SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: DNA FISH Probe Kit

Device Trade Name: Vysis CLL FISH Probe Kit

Device Procode: PNK

Applicant's Name and Address: Abbott Molecular Inc.
1300 E. Touhy Avenue
Des Plaines, IL 60018

Date of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P150041

Date of Notice of Approval: April 11, 2016

II. INDICATIONS FOR USE

The Vysis CLL FISH Probe Kit is a test to detect deletion of the LSI TP53 probe target via fluorescence in situ hybridization (FISH) in peripheral blood specimens from patients with B-cell chronic lymphocytic leukemia (CLL).

The test is indicated for detecting deletion of the LSI TP53 probe target (17p-) as an aid in identifying those patients with CLL for whom treatment with VENCLEXTA® (venetoclax) is indicated.

Vysis CLL FISH Probe Kit is not intended for monitoring of residual disease.

The test is for prescription use only.

Note:
The test was also previously cleared with the following indication (k100015):
The Vysis CLL FISH Probe Kit is intended to detect deletion of the LSI TP53, LSI ATM, and LSI D13S319 probe targets and gain of the D12Z3 sequence in peripheral blood specimens from untreated patients with B-cell chronic lymphocytic leukemia (CLL). The assay may be used to dichotomize CLL (the 13q-, +12, or normal genotype group versus the 11q- or 17p- group) and may be used as an aid in determining disease prognosis in combination with additional biomarkers, morphology and other clinical information.

III. CONTRAINDICATIONS

The Vysis CLL FISH Probe Kit is not intended for use in in monitoring of residual disease.

IV. WARNINGS AND PRECAUTIONS

Warnings and precautions can be found in the Vysis CLL FISH Probe Kit product labeling.

V. DEVICE DESCRIPTION

A. Kit Components

The Vysis CLL FISH Probe Kit contains two vials withfive reagents sufficient to process 20 assays. Vial 1 contains the probe reagent that is used in conjunction with the counterstain and buffers for this indication. Each assay uses 10 µL vial 1 applied to a hybridization target area 22 mm x 22 mm. . The quantity, concentration and storage conditions of the Kit reagents supplied are shown below.

Vial 1			
Probe Name**	**Locus**	**Quantity/Concentration**	**Storage**
LSI TP53 Spectum Orange	17p13.1	1 vial, 200 µL/vial (300 and 200ng/10 µL)	-20◦C (±5◦C) and protected from light
LSI ATM Spectrum Green*	11q22.3		
Vial 2			
Probe Name**	**Locus**	**Quantity/Concentration**	**Storage**
LSI D13S319 SpectrumOrange*	13q14.3	1 vial, 200 µL/vial (200, 400 and 25 ng/10 µL)	-20◦C (±5◦C) and protected from light
CEP 12 SpectrumGreen*	12p11.1-q11		
Vysis CEP 12 SpectrumGreen*	13q34		

* These reagents are included in the marketed kit. However, their function is related only to the indication cleared under k100015 and they are not part of this PMA review.
**(Locus Specific Identifier (LSI); Chromosome Enumeration Probe (CEP)

Other Reagents		
DAPI II Counterstain (4 ,6-diamidino-2-phenylindole•2HCl) and 1,4-phenylenediamine in a glycerol and phosphate buffered saline mixture	1 vial, 600 µL/vial (125 ng/mL)	-20◦C (±5◦C) and protected from light
NP-40 (non-ionic detergent)	2 vials, 2000 µL/vial	-25◦C to 30◦C
20X SSC Salt (sodium chloride and sodium citrate)	1 bottle, 66g	-25◦C to 30◦C

B. Description of Probe Used for 17p Deletion Testing

Vysis LSI TP53 SpectrumOrange/ATM SpectrumGreen Probes

- The SpectrumOrange-labeled LSI TP53 probe, approximately 172 Kb in length (chr17:7494395-7666098; February 2009 Assembly University of California, Santa Cruz [UCSC] Human Genome Browser), is located at 17p13.1 and contains the complete TP53 gene. It is used to detect p53 deletion on chromosome 17

C. Test Principle

Fluorescence In Situ Hybridization (FISH) is a technique that allows visualization of specific nucleic acid sequences within a cellular preparation. Specifically, FISH involves precise annealing of a single-stranded, fluorophore-labeled DNA probe to a complementary target sequence. Hybridization of the probe with the cellular DNA site is visible by direct detection using fluorescence microscopy. The Vysis CLL FISH Probe Kit uses FISH DNA probe technology to determine the deletion status of probe targets for TP53 (tumor protein p53 gene location chromosome 17p), and ATM (ataxia telangiectasia mutated gene-location at chromosome 11q), as a control.

Peripheral blood cells from CLL patients are attached to microscope slides using standard cytogenetic procedures. The resulting specimen DNA is denatured to single-stranded form and subsequently allowed to hybridize with the probes of the Vysis CLL FISH Probe Kit. Following hybridization, the unbound probe is removed by a series of washes, and the nuclei are counter-stained with DAPI, a DNA-specific stain that fluoresces blue. Hybridization of the Vysis LSI TP53 SpectrumOrange, LSI ATM SpectrumGreen, is viewed using a fluorescence microscope equipped with appropriate excitation and emission filters, allowing visualization of the orange, green, and aqua fluorescent signals.

