510(k) Summary

AUG - 9 2011

510(k) Number: K100015

Device Name: Vysis CLL FISH Probe Kit

Purpose of the Submission

The purpose of this 510(k) is to gain clearance to market the Vysis CLL FISH Probe Kit (List No. 4N02-020).

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Date of Preparation

August 9, 2011

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Intended Use

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

Trade Name

Vysis CLL FISH Probe Kit

Common Name

Fluorescence In Situ Hybridization (FISH) reagents

Classification

Class II

Regulation Number

21 CFR 866.6040 Gene expression profiling test system for breast cancer prognosis

Product Code

OVQ, Chronic Lymphocytic Leukemia FISH Probe Kit

Predicate Devices

Agendia BV MammaPrint® (k062694)

Comparison with Predicate

Not applicable. Clearance is supported by published clinical study and method concordance bridging studies.

Device Description

The Vysis CLL FISH Probe Kit uses fluorescence in situ hybridization (FISH) DNA probe technology to determine deletions of the locus-specific identifier (LSI) TP53, LSI ATM, and LSI D13S319 probe targets and gain of the D12Z3 sequence.

The Vysis CLL FISH Probe Kit (List No. 4N02-020) consists of two DNA FISH probe sets and three general purpose reagents sufficient to process 20 assays.

- LSI TP53 SpectrumOrange/ATM SpectrumGreen Probe
- LSI D13S319 SpectrumOrange/13q34 SpectrumAqua/CEP 12 SpectrumGreen Probe
- DAPI II Counterstain
- NP-40
- 20X SSC Salt

Background on Chronic Lymphocytic Leukemia (CLL)

Currently, most patients diagnosed with CLL have early-stage disease (Rai stage 0 or 1). Patients with early-stage CLL are a heterogeneous group; approximately 30% to 50% are at high risk of accelerated disease progression, and the remainder may live for decades and possibly never require therapy. Recent insights into the biological characteristics of leukemic B cells have led to the discovery of new prognostic tools (immunoglobulin variable-region heavy chain gene mutation status, cytogenetic abnormalities assessed by FISH, and Z-chain-associated protein kinase-70 protein expression) that can contribute to the identification of patients with early-stage disease who are at high risk for early disease progression.[1]

Routine karyotype analysis only detects chromosomal aberrations associated with CLL in 40% to 50% of the cases. Use of FISH and other technologies have detected genomic

abnormalities in over 80% of cases of CLL. The common genomic aberrations seen are trisomy 12 and deletions of 13q, 17p, and 11q.[2-4]

Several published studies suggest that some of these chromosomal abnormalities may be correlated with various disease parameters.[5-8]

The Vysis CLL FISH Probe Kit uses FISH DNA probe technology to determine deletion status of probe targets for LSI TP53 (containing tumor protein p53 gene, located on chromosome 17p), LSI ATM (containing ataxia telangiectasia mutated gene, located on chromosome 11q), and LSI D13S319 (containing marker D13S319, located on chromosome 13q), as well as determining trisomy 12 with CEP12 (D12Z3 alpha satellite-location chromosome 12).

The Vysis CLL FISH Probe Kit includes LSI 13q34 (containing lysosomal-associated membrane protein 1 gene, located on chromosome 13q) as a quality control probe.

Technological Description of the Device

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. Interpretation of FISH results should be made utilizing appropriate controls and analytical techniques as well as taking into consideration other clinical and diagnostic test data.[9]

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 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, LSI D13S319 SpectrumOrange, LSI 13q34 SpectrumAqua, and CEP 12 SpectrumGreen probes is viewed using a fluorescence microscope equipped with appropriate excitation

and emission filters, allowing visualization of the orange, green, and aqua fluorescent signals.

In a cell with normal copy numbers of the LSI TP53 SpectrumOrange and LSI ATM SpectrumGreen probe targets, two orange and two green signals will be expected. In a cell with normal copy numbers of Vysis LSI D13S319 SpectrumOrange and CEP 12 SpectrumGreen probe targets, two orange signals and two green signals will be expected. Enumeration of the Vysis LSI TP53 SpectrumOrange, LSI ATM SpectrumGreen, LSI D13S319 SpectrumOrange, and CEP12 SpectrumGreen signals provides a mechanism for determining absolute copy number of the probe targets and the presence of the aberrations of interest. Any aberrations detected are used to determine Döhner Classification and prognostic category.

