More than 30 years ago, John Mendelsohn and Gordon Sato collaborated to generate monoclonal antibodies that antagonize the function of the human epidermal growth factor receptor (EGFR; refs. 1 and 2). One of these antibodies, murine mAb 225, was eventually engineered as a human–mouse chimeric IgG1 mAb (referred to as C225, and later IMC-C225 and cetuximab). C225 binds the human EGFR with high affinity and competitively blocks ligand binding, which leads to inhibition of receptor phosphorylation and downstream signaling pathways important for controlling a number of cellular processes (3, 4).

It has been recognized for several decades that human tumors express high levels of growth factors and their receptors and utilize these autocrine- and paracrine-stimulated signaling pathways to sustain malignant growth. By far, the most studied growth factor receptor system in cancer has been the EGFR family, which is composed of four receptors: EGFR, ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4; ref. 5). Mendelsohn and Sato were at the forefront of this research recognizing the potential of EGFR as a therapeutic target. Their early preclinical studies with mAb 225 demonstrated antitumor activity of mAb 225 on human colorectal tumor cell lines and in human mCRC tumor xenografts in murine models (6). Even with these promising early preclinical data, the notion of EGFR as a cancer target was met with significant cynicism due to its lack of tumor specificity, i.e., EGFR is broadly expressed on normal epithelial cell populations. It was argued at the time that this lack of tumor specificity would lead to unmanageable toxicities, a concern that would eventually be refuted in later clinical testing. Determined, Mendelsohn and collaborators pressed on continuing their work to understand the role of EGFR in cancer and with a clear goal of advancing mAb 225 into the clinic.

In 1994, C225 was licensed to ImClone Systems, where research and development with this molecule continued, and shortly thereafter, C225 entered clinical testing. In the early development of C225 as a therapeutic agent, a number of potential cancer indications were considered based on its observed preclinical activity across a variety of human tumors of epithelial origin. Initial phase I clinical studies with C225 were showing particularly promising activity in squamous cell carcinoma of the head and neck (SCCHN), in particular in combination with radiation, which recapitulated preclinical findings from several laboratories (4). Another indication of particular interest was advanced, metastatic colorectal cancer (mCRC). It was hypothesized that the EGFR plays an important role in the growth and survival of human colorectal tumors. This hypothesis was largely based on the observation that EGFR and several of its ligands, e.g., EGF, TGFβ, epiregulin, and amphiregulin, are coexpressed in human mCRC, suggesting the potential existence of an autocrine loop (7, 8). Moreover, a number of preclinical studies had demonstrated antitumor activity of mAb 225 or C225 on human colorectal tumor cell lines and in human mCRC tumor xenograft mouse models (4).

The clinical development proposal that began to emerge for C225 in the late 1990s was a combination therapy approach in relapsed or refractory mCRC. At that time, the topoisomerase I inhibitor irinotecan, a semisynthetic derivative of camptothecin, had emerged as an important chemotherapy used in the treatment of mCRC. Irinotecan was initially approved in 1996 for second-line treatment in patients with mCRC for whom first-line 5-fluorouracil (5-FU)/leucovorin therapy was not successful. Irinotecan also demonstrated promising activity as a component of a three-drug regimen along with 5-FU and leucovorin. A clinical study was proposed for the treatment of irinotecan-refractory mCRC with a combination of irinotecan and C225. At the time, this was a bold hypothesis and a clinical development strategy viewed with a significant degree of skepticism. In oncology practice at the time, dogma dictated that patients not receive
further treatment with a particular chemotherapy regimen where resistance to the therapy had been demonstrated. Moreover, there was substantial concern that embarking on such an unconventional clinical study might delay the development of C225 for several years. Finally, no preclinical data were available to support the combination of C225 and irinotecan in mCRC.

