SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: DNA FISH Probe Assay

Device Trade Name: Vysis ALK Break Apart FISH Probe Kit, Vysis Paraffin Pretreatment IV & Post Hybridization Wash Buffer Kit, ProbeChek ALK Negative Control Slides, and ProbeChek ALK Positive Control Slides

Applicant's Name and Address: Abbott Molecular, Inc.
1300 E. Touhy Avenue
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Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P110012

Date of FDA Notice of Approval: August 26, 2011

Expedited: Granted expedited review status on April 21, 2011 because it was believed through literature support that the Vysis Anaplastic Lymphoma Kinase (ALK) Break Apart FISH Probe Kit would aid in the selection of previously treated non-small cell lung cancer patients for treatment with the Pfizer drug Xalkori® (crizotinib), which may result in a life-prolonging survival benefit and is therefore in the best interest of those patients. Because no other legally marketed device is available, the FDA decided to grant expedited review to the Vysis ALK Break Apart FISH Probe Kit.

II. INDICATIONS FOR USE

The Vysis ALK Break Apart FISH Probe Kit is a qualitative test to detect rearrangements involving the ALK gene via fluorescence in situ hybridization (FISH) in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissue specimens to aid in identifying patients eligible for treatment with Xalkori® (crizotinib).
This is for prescription use only.

The Vysis Paraffin Pretreatment IV & Post Hybridization Wash Buffer Kit is used to prepare paraffin-embedded lung cancer tissue sections fixed on positively charged slides for use in fluorescence in situ hybridization (FISH) with Vysis DNA FISH probes.

The ProbeChek ALK Negative Control Slides are intended for use as an assay control for appropriate hybridization conditions during routine use of the Vysis ALK Break Apart FISH Probe Kit (List No. 06N38-020). The ProbeChek ALK Negative Control Slides should be assayed in conjunction with the user's specimen slides according the package insert for the Vysis ALK Break Apart FISH Probe Kit (List No. 06N38-020).

The ProbeChek ALK Positive Control Slides are intended for use as an assay control for appropriate hybridization conditions during routine use of the Vysis ALK Break Apart FISH Probe Kit (List No. 06N38-020). The ProbeChek ALK Positive Control Slides should be assayed in conjunction with the user's specimen slides according the package insert for the Vysis ALK Break Apart FISH Probe Kit (List No. 06N38-020).

III. CONTRAINDICATIONS

None

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the Vysis ALK Break Apart FISH Probe Kit, Vysis Paraffin Pretreatment IV & Post Hybridization Wash Buffer Kit, ProbeChek ALK Negative Control Slides, and ProbeChek ALK Positive Control Slides labeling.

V. DEVICE DESCRIPTION

The Vysis ALK Break Apart FISH Probe Kit uses FFPE tissue sections which have been mounted to glass slides as the specimen. The tissue sections are deparaffinized and then treated with components of the Vysis Paraffin Pretreatment IV & Post-Hybridization Wash Buffer Kit. The DNA contained within the nuclei of the FFPE tissue sections is denatured to the single-stranded form and subsequently allowed to hybridize with the locus-specific indicator Vysis ALK Break Apart FISH Probes. Following hybridization, the unbound probe is removed by a series of washes using Vysis Wash Buffers I and II and the nuclei are counterstained with DAPI Counterstain (4,6 diamidino-2-phenylindole), a DNA-specific stain that fluoresces blue. Hybridization of the Vysis ALK Break Apart FISH Probes is viewed using a fluorescence microscope equipped with appropriate excitation and emission filters, allowing visualization of the orange and green fluorescent signals.

The Vysis ALK Break Apart FISH Probe Kit assay requires four kits:

- Vysis ALK Break Apart FISH Probe Kit
- Vysis Paraffin Pretreatment IV & Post Hybridization Wash Buffer Kit
- ProbeChek ALK Negative Control Slides
- ProbeChek ALK Positive Control Slides

The Vysis ALK Break Apart FISH Probe Kit:

The Vysis ALK Break Apart FISH Probe Kit consists of one 200 µL vial of Vysis LSI ALK Dual Color Break Apart FISH Probe and a 300 µL vial of DAPI I Counterstain. It contains sufficient reagents to process 20 assays. Each assay uses 10 µL of Vysis ALK Break Apart FISH Probe Kit applied to a hybridization target area of 22 mm x 22 mm. The probe requires storage at -30°C to -10°C upon receipt.

The VYSIS ALK BREAK APART FISH PROBE KIT, as shown in Figure 1, is a mixture that consists of two DNA probes directly labeled with fluorophores in hybridization buffer containing dextran sulfate, formamide, and SSC with blocking DNA:

- Vysis LSI 3'-ALK SpectrumOrange
- Vysis LSI 5'-ALK SpectrumGreen

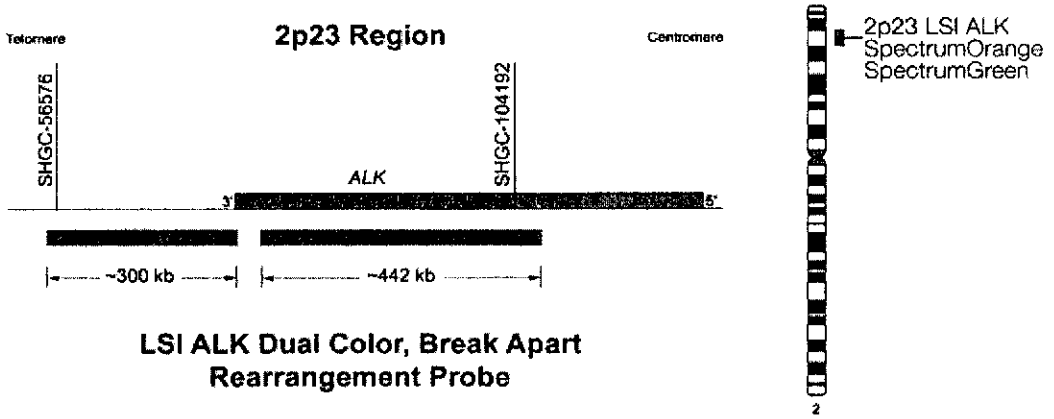

Figure 1. Probe map and ideogram for the Vysis ALK Break Apart FISH Probe Kit probes.

The hybridization targets of these probes are on opposite sides flanking the breakpoint region of the ALK gene. The 3'-ALK probe that hybridizes telomerically of the breakpoint is approximately 300 kb and is labeled with the SpectrumOrange (SO) fluorophore. The 5'-ALK probe that hybridizes centromerically of the breakpoint is approximately 442 kb and is labeled with the SpectrumGreen (SGn) fluorophore.

Vysis Paraffin Pretreatment IV & Post Hybridization Wash Buffer Kit:
The Vysis Paraffin Pretreatment IV & Post Hybridization Wash Buffer Kit consists of five 50 mL bottles of pretreatment solution (1N Sodium thiocyanate); five bottles Protease Buffer IV (0.1N hydrochloric acid); five bottles Protease IV (Pepsin 2500-4000 U/mg); one 250 mL bottle of Wash Buffer I (0.3% NP-40/0.7XX SSC pH 7); and one 250 mL bottle of Wash Buffer II (0.1% NP-40/2X SSC, pH 7.0). The Protease IV reagent requires storage at -20°C to ±10°C upon receipt and one vial the Protease is added to the contents of one bottle of Protease IV Buffer on the day of use. All other reagents are provided ready-to-use.

ProbeChek ALK Positive and Negative Control Slides:
A negative control (ProbeChek ALK Negative Control Slides) and positive control (ProbeChek ALK Positive Control Slides) are provided separately with five slides per kit. Both controls consist of FFPE cultured cell line specimens which have been sectioned and applied onto glass microscope slides. The cell line used for the negative control shows no chromosomal rearrangement at the ALK breakpoint and provides ALK signal enumeration consistent with a negative classification. The cell line mixture used for the positive control provides ALK signal enumeration consistent with a positive classification (20 – 62%). Control slides must be run concurrently with patient slides to monitor assay performance and to assess accuracy of signal enumeration. Control slides are introduced at the initiation of the slide deparaffinization procedure and evaluated to determine assay validity. Control slides are run on each day of FISH testing and with each new kit lot.

Specimen Preparation:
The Vysis ALK Break Apart FISH Probe Kit has been optimized only for identifying and quantifying rearrangements of the ALK gene from FFPE human NSCLC tissue specimens. The assay should be performed only on 10% neutral buffered formalin FFPE human lung tumor tissue. Other types of specimens or fixatives should not be used.

Signal Enumeration:
For each nucleus, the number of fused (adjacent) signals, single orange signals, and single green signals are recorded. An individual cell is counted only once regardless of the number of rearrangements and/or deletions that it contains. Users are instructed to not score nuclei with no signals or with signals of only one color (without a fused and/or broken apart signal). Only those nuclei with one or more FISH signals of each color are scored. A nucleus that contains signals that are weak or overly diffuse should not be enumerated.

Cells are considered negative (non-rearranged) when:

- Orange and green signals are adjacent or fused. Orange and green signals that are less than two signal diameters apart are considered as a single fused signal.
- There is a single green signal without a corresponding orange signal.

Cells are considered positive (rearranged) when:

- At least one set of orange and green signals are two or more signal diameters apart.
- There is a single orange signal without a corresponding green signal in addition to fused and/or broken apart signals.

Table 1. Classification of Cells as Positive or Negative

No. of Adjacent or Fused Signals	No. of Single Orange Signals	No. of Single Green Signals	Cell Classification
≥1	0	0	Negative
≥1	0	≥1	Negative
≥0	≥1	≥1	Positive
≥1	≥1	0	Positive

The number of cells classified as positive or negative for ALK rearrangements or deletions are calculated according to the table above.

- A sample is considered negative if <5 cells out of 50 (<5/50 or<10%) are positive.
- A sample is considered positive if >25 cells out of 50 (>25/50 or >50%) are positive.
- A sample is considered equivocal* if 5-25 cells (10-50%) are positive. If the sample is equivocal, a second reader should evaluate the slide.
  - The first and second cell count readings are added together and a percent is calculated out of 100 cells (average percent of positive cells).
  - If the average percent positive cells is <15% (<15/100), the sample is considered negative.
  - If the average percent positive cells are ≥15% (≥15/100), the sample is considered positive.

*Specimens whose results fall into the equivocal zone (10 – 50% positive), based on the enumeration by the first reader, should be enumerated by a second reader to confirm results.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently no alternative approved methods to the Vysis ALK Break Apart FISH Probe Kit assay for detecting ALK rearrangements in NSCLC.

VII. MARKETING HISTORY

The Vysis ALK Break Apart FISH Probe Kit, ProbeChek ALK Negative Control Slides, and ProbeChek ALK Positive Control Slides have not been marketed in the United States or any foreign country. The Vysis Paraffin Pretreatment IV & Post Hybridization Wash Buffer Kit has not been marketed in the United Stated but is marketed in Europe.

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 subsequently improper patient management decisions in NSCLC treatment.

For the specific adverse events that occurred in the clinical studies, please see the Adverse Event section the approval documents for NDA 202570.