Summary of Nonclinical Studies

Analytical Specificity

Analytical specificity is defined as the percentage of signals that hybridize to the correct locus and no other location. This test is a visual demonstration that each probe hybridizes specifically to the expected chromosome location. The analytical specificity of the probes in the Vysis CLL FISH Probe Kit for their respective chromosome target loci was established using metaphase chromosomes prepared from peripheral blood cultures of five karyotypically normal males on microscope slides. The hybridization location of each FISH signal on chromosomes of 20 consecutive metaphase nuclei on each of 5 slides was evaluated by one technologist for a total of 200 target loci.

For each probe and sample, the number of metaphase chromosome FISH signals hybridized to the correct locus and the number of metaphase chromosome FISH signals hybridized to the incorrect locus were enumerated. The specificity of each probe was calculated as the number of metaphase chromosome FISH signals hybridized to the correct locus divided by the total number of metaphase chromosome FISH signals hybridized and multiplied by 100 to give a percentage. The analytical specificity for each probe of the Vysis CLL FISH Probe Kit was 100% (200/200) (Table 1).

Table 1. Analytical Specificity of Probes in Vysis CLL FISH Probe Kit

Probe	Correct Cytogenetic Target	No. of Metaphase Chromosome Signals: Hybridized to the Correct Target Locus	No. of Metaphase Chromosome Signals: Total Hybridized Signals	Specificity (%)	95% Confidence Interval (%)
Vysis LSI TP53 SpectrumOrange	17p13.1	200	200	100	98.17, 100
Vysis LSI ATM SpectrumGreen	11q22.3	200	200	100	98.17, 100
Vysis LSI D13S319 SpectrumOrange	13q14.3	200	200	100	98.17, 100
Vysis LSI 13q34 SpectrumAqua	13q34	200	200	100	98.17, 100
Vysis CEP 12 SpectrumGreen	12p11.1-q11	200	200	100	98.17, 100

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 all probes in the Vysis CLL FISH Probe Kit is two signals per nucleus.

The analytical sensitivity of the probes in the Vysis CLL FISH Probe Kit for their respective chromosome target loci was established using interphase nuclei prepared from peripheral blood cultures of 25 karyotypically normal patients. For each specimen, the signal patterns of 200 nuclei were evaluated by counting the number of orange and green signals present for each probe target. Each technologist evaluated 100 nuclei per specimen for a total of 200 nuclei per specimen and 5000 scoreable nuclei from normal specimens.

The sensitivity (with 95% confidence intervals based on binomial distribution) was calculated as the percentage of scoreable interphase nuclei with the expected signal pattern of two signals per nucleus.

The Vysis CLL FISH Probe Kit has a sensitivity of 97.98% for the LSI TP53 SpectrumOrange probe, 98.68% for the LSI ATM SpectrumGreen probe, 98.60% for the LSI D13S319 SpectrumOrange probe, and 98.94% for the CEP 12 SpectrumGreen probe (Table 2).

Table 2. Analytical Sensitivity and Scoreable Percentage for Each Probe in the Vysis CLL FISH Probe Kit

Probe	Number of Interphase Nuclei		Sensitivity	
	With Expected Signal Pattern	Scoreable Signals	Percent (%)	95% Confidence Interval (%)
Vysis LSI TP53 SpectrumOrange	4899	5000	97.98	97.55, 98.35
Vysis LSI ATM SpectrumGreen	4934	5000	98.68	98.32, 98.98
Vysis LSI D13S319 SpectrumOrange	4930	5000	98.60	98.23, 98.91
Vysis CEP 12 SpectrumGreen	4947	5000	98.94	98.62, 99.21

Analytical Characterization of Normal Cut-off Values

The normal cut-off value, in association with FISH DNA probes, is defined as the maximum percentage of scoreable interphase nuclei with a specific abnormal signal pattern at which a specimen is considered normal for that signal pattern. The normal cut-off value is expressed in terms of a percentage or the actual number of abnormal nuclear FISH patterns per the standard number of nuclei tested. The criteria used to classify a nucleus as scoreable is located in the **Interpretation and Result Reporting** section of the package insert. The **Quality Control** and **Signal Enumeration** sections and the Dual Color Signal Counting Guide instruct the end user to determine whether an entire slide is adequate for signal enumeration and which types of cells/signals can be enumerated.

