

SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. GENERAL INFORMATION

Device Generic Name: DakoCytomation Mouse Anti-Human EGFR Immunohisto-chemistry Kit

Device Trade Name: DakoCytomation EGFR pharmDx™

Applicant Name and Address: DakoCytomation California, Inc.
6392 Via Real
Carpinteria, CA 93013

Premarket Approval Application Number: P030044

Date of Panel Recommendation: None

Date of Notice of Approval to the Applicant: February 12, 2004

II. INDICATIONS FOR USE

The EGFR pharmDx™ assay is a qualitative immunohistochemical (IHC) kit system to identify epidermal growth factor receptor (EGFR) expression in normal and neoplastic tissues routinely-fixed for histological evaluation. EGFR pharmDx specifically detects the EGFR (HER1) protein in EGFR-expressing cells.

EGFR pharmDx is indicated as an aid in identifying colorectal cancer patients eligible for treatment with ERBITUX™ (cetuximab).

III. CONTRAINDICATIONS: None

IV. WARNINGS AND PRECAUTIONS

Warnings and Precautions for use of the device are stated in the product labeling.

V. DEVICE DESCRIPTION

The DakoCytomation EGFR pharmDx™ assay is a standard immunohistochemical (IHC) kit that specifically detects the epidermal growth factor receptor (EGFR) gene product expressed on the cell surface of normal tissues and tumors.

The DakoCytomation EGFR pharmDx™ kit contains 8 vials of immunohistochemical reagents, one bottle of wash buffer, and 5 control slides bearing two formalin-fixed, paraffin-embedded cell line preparations. EGFR positive and negative cell lines are provided on each slide to be used as performance controls for the kit reagents.

The kit is available in manual and automated configurations.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently no other in vitro diagnostic devices indicated for assessment of patients suffering from colorectal cancer considered for EGFR targeted therapy.

VII. MARKETING HISTORY

The DakoCytomation EGFR pharmDx™ assay is currently marketed as a standard, immunohistochemical assay intended to identify EGFR expression in routinely processed normal and neoplastic tissue specimens in the United States, Europe, Japan, and South America. Less than 2000 kits have been distributed world wide since the summer of 2002. The device has not been withdrawn from any market for any reason of safety and effectiveness.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The DakoCytomation EGFR pharmDx™ assay is indicated as an aid in determining treatment with EGFR targeted therapy. Patients falsely assigned as positive following assessment would be considered eligible for treatment. Because the design of the clinical studies did not include treatment of patients with negative assay results, the risks or benefits of treatment in this patient population are unknown. The risks of Erbitux™ treatment to EGFR-positive patients included mild, moderate and severe infusion reactions, low incidence of pulmonary toxicity, and dermatologic toxicity.

Patients falsely assigned as negative would not be considered eligible for treatment based on their EGFR status.

IX. SUMMARY OF PRECLINICAL STUDIES

Preclinical testing of the DakoCytomation EGFR pharmDx™ assay included optimization, selection, verification, and validation of device reagents, reagent stability/shelf life, specificity, and reproducibility studies.

1. Optimization, Selection, Verification, and Validation of the Kit Contents.

The objective of these studies was to optimize test performance.

Optimal Specimen Pre-treatment

Enzymatic pre-treatment was compared with heat induced epitope retrieval (HIER) in immunohistochemical studies on a variety of formalin-fixed, paraffin-embedded tissues and cell lines. The results demonstrated that optimal performance was obtained by incubating the specimen slide with <0.1% Proteinase K for 5 minutes, which was incorporated into the test procedure.

Secondary Staining System

The commercially available visualization systems, EnVision+, was chosen as the secondary staining system because it required fewer steps in the process, and the EnVision polymer has no biotinylation step. This reduces the potential for background staining from endogenous

biotin. The secondary EnVision+ staining system was selected for incorporation into the EGFR assay due to design criteria.