IX. SUMMARY OF PRECLINICAL STUDIES

Analytical performance was assessed to evaluate the safety and effectiveness of the Vysis ALK Break Apart FISH Probe Kit assay on NSCLC tissue. The studies included probe concentration optimization, hybridization quality evaluation, establishment of the normal cutoff, probe localization, FISH success rate, analytical sensitivity, analytical specificity, microbial contamination, robustness, kit shelf-life stability, reproducibility, and repeatability testing. The robustness studies included: slide thickness, slide stability (pre-hybridization and post-hybridization), water bath section flotation temperature and time, slide baking, deparaffinization, pretreatment conditions, enzyme digestion, probe and target co-denaturation, hybridization time and temperature, post-hybridization wash, and DAPI staining.

A. Laboratory Studies

Evaluation of hybridization quality:

In all studies, the quality of the hybridization of each probe is evaluated for each hybridization target. Each hybridization target area within the assay is rated on a scale of 1 to 5 according to four attributes: probe signal intensity, nuclear and chromosomal specificity, target background, and cross-hybridization. An overall rating is then assigned to each slide based upon the lowest score of the attributes evaluated. For each slide, an overall rating of greater than or equal to three (3) indicates a disposition of "Pass", otherwise the disposition is "Fail."

Probe Concentration Optimization:

The Vysis ALK Break Apart FISH Probe Kit is composed of two individual probes. In order for the probe to be enumerated properly, the signal intensities of the two probes need to be balanced so the intensity (brightness) of the two probes is optimal for visualization. Two NSCLC FFPE tissue specimens were processed according to the package insert which included pretreatment, denaturation, hybridization and post-hybridization washes. Four readers were utilized to evaluate the results according to the qualitative/ quantitative assessments described above with signal intensity being the primary variable.

The concentration of the 5'ALK SGn probe was evaluated at three different starting concentrations. The 5'ALK SGn probe was fixed at one concentration after no significant difference was noted between the concentrations tested. The 3'ALK SO Probe concentration was added and tested at using various different concentrations. Quality scores with respect to intensity ≥ 3 were considered acceptable. One concentration of the 3'ALK SO probe was selected as it yielded the optimal balance of probe intensities with the intensity quality score of both the SGn and SO probes of ≥3. Once the two probes were optimized, the probe was tested using slides from 16 individual ALK-positive and negative NSCLC FFPE blocks and two lots of ProbeChek ALK Control slides (in duplicate). The 5'ALK SGn probe had 93% (56/60) success rate and 3'ALK SO Probe had 97% (58/60) success rate.

Normal Cutoff

The normal cutoff value was defined as the maximum amount of scoreable interphase nuclei with a specific abnormal signal pattern at which a specimen is considered negative for that signal pattern. The normal cutoff value is expressed in terms of a percentage, or the actual number of nuclear FISH patterns positive for rearrangement per the standard number of nuclei tested. The normal cutoff was established as 15% using NSCLC FFPE tissue specimens.

Probe Localization on Metaphase Chromosomes

To verify that the probes hybridized only to the intended locus, the location of hybridization of the Vysis ALK Break Apart FISH Probe Kit was evaluated on eight metaphase spreads prepared from cultured lymphocyte slide preparations in conjunction with the inverted DAPI chromosome banding technique. Both probes which make up the final probe mixture were shown to hybridize to the intended locus (2p23) on all eight metaphase spreads and to no other locations.

Analytical Sensitivity and Specificity

Analytical sensitivity was defined as the percentage of chromosome targets with the expected normal signal pattern. Analytical specificity was defined as the percentage of signals that hybridize to the correct locus and no other location. The sensitivity and specificity of the LSI 3'-ALK SpectrumOrange and LSI 5'-ALK SpectrumGreen FISH probes were evaluated using metaphase chromosomes prepared from 6 slide lots created from peripheral blood cultures of karyotypically normal specimens from 5 individual donors. The analytical sensitivity was calculated to be 100.0% (240/240) (95% CI 98.5-100.0%) for each probe.

The sensitivity calculation, the signals for LSI 3'-ALK SO and LSI 5'-ALK SGn FISH probes, were enumerated for each metaphase spread (normal = 2 signals). In total, 240 signals were expected for each probe (2 signals per cell x 20 metaphase spreads per lot x 6 slide lots). For the specificity calculation, the number of metaphase spreads with the expected signal pattern was enumerated. In total, 120 metaphase spreads were evaluated (20 metaphase spreads x 6 slide lots). The analytical specificity was calculated to be 100.0% (120/120) (95% CI 97.0-100.0%) for each probe.

FISH Success Rate on FFPE NSCLC Specimens
The FISH success rate on FFPE lung tumor specimens was evaluated using FFPE tumor tissue specimens. The assay success rates when using a dual bandpass green/orange filter v2 and the single bandpass green was 99.2 % (119/120) and the lower-bound of the 95% confidence interval was 96.1%. The assay success rates for the single bandpass orange were 98.3% (118/120) and the lower-bound of the 95% confidence interval was 94.8%.

Microbial Contamination
The Vysis ALK Break Apart FISH Probe Kit met the requirements for a microbiologically uncontrolled product per "Guideline for the Manufacture of In Vitro Diagnostic Products", 1/10/1994, as none of the reagents would sustain growth of the selected microorganisms and in fact killed the applied inoculum of microorganisms as referenced by the lack of growth upon subculture. Additionally upon testing the reagents in the normal QC procedure, all the reagents performed satisfactorily even after three days of incubation with the selected organisms at 35-37°C.

Robustness Studies
The objective of the guard banding studies was to establish the robustness of the Vysis ALK Break Apart FISH Probe Kit for detection of ALK gene rearrangements by FISH in NSCLC FFPE tissues. These studies included evaluation of specimen preparation (section thickness and water bath temperature), specimen pretreatment (slide baking, deparaffinization, pepsin digestion time), probe and target co-denaturation (time and temperature), wash conditions (wash solutions, time, temperature), DAPI staining, and slide stability (Pre-Hybridization and Post-Hybridization) using both NSCLC FFPE tissue samples and cell lines (ProbeChek ALK Control Slides). For all studies, a qualitative assessment of hybridization and/or a quantitative assessment of fluorescent signals were used to determine the acceptable guard banding condition.

A brief description of each characterization/robustness study is described below. For all, the interpretation of the FISH results included both qualitative assessment of hybridization and quantitative assessment of fluorescent signals. All slides were processed through all assay steps and evaluated. A slide was considered of acceptable quality (passing) if the overall qualitative score was ≥ 3. A slide was classified as "Fail" if either one of two readers gave it an overall quality score of < 3.

1. Section Thickness – Section thickness of the FFPE tissue was evaluated within the range of variability for slide preparation as thickness of the FFPE tissue sections may affect signal enumeration results for the Vysis ALK Break Apart FISH Probe Kit due to nuclear truncation or the degree of cell overlap. The three NSCLC specimens consisted of two ALK-positives and one ALK-negative and each specimen was sectioned into 4 µm, 5 µm and 6 µm thicknesses. Six sections were cut for thickness and the slides were blinded, processed, and enumerated by two readers, resulting in a total of 108

enumerations (3 specimens, 3 section thicknesses, 6 slides, 2 readers). The percentage of ALK-positive cells was determined for each slide. The statistical significance of the analyses was defined at the 5% level and variance assessed by ANOVA. From the results, there is little statistical impact on the positive cell enumeration for the Vysis ALK Break Apart FISH Probe Kit assay between 4 – 6 μm and therefore the recommended thickness is 5 ± 1 μm.

2. Slide Stability – Pre-Hybridization - Archived tissue specimens stored for approximately 2 years were evaluated with respect to assay performance to determine if up to 2 years of storage time is acceptable for this assay. Twenty (20) sections from 4 different NSCLC FFPE tissue specimen blocks were sectioned at 5 μm and tested between 23.5 – 24.5 months. The slides were enumerated by two independent readers. There were a total of 40 individual readings (20 per reader). Out of 40 readings, 36 (90%) indicated an overall quality passing rate.

3. Sample Preparation - Water bath Section Flotation Temperature – NSCLC FFPE tissue specimens and control slides were processed for up to 30 minutes and evaluated using eight flotation water bath conditions at four temperatures ranging between 37°C to 50°C and evaluated for flotation times of <1 min and 30 min at each temperature and evaluated by two readers. There was no difference seen in sections which remained in the flotation water bath for 30 minutes at 37-50°C.

4. Slide Baking - Prior to deparaffinization, NSCLC FFPE tissue sections are baked to assure adherence of FFPE sections to the slides. A guardbanding study was conducted using the ThermoBrite to characterize the slide baking step of the assay on NSCLC FFPE tissue specimens. NSCLC FFPE tissue specimens were each processed and evaluated using four slide baking conditions: 56°C for 2 hours, 60°C for 2 hours, 60°C for 24 hours, and 68°C for 24 hours. All of the slides evaluated resulted in scores with acceptable hybridization quality scores.

5. Deparaffinization (Hemo-De) Exposure Time – The purpose of this study was to evaluate a range of exposure times of the NSCLC FFPE tissue slides to Hemo-De (xylene substitute) as a deparaffinization pretreatment process. NSCLC FFPE tissue specimens and cell lines (ALK Negative and Positive Controls) were processed and evaluated using three deparaffinization conditions times. All slides were read by two independent readers. All of the slides evaluated resulted in scores with acceptable hybridization quality scores.

6. Pretreatment Conditions – Pretreatment is utilized to reverse the effects of cross-linking induced by fixation, to make the tissue permeable, to remove cytoplasm, and to digest proteins in order to make the genomic target DNA in the nuclei assessable for hybridization, as well as to reduce background

autofluorescence of cells and tissue. The pretreatment procedures used in FISH involve the application of high heat, chaotropic agents such as sodium thiocyanate, and protein digestion. Following deparaffinization, the cross-links formed during tissue fixation are broken by incubating sections at high temperature with sodium thiocyanate followed by protein digestion by a protease (pepsin). The effect of the temperature and time of incubation in Pretreatment Solution (1N sodium thiocyanate, NaSCN) on tissue morphology and hybridization signal quality were evaluated using the Vysis ALK Break Apart Probe Kit. NSCLC FFPE tissue specimens and cell lines were each processed and evaluated using three pretreatment solution incubation conditions:

- 9 minutes at 77°C
- 12 minutes at 80°C
- 15 minutes at 83°C

All slides were evaluated by two independent readers. All of the slides evaluated resulted in scores with acceptable hybridization quality scores.

7. Enzyme Digestion (Pepsin Concentration and Time) – Enzyme concentration is important for the performance of the assay, since inadequate enzymatic digestion of tissue could lead to weak signal and background autofluorescence, while over-digestion could damage nuclear morphology, and decrease signal intensity. NSCLC FFPE tissue specimens and cell lines were each processed and digested for 20 minutes at various concentrations to determine the optimal digestion times. All slides were evaluated by two independent readers and a pepsin concentration range which demonstrated acceptable hybridization quality was determined.

   Digestion time was evaluated using a specific pepsin concentration (which fell within the acceptable range) at 16, 18, 20, 22, and 24 minutes at 37°C. All slides were evaluated by two independent readers. In this experiment, greater than 85% of evaluations for each time point were necessary to "Pass."