The normal cut-off values of the probes in the Vysis CLL FISH Probe Kit for their respective chromosome target loci were established using interphase nuclei prepared from peripheral blood cultures of 25 karyotypically normal patients. For each specimen, the signal patterns of 200 nuclei were evaluated by counting the number of orange and green signals present for each probe target.

The expected normal interphase signal pattern for all probes in the Vysis CLL FISH Probe Kit was two signals per nucleus. Since the specimen population does not fit a Gaussian distribution, the normal cut-off value was calculated using the beta inverse function.[9]

The Vysis CLL FISH Probe Kit was shown to have normal cut-off values of 14 out of 200 nuclei evaluated (7.0%) for the LSI TP53 SpectrumOrange probe (1 signal), 12 out of 200 nuclei evaluated (6.0%) for the LSI ATM SpectrumGreen probe (1 signal), 11 out of 200 nuclei evaluated (5.5%) for the LSI D13S319 SpectrumOrange probe (1 signal), 3 out of 200 nuclei evaluated (1.5%) for the LSI D13S319 SpectrumOrange probe (0 signal), and 5 out of 200 nuclei evaluated (2.5%) for the CEP 12 SpectrumGreen probe (3 signals) (Table 3).

Table 3. Analytical Characterization of Normal Cut-off Values

Probe (Abnormal Signal Pattern of Interest)	Number of Nuclei Evaluated (n)	Maximum Number of False-Positive Patterns	Normal Cut-off Value (per 200 nuclei)	Normal Cut-off Value (%)
Vysis LSI TP53 SpectrumOrange (1 signal)	200	8	14	7.0 (14/200)
Vysis LSI ATM SpectrumGreen (1 signal)	200	6	12	6.0 (12/200)
Vysis LSI D13S319 SpectrumOrange (1 signal)	200	5	11	5.5 (11/200)
Vysis LSI D13S319 SpectrumOrange (0 signal)	200	0	3	1.5 (3/200)
Vysis CEP 12 SpectrumGreen (3 signals)	200	1	5	2.5 (5/200)

Precision

The precision of the probes in the Vysis CLL FISH Probe Kit was established using interphase nuclei prepared from two separate peripheral blood specimens lacking del(17p13.1), del(11q22.3), del(13q14.3), and trisomy 12, and eight additional specimens of which at least two specimens had one of the listed abnormalities (Precision Study 1).

This blinded 10-member slide panel consisting of both negative and positive specimens was used to test three lots of Vysis LSI TP53 SpectrumOrange/ATM SpectrumGreen and LSI D13S319 SpectrumOrange/13q34 SpectrumAqua/CEP 12 SpectrumGreen Probes on each of two days, and one of the three lots on a third day.

For each specimen, the FISH signal patterns of 200 nuclei were evaluated by counting the number of orange and green signals present for each probe target.

Precision was analyzed separately for each specimen per probe using the percentage of abnormal cells with the signal pattern(s) of interest.

A blinded panel created from eight different patient specimens was tested using three different DNA FISH probe lots on three days (the days were not required to be consecutive) (Precision Study 2). Precision analyses to challenge the normal cut-off values were performed using the percentage of cells with the signal pattern of interest and were analyzed separately for each panel member.

Precision Study 1 and Precision Study 2 mean and standard deviations of the observed percentages of abnormal cells of the negative, positive, and specimens near the normal cut-off are shown in Tables 4-8.