D. Interpretation and Result Reporting

1. Quality Control (Assessing Slide Adequacy)

Slide hybridization adequacy is evaluated using the following criteria. If the criteria are not met, the specimen slide will not be enumerated.

- Nuclear Morphology: Borders of cell nuclei should generally be distinguishable and be intact.
- Background: The background should appear dark or black and be relatively free of fluorescent particles or haziness.
- Probe Signal Intensity: The signals should be bright, compact, round or oval shapes, distinct and easily evaluable.

2. Signal Enumeration

Using the appropriate filters, two technologists (readers) each score 100 nuclei for each hybridization target by counting and recording the number of orange and green signals present in each nucleus.

In a cell with normal copy numbers of the LSI TP53 SpectrumOrange and LSI ATM SpectrumGreen probe targets, two orange (2R) and two green (2G) signals will be expected. The abnormal pattern for TP53 deletion (17p-) is one orange signal (1R). Any pattern containing a single orange signal, regardless of the number of G signals is considered an abnormal pattern for TP53 deletion (17p-).

A third reader may be required if any of the following apply:

- One reader has an abnormal signal pattern count at or below the cut-off for 100 nuclei and the other reader has a count above this value.
- The abnormal signal pattern counts determined by the two readers differ by more than 5 per 100 nuclei evaluated, and either of the two readers have abnormal signal pattern counts less than or equal to twice the cut-off value.
- The two readers have abnormal signal pattern counts which differ by more than 15 per 100 nuclei evaluated.

The number of observed orange and green signal patterns, from each of two readers are added together to generate the count for 200 nuclei per specimen. When a third reader is employed to complete the evaluation, the two scores closest to one another will be combined to generate the count for 200 nuclei. If any two scores are equidistant from the third score, the median value is doubled and used as the count for 200 nuclei.

3. Interpretation of Results

For each specimen, a normal/abnormal determination is made by comparing the number of nuclei observed (per 200 scoreable nuclei) with the 17p- signal patterns to the normal cut-off value. The normal cut-off value per 200 nuclei is 14 (7%).

If the number of such nuclei is less than or equal to 7%, the result is normal.

If the number of nuclei with 17p- exceeds 7%, the result is abnormal. The patient is eligible for VENCLEXTA® (venetoclax) treatment.

The Vysis CLL FISH Probe Kit is intended to be used in combination with additional biomarkers, morphology, and other clinical information.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently no alternative cleared or approved methods for detecting chromosome 17p deletions to direct treatment options for relapsed or refractory CLL patients.

VII. MARKETING HISTORY

Abbott Molecular Vysis CLL FISH Probe Kit (List No. 04N02-020) has been commercially marketed in United States, Singapore and Hong Kong with the indication cleared under (k100015) since 2011.

The kit is also commercially available in the following countries: European Union (EU), Australia, Belarus, Canada, China (as RUO), Egypt, Hong Kong, Israel, India, Jordan, Korea, Kuwait, Malaysia, Russia (as RUO), Saudi Arabia, Singapore, South Africa, Turkey and Taiwan.

This product has not been withdrawn from the market for any reason related to safety or effectiveness.

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 results and subsequently improper patient management decisions in CLL patients. For adverse effects during the clinical studies, see the VENCLEXTA drug label, and Section XII below.

IX. SUMMARY OF PRECLINICAL STUDIES

The analytical performance of the Vysis CLL FISH Probe Kit for detecting deletions in chromosome 17p in peripheral blood specimens from patients with CLL was assessed as part of the evaluation of the the safety and efficacy of the test as an aid in identifying those patients with CLL for whom treatment with VENCLEXTA® (venetoclax) is indicated.

Evaluation of hybridization quality:

For all studies, the quality of the hybridization of each probe was evaluated for each hybridization target using a Quality Scoring Method (Q score). The quality scoring (Q score) method is a quantitative assessment of the parameters described in Quality Control section of the Vysis CLL FISH Kit draft package insert and associated clinical brochure. Each hybridization target area is rated on a scale of 1 to 5 according to 4 attributes: probe signal intensity, nuclear and chromosomal specificity, target background, and cross-hybridization. The hybridization is considered adequate ("Pass") if scores for all evaluated parameters are ≥ 3, otherwise the slide was "Fail". The quality scoring method was applied throughout all studies to both lymphocyte slides, and slides prepared from peripheral blood samples.

Lymphocyte Slides:

Lymphocyte slides are made from PHA-stimulated peripheral blood of normal individuals in culture, and are manufactured at Abbott Molecular using standard cytogenetic techniques. The neutrophil and monocyte populations do not divide in culture and diminish in numbers, while lymphocytes proliferate. Lymphocyte slides are made from cultured normal blood matrix which represents direct peripheral blood of CLL patients. Both specimen types are enriched for lymphocyte population. In order to use

lymphocyte slides in some of the analytical studies described below, an equivalency study between lymphocyte slides and CLL peripheral blood specimen slides was assessed for hybridization validity. The results demonstrated that the validity rate (95% CI) for lymphocyte slides was 97.8% (95% CI 95.6 - 99.1%)

A. Laboratory Studies -17p deletion

1. Analytical Sensitivity

Analytical sensitivity is defined as the percentage of scoreable interphase nuclei with the expected normal signal pattern. The expected normal interphase signal pattern for vial 1 in the Vysis CLL FISH Probe Kit is two orange and two green signals (2R2G) for the Vysis LSI TP53 SpectrumOrange and Vysis LSI ATM SpectrumGreen Probe targets. The analytical sensitivity was established using interphase nuclei prepared from peripheral blood cultures of 25 male karyotypically normal specimens, 1 slide per specimen (200 nuclei from each specimen x 25 specimens = 5000 nuclei counted to calculate analytical sensitivity). The Vysis CLL FISH Probe Kit was shown to have a sensitivity of 97.98% for the LSI TP53 SpectrumOrange probe, 98.68% for the LSI ATM SpectrumGreen probe.

