Synergy Between Cetuximab and Chemotherapy in Tumors of the Gastrointestinal Tract

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ABSTRACT
Cetuximab is a recently approved monoclonal antibody that targets the epidermal growth factor receptor, a receptor tyrosine kinase involved in the development and progression of colorectal cancer (CRC) and other solid tumors. Cetuximab, as a single agent or in combination with chemotherapy, has demonstrated significant clinical efficacy against CRC. Combinations of cetuximab with chemotherapy have proven to be well tolerated, with minimal overlap of toxicities between agents; and the anticancer synergy between cetuximab and traditional chemotherapy agents has made cetuximab a vital treatment for patients who are no longer responsive to chemotherapy alone. The U.S. Food and Drug Administration approved cetuximab in combination with irinotecan for the treatment of irinotecan-refractory metastatic CRC or as monotherapy for treating patients intolerant to irinotecan. Combination chemotherapies involving cetuximab as well as combinations involving cetuximab and other targeted agents, such as bevacizumab, an anti–vascular endothelial growth factor monoclonal antibody, constitute powerful new treatment options for the management of CRC. This review discusses recent clinical studies that have further defined this synergy, focusing primarily on tumors of the gastrointestinal tract. The Oncologist 2008;13:39–50

INTRODUCTION
The human epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptor tyrosine kinases [1] and is the target of several recently approved anticancer agents (reviewed in Dutta and Maity [2]). It is predominantly expressed as a 1,186-amino acid transmembrane glycoprotein. EGFR consists of an extracellular region that comprises two ligand-binding domains, a single α-helical transmembrane domain, and the intracellular carboxy terminus, which is the site of tyrosine phosphorylation that regulates EGFR signal transduction [3]. EGFR is a receptor for numerous ligands, including EGF, transforming growth factor (TGF)-α, and heparin-binding EGF [3, 4]. Following ligand binding, EGFR either homodimerizes or heterodimerizes with an additional member of the ErbB receptor family [5, 6]. Receptor dimerization triggers tyrosine kinase activity and the subsequent autophosphorylation of EGFR and its dimerization partner. Multiple downstream signaling pathways regulating cell growth and survival, including the Ras/mitogen-activated protein kinase, phosphatidylinositol 3’ kinase/Akt, protein kinase C–mediated, and signal transducer and activator of transcription pathways

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[1, 3, 5], are thus activated. Although dimerization can occur in the absence of ligand binding, dimerization is not sufficient for receptor activation, and a ligand-induced conformational change appears to be required for receptor phosphorylation [7]. The bivalent nature of the EGF-like ligands stabilizes specific heterodimer pairs and determines their fate upon subsequent internalization. Depending on the ligand, the EGFR is either degraded in the endosome or lysosome or recycled back to the cell surface [1], thus modulating the potency and duration of EGFR signaling.

Overexpression of the EGFR occurs in many epithelial tumors, including those of the prostate, breast, head and neck, and gastrointestinal (GI) tract [8–11]. In colorectal carcinomas (CRCs), EGFR expression is significantly higher than in normal colorectal mucosa [12–14]. In addition, several studies have shown EGFR expression to be linked to advanced tumor stage, greater metastatic potential, and shorter survival in patients with CRC [12]. In pancreatic cancer, coexpression of EGFR and either EGF or TGF-α has been correlated with greater tumor size, advanced tumor stage, and shorter survival, suggesting that expression levels of EGFR and one of its ligands contribute to the advancement of pancreatic cancer [15]. In addition to EGFR overexpression, EGFR mutations may contribute to cancer development and affect tumor response to therapeutics.

The Mechanism of Action of Cetuximab

Cetuximab (Erbitux®; ImClone Systems, Inc., New York) is a recombinant, human–mouse chimeric monoclonal IgG1 antibody that specifically targets EGFR. It is approved by the U.S. Food and Drug Administration for the treatment of metastatic CRC (mCRC), either alone or in combination with irinotecan, in patients who are refractory to irinotecan-based chemotherapy [16]. In preclinical studies, EGFR targeting by cetuximab inhibited cell growth and proliferation in head and neck cancer, pancreatic cancer, and vulvar squamous cell carcinoma cell lines [17–19]. Cetuximab also inhibited the growth of prostate cancer, renal cell carcinoma, and pancreatic cancer xenografts in mice [20, 21] and resulted in a longer lifespan in host animals relative to that of control animals, suggesting that cetuximab can confer a survival benefit in vivo [21].

Cetuximab binds specifically to the extracellular ligand-binding domain of EGFR with a binding affinity that is two log units greater than that of EGF or TGF-α [11, 22], and it competitively inhibits binding of these ligands [23–25]. Cetuximab binding of EGFR induces receptor internalization and degradation, thus reducing EGFR binding capacity and potential for downstream growth and survival signaling [17, 22, 26, 27].