To inform the clinical development of C225 in the clinic, our laboratory initiated a series of studies to specifically ask the question of whether a combination of C225 and irinotecan would have improved antitumor activity compared with either treatment alone in preclinical models of colorectal cancer (9). This hypothesis was based on the work of several laboratories that had shown enhanced antitumor activity when combining EGFR blockade with other chemotherapeutic agents (4). To test the combination of C225 and irinotecan, we utilized human colon tumor xenograft models that were poorly responsive to irinotecan therapy. Unfortunately, preclinical models of human mCRC with acquired resistance to irinotecan were not available at the time these studies were conducted. Nevertheless, we designed a set of studies utilizing human colon tumor xenografts that responded poorly even to maximally tolerated doses of SN-28 in vitro or irinotecan in vivo. Moreover, in some studies, we conducted experiments with randomized, established tumors that were selected in vivo based on their ability to continue growth through multiple treatments of irinotecan. Treatment with irinotecan or C225 alone only modestly delayed tumor growth, whereas combined therapy led to significant regression of established tumors in a high percentage of animals. In subsequent studies using human colorectal cancer models selected for oxaliplatin resistance, similar results were obtained showing that a combination of cetuximab and oxaliplatin resensitized tumors, leading to enhanced antitumor efficacy compared with antibody or chemotherapy alone (10).

An additional major question at the onset of these studies was to address the proposed optimal schedule for the combination of C225 and irinotecan treatment. Based on the cell-cycle dependency of irinotecan and the known antiproliferative effects of C225, our initial hypothesis was that there would be sequence dependency of the combination for optimal antitumor activity. Much to our surprise, we were unable to demonstrate such a dependency. Rather, we observed optimal efficacy in preclinical models when C225 and irinotecan were administered together. Co-administration of C225 and irinotecan as a therapeutic regimen would ultimately be supported in later clinical studies.

In these and subsequent investigations in our laboratory, we, unfortunately, were unable to completely elucidate the mechanisms responsible for the C225 and irinotecan combination antitumor activity. In other model systems, C225 blockade of EGFR-mediated DNA repair and survival mechanisms in tumors has been demonstrated, and, ostensibly, these mechanisms also play a role in the enhanced antitumor activity observed with combined C225 and irinotecan therapy. However, the mechanism drivers of EGFR blockade and irinotecan combination activity remain unclear to this day.

These preclinical studies supported the clinical investigation of C225 mCRC. ImClone and its corporate partner Merck KGaA initiated clinical studies to investigate the safety and efficacy of C225 as a monotherapy and in combination with irinotecan in irinotecan-refractory mCRC. An initial phase II study demonstrated activity of cetuximab combined with irinotecan in irinotecan-refractory patients with a 22.5% response rate, compared with a 10% response rate for cetuximab treatment alone (11). Subsequently, a larger randomized trial was performed in 329 patients with advanced, irinotecan-refractory mCRC, which demonstrated a remarkably similar response rate of 23% for the cetuximab/irinotecan combination and an 11% response rate for cetuximab monotherapy (12). These results led to the FDA approval in 2004 of cetuximab for the treatment of patients with advanced colon cancer refractory to irinotecan.

These were landmark studies not only as the first application of an EGFR-targeted antibody for cancer but also in the reexamination of oncology practice for the management of refractory disease. As mentioned earlier, the established dogma in oncology for managing chemoresistant disease prescribed that therapy is changed assuming future drug resistance. Combination studies with cetuximab and irinotecan provided convincing clinical evidence for the concept of resensitizing “resistant” tumors to additional chemotherapy by combining it with EGFR-targeted therapy. This concept is now well established in clinical practice and has been applied to other targeted therapy paradigms.

Further clinical studies led to additional approvals for cetuximab as a single agent in patients for whom irinotecan- or oxaliplatin-based therapy was not successful. Most recently, cetuximab has been approved for the first-line treatment of mCRC based on the results of the CRYSTAL study (Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer), a randomized phase III trial that evaluated the efficacy of combining cetuximab with FOLFIRI (5-FU, leucovorin, and irinotecan) compared with FOLFIROI alone (13). In parallel clinical studies, cetuximab also showed significant activity in SCCHN and is now approved for the treatment of this disease as a monotherapy, in combination with radiation or in combination with platinum-based therapy and 5-FU. Two years after the approval of cetuximab, another anti-EGFR mAb, panitumumab, was also approved as a monotherapy for mCRC.