2. Analytical Specificity

Analytical specificity is defined as the percentage of signals that hybridize to the correct locus and no other location. The analytical specificity of the Vysis LSI TP53 SpectrumOrange/ATM SpectrumGreen Probes for their chromosome target loci (17p13.1, 11q22.3, respectively) was established using metaphase chromosomes prepared from peripheral blood cultures of 5 male karyotypically normal specimens. The hybridization location of each FISH signal on chromosomes of 20 consecutive metaphase nuclei from each of 5 specimens was evaluated by 1 technologist for a total of 200 target loci per probe. The analytical specificity for each probe in the Vysis CLL FISH Probe Kit was 100% (200/200) (95% CI 98.17-100%).

3. Normal Cutoff

Deleted 17p status was based on the normal cut-off. The normal cutoff is defined as the maximum quantity of scoreable interphase nuclei with a specific abnormal signal pattern at which a specimen is karyotypically normal (negative) for that signal pattern. The normal cutoff is expressed in terms of a percentage, or the actual number of specific abnormal nuclear FISH signal pattern per the standard number of nuclei tested. The normal cut-off values for the LSI TP53 SpectrumOrange/ATM SpectrumGreen probes in the Vysis CLL FISH Probe Kit were established by enumerating the number of abnormal signal patterns in 200 nuclei for each of 25 peripheral blood specimens from 25 different individuals

previously determined to be karyotypically normal for the genomic regions assessed by the Vysis CLL FISH Probe Kit.

The normal cutoff for 17p deletion was established as 14 per 200 cells or 7.0%

4. Precision

Precision of the assay was defined as the ability to obtain the same result for multiple runs and on different days with different reagent lots. Precision was evaluated in two parts for a total assessment of 12 CLL specimens (8 negative for 17p- and 4 positive for 17p-). CLL specimens represented a range of % cells including near the cutoff of 7%. The panel was tested according to the instructions for use with 3 non-consecutive days using 1 of 3 lots for a total of 7-9 replicates. The panel members were randomly blinded to the technologist.

The acceptance criteria for all specimens were met. At least 95% or greater of all replicates of the high positive samples had the abnormal signal pattern of interest above the normal cutoff. At least 95% or greater of all replicates of the negative samples did not have the abnormal signal pattern of interest above the normal cutoff. Total SD (standard deviation) for specimens near the cut-off were less than the pre-specified acceptance criterion SD (SD<10). The results are shown in Table 1 below:

Table 1. Precision Analysis of Abnormal Signal Patterns for Vysis LSI TP53 SpectrumOrange Probe [del(17p13.1)(1 signal)][a]

Sample	Category	N	Mean	Between-Day (Within	Between-Lot	Total SD[b]
1	Negative	7	2.6	1.23	0.00	1.23
2	Negative	7	2.9	1.58	1.11	1.94
3	Negative	7	3.8	3.48	0.00	3.48
4	Negative	7	3.1	2.00	0.00	2.00
5	Negative	7	2.7	0.76	0.65	1.00
6	Negative	7	2.4	2.90	0.00	2.90
7	Negative	7	2.1	1.38	0.00	1.38
8	Negative	7	2.8	1.29	0.00	1.29
9	Positive	7	29.8	5.15	4.54	6.87
10	Positive	7	73.2	5.29	0.00	5.29
11	Positive[c]	9	13.6	3.11	0.00	3.11
12	Positive[c]	9	16.9	3.90	0.00	3.90

[a] The mean and standard deviations are represented as percentages of abnormal signal patterns.
[b] Total variance is the sum of the other variance components.
[c] Positive specimen near the normal cut-off. the panel consisted of ten slides

5. Reproducibility

Reproducibility of the Vysis CLL FISH Probe Kit was evaluated at 3 external laboratories by testing a randomized 8-member specimen panel that consisted of 6 unique 17p deletion positive peripheral blood specimens with varying target levels of positivity (2 each), and 2 unique 17p deletion negative peripheral blood specimens as summarized in Table 2 below.

Table 2. Specimen representing the 8-member panel in the reproducibility study:

Specimen Type	Target Percent Positive Cells	Observed for each panel member[a]
(2) normal	< 7%	3.5%; 3.4%
(2) near cutoff positive (17p-)	7% to 19%	12.9%; 13.3%
(2) low positive (17p-)	20% to 50%	20.8%; 52.6%[b];
(2) high positive (17p-)	> 50%	63.5%; 82.1%

[a] Observed results are the mean of 90 replicates.
[b] Panel member was originally determined to be 40%

Three lots of the Vysis CLL FISH Probe Kit reagents were used for the evaluation at each of the 3 sites. Testing for each reagent lot was for 5 non-consecutive days over a time period of approximately 25 calendar days. Each site evaluated 240 specimen slides for a total of 720 (8 specimens x 2 replicates per day x 5 days x 3 lots x 3 sites). For each panel member slide, the signal patterns of 100 nuclei were enumerated by two readers for a combined total of 200 nuclei.