Cetuximab treatment of tumor cell lines leads to cell cycle arrest and the accumulation of cells in the G1 phase of the cell cycle, the result of increased expression of the clin-dependent kinase (CDK) inhibitors p27Kip1 and p15INK4B. In turn, the activities of CDK2, CDK4, and CDK6 are suppressed, thus preventing entry of cells into the S phase of the cell cycle [28–30]. Cetuximab also induces apoptosis in a variety of tumor cell lines. Cetuximab treatment of the human CRC cell line DiFi for 48 hours resulted in morphologic changes consistent with apoptosis: reduction in cell volume, condensation of nuclear chromatin, and fragmentation of nuclear material [31]. In a squamous cell carcinoma of the head and neck cell line, cetuximab treatment resulted in apoptosis associated with increased expression of the proapoptotic protein Bax, with a concomitant decreased expression level of the antiapoptotic protein Bcl-2 [18]. Cetuximab has also been shown to sensitize cells to radiation and chemotherapy, potentially through blocking EGFR nuclear import and the associated activation of DNA protein kinase enzymes necessary for repairing radiation- and chemotherapy-induced DNA damage [32].

Other mechanisms of action of cetuximab may include inhibition of tumor angiogenesis and activation of immune functions. Cetuximab downregulated vascular endothelial growth factor (VEGF) expression in a dose-dependent manner in a human CRC cell line and in human CRC mouse xenografts [33]. The xenografts also showed a significant reduction in blood vessel counts following several rounds of cetuximab treatment [33], indicating that the tumor-promoting effects of EGFR overexpression may be mediated through VEGF stimulation and tumor angiogenesis. In recent ex vivo studies, cetuximab induced antibody-dependent cellular cytotoxicity in patients’ peripheral blood mononuclear cells against EGFR-expressing esophageal squamous cell carcinoma cell lines [34] and in natural killer cells from normal subjects against a panel of head and neck, colon, and breast cancer cell lines [35].

Panitumumab (Vectibix®; Amgen, Inc., Thousand Oaks, CA) is another EGFR-targeted monoclonal antibody that has not worked as well in combination with chemotherapy for the treatment of CRC. In fact, a large phase III trial, the Panitumumab Advanced Colorectal Cancer Evaluation trial, comparing oxaliplatin- or irinotecan-based chemotherapy plus bevacizumab (an antiangiogenic antibody) with or without panitumumab, closed early as a result of a higher incidence of pulmonary embolisms and grade 3 diarrhea, dehydration, and infections in the panitumumab-containing arm [36]. Nevertheless, other combination trials with other regimens for mCRC—for example, the Panitumumab Randomized Trial in Combination with Chemotherapy for Metastatic Colorectal...
Cancer to Determine Efficacy (first-line 5-fluorouracil [5-FU], leucovorin [LV], and oxaliplatin, FOLFOX, with or without panitumumab) and NCT0039183 (second-line 5-FU, LV, and irinotecan, FOLFIRI, with or without panitumumab)—are ongoing [37].

Rationale for Combining Cetuximab and Chemotherapy
Numerous lines of evidence support the rationale for combining targeted cetuximab therapy with traditional chemotherapy. In vitro cell culture and in vivo animal studies have demonstrated the cytostatic activity of cetuximab, whereas chemotherapy has robust cytotoxic properties [38]. In combination, these two therapies may have an additive effect of destroying existing tumor cells and suppressing the proliferation of new tumor cells. Moreover, cetuximab induction of proapoptotic molecules and inhibition of antiapoptotic molecules may render tumor cells more vulnerable to the cytotoxic effects of chemotherapeutic agents [38, 39]. In addition, cetuximab may also augment the activity of cytotoxic agents by suppressing the chemotherapy-induced autocrine stimulation of EGFR in tumor cells and disrupting the maintenance of malignant cells [40], as well as by inhibiting DNA-repair mechanisms of tumor cells [32].

Mouse xenograft experiments have demonstrated that cetuximab can work additively or synergistically with a variety of chemotherapy regimens. In one study, the combination of cetuximab or another anti-EGFR antibody (MAb 528) with doxorubicin resulted in substantially greater antitumor activity against human squamous cell carcinoma (40–100% eradication) or breast adenocarcinoma xenografts than with single-agent therapy with either doxorubicin or an anti-EGFR antibody [41]. Cetuximab plus irinotecan, 5-FU, and LV inhibited tumor growth in >75% of human CRC xenografts in mice; combination chemotherapy alone or cetuximab alone affected only 25% or 48% of tumors at most, respectively [42]. Finally, the combination of cetuximab plus oxaliplatin showed synergistic antitumor effects in mice bearing one of two oxaliplatin-resistant colon carcinoma xenografts (HT29-OxR and KM12-OxR) [43]. Growth rates of these tumor xenografts, respectively, were 73% and 78% with cetuximab, 87% and 84% with oxaliplatin, and 33% and 55% with cetuximab plus oxaliplatin.

