# First-Line Treatment of Metastatic Colorectal Cancer: Focus on Cetuximab in Combination with Chemotherapy

Allison L. Balko[1], Esther P. Black[2] and Justin M. Balko[3]

[1]Department of Pharmaceutical Services, Vanderbilt University Medical Center, Nashville TN, USA. [2]Department of Pharmaceutical Sciences, University of Kentucky, Lexington KY, USA. [3]Department of Medicine, Vanderbilt University, Nashville, TN, USA. Corresponding authors email: justin.balko@vanderbilt.edu

**Abstract:** Metastatic colorectal cancer (mCRC) is primarily treated with cytotoxic chemotherapy. However, limitations in efficacy and tolerability clearly exist with the use of these agents. Molecularly targeted agents offer alternatives to chemotherapy or in many cases, can be used in combination with chemotherapy to synergistically enhance responses and patient survival. Monoclonal antibodies targeting the epidermal growth factor receptor (EGFR) have emerged as a therapeutic option to potentiate chemotherapeutic response and outcome in mCRC patients. However, in unselected populations, the advantages are modest. Thus, biomarkers that predict patients who will benefit from combination regimens employing both EGFR-targeted agents and chemotherapy are necessary to limit unnecessary toxicity and healthcare costs. This review will discuss the use of the EGFR monoclonal antibody cetuximab in combination with chemotherapy in mCRC patients with respect to toxicity, response, and predictive biomarkers of activity.

**Keywords:** cetuximab, epidermal growth factor receptor, chemotherapy, colorectal cancer

## Introduction

Colorectal cancer is the third most commonly occurring cancer worldwide and at least one-fourth of patients present with disseminated (metastatic) disease. First line treatment of metastatic colorectal cancer (mCRC) consists of cytotoxic chemotherapy, using fluoropyrimidines (capecitabine or 5-fluorouracil; 5-FU) in combination with either oxaliplatin or irinotecan. Bevacizumab, a monoclonal antibody (mAB) can be added to therapy and has demonstrated therapeutic benefit in terms of progression-free survival and overall survival.[1] Recently, anti-epidermal growth factor receptor (EGFR) mABs including the chimeric cetuximab (Erbitux™) and fully humanized panitumumab (Vectabix™) have been shown to have significant activity in mCRC and can be used as monotherapy or in combination with irinotecan as a second or third line agent, with substantial gains in clinical benefit. Both panitumumab and cetuximab are specific for EGFR and both demonstrate activity in mCRC. However, variable results in similarly-designed studies suggest that the agents are not interchangeable. Furthermore, the data are not directly comparable in many cases, since the majority of phase III studies performed with panitumumab were performed using response-predictive biomarkers identified in the cetuximab trial post-hoc analyses (such as KRAS status) integrated into the study design.[2,3] Because cetuximab was approved first, more data exist to support its use and its clinical utility in therapy is therefore better established. Cetuximab has been studied in a number of large phase II and phase III studies to determine if the addition of cetuximab to first line therapy can improve survival in mCRC. The outcome of these studies and their impact on the treatment of mCRC will be discussed.

## Mechanism of Action, Metabolism and Pharmacokinetic Profile

Cetuximab is a monoclonal, chimeric, IgG1 antibody specific for EGFR. Upon binding to EGFR, cetuximab blocks ligand-dependent hetero- or homo-dimerization of EGFR with other ErbB family members (HER2, ErbB3, or ErbB4) or EGFR monomers and results in enhanced receptor internalization and degradation. Down regulation of effector pathway activity, including the PI3 K/Akt, Ras/MAPK, and STAT3 modules, is a consequence of impaired receptor dimerization.[4] Expression of EGFR and its ligands are frequently observed in colorectal carcinomas, suggesting the EGFR pathway as a potential target in CRC patients.[5,6] Autocrine and paracrine binding of EGFR by its ligands have been well documented in human cancers, contributing to enhanced tumor cell proliferation, survival, and angiogenesis via these effector pathways.[4,7]

