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# Integration of novel agents in the treatment of colorectal cancer

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**Abstract** Two of the most promising new targets in the treatment of colorectal cancer are the epithelial growth factor receptor (EGFR) and the vascular endothelial growth factor (VEGF). Agents that inhibit the EGFR or bind to VEGF have demonstrated clinical activity as single agents and in combination with chemotherapy in phase II and phase III clinical trials. The most promising of these agents are cetuximab, which blocks the binding of EGF and transforming growth factor α (TGF-α) to EGFR, and bevacizumab, which binds free VEGF. Cetuximab and irinotecan have been evaluated in two clinical studies in the USA (IMCL CP02-0141 and IMCL CP02-9923). Study IMCL CP02-0141 evaluated the antitumor activity of single-agent cetuximab in patients with irinotecan-refractory, EGFR-positive metastatic colorectal carcinoma. There were 6 partial responses in 57 treated patients, for a response rate of 10.5%. Study IMCL CP02-9923 evaluated the combination of cetuximab and irinotecan in a total of 139 patients enrolled at 27 study sites. In this trial 22.5% of patients with progressive disease on irinotecan achieved an objective response (19% by investigator assessment) showing that the combination of cetuximab and irinotecan has antitumor activity in this population. A large randomized phase II trial evaluating similar study populations in Europe confirmed these findings, demonstrating response rates for cetuximab/irinotecan and cetuximab alone of 22.9% and 10.8%, respectively. The other promising agent bevacizumab is a humanized variant of the anti-VEGF monoclonal antibody. VEGF is produced by healthy and neoplastic cells. Its activities are mediated by two receptor tyrosine kinases. VEGF signaling is often a rate-limiting step in physiologic and pathologic angiogenesis. Bevacizumab has been studied as an antiangiogenic cancer therapeutic as a single agent and in combination with chemotherapy in patients with stage III and IV colon cancer. In addition to its direct antiangiogenic effects, bevacizumab may allow more efficient delivery of chemotherapy by altering tumor vasculature and decreasing the elevated interstitial pressure common in tumors. In this regard, some of the most robust phase II data using bevacizumab are from a randomized study of chemotherapy [fluorouracil (5-FU) and leucovorin (LV)] with or without bevacizumab in metastatic colorectal cancer. In this study, treatment with bevacizumab plus 5-FU/LV resulted in higher response rates, longer median time to disease progression, and longer median survival. Recently, a phase III, multicenter, double-blind, randomized, placebo-controlled trial was designed to investigate the addition of bevacizumab to first-line irinotecan, 5-FU, and LV chemotherapy (IFL). The trial showed a higher response rate, longer time to tumor progression, and prolonged overall survival in patients with metastatic colorectal cancer. It was the first large, randomized, phase III survival trial to assess the importance of targeting VEGF and tumor angiogenesis for the treatment of human cancer. Integration of novel agents targeting VEGF and EGFR with irinotecan-based chemotherapy has shown clinical activity in patients with metastatic colorectal cancer. The goal in the future will be to predict which specific chemotherapy and targeted agent combination will most likely benefit individual patients.

**Keywords** VEGF · EGFR · Novel targets · Bevacizumab · Cetuximab

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## Introduction

The treatment of colorectal cancer has consisted of fluoropyrimidine-based chemotherapy for over 50 years. Recently, the standard of care for the treatment of metastatic colorectal cancer has shifted and consists of combination chemotherapeutic regimens containing irinotecan and oxaliplatin. These regimens have increased time to progression and overall survival in patients with metastatic colorectal cancer. Ongoing clinical trials are assessing the efficacy of these agents in the adjuvant setting.

Because various available chemotherapeutic agents have demonstrated activity in metastatic disease, the intergroup trial N9741 was designed to evaluate fluorouracil (5-FU), oxaliplatin, and irinotecan as first-line treatment for patients with metastatic colorectal cancer. N9741 effectively compared 5-FU/leucovorin (LV)/irinotecan (IFL) with 5-FU/LV/oxaliplatin (FOLFOX). The response rate, median time to progression, and overall survival for patients treated with FOLFOX was found to be superior to those treated with IFL. The median overall survival of patients receiving FOLFOX was 19.5 months vs 15.0 months for patients receiving IFL [10]. In patients receiving both agents sequentially and crossing over to the other at the time of progression, the overall survival has been reported as approximately 22 months [24].