   In addition, the percentage of tissue loss (as assessed by the percent of degraded nuclear morphology) was evaluated in this experiment. If the tissue had greater than 25% degraded nuclear morphology it was considered failing. In this experiment, all conditions tested resulted in less than 10% tissue loss per specimen slide. Three replicates (two from one ALK-negative and one from an ALK-positive) received a minimum quality score of two at the 20 minute time point. This resulted in an overall quality score indicating a failure at this time point; however more than 85% of the evaluations still passed the quality evaluations and resulted in less than 10% degradation in nuclear morphology, therefore it was considered to still pass.

8. Probe and Target Co-Denaturation (Denaturation Study) – Co-denaturation of probe and sample DNA, and hybridization of the probe to its genomic target are carried out simultaneously on the ThermoBrite hybridization platform

upon probe application to the target area of the slide. Denaturation time and temperature for probe and target DNA is critical for subsequent hybridization. NSCLC FFPE tissue specimens were each processed and evaluated using seven denaturation conditions:

- 3 min. at 71°C
- 5 min. at 71°C
- 2 min. at 73°C
- 3 min. at 73°C
- 5 min. at 73°C
- 3 min. at 75°C
- 5 min. at 80°C

All slides were evaluated by two independent readers. A trend towards decreased signal intensity and specificity was observed at 80°C and 75°C x 5 min, and a trend towards decreased signal intensity and higher background was observed at 71°C x 3 min. Therefore, assay limits were found to be minimum of 71°C x 5 min and maximum of 75°C x 3 min. This study was repeated using five denaturation temperatures:

- 5 min. at 71°C
- 2 min. at 73°C
- 3 min. at 73°C
- 5 min. at 73°C
- 3 min. at 75°C

All slides were evaluated by two independent readers. All of the slides evaluated resulted in scores with acceptable hybridization quality scores. The denaturation condition was demonstrated to result in acceptable hybridization quality for all 5 conditions.

9. Hybridization Time and Temperature – Hybridization time and temperature for the FFPE lung tissue digestion were tested with Vysis ALK Break Apart FISH Probe Kit on NSCLC FFPE tissue specimens and cell lines. Each specimen was hybridized at three different temperatures (34°C, 37°C and 40°C) and three time points at each temperature (14 hours, 19 hours and 24 hours) to determine acceptable ranges of conditions for probe hybridization on the Thermobrite Platform. An additional condition (14 hours at 36°C) was tested with the same specimen set to further define the acceptable range of hybridization conditions. Denaturation conditions were held constant at 73°C x 3 min. The slides were evaluated by two different readers, and increments of 0.5 were used to increase the discriminatory power of the quality evaluation. Hybridization for 14 hours at 34°C (low temperature and time) resulted in average overall quality ratings of <3 and was therefore excluded from the acceptable range of the assay.

Table 2. Hybridization Time and Temperature Results.

Temp. (°C)	Time (hrs)	Average Quality Rating (min, max)	No. Passing Results (≥3)	No. Failing Results (<3)
34°	14	2.83 (2.0, 4.0)	8	4
	19	3.00 (2.0, 4.0)	10	2
	24	3.50 (2.0, 4.0)	11	1
36°	14	3.13 (2.0, 4.0)	11	1

Temp. (°C)	Time (hrs)	Average Quality Rating (min, max)	No. Passing Results (≥3)	No. Failing Results (<3)
37°	14	3.17 (2.5, 4.0)	11	1
	19	3.17 (2.5, 4.0)	11	1
	24	3.08 (2.5, 4.0)	10	2
40°	14	3.08 (3.0, 3.5)	12	0
	19	3.04 (2.0, 4.0)	11	1
	24	3.08 (3.0, 3.5)	12	0

An additional hybridization condition, 14 hours at 36°C, was tested with the same specimen set to establish the lower temperature/time bound of the acceptable range. The hybridization condition with the low limit of 14 hours at 36°C and high limit of 24 hours at 40°C were determined to be acceptable.

10. Post-Hybridization Wash – Post-hybridization wash is employed after the completion of hybridization and consists of two washes, a room-temperature wash (to remove coverslips) and a high-temperature wash. In some assay protocols an additional room temperature wash is used. A stringency of the high temperature wash is critical for the removal of the un-specifically bound probe and unbound probe. The factors affecting the stringency of the high-temperature wash are salt concentration, temperature and time. These factors were evaluated in characterization and guard band experiments. The studies were performed in two stages with the hybridization temperature kept constant at 37°C for the entire experiment.

    The first stage used two denaturation conditions and three wash conditions, with four NSCLC FFPE 5 µm tissue specimens (three ALK-negatives and one ALK-positive) and two lots of the ProbeChek ALK Negative Control. Three readers each evaluated six slides yielding 18 results per condition. The slides after pretreatment, hybridization and post-hybridization processing using an internal quality procedures with increments of 0.5, were used to increase discriminatory power of assay quality evaluation. Wash 1 condition remained constant for all conditions at 2X SSC/0.1% NP40.

    An additional room temperature wash following the high temperature 2X SSC/0.1% NP40 wash at 73°C for 2 minutes was determined not to be required for the Vysis ALK Break Apart FISH Probe Kit.

    As post-hybridization wash and denaturation are critical steps affecting probe intensity, specificity and background, the second stage of the experiment evaluated the best condition from the stage one.

    The second stage utilized four NSCLC FFPE tissue specimens (ALK-negative), two ALK-positive cultured cell lines, and one lot of the ProbeChek ALK Positive Control which was evaluated by three independent readers to confirm the condition selected. Additionally, for the slides washed at high temperature in 2X SSC/0.1% NP40, the 0.7X SSC/0.3% NP40 was used as the

room temperature wash. The use of 0.7X SSC/0.3% NP40 as the room temperature wash condition was incorporated into the final protocol. Increments of 0.5 were used to increase discriminatory power of assay quality evaluation. As shown in Table 3, Condition 5 (73°C x 3 min denaturation followed by 74°C, 2 min, 2X SSC/0.1% NP40 wash and 0.7X SSC/0.3% NP40 room temperature wash) was superior to the starting condition in overall quality scores and the percentage individual readings that passed hybridization quality assessment (overall quality rating of ≥3).

Table 3. Denaturation and Wash Condition Optimization Stage 2.

Condition	Denaturation	Wash 1 Condition (ambient)	Wash 2 Condition (high temperature)	Sample type	Avg. Quality Rating (min, max)	% Passing (≥3)
1	71°C x 5 min	2X SSC/ 0.1% NP40	73°C x 2 min in 0.7X SSC/0.3% NP40	FFPE cell line	2.92 (2.00, 4.00)	66.67
5	73°C x 3 min	0.7X SSC/ 0.3% NP40	74°C x 2 min in 2X SSC/0.1% NP40	FFPE cell line	3.25 (2.00, 4.00)	83.33
1	71°C x 5 min	2X SSC/ 0.1% NP40	73°C x 2 min in 0.7X SSC/0.3% NP40	NSCLC Tissue	2.64 (2.00, 3.50)	58.33
5	73°C x 3 min	0.7X SSC/ 0.3% NP40	74°C x 2 min in 2X SSC/0.1% NP40	NSCLC Tissue	3.11 (2.00, 4.00)	86.11

A guard band study was performed to assess the acceptable range surrounding the high temperature wash conditions. A range of wash temperatures (72 – 76°C) and times (1 – 6 min.) with NSCLC FFPE specimens and cell lines were used in the evaluation by three readers. Increments of 0.5 were used to increase discriminatory power of assay quality evaluation. From the data, signal quality decreased at for the 6 minute time point at 74°C and all time points for the 76°C temperature. More than 90% of the evaluations were successful for post-hybridization wash conditions between 72 – 74°C for 1 – 4 minutes.

A final confirmatory experiment at the selected conditions deemed to be optimal, 2 min at 74°C and the temperature range of 73°C – 75°C using NSCLC FFPE tissue specimens and cell lines were used in the evaluation by twp readers. The results confirmed the recommended wash range of 2 minutes at 74±1°C as all slides tested passed hybridization quality assessment by both readers, and all of the overall hybridization quality scores were acceptable.

11. DAPI Staining Time Course –FFPE NSCLC specimens were stained with DAPI/anti-fade solution which was added to the slides (1 at a time), coverslipped, and the time was started. The slides were photographed with the camera attached to a microscope at 1 minute intervals until 10 minutes, then 5 minute intervals to 25 minutes to assess saturation of DAPI staining in a pictorial record. By visual assessment without the aid of photography, 2 minutes post-DAPI staining appeared to be sufficient to visualize tissue morphology and nuclear boundaries. No change in DAPI intensity was

detected after 7 minutes, even when compared to a slide at 25 – 27 minutes post-staining or greater. Morphology could be assessed with ease at 5 – 6 minutes post-DAPI staining. Staining reached saturation at 15 – 20 minutes by visualization of the photographic images. No reduction in intensity after 25 minutes was noted.

12. Slide Stability: Post-Hybridization – Signal stability after hybridization was evaluated since fluorescence signals will fade over time. The FISH hybridization success rate on FFPE lung tumor specimens was evaluated using thirty different FFPE tumor tissue specimens. Each FFPE specimen block was sectioned into four sections, each 5 μm in thickness, and mounted on a glass slide. The specimens were processed and stored at -20°C for 5, 6, and 7 days.

    The FISH success rate on FFPE was evaluated for the dual and single bandpass filter sets. Results are shown below in Tables 4 and 5. Based on the data, the slide stability was demonstrated for a maximum of seven days post-hybridization when stored at -20°C.

Table 4. Post-hybridization storage stability – overall.

Filter Set	# Hybridizations	# Hybridizations with Passing Overall Quality Rating (≥3)	Assay Success Rate (%)	Lower Bound 95% Confidence Limit
Dual bandpass green/orange v2	120	119	99.2	96.1
Single bandpass green	120	119	99.2	96.1
Single bandpass orange	120	118	98.3	94.8

Table 5. Post-hybridization storage stability – by day.

Filter Set	Percentage of Passing Results (%)		
	Day 5 n=72 slides	Day 6 n=28 slides	Day 7 n=20 slides
Dual bandpass green/orange v2	98.6 (71/72)	100 (28/28)	100 (20/20)
Single bandpass green	98.6 (71/72)	100 (28/28)	100 (20/20)
Single bandpass orange	97.2 (70/72)	100 (28/28)	100 (20/20)

Conclusions

The following results were observed to be acceptable:

- Section thickness for 4 μm, 5 μm, and 6μm.
- Slide stability (FFPE tissue section) pre-hybridization for up to 24 months.
- Flotation water bath temperature at 37°C to 50°C during specimen preparation.
- Slide baking 56°C to 68°°C for 2 hours to 24 hours.
- Deparaffinization in Hemo-De for three (3) 5-minute exposures.
- Pretreatment at 75°C to 85°C for 9 min. to 15 min.
- Pepsin digestion time for 16 min. to 24 min.
- Denaturation for 5 min. at 71°C (lower limit) and 3 min. at 75°C (upper limit).
- Hybridization 36°C to 40°°C for 14 to 24 hours.

- Post-Hybridization Wash Solution 2 conditions at 72°C to 76°C for 1 – 4 min.
- DAPI staining for a minimum of 3 min.
- Slide stability post-hybridization for up to 7 days at -20°C (±10°C).

Note: It is recommended to adhere to the time and temperatures indicated in the staining procedure provided in the package insert.