Primary Antibody Selection

Two antibodies were evaluated for EGFR pharmDx™. The antibodies were evaluated for IHC staining of EGFR (specificity) and the ability to provide immunostaining scores across levels of EGFR expression (sensitivity). Clone 2-18C9 was selected after immunohistochemical comparisons demonstrated that the antibody specificity was similar to other commercially available antibodies to EGFR.

Additional flow cytometric and Western Blotting analyses were performed to determine the specificity and sensitivity of the chosen primary antibody (Clone 2-18C9). In Western blots of SKBR3 and A431 cell lysates, 2-18C9 recognized a 170 kD band which is consistent with the known molecular weight of EGFR. Clone 2-18C9 has also been found to recognize the EGFRvIII (145 kD) form of the receptor in immunohistochemistry, flow cytometry and Western blotting of EGFRvIII transfected cell lines. In Western blotting experiments, 2-18C9 was unreactive with HER2 positive CAMA-1 cell lysates, HER3-transformed *E. coli* BL-21 protein extracts and CHO-HER4 transfected cell lysates. Additionally, Chinese Hamster Ovary (CHO) transfectants expressing myc (vector tag), either alone or coexpressed with one of the HER family members, were grown in chamber slides that were formalin-fixed and paraffin-embedded, and stained with anti-myc and 2-18C9. The myc antibody stained all five CHO transfectants, whereas 2-18C9 only stained the CHO cells transfected with HER1. This further demonstrated that the 2-18C9 antibody reacts specifically with HER1 (EGFR) and does not cross-react with the other closely related growth factor receptors, HER2, HER3 or HER4, as well as the vector tag, myc.

Assay Design Verification

The assay component configuration was verified to the criteria set forth in the project design specifications. The assay design verification testing demonstrated that EGFR pharmDx™ performs to the established design specifications for: staining sensitivity, staining specificity to the targeted EGFR molecule, precision, and assay reagent working stability under typical laboratory conditions.

Assay Validation

Subsequent to the verification of the EGFR assay design, three manufacturing lots of the DakoCytomation EGFR pharmDx™ assay were produced to validate the established performance characteristics of the final kit configuration.

The results of the assay validation testing demonstrated that the final design configuration of DakoCytomation EGFR pharmDx™ assay performs to the established specifications with regard to sensitivity at various levels of EGFR expression, staining specificity to the targeted EGFR molecule, precision of staining scores, working assay reagent stability under normal laboratory conditions, and the robustness of the assay when performed at maximum and

minimum time tolerances established for the procedure.

2. Device Stability/Shelf Life.

The objective of this study was to determine the expiration date of the kit.

The shelf lives of the assay components (DakoCytomation EnVision+ visualization system, Wash Buffer, Proteinase K, and Peroxidase Block) were established during the development of the individual products. In addition, a real time shelf life and stability study for the DakoCytomation EGFR pharmDx™ finished product configuration was conducted.

Three validation lots of DakoCytomation EGFR pharmDx™ were stored at 2 – 8°C and tested at 3-month intervals. Results of this testing indicated that the test kit was stable for up to 6 months at 2° - 8°C.

3. Antibody Specificity - Normal Tissue Testing

To further establish the specificity of the EGFR pharmDx test, normal tissue testing was performed in accordance with the FDA Guidance Document For Immunohistochemical Product Premarket Submissions (June 6, 1998). Thirty normal tissues from three independent sources were embedded into multi-tissue blocks and evaluated using EGFR pharmDx. A summary of the EGFR pharmDx immunoreactivity on the recommended panel of normal tissues is presented in the following table.

