 (including those that were ALK FISH-) at the four screening laboratories were provided to Ventana from the Pfizer Tissue Bank and then transferred to the single diagnostic investigation site, where the specimens were sequentially enrolled in the diagnostic study for testing with the ALK IHC Assay. A total of 184 ALK FISH+, 782 ALK FISH- cases, and 52 ALK FISH-uninformative (FISHu) cases screened for Study 1014, as well as 81 cases submitted for Study 1014 but never tested with the ALK FISH assay, were stained with the VENTANA ALK (D5F3) CDx Assay in the diagnostic study (n=1099, Diagnostic Study 1014 IHC Population, **Table 15**). Among the 1099 specimens screened with the ALK IHC Assay, 194 were ALK IHC+, 825 were ALK IHC- and 80 cases were without a valid ALK IHC status. Notably, 894 of the 2217 patients screened for Study 1014 were never tested with the ALK IHC Assay (Figure 1, purple boxes).

Table 15: Disposition of Patients in the Diagnostic Study 1014 IHC Population

Diagnostic IHC Population	Valid ALK IHC[a]	Invalid ALK IHC
ALK IHC+	194	--
ALK IHC-	825	--
Total	1019	80
	1099	

[a] For an ALK status from a testing attempt to be considered valid, the record had to indicate an evaluable ALK IHC slide, an evaluable and appropriately stained negative control slide, a valid tissue run control, a positive identification of the patient specimen as NSCLC, and a positive or negative ALK status.

Figure 1: Study 1014 Specimen Accountability



* Protocol D033144 noted in green boxes indicates that a subset samples from Study 1014 were tested in a Ventana internal verification protocol during assay development, which used the same assay and scoring methodology as the diagnostic study protocol D032361 (blue boxes).

D. Study Population Demographics and Baseline Parameters

Table 16 below summarizes demographic characteristics and ALK IHC status of all cases in the Study 1014 FISH Screening Population. Additional comparisons between patients with valid vs. invalid ALK IHC results, and between patients with positive vs. negative ALK IHC results were also conducted (data not shown). Results suggest that ALK IHC results may be correlated with some patient characteristics (i.e., ALK FISH status, sex and age), which were included when imputing missing ALK IHC results. Some other factors, e.g., presence of brain metastases, ECOG status and smoking status may also be correlated with the ALK IHC results.

However, these patient characteristics data are not collected from any ALK FISH negative patients. Hence they are not considered when imputing the missing data.

Table 16: Demographic Characteristics and ALK IHC Status of All Cases: FISH Screening Population in Study 1014

Parameter		ALK Status [a] for Cases Stained Under Study Protocol D032361			ALK Status [b] for Cases Stained Under Study Protocol D033144			Not Screened with IHC	All A8081014 FISH Screened Cases
		Positive	Negative	N/D	Positive	Negative	N/D		
Age, years	N	185	793	40	51	245	9	894	2217
	Mean (SD)	56.5 (13.7)	63.3 (11.1)	62.4 (10.9)	53.4 (12.9)	63.1 (11.3)	57.2 (16.0)	60.3 (12.1)	61.2 (12.1)
	Median	56.0	64.0	63.0	55.0	64.0	50.0	61.0	62.0
	Min, Max	22, 82	23, 93	36, 82	22, 75	25, 90	38, 83	20, 90	20, 93
	Missing	0	0	0	0	0	0	0	0
Sex, n (%)	Male	70 (37.8)	434 (54.7)	22 (55.0)	15 (29.4)	122 (49.8)	4 (44.4)	457 (51.1)	1124 (50.7)
	Female	115 (62.2)	359 (45.3)	18 (45.0)	36 (70.6)	123 (50.2)	5 (55.6)	437 (48.9)	1093 (49.3)
Race, n (%) [c]	Asian	26 (14.1)	6 (0.8)	3 (7.5)	13 (25.5)	4 (1.6)	2 (22.2)	103 (11.5)	157 (7.1)
	Black	1 (0.5)	0	0	0	0	0	3 (0.3)	4 (0.2)
	White	84 (45.4)	11 (1.4)	1 (2.5)	16 (31.4)	4 (1.6)	0	60 (6.7)	178 (7.9)
	Other	1 (0.5)	0	0	0	0	0	5 (0.6)	6 (0.3)
	Missing	73	776	36	22	237	7	723	1874