Importantly, cetuximab, like other monoclonal therapies, can elicit antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC), which depend upon an intact host immune response. The biological processes underlying ADCC and CDC are reviewed elsewhere.[8,9] The relative contributions of these mechanisms to the *in vivo* activity are largely unknown, particularly because preclinical studies are commonly performed in xenograft mouse models using immune-compromised nude mice. However, ADCC following cetuximab administration has been demonstrated in various cancer cell/immune cell co-culture models.[10,11] Even low levels of expression of EGFR was sufficient for cetuximab-mediated ADCC activity,[10] offering a plausible scientific basis for the clinical finding that cetuximab treatment of CRCs which do not over-express EGFR may still demonstrate clinical benefit.[12] The contribution of CDC to anti-EGFR antibody therapy has also been demonstrated in various *in vitro* and *in vivo* models, and is hypothesized to contribute to positive outcome as well.[13,14]

Therapeutically, cetuximab has been shown to synergize with topoisomerase I inhibitors such as irinotecan (CPT-11) and topotecan in *in vivo* mouse models, which is the basis for its initial combinatorial use with irinotecan in clinical studies.[7,15] Enhanced cytotoxicity with other chemotherapeutic agents was previously observed with murine mAB 225, the prototype monoclonal to cetuximab prior to chimerization.[16] As such, further clinical studies have demonstrated enhancement of therapeutic response when cetuximab was combined with non-topoisomerase-containing regimens such as FOLFOX, but only in patient populations defined by mutation status, as will be discussed later.[17]

The metabolism of cetuximab is markedly different from other cancer therapies, primarily due to the nature of the molecule. Monoclonal antibodies exhibit a categorically long half-life, ranging from several

days to several weeks *in vivo*. The Fc region of IgG contained in therapeutically administered monoclonal antibodies as well as endogenous immunoglobulins mediate binding to protective receptors (the neonatal Fc receptor), thereby shielding the molecule from degradation. Thus, given their generally well-tolerated safety profile and specificity, they represent ideal pharmacologic agents. The primary mechanism of IgG metabolism is degradation in the endosome, which occurs following binding of the antibody to its polypeptide target.

A recent study of the pharmacokinetics (PK) of cetuximab in mCRC patients reported a steady-state half-life of approximately 4 days (range: 41.4–159.1 hours) after 5 weeks of therapy, with once weekly dosing (400 mg/m$^2$ load, followed by 250 mg/m$^2$ weekly).[18] Clearance ranges from 15 to 44 mL/hr with a mean steady state volume of distribution of 3.8 L.[18] Dose-finding studies with cetuximab have failed to identify a maximum tolerated dose in patients. Thus, coupled with its long half-life, treatment regimens are likely to be highly flexible, provided they are appropriately validated in patients prior to efficacy studies. Tabernero and colleagues recently compared the PK profile of cetuximab administered every two weeks to the once weekly dose.[18] PK parameters were comparable in patients treated every two weeks with 500 mg/m$^2$ versus every week with 250 mg/m$^2$ and this regimen was well-tolerated. Doses up to 700 mg/m$^2$ every 2 weeks did not result in dose-limiting toxicity. Further, no differences in EGFR signaling activity, assessed by immunohistochemistry, were observed between any of the regimens or doses tested. These data suggest that the activity of cetuximab is likely due to the specific antibody-tumor-host immune interactions rather than PK profile.

## Clinical Studies

Combining cetuximab with chemotherapy has been explored in a number of landmark phase II and phase III settings. Addition of cetuximab to chemotherapy has been demonstrated to provide a survival advantage in third or greater, second, and recently, first-line settings.[17,19–22] In this review, we will focus on the addition of cetuximab to chemotherapy in the first line setting, specifically where data include a comparator arm. First line therapy in mCRC consists predominantly of FOLFOX (5-FU, folinic acid, and oxaliplatin) or FOLFIRI (5-FU, folinic acid, and irinotecan) regimens, although neither regimen appears to be superior.[23] Recently, the benefit of adding cetuximab to standard chemotherapeutic regimens was assessed in several large trials (Table 1).

The OPUS trial was a large randomized Phase II study assessing whether the best overall response rate (ORR) of cetuximab combined with FOLFOX (n = 169) was superior to that of FOLFOX alone (n = 168) as first-line treatment for EGFR IHC+ mCRC.[17] Cetuximab was administered as a 400 mg/m$^2$ initial dose followed by 250 mg/m$^2$/wk plus FOLFOX-4 (oxaliplatin 85 mg/m$^2$ on day 1, plus leucovorin 200 mg/m$^2$ and 5-FU as a 400 mg/m$^2$ bolus followed by a 600 mg/m$^2$ infusion over 22 hours on days 1 and 2). The primary endpoint was overall response rate (ORR), while progression-free survival (PFS) was also assessed in the intent-to-treat (ITT) population. Overall survival (OS) was not assessed.

