Murine mAb 225 was effective against the EGFR tyrosine kinase and inhibited tumor growth in preclinical studies. A phase I trial showed safety, tumor localization, and satisfactory pharmacokinetics. Human:murine chimeric C225 retained biologic activity, which was essential for the conduct of subsequent combination therapy trials and eventual regulatory approval.

mAbs 225 and 528 with the desired capacity to inhibit EGFR tyrosine kinase activity were successfully produced and identified by selecting for their capacity to reduce incorporation of 32P into cellular lysates. Their capacities to bind to the receptor with high affinity, compete with EGF and TGFβ for binding, inhibit receptor tyrosine kinase activity, and inhibit cell proliferation in culture and in xenografts were reported in 1983 and 1984 (2–4). This was the first report of an antiproliferative agent acting on a growth factor receptor and on its tyrosine kinase. Between that time and 1996, preclinical investigation in the Mendelsohn research laboratory characterized the molecular events associated with mAb-mediated receptor internalization, mechanisms that inhibit cell proliferation, and additive effects of combining anti-EGFR mAb therapy of human tumor xenografts with chemotherapeutic agents, including cisplatin, paclitaxel, and doxorubicin. Others demonstrated that mAb 225 binds to and constrains domain III of the antibody, C225, leading to the demonstration of its efficacy and regulatory approval for the treatment of some human cancers.

**Background and Previous Research**

In 1980, Drs. John Mendelsohn and Gordon Sato joined forces at the University of California, San Diego (UCSD) Cancer Center to pursue the novel hypothesis that a mAb that bound to the EGFR and prevented ligand binding might inhibit activation of the receptor’s tyrosine kinase and prevent cancer cell proliferation. Why was this research undertaken? At the time, the EGFR was one of only three known tyrosine kinases, another being oncogenic src, which was known to cause cancer in murine models. EGFRs were being reported to be commonly overexpressed in human cancers, and circulating antibodies against cell surface receptors were known to produce stable physiologic change (disease) in patients with myasthenia gravis, rare forms of insulin resistance, and thyroid disorders.

We also were aware that cultures of malignant cells were less able to tolerate deprivation from essential nutrients and growth-promoting agents than cultured nonmalignant cells, suggesting the possibility that EGFR inactivation would selectively affect cancer cells.

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Extensive research has suggested that, except for situations in which the EGFR is mutated, it is not usually a primary “driver” of malignancy. Nevertheless, the results noted above and from many other laboratories strongly suggest that EGFR activity is an important contributor to the malignant behavior of many cancers. This is confirmed by the results of clinical trials with 225 mAb, which began in 1989.
against the murine 225 Ab within 2 weeks. (i) mAb 225 in a single dose up to 300 mg was safe, without serious adverse events; (ii) the antibody localized in the patients' lung cancers and metastases at doses ≥40 mg; (iii) at a dose ≥120 mg, serum levels were maintained for more than 3 days at levels that can saturate EGFRs; and (iv) all patients produced human antibodies against the murine 225 Ab within 2 weeks.

This was the first clinical trial investigating an inhibitor of a growth factor receptor and a tyrosine kinase. On the basis of these results, the NCI arranged for creation of a chimeric human/murine version of 225 to obviate an immune response to repeated doses. C225 (chimeric 225, cetuximab) was made available for further investigation.

The original license for C225 was released, and in 1994 the mAb was licensed to ImClone Systems, which immediately began laboratory studies to demonstrate that the C225 chimeric mAb retained efficacy. The results of these experiments were reported in the 1995 CCR article under discussion. The findings were favorable for moving forward in clinical testing: C225 bound to EGFRs with a 5-fold tighter affinity ($K_d = 0.39 \text{ mmol/L}$) and produced greater antitumor effects against xenografts, compared with murine 225.

**Subsequent Research**

On the basis of these findings, ImClone was able to move forward with developing clinical trials of cetuximab therapy, first administered alone in single and multiple doses and in combination with cisplatin, and then explored in phase II trials in combination with chemotherapies for a variety of solid tumors. In the initial series of phase I trials, only 1 of the 19 patients whose sera were assayed developed human antichimeric antibodies (HACA).

It could not be determined whether these affected bioavailability, but there were no sequelae related to the HACA. Cetuximab-associated toxicities were minimal. Many of the patients receiving multiple courses of therapy had stabilization of disease through one or more courses of therapy. Thirteen patients treated with cetuximab plus cisplatin completed 12 weeks of therapy, with 2 partial responses in patients with head and neck cancer.

Bristol-Myers Squibb and Merck KGaA became partners with ImClone and carried out additional trials in the United States and Europe, respectively. A pivotal trial, which established the efficacy of cetuximab plus irinotecan in patients with advanced colorectal cancer who had progressed on irinotecan, led to the FDA approval in 2004 (8). Modest single-agent activity of cetuximab against colon cancer (11%) was confirmed in the registration trial. Recent trials have demonstrated the efficacy of cetuximab with two chemotherapy regimens in the first-line setting for metastatic colon cancer. Importantly, it was demonstrated in retrospective studies of patients’ tumor specimens that treatment with cetuximab is not indicated for colorectal cancer with a mutation of the $\text{ras}$ gene, which is common in this form of cancer.