Table 4. Precision Analysis of Percentages of Abnormal Signal Patterns for Vysis LSI TP53 SpectrumOrange Probe [del(17q13.1(1 signal))][a]

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

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

Table 5. Precision Analysis of Percentages of Abnormal Signal Patterns for Vysis LSI ATM SpectrumGreen Probe [del(11q22.3)(1 signal)][a]

Study	Sample	Category	n	Mean	Between-Day (Within Lot) Component SD	Between-Lot Component SD	Total SD[b]
1	1	Negative	7	1.6	0.54	0.59	0.80
1	2	Negative	7	1.8	0.59	0.00	0.59
1	3	Negative	7	2.6	0.81	1.54	1.74
1	4	Negative	7	2.1	2.25	1.08	2.50
1	5	Negative	7	2.9	1.71	1.64	2.37
1	6	Negative	7	1.1	1.34	0.00	1.34
1	7	Positive	7	64.1	3.18	0.00	3.18
1	8	Positive	7	13.7	4.31	5.10	6.68
1	9	Negative	7	1.6	0.99	0.00	0.99
1	10	Negative	7	1.5	1.51	0.00	1.51
2	1	Positive[c]	9	8.4	3.12	0.91	3.25
2	2	Positive[c]	9	7.8	3.00	1.89	3.55
2	8	Positive[c]	9	18.7	3.46	0.00	3.46

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

Table 6. Precision Analysis of Percentages of Abnormal Signal Patterns for Vysis LSI D13S319 SpectrumOrange Probe [del(13q14.3) (1 signal)][a]

Study	Sample	Category	n	Mean	Between-Day (Within Lot) Component SD	Between-Lot Component SD	Total SD[b]
1	1	Negative	7	3.5	1.12	1.10	1.57
1	2	Negative	7	2.2	1.69	0.00	1.69
1	3	Positive	7	66.1	6.13	0.00	6.13
1	4	Positive	7	20.1	4.88	0.00	4.88
1	5	Negative	7	0.6	0.83	0.00	0.83
1	6	Negative	7	0.4	0.64	0.00	0.64
1	7	Positive	7	84.9	4.14	0.00	4.14
1	8	Negative	7	0.6	0.97	0.00	0.97
1	9	Negative	7	2.3	2.08	0.00	2.08
1	10	Positive	7	53.6	8.57	0.00	8.57
2	5	Positive[c]	9	5.8	2.49	0.00	2.49
2	6	Positive[c]	9	14.9	2.46	1.15	2.72

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

Table 7. Precision Analysis of Percentages of Abnormal Signal Patterns for Vysis LSI D13S319 SpectrumOrange Probe [del(13q14.3) (0 signals)][a]

Study	Sample	Category	n	Mean	Between-Day (Within Lot) Component SD	Between-Lot Component SD	Total SD[b]
1	1	Negative	7	0.0	0.00	0.00	0.00
1	2	Negative	7	0.0	0.00	0.00	0.00
1	3	Negative	7	0.2	0.21	0.38	0.44
1	4	Negative	7	0.1	0.40	0.00	0.40
1	5	Negative	7	0.0	0.00	0.00	0.00
1	6	Negative	7	0.0	0.00	0.00	0.00
1	7	Negative	7	0.2	0.32	0.00	0.32
1	8	Negative	7	0.0	0.00	0.00	0.00
1	9	Negative	7	0.0	0.00	0.00	0.00
1	10	Positive	7	18.4	6.32	0.87	6.38
2	8	Positive[c]	9	19.8	2.53	0.00	2.53

[a] The mean and standard deviations are represented as percent abnormal signal patterns.

[b] Total variance is the sum of the other variance components.

[c] Positive specimen near the normal cut-off.

Table 8. Precision Analysis of Percentages of Abnormal Signal Patterns for Vysis CEP 12 SpectrumGreen Probe [trisomy 12 (3 signals)][a]

Study	Sample	Category	n	Mean	Between-Day (Within Lot) Component SD	Between-Lot Component SD	Total SD[b]
1	1	Negative	7	0.2	0.42	0.00	0.42
1	2	Negative	7	0.2	0.60	0.00	0.60
1	3	Negative	7	0.0	0.00	0.00	0.00
1	4	Negative	7	0.1	0.40	0.00	0.40
1	5	Positive	7	21.6	6.07	0.00	6.07
1	6	Positive	7	70.7	4.06	1.07	4.19
1	7	Negative	7	0.0	0.00	0.00	0.00
1	8	Positive	7	67.3	4.84	0.00	4.84
1	9	Negative	7	0.0	0.00	0.00	0.00
1	10	Negative	7	0.1	0.20	0.00	0.20
2	3	Positive[c]	9	8.3	2.77	0.00	2.77
2	4	Positive[c]	9	3.6	3.05	0.00	3.05