Reproducibility

1. Control Slide Reproducibility – Control slide reproducibility was evaluated using three lots of both the ProbeChek ALK Negative Control Slides and ProbeChek ALK Positive Control Slides. Each lot was run on 5 non-consecutive days over a 23-day time period and evaluated by three readers for a total of 90 data points (3 lots x 5 runs x 3 readers = 45 evaluations per control slide type). For each specimen, the signal patterns of 50 nuclei were evaluated by counting the number of fused signals, single orange signals and single green signals present for each target by each reader. The control slides are described in Tables 6 and 7.

Table 6. ProbeChek ALK Positive Control Slides Descriptive Statistics

Control Slide Lot	N	Reader 1				Reader 2				Reader 3				Total				
		Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	n	Mean	SD	Min	Max
1	5	41.6	9.32	28	54	40.4	5.90	32	48	50.4	21.61	34	76	15	44.1	13.76	28	76
2	5	36.4	5.90	28	44	42.4	2.61	38	44	46.8	23.35	24	74	15	41.9	13.68	24	74
3	5	46.0	9.59	36	56	45.6	4.98	38	50	50.8	21.94	28	74	15	47.5	13.30	28	74
Total	15	41.3	8.80	28	56	42.8	4.89	32	50	49.3	20.74	24	76	45	44.5	13.47	24	76

Mean= mean percentage of cells with ALK rearrangements SD = standard deviation between percentage of cells with ALK rearrangements
Min = minimum percentage of cells with ALK rearrangements Max = maximum percentage of cells with ALK rearrangements

Table 7. ProbeChek ALK Negative Control Slides Descriptive Statistics.

Control Slide Lot	n	Reader 1				Reader 2				Reader 3				Total				
		Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	n	Mean	SD	Min	Max
1	5	4.0	2.83	2	8	1.2	1.10	0	2	1.2	1.79	0	4	15	2.1	2.33	0	8
2	5	3.6	3.29	0	8	1.2	1.10	0	2	1.2	1.10	0	2	15	2.0	2.27	0	8
3	5	2.0	1.41	0	4	1.2	1.79	0	4	1.6	1.67	0	4	15	1.6	1.55	0	4
Total	15	3.2	2.60	0	8	1.2	1.26	0	4	1.3	1.45	0	4	45	1.9	2.04	0	8

Mean= mean percentage of cells with ALK rearrangements SD = standard deviation between percentage of cells with ALK rearrangements
Min = minimum percentage of cells with ALK rearrangements Max = maximum percentage of cells with ALK rearrangements

There was no statistical difference in FISH classification between 3 readers by the Fisher-Freeman-Halton test at the significance level of 0.05 for either the ProbeChek ALK Positive or Negative Control slides as shown in Tables 8 and 9. Therefore, it was demonstrated that ProbeChek ALK Negative Control Slides and ProbeChek ALK Positive Control Slides could be reproducibly classified. All slides in this study were found to be within specifications.

Table 8. Reproducibility of ProbeChek ALK Positive Control Slides-By Reader

Readers	# Observations with the % ALK Rearrangement		Total
	Within Specification* (≥20%)	Outside Specification (<20%)	
1	15	0	15
2	15	0	15
3	15	0	15
Fisher-Freeman-Halton p-value = 1.00			

* Specification refers to the lower limit for the range of ALK rearrangements observed in the positive control cell line.

Table 9. Reproducibility of ProbeChek ALK Negative Control Slides-By Reader

Readers	# of Observations with the % ALK Rearrangement		Total
	Within Specification* (≤8%)	Outside Specification (>8%)	
1	15	0	15
2	15	0	15
3	15	0	15
Fisher-Freeman-Halton p-value = 1.00			

* Specification refers to the upper limit for the range of ALK rearrangements observed in the negative control cell line.

Some variation in the percentage of cells with the ALK rearrangement for within-readers (day-to-day), between-readers, and between-lots is evident. However, the variability did not affect the slide classification as demonstrated by the primary analysis of reader reproducibility and a secondary analysis of reproducibility by lot. All positive control slides were correctly classified.

2. Tissue Reproducibility – Tissue reproducibility was designed to evaluate the reader component of reproducibility by evaluation of between-reader and within-reader using FFPE lung tumor sections. This study was conducted using six serial sections (5 μm) prepared from twenty NSCLC FFPE specimen blocks. The panel included three ALK-positive specimens with >50% of the cells with ALK rearrangement, three specimens falling within the range of 10% to 50% cells with the ALK rearrangement and fourteen ALK-negative specimens with <10% cells with the ALK rearrangement. Three readers participated in the three study arms: between reader (Arm 1A), within reader (Arm 2), and between slide reproducibility (Arm 1B). The Vysis ALK Break Apart FISH Probe Kit was shown to be reproducible based upon the between-reader and between-slide analyses resulting in a Fisher-Freeman-Halton p-value of 1.00.

   a. Between Reader Reproducibility (Arm 1A) – Three readers evaluated the same set of slides prepared from 20 patient specimens resulting in a total of 60 evaluations. Each evaluation involved two enumerations of the same randomized, blinded slide by each reader for the purpose of determining the final classification (e.g., positive or negative) via averaging of the two enumerations. The final classification (negative or positive) was used for primary data analysis using kappa statistic.

This statistic measures the degree to which interpretation variability arises from differences among panel members relative to differences among readers interpreting the same panel member. For each panel member, the proportion of all possible pairings on which readers agree is calculated. An overall mean agreement of all panel members is determined and a correction for chance agreement is made to then determine a final kappa coefficient (K) and standard error (SE). Based on literature, an interpretation for the strength of agreement was assigned for various ranges of kappa. To show statistical significance, a test for the null hypothesis that the kappa coefficient is not different from zero (*i.e.*, no better than chance), the generalized kappa statistic is compared with the standard normal distribution by creating a Z-Score (K/SE) and compared to 1.96 (alpha level of 0.05) for significance. An analysis of between reader reproducibility, based on the final FISH classification, with and without the equivocal calls is shown in Table 10 from a secondary analysis. This analysis used the initial classification, the first reading out of two for each slide by each reader, resulting in a positive, negative or equivocal classification.

Table 10. Between-reader reproducibility – Classification

Not including "equivocal" results	Kappa Statistic: 1.00 (0.66, 1.00), SE = 0.171 Strength: Almost Perfect, z-score: 5.84
Including "equivocal" results	Kappa Statistic: 0.72 (0.41, 1.00), SE = 0.162 Strength: Substantial, z-score: 4.46

The Fisher-Freeman-Halton test was also used for reproducibility analyses of the data. The null hypothesis for this test showed no association between two variables as displayed in an r x c contingency table, where r is the number of rows and c is the number of columns. The alternative hypothesis (if the null is rejected with a significant p-value) shows that there is some statistically significant association, *i.e.*, some effect, of the row variable on the column variable. The test was performed at a 0.05 level of significance. Table 11 shows the reproducibility between readers with the inclusion of the equivocal zone results. When the equivocal results are resolved into positive or negative results the Fisher-Freeman-Halton p-value was 1.00.

Table 11. Between-Reader Reproducibility – Fisher-Freeman-Halton Analysis

	FISH Classification Number of Panel Members			
Reader	Negative	Equivocal	Positive	Total
1	13	3	4	20
2	14	2	4	20
3	14	4	2	20

Fisher-Freeman-Halton p-value: 0.85

b. Between-Slide Reproducibility (Arm 1B) – For this study, one reader was selected at random out of the three readers in the comparison between

readers (Arm 1A) and evaluated two additional serial sections from each of the patient specimens evaluated in Arm 1A (20 patient specimens, 2 slides each). The two additional evaluations were combined with the evaluation from the same reader for those patient specimens in Arm 1A. The resulting three evaluations from the same reader for each patient specimen yielded a total of sixty evaluations. Each evaluation involved two enumerations of the same randomized, blinded slide by the same reader for the purpose of determining the final classification (e.g. positive or negative) via averaging of the two enumerations. Table 12 shows the reproducibility between slides with the inclusion of the equivocal zone results.

The final classification (negative or positive) was used for primary data analysis. A secondary analysis was conducted using the initial classification, the first reading out of two for each slide by each reader, resulting in a positive, negative or equivocal classification. When the equivocal results are resolved into positive or negative results the Fisher-Freeman-Halton p-value was 1.00.

Table 12. Between-Slide Reproducibility – Fisher-Freeman-Halton Analysis.

	FISH Classification – Number of Panel Members			
Slide	Negative	Equivocal	Positive	Total
1	14	2	4	20
2	15	4	1	20
3	14	4	2	20

Fisher-Freeman-Halton p-value: 0.60

c. Within-Reader Reproducibility (Arm 2) – This study assessed reproducibility of the evaluation and classification of the same slide hybridized with the Vysis ALK Break Apart FISH Probe Kit over successive reading by the same reader. Three slides from twenty patient specimens were hybridized with the Vysis ALK Break Apart FISH Probe Kit and each of the three readers evaluated one slide exclusively three times in a random, blinded setup. To determine the final classification of samples (e.g., positive or negative), the following pairs of enumerated results were averaged together: 1) readings A and B, 2) readings B and C, and 3) readings A and C. This resulted in three evaluation results per slide for a total 180 evaluations. The final classification (negative or positive) was used for primary data analysis. A secondary analysis was conducted using the initial classification, the first reading out of two for each slide by each reader, resulting in a positive, negative or equivocal classification.

For each arm (Arm 1A, Arm 1B, Arm 2), two-by-two contingency tables were constructed with the Final FISH classification (Positive, Negative) as the row, the agreement status (Agree, Disagree) as the columns, and the cells representing the number of results for each specimen which are found to agree or disagree. Positive Percent Agreement (PPA) and

Negative Percent Agreement (NPA) were calculated with corresponding 95% confidence intervals. With the exception of the PPA and OA for Arm 1B (Between-slides/within one reader), all other comparisons had an NPA of 100% (14/14) ($CI_{95\%}$ 78.47, 100) and PPA was100% (6/6) ($CI_{95\%}$ 60.97, 100) and an overall percent agreement (OA) of 100% (20/20) ($CI_{95\%}$ 83.89, 100). The PPA and OA for Arm 1B was 83.33% (5/6) ($CI_{95\%}$ 43.65, 96.99) and the OA was 95% (19/20) ($CI_{95\%}$ 76.39, 99.11).

External Reproducibility

Reproducibility of the Vysis ALK Break Apart FISH Probe Kit was evaluated at three external laboratories by testing a coded, randomized 12-member specimen panel (6 unique specimens, 2 slides each) that consisted of four unique ALK-positive with varying levels of positivity (Panel Member 1, 2, 3, and 6) and two unique ALK-negative NSCLC FFPE tissue specimens (Panel Member 4 and 5).

Three lots of the Vysis ALK Break Apart FISH Probe Kit reagents were used in the evaluation. A run consisted of one replicate each of a ProbeChek ALK Negative Control slide, a ProbeChek ALK Positive Control slide and each panel member. Each of the three clinical sites tested the Reproducibility Panel using two of the three clinical lots. Lot 1 was tested at sites 1 and 3; Lot 2 at sites 1 and 2; and Lot 3 at sites 2 and 3. Each of the two technologists at each of the three testing sites enumerated the 6 study specimens and control slides once a day, for 5 non-consecutive days, per reagent lot over a period of 20 days.