Evaluation of Normal Tissue Staining by DAKO EGFR pharmDx™

<i>Tissue Type (# tested)</i>	<i>Positive Tissue Element Staining and Staining Pattern</i>
Adrenal (2)	Cortical cells (2+): Cytoplasmic
Bone Marrow (3)	None
Breast (2)	Lobular epithelial cells (2+): Membrane and cytoplasmic
Brain/Cerebellum) (3)	Molecular layer (1+): extracellular
Brain/Cerebrum (3)	None
Cervix (3)	Basalar squamous epithelial cells (2+): Membrane
Colon (3)	None
Esophagus (2)	Basalar squamous epithelial cells (2+): Membrane
Heart (3)	None
Kidney (3)	Tubules (1+): Cytoplasmic staining (granular)
Liver (3)	Hepatocytes (sinusoids) (3+):Membrane Bile ducts (3+): Membrane and cytoplasmic
Lung (3)	Alveolar lining cells/ basalar bronchial cells (myoepithelial cells) (2+): Membrane and Cytoplasmic
Mesothelial Cells (3)	Mesothelial cells (2+): Membrane & cytoplasmic
Ovary (3)	None

<i>Tissue Type (# tested)</i>	<i>Positive Tissue Element Staining and Staining Pattern</i>
Pancreas (3)	Ducts (2+): Membrane
Parathyroid (1)	None
Peripheral Nerve (3)	Nerve cell processes (1+): Fibrous
Pituitary (3)	None
Prostate (3)	Glandular epithelial cells (2+): Membrane
Salivary Gland (3)	Ductal elements (1+): Cytoplasmic
Skeletal Muscle (3)	None
Skin (3)	Squamous cells, adnexal structures (2+): Membrane and cytoplasmic
Small Intestine (3)	None
Spleen (3)	None
Stomach (3)	None
Testis (3)	None
Thymus (3)	None
Thyroid (3)	None
Tonsil (3)	Squamous epithelium (3+): Membrane and cytoplasmic
Uterus (3)	Endometrial gland epithelium (2+): Membrane and cytoplasmic Endometrial stromal cells (2+): Membrane and cytoplasmic Myometrium: None

*The majority of tissues tested had positive staining of fibroblasts in stromal tissue (1+, fibrous) as well as perineural fibroblasts and myoepithelial cells. Endogenous peroxidase-induced staining of eosinophils has been observed occasionally.

Conclusion. DakoCytomation has met one of the requirements set forth in accordance with the FDA Guidance Document For Immunohistochemical Product Premarket Submissions (June 6, 1998). They have characterized the performance of this product with thirty normal tissues from three independent sources as can be seen in the preceding table.

X. REPRODUCIBILITY

Inter-run reproducibility

The protocol was designed to demonstrate DakoCytomation EGFR pharmDx™ intra-laboratory reproducibility (within day and between technicians) of staining results. Inter-run reproducibility was tested using manual methodology at two laboratories by two technicians in each laboratory over 3 days with 5 different specimens (4 positive, 1 negative in each lab), of different staining intensity scores randomized and masked. Excellent reproducibility (100%) was seen for positive versus negative results (0 vs. 1+, 2+ and 3+). Staining intensity varied by 1+ in two of the positive specimens and by 2+ in one specimen (in one of the tests, the positive tissue element was mostly washed off the slide). The two negative specimens remained negative. In conclusion, EGFR pharmDx™ provided reproducible results between days and between technicians within each of two laboratories.

Inter-laboratory reproducibility of staining

The protocol was designed to demonstrate DakoCytomation EGFR pharmDx™ reproducibility between laboratories using the manual and automated procedures. Inter-laboratory reproducibility was tested at three geographically separated laboratories with 30 randomized and masked specimens of various IHC staining intensity scores. Freshly cut slides were forwarded to each testing laboratory for manual and automated staining and evaluation by a pathologist. Inter-laboratory percent agreement was 100% for a dichotomous positive/negative determination where zero (0) was negative and 1+, 2+ and 3+ were positive for EGFR protein expression for both manual and automated testing procedures. This study, thus, demonstrated agreement in reproducibility of results between three laboratories and concordance between manual and automated assay procedures.

