510(k) Summary

AUG 2 9 2011

510(k) Number K091960

Device Name: Vysis EGR1 FISH Probe Kit (Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes) (List No. 4N37-020)

Purpose of the Submission

The purpose of this 510(k) is to gain clearance to market the Vysis EGR1 FISH Probe Kit (List No. 4N37-020).

Official Correspondent to the File

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Date of Preparation

August 25, 2011

Manufacturer

Abbott Molecular Inc. is the legal manufacturer of the Vysis EGR1 FISH Probe Kit (List No. 4N37-020).

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Intended Use

The Vysis EGR1 FISH Probe Kit is intended to detect deletion of LSI EGR1 probe target on chromosome 5q in bone marrow specimens and to be used, in addition to cytogenetics, other biomarkers, morphology and other clinical information, at the time of acute myeloid leukemia (AML) diagnosis as an aid in determining prognosis. Deletion of chromosome 5q has been associated with an unfavorable prognosis in AML patients.

Trade Name

Vysis EGR1 FISH Probe Kit (Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes) (List No. 4N37-020)

Common Name

Vysis EGR1 Fluorescence In Situ Hybridization (FISH) Probe Kit

Classification

Class II

Regulation Number

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

Product Code

OWK, Early growth response 1 (EGR1) kit

Predicate Device(s)

81, Immunology

Comparison with Predicate Device(s)

Similarities

Item	Device	Predicate (k100015)
Intended Use	Determination of deletion status as an aid in determining prognosis	Same
Technology	FISH	Same

Differences

Item	Device	Predicate (k100015)
Patient Population	Acute myeloid leukemia (AML) patients	Chronic lymphocytic leukemia (CLL) patients
Probe Targets	LSI EGR1	LSI TP53 LSI ATM LSI D13S319 CEP 12

Device Description

The Vysis EGR1 FISH Probe Kit uses fluorescence in situ hybridization (FISH) DNA probe technology to determine deletion status of the LSI EGR1 (containing early growth response 1 gene; location chromosome 5q31) probe target in AML specimens. The Vysis EGR1 FISH Probe Kit also contains the LSI D5S23, D5S721 probe (location chromosome 5p15.2) and serves as a control.

DNA Probe Description

Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes:

The SpectrumOrange-labeled LSI EGR1 probe, approximately 209 kb in length (chr5:137682107-137890637; March 2006 Assembly; UCSC Human Genome Browser[1]), is located at 5q31 and contains the complete EGR1 gene.

The SpectrumGreen-labeled LSI D5S23, D5S721 probe, approximately 561 kb in length (chr5:9450109-10011407; March 2006 Assembly; UCSC Human Genome Browser[1]), is located at 5p15.2.

The Vysis EGR1 FISH Probe Kit (List No. 4N37-020) consists of one vial containing two DNA FISH probes and four general purpose reagents sufficient to process 20 specimens.

- Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes
- Vysis LSI/WCP Hybridization Buffer
- DAPI II Counterstain
- NP-40
- 20X SSC Salt

Background on Acute Myeloid Leukemia (AML) Disease

Deletion of chromosome 5q as detected by cytogenetics is a recurring abnormality in AML. A commonly deleted segment on chromosome band 5q31 has been identified and the early growth response 1 (EGR1) gene is among the candidate genes in this segment.[2] A study suggests that haploinsufficiency of EGR1 may play a role in leukemogenesis.[3] The Vysis locus-specific identifier (LSI) EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes, components of the Vysis EGR1 FISH Probe Kit, have been used in several studies to detect EGR1 deletions.[4-7] A study, conducted as part of an Eastern Cooperative Oncology Group (ECOG) clinical trial, demonstrated the utility of the interphase fluorescence in situ hybridization (FISH) technique to stratify patients into cytogenetic risk categories at diagnosis of AML using the Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes in conjunction with several additional FISH probes.[4] In addition, the prognostic importance of chromosome 5 abnormalities has been established in several large clinical studies.[8,9] The prognostic clinical utility of detecting specific chromosomal abnormalities in bone marrow specimens from patients diagnosed with AML is firmly established in both standard medical practice guidelines and in the medical literature. The National Comprehensive Cancer Network (NCCN) Practice Guidelines™ for Acute Myeloid Leukemia, which are

the consensus recommendations of leading US AML experts, states that cytogenetics is the single most important prognostic factor in AML.[10] More specifically, the guidelines provide different risk categories for patients depending upon cytogenetic or molecular abnormalities. As an example, patients with deletion of chromosome 5q are in the 'poor risk' category and may have an unfavorable prognosis.

The Vysis EGR1 FISH Probe Kit uses FISH DNA probe technology to determine deletion status of the probe target for LSI EGR1, and the LSI D5S23, D5S721 probe serves as a control.

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.