Parameter		ALK Status [a] for Cases Stained Under Study Protocol D032361			ALK Status [b] for Cases Stained Under Study Protocol D033144			Not Screened with IHC	All A8081014 FISH Screened Cases
		Positive	Negative	N/D	Positive	Negative	N/D		
Presence of Brain Metastases, n (%) [c]	Absence	80 (43.2)	14 (1.8)	2 (5.0)	24 (47.1)	7 (2.9)	2 (22.2)	122 (13.6)	251 (11.3)
	Presence	32 (17.3)	3 (0.4)	2 (5.0)	5 (9.8)	1 (0.4)	0	49 (5.5)	92 (4.1)
	Missing	73	776	36	22	237	7	723	1874
ECOG PS [d] at Baseline, n (%) [c]	PS 0-1	106 (57.3)	15 (1.9)	4 (10.0)	29 (56.9)	8 (3.3)	2 (22.2)	160 (17.9)	324 (14.6)
	PS 2	6 (3.2)	1 (0.1)	0	0	0	0	11 (1.2)	18 (0.8)
	Missing	73	777	36	22	237	7	723	1875
Smoking History, n (%) [c]	Smoker	4 (2.2)	3 (0.4)	1 (2.5)	0	0	0	7 (0.8)	15 (0.7)
	Ex Smoker	32 (17.3)	7 (0.9)	2 (5.0)	8 (15.7)	5 (2.0)	1 (11.1)	55 (6.2)	110 (5.0)
	Never Smoked	76 (41.1)	7 (0.9)	1 (2.5)	21 (41.2)(	3 (1.2)	1 (11.1)	109 (12.2)	218 (9.8)
	Missing (n)	73	776	36	22	237	7	723	1874

[a] Uses ALK IHC results obtained under diagnostic protocol D032361 for cases screened for Study 1014 by FISH.
[b] Uses ALK IHC patient-level results obtained during internal verification testing performed under Ventana protocol D033144.
[c] This information was collected only for ALKFISH+ patients enrolled in Study 1014.
[d] ECOG PS = Eastern Cooperative Oncology Group Performance Status.
Note: Not all of these patients were enrolled into Study 1014 due to Study 1014 inclusion/exclusion criteria unrelated to the ALK IHC result.

In addition, demographic and baseline characteristics for the two treatment arms were compared in ALK FISH+/ALK IHC+ study population (**Table 17**). The resulting analysis demonstrates that these two treatment arms were balanced for the observed demographics and baseline characteristics. The randomization ratios were maintained with the ALK IHC retrospective testing cohort.

Table 17: Demographic Characteristics by Treatment Arms in ALK FISH+/ALK IHC+ Patients

Parameter		Treatment Arm		Overall
		Chemo	Crizotinb	
Age, years	N	63	78	141
	Mean (SD)	55.0 (13.9)	55.0 (12.6)	55.0 (13.1)
	Median	56.0	55.5	56.0
	Min, Max	22, 80	24, 78	22,80
				P-value [a] 0.989
Sex, n (%)	Male	24 (38.1)	24 (30.8)	48 (34.0)
	Female	39 (61.9)	54 (69.2)	93 (66.0)
				P-value [b] 0.377
Race, n (%)	Asian	19 (30.2)	20 (25.6)	39 (27.7)
	Black	1 (1.6)	0	1 (0.7)
	White	43 (68.3)	57 (73.1)	100 (70.9)
	Other	0	1 (1.3)	1 (0.7)
				P-value [b] 0.620
Presence of Brain Metastases, n (%)	Absence	Absence	45 (71.4)	59 (75.6)
	Presence	Presence	18 (28.6)	19 (24.4)
				P-value [b] 0.701
ECOG PS at Baseline, n (%)	PS 0-1	61 (96.8)	74 (94.9)	135 (95.7)
	PS 2	2 (3.2)	4 (5.1)	6 (4.3)
				P-value [b] 0.692
Smoking History	Smoker	0	4 (5.1)	4 (2.8)
	Ex Smoker	16 (25.4)	24 (30.8)	40 (28.4)
	Never Smoked	47 (74.6)	50 (64.1)	97 (68.8)
				P-value [b] 0.153

