

First-line targeted therapies in the treatment of metastatic colorectal cancer – role of cetuximab

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Abstract: Worldwide, colorectal cancer (CRC) is the fourth most commonly diagnosed malignant disease and the second leading cause of cancer-related death in Western nations. In 2008 there were an estimated 148,810 new cases and 49,960 deaths in the US. For several years different chemotherapeutic regimens, based on fluoropyrimidines, irinotecan and oxaliplatin, have been used in advanced CRC, but survival is still unsatisfactory. New targeted therapies, including drugs and monoclonal antibodies (MoABs), show great promise in the fight against CRC and have shown activity in different disease settings. Cetuximab, a chimeric IgG1 monoclonal antibody that binds to the extracellular domain of epidermal growth factor receptor (EGFR), is active in metastatic colorectal cancer (mCRC). As an IgG1 antibody, cetuximab may exert its antitumor efficacy through both EGFR antagonism and antibody-dependent cell-mediated cytotoxicity. The combination of this drug with classical chemotherapies has shown better clinical profiles reflected in an improvement in overall and progression-free survival. Clinical trials established the role of cetuximab, particularly with irinotecan, in irinotecan-refractory/heavily pretreated patients. Whereas cetuximab has a clear indication in the salvage setting, its role in first-line therapy remains investigational. It is particularly encouraging that cetuximab may enhance curative opportunities in patients with early metastatic disease, suggesting that adding cetuximab in first-line therapy may downstage disease in some patients, and, as a result, allow potentially curative resection of previously unresectable metastases. In this review we will focus on the main epidermal growth factor receptor inhibitors demonstrating clinical benefit, and the role of cetuximab in first-line treatment of metastatic CRC.

Keywords: cetuximab, colorectal cancer, clinical trials

Introduction

In Europe, colorectal cancer (CRC) was estimated to be the second most frequent cause of cancer-related death in 2006.¹ Although there have been substantial advances in the treatment of metastatic CRC (mCRC), median survival remains less than 2 years and less than 5% of patients survive for more than 5 years.^{2,3,4,5,6,7}

Chemotherapy (CT) is the mainstay of the treatment of mCRC, and, except for a minority of patients who are candidates for salvage surgery, in most cases its goal is palliation. The standard chemotherapy for patients with advanced CRC (ACC) consists of fluoropyrimidines, irinotecan and oxaliplatin.⁸ Since the introduction of 5-fluorouracil (5-FU), CT has proved to prolong median survival time (MST) of patients with advanced disease. With optimal scheduling, 5-FU doubled MST compared with best supportive care alone.^{9,10} Recent advances in combination CT, by addition of either oxaliplatin or irinotecan to 5-FU, resulted in improvement in both response rate (RR) and overall survival (OS),¹¹ with maximal benefit obtained when patients receive all three agents during the course of their disease.¹² Moreover, combination CT has improved the possibility of downstaging metastatic patients, thereby making resectable an additional 12% to 20% of patients initially considered unresectable.¹³ When these drugs are made available to patients during the course of their disease, a median survival of 17 to 20 months may be achieved.

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The treatment of ACC has made considerable progress in recent years. The newest developments are monoclonal antibodies (MoABs) such as cetuximab, which have shown activity in different disease settings.^{14,15} The epidermal growth factor receptor (EGFR) is a 170-kDa transmembrane glycoprotein, which is a member of the ErbB family of receptors. Ten different ligands can selectively bind to each receptor. After a ligand binds to a single-chain EGFR, the receptor forms a dimer¹⁶ that signals within the cell by activating receptor autophosphorylation through tyrosine kinase activity.¹⁷ Autophosphorylation triggers a series of intracellular pathways that may result in cancer cell proliferation, differentiation, migration, and adhesion, and enhances critical tumor growth and progression, including angiogenesis, apoptosis inhibition, tumor invasiveness, and metastatic spread (Figure 1).

Mechanisms of action of anti-EGFR drugs in cancer cells

The first anti-EGFR drugs were been developed in the 1980s.¹⁸ Anti-EGFR monoclonal antibodies such as cetuximab

bind to the EGFR extracellular domain during the inactive configuration. The occlusion of the ligand-binding region prevents the interaction between the receptor and the ligand, inhibiting ligand-induced EGFR tyrosine kinase activation.^{19,20}

Cetuximab, an IgG1 monoclonal antibody, specifically targets EGFR with a higher affinity than its natural ligands.²¹ Cetuximab prevents EGFR from binding to these endogenous ligands and therefore from adopting the extended configuration necessary for dimerization and signal transduction.²² Monoclonal antibody binding also stimulates receptor internalization and degradation.^{23,24} In preclinical models, cetuximab has been shown to increase tumor cell apoptosis,^{25,26} suppress invasion and metastasis,²⁷ inhibit proliferation,²⁸ and downregulate the production of proangiogenic factors (Figure 2).²⁹