XI. SUMMARY OF CLINICAL STUDIES

The DakoCytomation EGFR pharmDx test system was used in three cetuximab drug clinical trials. A positive test result in patients with colorectal cancer enabled enrollment in the drug trials.

Objectives Of The Studies

The primary objective of the clinical trials was to demonstrate that the DakoCytomation EGFR pharmDx test is useful for choosing patients to receive Erbitux™ (cetuximab) therapy. If the clinical drug trials demonstrated that DakoCytomation EGFR-positive colon cancer patients treated with Erbitux™ had a clinically significant improvement in response rate compared to those who do not receive Erbitux™ therapy, then it was concluded that the DakoCytomation test is a useful adjunct to Erbitux™ therapy.

A secondary objective of the clinical trials was to evaluate scoring elements of the assay to decide where to define the cutoff between EGFR positive vs. negative results.

Study 1 –Pivotal Clinical Drug Trial - All samples were tested at the Institute fur Pathologie, Klinikum Barmen, Wuppertal, Germany.

Study 2 -Phase 2 Supportive Study - All samples were tested at the IMPATH Laboratories, Los Angeles, CA.

Study 3 - Supportive Study with Prototype EGFR Assay - All samples were tested at the IMPATH Laboratories, Los Angeles, CA.

Study Populations

Study 1 –Pivotal Clinical Drug Trial - In the pivotal trial (EMD 62202-007), patients with EGFR pharmDx positive test results were treated with cetuximab in combination with irinotecan or with cetuximab alone. 577 tumor specimens were tested. Four hundred seventy-four (474/577) (82%, 95% CI = 78.1%, 86.1%) of the colorectal carcinoma specimens tested were positive for EGFR pharmDx expression. Three hundred twenty-nine (329) EGFR pharmDx patients were available for 2:1 randomization to the two arms of the pivotal drug trial.

Study 2 -Phase 2 Supportive Study - In one supportive study (IMCL CP02-0141) during cetuximab development, 140 specimens were tested. Of these specimens, 105 /140 (75%, 95% CI = 66.9%, 83.1%) specimens had tumors that were identified as EGFR pharmDx positive. A total of 61 patients were enrolled in this study; 57 patients received cetuximab.

Study 3 - Supportive Study with Prototype EGFR Assay - In an additional supportive study (IMCL CP02-9923), a prototype EGFR pharmDx assay (composed of the same primary antibody and detection system as above), was used to enroll patients. A total of 412 specimens from 401 patients were tested. 292/401 (72.8%, 95% CI = 68.0%, 77.6%) patients had a positive test result. 139 patients were enrolled; 138 received cetuximab plus irinotecan.

All of these studies taken together help to characterize the percent positivity to be expected of an acceptable EGFR immunohistochemistry test.

Summary of EGFR Percent Positivity in Colon Cancer Patients

Study ID	Positive Ratio (# positive/# tested)	% Positive	95% Confidence Intervals
Pivotal Trial EMD 62202-007	474/577	82.1%	78.1 – 86.1%
Supportive Study IMCL CP02-0141	105/140	75.0%	66.9 – 83.1%
Prototype EGFR Study IMCL CP02-9923	292/401	72.8%	68.0 – 77.6%

Study demographics and baseline characteristics

The demographic variables for enrolled cases were similar in the 3 studies. The median age was between 56 and 60 years. There was a higher proportion of men than women, but the distributions of males and females were similar in all studies. Ethnicity was predominately white.

XII. STUDY SUMMARIES

Study 1 –Pivotal Clinical Drug Trial

Study 1 was an open label trial of metastatic colorectal carcinoma patients with progressive disease during or after treatment with irinotecan. Patients were randomized to one of two treatment groups, irinotecan plus cetuximab or cetuximab alone.