The CRYSTAL trial was a Phase III trial comparing FOLFIRI (n = 599) with FOLFIRI plus cetuximab (n = 599) as first line treatment in EGFR IHC+ mCRC.[22] FOLFIRI was administered as irinotecan as a 180 mg/m$^2$ infusion, leucovorin or L-leucovorin as a 400 mg/m$^2$ or 200 mg/m$^2$ infusion, respectively, and 5-FU in a bolus of 400 mg/m2 followed by a continuous 46 hour infusion of 2400 mg/m$^2$. Treatment was administered on day 1 of a 14 day cycle. Cetuximab was administered identically to the OPUS trial (above). The primary endpoint of the study was progression-free survival (PFS), with secondary efficacy endpoints of ORR and OS.

CAIRO II tested the benefit of adding cetuximab to CAPOX + bevacizumab in first-line treatment of mCRC.[21] Three hundred and eighty-six eligible patients were enrolled to each arm. Patients were not selected based on EGFR IHC status, although it was determined in the course of the study. Treatment for the CAPOX-bevacizumab group consisted of a 3-week cycle of 1000 mg/m$^2$ capecitabine given orally twice daily on days 1 to 14, 130 mg/m$^2$ oxaliplatin on day 1, and 7.5 mg/kg bevacizumab on day 1. In the experimental arm, cetuximab was added in an identical fashion to the OPUS and CRYSTAL trials (above). The primary endpoint was PFS, recorded based on ITT principles. ORR and OS were also assessed.

**Table 1.** Phase III and selected phase II trials of cetuximab in mCRC.

| Trial | Phase | Patients | Setting | Treatment | N | ORR (%) | Statistic | Median PFS (months) | Statistic | Median OS (months) | Statistic |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CRYSTAL | III | mCRC, EGFR IHC+ | First line | FOLFIRI | 599 | 38.7 | P = 0.004 | 8 | P = 0.048 | 18.6 | P = 0.31 |
|  |  |  |  | FOLFIRI + cetuximab | 599 | 46.9 |  | 8.9 |  | 19.9 |  |
| CAIRO2 | III | mCRC | First line | CAPOX+ bevacizumab | 368 | 50 | P = 0.49 | 10.7 | P = 0.01 | 20.3 | P = 0.16 |
|  |  |  |  | CAPOX + bevacizumab + cetuximab | 368 | 52.7 |  | 9.4 |  | 9.4 |  |
| OPUS | II | mCRC, EGFR IHC+ | First line | FOLFOX | 168 | 36 | P = 0.064 | 7.2 | P = 0.62 | NA | NA |
|  |  |  |  | FOLFOX + cetuximab | 169 | 46 |  | 7.2 |  | NA |  |
| COIN | III | mCRC | First Line | FOLFOX or XELOX |  |  |  | Data not formally reported |  |  |  |
|  |  |  |  | FOLFOX or XELOX + cetuximab |  |  |  |  |  |  |  |
| BOND | II | mCRC, EGFR IHC+ | 2nd line or greater | IRI + cetuximab | 218 | 22.9 | P = 0.007 | 4.1 | P < 0.001 | 8.6 | P = 0.48 |
|  |  |  |  | cetuximab | 111 | 10.8 |  | 1.5 |  | 6.9 |  |
| EPIC | III | mCRC, EGFR IHC+ | 2nd line or greater | IRI |  | 4.2 | P < 0.0001 | 2.6 | P < 0.0001 | 10 | P = 0.71 |
|  |  |  |  | IRI + cetuximab |  | 16.4 |  | 4 |  | 10.7 |  |