Additional improvements in treatment are likely to be facilitated by the use of rationally selected therapeutic agents that target functionally important proteins in tumor cells, such as EGFR, expressed in 75% to 89% of CRCs.^{30,31} EGFR is often overexpressed in CRC and EGFR expression levels have been shown to be related to prognosis: higher EGFR

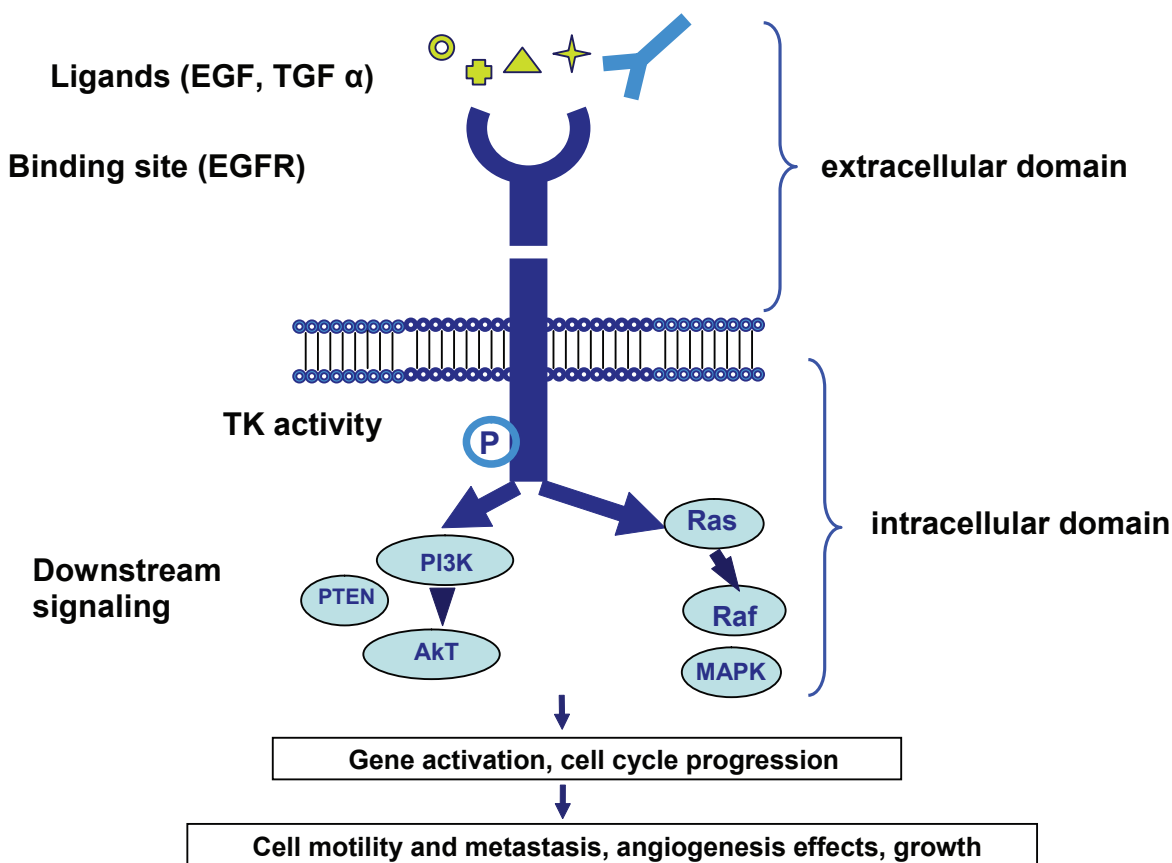


Figure 1 Signal transduction pathways controlled by the activation of EGFR.

a) The binding of a receptor-specific ligand occurs in the extracellular portion or of one of the EGFR-related receptors (HER2, HER3, HER4). b) The information of a functionally active EGFR-EGFR dimer causes the ATP-dependent phosphorylation of specific tyrosine residues in the EGFR intracellular domain. c) This phosphorylation triggers a complex program of intracellular signals to the cytoplasm and then to the nucleus to activate a cascade of anti-apoptotic and pro-survival signals.