Cetuximab in Combination with Chemotherapy in CRC

Second- or Third-Line Combination Therapy in Refractory CRC
Numerous trials have assessed the efficacy and safety of cetuximab in combination with chemotherapeutic agents in patients with mCRC (Table 1) [44–65]. One of the earliest studies of cetuximab–irinotecan combination therapy demonstrated its efficacy in patients with CRC refractory to both 5-FU and irinotecan (n = 121) [47], with 21 patients (17%) achieving partial responses (PRs) lasting a median of 84 days. Response to therapy was strongly correlated with the presence of rash [66].

Subsequently, in the controlled phase II Bowel Oncology with Cetuximab Antibody (BOND) study, patients with irinotecan-refractory mCRC were randomized to receive either cetuximab alone (n = 111) or cetuximab plus irinotecan (n = 218) [44]. Results showed a significantly higher overall response rate (ORR) in the cetuximab–irinotecan group compared with the cetuximab group (22.9% versus 10.8%; p = .007), as well as a longer median time to progression (TTP) (4.1 months versus 1.5 months, respectively).

In the Monoclonal Antibody Erbitux in a European Pre-Licence (MABEL) study involving 1,147 heavily pretreated (1,123 evaluable) patients, cetuximab in combination with four different irinotecan regimens resulted in overall progression-free survival (PFS) rates of 61% at 12 weeks and 34% at 24 weeks, both meeting the primary endpoint. The median overall survival (OS) time was 9.2 months (range, 8.7–9.9), and treatment was generally well tolerated [67]. Comparable results with cetuximab plus irinotecan versus irinotecan alone have been reported from the Latin American Erbitux Pre-Licence phase III study [46].

Another large (n = 1,298) multinational phase III study, the Erbitux Plus Irinotecan in Colorectal Cancer trial, has shown that adding cetuximab to irinotecan resulted in a higher PFS rate and ORR than with irinotecan alone in patients who had failed prior oxaliplatin-based therapy with or without the anti-VEGF antibody bevacizumab [45]. The OS rate did not differ significantly between the two arms, although results may have been confounded by the high rate of crossover into the dual therapy arm. Nevertheless, the PFS time was significantly longer, 4.0 months versus 2.6 months, the ORR was significantly higher, 16.4% versus 4.2% (p < .0001), and the disease control rate was significantly greater, 60.4% versus 45.8%, with the addition of cetuximab [45]. Moreover, the combination arm showed better preservation of health-related quality of life with less deterioration in symptom scores (pain, nausea, and insomnia) and global health status [68].

The cetuximab–oxaliplatin plus capecitabine (CAPOX) combination was evaluated in a phase II study of 15 mCRC patients refractory to standard chemotherapy (including 5-FU, oxaliplatin, and irinotecan) [58]. The efficacy analysis indicated PR in 27%, stable disease (SD) in 27%, and
Table 1. Selected clinical studies of cetuximab in combination with chemotherapy