Lot-to-lot and site-to-site pairwise comparisons were conducted. For the lot-to-lot comparison the pairs consisted of Lot 1 vs. Lot 2, Lot 1 vs. Lot 3, and Lot 2 vs. s Lot 3. For Lot A vs. Lot B, lot A was 1 or 2 and lot B was 2 or 3 (Table 3). A similar analysis was done for the site-to-site comparison but with the pairs consisting of sites (Table 4).

Table 3. 17p Deletion: Reproducibility Lot-to-Lot Pairwise Comparisons

			First Lot Result Second Lot Result				Point Estimate and 95% CI		
Panel	Descript.	n	Abnormal Abnormal	Normal Normal	Abnormal Normal	Normal Abnormal	ANA	APA	OA
Normal 1	Lot 1,2	300	0	290	10	0	98% (95%,100%)	N/A	97% (90%,100%)
	Lot 1,3	300	3	263	7	27	94% (89%,98%)	15% (0%,30%)	89% (80%,97%)
	Lot 2,3	300	0	270	0	30	95% (89%,99%)	N/A	90% (80%,98%)
	Lot A vs. B	900	3	823	17	57	96% (92%,99%)	8% (0%,15%)	92% (85%,98%)
Normal 2	Lot 1,2	300	1	281	9	9	97% (92%,100%)	10% (0%,24%)	94% (86%,100%)
	Lot 1,3	300	1	281	9	9	97% (93%,100%)	10% (0%,24%)	94% (87%,100%)
	Lot 2,3	300	1	281	9	9	97% (93%,100%)	10% (0%,20%)	94% (87%,100%)
	Lot A vs. B	900	3	843	27	27	97% (94%,100%)	10% (0%,18%)	94% (88%,100%)
Near Cutoff 1	Lot 1,2	300	253	3	27	17	12% (0%,29%)	92% (85%,98%)	85% (75%,97%)
	Lot 1,3	300	280	0	0	20	N/A	97% (91%,100%)	93% (83%,100%)
	Lot 2,3	300	270	0	0	30	N/A	95% (89%,100%)	90% (80%,100%)
	Lot A vs. B	900	803	3	27	67	6% (0%,14%)	94% (90%,99%)	90% (82%,98%)
Near Cutoff 2	Lot 1,2	300	280	0	10	10	N/A	97% (91%,100%)	93% (83%,100%)
	Lot 1,3	300	290	0	0	10	N/A	98% (95%,100%)	97% (90%,100%)
	Lot 2,3	300	290	0	0	10	N/A	98% (95%,100%)	97% (90%,100%)
	Lot A vs. B	900	860	0	10	30	N/A	98% (94%,100%)	96% (89%,100%)

Table 3. 17p Deletion: Reproducibility Lot-to-Lot Pairwise Comparisons

			First Lot Result Second Lot Result				Point Estimate and 95% CI		
Panel	Descript.	n	Abnormal Abnormal	Normal Normal	Abnormal Normal	Normal Abnormal	ANA	APA	OA
Low Pos. 1*	Lot 1,2	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Lot 1,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Lot 2,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Lot A vs. B	900	900	0	0	0	N/A	100% (99%, 100%)	100% (99%, 100%)
Low Pos. 2	Lot 1,2	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Lot 1,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Lot 2,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Lot A vs. B	900	900	0	0	0	N/A	100% (99%, 100%)	100% (99%, 100%)
High Pos. 1	Lot 1,2	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Lot 1,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Lot 2,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Lot A vs. B	900	900	0	0	0	N/A	100% (99%, 100%)	100% (99%, 100%)
High Pos. 2	Lot 1,2	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Lot 1,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Lot 2,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Lot A vs. B	900	900	0	0	0	N/A	100% (99%, 100%)	100% (99%, 100%)

N/A = not applicable; no observations in these categories

*Low Positive 1 was initially measured at 40% positive cells at the screening stage. Upon analysis of reproducibility study data, the mean percentage of positive cells was measured to 52.6%.