The most recent study assessing the addition of cetuximab to standard chemotherapy regimens was the MRC COIN trial.[24,25] MRC COIN (N = 1630) was a phase III randomized controlled trial with tested the benefit of adding cetuximab to oxaliplatin-containing regimens. The regimen was a physician/patient choice of either 5-FU or oral capecitabine combined with oxaliplatin. MRC COIN was more complicated in design, and included 3 study arms. The control arm utilized either leucovorin or L-leucovorin as a 350 mg/m$^2$ or 175 mg/m$^2$ infusion, respectively, oxaliplatin as a 85 mg/m$^2$ infusion, and 5-FU in a bolus of 400 mg/m2 followed by a continuous 46 hour infusion of 2400 mg/m$^2$ every 2 weeks OR oxaliplatin as a 130 mg/m$^2$ infusion on day 1 and capecitabine 1000 mg/m$^2$ twice daily, orally on days 1–14 of a three week cycle. For the first experimental arm cetuximab was added to one of the regimens above. An additional experimental arm was included testing the effects of using an intermittent strategy to the control chemotherapy-alone arm. The primary endpoint in MRC COIN was OS.

## Side Effects and Treatment Strategies

The most frequently observed toxicities associated with anti-EGFR therapy, including cetuximab, are skin rash, diarrhea, electrolyte abnormalities, and rare interstitial lung disease ($<$1%). Infusion related reactions are also frequent with cetuximab, but are infrequently associated with the administration of the fully humanized anti-EGFR mAB, panitumumab.

Dermatologic toxicity is the most common adverse event associated with cetuximab therapy, occurring in up to 88% of patients.[26] This can present as an acneform rash, pruritus, dry skin, hyperkeratosis, and nail changes. These adverse reactions are most likely due to the expression of EGFR in the skin and hair follicles. Although the dermatologic toxicities of anti-EGFR therapeutics can decrease quality of life, they can usually be managed. Treatment strategies consist of reducing sun exposure, topical steroids or moisturizers and oral antibiotics. Response and survival rates have been linked to the presence and severity of acneform rash, therefore it is advisable to attempt to manage this reaction prior to implementation of dose reductions or discontinuation of cetuximab therapy altogether.[27] This rash most often appears within the first few weeks of treatment and usually resolves after therapy is stopped.

Another common side effect of cetuximab is hypomagnesemia, which occurs in approximately one-half of cetuximab treated patients. Electrolytes should be monitored throughout cetuximab therapy and replaced as necessary. Hypomagnesemia may be due to the blockade of EGFR in the kidney resulting in magnesium wasting.[28] It is also thought that this could be a result of cetuximab induced diarrhea.

Infusion related reactions have been observed with the administration of cetuximab resulting in fever, chills, dyspnea, bronchospasm, hypertension, and hypotension. The majority of infusion reactions occur during the first infusion of cetuximab. Although these reactions are generally uncommon, there is a higher incidence reported in the southeastern part of the United States.[29] The reason for this is unknown, but it is hypothesized that this could be due to exposure to an antigen that is local to this region. A recent study found IgE antibodies to cetuximab exist in the serum of southeastern patients prior to treatment. The IgE antibodies are specific galactose-alpha-1,3-galactose, an oligosaccharide which is present on the Fab portion of the heavy chain of cetuximab.[30] Patients treated with cetuximab, particularly those from this geographical region, should be pre-medicated with an H1 antagonist 30–60 minutes before cetuximab administration and monitored for one hour after the end of the infusion. If a patient experiences a severe reaction, it is generally not recommended to rechallenge with cetuximab. Panitumumab may offer an alternative this scenario, as the incidence of infusion –related reactions is substantially reduced ($<$1%).[2] However, one should be cautioned that insufficient data exist to consider the two mABs directly interchangeable.

When cetuximab is added to chemotherapy regimens it can impact on the safety profile of that regimen. In the OPUS trial, the most common adverse events seen in the cetuximab plus FOLFOX-4 group were skin disorders (90%) and GI disorders (78%). It was concluded that the combination therapy was well tolerated with no additive effect on previously known toxicities associated with FOLFOX-4. The COIN trial showed an increase in grade 3/4 nausea and vomiting, diarrhea, skin rash and lethargy with the addition of cetuximab to oxaliplatin and fluoropyrimidine based chemotherapy. There were

similar findings in the CRYSTAL trial. The addition of cetuximab to FOLFIRI resulted in higher incidence of grade 3/4 diarrhea and skin reactions. It is important to note that the dose-limiting toxicity of irinotecan is diarrhea. However, adding cetuximab to FOLFIRI in the CRYSTAL trial did not appear to cause synergistic increase in rates of grade 3/4 diarrhea, and instead appeared to be additive.[22] The CAIRO II trial also indicated an increase in grade 3/4 adverse events with the addition of cetuximab to capecitabine, oxaliplatin, and bevacizumab. However, they found that removing adverse cutaneous effects from their analysis resulted in a similar incidence of grade 3/4 adverse events.[21]

Thus, adding cetuximab to chemotherapy has been shown to increase the incidence of adverse events. However, rather than increase the toxicities associated with those regimens, cetuximab adds a unique profile of toxicity, primarily including skin rash and diarrhea. In the majority of trials to date, these effects were considered manageable with treatment modifications. With appropriate management, the addition of cetuximab to chemotherapy can be beneficial in selected populations.