[a] Only includes cases that were observed to be IHC+ in D032361 or D033144, cases with imputed IHC status are not included

[b] Mean differences were compared using the t-test. The Satterthwaite approximation was used to determine degrees of freedom.

Note: ECOG PS = Eastern Cooperative Oncology Group Performance Status.

E. Safety and Effectiveness Results

1. Safety Results

As an *in vitro* diagnostic test, the VENTANA ALK (D5F3) CDx Assay involves testing on FFPE NSCLC sections. These tissues are routinely removed as part of the practice of medicine for the diagnosis of NSCLC by pathologists. Removal of these tissues; therefore, presents no additional safety hazard to the patient being tested.

The safety with respect to treatment with crizotinib will not be addressed in detail in the SSED for the VENTANA ALK (D5F3) CDx Assay. The most common adverse reactions (≥ 25%) in clinical trials of crizotinib are vision disorders, nausea, diarrhea, vomiting, constipation, edema, elevated transaminases, and fatigue. These adverse events (AEs) were primarily mild to moderate in severity and were generally clinically manageable through dosing interruption, dose reduction, and/or standard medical therapy, as the rate of permanent treatment discontinuations and deaths associated with AEs was low. The common adverse events associated with crizotinib in Study 1014 were consistent with those described in the crizotinib product label information.

2. Effectiveness Results

Agreement Analysis – ALK FISH vs. ALK IHC

A total of 933 samples with both valid ALK FISH and ALK IHC results are included in the following agreement analysis. The positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) were calculated to compare ALK diagnostic status results as determined by using the ALK IHC Assay vs. the ALK FISH assay for the same NSCLC cases from Study 1014 under diagnostic study protocol D032361 (**Table 18**).

There were 25 ALK FISH+/ALK IHC- discordant cases, where the median ALK FISH score (% tumor cells positive for gene rearrangement) was 20.0% (mean 31.6%, SD 21.6%), which was lower than the median ALK FISH score of 58.0% (mean 56.9%, SD 22.0%), observed for all enrolled patients tested with ALK IHC. Fourteen out of the 25 cases had a FISH score of 25% or less, which is above the cut-off for ALK FISH+ (cut-off of ≥ 15% positive cells), and also within the ALK FISH equivocal zone (10-50% positive cells). Six discordant cases were randomized into the crizotinib arm; the clinical outcomes for these cases are noted below in "*Overall Efficacy*" section below.

There were also 28 ALK FISH-/ALK IHC+ discordant cases. No clinical outcome data are available on these discordant cases, since only ALK FISH+ cases were enrolled into Study 1014.

Table 18: ALK IHC vs. ALK FISH Agreement

Study 1014		ALK FISH Status			Agreement Rates			
		Pos.	Neg.	Total	Rate	n/N	%	95% CI
ALK IHC Status	Pos.	154	28	182	PPA	154/179	86.0	80.2-90.4
	Neg.	25	726	751	NPA	726/754	96.3	94.7-97.4
	Total	179	754	933	OPA	880/933	94.3	92.6-95.6