Table 4. 17p Deletion: Reproducibility Site-to-Site Pairwise Comparisons

			First Site Result Second Site Result				Point Estimate and 95% CI		
Panel	Descript.	n	Abnormal Abnormal	Normal Normal	Abnormal Normal	Normal Abnormal	ANA	APA	OA
Normal 1	Site 1,2	300	0	260	0	40	93% (85%,98%)	N/A	87% (73%,97%)
	Site 1,3	300	0	300	0	0	100% (98%,100%)	N/A	100% (98%,100%)
	Site 2,3	300	0	260	40	0	93% (85%,98%)	N/A	87% (73%,97%)
	Site A vs. B	900	0	820	40	40	95% (90%,99%)	N/A	91% (82%,98%)
Normal 2	Site 1,2	300	0	270	0	30	95% (87%,100%)	N/A	90% (77%,100%)
	Site 1,3	300	0	300	0	0	100% (98%, 100%)	N/A	100% (98%, 100%)
	Site 2,3	300	0	270	30	0	95% (87%,100%)	N/A	90% (77%,100%)
	Site A vs. B	900	0	840	30	30	97% (92%,100%)	N/A	93% (84%,100%)
Near Cutoff 1	Site 1,2	300	290	0	10	0	N/A	98% (95%,100%)	97% (90%,100%)
	Site 1,3	300	260	0	40	0	N/A	93% (85%,98%)	87% (73%,97%)
	Site 2,3	300	251	1	39	9	4% (0%,15%)	91% (82%,97%)	84% (70%,93%)
	Site A vs. B	900	801	1	89	9	2% (0%,8%)	94% (89%,98%)	89% (80%,96%)
Near Cutoff 2	Site 1,2	300	290	0	10	0	N/A	98% (95%,100%)	97% (90%,100%)
	Site 1,3	300	290	0	10	0	N/A	98% (95%,100%)	97% (90%,100%)
	Site 2,3	300	280	0	10	10	N/A	97% (91%,100%)	93% (83%,100%)
	Site A vs. B	900	860	0	30	10	N/A	98% (94%,100%)	96% (89%,100%)

Table 4. 17p Deletion: Reproducibility Site-to-Site Pairwise Comparisons

			First Site Result Second Site Result				Point Estimate and 95% CI		
Panel	Descript.	n	Abnormal Abnormal	Normal Normal	Abnormal Normal	Normal Abnormal	ANA	APA	OA
Low Pos. 1*	Site 1,2	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Site 1,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Site 2,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Site A vs. B	900	900	0	0	0	N/A	100% (99%, 100%)	100% (99%, 100%)
Low Pos. 2	Site 1,2	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Site 1,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Site 2,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Site A vs. B	900	900	0	0	0	N/A	100% (99%, 100%)	100% (99%, 100%)
High Pos. 1	Site 1,2	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Site 1,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Site 2,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Site A vs. B	900	900	0	0	0	N/A	100% (99%, 100%)	100% (99%, 100%)
High Pos. 2	Site 1,2	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Site 1,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Site 2,3	300	300	0	0	0	N/A	100% (98%, 100%)	100% (98%, 100%)
	Site A vs. B	900	900	0	0	0	N/A	100% (99%, 100%)	100% (99%, 100%)

N/A = not applicable; no observations in these categories

*Low Positive 1 was initially measured at 40% positive cells at the screening stage. Upon analysis of reproducibility study data, the mean percentage of positive cells was measured to 52.6%.

6. Robustness Studies:

Robustness studies were performed to demonstrate robust performance of the 17p- probe set in the Vysis CLL FISH Probe Kit and to confirm the adequacy of assay specifications for the following assay procedures: peripheral blood treatment with hypotonic solution (KCl), CLL blood processing, slide preparation conditions, slide baking, temperature and time, slide baking and aging, 2XSSC treatment temperature and time with ethanol dehydration time slide denaturation, post-denaturation dehydration in ethanol, manual probe denaturation temperature and time, slide warmer temperature and time, hybridization temperature and time, coverslip seal with rubber cement, and wash procedures including 0.4X SSC / 0.3% NP-40 wash Temperature and time, 2X SSC/ 0.1% NP-40 wash time, and 0.4X SSC/ 0.3% NP-40 wash solution pH study.

For all studies, the interpretation of the FISH results included both qualitative assessment of hybridization and where applicable, quantitative assessment of fluorescent signals. A slide was considered passing if the overall score was ≥3. All studies passed the pre-specified criteria of 67% passing rate. Enumeration data on CLL patient specimens including 17p- specimens were also provided for studies concerning specimen/target preparation. 100% agreement with expected result (abnormal/normal) was observed for all studies.

7. Probe Concentration Optimization/Limits

Four probe concentrations (2X, 1X, 0.5X, and 0.33X where X is the concentration supplied in the kit) were tested to evaluate the limits of the probe concentration used in the Kit. For each probe concentration, lymphocyte slides from one specimen were prepared in triplicate and evaluated by 2 readers for quality (Q score). The passing rate was 100% for all concentrations except at the 0.33X concentration. The results demonstrated optimal performance at the supplied concentration. Each probe combination is pre-formulated in hybridization buffer at optimal concentration and should not be diluted. Dilution may lead to erroneous assay results.

8. Photostability

Probes and DAPI II of the Vysis CLL FISH Probe Kit were tested for photostability by exposing to white fluorescent light at room temperature for up to 72 hours. Three replicates from a single lot of lymphocyte slides were evaluated per light exposure condition using Q score. The passing rate was 100% for 0, 3, 8, 24, 48 hours light exposure and 67% for 72 hours. All steps of the Vysis CLL FISH Probe Kit which do not require light for manipulation (incubation periods, washes, etc.) should be carried out in the dark or reduced light.

9. Peripheral Blood Success Rate

A study was conducted to verify the Vysis CLL FISH Probe Kit hybridization success rate on peripheral blood (PB) slides was greater than or equal to 90%. FISH hybridization success rate on 15 CLL PB specimens was determined by the total number of slides tested (45) with the Vysis CLL FISH Probe Kit, and the number of these slides resulting in an overall passing score for slide quality. The success rate for PB specimen slides was 100% (45/45) with each slide passing with each filter.