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<th>Cancer typea</th>
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<th>Study details</th>
<th>ORR (combination versus CT only)</th>
<th>TTP</th>
<th>Grade 3 or 4 rash</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal (+) Second/third line</td>
<td>Irinotecan</td>
<td>Cetuximab (n = 111) versus combination (n = 218)</td>
<td>10.8% versus 22.9%</td>
<td>1.5 mos versus 4.1 mos</td>
<td>5.2% versus 9.4%</td>
<td>Cunningham et al. [44]</td>
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<tr>
<td>Colorectal (+) Second/third line</td>
<td>Irinotecan</td>
<td>Irinotecan (n = 650) versus combination (n = 648)</td>
<td>4.2% versus 16.4%</td>
<td>PFS, 3.98 mos versus 2.56 mos</td>
<td>0.2% versus 8%</td>
<td>Sobrero et al. [45]</td>
<td></td>
</tr>
<tr>
<td>Colorectal (+) Second/third line</td>
<td>Irinotecan</td>
<td>(n = 79)</td>
<td>25.3%</td>
<td>PFS, 4.0 mos</td>
<td>9%</td>
<td>Mathias et al. [46]</td>
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<tr>
<td>Colorectal (+) Second/third line</td>
<td>Irinotecan</td>
<td>Open label (n = 121)</td>
<td>17%</td>
<td>84 days</td>
<td>8%</td>
<td>Saltz et al. [47]</td>
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</tr>
<tr>
<td>Colorectal (±) Second/third line</td>
<td>Irinotecan</td>
<td>Retrospective; cetuximab (n = 2), combination (n = 14)</td>
<td>25% (both groups)</td>
<td>NA</td>
<td>NA</td>
<td>Chung et al. [48]</td>
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<tr>
<td>Colorectal (±) Second/third line</td>
<td>Irinotecan</td>
<td>Retrospective; cetuximab (n = 3), combination (n = 15)</td>
<td>21.4% (n = 4)</td>
<td>3.9 mos</td>
<td>NA</td>
<td>Seitz et al. [49]</td>
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<tr>
<td>Colorectal (+) First line</td>
<td>FOLFIRI</td>
<td>Open label (n = 20)</td>
<td>CR or PR, 67%; SD, 29%</td>
<td>9.9 mos</td>
<td>38%</td>
<td>Folprecht et al. [50]</td>
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<tr>
<td>Colorectal (+) First line</td>
<td>FOLFIRI</td>
<td>Open label (n = 1,217)</td>
<td>46.9% versus 38.7%</td>
<td>PFS, 8.9 mos versus 8.0 mos</td>
<td>18.7% versus 0.2%</td>
<td>Van Cutsem [51]</td>
<td></td>
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<tr>
<td>Colorectal (+) First line</td>
<td>IFL</td>
<td>Open label (n = 25)</td>
<td>44%</td>
<td>NA</td>
<td>19%</td>
<td>Rosenberg et al. [52]</td>
<td></td>
</tr>
<tr>
<td>Colorectal (+) First line</td>
<td>FOLFOX-4</td>
<td>Open label (n = 43)</td>
<td>72%</td>
<td>NA</td>
<td>30.2%</td>
<td>Diaz-Rubio et al. [53]</td>
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</tr>
<tr>
<td>Colorectal (+) First line</td>
<td>FOLFOX-4</td>
<td>Open label (n = 43)</td>
<td>72%; CR + PR = 9%</td>
<td>PFS, 12.3 mos</td>
<td>28%</td>
<td>Andre et al. [54]</td>
<td></td>
</tr>
<tr>
<td>Colorectal (+) First line</td>
<td>FOLFOX-4</td>
<td>Open label (n = 337)</td>
<td>45.6% versus 35.7%</td>
<td>In progress</td>
<td>In progress</td>
<td>Boke et al. [55]</td>
<td></td>
</tr>
<tr>
<td>Colorectal (+) First line</td>
<td>FUFOX</td>
<td>Low dose (n = 7) versus high dose (n = 41)</td>
<td>71% versus 54%</td>
<td>NA</td>
<td>17%</td>
<td>Lordick et al. [56]</td>
<td></td>
</tr>
<tr>
<td>Colorectal (±) First line</td>
<td>FOLFOX-6</td>
<td>Open label (n = 26)</td>
<td>NA</td>
<td>NA</td>
<td>12%</td>
<td>Scott et al. [57]</td>
<td></td>
</tr>
<tr>
<td>Colorectal (+) Second/third line</td>
<td>CAPOX</td>
<td>Open label (n = 15)</td>
<td>27%</td>
<td>2.5 mos</td>
<td>13%</td>
<td>Grothe et al. [58]</td>
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</tr>
<tr>
<td>Colorectal (±) Second/third line</td>
<td>CAPOX</td>
<td>(n = 40)</td>
<td>20%</td>
<td>3 mos</td>
<td>15%</td>
<td>Souglakos et al. [59]</td>
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(continued)
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Colorectal (±)</td>
<td>Second/third line</td>
<td>Irinotecan</td>
<td>Randomized; A, bevacizumab + irinotecan, n = 40; B, bevacizumab alone (n = 35)</td>
<td>PR, 35% versus 23%; SD, 43% versus 54%</td>
<td>5.8 mos (A) versus 4.0 mos (B)</td>
<td>NA</td>
<td>Saltz et al. [60]</td>
</tr>
<tr>
<td>Gastric</td>
<td>First line</td>
<td>FUFOX</td>
<td>Multicenter, phase II; (n = 52; 46 evaluable)</td>
<td>65.2%</td>
<td>7.6 mos</td>
<td>23.5%</td>
<td>Lordick et al. [61]</td>
</tr>
<tr>
<td>Pancreatic (+)</td>
<td>First line</td>
<td>Gemcitabine</td>
<td>Open label (n = 41)</td>
<td>12%</td>
<td>3.8 mos</td>
<td>12.2%</td>
<td>Xiong et al. [62]</td>
</tr>
<tr>
<td>Colorectal</td>
<td>Neoadjuvant</td>
<td>Irinotecan or oxaliplatin, or both</td>
<td>Preoperative treatment (median 6 cycles) (n = 151)</td>
<td>25/151 resected (9/133 or 7% treated referred); 24/25 (96%) disease free at 16 months</td>
<td>NA</td>
<td>NA</td>
<td>Aloia et al. [63]</td>
</tr>
<tr>
<td>Rectal</td>
<td>Neoadjuvant</td>
<td>Irinotecan + capecitabine + RT</td>
<td>Preoperative CRT (n = 40)</td>
<td>CR, 5%; PR, 5%</td>
<td>NA</td>
<td>NA</td>
<td>Hong et al. [64]</td>
</tr>
<tr>
<td>Rectal</td>
<td>Neoadjuvant</td>
<td>CAPOX + RT</td>
<td>(n = 60)</td>
<td>CR, 8%; &gt;50% regression, 42%</td>
<td>NA</td>
<td>3% (grade 3)</td>
<td>Arnold et al. [65]</td>
</tr>
</tbody>
</table>

(+) denotes EGFR-positive tumors; (±) denotes EGFR-positive and/or -negative tumors.

