

Buddhist monks, evening relaxation, Angkor Wat, Cambodia. Photograph courtesy of J. Bryan Murphy, MD, Clearwater, Florida.

Therapeutic targeting of molecular structures may enhance the treatment of colorectal cancer.

Targeted Therapies in the Treatment of Colorectal Cancers

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Background: *In solid organ malignancies, no tumor type has seen a greater impact from the development of novel targeted therapies in 2004 than metastatic colorectal cancer.*

Methods: *We review the current progress to date with the use of monoclonal antibodies in colorectal cancer and look at newer therapies under investigation.*

Results: *Two monoclonal antibodies received Food and Drug Administration approval in early 2004, both for the indication of advanced, metastatic colorectal cancer. A large, randomized, placebo-controlled study demonstrated that the addition of a monoclonal antibody to vascular endothelial growth factor, bevacizumab, led to a statistically significant improvement in overall survival, with tolerable additional toxicity. Chimeric monoclonal antibody therapy directed at the epidermal growth factor receptor was associated with radiographic responses in a significant minority of patients with irinotecan-refractory colon cancer in a randomized phase II study of patients with irinotecan-refractory disease.*

Conclusions: *These dramatic successes have led to further clinical studies of targeted therapy in colorectal cancer, making it one of the most promising areas of cancer research.*

Background

The relationships between growth factors, cell surface receptors, and their second messengers in the development and progression of human malignancies are not new concepts. However, recent advances in molecular and cell biology techniques have allowed for rapid advances in rational drug design and targeted therapies for the treatment of human malignancies. Among the most recent advances in treatments for colorectal cancer are therapies that target novel cellular entities such as EGFR and VEGF (Figure). The uses of therapeutic targeting of these molecular structures in colorectal cancer are enhancing the

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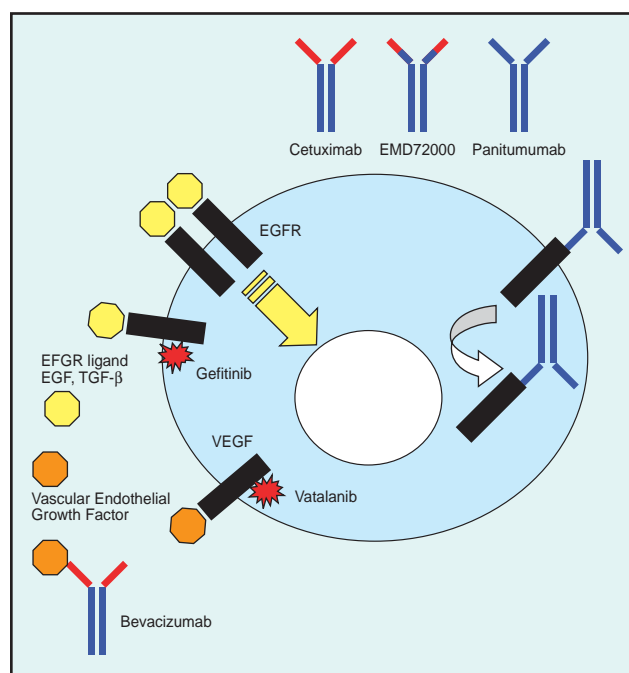
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treatment of colorectal cancer. This review summarizes targeted therapies for treating colorectal cancer.

Epidermal Growth Factor

Growth factors and their receptors are vital to normal cellular dynamics, as they regulate cellular growth, proliferation, differentiation, migration, angiogenesis, and cell death. Six subclasses of tyrosine kinase receptors have been isolated and fully characterized. These include receptors for the following unique ligands: (1) insulin, (2) platelet-derived growth factor, (3) epidermal growth factor, (4) fibroblast growth factor, (5) ephrins, and (6) growth hormone.¹ EGFR is a monomeric 170-kD transmembrane glycoprotein that possesses intrinsic tyrosine kinase activity. EGFR belongs to the family ErbB, of which four receptors have been characterized: HER-1 (EGFR or *c-erb B-1*), HER-2 (*neu* or *c-erb B-2*), HER-3 (*c-erb B-3*), and HER-4 (*c-erb B-4*). EGFR is encoded by the *c-erb B-1* protooncogene and is expressed on both normal and malignant cells, primarily of epithelial origin. EGFR is a transmembrane entity that consists of three important regions to help serve its function as a cell-signaling receptor. Epithelial growth factor (EGF) and transforming growth factor- α are the two major extracellular endogenous ligands that activate EGFR. However, other endogenous lig-