## Efficacy

Cetuximab has shown efficacy when added to irinotecan or as monotherapy in 2nd line or greater mCRC treatment.[19,20] These studies have thus prompted the investigation of the benefit of adding cetuximab to first line therapy in metastatic disease. Currently, the evidence does not clearly support the broad addition of cetuximab to chemotherapy in first line treatment of mCRC in unselected patient populations. The CRYSTAL trial, which examined the addition of cetuximab to irinotecan based regimen (FOLFIRI) demonstrated a clear improvement in ORR and a modest improvement in PFS, but no change in OS. However, treatment arm cross-overs were permitted in the trial, and may have contributed to the lack of improvement in OS.[22] In contrast, the addition of cetuximab to FOLFOX containing regimens in the OPUS trial did not improve responses or PFS. The OPUS trial tested the benefit of adding cetuximab to FOLFOX-4 in first line treatment of EGFR+ mCRC patients. Cetuximab plus FOLFOX appeared to increase the ORR compared to FOLFOX-4 alone (46% versus 36%, respectively), although this difference was not found to be statistically significant ($P = 0.064$).[17] Likewise, there was

no difference in PFS in the intent to treat population with the addition of cetuximab. The ORR to FOLFOX (36%) in this trial was lower compared to previously reports in similarly designed studies. Thus, the response rate to the combined treatment may have been lower due to an unidentified confounding factor in this particular patient group. Indeed, a single-arm phase II study combining the same FOLFOX regimen as OPUS with cetuximab found a 76% ORR compared to the 46% ORR observed in OPUS.[31] Therefore, the OPUS findings will need to be substantiated in the context of a full phase III study. The MRC COIN study which assessed cetuximab in combination with either CAPOX or FOLFOX regimens in select mutational subgroups reportedly failed to identify a benefit as well, although the formal data from this trial have not been published.[24,25] Furthermore, The CAIRO2 study, which assessed the benefit of adding cetuximab to CAPOX + bevacizumab actually found a detrimental effect of cetuximab in this setting.[21] In CAIRO2, median PFS was decreased from 10.7 to 9.4 months ($P = 0.01$) with the addition of cetuximab ($P = 0.01$). OS was also decreased (20.3 months vs. 9.4 months, $P = 0.16$) although this change was not significant. In light of the findings of OPUS, MRC COIN, and CAIRO2, the benefit of adding cetuximab to oxaliplatin containing regimens (regardless of the fluoropyrimidine utilized) cannot be confirmed at this time in unselected patients.

## Biomarkers of Response

Significant data are now available demonstrating that biomarkers can predict benefit of cetuximab in mCRC patients. The most promising of these biomarkers is KRAS mutational status, which has been assessed in post-hoc analyses of the existing data. KRAS is mutated in approximately 40%–50% of colorectal cancers and its role in activating the downstream PI3K and MAPK pathways has been well established. Thus, mutational activation of KRAS or components of these downstream pathways could biologically preclude the inhibitory activity of mABs targeting upstream growth factor receptors by uncoupling downstream activation from the growth factor receptor. A number of retrospective studies and post-hoc analyses have demonstrated that KRAS mutant mCRC tumors do not respond to EGFR mAbs regardless of line of treatment, and therefore

it is recommended that all patients undergo KRAS sequencing before treatment with EGFR-targeted mAbs.[3,17,21,22,24,32,33] Mutations in downstream BRAF (5% of colorectal cancers) are observed more rarely, but have also been associated with cetuximab resistance.[32] Of particular note, mutations in the catalytic region (exon 20) of PIK3CA appear to be associated with cetuximab resistance, while mutations in the regulatory domain (exon 9) do not.[32] Thus, mutational analysis of KRAS, BRAF and PI3KCA is likely to become standard of care in selecting patients for treatment with cetuximab in mCRC.