Cetuximab also has demonstrated efficacy against squamous cell carcinoma of the head and neck (SCCHN), both as a single agent and in combination with radiotherapy. In the registration trial, adding cetuximab to first-line radiotherapy improved median overall survival (OS) from 29.3 to 49.0 months, and 3-year OS from 36.4% to 45.6% (9). In a separate clinical study, cetuximab as a single agent produced an objective response rate of 13%, which is comparable with that observed in colorectal cancer. Other studies confirmed previous reports that cetuximab can overcome resistance to cisplatin in some patients, producing clinical responses and prolonged survival.

On the basis of these findings, the FDA approved cetuximab for treatment of SCCHN in combination with radiotherapy, and for treatment of platinum-refractory advanced SCCHN (in practice, often in combination with platinum-based chemotherapy). More recent studies demonstrated that addition of cetuximab to first-line platinum-based chemotherapy for recurrent locoregional or metastatic SCCHN improved median survival from 7.4 to 10.1 months, leading to the FDA approval for this indication in 2011.

In all reported studies with cetuximab, patients who experienced an acneiform rash were far more likely to receive benefit from treatment, and this has been found to be true with nearly all of the anti-EGFR agents in the clinic. Of course, the rash is not a satisfactory biomarker for selecting patients appropriate for anti-EGFR therapy, as it appears after treatment is initiated.

The search for biomarkers prospectively predicting sensitivity of a cancer to an anti-EGFR agent has been frustrating. The one important exception is the finding that non–small-cell lung cancer (NSCLC) with mutations in the EGFR has increased sensitivity to the oral tyrosine kinase inhibitors (TKI) erlotinib and gefitinib, but not to anti-EGFR mAbs. These oral TKIs are now standard-of-care therapy for patients with advanced NSCLC with mutated EGFRs, and this treatment is now being explored for far rarer instances of mutated EGFRs in other solid tumors.

There is no correlation between sensitivity to cetuximab, or the oral TKIs, and level of EGFR expression in the tumor, in contrast with the positive predictive value of high levels of HER2 for the response to trastuzumab (Herceptin; Genentech). A few reports have suggested that high expression of epiregulin and amphiregulin in tumors correlates with response to cetuximab in patients with colorectal cancer (10). However, patients whose cancers expressed high levels of TGFα (which binds more tightly to EGFR) had poor responses to cetuximab (9).

To enable delivery of personalized/precision cancer therapy with cetuximab and other anti-EGFR therapies to patients most likely to receive benefit, the search for biomarkers predicting sensitivity must continue.

Recent publications have described a number of mechanisms for developing resistance to anti-EGFR therapy, which invariably is observed in the clinic (11). One report demonstrated that amplification of MET and increased activity of this receptor caused resistance to gefitinib by driving HER3-dependent activation of the PI3K/AKT–mTOR pathway (12). Overexpression of the IGF1 receptor has been shown to contribute to resistance to cetuximab in $K_{ras}$ wild-type colorectal cancer by activating the same pathway. These observations have led to preclinical studies and clinical trials of relevant combination therapies.

A recent example of potential combination therapies followed up observations that gene silencing of HER3 by siRNA in cultured cetuximab-resistant cells can restore sensitivity to the mAb. In experiments with SCCHN cell lines, treatment with the combination of cetuximab and the MM-121 mAb against HER3 produced enhancement of cell kill in culture and in xenografts (13). The mechanism involved enhanced suppression of the PI3K/AKT.
and ERK pathways by the mAbs against HER3 and the EGFR, respectively.

In the future, it is likely that there will be an increase in preclinical and clinical studies that explore rational combinations of therapies against targets in the same signaling pathway and in different interacting pathways. Combinations of immunotherapy or proapoptotic agents with EGFR inhibition are also being explored. In these experiments, special attention must be paid to the timing of administering agents that act through entirely different mechanisms.

Recently, ImClone was acquired by Eli Lilly. Collaborative studies of cetuximab continue, involving clinical investigators and the involved pharmaceutical companies. We were not able to review the numerous other antibodies and drugs that act on the EGFR in this brief commentary.

Disclosure of Potential Conflicts of Interest
J. Mendelsohn reports receiving licensing fees for a patent on cetuximab that is owned by the University of California San Diego and licensed to ImClone; is a consultant/advisory board member for Capital Royalty and AIMM Therapeutics; and is a board member for Merrimack Pharmaceuticals. M. Prewett is an employee of and has ownership interest in Eli Lilly. N.I. Goldstein is the chief operating officer of Helio Genetics. No potential conflicts of interest were disclosed by the other author.

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