Overall Efficacy

Study 1014 was aimed at demonstrating that crizotinib is superior to first-line chemotherapy, pemetrexed/cisplatin or pemetrexed/carboplatin, in prolonging progression-free survival (PFS) in patients with advanced NSCLC whose tumors harbor a translocation or inversion event involving the ALK gene locus based on the ALK FISH assay results. The primary efficacy outcome was PFS as assessed by independent radiology review (IRR). PFS was defined as the time from the date of randomization to the date of the first documentation of objective tumor progression (by IRR) or death on study due to any cause, whichever occurred first. Based on the positive ALK FISH assay results, 343 patients were in the randomized set (172 in the crizotinib arm and 171 in the chemotherapy arm). Retrospective testing of tissue specimens from Study 1014 was conducted using the alternative companion diagnostic test for crizotinib, VENTANA ALK (D5F3) CDx Assay. Among all the NSCLC tumor samples tested retrospectively, a total of 166 patients (86 randomized to crizotinib and 80 patients randomized to chemotherapy) had valid results from both ALK IHC and ALK FISH assays which are included in the analysis shown below. The overall efficacy results are summarized in **Table 19**.

Table 19: Clinical Benefit of ALK FISH+ Patient Population in Study 1014

	ALK Status	HR[a]	SE[a]	95% CI[a]	Sample Size	
					Chemo	Crizotinib
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] HR, Hazard Ratio of crizotinib versus chemotherapy; SE, standard error; and 95% CI, 2-sided 95% confidence interval. Results were estimated using a stratified Cox model with the following stratum: race, brain metastasis, and ECOG score.
[b] Two ALK FISH+ patients in the chemotherapy arm and four patients in the crizotinib arm had invalid ALK IHC test results.

There were 25 cases from Study 1014 evaluated as ALK FISH+/ALK IHC- (last row of **Table 19**). For the eight of the 25 ALK FISH+/ALK IHC- cases randomized to the crizotinib arm, the following clinical observations were noted:

- Five patients had ALK FISH scores very close to the FISH cut-off (15-18% of tumor cells positive for gene rearrangement), and showed objective progression or stable disease/no response. (Four of the five patients are the discordant ALK FISH+/ALK IHC- cases in the agreement analysis above.)
- Two patients had ALK FISH scores that were outside the FISH equivocal zone (66% and 72% of tumor cells positive for gene rearrangement). Both patients experienced partial objective tumor response. (Both patients are the discordant ALK FISH+/ALK IHC- cases in the agreement analysis above.)
- One patient was enrolled erroneously and was determined as ALK FISH-; hence, the ALK IHC result is concordant with the ALK FISH result. Indeterminate response was noted for this patient.

Efficacy in ALK IHC Positive (+) Population & Sensitivity Analysis
The ALK IHC positive population included two sub-populations in Study 1014. One population defined as ALK FISH+/ALK IHC+ had clinical outcome (see **Table 19**). The other population defined as ALK FISH-/ALK IHC+ had no clinical outcome because the ALK FISH- patients were excluded from the trial study design. Thus, additional efficacy analyses were conducted to consider patients who tested positive by the ALK IHC Assay, but were negative by the ALK FISH assay (i.e., ALK FISH-/ALK IHC+), in the Study 1014 screen population. A logistic model was used to impute missing ALK IHC results for samples with ALK FISH status (i.e., positive, negative or unknown). The clinical

outcomes of the ALK FISH-/ALK IHC+ patients are not observed but imputed using bootstrap resampling techniques. Hence, the hazard ratio of PFS between the crizotinib-treated arm and the chemotherapy-treated arm for the ALK IHC+ patients are calculated and presented in **Table 20** below, with Scenario 1 assuming the best case scenario (i.e., 100% of these patients respond as ALK FISH+/ALK IHC+), and Scenario 5 assuming the worst case scenario (i.e., 0% of these patients respond as ALK FISH+/ALK IHC+).