Even with the significant improvement in traditional chemotherapy, there remain limitations with this treatment. Therefore, several novel targets are being investigated both as single agents and in combination with chemotherapy to assess the potential for increased efficacy. Some of the most promising targets include the epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF). Two agents that have demonstrated activity in phase II and III trials include cetuximab, which blocks the binding of EGF and transforming growth factor α (TGF-α), and bevacuzimab, which binds free VEGF.

## Epidermal growth factor receptor

The EGFR is composed of four homologous receptors: the EGFR (ErbB1/EGFr/HER1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4) [2]. EGFR is a plasma membrane glycoprotein that is composed of an extracellular ligand-binding domain, a transmembrane lipophilic region that anchors the receptor to the plasma membrane, and a cytoplasmic region containing a tyrosine kinase domain with a regulatory carboxyl terminal segment. There are at least six different ligands known as EGF-like ligands that bind to the EGFR (ErbB1). The main ligands responsible for activation are EGF and TGF-α. After ligand binding, EGFR dimerization occurs, which results in high-affinity ligand binding, activation of the intrinsic protein tyrosine kinase activity, and tyrosine autophosphorylation [16]. The EGFR is overexpressed in many epithelial tumors, including 25–77% of colorectal cancers. Overexpression of EGFR has been associated with a poor prognosis and has been suggested as a potential target for antitumor agents. Studies have shown that monoclonal antibodies (mAbs) directed at the EGFR inhibit the growth of EGFR-expressing cancer cells [2, 18].

## Cetuximab

Cetuximab is a recombinant DNA-derived, chimerized mAb engineered by attaching the murine Fv regions of M225 (a murine antibody to block the ligand binding site of EGFR) to a human IgG1 constant region gene segment. It binds to the receptor with affinity comparable to that of the natural ligand (Kd 1 nmol/l) and competes with ligand binding and receptor tyrosine kinase activation. It also induces antibody-mediated receptor dimerization (without activation of the tyrosine kinase) [14]. This results in downregulation of EGFR, which prevents further receptor binding and activation by the ligands. This appears to be important for inhibition of growth.

There are multiple mechanisms that are thought to contribute to the antitumor activity of cetuximab. The addition of mAb 225 to tumor cell cultures has been shown to result in a concentration-dependent direct inhibition of receptor tyrosine kinase activity [14]. Cetuximab also interrupts the EGFR-ligands' autocrine pathway resulting in $G_1$ phase arrest. The accumulation of cells in $G_1$ phase is accompanied by elevated levels of p27Kip1 inhibitor of cyclin-dependent kinases, which suggests that reduction in cell proliferation may be caused by the modulation of molecules that regulate cell-cycle progression. It has also been shown that a variety of proapoptotic mechanisms are activated when the EGFR signaling pathway is blocked by mAb 225. Inhibiting EGFR may also induce antimetastatic properties and antiinvasive properties. Another significant mechanism is inhibition of angiogenesis. This was demonstrated preclinically when orthotopic xenografts of bladder cancer cells were excised and examined histologically—3 weeks of treatment with cetuximab produced a marked decrease in the presence of new blood vessels and a marked reduction in the amounts of VEGF, interleukin 8, and basic fibroblast growth factor in the tumor cells. It appears that activity of the EGFR signal transduction pathway is required for stimulation of angiogenesis by these malignant cells.

Studies have demonstrated that cetuximab treatment alone effectively inhibits proliferation of EGFR-positive tumor cells in vitro and tumor growth in xenograft models. In preclinical studies, cetuximab has shown increased activity in combination with chemotherapeutic agents including cisplatin, doxorubicin, and paclitaxel. In an irinotecan-refractory colorectal cancer patient,

cetuximab was used in combination with irinotecan as a compassionate-use agent. This patient had a significant response to therapy that resulted in a trial combining these agents.