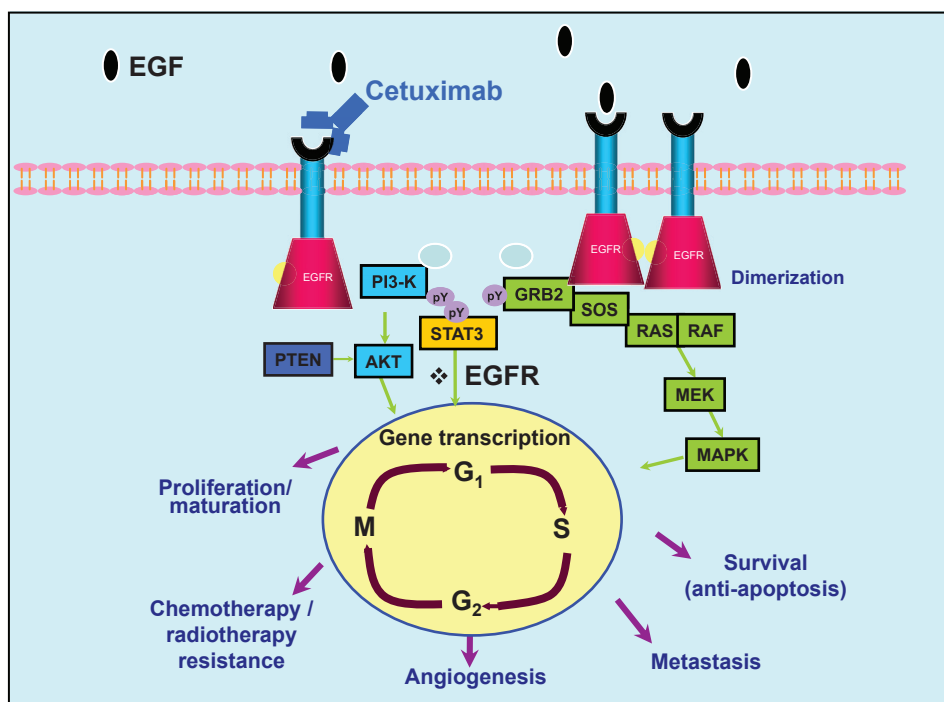


Figure 2 Monoclonal antibody cetuximab blocks EGFR. The anti-EGFR monoclonal antibody cetuximab, which is an IgG1 immunoglobulin, could elicit host antitumor immune responses, including antibody-dependent, cell-mediated cytotoxicity and can induce EGFR cellular internalization and downregulation, thereby enhancing receptor degradation. These two mechanisms could make an important contribution to antitumor activity.

expression levels correlating with a shorter OS and greater metastatic potential.³²

Cetuximab in first-line treatment of metastatic colorectal cancer

Preclinical cancer models

Experimental and clinical studies support the hypothesis that the EGFR represents a relevant target for cancer therapy.¹⁶ The possibility of combining conventional cytotoxic drugs with novel agents that specifically interfere with key pathways in controlling cancer cell survival, proliferation, invasion and/or metastatic spreading has generated a wide interest. Since the cellular targets of new agents are different from those of cytotoxic drugs, it is possible to combine them with standard chemotherapy without the risk of cross-resistance. The modification of the expression and/or the activity of genes regulating mitogenic signals can directly cause a perturbation of cell growth, but can also affect cancer cell sensitivity to conventional chemotherapy and radiotherapy. The EGFR autocrine pathway is one of the most active growth factor-driven mechanisms implied in the development and in the progression of human epithelial cancers. For this reason, in the past few years several preclinical and clinical studies have been conducted to test the antitumor activity of selective EGFR antagonists and cytotoxic therapies.

Morelli et al³³ demonstrated, through an in vitro model of human cancer epithelial cell line with a functional EGFR-dependent autocrine growth pathway, that antiproliferative activity potentiation of selected cytotoxic drugs (such as platinum derivatives and taxanes) combined with EGFR inhibitors was both schedule- and sequence-dependent. This model clearly demonstrated how a synergistic effect in inhibiting cancer growth could be observed only in those neoplastic cells first exposed to a cytotoxic drug and then treated with an EGFR inhibitor. Moreover, cancer cells death was significantly increased and the surviving cells tended to accumulate in G2/M phases, showing a modification in cell cycle distribution.

Many preclinical studies have been done to understand the mechanisms through which cetuximab succeeds in enhancing oxaliplatin's antiproliferative effect. In the Balin-Gauthier et al³⁴ study, the interaction between cetuximab and oxaliplatin antitumoral activities was tested on a panel of human CRC cell lines (HCT-8, HT-29, SW620, HCT-116). It was shown that observed responses were strictly dependent on cell type and on phospho-EGFR basal level, without any significant correlation with EGFR expression level. Cetuximab was also proven to lower oxaliplatin IC₅₀ values when tested, in vitro, with HCT-8 (EGF-R moderate) and

HT-29 (EGF-R weak) cell lines, while no major modification was found with HCT-116 or SW620.