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

Reproducibility

Three individual laboratories tested a blinded 20-member slide panel consisting of specimens representing each of the five Döhner classifications:

- 13q- (monosomy or nullisomy) as sole abnormality
- No cytogenetic abnormality
- +12 without 17p- or 11q-
- 11q- without 17p-
- 17p-

The analysis was conducted with 10 slides per day for two days for a total of six runs across three testing sites. The same lots of Vysis LSI TP53 SpectrumOrange/ATM SpectrumGreen and LSI D13S319 SpectrumOrange/13q34 SpectrumAqua/CEP 12

SpectrumGreen Probes were tested at each laboratory. Results shown in Table 9 show an overall agreement with specimens representing each of the five Dohner classifications.

Table 9. Overall Agreement, Site to Site by Probe

	Disagree[a]			Overall		
Probe (Abnormality)	**Site 1**	**Site 2**	**Site 3**	**Number Agree[a]**	**Number Tested**	**Percent Agreement**
TP53 (17p-)	0	0	0	20	20	100
ATM (11q-)	2	0	0	18	20	90
CEP 12 (+12)	0	0	0	20	20	100
D13S319 1x (13q-)	2	0	0	18	20	90
D13S319 2x (13q-)	0	1	1	18	20	90

[a] Disagree = Number of specimens for which one's site result did not agree with the other sites' results.

Agree = Number of specimens for which all 3 sites agreed on results.

The reproducibility of the Vysis CLL FISH Probe Kit for each Prognostic Category demonstrated almost perfect agreement[14] using a generalized kappa statistic among the three testing sites (Table 10).

Table 10. Vysis CLL FISH Probe Kit Reproducibility Based on Generalized Kappa Statistic

	Prognostic Category	
Sample	**Intermediate/Good Prognosis[a]**	**Poor Prognosis[a]**
1	3	0
2	3	0
3	3	0
4	3	0
5	3	0
6	3	0
7	3	0
8	3	0
9	3	0
10	3	0
11	3	0
12	3	0
13	0	3
14	1	2
15	0	3
16	1	2
17	0	3
18	0	3
19	0	3
20	0	3
Kappa	0.86	
Strength	Almost Perfect[14]	

[a] Number of sites based on Prognostic Category.

Additionally, the data were analyzed by site and probe using the Fisher's Exact Test (Tables 11 and 12).

Table 11. Vysis CLL FISH Probe Kit Reproducibility by Site Based on Fisher's Exact Test

Site	Prognostic Category	
	Intermediate/Good[a]	Poor[a]
1	12	8
2	12	8
3	14	6
p-value	0.8396	

[a] Number of panel members based on Prognostic Category.

Table 12. Vysis CLL FISH Probe Kit Reproducibility by Probe Based on Fisher's Exact Test

Probe (Abnormality)	Site	Abnormality Detected	No Abnormality Detected	*p*-value
TP 53 (17p-)	1	4	16	1.0000
	2	4	16	
	3	4	16	
ATM (11q-)	1	2	18	0.7495
	2	4	16	
	3	4	16	
D13S319 1X (13q- [x1])	1	6	14	0.8396
	2	8	12	
	3	8	12	
D13S319 2x (13q- [x2])	1	2	18	1.000
	2	3	17	
	3	3	17	
CEP 12 (+12)	1	5	15	1.000
	2	5	15	
	3	5	15	

Clinical Utility

The traditional Rai and Binet clinical CLL staging systems are based on disease burden and have been useful for assigning patients to groups having similar survival times.[10,11] These systems, however, are not effective in predicting survival in early-stage disease when most CLL cases are diagnosed. This has led to development of newer molecular markers in an attempt to differentiate those patients who are prone to rapid progression from those who have indolent disease.

