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Immunohistochemistry

Epidermal Growth Factor Receptor and Erbitux™

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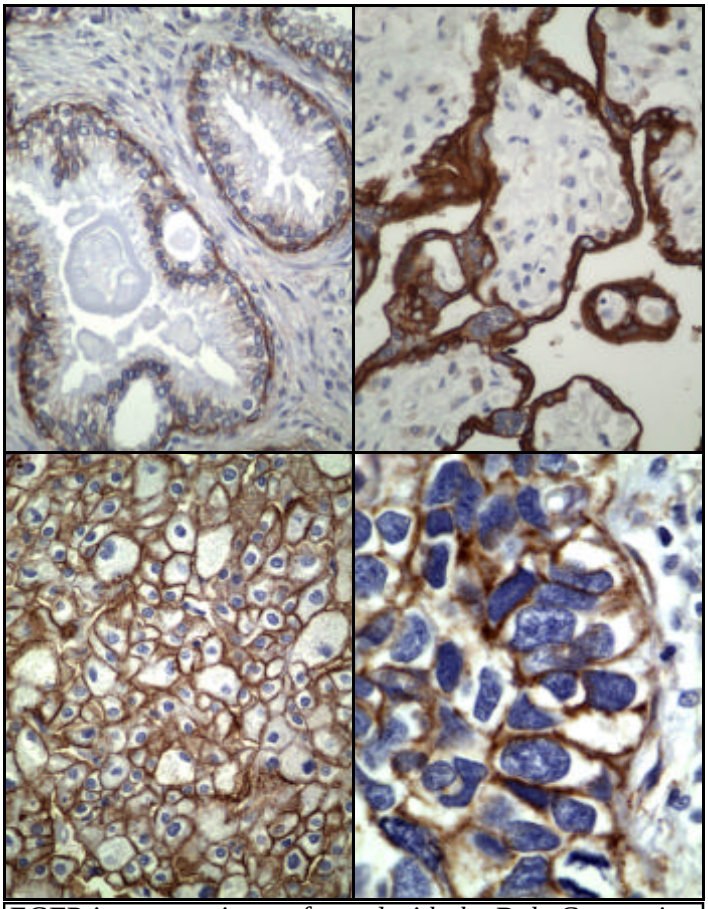
Epidermal Growth Factor Receptor (EGFR, also known as HER1 or c-erbB-1), a member of the subfamily of type 1 tyrosine kinase receptors, is a cytoplasmic membrane protein that has an important role in regulating cell proliferation. It is normally expressed in many benign epithelial cells, and is overexpressed in many types of tumors.

As many of you know, on 2/12/2004, the FDA approved the use of Erbitux™ (cetuximab), a monoclonal antibody raised against EGFR, for the treatment of metastatic colorectal adenocarcinoma. For that reason, many of you have undoubtedly received phone calls from oncologists requesting that patients with colonic carcinoma (and sometimes other carcinomas) have EGFR immunostains performed on their tumor tissue, as a positive EGFR immunostain is required before patients are considered for Erbitux™ therapy.

In both in vitro and in vivo animal models, Erbitux™ has been found to inhibit growth and survival of tumors that overexpress EGFR. Human tumor xenografts that lack EGFR expression show no effect. In animal studies, the addition of Erbitux™ to irinotecan or irinotecan and 5-fluorouracil increased antitumor effects when compared to chemotherapy alone.

Erbitux™ was evaluated in a randomized controlled trial of 329 patients with colorectal cancer, with 111 patients receiving Erbitux™ alone, and 218 patients receiving Erbitux™ plus irinotecan. As a group, the objective response rate was 22.9% in those patients that received both Erbitux™ plus irinotecan, and 10.8% in those that received Erbitux™ alone. The median time to progression following therapy was 4.1 months in those who received both Erbitux™ and irinotecan, and 1.5 months in those that received Erbitux™ alone. Although Erbitux™ can shrink tumors and delay tumor growth in some patients, it has not been shown to extend patient's survival. In order to be enrolled in the study, patients were required to demonstrate immunohistochemical evidence of EGFR expression, and testing was performed using the DakoCytomation EGFR pharmDx™ kit. The results were scored based on the intensity of expression (barely/faint, weak to moderate, strong) and on the percent-