Each site evaluated 120 specimen slides for a total of 360. This resulted in 240 enumerations at each site for a minimum of 720 enumerations. Each site evaluated 40 controls slides (20 positive and 20 negative slides) for a total of 120. This resulted in 80 enumerations at each site for a minimum of 240 enumerations. For each panel member and control slides, the signal patterns of 50 nuclei were enumerated by two readers. An analysis of the data in Table 13 resulted in an overall kappa coefficient of 0.92 (95% CI 0.85 – 0.98) and standard error of 0.034. The Z-Score of 27.08, which is greater than 1.96, showed the kappa coefficient is significantly different from zero at a 0.05 level of significance. The kappa coefficient demonstrated the reproducibility for each site, ranging from 0.83 – 0.96, and for each lot, ranging from 0.86 – 0.96. The results are found in Tables 14 and 15, respectively.

Table 13. Final FISH Classification – Kappa Analysis for Overall Reproducibility.

Panel Member	Number of Slides Across Sites/Lot/Runs/Readers		
	Negative	Positive	Total
1	1	59	60
2	0	60	60
3	2	58	60
4	60	0	60
5	60	0	60
6	4	56	60

Table 14. Final FISH Classification - Kappa Analysis, Reproducibility by Site

Site	Panel Member	Final FISH Classification No. of Slides Across Sites/Lot/Runs/Readers		Kappa Statistics				
		Negative	Positive	Kappa	$CI_{95\%}$	Strength	Standard Error	Z-score
1	1	0	20	0.96	0.83, 1.00	Almost Perfect	0.068	14.21
	2	0	20					
	3	0	20					
	4	20	0					
	5	20	0					
	6	1	19					
2	1	0	20	0.96	0.83, 1.00	Almost Perfect	0.068	14.21
	2	0	20					
	3	0	20					
	4	20	0					
	5	20	0					
	6	1	19					
3	1	1	19	0.83	0.72, 0.94	Almost Perfect	0.056	14.90
	2	0	20					
	3	2	18					
	4	20	0					
	5	20	0					
	6	2	18					

Table 15. Final FISH Classification - Kappa Analysis, Reproducibility by Lot

Lot	Panel Member	Final FISH Classification No. of Slides Across Sites/Lot/Runs/Readers		Kappa Statistics				
		Negative	Positive	Kappa	$CI_{95\%}$	Strength	Standard Error	Z-score
1	1	0	20	0.86	0.75, 0.98	Almost Perfect	0.059	14.75
	2	0	20					
	3	0	20					
	4	20	0					
	5	20	0					
	6	2	18					
2	1	0	20	0.96	0.83, 1.00	Almost Perfect	0.068	14.21
	2	0	20					
	3	0	20					
	4	20	0					
	5	20	0					
	6	1	19					
3	1	1	19	0.93	0.80, 1.00	Almost Perfect	0.065	14.34
	2	0	20					
	3	0	20					
	4	20	0					
	5	20	0					
	6	1	19					

There was no significant association between the sites for the overall analysis (p-value of 0.8354) and by panel member (p-value ranged from 0.3220 to 1.00) by Fisher-Freeman-Halton analysis on the final FISH classification. For the analyses on the individual FISH classifications, there was a significant association between the sites for the overall analysis (p-value of <0.0001) and for the positive panel members 1, 2, 3, and 6 (all p- values <0.0001) and no significant association for the negative panel members 4 and 5 (both p-values of 1.00).

The results for final FISH classification and individual FISH classification overall and individual FISH classification for each panel member are shown in Table 16. There was a statistically significant difference between sites in terms of reproducibility based on the raw results before any resolution of equivocal results. When equivocal results are resolved, the p-value of the Fisher-Freeman-Halton statistic changes to 0.8354, which indicates that there is not enough evidence to conclude that there are differences between sites.

Table 16. Between site results for external reproducibility study.

Panel Member	Site	Neg.	Equivocal	Positive	Total
All	Between Site Overall - Final FISH Classification Number of Slides Across Lots/Runs/Readers				
	1	41		79	120
	2	41		79	120
	3	45		75	120
	Fisher-Freeman-Halton p-value: 0.8354				
All	Between Site Overall - Individual FISH Classification Number of Slides Across Lots/Runs/Readers/Enumerations				
	1	80	94	66	240
	2	83	1	156	240
	3	87	83	70	240
	Fisher-Freeman-Halton p-value: <0.0001				
1	Individual FISH Classification Number of Slides Across Lots/Runs/Readers/Enumerations				
	1	0	30	10	40
	2	0	1	39	40
	3	2	33	5	40
	Fisher-Freeman-Halton p-value: <0.0001				
2	Individual FISH Classification Number of Slides Across Lots/Runs/Readers/Enumerations				
	1	0	18	22	40
	2	0	0	40	40
	3	0	2	38	40
	Fisher-Freeman-Halton p-value: <0.0001				
3	Individual FISH Classification Number of Slides Across Lots/Runs/Readers/Enumerations				
	1	0	12	28	40
	2	0	0	40	40
	3	3	26	11	40
	Fisher-Freeman-Halton p-value: <0.0001				

Panel Member	Site	Neg.	Equivocal	Positive	Total
4	Individual FISH Classification Number of Slides Across Lots/Runs/Readers/Enumerations				
	1	40	0	0	40
	2	40	0	0	40
	3	40	0	0	40
	Fisher-Freeman-Halton p-value: 1.00				
5	Individual FISH Classification Number of Slides Across Lots/Runs/Readers/Enumerations				
	1	40	0	0	40
	2	40	0	0	40
	3	39	1	0	40
	Fisher-Freeman-Halton p-value: 1.00				
6	Individual FISH Classification Number of Slides Across Lots/Runs/Readers/Enumerations				
	1	0	34	6	40
	2	3	0	37	40
	3	3	21	16	40
	Fisher-Freeman-Halton p-value: <0.0001				

A standard agreement analysis was provided comparing sites and demonstrated good agreement between sites.

Table 17. Agreement Between Site A and Site B, Pair-wise

Panel	Site A	Site B	N	Called				% Agreement		
				Pos by both readers	Neg by R1 & Pos by R2	Pos by R1 & Neg by R2	Neg by both readers	PPA	NPA	Overall
All	1	2	120	79	0	0	41	100.00	100.00	100.00
	1	3	120	75	0	4	41	94.94	100.00	96.67
	2	1	120	79	0	0	41	100.00	100.00	100.00
	2	3	120	75	0	4	41	94.94	100.00	96.67
	3	1	120	75	4	0	41	100.00	91.11	96.67
	3	2	120	75	4	0	41	100.00	91.11	96.67

R1 = Reader 1; R2 = Reader 2

Between-Lot Reproducibility – For the analyses on the final FISH classification, there was no significant association between the lots for the overall analysis (p-value of 0.9418) and by panel member (p-value ranged from 0.3220 to 1.00) by Fisher-Freeman-Halton (Table 18). A pair-wise agreement analysis was also provided (Table 19) which indicated good agreement between lots.

Table 18. Between-lot results for external reproducibility study.

Panel Member	Lot	Neg.	Equivocal	Positive	Total
All	**Between Lot Overall - Final FISH Classification Number of Slides Across Lots/Runs/Readers**				
	1	44		76	120
	2	41		79	120
	3	42		78	120

Panel Member	Lot	Neg.	Equivocal	Positive	Total
	Fisher-Freeman-Halton p-value: 0.9418				
All	**Probe Lot Overall - Individual FISH Classification Number of Slides Across Lots/Runs/Readers/Enumerations**				
	1	85	88	67	240
	2	82	47	111	240
	3	83	43	114	240
	Fisher-Freeman-Halton p-value: <0.0001				
1	**Probe Lot - Individual FISH Classification Number of Slides Across Lots/Runs/Readers/Enumerations**				
	1	1	32	7	40
	2	0	15	25	40
	3	1	17	22	40
	Fisher-Freeman-Halton p-value: <0.0001				
2	1	0	9	31	40
	2	0	10	30	40
	3	0	1	39	40
	Fisher-Freeman-Halton p-value: 0.0070				
3	1	3	21	16	40
	2	0	6	34	40
	3	0	11	29	40
	Fisher-Freeman-Halton p-value: 0.0001				
4	1	40	0	0	40
	2	40	0	0	40
	3	40	0	0	40
	Fisher-Freeman-Halton p-value: 1.00				
5	1	39	1	0	40
	2	40	0	0	40
	3	40	0	0	40
	Fisher-Freeman-Halton p-value: 1.00				
6	1	2	25	13	40
	2	2	16	22	40
	3	2	14	24	40
	Fisher-Freeman-Halton p-value: 0.0949				

Table 19. Agreement Between Lot A and Lot B, Pair-wise

Panel	Lot A (LA)	Lot B (LB)	N	Called				% Agreement		
				Pos by both Lots	Neg by LA & Pos by LB	Pos by LA & Neg by LB	Neg by both Lots	PPA	NPA	Overall
All	2	3	120	78	0	1	41	98.73	100.00	99.17
	2	1	120	76	0	3	41	96.20	100.00	97.50
	3	2	120	78	1	0	41	100.00	97.62	99.17
	3	1	120	76	0	2	42	97.44	100.00	98.33
	1	2	120	76	3	0	41	100.00	93.18	97.50
	1	3	120	76	2	0	42	100.00	95.45	98.33

Between-Reader Results – A pair-wise between-reader agreement analysis, stratified by site, was performed for is shown in Table 20.

Table 20. Agreement Between Reader 1 and Reader 2 by site

Panel	Testing Site	N	Called				% Agreement		
			Pos by both readers	Neg by R1 & Pos by R2	Pos by R1 & Neg by R2	Neg by both readers	NPA	PPA	Overall
All	1	120	79	0	0	41	100.00	100.00	100.00
	2	120	77	1	2	40	97.56	97.47	97.50
	3	120	69	0	10	41	100.00	87.34	91.67
	ALL	360	225	1	12	122	99.19	94.94	96.39

R1 = Reader 1; R2 = Reader 2

An analysis between readings for each panel member by reading the results over 2 lots, 5 runs, and 2 slides (20 readings) using the Gini Index is shown in Table 21. The Gini Index is a measure of the variability of repeated readings and is the probability that a pair of randomly selected readings will fall into different categories.

Table 21. Gini Index analysis.

Panel Member	Total (n)	Gini Index		
		Minimum	Median	Maximum
1	6	0.00	0.25	0.42
2	6	0.00	0.09	0.50
3	6	0.00	0.37	0.56
4	6	0.00	0.00	0.00
5	6	0.00	0.00	0.10
6	6	0.10	0.26	0.58

The percent agreement across all readers at all of the sites with the expected results is shown in Table 22 below.

Table 22. Percent agreement between all readers with expected results.

		Expected Results		
		Positive	Negative	Total
Observed	Positive	463	0	463
	Negative	17	240	257
	Total	480	240	720

PPA: 463/480 = 96.46% (94.40, 97.78)
NPA: 240/240 = 100.00% (98.42, 100.00)
OA: 703/720 = 97.64% (96.25, 98.52)

Between-Reading – The end point for this analysis was the individual FISH classifications (positive/equivocal/negative) as determined by the scoring algorithm indicated in the package insert. A comparison of all of the readings of all the sections

of a tissue specimen for each panel member by reader over 2 lots, 5 runs, and 2 slides (20 readings) was calculated for inter-reader variability using the Gini Index. This analysis combines all panel members, while stratifying by site and reader.