In this study, a positive test result was defined as complete or incomplete circumferential membrane staining at any intensity ($\geq 1+$ intensity in $>1\%$ of tumor cells). In this trial, patients who received irinotecan plus cetuximab achieved a response rate of 50/218 (22.9%, 95% CI = 17.5%, 29.1%). Patients who received cetuximab alone achieved a response rate of 12/111 (10.8%, 95% CI = 5.7%, 18.1%). Only DakoCytomation EGFR positive persons received cetuximab treatment. The response rate for EGFR negative persons is unknown, and therefore cannot be compared. There was no correlation between the degree of tumor response and the percentage of EGFR-positive cells or EGFR-staining intensity.

Cetuximab Pivotal Trial (EMD 62202-007) Response Rates

	<i>Total number of patients tested</i>	<i>Response Rate[#] of cases treated with cetuximab and irinotecan</i>	<i>Response Rate[#] of cases treated with cetuximab alone</i>
EGFR pharmDx +	474*	50/218 (22.9%, 95% CI = 17.5%, 29.1%)	12/111 (10.8%, 95% CI = 5.7%, 18.1%)
EGFR pharmDx -	103	None treated	None treated

*329 Patients were available for 2:1 randomization to the two arms of the drug trial

Response rate was the proportion of patients in the entire study population with a decrease by $\geq 50\%$ in the sum of the perpendicular diameter of all measurable tumor (i.e., a 50% or more decrease in tumor by surface area) that persisted for at least 28 days.

Study 2 -Phase 2 Supportive Study

Preliminary clinical experience suggested that cetuximab alone may have activity. Therefore, Study 2 was initiated as an open label trial of metastatic colorectal cancer patients with progressive disease after or while being treated with an irinotecan-containing regimen. Patients were treated with cetuximab alone. The DakoCytomation EGFR pharmDx kit was used to demonstrate expression of EGFR protein on 140 specimens. Patients whose tumor or metastasis demonstrated positive expression were enrolled in the trial. Positive expression was defined as $\geq 10\%$ of tumor staining at any intensity. In Study 2, 57 patients with metastatic colorectal cancer (CRC) refractory to irinotecan were treated with cetuximab alone, resulting in a response rate of 10.5% according to the investigators' assessment.

Study 3 - Supportive Study with Prototype EGFR Assay

Study 3 was an open label trial of metastatic colorectal cancer patients with progressive disease during or after treatment with irinotecan. Patients were treated with irinotecan plus cetuximab. The DakoCytomation EGFR pharmDx prototype kit was used to assess the EGFR protein expression on specimens from 401 patients. Patients whose tumor or metastasis demonstrated positive expression were enrolled in the trial. Per the protocol, 10 percent membrane staining was required for a positive EGFR test result. The pathologist report forms documented staining intensity and percent of tumor staining. The oncologists interpreted the staining results for eligibility and as a consequence, several patients were enrolled with less than 10 percent positive staining. In this trial 138 patients were treated. According to the investigators' assessment, 15.2% of the patients who had progressive disease after or while being treated with an irinotecan-containing regimen showed a response to the combination of irinotecan and cetuximab.

Examination of EGFR pharmDx parameters to determine the cutoff between positive and negative results.

The secondary objective of evaluation of multiple specimen parameters in all three clinical studies demonstrated that semi-quantitative scoring provides no further benefit with respect to the study outcome of response rate. Membrane staining, whether complete or incomplete, correlated with the therapy response. In reviewing results from the three studies, it was established that cases with membrane staining of $\geq 1+$ intensity of $\geq 1\%$ of tumor cells should be considered as positive for

EGFR expression.

Evaluation of the staining pattern of EGFR in colorectal tissue showed also that colorectal tissue has a predominance of heterogeneous EGFR expression. Other assessed parameters, including histological diagnosis, percent of tumor in the specimen, total membrane staining, and cytoplasmic staining, did not add any additional useful information to the analysis.

Conclusion of the Clinical Studies

By identifying EGFR positive colorectal cancer tissue, EGFR pharmDx™ was demonstrated to be an effective aid in the assessment of patients being considered for cetuximab therapy.