Monoclonal antibodies cetuximab (chimeric), EMD72000 (humanized), and panitumumab (fully human) bind cell surface epidermal growth factor receptor (EGFR) and inhibit binding of natural ligands epidermal growth factor (EGF) and transforming growth factor beta (TGF- β), in addition to causing receptor internalization and proteosomal degradation. The humanized antibody bevacizumab binds circulating vascular endothelial growth factor (VEGF) preventing binding to receptor. Receptor tyrosine kinase inhibitors gefitinib and vatalanib work intracellularly to inhibit the EGFR and VEGFR receptor, respectively.

ands that may function to activate EGFR include amphiregulin, heparin binding-epithelial growth factor, and betacellulin. Once a ligand binds to the extracellular domain of EGFR, then receptor dimerization occurs. Internalization of both receptor and ligand induces autophosphorylation and subsequent activation of tyrosine kinase. As a consequence, signaling pathways such as Ras-Raf-MAP kinases, phosphatidylinositol 3-kinase, Akt, Jak/Stat kinases, and protein kinase C are activated and gene transcription is regulated.

EGFR gene expression occurs in 25% to 77% of colorectal neoplasms.² The wide range of EGFR expression seen between the different studies reflects the technical difficulties of antigen retrieval required in immunohistochemical (IHC) studies. Those EGFR-expressing colorectal neoplasms have been shown in retrospective studies to behave more aggressively and prognosticates for poorer survival rates.³

Cetuximab

Cetuximab is a chimeric immunoglobulin IgG1 monoclonal antibody that was initially developed from the murine antibody M225 developed by Mendelsohn and colleagues.⁴ It functions as a competitive antagonist that binds to the extracellular endogenous binding domain of the EGFR with a 5- to 10-fold greater affinity than its natural ligands, epidermal growth factor and transforming growth factor- α . Cetuximab acts primarily by downregulating EGFR expression and thereby limits the Ras-Raf-MAP, phosphatidylinositol 3-kinase, Akt, Jak/Stat kinases, and protein kinase C signal transduction pathways. Inhibiting cell cycle progression by causing G₁ phase cell cycle arrest via Rb protein hypophosphorylation, activation of pro-apoptotic molecules, inhibition of angiogenesis, downregulation of vascular endothelial growth factor expression, and inhibition of metastasis by reducing expression of matrix metalloproteinases are among other postulated mechanisms by which cetuximab inhibits tumorigenesis.⁵ This competitive monoclonal antibody is not effective against mutant forms of EGFR, as it requires an intact extracellular binding region to remain effective. Unlike the predictable correlation of response to trastuzumab in HER-2/*neu*-expressing breast cancers (as determined by IHC), responses to cetuximab in EGFR-expressing colorectal cancers is not predictable.

An open-label, multicenter study (the "BOND" trial) randomized 329 patients with stage IV, EGFR-expressing colorectal adenocarcinomas to receive either a combination irinotecan plus cetuximab or cetuximab monotherapy (400 mg/m² as initial dose, then 250 mg/m² weekly thereafter).⁶ Irinotecan was administered in the same dose and schedule that the patients had previously received their therapy (weekly, fortnightly, or once every 21 days). All patients had documented radiographic evidence of disease progression while receiving an irinotecan-based regimen and were EGFR-positive by IHC (DAKO

kit). The overall response rate for 218 patients who received combination irinotecan plus cetuximab was 22.9% ($P=.007$), while 111 patients who received cetuximab alone demonstrated a 10.8% response rate ($P=.007$). The median time to progression was 4.1 months for the combination group and 1.5 months for the cetuximab monotherapy group. Severe anaphylaxis occurred in approximately 2%, but there were no treatment-related deaths. Fifty-six patients who received monotherapy developed disease progression and crossed over; the outcome of these patients has not yet been reported. An acneiform maculopapular rash developed in 80%. The development of rash correlated with a higher survival rate and was associated with higher radiographic response rates compared with those who did not develop a rash. The authors also concluded that predictability of cetuximab response does not correlate with EGFR expression. Induction of apoptosis, DNA impairment, and reduction in irinotecan efflux are some of the proposed mechanisms by which cetuximab augments irinotecan and is able to overcome irinotecan resistance.