Precision (Repeatability):

A precision analysis, the variance (standard deviation) and percent coefficient of variation (%CV) were estimated for within-reader component, between-reader component, between-run component, and between-site component for each lot and panel member. All the effects were considered as random for the analysis. The total lot variability was defined as the accumulation of within-reader component, between-reader component, between-run component, and between-site component. Overall precision by site, panel member, and by kit lot are provided in Tables 23-25. Some very high %CV values were noted; however this observation is not unexpected with FISH, particularly for specimens with low counts. In general, a higher degree of variance is to be expected in negative or low positive specimens due to the fewer signals.

Table 23. Vysis ALK Break Apart FISH Probe Kit overall precision analysis by panel member.

Panel Member	n	Mean % Cells with ALK Rearrangement	Within-Reader Component		Between-Reader Component		Between-Run (Day) Component		Between-Lot Component		Between-Site Component		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	120	47.6	8.86	18.60	6.17	12.96	2.76	5.78	0.00	0.00	15.17	31.84	18.82	39.51
2	120	61.5	7.91	12.87	6.80	11.06	0.00	0.00	0.00	0.00	10.00	16.27	14.46	23.51
3	120	53.2	7.90	14.87	9.28	17.46	0.00	0.00	5.73	10.77	14.91	28.05	20.10	37.80
4	120	1.3	1.52	118.17	0.84	65.19	0.00	0.00	0.00	0.00	0.79	61.93	1.91	148.50
5	120	2.1	2.24	106.48	0.41	19.44	1.06	50.69	0.00	0.00	0.79	37.52	2.63	125.27
6	120	46.2	15.15	32.76	0.00	0.00	9.07	19.62	1.81	3.92	10.24	22.14	20.49	44.32

Table 24. Vysis ALK Break Apart FISH Probe Kit precision analysis by site.

Site	Panel Member	n	Mean % Cells with ALK Rearrangement	Within-Reader Component		Between-Reader Component		Between-Run (Day) Component		Between-Lot Component		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	1	40	47.5	6.68	14.06	0.00	0.00	2.41	5.06	0.00	0.00	7.10	14.94
	2	40	50.3	6.67	13.28	3.81	7.58	2.85	5.67	0.00	0.00	8.19	16.30
	3	40	54.0	6.04	11.20	1.67	3.10	1.97	3.65	0.00	0.00	6.57	12.18
	4	40	1.6	1.48	92.70	0.00	0.00	0.74	46.35	0.00	0.00	1.66	103.64
	5	40	2.2	1.97	91.85	0.32	14.71	0.91	42.25	0.00	0.00	2.20	102.17
	6	40	38.7	10.39	26.85	0.00	0.00	10.28	26.57	0.00	0.00	14.62	37.77
2	1	40	63.0	4.97	7.90	13.08	20.79	0.00	0.00	0.11	0.18	14.00	22.23
	2	40	69.5	8.31	11.95	11.05	15.91	0.00	0.00	0.00	0.00	13.83	19.90
	3	40	68.4	5.67	8.29	14.69	21.49	0.00	0.00	3.00	4.39	16.02	23.44
	4	40	1.9	2.00	105.26	1.58	83.22	0.00	0.00	0.00	0.00	2.55	134.18
	5	40	2.9	2.61	89.92	0.00	0.00	1.35	46.58	0.00	0.00	2.94	101.27
	6	40	58.8	14.65	24.94	8.29	14.12	5.37	9.13	3.33	5.66	17.98	30.61
3	1	40	32.5	12.89	39.72	0.00	0.00	9.37	28.88	0.00	0.00	15.93	49.10
	2	40	64.7	8.63	13.33	1.41	2.19	4.79	7.40	0.00	0.00	9.97	15.40

Site	Panel Member	n	Mean % Cells with ALK Rearrangement	Within-Reader Component		Between-Reader Component		Between-Run (Day) Component		Between-Lot Component		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
	3	40	37.2	10.90	29.30	6.32	17.00	6.04	16.22	9.61	25.83	16.96	45.58
	4	40	0.4	0.84	239.05	0.00	0.00	0.34	95.83	0.00	0.00	0.90	257.54
	5	40	1.3	2.07	165.89	0.71	56.57	0.87	69.28	0.00	0.00	2.36	188.47
	6	40	41.3	19.12	46.35	0.00	0.00	10.60	25.71	3.02	7.31	22.07	53.50

Table 25. Vysis ALK Break Apart FISH Probe Kit precision analysis by lot.

Lot	Panel Member	n	Mean % Cells with ALK Rearrangement	Within-Reader Component		Between-Reader Component		Between-Run (Day) Component		Between-Site Component		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	1	40	40.8	11.23	27.53	0.00	0.00	5.58	13.67	8.16	20.01	14.97	36.68
	2	40	57.5	7.94	13.83	4.37	7.61	0.00	0.00	9.04	15.73	12.80	22.28
	3	40	41.9	8.38	20.00	7.65	18.25	0.00	0.00	16.96	40.47	20.40	48.69
	4	40	0.8	1.10	136.93	0.00	0.00	0.64	80.28	0.63	78.81	1.42	177.22
	5	40	1.8	1.84	102.44	1.18	65.73	1.13	62.73	0.00	0.00	2.46	136.93
	6	40	40.9	15.27	37.37	0.00	0.00	12.07	29.54	3.68	9.00	19.81	48.48
2	1	40	54.8	4.56	8.32	8.64	15.77	0.00	0.00	9.29	16.96	13.49	24.61
	2	40	59.5	7.10	11.93	5.49	9.22	4.69	7.88	13.64	22.93	16.99	28.55
	3	40	59.8	7.13	11.92	5.81	9.71	0.00	0.00	8.22	13.74	12.33	20.62
	4	40	2.0	1.90	94.87	0.84	41.83	0.42	20.92	0.00	0.00	2.12	105.77
	5	40	2.7	2.47	93.20	0.00	0.00	1.11	41.77	0.61	23.11	2.77	104.71
	6	40	48.0	13.56	28.28	4.74	9.89	8.30	17.31	7.95	16.58	18.40	38.37
3	1	40	47.3	9.40	19.88	9.34	19.74	4.13	8.73	23.84	50.40	27.58	58.32
	2	40	67.5	8.63	12.78	9.46	14.02	0.00	0.00	0.00	0.00	12.80	18.97
	3	40	57.8	8.15	14.10	12.90	22.31	0.00	0.00	18.18	31.46	23.74	41.07
	4	40	1.1	1.45	138.01	1.34	127.78	0.00	0.00	0.88	83.57	2.16	205.81
	5	40	1.9	2.35	126.77	0.00	0.00	0.95	51.28	0.63	34.19	2.61	140.96
	6	40	49.9	16.47	33.00	0.00	0.00	5.69	11.41	17.86	35.79	24.95	50.00

Conclusions

Variability between readers was observed within and between sites, particularly prior to the resolution of the equivocal cases. Based on the Gini Index results, readers at the same site seemed to perform more similarly than between sites. Site 3 seemed to show the greatest differences between readers which could not be fully accounted for and specimens (panels) one and three showed more variability in results than the other four specimens from the external reproducibility study. This could result from the observation that if the readers do not enumerate the same areas and/or cells, variability in results is expected. Overall the reproducibility between readers, sites, tissue specimens, and the ProbeChek ALK Control slides was determined to be good.

B. <u>Animal Studies</u>

None

C. Additional Studies

Assay Run Validity Rate – Failure Rate

The assay run informative rate, defined as the number of passing slides divided by the number of total slides multiplied by 100, was calculated from the reproducibility studies. Based on the results of the ProbeChek ALK Negative and Positive Control slides, the assay run failure rate was calculated to be 3.08% and the passing rate was calculated to be 96.92%. In a total of four invalid assay runs, both the ProbeChek ALK Negative and Positive Control slides were invalid resulting in a total frequency of 8 failures.

Table 26. ProbeChek ALK Control Slides - Pass and Fail rate.

ProbeChek ALK Control slides	Disposition	Frequency	Rate (%)
Positive and Negative slides	Fail	8	3.08
	Pass	252	96.92
Positive slides	Fail	4	3.08
	Pass	126	96.92
Negative slides	Fail	4	3.08
	Pass	126	96.92

Rates of Information and Uninformative Slides

The rates of informative (successful hybridization) and uninformative (failed hybridization) slides were calculated from the reproducibility studies which used primarily non-clinical trial specimens. The rate of uninformative slides was defined as the number of uninformative slides divided by the number of total slides multiplied by 100. The rate of informative slides was calculated to be 95.24% and the rate of uninformative slides was calculated to be 4.76%.

Table 27. External reproducibility study uninformative slide rate.

Disposition	Frequency	Rate (%)
Informative	720	95.24
Uninformative	36	4.76
Total	756	100.00

In order to collect the planned sample size of 390 samples for the Concordance study (discussed in Sect. III and not used to support the PMA), Abbott Molecular reviewed 425 slides from the 1005 trial. The uninformative rate of the Vysis ALK Break Apart FISH Probe Kit for this subset of samples was 8.47%. The results are shown in Table 28.

Table 28. Uninformative rate with a subset of 1005 clinical trial specimens.

Disposition	Frequency	Rate (%)
Informative	389*	91.53
Uninformative	36	8.47
Total	425	100.00

* One slide was mistakenly included in the randomization as a negative specimen however the actual result was uninformative.

Some observed causes for uninformative results are listed below:

- Control slide(s) uninformative or failed
- Less than 50 nuclei are evaluable
- Fluorescent haze/glow covering nuclei
- No FISH signals
- Weak FISH signals
- Debris, bacteria, non-specific signals or high background
- Borders of nuclei are overlapping or not distinguishable
- Poor nuclear integrity
- Wrong specimen type

The uninformative rate with the clinical specimens was nearly twice that as seen with the reproducibility study specimens. The precise reason for this difference is not clear as the participating sites from the external reproducibility study are three of the four clinical trial testing sites and the only difference are the specimens which were used in the two studies. If the cause for the higher uninformative rate is due to differences in specimens, then the rate seen with the clinical trial specimens may be more representative.

Vysis ALK Break Apart FISH Probe Kit Shelf life and Stability

Real-time kit stability was assessed under the intended storage conditions (ISC), inverted storage (INVERT), and combined transport and temperature extremes conditions (TTE) for the Vysis ALK Break Apart FISH Probe Kit and DAPI I Counterstain to provide evidence of ruggedness to various stresses for the material.

The expiration date assigned for the kit is based on the expiration data of the shortest dated component. Stability was measured at 0, 3, 6, 9, and 12 months for ISC conditions and 0, 6, and 12 months for TTE/INVERT conditions. The zero (0) month time point for ISC storage condition served as the baseline for both the ISC and TTE/INVERT conditions.