10. Specimen Stability

A study was conducted to verify the 17p- probe set in the Vysis CLL FISH Probe Kit can be used to test whole blood processed immediately, or stored and shipped at 2 to 8°C for a minimum of 96 hours. Three normal specimens and 2 CLL specimens with 17p deletion were tested with 9 replicates (3 replicates x 3 lots) per time points. The enumeration of 17p probe signal and percent of intact cells support blood stability for up to 96 hours at 2 to 8ºC.

11. Cell Pellet Stability

A study was conducted to verify fixed pellets can be stored at -20°C (± 5°C) for up to 24 months. Fixed cell pellets were tested for one healthy blood specimen, three normal CLL specimens, and two 17p- abnormal specimens. The four normal specimens were tested at baseline, multiple interim points, and 25 or 26 months. The two additional 17p- specimens were tested at baseline and 31or 35 months. All interim time points and the final time point (2-3 replicates at ≥25 month) demonstrated 100% agreement (abnormal/normal) with baseline.

12. Hybridized Slide Stability

A study was conducted to demonstrate stability of archived slides hybridized with Vysis CLL FISH Probe Kit within and greater than 3 weeks under intended storage (-20°C), protected from light. A total of 18 slides from 1 normal specimen and 5 CLL patient specimens including 2, 17p deletion positive specimens, each tested in triplicate, were stored at -20°C for 3 and 6 weeks protected from light. All slides were enumerable and 100% agreement with baseline (abnormal/normal) was observed.

13. Kit stability

A stability study was conducted to confirm the 12 month expiration dating of the Vysis CLL FISH Probe Kit under intended storage conditions (ISC) and to support dry ice shipping conditions. Three kit lots were tested under ISC conditions: -20ºC (± 5ºC) for the probes and DAPI II, and at 20ºC (± 5ºC) and +30ºC (± 2ºC) for the 20X SSC salt and NP-40. One of the above three kit lot was also subjected to Transport and Temperature Extreme (TTE) and then followed by

the inverted storage (INV) at -20ºC (± 5ºC) for the probes and DAPI II, and at -20ºC (± 5ºC) and +30ºC (±2ºC) for the 20X SSC salt and NP-40. A total of 3 to 9 lymphocyte slides were examined for acceptable Q score criteria per lot per test condition.

14. In-Use Stability

A study was conducted to verify that Vysis CLL FISH Probe Kit maintains stability after multiple freeze/thaw cycles in simulated customer use conditions. The kit provides sufficient material for 20 assays. 3 replicate lymphocyte slides were examined for Q score before and after 21 freeze/thaw cycles. 100% slide passing rate was observed.

15. Working Reagents Stability

A study was conducted to confirm the expiration dating of the Vysis CLL FISH Probe Kit working reagent solutions shown in Table 5, below.

Table 5. Working Reagent Expiration Dating

Reagent	Expiration Date	ISC
Ethanol Solutions (70%, 85% and 100%)	1 week	Ambient
20X SSC Solution	6 months	Ambient
2X SSC Solution	6 months	Ambient
Denaturation Solution (70% formamide/2X SSC Solution)	1 week	2 to 8°C
0.4X SSC/0.3% NP-40 Wash Solution	6 months	Ambient
2X SSC/0.1% NP-40 Wash Solution	6 months	Ambient

A total of 3 replicate lymphocyte slides and 1 negative control per each working solution lot tested. Three lots of SSC and NP40 were used to make working reagents, which were tested at 1 time unit past expiry date. One hundred percent Q score passing rate was observed for all test conditions.

16. Microbial Interference Characterization

A study was conducted to determine the effect of bio-burden challenge on performance of the Vysis CLL FISH Probe Kit components. Microbial Interference Characterization (MIC) testing was conducted by inoculating a single lot of Vysis CLL FISH Probe Kit components with selected microorganisms (10^3 to 10^4 CFU/mL), evaluating the assay performance by Q score, storing (at their intended storage condition (ISC)), and then evaluating the performance again after 32 days of storage. Three replicate lymphocyte slides (single lot) and one negative control were tested per each microorganism, reagent kit component, and testing time point. All test conditions passed the pre-specified criteria of 67% passing rate.

Inoculated Organism	Organism Type
Candida albicans	Fungal
Aspergillus brasiliensis	Fungal
Escherichia coli	Bacterial
Pseudomonas aeruginosa	Bacterial
Pseudomonas species (*fluorescens*	Bacterial
Staphylococcus aureus	Bacterial
Saline	None

B. Animal Studies

None.

C. Additional Studies

None.

X. SUMMARY OF PRIMARY CLINICAL STUDY

Clinical performance of the Vysis CLL FISH Probe Kit as an aid in identifying those patients for whom treatment with VENCLEXTA® (venetoclax) is indicated was evaluated in study M13-982, conducted by AbbVie Inc. in collaboration with Abbott Molecular, Inc. Study M13-982 required that a 17p deletion must have been documented using the Vysis CLL FISH Assay for enrollment. The overall response rate data from this study was used to support approval of VENCLEXTA® (venetoclax). A summary of the clinical study is presented below.

A. Study Design

The M13-982 clinical trial was a Phase II, multi-center, single-arm study completed at three sites located in Florida (Fort Meyers), Belgium (Antwerp), and Australia (Adelaide). The efficacy study screened 167 patients with relapsed or refractory CLL using peripheral blood specimens. A total of 106 patients with 17p deletion were enrolled. VENCLEXTA was administrated at 400 mg daily dose following a dose ramp-up schedule. The median time on treatment at the time of evaluation was 12.1 months (range: 0-21.5 months). The efficacy endpoint was overall response rate (ORR) assessed by an Independent Review Committee (IRC).

