CCR 20th Anniversary Commentary: A Chimeric Antibody, C225, Inhibits EGFR Activation and Tumor Growth

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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. *Clin Cancer Res; 21(2); 227–9. ©2015 AACR.*

See related article by Goldstein et al., Clin Cancer Res 1995;1(11) November 1995;1311–8

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It is a pleasure to provide a commentary on the article by Goldstein and colleagues, "Biological Efficacy of a Chimeric Antibody to the Epidermal Growth Factor Receptor in a Human Tumor Xenograft Model," which was published in *Clinical Cancer Research* in 1995 (1). This research provided an essential and critical bridge, building on earlier preclinical and clinical studies with murine mAb 225 against the EGFR. Subsequent clinical trials with the human:murine chimeric form of the antibody, C225, led 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.

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 ^{32}P into cellular lysates. Their capacities to bind to the receptor with high affinity, compete with EGF and TGFα 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 EGFR, thereby preventing domain II from forming active dimers with other EGFRs.

Mendelsohn (5) described a number of mechanisms for blocking cell proliferation. mAbs induce G_1 arrest in the cell cycle, mediated by increased levels of the CDK2 inhibitor $p27^{kip1}$ and resulting in Rb protein hypophosphorylation. The mAb treatment also activates apoptosis by induction of Bax and activation of caspase-8. Angiogenesis is inhibited by a reduction in EGFR-induced production of VEGF and IL8 by the malignant cells, whereas tumor cell invasion and metastases are inhibited by reduced production of matrix metalloproteinase 9. In fact, nearly all of the hallmarks of cancer described by Hanahan and Weinberg in their landmark publication in 2000 were attenuated when EGFR activation was blocked by treating cells and human tumor xenografts with these anti-EGFR mAbs (6).

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.

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doi: 10.1158/1078-0432.CCR-14-2491

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Hybritech, Inc. licensed anti-EGFR mAb 225 from UCSD in 1986 and carried out scaled-up production in mouse ascites and the additional studies required for FDA approval of a phase I study with the murine mAb. On the basis of preclinical studies in Mendelsohn's laboratory, the initial phase I clinical trial was performed with indium-III–labeled 225 to enable pharmacologic and localization studies (7). The trial in patients with advanced lung cancer produced a number of conclusions: (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 $\geq$40 mg; (iii) at a dose $\geq$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 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 *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 5-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

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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 differently 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.

Authors' Contributions

Conception and design: J. Mendelsohn, M. Prewett, P. Rockwell, N.I. Goldstein
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Received September 25, 2014; accepted November 7, 2014; published online January 15, 2015.

References

1. Goldstein NI, Prewett M, Zuklys K, Rockwell P, Mendelsohn J. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. Clin Cancer Res 1995;1:1311–8.
2. Kawamoto T, Sato JD, Le A, Polikoff J, Sato GH, Mendelsohn J. Growth stimulation of A431 cells by epidermal growth factor: identification of high-affinity receptors for epidermal growth factor by an anti-receptor monoclonal antibody. Proc Natl Acad Sci U S A 1983;80:1337–41.
3. Gill GN, Kawamoto T, Cochet C, Le A, Sato JD, Masui H, et al. Monoclonal anti-epidermal growth factor receptor antibodies which are inhibitors of epidermal growth factor binding and antagonists of epidermal growth factor binding and antagonists of epidermal growth factor-stimulated tyrosine protein kinase activity. J Biol Chem 1984;259:7755–60.
4. Masui H, Kawamoto T, Sato JD, Wolf B, Sato G, Mendelsohn J. Growth inhibition of human tumor cells in athymic mice by anti-epidermal growth factor receptor monoclonal antibodies. Cancer Res 1984;44:1002–7.
5. Mendelsohn J. Blockade of receptors for growth factors: an anticancer therapy–the fourth annual Joseph H. Burchenal American Association of Cancer Research Clinical Research Award Lecture. Clin Cancer Res 2000;6:747–53.
6. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.
7. Divgi CR, Welt S, Kris M, Real FX, Yeh SD, Gralla R, et al. Phase I imaging trial of indium 111-labeled anti-epidermal growth factor receptor monoclonal antibody 225 in patients with squamous cell lung carcinoma. J Natl Cancer Inst 1991;83:97–104.
8. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004;351:337–45.
9. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 2006;354:567–78.
10. Tabernero J, Cervantes A, Rivera F, Martinelli E, Rojo F, von Heydebreck A, et al. Pharmacogenomic and pharmacoproteomic studies of cetuximab in metastatic colorectal cancer: biomarker analysis of a phase I dose-escalation study. J Clin Oncol 2010;28:1181–9.
11. Ratti M, Tomasello G. Emerging combination therapies to overcome resistance in EGFR-driven tumors. Anticancer Drugs 2014;25:127–39.
12. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitnib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039–43.
13. Jiang N, Wang D, Hu Z, Shin HJ, Qian G, Rahman MA, et al. Combination of anti-HER3 antibody MM-121/SAR256212 and cetuximab inhibits tumor growth in preclinical models of head and neck squamous cell carcinoma. Mol Cancer Ther 2014;13:1826–36.

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