In a pivotal study conducted by Döhner et al, titled "Genomic Aberrations and Survival in Chronic Lymphocytic Leukemia," genomic alterations as determined by FISH were found to be predictive for disease progression and overall survival.[5] Multiple studies support the conclusion of Döhner et al that loss of 17p and of 11q markers predicts reduced survival times as compared to other Döhner groups as determined by FISH aberrations.[6-8] Such studies have led to the inclusion of FISH testing in the National Comprehensive Cancer Network (NCCN) practice guidelines as a means to determine CLL prognosis.[12]

In a 2006 prospective study of 151 patients by Shanafelt, et al. utilizing Vysis FISH probes, a correlation was established between overall survival and FISH risk category for CLL at diagnosis.[8] Patients were divided into two prognostic groups. They were assigned to the good/intermediate FISH prognosis group if there were no chromosomal aberrations or if only 13q- and/or +12 aberrations were present. If a chromosomal aberration of 17p- or 11q- was present, the patient was placed in the poor FISH prognosis group. Poor vs. good/intermediate FISH (P=0.004), age at diagnosis (P=0.0006), and Rai stage (P=0.0026) were each significantly associated with overall survival from diagnosis in univariate analysis. When all factors were included in multivariable Cox regression model, each of three factors still remained significant: poor vs. good/intermediate FISH (P=0.00022), age at diagnosis (P=0.000024); and Rai stage (P=0.00012).

The clinical utility of the Vysis CLL FISH Probe Kit has been established primarily from its high concordance with the assay employed in the publication of Shanafelt et al.[8]

Also, as noted in the Shanafelt study, all patients with the 17p- abnormality had between 24-94% of cells with this abnormality.[8] Therefore, the effect of 17p- at very low levels could not be determined. In a recent publication on untreated 17p- CLL patients, Tam et al reported a 3-year overall survival of 92% for patients with < 25% 17p-deleted nuclei, vs. 54% for patients with ≥ 25% 17p-deleted nuclei (P=0.007).[12]

The NCCN CLL practice guideline (v.2.2011) states that prognostic CLL FISH abnormalities noted on page CLLS-A of that document (including abnormalities tested for by this kit) may not have the noted clinical prognostic impact when present in less than 10% of cells.[13]

Method Concordance

This study establishes the clinical validity of the AMT by demonstrating concordance to the Reference FISH Test (RFT) used in the Shanafelt study.[8] The clinical validity of the RFT is documented via peer-reviewed literature.

This study analyzed 64 specimens whose Döhner classifications were based on previous results using the RFT. Table 13 shows the distribution of specimens by Döhner classification.

Table 13. Döhner Classification of Specimens Analyzed in Concordance Study

Döhner Classification	Number of Specimens Analyzed
13q- (monosomy or nullisomy) as sole abnormality	13
No cytogenetic abnormality	12
+12 without 11q- or 17p-	14
11q- without 17p-	18
17p-	7

The specimens were de-identified and randomized before testing was conducted with the AMT and the RFT.

A Döhner classification was assigned to each AMT and RFT result, and the Prognostic Category was determined.

The percent agreement between the AMT and RFT for Prognostic Category was 97% (62/64) with a lower bound of the one-sided 95% confidence interval of 90% (Table 14). Overall agreement was defined as the percentage of specimens that had the same Prognostic Category when testing using the RFT and the AMT.

Table 14. Concordance between AMT and RFT for Prognostic Category[a]

	Reference FISH Test (RFT)		
Abbott Molecular Test (AMT)	**Intermediate/Good**	**Poor**	**Total**
Intermediate/Good	38	1	39
Poor	1	24	25
Total	39	25	64

[a] The values in this table represent the number of specimens.

References

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

Abbott Molecular Inc.
c/o Ms. Nancy Bengtson
Manager, Global Regulatory Affairs
1300 E. Touhy Avenue
Des Plaines, IL, 60018

AUG 0 9 2011

Re: k100015

Trade/Device Name:	Vysis CLL FISH Probe Kit
Regulation Number:	21 CFR §866.6040
Regulation Name:	Gene expression profiling test system for breast cancer prognosis
Regulatory Class:	Class II
Product Code:	OVQ
Dated:	July 21, 2011
Received:	July 22, 2011

Dear Ms. Bengtson:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed

predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Maria M. Chan

Maria M. Chan, Ph.D
Director
Division of Immunology and Hematology Devices
Office of *In Vitro* Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use Statement

510(k) Number: K100015

Device Name: Vysis CLL FISH Probe Kit

Indications for Use:

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

Prescription Use X (Part 21 CFR 801 Subpart D) AND/OR Over-The-Counter Use _____ (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE OF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) K100015