1. Clinical Inclusion and Exclusion Criteria

Inclusion Criteria

- Subject voluntarily signed and dated an informed consent
- Subject was ≥18 years of age
- Subject had diagnosis of CLL

- Subject had the 17p deletion as determined by Vysis CLL FISH Probe Kit
- Subject had an Eastern Cooperative Oncology Group (ECOG) performance score of ≤ 2
- Subject had adequate bone marrow function at Screening
- Subject had adequate coagulation, renal, and hepatic function per laboratory reference range at Screening
- Female subjects of childbearing potential and non-sterile male subjects must have practiced at least 1 method of birth control with partner(s) beginning with initial study drug administration and continuing to 30 days after the last dose of study drug
- Females of childbearing potential had negative results for pregnancy test performed
- Male subjects agreed to refrain from sperm donation from initial study drug administration until 90 days after the last dose of study drug
- For high-risk subjects preapproval by the AbbVie medical monitor was required prior to enrollment.

Exclusion Criteria

- Specimen is not handled, stored, or transported in accordance with the instructions. .
- Subject had previously received VENCLEXTA® (venetoclax).
- Subject had undergone an allogeneic stem cell transplant
- Subject had developed Richter's transformation
- Subject had prolymphocytic leukemia
- Subject had active and uncontrolled autoimmune cytopenias
- Subject was known to be positive for human immunodeficiency virus
- Subject had received a biologic agent (i.e., monoclonal antibodies) for anti-neoplastic intent within 8 weeks prior to the first dose of study drug
- Subject had received any anticancer therapy including chemotherapy or radiotherapy, or investigational therapy including targeted small molecule agents, within 14 days prior t the first dose of study drugs, or had not recovered to less than National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) grade 2 clinically significant adverse effect(s)/toxicity(s) of the previous therapy
- Subject had received the following within 7 days prior to the first dose of study drug:
    - Steroid therapy for anti-neoplastic intent
    - Cytochrome P450 (CYP) 3A inhibitors (such as fluconazole, ketoconazole, and clarithromycin)
    - Potent CYP3A inducers (e.g., rifampin, phenytoin, carbamazepine, or St. John's Wort)
    - Warfarin, or required the use of warfarin
    - Antiretroviral medications

- Subject had consumed the following within 3 days prior to the first dose of study drug
    - Grapefruit or grapefruit products
    - Seville oranges (including marmalade-containing Seville oranges)
    - Star fruit
- Subject had a known allergy to both xanthine oxidase inhibitors and rasburicase
- Subject had a cardiovascular disability status of New York Heart Association Class ≥ 2
- Subject exhibited evidence of other clinically significant uncontrolled condition(s)
- Subject had a significant history of renal, pulmonary neurologic, psychiatric, endocrinologic, metabolic, immunologic, cardiovascular, or hepatic disease that in the opinion of the investigator would adversely affect his/her participating in this study. For subjects who required an intervention for any above diseases within the past 6 months, correspondence with the investigator and the AbbVie medical monitor had to occur.
- A female subject was pregnant or breastfeeding
- Subject had a history of active malignancies other than CLL within the past 2 years prior to study entry, with the exception of
    - Adequately treated in situ carcinoma of the cervix uteri
    - Adequately treated basal cell carcinoma or localized squamous cell carcinoma of the skin
    - Previous malignancy confined and surgically resected (or treated with other modalities) with curative intent
- Subject had malabsorption syndrome or other condition that precluded enteral route of administration

2. <u>Follow-up Schedule</u>

A Safety Follow-Up Visit was performed for all subjects approximately 30 days following discontinuation of study drug for any adverse event.

The most common drug adverse reactions (≥20%) of any grade were neutropenia, diarrhea, nausea, anemia, upper respiratory tract infection, thrombocytopenia, and fatigue. Serious adverse reactions were reported in 43.8% patients. The most frequent serious adverse reactions (≥2%) were pneumonia, febrile neutropenia, pyrexia, autoimmune hemolytic anemia, anemia, and TLS.

3. <u>Clinical Endpoints</u>

The efficacy endpoint was overall response rate (ORR) assessed by an independent Review Committee IRC.

B. <u>Accountability of PMA Cohort</u>

A total of 272 peripheral blood specimens were collected and shipped to Abbott Molecular clinical testing sites. Two specimens were excluded from the data set due to the testing not being performed due specimen mishandling. Of the remaining 270 specimens, 261 were tested. For the 9 specimens that were not tested, 8 did not meet the inclusion criteria for testing and 1 was a duplicate specimen. Of the 261 specimens tested, 196 were from the main cohort and 65 were from the safety cohort. For some subjects, more than one specimen was tested.

Of the 167 subjects screened for the main cohort, a total of 144 had an abnormal 17p- result. Of the 144 subjects which had an abnormal 17p- result, 106 were enrolled in the M13-982 study. The 38 subjects that had an abnormal 17p- result but were not enrolled were screen failures based on M13-982 criteria.

C. Study Population Demographics and Baseline Parameters

A total of 106 17p- subjects were enrolled in Study M13-982. Demographic and disease characteristics of the study population for Study M13-982 are provided in Table 6.