In a recent study Balin-Gauthier et al³⁵ used responsive (HCT-8) and non-responsive (HCT-116) human colon cancer cell lines to analyze the effect of cetuximab on oxaliplatin intracellular detoxification, DNA adduct formation, cell cycle distribution, and induction of apoptosis. In responsive HCT-8 CRC cell line, cetuximab induces platinum–DNA adducts formation. This promotion is associated with a decreased expression of ERCC1 enzyme (excision repair cross complementation group 1) that seems to have a key role in the oxaliplatin–DNA platinum adduct repair process, the nucleotide excision repair (NER). They also observed a reduced expression of factors involved in DNA replication initiation, which correlates with an enrichment of G1 phase cells, and the stimulation of apoptosis pathway with an acceleration of cells death. None of these changes occurred in the non-responsive HCT-116 cell line, showing multiple interactions between oxaliplatin pharmacology and the EGFR signaling transduction pathway. These results suggested that under oxaliplatin treatment, the presence of adducts prevents cells from replication, decreasing the number of S phase cells. On the other hand, cetuximab combination therapy modified oxaliplatin adduct repair and induced modifications in the early stages of HCT-8 DNA.

Further studies by Prewett et al³⁶ investigated the effects of cetuximab and oxaliplatin treatment, single-agent or in combination, *in vitro* and *in vivo*, by using human CRC cell lines selected for oxaliplatin resistance, as well as parental control cell lines. Subcutaneous xenograft tumor growth in nu/nu athymic mice was evaluated, as well as activation of mitogen-activated protein kinase (extracellular signal-regulated kinase 1/2) and AKT, expression of DNA repair genes, density of apurinic/apyrimidinic DNA damage, and accumulation of platinum–DNA adducts *in vitro*. Oxaliplatin and cetuximab combination efficacy in murine subcutaneous xenograft models was greater than the respective monotherapy activity without any correlation with oxaliplatin monotherapy responsiveness. *In vitro*, cetuximab reduced expression of excision repair cross-complementation group 1 and XPF, which are key components of the nucleotide excision repair pathway involved in the cutting of platinum–DNA adducts. In addition, cetuximab reduced expression of XRCC1, a component of the base excision repair pathway responsible for repairing apurinic/apyrimidinic sites. Effects of cetuximab on DNA reparation proteins were downstream to the effects on mitogen-activated protein kinase and AKT

pathway activation. In line with effects on DNA repair protein expression, cetuximab increased the accumulation of platinum and apurinic/apyrimidinic sites on DNA during oxaliplatin treatment.

In summary, these preclinical studies illustrate cetuximab activity in the restoration of chemosensitivity to cytotoxic agents and provide a rationale for the evaluation of a potentially synergistic sequence of cytotoxic drugs and EGFR inhibitors in a clinical setting.

Clinical application

Building upon the effectiveness of cetuximab in refractory mCRC, several studies were conducted to explore whether adding cetuximab to first-line therapy would improve patients outcome.

A single-arm phase Ib/II study was conducted by Arnold et al³⁷ in order to investigate the feasibility of administering cetuximab in combination with oxaliplatin and infusional 5-fluorouracil (5-FU)/folinic acid (FA) in a weekly schedule (Association of Internal Oncology FUFOX protocol) as first-line treatment in patients with EGFR-positive advanced CRC. Cetuximab was administered weekly: 400 mg/m² initial dose, then 250 mg/m², and FUFOX: oxaliplatin 50 mg/m², FA 500 mg/m² and 5-FU as a 24-h infusion at either 1500 or 2000 mg/m² administered for 4 weeks followed by a 1-week rest (one cycle). Dose-limiting toxicity (grade 3 diarrhea) occurred in 3 of 14 assessable patients receiving 5-FU at standard 2000 mg/m². Cetuximab combined with FUFOX was generally well tolerated with the most common adverse events being diarrhea (27%) and paresthesia (16%) grade 3/4. The confirmed response rate in patients receiving 5-FU at standard 2000 mg/m² (N = 41) was 56%, with a median response time of 9.3 months. Median progression-free survival (PFS) and OS were, respectively, 8.1 (95% confidence interval [CI] 6.0–9.7) and 28.2 months, including all 49 patients. Cetuximab pharmacokinetics did not seem to differ in the FUFOX combination compared with cetuximab/irinotecan combinations.

The ACROBAT study, investigating cetuximab combined with FOLFOX-4 in 43 previously untreated ACC patients, reported similar grade 3–4 AEs, with diarrhea in 26% and paresthesia in 19% of patients, but a higher rate of neutropenia (23%, probably due to 5-FU bolus administration in FOLFOX-4 regimen) and rash (16%). The results showed a 77% RR, a median survival of 30 months and a median PFS of 12.3 months. Twenty-three percent of these patients had their liver metastases resected subsequently.³⁸ The favorable results of the ACROBAT trial are due merely

to selection of patients with favorable prognostic factors for cetuximab-based combination chemotherapy. In addition to clinical factors (eg, smaller metastases load), this could be related to molecular prognostic or predictive factors.