Khambata-Ford and colleagues demonstrated that upregulated expression of the EGFR ligands amphiregulin (AREG) and epiregulin (EREG) has been shown to be predictive of response to cetuximab when used as a single agent in mCRC.[34] However, in this study, the assessment of the predictive power of ligand expression on response was confined to the dataset from which the hypothesis was generated. However, Jacobs and colleagues corroborated this finding in a retrospective analysis of 220 FFPE archival specimens from mCRC patients treated with irinotecan and cetuximab.[35] The investigators found that AREG and EREG mRNA expression were both predictive of response and PFS in this cohort.

In addition to mutational analysis and EGFR ligand expression, our group has hypothesized that gene expression patterns in colorectal tumors may be indicative of EGFR pathway dependency and thus could be more effective than mutational status alone or expression of AREG/EREG in capturing patient/tumor heterogeneity to predict response. Moreover, we developed a gene expression predictor of response (GEPR) to the small molecule inhibitor of EGFR, erlotinib, in NSCLC.[36] This diagonal-linear discriminant function quantitatively employs the expression patterns of 180 genes ascertained by microarray. When the model was applied to human mCRC tumor biopsies prior to treatment with cetuximab, we found that the GEPR was also capable of predicting both patient response and PFS.[37] A 26-gene subset of these 180 genes further refined the model and improved predictive capacity, particularly in KRAS-wildtype patients.[37] This signature is currently under more extensive validation and is being migrated to a more clinically-utilizable platform (quantitative real-time PCR). Future studies will confirm the utility of the GEPR in predicting response to cetuximab, and possibly panitumumab, in mCRC.

## Quality of Life

Quality of life (QOL) is an important measurement when considering combinatorial treatment regimens, particularly in metastatic disease. QOL, as measured by patient-response surveys informs on aspects of care which are not otherwise tangible, and is often considered to be as important, or more important, than survival endpoints in late-stage disease. When the goals of therapy include palliative care (particularly in later stage disease and in second or third line therapy), single-agent cetuximab has been shown to improve QOL measures over best supportive care.[38] The phase III EPIC trial also demonstrated that when cetuximab was added to single-agent irinotecan, QOL measures were also markedly improved.[20] In the CRYSTAL trial (FOLFIRI ± cetuximab), cetuximab did not significantly alter QOL scores, while increasing PFS in KRAS-wildtype patients. Collectively, these results suggest that cetuximab does not adversely impact QOL in mCRC patients when added to irinotecan-based chemotherapy regimens or when used as a single agent. The OPUS trial (FOLFOX + cetuximab) did not assess quality of life measures, or have not yet reported them.[17] Thus, it is unknown at this time whether cetuximab impacts patient QOL when combined with FOLFOX. In contrast, the addition of cetuximab to CAPOX + bevacizumab (CAIRO2) resulted in both inferior QOL scores and reduced PFS further substantiating the lack of benefit of cetuximab in this regimen.[21]

## Place in Therapy

The results of the OPUS and CRYSTAL trials suggest that cetuximab can be safely added to FOLFOX and FOLFIRI regimens without a substantial increase in drug-related toxicity. However, the results of these trials have raised significant red flags as to the benefits of cetuximab in unselected patients. Subgroup analyses have found that patients harboring mutated KRAS in the tumor genome do not benefit from the addition of cetuximab. In the case of the OPUS trial, the addition of cetuximab to FOLFOX was actually reduced RFS in KRAS-mutant mCRC patients. Further, the addition of cetuximab to bevacizumab-containing regimens in KRAS-status unknown patients

was likewise found to produce antagonistic effects. This finding clearly demonstrates that regimens combining cetuximab and bevacizumab should not be used clinically until further data regarding the benefit of cetuximab in mutational subgroups and with other chemotherapy regimens are made available. Thus, the present consensus is that all patients who are candidates for cetuximab therapy in mCRC, regardless of line of treatment, should undergo routine KRAS mutational testing to assess status of the gene prior to initiating therapy with anti-EGFR mABs wherein only patients with wild-type KRAS should go forward with therapy. The utility of other biomarkers for patient selection remain to be validated, but hold promise for further personalization of colorectal cancer pharmacotherapy.