Three lots of the Vysis ALK Break Apart FISH Probe Kits were stored upright at -20°C while protected from light for the duration of the stability study. To evaluate temperature and transport extremes and inverted storage (TTE/INVERT), one lot of the Vysis ALK Break Apart FISH Probe Kit was removed from storage and subject to the conditions below:

- Removed from -20°C freezer
- 1 cycle of dry ice for 48 hours ±2 hours
- 1 cycle of 25°C for 72 hours ±2 hours
- 1 cycle of 40°C for 72 hours ±2 hours
- 1 cycle of –20°C for 24 hours ±2 hours
- 3 freeze/thaw cycles**
- Return to ISC
- Store inverted (INVERT)

For the freeze/thaw cycles, the kits for TTE/INVERT testing were removed from -20°C freezer and left at room temperature for ≤ 8 hours at room temperature until thawed, protected from the light, then returned to -20°C for ≥ 16 hours. 3 cycles of freeze/thaw were performed for each TTE/INVERT simulation.

At the time of approval the Vysis ALK Break Apart FISH Probe Kit has been tested at the 0 (Baseline), 3, 6, 9, and 12 months and expiration dating is valid for 12 months from the date of manufacture. Stability studies are ongoing to support 24 months.

ProbeChek ALK Negative and Positive Control Shelf life Stability
Real-time studies to establish shelf life integrity and expiration dating for the ProbeChek ALK Negative and Positive Control slides will be conducted in accordance with the ISC and TTE testing schedule described above. Three lots each of the control slides were stored upright at 15-30°C (ISC) for the duration of the stability study and one lot of each control slide tested according to the following parameters:

- Removed from ISC
- 1 cycle of –20°C for 72 hours ±2 hours
- Return to ISC for 24 hours
- 1 cycle of 45°C for 72 hours ±2 hours
- Return to ISC

Data was provided for only the baseline time point. Because the ProbeChek ALK Control slide lots ranged from 4-8 months from the original manufacturing date at baseline testing and data from an additional functional testing study will allow for initial expiration dating for six months. A real-time stability protocol was submitted and approved to allow for the establishment the stability dating to 24 months from the date of manufacture.

Paraffin Pretreatment IV & Post-Hybridization Wash Buffer Kit Shelf life Stability
Real-time studies to establish shelf life integrity and expiration dating for the Vysis Paraffin Pretreatment IV & Post-Hybridization Wash Buffer Kit will be conducted under four conditions. The ISC condition (2-8°C, upright) and an alternative ISC condition [in accordance with the conditions recommend to the customer after receipt, where the pepsin is separately stored at -20°C (±10°C)] will be tested on 3 separate kit lots and tested at 0 month (baseline) and 6, 13 18, 24 and 25 (final time point) month time points. TTE/INVERT (combined) and 15-30°C (upright) testing will be conducted on a single kit lots at 0 (baseline), 13, 18 and 25 month time points. The TTE/INVERT condition according to the following parameters:

- Removed from 2-8°C storage,
- 1 cycle of 15-30°C for 168 hours ±2 hours
- 1 cycle of 25°C for 72 hours ±2 hours
- 1 cycle of 40°C for 24 hours ±2 hours
- 1 cycle of –20°C for 24 hours ±2 hours

- Return to 2-8°C
- Store inverted (INVERT)

Data was provided for only the baseline time point. Because one kit was at 1.5 months after kit manufacture at baseline testing and data from an additional functional testing study will allow for initial expiration dating for six months. However a real-time stability protocol was submitted and approved to allow for the establishment the stability dating to 24 months from the date of manufacture.

X. SUMMARY OF PRIMARY CLINICAL STUDY

Study A8081005 (1005), conducted by Pfizer, Inc. (NCT00932451) in collaboration with Abbott Molecular, Inc. was used to established a reasonable assurance of safety and effectiveness of the Vysis ALK Break Apart FISH Probe Kit with regard to identifying ALK gene rearrangements in the tumors of patients with previously treated, advanced (locally or metastatic) non-small cell lung cancer, for whom Xalkori® (crizotinib) is being considered. Data from Study 1005 required that an ALK translocation must have been documented using the Vysis ALK Break Apart FISH Assay for enrollment. It was submitted to CDER as part of NDA 202570 and used to support the accelerated approval of Xalkori for anaplastic lymphoma kinase (ALK)-positive advanced non-small cell lung cancer.

Data from clinical study 1005 were the basis for the PMA approval decision. A summary of the clinical study is presented below.

A. Study Design

The 1005 study is an ongoing Phase II, multicenter, multinational, open label, single-arm study evaluating the safety and efficacy of crizotinib in patients with advanced NSCLC harboring a translocation or inversion involving the anaplastic lymphoma kinase gene locus. The study was initiated in January 2010 and the original NDA submission included clinical data through the target last visit date of September 15, 2010 with a database snapshot date of October 29, 2010. A 60-day safety update, with a cutoff date of 01 February 2011 from a snapshot made on 17 March 2011 of the active clinical database was provided and included clinical data from 136 patients in this study. Table 29 provides the study design criteria for Study 1005.

The study was conducted at 57 sites, including Australia, Canada, France, Germany, Hong Kong, Italy, Japan, Republic of Korea, Poland, Russian Federation, Spain, and 27 centers in the United States. Four central laboratories (LabCorp of America Holding; Research Triangle Park, NC, US; US Labs, Irvine, CA, US; Peter MacCallum Cancer Center, Melbourne, Australia; Antwerp University Hospital, Edegem, Belgium) served as regional testing sites and performed ALK testing on tumor specimens under IDE #G090233 using the Vysis ALK Break Apart FISH Probe Kitunder the direction of Abbott Molecular, Inc.

Table 29. Study Design – Study 1005

Eligibility Criteria	1. Locally advanced or metastatic NSCLC containing an ALK translocation or inversion event by the Vysis ALK Break Apart Assay 2. Progressive disease on the control arm of a randomized study of crizotinib OR ineligible for a randomized study 3. Measurable disease 4. Prior chemotherapy for NSCLC; erlotinib, gefitinib not considered prior therapy 5. Performance status 0-3 6. No grade 1-2 cardiac arrhythmia, uncontrolled atrial fibrillation, QTc > 470 msec
Treatment	Crizotinib 250 mg orally, twice a day; tablets. Cycles defined as 21 d.
Safety Monitoring	NCI CTCAE v 4.0 CBC, Chemistries: baseline, C1D15, every cycle EKG: baseline, then C1D1, C2D1 prior to dose and at 2 h, 6 h post dose Ophthalmology evaluation: baseline, w/ visual disorder; visual symptom assessment questionnaire
Endpoint Evaluation	Imaging: baseline, every 8 wks until INV- determined progression or start of a new cancer therapy
Primary Endpoint	Response rate by Investigator using RECIST v 1.0 with 95% confidence intervals
Secondary Endpoints	Duration of response; Time to response; Disease control rate at 8 and 16 wks; Progression free survival; 6 mo PFS; Overall Survival; 6 and 12 mo OS
Statistical Plan	The primary analysis population includes patients who: 1) received crizotinib, 2) had an adequate baseline tumor assessment, and 3) had $\geq$ 1 adequate post-baseline tumor assessment. Patients who died or withdraw due to disease progression are included in this population. The post-baseline assessment must be $\geq$ 6 wks from the 1st dose. The primary endpoint will be confirmed by an assessment of response by an Independent Radiology Committee. The primary analysis population is the same.

Abbreviations: NCI CTCAE = NCI Common Terminology Criteria for Adverse Events; C#D# = cycle # day # (*e.g.*, C1D1 = Cycle 1 Day 1)

1. Clinical Inclusion and Exclusion Criteria

Patients were permitted to enroll in the 1005 trial if they met the following criteria: histologically or cytologically proven diagnosis of advanced NSCLC; positive for an ALK translocation or inversions as determined by an ALK break apart FISH assay and defined by an increase in the distance of 5' and 3' ALK probes or the loss of the 5' probe; as well as one of the following criteria: a) randomized to Arm B (pemetrexed or docetaxel) of Study A8081007 (1007, NCT00932893) and was discontinued from treatment due to RECIST version 1.1-defined disease progression as determined by independent radiology review, or b) ineligibility for 1007 due to (1) prior treatment for advanced disease with more than one chemotherapy regimen, (2) prior treatment with only one chemotherapy regimen for advanced disease and that regimen was not platinum-based, (3) prior treatment with pemetrexed as part of their platinum-based chemotherapy and did not meet the docetaxel eligibility requirements from Protocol 1007 or (4) treated

with docetaxel as part of their platinum-based prior chemotherapy but have NSCLC that is predominantly squamous cell carcinoma and thus, not eligible to be dosed with pemetrexed; Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 to 3; patients with brain metastases are eligible if treated and neurologically stable for at least 2 weeks and are not taking any medications contraindicated in Exclusion Criteria; any prior treatment (chemotherapy, radiation or surgery) completed at least 2 weeks prior to initiation of study medication; any acute toxicity must have been recovered to ≤ Grade 1 (except alopecia); measurable tumors per RECIST (version 1.1); female or male, 18 years of age or older (for patients enrolled in Japan: consent from a legally acceptable representative is required for all patients who are under 20 years old); signed and dated informed consent document; willingness and ability to comply with all requirements; and (for patients enrolled in Japan) agree to use effective contraception during the study period and for at least 90 days after completion of the study treatment (excludes surgically sterile male patients or surgically sterile or postmenopausal female patients).

Patients were not permitted to enroll if they were: eligible for Protocol 1007; received no prior chemotherapy for advanced NSCLC, or erlotinib or gefitinib as the only prior treatment for advanced NSCLC; were under current treatment on another therapeutic clinical trial; received prior therapy specifically directed against ALK; suffered from spinal cord compression unless treated with the patient attaining good pain control and stable or recovered neurologic function, carcinomatous meningitis, or leptomeningeal disease; suffered from any of the following within the 3 months prior to starting study treatment: myocardial infarction, severe/unstable angina, coronary/peripheral artery bypass graft, congestive heart failure, or cerebrovascular accident including transient ischemic attack; suffering from ongoing cardiac dysrhythmias of NCI CTCAE Grade ≥2, uncontrolled atrial fibrillation of any grade, or QTc interval >470 msec; received crizotinib previously; were pregnant or currently breastfeeding; using drugs or consuming foods that are known potent CYP3A4 inhibitors; using drugs that are known potent CYP3A4 inducers; using drugs that are CYP3A4 substrates with narrow therapeutic indices; prior malignancy (other than current NSCLC): patients will not be eligible if they have evidence of active malignancy (other than non-melanoma skin cancer or localized cervical cancer, or localized and presumed cured prostate cancer) within the last 3 years; for Japan only: patients who have following complications or symptoms: serious wound such as chronic wound, or grade >3 gastrointestinal ulcer or serious gastrointestinal symptoms such as grade >3 diarrhea; suffer from other severe acute or chronic medical or psychiatric conditions, or laboratory abnormalities that would impart, in the judgment of the investigator and/or sponsor, excess risk associated with study participation or study drug administration, and which would, therefore, make the patient inappropriate for entry into this study; and patients with known interstitial fibrosis or interstitial lung disease.

2. Follow-up Schedule

Patients received a full medical exam, ECOG performance status, all laboratory testing and other clinical assessments as defined in the protocol during screening (≤28 days prior to first receiving treatment. Baseline signs/symptoms were assessed between 2 days before beginning the first cycle to two days after receiving the first dose. A physical exam, ECOG status, laboratory studies (hematology and chemistry), clinical assessment, and 12-lead ECG were performed on the first day (±2 days) of each cycle, at the end of treatment or withdrawal. Tumor assessments were conducted every six weeks (±1 week) and at the end of treatment or withdrawal.