XIII. CONCLUSIONS DRAWN FROM ALL OF THE STUDIES

The results of the pre-clinical and clinical testing performed on DakoCytomation EGFR pharmDx™ demonstrate that the assay is reproducible and is specific to EGFR expression with performance characteristics appropriate to aid in the assessment of patients considered for Erbitux™ (cetuximab) therapy.

Risk Benefit Analysis

The testing performed on DakoCytomation EGFR pharmDx™ indicates that the assay performs consistently and that the assay results are clinically relevant in the assessment of patients considered for EGFR targeted therapy.

Patients falsely assigned as EGFR-positive following assessment would be considered eligible for treatment. Because the design of the clinical studies did not include treatment of patients with negative assay results, the risks or benefits of treatment in this patient population are unknown. The risks of Erbitux™ treatment to EGFR-positive patients included mild, moderate and severe infusion reactions, low incidence of pulmonary toxicity, and dermatologic toxicity.

False negative test results would potentially exclude the patient from treatment. Because the design of the clinical studies did not include treatment of patients with negative assay results, the risks or benefits of treatment in this patient population are unknown.

Based on the information in the studies provided, the FDA has concluded that the benefits of using the DakoCytomation EGFR pharmDx test for its intended use outweigh the risks associated with using it.

Safety

The DakoCytomation EGFR pharmDx™ assay is an *in vitro* diagnostic test and does not contact the patient. Instructions for the safe use of the product are included in the package insert.

Effectiveness

The results of testing performed on DakoCytomation EGFR pharmDx™ indicate that the assay is

effective in aiding in the assessment of patients considered for EGFR targeted therapy.

CDRH has, therefore, concluded that the device is safe and effective for the stated indication.

XIV.. PANEL RECOMMENDATIONS

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Immunology Devices Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XV. CDRH DECISION

CDRH issued an approval order for the applicant's EGFR pharmDx Test on February 12, 2004. This order included the following post approval requirements:

1. Since the EGFR pharmDx™ kit is to be shipped at ambient temperatures and to cover expected temperatures, please demonstrate that the product can withstand shipping temperatures of at least 37°C in addition to the testing that was done to support shipping at 30°C.
2. To assure that pathologists interpret results using criteria identical to those used to determine the performance characteristics declared in the product labeling, please provide also an interpretation guide that includes enlarged pictures of correct and unacceptable staining of the 2+ tissue culture control provided in the EGFR pharmDx™ kit.
3. Because there was no difference in clinical outcome that depended on the intensity of EGFR staining or any other interpretation feature and persons with EGFR test negative results were not treated and followed, it is not known that EGFR-negative persons will not respond to Erbitux™ therapy. To determine whether the assumption that EGFR-test negative persons will not respond to Erbitux™ therapy is true, please submit a post approval study for approval by FDA to determine the clinical outcomes of EGFR-negative patients. Conduct one or more studies in EGFR-negative patients to further evaluate and confirm the value of EGFR expression in tumors as a selection criterion for Erbitux™ therapy in patients with metastatic colorectal cancer.
 - a) Include the results of a Phase 2 study, enrolling 50-60 patients with refractory, EGFR-negative, metastatic colorectal cancer designed to estimate the overall response rate and duration obtained with single agent Erbitux™ in this population.
 - b) Provide the data and analyze the results obtained in a subset of patients with EGFR-negative metastatic colorectal cancer enrolled in the protocol entitled CALGB 80203 "A Phase III Trial of Irinotecan/5-FU/Leucovorin or Oxaliplatin/5-FU/Leucovorin with and without Cetuximab (C225) for Patients with Untreated Metastatic Adenocarcinoma of the Colon or Rectum".

The applicant's manufacturing and control facilities were inspected on July 3, 2003, and the facilities were found to be in compliance with the Quality System Regulation (21 CFR 820).

XVI. APPROVAL SPECIFICATIONS

Directions for use: See labeling

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions and Adverse Events in the labeling.