Table 6. Demographic and Disease Characteristics in Study 1

		N=106	
Characteristics			
Age (Years)	Median (Range)	67	(37-83)
Race, n (%)	Asian	0	0.0% (0/106)
Race, n (%)	Black	3	2.8% (3/106)
Race, n (%)	White/Unknown [a]	103	97.2% (103/106)
Gender, n (%)	Male	69	65.1% (69/106)
Gender, n (%)	Female	37	34.9% (37/106)
ECOG, n (%)	0	42	39.6% (42/106)
ECOG, n (%)	1	55	51.9% (55/106)
ECOG, n (%)	2	9	8.5% (9/106)
Number of Prior Therapies	Median (Range)	2.5	(1-10)

[a] Race unknown for one patient.

D. Safety and Effectiveness Results

1. Safety Results

For safety with respect to treatment with VENCLEXTA® (venetoclax) refer to the VENCLEXTA® (venetoclax) drug label; this information is not fully addressed

in this SSED for the Vysis CLL FISH Probe Kit. Overall, the most common adverse reactions (≥20%) with VENCLEXTA® (venetoclax) were neutropenia, diarrhea, nausea, anemia, upper respiratory tract infection, thrombocytopenia, and fatigue.

2. Effectiveness Results

The efficacy of VENCLEXTA® (venetoclax) was established in a clinical trial of 106 patients with CLL with 17p deletion who had received at least one prior therapy. In the study, 17p deletion was confirmed in specimens from patients using Vysis CLL FISH Probe Kit.

The overall response rate (ORR) of the 106 CLL patients with 17p deletion was 80.2%. Efficacy data from Study 1 are provided in Table 7.

Table 7. Efficacy Results for Patients with Previously Treated CLL with 17p- assessed by an Independent Review Committee (IRC)

	VENCLEXTA N (%) N=106
ORR, n (%) (95% CI)	85 (80.2) (71.3, 87.3)
CR + CRi, n (%)	8 (7.5)
CR, n (%)	6 (5.7)
CRi, n (%)	2 (1.9)
nPR, n (%)	3 (2.8)
PR, n (%)	74 (69.8)
CI = confidence interval; CR = complete remission; CRi = complete remission with incomplete marrow recovery; IRC = Independent review committee; nPR = nodular partial remission; ORR = overall response rate (CR + CRi + nPR + PR); PR = partial remission.	

E. 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 clinical study included 3 investigators. None of the clinical investigators had 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. 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 an FDA advisory committee for review and recommendation.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The Vysis CLL FISH Probe Kit was used to identify the CLL 17p deletion positive patient population enrolled into Study M13-982. The response rate data from this study was used to support the accelerated approval of VENCLEXTA® (venetoclax). Patients tested positive for 17p- by the Vysis CLL FISH Kit and treated with VENCLEXTA demonstrated an overall response rate (ORR) 80.2%. Analytical performance studies were reviewed and found to be in support of the device indications for use (see Section IX, Summary of Preclinical studies).

B. Safety Conclusions

Adverse effects are based on data collected in the clinical study conducted to support PMA approval as described above. (See VENCLEXTA® labeling regarding adverse events related to the drug). As a diagnostic test, the Vysis CLL FISH Probe Kit involves testing on CLL patient blood. The test, therefore, presents no additional direct safety hazard to the patient being tested.

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect 17p deletion results, and consequently improper treatment decisions for CLL patients (see Benefit-Risk Section below).

C. Benefit-Risk Conclusions

The probable benefits of the Vysis CLL FISH Probe Kit are based on evaluation that the test performs consistently and provides clinically relevant results for assessing patients with previously treated CLL being considered for VENCLEXTA® (venetoclax) therapy.

The risks of the Vysis CLL FISH Probe Kit are associated with the potential mismanagement of patients resulting from false results of the test. Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect 17p deletion results, and consequently improper treatment decisions for CLL patients. A false positive test result may lead to treatment being administered to a patient who may not benefit, and potentially any adverse side effects associated with treatment. A false negative test result may lead to treatment being withheld from

a patient who might have benefitted. The device is a key part of diagnostic evaluation for previously treated CLL patients in decisions regarding treatment with VENCLEXTA® (venetoclax).

In conclusion, the data support the use of Vysis CLL FISH Probe Kit as an aid in selecting previously treated CLL patients for VENCLEXTA® (venetoclax) based on a Vysis CLL FISH Probe Kit Test 17p- "Abnormal" result, and the probable benefits outweigh the probable risks.

Patient Perspective Information

FDA is unaware of patient perspective information relevant to review of this device.

D. Overall Conclusions

Based on the preclinical and clinical data in this application, FDA concludes that there is reasonable assurance of safety and effectiveness of this device for use in the detection of 17p deletions in peripheral blood specimens from relapsed or refractory CLL patients and there is reasonable assurance of safety and efficacy for use as indicated to identify appropriate patients for VENCLEXTA® (venetoclax) therapy.

XIII. CDRH DECISION

CDRH issued an approval order on April 11, 2016.

The applicant's manufacturing facilities have been inspected 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, Precautions and Warnings, and Limitations in the device labeling. Refer to the drug label for VENCLEXTA® (venetoclax) for additional information related to the drug.

Post-approval Requirements and Restrictions: See approval order.