The study of Rosenberg et al in 2002³⁹ was designed as a phase II study enrolling 27 EGFR-positive patients treated with irinotecan, 5-fluorouracil/leucovorin (IFL) and cetuximab as frontline. The data showed a 44% partial response (PR) rate with a 20% of patients showing minor responses. Rash occurred in 26 out of 27 patients, but only 19% were grade 3.

A similar chemotherapeutic regimen was used in the study of Folprecht et al⁴⁰ in 2005, in which 21 patients with EGFR-expressing mCRC underwent cetuximab treatment in combination with weekly irinotecan and a low or high dose of infusional 5-FU. Among recruited patients 67% obtained a RR, 29% stable disease and in 20% of the cases liver metastases were resected after treatment. High and normal doses of 5-fluorouracil/leucovorin were used: in the group of high dose (2000 mg/m²) 3 out of 15 patients presented dose limiting toxicities (DLT). Median survival was 33 months, and 4 patients (19%) underwent potentially curative surgery.

A relevant phase II study conducted by Tabernero et al⁴¹ investigated the efficacy and the safety of cetuximab combined with standard oxaliplatin-based chemotherapy (infusional fluorouracil, leucovorin, and oxaliplatin [FOLFOX-4]) in the first-line treatment of EGFR-expressing mCRC. Patients with mCRC received on day 1 of a 14-day cycle, cetuximab (initial dose 400 mg/m² during week 1, then 250 mg/m² weekly) followed by FOLFOX-4 (oxaliplatin 85 mg/m² on day 1; leucovorin 200 mg/m² on days 1 and 2, followed by fluorouracil 400 mg/m² bolus then 600 mg/m² intravenous infusion during 22 hours on days 1 and 2).

The preclinical studies confirmed the supra-additive activity of cetuximab to oxaliplatin. In the clinical phase, 43 patients were included, with a median age of 65 years (range, 43 to 78 years). RRs were 79% (unconfirmed) and 72% (confirmed), with 95% of disease control. Median PFS (mPFS) and median duration of response were respectively 12.3 and 10.8 months. Ten patients (23%) with previously unresectable metastases underwent curative resection. After a median follow-up of 30.5 months, median OS (mOS) was 30.0 months. Cetuximab did not increase FOLFOX-4 characteristic toxicity and it was generally well tolerated.

A further study with FOLFOX-6 plus cetuximab in chemo-naïve patients showed a preliminary 53% overall RR (ORR) with 3 CR.⁴² This was a phase II study with 82 mCRC with positive or undetectable EGFR expression. Fourteen patients

left the study due to toxicity and 10% of the patients had grade 4 neutropenia and 2% grade 4 sepsis (Table 1).

The CALGB 80203 study was designed to compare first-line FOLFIRI vs FOLFOX, both with and without cetuximab. The study was closed to accrual after a total of 238 of the planned 2,200 patients were enrolled.⁴³ Preliminary results showed that response rates were higher in the cetuximab arms, especially when administered with FOLFOX: 60% for FOLFOX plus cetuximab; 40% for FOLFOX alone; 44% for FOLFIRI plus cetuximab; and 36% for FOLFIRI alone. Overall, adding cetuximab significantly increased RRs compared to treatment without cetuximab (52% vs 38%; $p = 0.0029$).

A more recent phase III clinical trial, the CRYSTAL study, enrolled a total of 1220 patients with untreated EGFR-expressing mCRC and randomly assigned them to cetuximab plus FOLFIRI (arm A) or FOLFIRI alone (arm B) treatment. Median PFS was significantly longer for arm A compared to arm B (8.9 months [CI: 8–9.5] for group A vs 8 months [CI: 7.6–9] for group B, $P = 0.036$). RR was also significantly increased by cetuximab (46.9% vs 38.7%, $p = 0.005$) compared to FOLFIRI alone.⁴⁴ The most common toxicities were neutropenia (26.7% in group A, 23.3% in group B), diarrhea (15.2% and 10.5% respectively) and skin reactions (18.7% and 0.2% respectively). The benefit derived by patients with liver disease only is particularly interesting: the resectability rates of RO (radical resection) increased 3-fold with the addition of cetuximab. These findings suggest that cetuximab is active in first-line setting, and especially valuable for patients with synchronous liver disease (Table 2).