## Conclusions

In conclusion, the addition of cetuximab in both FOLFOX and FOLFIRI first line therapy is a promising advance in the treatment of mCRC. However, post-hoc and retrospective subpopulation analyses have clearly demonstrated that, as has been observed with many molecularly targeted agents, biomarker identification and patient selection strategies will be necessary to fully realize the potential impact on outcomes. Activating mutations in *KRAS* have been well documented in a number of studies to be a marker of resistance to cetuximab and routine testing for *KRAS* mutational status in recommended in clinical guidelines. In light of current evidence, the most apparent benefit of adding cetuximab to first line chemotherapy has been observed in KRAS-wildtype patients, using FOLFOX or FOLFIRI regimens. Capecitabine regimens, particularly those also utilizing bevacizumab, should not include cetuximab, regardless of KRAS status, until more data become available. The role of *PIK3CA, PTEN* and *BRAF* mutations as biomarkers of cetuximab resistance is less convincing at this time, although activating mutations in these genes make biological sense as mechanisms of resistance. Validation efforts confirming the role of other biomarkers such as the gene expression predictor of response to EGFR inhibitors[36,37] and expression of amphiregulin and epiregulin are underway and promise to further personalize anti-EGFR treatment in mCRC.

## Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

## References

1. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med*. 2004 Jun 3;350(23):2335–42.
2. Peeters M, Price TJ, Cervantes A, et al. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. *J Clin Oncol*. 2010 Nov 1;28(31): 4706–13.
3. Douillard JY, Siena S, Cassidy J, et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol*. 2010 Nov 1;28(31):4697–705.
4. Kalyankrishna S, Grandis JR. Epidermal growth factor receptor biology in head and neck cancer. *J Clin Oncol*. 2006 Jun 10;24(17):2666–72.
5. Ito M, Yoshida K, Kyo E, et al. Expression of several growth factors and their receptor genes in human colon carcinomas. *Virchows Arch B Cell Pathol Incl Mol Pathol*. 1990;59(3):173–8.
6. Yasui W, Sumiyoshi H, Hata J, et al. Expression of epidermal growth factor receptor in human gastric and colonic carcinomas. *Cancer Res*. 1988 Jan 1;48(1):137–41.
7. Prewett MC, Hooper AT, Bassi R, Ellis LM, Waksal HW, Hicklin DJ. Enhanced antitumor activity of anti-epidermal growth factor receptor monoclonal antibody IMC-C225 in combination with irinotecan (CPT-11) against human colorectal tumor xenografts. *Clin Cancer Res*. 2002 May;8(5):994–1003.
8. Iannello A, Ahmad A. Role of antibody-dependent cell-mediated cytotoxicity in the efficacy of therapeutic anti-cancer monoclonal antibodies. *Cancer Metastasis Rev*. 2005 Dec;24(4):487–99.
9. Kojouharova M, Reid K, Gadjeva M. New insights into the molecular mechanisms of classical complement activation. *Mol Immunol*. 2010 Aug;47(13):2154–60.
10. Kurai J, Chikumi H, Hashimoto K, et al. Antibody-dependent cellular cytotoxicity mediated by cetuximab against lung cancer cell lines. *Clin Cancer Res*. 2007 Mar 1;13(5):1552–61.
11. Kimura H, Sakai K, Arao T, Shimoyama T, Tamura T, Nishio K. Antibody-dependent cellular cytotoxicity of cetuximab against tumor cells with wild-type or mutant epidermal growth factor receptor. *Cancer Sci*. 2007 Aug;98(8):1275–80.
12. Chung KY, Shia J, Kemeny NE, et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J Clin Oncol*. 2005 Mar 20;23(9):1803–10.
13. Dechant M, Weisner W, Berger S, et al. Complement-dependent tumor cell lysis triggered by combinations of epidermal growth factor receptor antibodies. *Cancer Res*. 2008 Jul 1;68(13):4998–5003.
14. Hsu YF, Ajona D, Corrales L, et al. Complement activation mediates cetuximab inhibition of non-small cell lung cancer tumor growth in vivo. *Mol Cancer*. 2010;9:139.
15. Ciardiello F, Bianco R, Damiano V, et al. Antitumor activity of sequential treatment with topotecan and anti-epidermal growth factor receptor monoclonal antibody C225. *Clin Cancer Res*. 1999 Apr;5(4):909–16.