The radiographic response rates seen in this large randomized phase II study were remarkably similar to two prior nonrandomized phase II studies conducted in the United States. These studies were initially critiqued for having insufficient source documentation to justify the eligibility criterion that subjects have documented disease progression while receiving irinotecan therapy. However, subsequent work was able to retrieve the supporting documentation to sustain the assertion that the population studies were irinotecan refractory in over half of the cases. In a multicenter study conducted in the United States, with documented evidence of irinotecan resistance radiographically, 15% developed a radiographic partial response (confirmed by independent radiographic review) to therapy with irinotecan plus cetuximab.⁷ A subsequent multicenter study of cetuximab monotherapy in irinotecan-refractory colorectal cancer demonstrated a 9% radiographic response rate (5 of 57 cases).⁸ These two studies, interpreted with the BOND trial, show that cetuximab has significant activity in a subset of patients with metastatic colorectal cancer. Although the frequency of activity as judged by radiographic response rates is low, a higher percentage of patients in all three studies show a failure to progress following 3 months of therapy, suggesting a clinically useful biologic effect in a larger percentage of patients. It is important to note that the use of the drug is associated with modest and acceptable toxicity. Less than 2% of patients experience a severe hypersensitivity reaction (grade 3 or greater toxicity as measured by the Common Toxicity Criteria for Adverse Events⁹).

Cetuximab was provisionally approved by the FDA in February 2004 based on a surrogate marker for survival, radiographic response. Studies are ongoing to document that cetuximab, when added to conventional second-line

chemotherapy in patients whose tumors are EGFR-positive by IHC, will lead to a survival benefit. The ongoing multicenter phase III EXPLORE study randomizes subjects with EGFR-positive tumors by IHC to either FOLFOX4 (5-fluorouracil, leucovorin, oxaliplatin), or FOLFOX4 plus cetuximab. This study began accrual in March 2003 and plans to continue accrual until a target of 1,100 subjects are enrolled. Preliminary safety data on 40 patients suggest that the addition of cetuximab to FOLFOX4 does not increase the toxicity of chemotherapy.¹⁰ A second phase III study, the EPIC trial (CA225006), seeks to evaluate the impact on overall survival when cetuximab is added to irinotecan compared with single-agent irinotecan alone in patients with EGFR-positive tumors who have developed disease progression on first-line oxaliplatin-based therapy.

Clinical trials initially were based on the assumption that presence of the target (in this case, cell-surface EGFR) would be necessary before seeing activity of the drug. This presumption was based largely on the prior clinical experience of trastuzumab, another chimeric antibody in treatment of breast cancer, targeting the another EGFR subclass receptor, HER-2/*neu*. However, in contradistinction to trastuzumab, quantitative levels of receptor (as judged by IHC) were not correlated with radiographic response rates. In fact, of 9 subjects who were EGFR-negative by IHC treated with cetuximab monotherapy, 2 had a partial response to therapy, 1 confirmed.¹¹ In a second study of 16 patients with metastatic colorectal cancer, whose tumors were EGFR-negative by IHC, four major objective responses were seen with the use of cetuximab therapy.¹² These preliminary data suggest that cetuximab may have similar activity in EGFR-negative and EGFR-positive tumors when assessed by IHC. This assertion has to be validated by additional ongoing studies.

Panitumumab (ABX-EGF, clone E7.6.3)

Since the most serious grade 4 toxicity from cetuximab relates to hypersensitivity reactions, fully humanized antibodies to the EGFR extracellular domain have been developed. Of these, ABX-EGR and EMD72000 are the furthest along in clinical development. ABX-EGF is a fully humanized IgG2 subclass monoclonal EGFR antibody, with high affinity ($K_D = 5 \times 10^{-11}M$) for the receptor. Preclinical studies show tumor growth inhibition and chemosensitization as similar to those demonstrated with cetuximab.¹³ A large phase II study of the compound's activity in patients with advanced colorectal cancer in whom therapy with 5-fluorouracil, irinotecan, and oxaliplatin has failed was commenced in 2003. Over 200 patients have been accrued to this study. Preliminary data on 148 subjects presented showed similar activity in terms of radiographic response rate by RECIST criteria compared with historical cetuximab data: 10.1% had confirmed partial response rates and 36.5% had stable disease.¹⁴ Greater than 90% developed skin rash. Only 1 subject developed a grade 3 hypersensitivity reaction that was believed to be

attributable to drug; the patient subsequently received therapy with premedication without further reaction.

EMD72000

EMD72000 is a genetically engineered monoclonal antibody with a similar high affinity for the EGFR receptor ($K_D = 3.4 \times 10^{-10}M$) as cetuximab and ABX-EGF.¹⁵ A phase I study of subjects with predominantly gastrointestinal and head and neck malignancies evaluated five absolute dose levels between 400 mg/week and 2,000 mg/week.¹⁶ The drug was well tolerated up to doses of 1,600 mg/week, with the dose-limiting toxicity at 2,000 mg/week being fever and rash. Investigators determined radiographic responses, using World Health Organization response criteria, occurred in 23% of patients, with stable disease being observed in 27%. These promising phase I data have led to a multicenter phase II study. Further studies will be needed to determine the efficacy of this antibody, both as monotherapy and in combination with chemotherapy, in the management of colorectal cancer.