The OPUS study is a phase III clinical trial⁴⁵ that randomized chemo-naïve patients to FOLFOX or FOLFOX plus cetuximab. Their primary end point was response rate and secondary goals were PFS, OS, and the R0 resection rate after metastatic surgery of curative intent. The preliminary results showed an RR of 35.7% and 45.6%, respectively, with 337 patients enrolled at that time. The most common

Table 1 Cetuximab in front-line phase II studies

	Cetuximab + FOLFIRI	Cetuximab + FOLFOX-4	Cetuximab + FOLFOX-6
Pts (n)	22	43	82
RR (%)	80	77	53
PFS (months)	10.9	12.3	–
OS (months)	–	30	–

Abbreviations: Pts, patients; RR, response rate; PFS, progression-free survival; OS, overall survival.

Table 2 Clinical studies of cetuximab in first-line treatment of EGFR-expressing mCRC

Study Type Phase II	Treatment (No of patients)	ORR (CR + PR) (%)	mTTP (months) (%)	mPFS (months) (%)	MOS (months) (%)
ACROBAT trial André et al ³⁸	Cetuximab + Folfox – 4 (43)	77	NR	12.3	30.0
Folprecht et al ³¹	Cetuximab + Irinotecan + 5-FU/FA (21)	67	9.9	NR	33
SAKK Group Borner et al ⁶⁴	Cetuximab + Xelox (37)	35.1	5.8	NR	16.5
	vs Xelox (74)	54	7.2		20.5
Study Type phase III	Treatment (no. of patients)	ORR (CR + PR) (%)	mTTP (months) (%)	mPFS (months) (%)	MOS (months) (%)
OPUS trial (on going) ⁴⁵	Folfox vs	35.7			
	Folfox + Cetuximab (337)	45.6			
Phase III CALGB 80203 ⁴³	Folfiri (61)	36	38	8.4	NR
	Folfiri + Cetuximab (59)	44	32	10.6	
	Folfox (60)	40	30	9.8	
	Folfox + Cetuximab (58)	60	26	8.2	
Phase III COIN trial (ongoing) ⁴⁶	Continuous Folfox	NR	NR	NR	NR
	Continuous Folfox + Cetuximab CT for 12 weeks followed by its interruption and resumption on PD				
Phase III CRYSTAL ⁴⁴	Folfiri (600) vs	38.7		8	NR
	Folfiri + Cetuximab (602)	46.9	N.R	8.9	
		(p = 0.004)		(p < 0.04)	

Abbreviations: CRC, colorectal cancer; ORR, overall response rate; CR, complete response; PR, partial response; mPTT, median time to progression; mPFS, median progression-free survival; MOS, median overall survival; NR, not reported.

grade 3–4 adverse events were neutropenia (27.6% in A; 31.5% in B), diarrhea (7.1% and 6.0%), leucopenia (7.1% and 5.4%) and rash (9.4% in the cetuximab arm only).

The ongoing UK Medical Research Council's COIN phase III trial⁴⁶ in mCRC is going to randomize approximately 2,400 first-line patients, whose EGFR status has not been previously established, to one of three arms: continuous oxaliplatin and fluoropyrimidine chemotherapy, continuous chemotherapy plus cetuximab, or chemotherapy for 12 weeks followed by its interruption and resumption on disease progression. The addition of cetuximab to oxaliplatin/fluoropyrimidine combinations results in increased grade 3–4 toxicities, and gastrointestinal (GI), skin rash and lethargy seem to be the most represented.

The results of these ongoing studies will elucidate the role of these combination in first-line treatment.

Cetuximab in combination with other novel agents

Animal models suggest that additive efficacy and sometimes synergy can be achieved using EGFR inhibitors in combination with agents that inhibit the vascular endothelial growth factor receptor.^{47,48} The EGFR pathway rules the production of vascular endothelial growth factor (and other

angiogenic factors); targeting both markers may therefore have a greater antitumor effect.

The addition of bevacizumab (a humanized mAb against the vascular endothelial growth factor) to fluoropyrimidine-containing chemotherapy⁴⁹ results in a significant survival benefit and it is currently considered as part of standard first-line therapy. In irinotecan monotherapy-resistant patients, the use of cetuximab improves mPFS. Cetuximab monotherapy improves the mOS in ACC patients in whom all available standard treatments have failed.⁵⁰ In ACC first-line treatment, cetuximab improves PFS when given in combination with bolus 5-FU plus FOLFIRI compared with FOLFIRI alone, although the absolute increase in mPFS is modest (8.9 vs 8.0 months). So far, limited data are available on the efficacy and safety of the combination of bevacizumab and anti-EGFR targeted agents in ACC. A randomized phase II study comparing bevacizumab, cetuximab and irinotecan with bevacizumab and cetuximab in irinotecan-resistant patients with ACC (BOND-2) showed an acceptable toxicity profile in both arms and a median time to progression of 7.9 vs 5.6 months.⁵¹