16. Baselga J, Norton L, Masui H, et al. Antitumor effects of doxorubicin in combination with anti-epidermal growth factor receptor monoclonal antibodies. *J Natl Cancer Inst*. 1993 Aug 18;85(16):1327–33.

17. Bokemeyer C, Bondarenko I, Makhson A, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol*. 2009 Feb 10;27(5):663–71.

18. Tabernero J, Ciardiello F, Rivera F, et al. Cetuximab administered once every second week to patients with metastatic colorectal cancer: a two-part pharmacokinetic/pharmacodynamic phase I dose-escalation study. *Ann Oncol*. 2010 Jul;21(7):1537–45.

19. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med*. 2004 Jul 22;351(4):337–45.

20. Sobrero AF, Maurel J, Fehrenbacher L, et al. EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. *J Clin Oncol*. 2008 May 10;26(14):2311–19.

21. Tol J, Koopman M, Cats A, et al. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. *N Engl J Med*. 2009 Feb 5;360(6): 563–72.

22. Van Cutsem E, Kohne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med*. 2009 Apr 2;360(14):1408–17.

23. Tournigand C, Andre T, Achille E, et al. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol*. 2004 Jan 15;22(2):229–37.

24. Maughan T, Adams RA, Smith CG, et al. Addition of cetuximab to oxaliplatin-based combination chemotherapy (CT) in patients with KRAS wild-type advanced colorectal cancer (ACRC): a randomised superiority trial (MRC COIN). *European Journal of Cancer Supplements*. 2009;7(3):4.

25. Adams RA, Meade AM, Madi A, et al. Toxicity associated with combination oxaliplatin plus fluoropyrimidine with or without cetuximab in the MRC COIN trial experience. *Br J Cancer*. 2009 Jan 27;100(2):251–8.

26. Saltz LB, Meropol NJ, Loehrer PJ Sr, Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J Clin Oncol*. 2004 Apr 1; 22(7):1201–8.

27. Jonker DJ, O'Callaghan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. *N Engl J Med*. 2007 Nov 15;357(20):2040–8.

28. Schrag D, Chung KY, Flombaum C, Saltz L. Cetuximab therapy and symptomatic hypomagnesemia. *J Natl Cancer Inst*. 2005 Aug 17;97(16):1221–4.

29. O'Neil BH, Allen R, Spigel DR, et al. High incidence of cetuximab-related infusion reactions in Tennessee and North Carolina and the association with atopic history. *J Clin Oncol*. 2007 Aug 20;25(24):3644–8.

30. Chung CH, Mirakhur B, Chan E, et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. *N Engl J Med*. 2008 Mar 13; 358(11):1109–17.

31. Tabernero J, van Cutsem E, Diaz-Rubio E, et al. Phase II trial of cetuximab in combination with fluorouracil, leucovorin, and oxaliplatin in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol*. 2007 Nov 20; 25(33):5225–32.

32. De Roock W, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol*. 2010 Aug;11(8):753–62.

33. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med*. 2008 Oct 23;359(17):1757–65.

34. Khambata-Ford S, Garrett CR, Meropol NJ, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. *J Clin Oncol*. 2007 Aug 1;25(22):3230–7.

35. Jacobs B, de Roock W, Piessevaux H, et al. Amphiregulin and epiregulin mRNA expression in primary tumors predicts outcome in metastatic colorectal cancer treated with cetuximab. *J Clin Oncol*. 2009 Oct 20;27(30): 5068–74.

36. Balko JM, Potti A, Saunders C, Stromberg A, Haura EB, Black EP. Gene expression patterns that predict sensitivity to epidermal growth factor receptor tyrosine kinase inhibitors in lung cancer cell lines and human lung tumors. *BMC Genomics*. 2006;7:289.

37. Balko JM, Black EP. A gene expression predictor of response to EGFR-targeted therapy stratifies progression-free survival to cetuximab in KRAS wild-type metastatic colorectal cancer. *BMC Cancer*. 2009;9:145.

38. Au HJ, Karapetis CS, O'Callaghan CJ, et al. Health-related quality of life in patients with advanced colorectal cancer treated with cetuximab: overall and KRAS-specific results of the NCIC CTG and AGITG CO.17 Trial. *J Clin Oncol*. 2009 Apr 10;27(11):1822–8.