## Cetuximab in combination with irinotecan

The clinical efficacy of the combination of cetuximab and irinotecan was assessed in a phase II trial in patients with metastatic colorectal cancer refractory to both 5-FU and irinotecan [20]. Eligible patients had measurable metastatic colorectal cancer with either documented progressive or stable disease while on irinotecan. The tumors of patients entered onto the trial must have tested positive by immunohistochemistry (IHC) for EGFR and were reported as 1+ to 3+. Of the patients evaluated, approximately 72% of the screened tumors tested positive for the EGFR. Patients were treated with cetuximab on day 1 with a 400 mg/m$^2$ loading dose, followed by a weekly dose of 250 mg/m$^2$ in addition to irinotecan at the same dose and schedule that the patient had previously been receiving when progression or stable disease was noted. Any dose adjustments that had been made with the previous administration of irinotecan were maintained. There were 120 patients with documented irinotecan failure and 18 patients on irinotecan with stable disease. The median time from irinotecan failure to initiation of cetuximab plus irinotecan was 30 days (mean 54 days). The main toxicities reported with the combination therapy were allergic reactions, of which 2% were grade 3 and 1% were grade 4, and an acne-like rash/folliculitis, of which 53% were grade 1/2 and 8% were grade 3 and were responsive to standard management. Other toxicities seen were those typically associated with irinotecan and appear not to have been exacerbated by cetuximab. Interestingly, in the patients with the skin rash, 26/89 (29%) had a response, whereas only 1/31 (3%) without the rash had a response. This association is statistically significant ($P < 0.001$).

Of the 121 patients, 27 (19.2%, 95% CI 13–27%) achieved a partial response and 32 (26.7%) had stable disease. The median duration of response was reported as 186 days and patients had stable disease for a minimum of 12 weeks. This study was one of the first to show that cetuximab with irinotecan can produce major objective responses, with manageable toxicity in patients with EGFR-positive, irinotecan-refractory colorectal cancer. This result suggests that cetuximab can overcome resistance to irinotecan [20]. In addition, response to cetuximab may be dependent on the presence of EGFR, but there does not appear to be variability in response to cetuximab dependent on the intensity of EGFR staining. Responses were analyzed as a function of the degree of positivity of the staining by IHC of the EGFR. IHC was reported as 1+ to 3+ and approximately 22% of patients from each group responded to therapy, revealing no differences based on degree of positivity (Table 1).

The findings from this study were exciting in that an agent had been added to a chemotherapeutic to which the tumor had developed resistance and overcame this resistance to achieve a response. Unfortunately, the main criticism of this trial was that the patients entered may not have demonstrated clear progression of disease with irinotecan alone prior to the addition of cetuximab to this treatment. As a result, an independent review committee evaluated these data. This process slowed the acceptance of cetuximab as an effective agent.

## Cetuximab monotherapy

Given the previous reports of cetuximab activity in combination with irinotecan, a follow-up trial evaluated the single-agent activity of this drug. A phase II trial of cetuximab was undertaken in patients with colorectal cancer refractory to both 5-FU and irinotecan, whose tumors tested positive for EGFR by IHC [21]. A total of 57 patients with documented progression on irinotecan or an irinotecan-based regimen were treated with cetuximab at standard doses (400 mg/m$^2$ loading dose over 2 h, then 250 mg/m$^2$ over 1 h weekly). The median time from irinotecan failure to initiation of cetuximab was 2.0 months (range 0.5–10.6 months). As in the previous trial, the most commonly encountered adverse events, regardless of relationship to cetuximab, were an acne-like skin rash, predominantly on the face and upper torso (86% any grade, 16% grade 3), and asthenia (53% any grade, 4% grade 3). Two patients (3.5%) experienced grade 3 allergic reactions requiring discontinuation of study treatment. Neither diarrhea nor neutropenia were dose-limiting in any of the 57 patients treated. Six patients (10.5%, 95% CI 4–22%) achieved a partial response and 20 additional patients (35.1%) had stable disease or minor responses. With a response rate of 10.5% (95% CI 4–22%), cetuximab demonstrated activity as a third-line agent for patients with metastatic colorectal cancer. These results were remarkable in a disease that, until recently, had few agents even demonstrating second-line activity (Table 1).