FOLFIRI consists of infusional 5-FU, LV, and irinotecan; IFL consists of irinotecan plus bolus 5-FU and LV; FOLFOX-4 consists of oxaliplatin plus 5-FU and LV; FOLFOX-6 consists of oxaliplatin plus 5-FU and LV; CAPOX consists of cetuximab and oxaliplatin plus capecitabine; FUFOX consists of infusional 5-FU and LV plus oxaliplatin.

Abbreviations: 5-FU, 5-fluorouracil; CR, complete response; CRT, chemoradiotherapy; CT, chemotherapy; LV, leucovorin; NA, not applicable; ORR, overall response rate; PR, partial response; RT, radiation therapy; SD = stable disease; TTP, time to progression.
disease progression in 46% of patients. The combination of CAPOX plus cetuximab also showed potential in a phase II study \( (n = 40) \) of mCRC patients who progressed under oxaliplatin-based chemotherapy [59]. One complete response (CR) and seven PRs were achieved (20% ORR); the median TTP was 3 months, median survival time was 10.7 months, and probability of 1-year survival was 53.4%.

**Cetuximab plus Bevacizumab: Combining Two Targeted Agents**

Cetuximab and bevacizumab attack different targets and disrupt tumor cells through different pathways; additionally, their respective toxicities are not expected to overlap if they are combined. Mouse xenograft experiments have shown synergistic responses from the combination of cetuximab plus an anti–VEGF receptor 2 antibody [69]. Based on this, the safety and efficacy of cetuximab plus bevacizumab, with or without irinotecan, were evaluated in the randomized phase II BOND-2 study. No unexpected toxicities were observed during a preliminary analysis of 64 irinotecan-refractory patients. Patients receiving cetuximab, bevacizumab, and irinotecan (CBI, \( n = 39 \)) experienced a 38% ORR, and patients on cetuximab and bevacizumab (CB, \( n = 35 \)) experienced a 23% ORR. The median TTP in the CBI group was 8.5 months, compared with 6.9 months in the CB group \( (p < .01) \) [60].

**First-Line Combination Therapy in CRC**

The addition of cetuximab to first-line chemotherapy regimens for mCRC has yielded encouraging results in several clinical studies. A phase I/II study has shown the safety and tolerability of cetuximab in combination with irinotecan, 5-FU, and LV (IFL, AIO schedule) in 20 patients with mCRC, with an ORR of 67% [50]. The median TTP was 9.9 months and median survival time was 33 months. The combination with chemotherapy had no effect on cetuximab pharmacokinetics.

Several phase II and III studies have investigated cetuximab with IFL, FOLFIRI, or FOLFOX-4 as a first-line treatment of EGFR-detectable mCRC. In a trial with cetuximab and IFL, 11 patients (44%) achieved PRs, and five additional patients (20%) achieved minor responses. Dose reductions were required for irinotecan and 5-FU during the first two cycles of therapy, which may have been a result of cetuximab-induced enhancement of IFL cytotoxicity [52]. In the more recently concluded phase III CRYSTAL (Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer) trial, which assessed and definitely established cetuximab plus FOLFIRI as first-line treatment in 1,217 patients with mCRC, the cetuximab–FOLFIRI combination met its primary endpoint of a significantly longer median PFS time (8.9 months) than with FOLFIRI alone (8 months, \( p = .036 \)). The response rate was also significantly higher with the addition of cetuximab (46.9% versus 38.7%; \( p = .005 \)) [51]. The most remarkable finding from this study was the longer PFS duration in the subgroup of patients with only liver metastases (21% of all patients) who received cetuximab plus FOLFIRI (11.4 months) versus patients in the same subgroup who received FOLFIRI alone (9.2 months). The cetuximab–FOLFIRI arm of this subgroup also showed twice as many patients with no residual tumor (9.8%) as the FOLFIRI arm (4.5%). These benefits were not associated with greater toxicity, with the exception of skin toxicity (84%) [51, 70]. Patients believed to be likely candidates for hepatic resection stand to benefit the most from this therapy, because the appropriate goal of neoadjuvant therapy is resectability instead of best response [71–73].