Gefitinib and Erlotinib

Preclinical studies suggested that gefitinib (Iressa), a receptor tyrosine kinase inhibitor of the EGFR, may have significant single-agent activity in colorectal cancer.¹⁷ In light of the activity of EGFR antibodies in colorectal cancer, activity might have been anticipated with this drug; however, a multicenter phase II study by the Eastern Cooperative Oncology Group (ECOG 6200) of 115 patients with metastatic colorectal cancer failed to demonstrate any single-agent activity when judged by radiographic response rates. No changes in EGFR activation were seen in markers of EGFR activation obtained before and 1 week after therapy when paired tumor samples were analyzed.¹⁸ Ongoing studies are evaluating the single-agent efficacy of erlotinib (Tarceva), a similar oral EGFR receptor tyrosine kinase inhibitor. Preliminary data from a phase II study indicate that the best response to therapy to date to be stable disease.¹⁹

Vascular Endothelial Growth Factor

Targeted therapies for treating colorectal cancer have expanded beyond inhibiting cell surface signaling receptors. Interfering with receptor stimuli, such as vascular endothelial growth factor (VEGF) inhibition, has added a new dimension to treating advanced colorectal cancers.

VEGF is an approximately 45-kD proangiogenic homodimeric glycoprotein that functions as a major contributor in stimulating pathologic angiogenesis. Although VEGF functions to stimulate angiogenesis and regulate vascular permeability as part of physiologic homeostasis, this review focuses only on pathologic angiogenesis. Similar to growth factor receptors, VEGF is considered part of a family of growth factors, of which six currently exist: VEGF

placental growth factor, VEGF-B, VEGF-C, VEGF-D, and VEGF-E. Two tyrosine kinase receptor subtypes, VEGFR-1 (Flt-1, *fms*-like tyrosine kinase) and VEGFR-2 (Flk-1/KDR) mediate the effects of VEGF via signal transduction, ultimately regulating angiogenesis.²⁰

Endothelial permeability is considered an important phase for tumor growth, propagation, and metastasis. VEGF increases permeability by creating endothelial fenestrations, thus allowing plasma proteins to leak into the extravascular space and create a fibrin-like microenvironment.²¹ This microenvironment serves as the foundation for microvessel and tumor growth.

Approximately 50% of colorectal neoplasms express VEGF and expression appears to be a poor prognostic marker in colorectal cancers.^{22,23} Levels of VEGF expression have been shown to be proportional to the likelihood of disease recurrence, extent of neovascularization, proliferation, and subsequent metastasis.^{24,25} Warren et al²⁶ reported increased VEGFR-1 and VEGFR-2 expression in liver metastasis for colorectal cancers.

Bevacizumab

Bevacizumab (Avastin) is a 149-kD recombinant humanized monoclonal IgG1 antibody that consists of approximately 93% human framework domains and 7% murine-derived binding domains.^{27,28} It is indicated as first-line therapy for treating metastatic colorectal cancer in combination with an intravenous 5-fluorouracil-based regimen. Bevacizumab selectively inhibits VEGF, thereby preventing VEGF activation of VEGFR-1 and VEGFR-2 receptors.

The solitary phase III study that evaluated bevacizumab in patients with advanced colorectal cancer was performed by Hurwitz et al.²⁹ This randomized, multicenter study evaluated the overall survival in 402 subjects with metastatic colorectal cancer who were randomized to receive irinotecan, 5-fluorouracil, leucovorin (IFL) plus bevacizumab (5 mg/kg every 2 weeks) compared with 411 patients who were randomized to receive IFL plus placebo. Twenty-eight percent of the patients who were randomized to the placebo group had received prior fluoropyrimidine (adjuvant or radiosensitizing) therapy, while 24% of the patients randomized to the bevacizumab group had received prior fluoropyrimidine therapy more than 1 year prior to study entry. Prior radiation therapy had also been given to 14% of those receiving IFL plus placebo and 15% of those receiving IFL plus bevacizumab. The median duration of overall survival, the primary end point of this study, was 20.3 months for those in the bevacizumab group and 15.6 months in the placebo group ($P < .001$). One-year survival rates were 74.3% and 63.4% for those who received IFL plus bevacizumab and IFL plus placebo, respectively. Secondary end points were a 10.6-month progression-free survival (IFL plus bevacizumab) compared with a 6.2-month progression-free survival (IFL plus placebo).