**Table 1** Background of cetuximab in colorectal cancer

| | Irinotecan + cetuximab[a] | Cetuximab[b] |
|---|---|---|
| No. of patients | 121 | 57 |
| Partial response (95% CI) | 19.2% (13–27%) | 10.5% (4–22%) |
| Stable disease | 26.7% | 35.1% |
| Median duration of response (days) | 186 | 164 |

[a]Reference 20
[b]Reference 21

## Randomized trial with cetuximab

Based on these previous studies, a trial was conducted in Europe that was designed to determine the objective confirmed response rate of the combination of cetuximab plus irinotecan, or of cetuximab as a single agent in EGFR-positive patients, who had progressed on irinotecan within 3 months of entering the trial [3]. Time to progression and survival were secondary endpoints. The design accounted for the potential difference in response rates with monotherapy and combination therapy with irinotecan. Thereby this trial allowed patients randomized to the monotherapy arm to cross over to combination therapy with irinotecan once they had progressive disease. Of 576 patients screened, 470 were EGFR-positive (82%). A total of 329 patients were randomized in a 2:1 ratio, to combination and monotherapy arms, respectively. Patients in arm A received cetuximab (400 mg/m$^2$ first infusion, then 250 mg/m$^2$ weekly) plus irinotecan at the same dose and schedule on which they had progressed. Patients in arm B received cetuximab alone with the option to switch to the combination of cetuximab with irinotecan after failure of cetuximab as a single agent. In arm A, 218 patients were accrued (75 female, 143 male, median age 71 years, 88% with Karnofsky performance status 80–100). In arm B, 111 were accrued (46 female, 65 male, median age 70 years, 85% with Karnofsky performance status 80–100). Over 70% of patients in both arms had received previous treatment with two or three lines of therapy, and over 60% had received oxaliplatin-based chemotherapy. The main toxicities noted in arm A were diarrhea (45%), asthenia, and acne-like rash, whereas in arm B the main side effect was the acne-like rash. This was consistent with previous reports. The additional diarrhea and asthenia were likely secondary to the added toxicity of irinotecan.

The response rate in the combination of cetuximab with irinotecan was 22.5% (95% CI, 17.5–29.1%), median time to progression 4.1 months for the combination regimen and 1.5 months for monotherapy. In the subgroup of 54 patients who crossed over to combination therapy with irinotecan with cetuximab after having progressive disease with single-agent cetuximab, 1 (1.9%, 95% CI 0.0–9.9%) achieved a partial response and 22 (40.7%, 95% CI 27.6–55.0%) achieved stable disease. The median survival with combination therapy was 8.6 months and 6.9 months with monotherapy ($P = 0.48$, hazard ratio 0.91, 95% CI 0.68–1.21). Again, there was no correlation between EGFR staining intensity and response. Consistent with previous reports, there appears to be a correlation between development of skin toxicity and response to therapy. Patients who received combination therapy with cetuximab and irinotecan had a better overall response rate and time to progression when compared with those who received monotherapy with cetuximab.

This trial confirmed the previously reported results by Saltz et al. for cetuximab as a single agent and in combination therapy with irinotecan [3, 20]. When comparing these results to those of the phase II studies, these patients had similar response rates and time to progression. There was no survival difference noted between the two arms, which may have been because of the crossover design. Cetuximab has clearly demonstrated activity in patients with metastatic colorectal cancer who have developed progressive disease on irinotecan therapy, both as a single agent and in combination with irinotecan. These trials imply that with the addition of cetuximab, patients overcome resistance to irinotecan-based chemotherapy. The patients who participated in this trial all had tumors that overexpressed EGFR, although the expression level of EGFR did not correlate with response. It is unclear whether the presence of EGFR expression is necessary for this therapy to be effective. Additionally, the method for the evaluation of EGFR is IHC, which is a limited technique with some inherent inconsistency. Methods to evaluate this marker more effectively are being investigated, specifically gene expression and genomic polymorphisms, which may help identify and delineate patients more effectively. Trials evaluating patients with EGFR(-) tumors are ongoing.

The rash that develops with cetuximab correlated with response in these trials and this issue has also been assessed in other trials with cetuximab [22]. An analysis investigated the relationship between the presence and the severity of the rash and survival in patients with various cancers including colorectal, head and neck, and pancreatic cancer in four phase II studies. In all four studies, patients who developed the acne-like rash survived significantly longer than those who did not develop a rash, and those with more intense rash survived significantly longer still. The consistency of this observation, in studies of three different malignancies, and in studies of cetuximab in combination with chemotherapy and as monotherapy, suggests that the skin rash may be an important clinical surrogate of a response. It may be a useful tool in future trials potentially to use this as a marker of effective dosing, i.e., potentially titrating cetuximab to the development of a rash. The molecular identification that links the rash to response may also be a prospective marker to response to therapy.