Oxaliplatin-based regimens have also been evaluated in combination with cetuximab. The international phase II ACROBAT (Phase I/II Study of First-Line Erbitux® + FOLFOX4 in Metastatic CRC) trial indicated the feasibility and efficacy of cetuximab in combination with FOLFOX-4 as first-line therapy in patients with mCRC [54]. The study resulted in a promising 72% confirmed ORR (31 of 43 patients), with CRs or PRs in 9% (4/43) and 63% (27/43) of patients, respectively, and SD in 23%. The median PFS time was 12.3 months; with a median follow-up of 30.5 months, the median OS time was 30.0 months. Recent reports on a larger, multicenter European study (OPUS [Phase I/II First-Line Erbitux® + FOLFOX4 in Metastatic CRC EMR-018 (ACROBAT)]) showed a higher ORR with first-line FOLFOX-4 plus cetuximab (45.6%) compared with FOLFIRI alone (35.7%); analysis of PFS and OS data is still under way [55].

Other cetuximab combination first-line therapies have been evaluated in more recent phase I, II, and III studies (Table 1). These include combinations with capcitabine-based XELIRI [Capecitabine (Xeloda®) plus Irinotecan] or XELOX [CAPOX, Capecitabine (Xeloda®) plus Oxaliplatin] [74, 75] and with capcitabine and bevacizumab (CAIRO2, Randomized Phase III Trial of Cetuximab plus Capecitabine, Oxaliplatin and Bevacizumab in Previously Untreated CRC) [76], mostly with encouraging significant improvements in ORR and TTP/PFS. Another study (the Cancer and Leukemia Group B/Southwest Oncology Group 80405 trial) involving ≥2,300 previously untreated patients is currently investigating three different combinations with standard chemotherapy—allowing patients with metastatic or locally advanced CRC who are receiving FOLFOX or FOLFIRI to receive either cetuximab, bevaciz-
zumab, or a combination of cetuximab and bevacizumab in addition to the chemotherapy [77].

**Cetuximab in Combination with Chemotherapy in Other GI Cancers**

The safety and tolerability of cetuximab in combination with irinotecan as salvage therapy have been demonstrated in a small phase II study of heavily treated metastatic gastric cancer patients. With a median number of 10 treatments per patient, the tumor control rate was 62% (8/13 patients), with PRs in five patients [78]. Another combination consisting of cetuximab plus oxaliplatin, 5-FU, and LV (FUFOX) has been shown to be highly active as first-line therapy for metastatic gastric cancer in a recent multicenter phase II study [61]. The ORR in 46 evaluable patients at seven study centers was 65.2%, including four CRs and 26 PRs. Activity was not correlated with EGFR detectability by immunohistochemistry (IHC); the response rate was 76.5% in undetectable and 54.5% in IHC-detectable tumors [61].

On the other hand, pancreatic cancer continues to frustrate efforts to substantially improve response rates and survival, although recent success has been achieved by combining erlotinib or capecitabine with gemcitabine, the traditional chemotherapeutic for pancreatic cancer [8, 79, 80]. Cetuximab in combination with gemcitabine [8] or with 5-FU [80] has been shown to significantly increase tumor cell apoptosis and significantly inhibit growth of human pancreatic cancer xenografts in mice. Notably, microvessel density was significantly reduced in tumors of mice treated with cetuximab or cetuximab and gemcitabine but not in those treated with gemcitabine alone, suggesting that cetuximab therapy affected angiogenesis in these tumors [8].

Preclinical studies of triple combination therapy with cetuximab, chemotherapy, and radiotherapy have shown greater antitumor activity than any monotherapy or dual combination therapies. In one set of xenograft studies using MiaPaCa-2 human pancreatic cancer cells, 100% of the mice treated with triple therapy (n = 8) experienced complete remission, with only one incident of tumor relapse. By comparison, none of the singly treated mice experienced complete remissions. A fraction of the mice treated with gemcitabine plus radiation and cetuximab plus radiation also experienced complete remissions but at much lower rates than did the triply treated animals (13% and 63%, respectively) [81]. Follow-up studies in cell culture showed that exposure of the MiaPaCa-2 human pancreatic cancer cells to cetuximab sensitized the cells to gemcitabine and radiation combination therapy.

Limited clinical data are available regarding the treatment of pancreatic cancer with cetuximab chemotherapy combinations in humans (Table 1). A phase II study involving 41 enrolled patients demonstrated cetuximab and gemcitabine combination therapy to have potential clinical benefit in the treatment of EGFR-positive patients with advanced pancreatic cancer [62]. However, in a large phase III trial of >700 patients with locally advanced unresectable or metastatic pancreatic cancer, cetuximab plus gemcitabine as first-line treatment did not show a significant clinical advantage in terms of the median OS time (6.5 months) compared with gemcitabine alone (6.0 months; p = .14) [82]. The combination arm also showed no superiority over gemcitabine alone in terms of the PFS duration (3.5 months vs. 3.0 months).