3. Clinical Endpoints:

The primary endpoint was objective response rate (ORR) as assessed by the investigator on a response-evaluable population. The response-evaluable population received crizotinib, an adequate baseline scan, and a follow up scan >6 weeks after starting crizotinib. Duration of Response (DR) was also evaluated.

B. Accountability of PMA Cohort

At the time of data cutoff (Feb. 1, 2011), the safety analysis population (#1) consisted of 261 patients which were included in the safety population and 136 patients for whom efficacy data was available with ALK-positive advanced NSCLC from Study 1005 were analyzed. Study is still ongoing and contains several populations with a variety of cutoff dates. The table below outlines these populations and provides information on the disposition for Safety Population 1. Information on all patient deaths and discontinuations in Safety Population 1 is included in the safety analysis below.

Table 30. Patient Disposition of Study 1005.

Efficacy Population (Data cutoff 2-1-11)	136
Safety Population 1 (Deaths, Discontinuations, SAEs)	261
Safety Population 2 (Grade 1-4 AEs)	136
Patients Treated	261
Ongoing	205
Discontinued	56
Adverse Events	9
Progressive Disease	31
Death	13
Lost to Follow Up/Patient Decision	1/2

C. Study Population Demographics and Baseline Parameters

Median age was 52 years and unlike most studies of NSCLC Study 1005 contained a nearly equal proportion of female and male patients. The table below shows that the majority of patients were non-smokers or former smokers. Among former smokers, the median time since discontinuation was 15 years. Given the late presentation of most patients with NSCLC, the median time from diagnosis on both studies was surprisingly. It is unclear if patients at the edge of this range (e.g., patients diagnosed

with NSCLC 13.7 years prior to study entry), in fact, had a second primary tumor. The sample date for ALK testing was examined in patients diagnosed more than 5 years prior to study entry and all samples dates were within the year prior of study entry. Finally, the majority of patients on both trials had an adenocarcinoma. Additional information on tumor histology is provided in the footnote below the table. Thirteen patients entered the study after disease progression on the control arm of randomized trials involving crizotinib.

Table 31. Baseline and Disease Characteristics Study 1005 (N = 136)

Sex, n (%)	
Male	64 (47)
Female	72 (53)
Age (years), n (%) Median (range)	52 (29-82)
Race, n (%)	
White	87 (64)
Black	5 (4)
Asian	43 (32)
Other	1 (1)
ECOG Performance Status, n (%)	
0	37 (27.2)
1	74 (54.4)
2 – 3[a]	25 (18.4)
Smoking Status	
Non-smoker	92 (67.6%)
Former Smoker	39 (28.7%)
Smoker	5 (3.7%)
Median Time Since Diagnosis	2 years (0.15-13.7)
Median Time Since Metastatic/Recurrent Disease	0.9 years (0.005-11.3)
Stage	
Locally Advanced	(5.9%)
Metastatic	(94.1%)
Histological Subtype	
Adenocarcinoma	130 (95.6%)
Large Cell Carcinoma	1 (0.7%)
Adenosquamous Carcinoma	3 (2.2%)
Other	2 (1.5%)
Prior Therapy	
Surgery	97.8%
Radiation Therapy	56.6%
Chemotherapy	99.3%

Table 32 below provides information on prior chemotherapy, both in terms of the number of prior regimens and the percentage of patients who received FDA-approved agents for NSCLC. A substantial numbers of patients have received each of the approved agents.

Table 32. Number and type of prior chemotherapy undergone.

Number of Prior Chemotherapy Regimens for Metastatic Disease	
0 Prior Regimens	0
1 Prior Regimen	9.6%
2 Prior Regimens	27.2%
3 Prior Regimens	27.2%
4-12 Prior Regimens	36.0%
Prior Adjuvant/Metastatic Chemotherapy	N = 136
Bevacizumab	40.4%
Erlotinib	47.8%
Gemcitabine	44.9%
Pemetrexed	88.2%
Platinum Compounds	95.6%
Taxanes (docetaxel, paclitaxel)	74.3%
Vinorelbine	20.6%

[1]Includes adenocarcinoma NOS (93), signet ring (11), acinar (7), bronchoalveolar (7), solid (6), papillary (5), mixed acinar and papillary (2), and large cell (1).

Table 33 below provides information on the baseline tumor characteristics of patients on Study 1005. Disease burden is assessed by the sum of the longest diameter (SLD) of the target lesions. The SLD in both trials is small and subset analyses of the primary endpoint will be conducted in patients with various degrees of tumor burden. The metastatic pattern in patients with ALK-positive NSCLC appears typical of patients with NSCLC as a whole.

Table 33. Baseline Tumor Characteristics.

IRC Sum of the Longest Diameter	N = 93
Median (range)	5.2 cm (1.0-19.6)
INV Sum of the Longest Diameter	N = 135
Median (range)	6.7 cm (1.1-62.5)
INV Sites of Target Lesions	N = 135
Lung	70.4%
Lymph Node	38.5%
Liver	35.6%
Adrenal	7.4%
Chest/Chest Wall	3.7%
Brain	2.2%

D. Safety and Effectiveness Results

The safety with respect to treatment with crizotinib (Xalkori®) will not be addressed in the SSED for the Vysis ALK Break Apart FISH Probe Kit. (For this information, please refer to the approval package for crizotinib.)

1. Effectiveness Results

One hundred thirty-six patients with locally advanced or metastatic ALK-positive NSCLC from Study A were analyzed at the time of data cutoff. The median duration of treatment was 22 weeks. Based on investigator assessments, there was

1 complete and 67 partial responses for an ORR of 50% (95% CI: 42%, 59%). Seventy-nine percent of objective tumor responses were achieved during the first 8 weeks of treatment. The median response duration was 41.9 weeks.

Table 34. Locally Advanced or Metastatic ALK-Positive NSCLC Efficacy Results

Efficacy Parameter	N=136
ORR (CR+PR)[a] [% (95% CI)]	50% (42%, 59%)
Number of Responders	68
Duration of Response[b] [Median (range) weeks]	41.9 (6.1+, 42.1+)

[a]One patient was not evaluable for response.
[b]Preliminary estimate using Kaplan-Meier method.
+Censored values
CR = complete response
PR = partial response

2. Subgroup Analyses

To further understand the response to crizotinib, a number of subset analyses were carried out and selected analyses are shown in the table below. There was no clear difference in response by performance status, sex, age, number of prior chemotherapeutic regimens, tumor burden, or in the percentage of cells found to have a rearrangement in the ALK gene by FISH. There was, however, a difference in response by race. This difference was examined and found that much of this difference is related to patient body size, with smaller patients receiving a larger dose of crizotinib on a mg/kg basis. It is unclear if there is also a difference in drug metabolism by race. This was of particular concern since the median SLD is very small in both studies. Finally, among the 11 patients with signet ring histology (an unusual histology for NSCLC, but previously reported in ALK-positive patients), 7 were responders.

Table 35. Subgroup Analyses for Study 1005 (N = 135)

Response by Performance Status	
0	54.1%
1	52.1%
≥ 2	36.0%
Response by Race	
Asian	60.5%
Non-Asian	44.6%
Response by Region	
US	44.3%
Non-US	55.4%
Response by Number of Prior Chemotherapy Regimens	
0 Prior Regimens	NA
1 Prior Regimen	50.0%
2 Prior Regimens	54.1%
3 Prior Regimens	42.5%
≥ 4 Prior Regimens	51.0%

Table 35. Subgroup Analyses for Study 1005 (N = 135)

Response by Disease Burden	
Baseline SLD ≤ Median	42.7%
Baseline SLD > Median	56.7%

In some patients a prolonged period between diagnosis and study entry was recorded. It is unclear if these patients, in fact, had a 2nd primary lesion. Among the 19 patients in whom the time between diagnosis and study entry was reported to be > 5 years, 6 (31.6%) were responders. Of more concern are the 48 patients on Study 1005 who were reported to have metastatic or recurrent disease >18 months prior to entry. In these patients, 24 (50.0%) were responders. This is consistent with the patient population as a whole.

ALK Negative Non-Small Cell Lung Cancer

Twenty-five (25) patients with locally advanced or metastatic ALK-negative NSCLC, by the Vysis ALK Break Apart FISH Probe Kit, were enrolled into a separate trial and received crizotinib. Preliminary response data on these patients were included in the 60-day safety update on June 10, 2011. After the March 2011 snapshot of the clinical database, it was determined that two of the 25 should not have been included due to testing of an inappropriate specimen type and a reporting error by the central testing laboratory. The baseline characteristics for ALK-negative advanced NSCLC patients were generally comparable with those reported for ALK-positive advanced NSCLC patients enrolled in Study 1005. However, 8 of 23 (34.8%) patients had not received prior chemotherapy for metastatic disease. Five of 19 response evaluable patients responded for an investigator response rate of 26.3% (95% CI 9.1%, 51.2%). Two additional patients have a single assessment of PR. If confirmed, the response rate in this population would be 7/20 (35.0%). This is similar to the RR in patients with ALK-positive NSCLC in Study 1005, 49.6%, which also used the Vysis ALK Break Apart FISH assay to determine ALK positivity; however these results are based on only a very small number of patients. It is unclear if this finding is related to the ability of crizotinib to target other genetic abnormalities associated with NSCLC such as MET, RON, or ROS. It may be that a substantial number of patients with NSCLC (or with other tumors) could benefit from crizotinib. The study of patients with ALK-negative NSCLC will be a post-marketing commitment for crizotinib.

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

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Device Panel, an FDA advisory committee, for review and recommendation.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

The percent agreement and Gini Index coefficient indicated good reproducibility between readers, sites, and overall reproducibility. No significant differences were noted between readers, sites, and days after resolution of the equivocal specimens. While a moderate to

high percent coefficient of variation (%CV), was observed for some values, this was not unexpected with FISH, particularly for specimens with low counts and if the readers do not enumerate signals from the same regions on the slides.

A. **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. As a diagnostic test, the Vysis ALK Break Apart FISH Probe Kit involves testing on formalin-fixed, paraffin embedded human NSCLC cancer tissue sections. These tissue sections are routinely removed for NSCLC cancer diagnosis. The test, therefore, presents no additional safety hazard to the patient being tested.

B. **Effectiveness Conclusions**

The Vysis ALK Break Apart FISH Probe Kit was used to identify the ALK-positive patient population enrolled into Study 1005. The response rate data from this study was used to support the accelerated approval of crizotinib.

C. **Overall Conclusions**

Based on the preclinical and clinical data, FDA concludes that there is reasonable assurance of safety and effectiveness of this device for use in the assessment of ALK gene rearrangements (e.g., translocations, inversions, and deletions) and is sufficient to effectively identify appropriate patients to be considered for crizotinib (Xalkori®) therapy.

XIII. CDRH DECISION

CDRH issued an approval order on August 26, 2011. The final conditions of approval cited in the approval order.

The applicant's manufacturing facility was inspected on May 16-24, 2011 and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling. Refer to the drug label for Xalkori® for additional information related to use of the drug.

Post-approval Requirements and Restriction: See device approval order and Xalkori® (crizitonib) approval order.

XV. REFERENCES

None.