The ideal combination with cetuximab is yet to be delineated. Currently this agent is combined with irinotecan, but there may also be a molecular basis to support the combination with oxaliplatin, given reports of the synergy of HER2 (EGFR2) and platinum-based therapy [17]. This is being tested in ongoing clinical trials.

## Vascular endothelial growth factor

The development of new blood vessels, a process known as angiogenesis, plays an important role in

many pathologic processes, including proliferative retinopathies, age-related macular degeneration, rheumatoid arthritis, psoriasis, and cancer [7]. Folkman et al. theorized that tumor growth beyond 1 to 2 mm depends on this process [8]. VEGF is a diffusible, homodimeric glycoprotein produced by healthy and neoplastic cells. The human VEGF-A variant is the predominant and most critical regulator of the development of the vascular system [6]. VEGF-A expression is regulated by differentiation and transformation, as well as by oxygen deprivation [4]. A number of cytokines, hormones, and growth factors (e.g. EGFR, keratinocyte growth factor, interleukin 1$\beta$, prostaglandin E2, and insulin-like growth factor-1) also increase VEGF-A expression and stimulate its secretion in various cell types [5]. The VEGF-A receptors VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR) both consist of an extracellular domain, a transmembrane region, and an intracellular domain that contains the consensus tyrosine kinase sequence. After binding VEGF-A, VEGFR-2 undergoes tyrosine autophosphorylation, while the VEGFR-1 phosphorylation is weak or undetectable. Activation of this receptor induces angiogenesis, and increases vascular permeability (theoretically by decreasing the elevated interstitial pressure) [9], mitogenesis, and chemotaxis in healthy endothelial cells. VEGFR-2 (KDR) is thought to play a significant role in the development of malignancies [15].

In human cancers, angiogenesis has been specifically linked to increased growth and metastatic potential. In vivo studies have shown that the growth of human tumor xenografts in mice is preceded by an increase in vascular density, indicating that neovascularization is required for rapid tumor growth. Expression of VEGF-A is increased in most human tumors, with contributions from both tumor and host cells. VEGF levels correlate with an increase in microvessel density, a decrease in apoptotic index, a decrease in overall survival, and an increase in the incidence of metastases, contributing to a poor prognosis overall [23].

In colorectal cancer, increased VEGF expression (mRNA) has been detected in human liver metastases from primary colorectal cancers and expression of two VEGF receptors was upregulated in liver metastases compared with nontumorous adjacent liver tissue [25]. In vitro studies with mAbs against VEGF-A have revealed a near-complete suppression of tumor-associated angiogenesis.

## Bevacizumab

Bevacizumab is a human mAb against VEGF-A derived from the murine mAb A4.6.1. This human antibody maintains the high specificity and affinity of the parent antibody for human VEGF-A, while reducing immunogenicity and providing a longer biologic half-life. The antibody is thought to prevent binding of all VEGF isoforms to all VEGF receptors [19].

Anti-VEGF mAbs have been reported to inhibit VEGF, block the growth of human tumor xenografts, and dramatically reduce the size and number of liver tumors in a mouse xenograft model of human colon cancer metastasis [25]. Additionally, the combination of the anti-VEGF antibody and chemotherapy in nude mice with human cancer xenografts has an increased activity compared with chemotherapy alone or antibody alone [1].

Phase I studies have found this to be a well-tolerated agent. The severe toxic effects that occurred in the phase I trials were infrequent, specifically intratumoral bleeding, pulmonary emboli, and peripheral venous thrombosis. The terminal elimination half-life was approximately 21 days and did not induce antibodies to bevacizumab [11].