A phase II trial of first-line cetuximab plus gemcitabine and oxaliplatin yielded a high response rate with acceptable toxicity [83]. An intent-to-treat analysis of 34 patients showed an ORR of 38% (one CR and 12 PRs), a median TTP of 155 days, and a preliminary estimated 54% 6-month survival rate. A number of smaller phase I and II trials with cetuximab and other chemotherapies including gemcitabine as first-line treatment for advanced/locally advanced metastatic pancreatic cancer are planned or ongoing (see http://www.clinicaltrials.gov). Ongoing studies of advanced/metastatic pancreatic cancer are investigating cetuximab in combination with various agents, including gemcitabine alone or with oxaliplatin; cyclophosphamide plus vaccine therapy; gemcitabine, capecitabine, and radiation therapy; bevacizumab and gemcitabine; irinotecan and docetaxel; and ixabepilone. At least one study has demonstrated therapeutic activity of a non–gemcitabine-containing combination, irinotecan plus docetaxel with cetuximab (I/D/C) or without cetuximab (I/D), in metastatic pancreatic cancer, resulting in a median OS time of 7.4 months for patients treated with I/D/C and 6.5 months for those treated with I/D [84].

**Cetuximab–Chemotherapy Combinations in Adjuvant and Neoadjuvant Therapy**

In addition to its indication for CRC, cetuximab has also been approved as monotherapy or in combination with radiation therapy for treating squamous cell carcinoma of the head and neck [16]. In colorectal and other cancers, studies have been initiated to evaluate cetuximab–chemotherapy combinations in the adjuvant or neoadjuvant setting. In the recently initiated Pan-European Trials in Alimentary Tract Cancer 8 study, approximately 2,000 patients with stage III CRC will be given FOLFOX-4 with or without cetuximab after radical surgery for 6 months, and will be monitored for recurrence-free survival at 3 and 5 years. The study was still recruiting patients as of February 2007 (see http://www.clinicaltrials.gov). In an earlier study involving 151 patients...
with unresectable colorectal liver metastases resistant to initial chemotherapy, 27 patients underwent surgery after rescue treatment. Cetuximab plus irinotecan produced significantly higher resectability rates (20/27 patients) compared with treatment with cetuximab plus oxaliplatin (4/27) or both irinotecan and oxaliplatin (1/27) [63]. Postoperative mortality (one patient, 3.7%) and liver abnormalities (nine patients, 33%) remained low, and complete tumor necrosis was observed in two patients (8%). Ten of the 26 surviving patients remained disease free after a median follow-up duration of 16 months (range, 6–39 months) [63].

In rectal cancer patients, two phase II neoadjuvant therapy studies have been initiated evaluating cetuximab in combination with preoperative radiotherapy plus CAPOX or with radiotherapy plus 5-FU [85, 86]. In both studies, the addition of cetuximab to preoperative chemoradiotherapy regimens was shown to be safe and well tolerated. Recently, it was demonstrated that cetuximab given concurrently with CAPOX neoadjuvant radiotherapy in 60 patients with untreated, advanced rectal cancer resulted in significant radiologic downstaging (35% for tumor category and 67% for node category) without requiring dose reduction of either chemo- or radiotherapy [65]. Similarly, a recently completed phase II study of preoperative cetuximab, irinotecan, and capecitabine chemotherapy with radiation showed promising pathologic responses with mild toxicity in patients with locally advanced resectable rectal cancer [64].

**THE SAFETY OF CETUXIMAB THERAPY**

A robust clinical studies program has shown cetuximab to be a well-tolerated therapeutic agent, with a low incidence of grade 3 or 4 adverse events and no exacerbation of the known toxicities of chemotherapeutic agents when used in combined regimens to treat patients with CRC, pancreatic cancer, or head and neck cancer [87] (Table 2). So far, there have been no reports of differences in the type or frequency of cetuximab-related adverse events resulting from combination with chemotherapy. The most frequently reported adverse event associated with cetuximab therapy is acneiform rash, which usually appears on the face, scalp, neck, or upper torso within the first 3 weeks of treatment. Although the rash is often mild, some individuals experience grade 3 or 4 rash, which appears to correlate with response rate and survival [88].

Infusion-related reactions (IRRs) have been observed in a small proportion of patients, particularly after the first infusion, and have been managed by slowing the infusion rate and the use of antihistamines [16]. In the MABEL study, the frequency of grade 3 or 4 IRRs was lower, 1% versus 4.7%, with the addition of corticosteroids to antihistamines administered for prophylaxis [89].