The Cancer and Leukemia Group B (CALGB/SWOG 80405) plans to randomize 2,289 mCRC patients for a first-line treatment with chemotherapy (FOLFOX or FOLFIRI) plus bevacizumab or cetuximab vs the combination of bevacizumab plus cetuximab. Patients and their

physicians will first choose either the FOLFOX or FOLFIRI chemotherapy regimen and then will be randomized to treatment with cetuximab, bevacizumab or both. The primary endpoint is OS, with RR, PFS, duration of response, and time to progression as secondary endpoints. Additionally, this study will explore which regimen is the most likely to allow patients to undergo metastases potentially curative surgery after chemotherapy.⁵²

CAIRO2 is a randomized phase III trial, evaluating the possibility of adding cetuximab to a regimen of capecitabine, oxaliplatin, and bevacizumab in order to improve PFS in previously untreated mCRC patient. Secondary end points are OS, tumor response, duration of response, quality of life, safety and translational research on factors that predict the response to treatment. 750 patients will be enrolled. Treatment consists of capecitabine 1000 mg/m² given orally twice daily on days 1–14, oxaliplatin 130 mg/m² iv on day 1 and bevacizumab 7.5 mg/kg iv on day 1 (arm A) or the same schedule plus cetuximab 400 mg/m² iv in week 1 of the first treatment cycle and 250 mg/m² iv weekly thereafter (arm B). All cycles are administered every 3 weeks. The study is still ongoing, but preliminary results on an interim analysis of toxicity show that the incidence of overall grade 3–4 toxicity is significantly higher in arm B compared with arm A (81% vs 72%, $p = 0.03$). This difference is fully attributed to cetuximab-related skin toxicity. The addition of cetuximab does not seem to result in an increase of gastrointestinal toxicity or treatment-related mortality.⁵³

Response to anti-EGFR drugs: clinical and pathological predictors

Since only a subgroup of patients with cancer have a clinical benefit from treatment with EGFR inhibitors, there is an urgent need for identification and clinical validation of useful criteria in selecting patients for such treatment.

A cetuximab-peculiar toxic effect is a papulopustular skin rash, generally on the face and upper torso, which is thought to be mechanism- and dose-related.⁵⁴ Findings suggest that there is a correlation between intensity of skin rash, response and survival.⁵⁵ This correlation is particularly striking in a subgroup analysis from the IMC 0144 trial reported by Pippas et al. In that trial, patients with no skin toxicity presented no objective responses and had a median survival of 1.7 months, whereas those who experienced grade 3 skin rash had a 20% RR and a mOS of almost 1 year.⁵⁶ This is the first reported observation of a clinical feature that may predict the clinical outcome of an antitumor agent. Dose-escalation schedules are currently under investigation in order to explore the

possibility of increasing cetuximab efficacy by inducing skin rash.

However, the presence and severity of cutaneous toxic effects are the most important clinical correlates of the efficacy of anti-EGFR therapy. The skin effect reflects the extent of EGFR blockade achieved in the tumor and correlates with EGFR saturation and with relevant drug concentration within the target.

EGFR expression, determined by immuno-histochemical methods, was the first biomarker investigated as a potential predictor of response. However, most studies have failed to show any relationship between EGFR expression and the clinical activity of anti-EGFR drugs.⁵⁷ Cetuximab has also been shown to have clinical activity in patients with EGFR-negative CRC.⁵⁸ Collectively, these data suggest that EGFR immuno-histochemical testing is not an optimal method for identifying patients who may have a response to anti-EGFR drug treatment.

Lievre et al recently reported that tumors with *KRAS* mutation are associated with resistance to cetuximab therapy.⁵⁹ In their study, 11 out of 30 patients (37%) responded to cetuximab therapy, which was mostly given in combination with irinotecan alone (75%) and in a multi-refractory setting (80%). *KRAS* mutation was detected in 13 of the 19 non-responder patients, but none of the responders was found to be positive ($p = 0.0003$).

KRAS activating mutation, which results in an increase in the mitogen-activated protein kinase pathway, is detected in approximately 40% to 45% of patients with CRC, and its presence seems to be correlated with a worse prognosis. *KRAS* mutations are frequently recorded codons 12 and 13 of exon 2 and usually eliminate the possibility of a concurrent EGFR mutation.