Following the initial approval of cetuximab, retrospective and prospective analyses of clinical samples revealed that KRAS mutations are associated with lack of response to cetuximab in patients with mCRC (13–16). Activating mutations in KRAS occur with high frequency (>40%) in mCRC and, in hindsight, constitutive activation of MAPK signaling via these mutations provides a strong rationale for resistance to EGFR blockade. However, preclinical studies did not predict mutant KRAS as a resistance factor for EGFR-targeted therapy, and clinical research was required to uncover this mechanism. Additional randomized trials confirmed that patients only with wild-type KRAS benefited from cetuximab or panitumumab therapy, and these agents are now indicated only for patients with wild-type KRAS mCRC. Moreover, recent post hoc mutation analyses of patient samples from cetuximab trials suggest that additional KRAS mutations, and mutations in the BRAF, NRAS, and PIK3CA genes, may also be associated with reduced clinical benefit to cetuximab therapy in mCRC (17, 18). These findings underscore the increasing importance of molecular testing for genetic alterations in mCRC and its role in the selection of patients that are most likely to benefit from EGFR-targeted therapy.

Since the initial generation of the murine mAb 225 and subsequent decades of extensive basic and translational research, we have learned a great deal about the utility of EGFR-targeted...
therapy. From the onset, there has been understandable excitement about the possibility to exploit the EGFR pathway broadly as a target for the treatment of a variety of human cancers. Indeed, preclinical studies from a number of laboratories suggested that EGFR blockade may be active in many tumor types of epithelial origin, including bladder, breast, esophageal, lung ovarian, pancreatic, and kidney cancers. Hence, it is somewhat disappointing that, to date, significant clinical benefit for cetuximab has been limited to mCRC and SCCCHN. There is, however, a growing body of clinical evidence for activity of cetuximab and other EGFR antibodies in non–small cell lung cancer (NSCLC). A randomized study of cetuximab in combination with cisplatin plus vinorelbine versus chemotherapy alone in the first-line treatment of patients with advanced NSCLC showed a statistically significant, albeit modest, improvement in survival benefit, which has not thus far led to cetuximab approval in this indication (19). Interestingly, retrospective analyses of this study identified EGFR levels as a predictive biomarker for overall survival in the cetuximab treatment group (20). More recently, a study in a metastatic squamous NSCLC with another anti-EGFR monoclonal antibody, necitumumab, showed a statistically significant improvement in overall survival of combining necitumumab with gemcitabine and cisplatin compared with chemotherapy alone as a first-line treatment (11.5 vs. 9.9 months; ref. 21). Whether an EGFR antibody will eventually be approved for the treatment of NSCLC remains to be determined.

Certainly, the experience with mCRC and identification of resistance mechanisms in this disease, and possibly emerging data in NSCLC, suggest that expanding the clinical benefit for cetuximab and other EGFR-targeted antibodies will require additional research to identify genetic alterations or biomarkers that may confer resistance or response in other epithelial cancers. The hope for the future is that patient selection strategies and rational drug combinations based on prespecified genetic determinants and biomarkers will open new doors for EGFR-targeted therapy, and most importantly, for patients.

**Disclosure of Potential Conflicts of Interest**

D.J. Hicklin is CEO of and has ownership interest in Potenza Therapeutics.

Received January 25, 2015; revised February 4, 2015; accepted February 4, 2015; published online April 1, 2015.

www.aacrjournals.org Clin Cancer Res; 21(7) April 1, 2015

---

**References**


CCR 20th Anniversary Commentary: In Search of Cetuximab’s First Indication—Combination Therapy with Irinotecan in Colorectal Cancer

Daniel J. Hicklin


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/21/7/1505

Cited articles
This article cites 21 articles, 9 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/21/7/1505.full.html#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.