The efficacy of bevacizumab, when used as neoadjuvant therapy, has not yet been established. Willett et al³⁰

conducted a phase I study and analyzed 6 patients with primary, locally advanced rectal adenocarcinomas who received bevacizumab (5 mg/kg) as an initial dose, followed by 2-week cycles times three of bevacizumab (5 mg/kg) concurrently with 5-fluorouracil and pelvic and pelvic external beam radiation therapy 2 weeks later. Seven weeks after the cessation of therapy, all underwent surgical resection. There were no dose-limiting toxicities or perioperative complications. Further studies will be needed to assess the effect of bevacizumab as monotherapy or in combination, when used in a neoadjuvant setting.

The addition of bevacizumab at a dose of 10 mg/m² per week in the second-line setting (irinotecan-refractory subjects) to palliative FOLFOX4 chemotherapy has been shown to improve survival in an ECOG randomized phase III study.³¹ Preliminary results showed a prolongation in median survival from 10.7 to 12.5 months. The combination of bevacizumab and cetuximab with or without irinotecan is being studied in an ongoing randomized phase II trial being conducted in irinotecan-refractory subjects.³²

Vatalanib (PTK787/ZK222584)

A small molecule receptor tyrosine kinase inhibitor of the VEGF receptor, vatalanib, has been developed and is the first VEGF receptor inhibitor suitable for oral administration.³³ Single-agent studies suggested that PTK787 had modest single-agent activity in colorectal cancer; 8 of 16 subjects had stable disease by Southwest Oncology Group (SWOG) criteria, with a significant reduction in their dynamic contrast magnetic resonance imaging enhancement in those subjects with stable disease.³⁴ This has led to combinations with FOLFOX4 chemotherapy³⁵ and FOLFIRI chemotherapy,³⁶ with tolerable and acceptable toxicity profiles. Whether the addition of this novel agent to first-line FOLFOX4 chemotherapy in metastatic colorectal cancer will lead to a survival benefit is the subject of an ongoing phase III randomized CONFIRM-1 trial (Colorectal Oral Novel Therapy for the Inhibition of Angiogenesis and Retarding Metastasis). This trial has recently completed accrual and will hopefully be reported sometime

this year. The CONFIRM-2 trial is a phase III study evaluating whether there is a survival benefit to the addition of vatalanib to FOLFOX-4 in the second-line setting (patients who have progressed on front-line irinotecan plus 5-fluorouracil therapy).

Conclusions

The significant unmet medical need posed by recurrent colorectal cancer that is not curable with surgery has prompted extensive clinical research in an effort to find agents with superior single-agent activity. Some of the ongoing and recently completed phase II trials in the United States are demonstrated in the Table. The recent advances are leading to efforts to determine the appropriate sequencing and combination of these agents for the optimal treatment of recurrent colorectal cancer. It is anticipated that the improved outcomes and survivals now being achieved in metastatic disease will lead to changes in the observed natural history of the treated disease, requiring new strategies to manage central nervous system and osseous relapses. Given that a substantial minority of patients with visceral relapse may be cured with surgical intervention, improved systemic therapies are altering the surgical management of advanced disease and requiring new approaches to the selection criteria of patients for metastasectomy with curative or palliative intent. All of these questions will require the continued participation of patients in clinical trials in order to devise the most appropriate and effective treatment strategies for patients.

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Experimental Compounds in Ongoing or Recently Completed Phase II Clinical Trials

Experimental Agent	Target	Research Sponsor	Phase I Experience
ABT-751	Colchicines binding site of β -tubulin	Abbott Laboratories	Kobayashi et al ³⁷ Cho et al ³⁸
Pelitrexol (AG2037)	Folate acid synthesis (guanyl aryl ribonucleotide transferase [GARFT] inhibitor	Pfizer Inc	Garrett et al ³⁹
DJ-927	β -tubulin (taxane)	Daiichi Pharmaceuticals	Syed et al ⁴⁰ Beeram et al ⁴¹
Epothilone D	β -tubulin	Kosan Biosciences	Holen et al ⁴²
Imatinib mesylate (STI-571)	Receptor tyrosine kinase inhibitor (<i>c-kit</i> , <i>bcr-abl</i> , platelet-derived growth factor)	M.D. Anderson Cancer Center	Peng et al ⁴³
Thalidomide	Angiogenesis	National Cancer Institute (randomized phase II following surgery)	

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