In several studies, *KRAS* mutations have been associated with lack of response to cetuximab in patient with advanced chemotherapy-refractory colorectal cancer.^{60,61} These studies suggest that constitutive activation of the *KRAS* signaling pathway could impair the response to anti-EGFR drugs.⁶²

Bokemeyer et al⁶³ evaluated the influence of *KRAS* mutation in patients treated with standard first-line therapy, whether or not associated with cetuximab. Best OR and PFS time (IRC evaluation) are linked to *KRAS* mutation presence. *KRAS* mutations were detected in 42% (99/233) of evaluable samples. Their data suggest that the benefit from addition of cetuximab to standard treatment is higher for the population with wild-type *KRAS*. For patients with *KRAS* mutations, no major benefit has been shown from adding cetuximab to FOLFOX regimen (Table 3).

Table 3 Clinical studies of cetuximab in combination with other novel agent in first-line treatment

Study type	Treatment	Patients	MOS (months) (%)
Randomized phase II study (BOND-2) Saltz et al ⁵¹	Cetuximab + Bevacizumab + Irinotecan	43	14.5
	vs Cetuximab + Bevacizumab	40	11.4
Randomized phase III study (CALGB/ SWOG 80405) ⁵²	Folfox/Folfiri + Bevacizumab or Cetuximab vs Folfox/ Folfiri + Bevacizumab + Cetuximab	Plans to randomize 2,289 patients	Still ongoing
Randomized phase III study (CAIRO2) ⁵³	Capecitabine + Oxaliplatin + Bevacizumab vs Capecitabine + Oxaliplatin + Bevacizumab + Cetuximab	Plans to randomize 750 patients	Still ongoing

Abbreviation: MOS, median overall survival.

These data suggest the possibility of choosing the most suitable treatment (cetuximab vs bevacizumab) based on KRAS analysis.

Conclusions

The results from the studies reviewed clearly point out the major role played by cetuximab in advanced CRC. Cetuximab has been used successfully in patients with refractory mCRC, and nowadays its role in the management of this tumor is growing. A defined role in the treatment of mCRC was established for regimens based on cetuximab plus irinotecan. Encouraging activity has also been observed in first-line treatment in combination with FOLFIRI and FOLFOX. Data from phase II trials in metastatic disease seem to indicate better results obtained with the addition of cetuximab to standard chemotherapy treatments (in particular, to oxaliplatin-containing regimens). On the basis of available evidence, the best interaction seems to be achieved when cetuximab is combined with oxaliplatin in first-line treatment and with irinotecan in refractory disease. The reason for these results is still unclear, but they suggest that interactions between cetuximab and oxaliplatin involve pathways different from those between cetuximab and irinotecan. Pending data from phase III trials will clarify their role in this setting.

It is also particularly evident that cetuximab may enhance the curative opportunities in patients with early metastatic disease. The early use of cetuximab combined with cytotoxic therapy during a treatment course could perhaps make a

higher fraction of mCRC fully resectable, as asserted by the significantly higher R0 resection rate reported for the cetuximab plus fluorouracil, leucovorin, and irinotecan arm of the Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer (CRYSTAL) study. Although most of the patients (91%) experienced grade 3–4 adverse events, the combination of cetuximab and FOLFOX-4 was generally well tolerated, and there was no evidence to suggest that cetuximab increased the frequency or the severity of usual FOLFOX toxicities.

Preliminary evidence suggests that adding cetuximab to first-line therapy may downstage disease in metastatic patients, allowing a potentially curative resection of previously unresectable lesions. The significant improvement in resection rate may increase OS.

Future utilization of cetuximab in mCRC treatment could combine the activity of the two different biologic agents: cetuximab and bevacizumab. The combination of cetuximab and bevacizumab, alone or together with other standard drugs, appears tolerable and active, and warrants investigation as front-line combination chemotherapy regimens. It is likely that some patients may benefit more from FOLFOX plus bevacizumab, others from FOLFIRI plus cetuximab, and still others from different combination of chemotherapy and biologic agents. The key will be to determine predictive factors, which will help us to identify the treatment that best suits the patient. The observation that *KRAS* gene mutation is associated with resistance to cetuximab is a promising first step.

Table 4 Median progression-free survival (mPFS) and overall response rate (RR) by *KRAS* mutation status

KRAS status	Median PFS (mo) Cetuximab + FOLFOX	Median PFS (mo) FOLFOX		Overall RR (%) Cetuximab + FOLFOX	Overall RR (%) FOLFOX
Wild-type	7.7 (n = 61)	7.2 (n = 73)	HR: 0.57 p = 0.02	61 (n = 61)	37 (n = 73) p = 0.01
Mutation	5.5 (n = 52)	8.6 (n = 47)	HR: 1.83 p = 0.02	33 (n = 52)	49 (n = 47) p = 0.11

Abbreviation: HR, hazard ratio.

All these data must encourage clinicians and basic researchers to maintain their efforts to untangle the EGFR network, in order to improve patient quality of life and survival.

Disclosures

None of the authors have conflicts of interest to disclose.

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