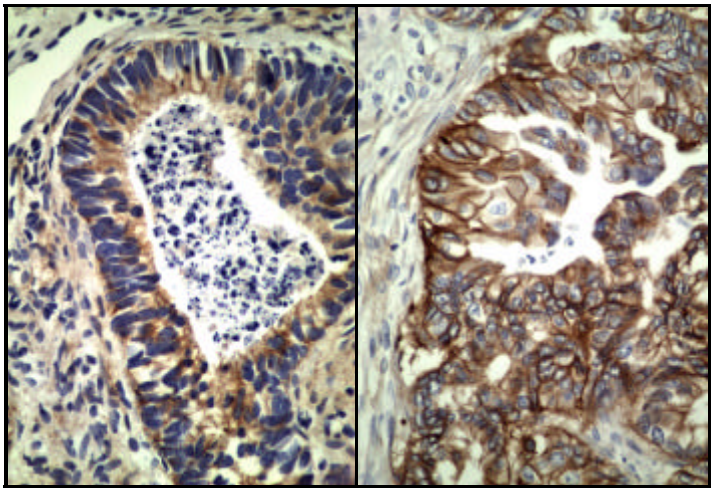


EGFR immunostains performed with the DakoCytomation kit. Note staining of prostate basal cells (upper left), placenta (upper right), chromophobe renal cell carcinoma (lower left), and lung adenocarcinoma (lower right).

age of tumor cells expressing EGFR. The intensity of expression and percentage of positive cells showed no correlation with response rate. On the same day that the FDA approved Erbitux™, it also approved the DakoCytomation EGFR pharmDx™ kit for EGFR testing. According to that kit, a "positive" immunohistochemical stain for EGFR is defined as "≥1% of tumor cells showing partial or complete circumferential membrane staining of any intensity (above background)". Staining is often very heterogeneous, and nonspecific cytoplasmic staining (which should be ignored for scoring purposes) is very common.

Unfortunately, patients scored as "negative" for EGFR expression were not offered Erbitux™. In my opinion, the line between "negative" and "positive" using the definition "≥1% partial or complete membrane staining of any intensity" is a very fuzzy line. It would not have surprised me at all if EGFR-negative patients had similar response rates to those who were scored as EGFR positive, particularly since there was no correlation between intensity of expression, percentage of positive cells, and therapeutic response. In my mind this begs the question of whether or not EGFR testing in these patients is really providing any useful information at all.

The DakoCytomation EGFR pharmDx kit is very expensive, and for those of us who have been performing EGFR testing for many years, the logical question that arises is whether or not we can use our "usual" EGFR assay for patient testing. The Erbitux™ package insert contains the following language in the "EGF Receptor Testing" section: *"Patients enrolled in the clinical studies were required to have positive immunohistochemical evidence of positive EGFr expression using the DakoCytomation EGFR pharmDx™ test kit. Assessment for EGFR expression should be performed by laboratories with demonstrated proficiency in the specific technology being utilized. Improper assay performance, including the use of suboptimally fixed tissue, failure to utilize specified reagents, deviation from specific assay instructions, and failure to include appropriate controls for assay validation, can lead to unreliable results. Refer to the DakoCytomation test kit package insert for full instructions on assay performance."* In my mind this language is somewhat ambiguous, so I contacted the FDA and spoke with several FDA representatives. I was told that using a different EGFR immunostain is not illegal. It would be considered an "off-label" situation by the FDA. It is then up to the individual lab performing the test to ensure that a reliable result is reported. The FDA would not provide a direct answer to the question *"Are we required to use the DakoCytomation kit or can we use the EGFR assay that we have been performing in our lab for many years?"*, as they say that determination is one involving medical judgment, and the FDA representatives tell me that it is not their role to tell physicians how to practice medicine.



EGFR stains using the DakoCytomation kit on colon carcinomas. Nonspecific cytoplasmic staining (left) is common, and should be ignored. Only membrane staining (right) is scored, even if it is very focal (≥1% of cells) and very faint.

ProPath has been doing EGFR testing for over 5 years, so we are experienced in the performance and interpretation of these assays. At the request of some of our clients, we are currently performing the assay using the FDA-approved DakoCytomation EGFR pharmDx™ kit.

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