Table 20: Observed and Simulated Outcome in ALK IHC+ Population

Scenario[a]	Mean HR	Median HR	SD of HR	20% Efficacy[b]	Avg LB 95 [c]	Avg UB 95 [c]	2-sided 95% confidence interval [d]	Mean sample size (SD)	
								Chemo	Crizotinib
1	0.447	0.446	0.03	1	0.342 (0.024)	0.584 (0.038)	(0.391,0.509)	180 (3.8)	180.5 (3.8)
2	0.463	0.462	0.031	1	0.355 (0.024)	0.604 (0.039)	(0.406, 0.527)	180 (3.8)	180.5 (3.8)
3	0.479	0.478	0.031	1	0.368 (0.025)	0.624 (0.039)	(0.421, 0.543)	180 (3.8)	180.5 (3.8)
4	0.496	0.495	0.031	1	0.381 (0.025)	0.644 (0.039)	0.438, 0.559)	180 (3.8)	180.5 (3.8)
5	0.512	0.511	0.031	1	0.395 (0.024)	0.665 (0.039)	(0.455, 0.575)	180 (3.8)	180.5 (3.8)

[a] The 5 scenarios represent different response to crizotinib for the (IHC+, FISH-/u) patients who are randomly assigned to the crizotinib treated arm. Scenario 1 assumes 100% of these patients respond as FISH+/IHC+ crizotinib treated patients do, scenario 2 assumes 75%, scenario 3 assumes 50%, scenario 4 assumes 25% and scenario 5 assumes 0% (i.e. that all patients have similar outcomes as chemotherapy treated patients).
[b] In each simulated trial, a 2-sided 95% CI of log(HR) was estimated and its upper limit was compared with 20% of log(HR) in the original FISH+ trial. If the upper limit was less than 20% of log(HR) in the original FISH+ trial, the simulated trial successfully achieved 20% of the original trial's efficacy. Otherwise, the simulated trial failed to maintain 20% efficacy. The proportion of successes among 100,000 simulated trials is presented.
[c] The Avg LB 95 is the mean lower confidence bound based on the 100,000 simulated 95% confidence bounds of the HR. The Avg UB 95 is the mean upper confidence bound based on the 100,000 simulated 95% confidence bounds of the HR.
[d] The 2-sided 95% CI is the 2.5th and 97.5th percentiles of the 100,000 simulated HRs.
Note: HR statistics are based on 100,000 simulated ALKIHC+ trials. ALK IHC status was imputed for those patients not tested with the ALK IHC Assay under the diagnostic protocol or during verification study D033144. In each simulated trial, the HR of crizotinib versus chemotherapy was estimated with a stratified Cox regression model with the following stratum: race, brain metastasis, and ECOG score.

Additional imputation analyses were performed to evaluate the robustness of study conclusions. Statistical analysis of discordant patients not enrolled in Study 1014 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.

F. Financial Disclosures

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The diagnostic clinical study included three investigators who did not have disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

An additional agreement study was conducted to determine concordance between the ALK IHC and the ALK FISH assays using FFPE patient samples from another Pfizer-sponsored study of crizotinib, i.e., Study A8081029 (Study 1029). Study 1029 is an ongoing Phase 3, randomized, open-label study of the efficacy and safety of crizotinib versus pemetrexed/cisplatin or pemetrexed/carboplatin in previously untreated East Asian patients with non-squamous carcinoma of the lung harboring a translocation or inversion event involving the alk gene locus. The ALK FISH test was used to determine ALK diagnostic status (ALK positive or ALK negative) for the patients who were enrolled into Study 1029. Patient specimens from this study were not transferred to Ventana for retrospective testing. These FFPE patient specimens were tested with the ALK IHC Assay at the Study 1029 central enrollment laboratories that performed the ALK FISH test for the clinical trial protocol. Both the ALK FISH and ALK IHC data were transferred from Pfizer to Ventana.

A total of 598 samples with both valid ALK FISH and ALK IHC results were included in the agreement analysis. The PPA, NPA, and OPA were calculated to compare ALK diagnostic status results as determined by using the ALK IHC Assay vs. the ALK FISH assay for the same NSCLC cases from Study 1014 (**Table 21**).