Cells attached to microscope slides using standard cytogenetic procedures are used for this assay. The resulting specimen DNA is denatured to single-stranded form and subsequently allowed to hybridize with the LSI EGR1 and LSI D5S23, D5S721 probes. Following hybridization, the unbound probe is removed by a series of washes, and the nuclei are counterstained with DAPI, a DNA-specific stain that fluoresces blue. Hybridization of the LSI EGR1 and LSI D5S23, D5S721 probes is viewed using a fluorescence microscope equipped with appropriate excitation and emission filters, allowing visualization of the orange and green fluorescent signals. In a cell with normal copy numbers of the LSI EGR1 and LSI D5S23, D5S721 probe targets, two SpectrumOrange signals (LSI EGR1) and two SpectrumGreen signals (LSI D5S23, D5S721) will be expected.

In a cell with the 5q deletion, one SpectrumOrange signal (LSI EGR1) and two SpectrumGreen signals (LSI D5S23, D5S721) will be expected. Enumeration of the orange LSI EGR1 and green LSI D5S23, D5S721 signals provide a mechanism for determining absolute copy number of the probe targets and the presence of the aberrations of interest.

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. The analytical specificity of the Vysis LSI EGR1 D5S23, D5S721 probes for their respective chromosome target loci was established using metaphase chromosomes prepared from peripheral blood cultures of five karyotypically normal males that were pooled prior to dropping on microscope slides. The hybridization location of each FISH signal on chromosomes of 100 consecutive metaphase nuclei 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 analytical 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 of the Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes was 100%, as shown in Table 1.

Table 1. Analytical Specificity

Probe	Number of Metaphase Chromosome Signals: Hybridized to the Correct Target Locus	Number of Metaphase Chromosome Signals: Total Hybridized Signals	Analytical Specificity (%)	95% Confidence Interval (%)
D5S23, D5S721	200	200	100	(98, 100)
EGR1	200	200	100	(98, 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 the probes in the Vysis EGR1 FISH Probe Kit is 2R2G per nucleus.

The analytical sensitivity of the Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes was established using interphase nuclei prepared from 25 bone marrow specimens that were either karyotypically normal or 5p15 and 5q31 deletion-free. The orange and green signal patterns of nuclei for 25 specimens were evaluated by two technologists. Each technologist evaluated 100 nuclei per specimen for a total of 200 nuclei per specimen and 5000 scoreable nuclei from normal specimens.

The analytical sensitivity was calculated as the percentage of scoreable interphase nuclei with the expected 2R2G signal pattern at the 95% confidence interval.

The Vysis EGR1 FISH Probe Kit has an analytical sensitivity of 99.6%, as shown in Table 2.

Table 2. Analytical Sensitivity

Probe Kit	Number of Interphase Chromosome Signals		Analytical Sensitivity (%)	
	With Expected Signal Pattern	Scoreable Signals	Point Estimate	95% Confidence Interval
LSI EGR1/D5S23, D5S721	4979	5000	99.6	(99.4, 99.7)

Verification of Normal Cut-off

The normal cut-off value is defined as the maximum quantity 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 a specific abnormal FISH signal pattern per the standard number of nuclei tested.

The normal cut-off value for this assay is 6% or 12 1R2G patterns per 200 scoreable interphase nuclei. Specimens exceeding 12 1R2G patterns per 200 scoreable nuclei are considered abnormal for deletion of the Vysis LSI EGR1 probe target. This 6% normal cut-off value was adopted from the publication of Vance et al, who utilized the Vysis LSI ERG1/D5S23, D5S721 probe set in a study that established a high level of agreement between cytogenetics and FISH in 237 blood and bone marrow specimens studied at AML diagnosis.[4]

In order to confirm that the 6% normal cut-off served well to prevent normal specimens from being called abnormal, the assay was performed on interphase nuclei from 25 bone marrow specimens from either karyotypically normal specimens or 5p15.2 and 5q31 deletion-free specimens. The signal patterns of 200 nuclei were evaluated by counting the number of orange and green signals. Each of two technologists evaluated 100 nuclei per specimen. Among the 25 normal specimens, none produced 1R2G signals at or above the 6% normal cut-off.

Reproducibility

Two replicates of the assay were run on 2 high-positive, 2 low-positive, and 2 normal specimens at three sites on 5 different days. The positive specimens for the site-to-site study were obtained by mixing positive bone marrow cells with normal bone marrow cells to obtain the desired levels of abnormality. The mean and the standard deviation of the percentage of cells with the 1R2G signal pattern was calculated. Results shown in Table 3 show the overall agreement with the normal/abnormal status of the test specimens. All sites obtained 100% agreement with the known status of all 6 specimens on all 5 days, except one site which had one discordant result for a normal specimen.

Table 3. Overall Agreement, Site to Site

	Number			
Category	**Agree**	**Disagree**	**Total**	**Percent Agreement**
High Positive	60	0	60	100
Low Positive	60	0	60	100
Normal	59	1	60	98

The analysis of variance components for the site-to-site study is shown in Table 4.