Occasionally, patients treated with cetuximab and other EGFR-targeted agents may develop hypomagnesemia

<table>
<thead>
<tr>
<th>Body system</th>
<th>Incidence of grade 3 or 4 AE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cetuximab + irinotecan (n = 354)</td>
</tr>
<tr>
<td>Whole body</td>
<td></td>
</tr>
<tr>
<td>Asthenia/malaise/somnolence</td>
<td>16</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>8</td>
</tr>
<tr>
<td>Fevera</td>
<td>4</td>
</tr>
<tr>
<td>Pain</td>
<td>6</td>
</tr>
<tr>
<td>Infusion reactionb</td>
<td>3</td>
</tr>
<tr>
<td>Back pain</td>
<td>3</td>
</tr>
<tr>
<td>Headache, infection</td>
<td>≤2</td>
</tr>
<tr>
<td>Digestive</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>22</td>
</tr>
<tr>
<td>Nausea</td>
<td>6</td>
</tr>
<tr>
<td>Vomiting</td>
<td>7</td>
</tr>
<tr>
<td>Anorexia</td>
<td>4</td>
</tr>
<tr>
<td>Constipation, stomatitis, dyspepsia</td>
<td>≤2</td>
</tr>
<tr>
<td>Hematic/lymphatic</td>
<td></td>
</tr>
<tr>
<td>Leukopenia</td>
<td>17</td>
</tr>
<tr>
<td>Anemia</td>
<td>5</td>
</tr>
<tr>
<td>Metabolic/nutritional</td>
<td></td>
</tr>
<tr>
<td>Weight loss, peripheral edema</td>
<td>≤1</td>
</tr>
<tr>
<td>Dehydration</td>
<td>6</td>
</tr>
<tr>
<td>Nervous</td>
<td></td>
</tr>
<tr>
<td>Insomnia, depression</td>
<td>0</td>
</tr>
<tr>
<td>Respiratory</td>
<td></td>
</tr>
<tr>
<td>Dyspneaa</td>
<td>2</td>
</tr>
<tr>
<td>Increased cough</td>
<td>0</td>
</tr>
<tr>
<td>Acneiform rashc</td>
<td>14</td>
</tr>
<tr>
<td>Skin, appendages</td>
<td></td>
</tr>
<tr>
<td>Alopecia, skin disorder, nail disorder, pruritus, conjunctivitis</td>
<td>≤1</td>
</tr>
</tbody>
</table>

*a*Includes cases reported as infusion reactions.

*b*Defined as an allergic reaction or anaphylactoid reaction, or as fever, chills, fever and chills, or dyspnea on the first day of dosing.

*c*Defined as acne, rash, maculopapular rash, pustular rash, dry skin, or exfoliative dermatitis.
and/or hypocalcemia [90]. Among 176 patients treated with cetuximab, 22% experienced hypocalcemia. Of the 47 patients tested for serum magnesium, eight grade 3 and three grade 4 toxicities were reported. In a separate study, after a median of 8 weeks on therapy, 65% developed hypomagnesemia [91]. Patients on cetuximab should therefore be monitored for calcium and magnesium deficiency.

**RELATIONSHIP BETWEEN ACNEIFORM RASH AND CETUXIMAB EFFICACY**

Recently, the randomized phase I/II EVEREST (Evaluation of Various Erbitux® Regimens by Means of Skin Tumor Biopsies) trial (n = 166) showed that patients who exhibit acneiform rash of grade 1 or 0 from the standard weekly dose of 250 mg/m² cetuximab may benefit from cetuximab dose escalation [92]. After 3 weeks of cetuximab plus irinotecan treatment, patients with minimal skin reactions were randomized to receive cetuximab for 24 weeks at the standard dose or with dose escalation. Cetuximab dose escalation up to 500 mg/m² resulted in a higher response rate, 30%, versus 13% in patients who received the standard cetuximab dose. The incidence of grade 3 or 4 skin rash was greater with dose escalation (9% versus 0%), yet the therapy was generally well tolerated.

**CONCLUSION**

Cetuximab combination therapies evaluated in colorectal and other GI cancers to date have shown impressive results in terms of both survival and tumor response rates, together with generally acceptable and manageable levels of toxicity (with the most commonly reported grade 3 or 4 events being acneiform rash, neutropenia, nausea, diarrhea, paresthesia, and asthenia). Two premises point to the likely benefit of earlier intervention with cetuximab treatment: (a) the higher response rates in heavily pretreated patients and (b) the encouraging safety results obtained with neoadjuvant treatment. Combinations of multiple targeted biologic agents with chemotherapy, such as cetuximab and bevacizumab plus either FOLFOX or FOLFIRI, are additional exciting possibilities currently being evaluated. It is noteworthy that the combination of cetuximab and irinotecan produced significant response rates in irinotecan-refractory patients, suggesting the existence of a mechanism for overcoming irinotecan resistance and resensitization of tumor cells to chemotherapy that has yet to be elucidated. Studies of cetuximab in combination with chemotherapy and/or other targeted therapies, and as first-line therapy, are likely to expand the indications for this agent. Further studies should address which combination therapies are most effective for specific cancers of the GI tract, which cetuximab–chemotherapy combinations can elicit the most favorable responses overall in various clinical settings, whether or not and how agents other than cetuximab can improve the efficacy of radiotherapy and DNA-damaging chemotherapy, and which, if any, biomarkers can be used to guide the design of treatment regimens for GI tract tumors.

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