## Bevacizumab in combination with 5-FU

Based on the preclinical data and the demonstrated tolerability of this agent in phase I studies, a clinical trial was conducted randomizing patients with previously untreated metastatic colorectal cancer to one of three treatment groups: 5-FU (500 mg/m$^2$)/LV (500 mg/m$^2$) alone; 5-FU/LV and low-dose bevacizumab (5 mg/kg every 2 weeks); and 5-FU/LV and high-dose bevacizumab (10 mg/kg every 2 weeks) [13]. 5-FU/LV was given weekly for the first 6 weeks of each 8-week cycle. Treatment with 5-FU/LV with bevacizumab at both dose levels compared with 5-FU/LV resulted in higher response rates [chemotherapy alone 17% (95% CI 7–34%), low dose 40% (95% CI 24–58%), high dose 24% (95% CI,12–43%)], longer median time to disease progression [chemotherapy alone 5.2 months (95% CI 3.5–5.6 months), low dose 9.0 months (95% CI 5.8–10.9 months), high dose 7.2 months (95% CI 3.8–9.2 months)] and longer median survival [chemotherapy alone 13.8 months (95% CI 9.1–23), low dose 21.5 months (95% CI 17.3 months to undetermined), high dose 16.1 months (95% CI 11–20.7 months)]. Patients were given the option to crossover after treatment with chemotherapy alone to bevacizumab 10 mg/kg every 2 weeks. Of 22 patients that crossed over, 2 had a partial response to bevacizumab alone. The most significant toxicities were fatal thrombosis in one patient, hypertension, proteinuria, and epistaxis.

This study found bevacizumab to be a well-tolerated addition to chemotherapy. Patients who received bevacizumab had a higher incidence of thrombosis than patients receiving chemotherapy alone. Further, patients who received chemotherapy in combination with 5-FU and bevacizumab (low dose) had an outcome similar to patients who received chemotherapy sequentially with oxaliplatin and irinotecan in combination with 5-FU. It is unclear why the patients receiving low-dose bevacizumab did better than those patients receiving

high-dose therapy. This may be due to the statistical imbalances in this randomized phase II trial (patient characteristics). These promising data led to a phase III trial essentially comparing IFL to IFL with low-dose bevacizumab

## Bevacizumab in combination with 5-FU/LV/irinotecan

Over 800 patients with previously untreated metastatic colorectal cancer were randomized to receive irinotecan, bolus 5-FU, and LV (IFL) plus bevacizumab (5 mg/kg every 2 weeks, $n = 411$) or IFL plus placebo ($n = 402$). A third group of patients (about 100) received 5-FU/LV chemotherapy plus bevacizumab, until safety was established and this arm was subsequently halted. At the time of disease progression, treatment was unblinded and patients on the bevacizumab arms could continue bevacizumab in combination with second-line chemotherapy. The primary endpoint was overall survival. In the intention-to-treat analysis, median survival significantly increased in subjects receiving IFL/bevacizumab compared with subjects receiving IFL/placebo (20.3 months vs 15.6 months; $P = 0.001$). Increases were observed in progression-free survival in the IFL/bevacizumab arm compared with the IFL/placebo arm (10.6 months vs 6.2 months; $P < 0.001$). Similar increases were seen for the IFL/bevacizumab arm compared to the IFL/placebo arm in response rate (45% vs 35%; $P = 0.004$), and duration of the response. There were no differences in adverse events leading to hospitalization, study discontinuation, or 60-day all-cause mortality. Grade 3 hypertension was more common during treatment with IFL/bevacizumab (11.0% vs 2.3%), but was easily managed and did not lead to significant clinical consequences [12].

The addition of bevacizumab to the IFL regimen in the first-line treatment of metastatic colorectal cancer provides significant clinical benefit to patients by increasing survival, progression-free survival, objective response rate, and duration of response [12]. The prolongation of survival and improvement in other markers of clinical benefit observed with the addition of bevacizumab to standard chemotherapy establishes the role of angiogenesis inhibition for the treatment of patients with colorectal cancer.

The addition of bevacizumab to IFL was well tolerated. The main toxicities in this trial were bleeding, thrombosis, proteinuria, and hypertension consistent with those toxicities reported in the phase II study. However, in this trial, thromboembolism and proteinuria were not noted to be significantly increased with the addition of bevacizumab to IFL. Gastrointestinal perforation was noted in this trial and was thought to be secondary to the addition of bevacizumab to IFL.

The survival reported is comparable to that reported with both chemotherapeutic combinations approved for metastatic colorectal cancer, in the 20-month range [24]. The impact of angiogenesis on the progression of disease in these patients has been confirmed in this trial [12].