Table 4. Site-to-Site Analysis of Variance Components

Sample	**N**	**Mean[a]**	**Within Day SD[b]**	**Between Day SD**	**Between Site SD**	**Total SD**
High Positive 1	30	70.0	3.28	4.01	5.44	7.51
High Positive 2	30	47.6	5.56	0.00	0.74	5.61
Low Positive 1	30	18.1	3.00	3.82	1.03	4.97
Low Positive 2	30	14.9	3.25	1.54	0.00	3.59
Normal 1	30	0.7	0.71	0.00	0.68	0.99
Normal 2	30	0.9	0.66	1.42	0.22	1.59

[a] Percentage of cells with 1R2G signal pattern
[b] SD = Standard deviation

Using the same specimens from the site-to-site study, four replicates of the assay were run on 2 high-positive, 2 low-positive, and 2 normal specimens using 3 different lots of probe at a single site. The overall agreement with the known normal/abnormal status of the test specimen is shown in Table 5. All replicates using the three probe lots for each of the 6 specimens produced agreement with the known status of the specimens.

Table 5. Overall Agreement, Lot to Lot

	Number			Percent
Category	**Agree**	**Disagree**	**Total**	**Agreement**
High Positive	24	0	24	100
Low Positive	24	0	24	100
Normal	24	0	24	100

The analysis of variance components for the lot-to-lot study is shown in Table 6.

Table 6. Lot-to-Lot Analysis of Variance Components

Sample	N	Mean[a]	Within Lot SD[b]	Between Lot SD	Total SD
High Positive 1	12	66.2	7.19	0.00	7.19
High Positive 2	12	47.4	3.69	3.04	4.78
Low Positive 1	12	12.7	4.29	0.00	4.29
Low Positive 2	12	12.3	1.84	1.12	2.15
Normal 1	12	0.0	0.00	0.00	0.00
Normal 2	12	0.1	0.14	0.20	0.25

[a] Percentage of cells with 1R2G signal pattern
[b] SD = Standard deviation

In these reproducibility studies, 84 assays were run on low positive specimens. None of the 84 low-positive assays resulted in a 1R2G signal pattern at or below 6%.

Clinical Utility

The clinical utility of the cytogenetic detection of the deletion of chromosome 5q is correlated with reduced 5-year overall survival in studies by Byrd et al[8] and Grimwade et

al[9]. The Byrd publication demonstrated the prognostic value in 86 patients with a -5/5q- abnormality by a median overall survival (OS) of 0.3 years compared to the median OS of 1.3 years associated with patients exhibiting a normal karyotype. Patients with a -5/5q- abnormality had a 5-year OS of 6% compared to a 5-year OS of 24% associated with patients exhibiting a normal karyotype. Patients with a -5/5q- abnormality also had a significantly lower complete remission (CR) rate of 31% than the normal karyotype, which resulted in a CR of 68% with a $p<0.001$. For del(5q), 42 patients had a 5-year OS of 5%, 95% CI (2-13%) and median overall survival of 0.3 years.

Grimwade et al, showed prognostic value in 28 patients for 5q-. Cytogenetic abnormalities in the Medical Research Council (MRC) AML 10 clinical trial for del(5q) patients showed 5-year OS of 11% and CR of 57% in the adverse risk group. These values varied significantly from the no abnormality, or normal karyotype group, which had 5-year OS of 42% and CR of 88% ($p<0.001$).

The publication "Utility of interphase FISH to stratify patients into cytogenetic risk categories at diagnosis of AML in an Eastern Cooperative Oncology Group (ECOG) clinical trial (E1900)" by Vance et al[4], establishes linkage between cytogenetic results and the Vysis EGR1 FISH Probe kit. When 181 bone marrow specimens were compared to cytogenetic results at the >6% cut-off, there was overall agreement of 98.90% (179/181) (95% CI-96.06%-99.70%), negative percent agreement of 100% (171/171) (95% CI-97.80%-100.00%) and positive percent agreement of 80% (8/10) (95% CI-49.02%-94.33%). Results are presented below:

		Karyotype -5/del5q		
		Positive	Negative	Total
FISH (1R2G- 5q deletion signal pattern)	Positive	8	0	8
	Negative	2[a,b]	171	173
	Total	10	171	181

[a] Cytogenetic result was -5/del(5q). FISH signal pattern was 44% 1R1G (monosomy of chromosome 5).
[b] Cytogenetic result was -5/del(5q). FISH signal pattern was 1% 1R2G. False negative results.

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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AUG 29 2011

Re: k091960
Trade/Device Name: Vysis EGR1 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: OWK
Dated: February 24, 2011
Received: February 25, 2011

Dear Ms. Swatkowski:

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 Chan, PhD
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: K091960

Device Name: Vysis EGR1 FISH Probe Kit (Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probe)

Indications for Use:

The Vysis EGR1 FISH Probe Kit is intended to detect deletion of the LSI EGR1 probe target on chromosome 5q in bone marrow specimens and to be used, in addition to cytogenetics, other biomarkers, morphology and other clinical information, at the time of acute myeloid leukemia (AML) diagnosis as an aid in determining prognosis. Deletion of chromosome 5q has been associated with an unfavorable prognosis in AML patients.

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) K091960