Table 21: ALK IHC vs. ALK FISH Agreement

Study 1029		**ALK FISH Status**			**Agreement Rates**			
		Pos.	**Neg.**	**Total**	**Rate**	**n/N**	**%**	**95% CI**
ALK IHC Status	**Pos.**	179	21	200	**PPA**	179/193	92.7	88.2-95.6
	Neg.	14	384	398	**NPA**	384/405	94.8	92.2-96.6
	Total	193	405	598	**OPA**	563/598	94.1	92.0-95.8

XII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Panel, an FDA advisory committee, for review and recommendation because

the information in the PMA substantially duplicates information previously reviewed by this panel.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The effectiveness of the VENTANA ALK (D5F3) CDx Assay was assessed by the agreement rates between the VENTANA ALK (D5F3) CDx Assay and the FDA-approved companion diagnostic for crizotinib, the Vysis ALK Break Apart FISH Probe Kit. The clinical benefit of the VENTANA ALK (D5F3) CDx Assay was demonstrated in a retrospective analysis for patients enrolled in Study 1014, in which the ALK status was determined using the Vysis ALK Break Apart FISH Probe Kit. Overall, a statistically significant efficacy benefit for crizotinib vs. chemotherapy was observed in the subset of NSCLC patients whose tumors had ALK protein expression, as detected by the VENTANA ALK (D5F3) CDx Assay. The observed clinical benefit in the subset of patients tested with the VENTANA ALK (D5F3) CDx Assay was comparable to that observed in the full study population. Additional case scenario analyses and sensitivity analyses also support the general conclusion observed.

B. Safety Conclusions

The adverse effects of the device are based on data collected in the clinical study conducted to support PMA approval as described above. In the context of an *in vitro* diagnostic test, there are no directly harmful events from testing FFPE NSCLC tissue sections. These tissue sections are routinely removed as part of the diagnosis of NSCLC by pathologists. The test, therefore, presents no additional safety hazard to the patient being tested.

The risks of the VENTANA ALK (D5F3) CDx Assay are associated with the potential mismanagement of patients resulting from false results of the test. A false negative test result may lead to crizotinib treatment being withheld from a patient who might have benefited. A false positive test result may lead to crizotinib treatment being administered to a patient who may experience adverse side effects associated with treatment without clinical benefit.

C. Benefit-Risk Conclusions

Screening and diagnosis of ALK positivity in the intended use population is a key part of diagnostic evaluation for NSCLC patients, in decisions regarding treatment with crizotinib. The probable benefits of the VENTANA ALK (D5F3) CDx Assay are based on evaluation that the test performs consistently and provides clinically relevant results for assessing ALK status in NSCLC patients who are being considered for crizotinib therapy. The risks of the VENTANA ALK (D5F3) CDx

Assay are associated with potential mismanagement of patients due to false test results as noted above.

In conclusion, results from the non-clinical and clinical studies presented in this original PMA application submission establish reasonable assurance that the VENTANA ALK (D5F3) CDx Assay is safe and effective for its intended use when used in accordance with product labeling. Clinical evaluation and performance is in line with the other FDA-approved companion diagnostic for crizotinib, the Vysis ALK Break Apart FISH Probe Kit. The totality of analytical and clinical data supports the use of the VENTANA ALK (D5F3) CDx Assay for a similar indication as the Vysis ALK Break Apart FISH Probe Kit. The probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use and product labeling. The provided studies support use of the VENTANA ALK (D5F3) CDx Assay as an aid in identifying patients eligible for treatment with crizotinib.

XIV. CDRH DECISION

CDRH issued an approval order on June 12, 2015.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Limitations in the device labeling. Refer to the drug label for XALKORI (crizotinib) for additional information related to use of the drug.

Post-approval Requirements and Restrictions: See approval order.