## Identification of targets

Accurately identifying patients who will benefit from therapy will allow more effective administration of therapeutics. For instance, some of these novel therapeutic agents require the presence or detection of a particular receptor, i.e. EGFR. Currently, the evaluation of EGFR is limited by the technology used to assess it. The assessment of EGFR by gene expression or genomic polymorphisms, potentially will allow a more accurate measure of this target.

## mRNA expression vs immunohistochemistry

The techniques that have been used to assess EGFR include IHC, fluorescence in situ hybridization (FISH), enzyme-linked immunoassay (ELISA), and reverse transcriptase polymerase chain reaction (RT-PCR), but no standard measurement has been established. Our group at the University of Southern California has evaluated protein expression, as measured by IHC, and compared it to the gene expression levels of EGFR in 24 patients with metastatic colorectal cancer. For quantitative gene expression, total RNA was isolated from microdissected paraffin-embedded tumor specimens. Expression levels were determined relative to the internal reference gene (beta)-Actin using real-time PCR (TaqMan; ABI, Foster City, Calif.). Of the 24 tumors, 11 (46%) had no detectable EGFR mRNA expression (but one of those specimens had been counted positive for the EGFR staining by IHC), 13 (58%) had detectable EGFR mRNA expression (range $0.36–142.4 \times 10^{-3}$ /beta-Actin), but 3 (12%) did not show positive staining. The EGFR mRNA expression levels for those samples that were negative by IHC were low ($1.18–2.53 \times 10^{-3}$ /beta-Actin). Five patients with positive staining results were included in this phase II study with the EGFR inhibitor cetuximab. Responders (two partial responses, one complete response) showed higher EGFR mRNA expression levels ($1.4–14.8 \times 10^{-3}$ /beta-Actin) compared to non-responders (one stable disease 0.36, one progressive disease $0.00 \times 10^{-3}$ /beta-Actin).

These preliminary results suggest that determination of EGFR expression by RT-PCR from microdissected tumor tissue may be more specific and more sensitive than IHC. Further, the data suggest that the EGFR mRNA level in tumor tissues may be a predictive marker for response to therapy with EGFR inhibitors [10]. Preliminary data (unpublished) from our group also indicate that a genomic polymorphism of the EGFR may be associated with EGFR gene expression, suggesting that this polymorphism may be of clinical significance.

## Conclusions

There are many novel targets that are currently being evaluated in clinical trials. The mAbs that have proven to be most effective thus far are cetuximab (EGFR inhibitor) and bevacizumab (VEGF inhibitor), both of which have demonstrated significant activity in randomized studies. These drugs are well tolerated both as single agents and in combination with chemotherapy. Clearly, evaluation of these agents in colorectal cancer has shown improved time to progression and survival in patients receiving these antibodies. These data are consistent with or better than progression and survival outcome from phase II and III trials utilizing the best chemotherapeutics available for this disease.

The activity of these novel targeted agents may be multifaceted in that, not only do they have antineoplastic effects, but they also stabilize qualities that may allow the chemotherapy to be more effective. This may explain the enhanced efficacy of these agents when combined with chemotherapy.

However, the approach of utilizing multiple targeted therapies (i.e. bevacizumab with cetuximab) in combination with each other and chemotherapeutics other than irinotecan is currently being evaluated. Different combination regimens are undergoing evaluation to assess the best regimen with which to combine these agents. Similarly, while these drugs are currently being utilized in patients with metastatic disease, the impact and utility of these drugs as adjuvant therapy for patients with colorectal cancer is ongoing.

One of the limitations of these agents, however, is the necessity to identify a particular receptor or target; for example, the suboptimal evaluation of the EGFR. More sensitive technologies could more accurately identify those who express the receptor thus increasing the accuracy of patient selection for participation in these trials and therefore the number of patients benefiting from cetuximab therapy. This may be accomplished by assessing genes within the tumor or even a simple blood test, or the more significant issue may be that the presence of these receptors may not be necessary for efficacy.

Ideally one would select patients for a particular chemotherapy or novel target based on their own genetic makeup and the genetic characteristics of their tumor. This could help identify those patients who may have the best response to therapy. Future clinical trials with strong correlative markers will allow us to delineate some of these issues.

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