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TOPIC HIGHLIGHT

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Epidermal growth factor receptor inhibitors in colorectal cancer treatment: What's new?

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Abstract

Colorectal cancer constitutes one of the most common malignancies and the second leading cause of death from cancer in the western world representing one million new cases and half a million deaths annually worldwide. The treatment of patients with metastatic colon cancer comprises different regimens of chemotherapeutic compounds (fluoropyrimidines, irinotecan and oxaliplatin) and new targeted therapies. Interestingly, most recent trials that attempt to expose patients to all five-drug classes (fluoropyrimidines, irinotecan, oxaliplatin, bevacizumab and cetuximab) achieve an overall survival well over 2 years. In this review we will focus on the main epidermal growth factor receptor inhibitors demonstrating clinical benefit for colorectal cancer mainly cetuximab, panitumumab, erlotinib and gefitinib. We will also describe briefly the molecular steps that lie beneath them and the different clinical or molecular mechanisms that are reported for resistance and response.

Key words: Epidermal growth factor receptor inhibitors; Cetuximab; Panitumumab; Erlotinib; Gefitinib; Metastatic colorectal cancer; Tyrosine kinase inhibitors; Monoclonal antibodies

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies and the second leading cause of death from cancer in Europe and North America. It is responsible for approximately one million new cases and half a million deaths per year worldwide[1].

Several options are currently available for the treatment of patients with metastatic colorectal cancer (mCRC), including different regimens of chemotherapeutic compounds (fluoropyrimidines, irinotecan and oxaliplatin) and targeted therapies such as bevacizumab and cetuximab. Interestingly, most recent trials that attempt to expose patients to all five drug classes (fluoropyrimidines, irinotecan, oxaliplatin, bevacizumab and cetuximab) target an overall survival (OS) well over 2 years.

In this review we will summarise state-of-the-art targeting of the epidermal growth factor receptor (EGFR) in the management of metastatic colorectal cancer.

BIOLOGY OF EGFR

EGFR belongs to the ErbB family[2]. This family is comprised by transmembrane proteins that form part of the tyrosine kinases receptor proteins which are activated by different kinds of ligands[3] (Figure 1). All the receptor tyrosine kinases share the same protein structure with an extracellular binding domain, a transmembrane domain and an intracellular domain where the catalytic domain is located. The autophosphorylation of tyrosine residues outside the catalytic domain stabilises the receptor in the active conformation and recruit different proteins required for signalling.

There are several ligands binding ErbB including EGF, TGF alpha, Neuregulin family and some others[4]. Not all the ligands 'fit' all the receptors and this feature also has its implications at a molecular level[2]. Once the ligand binds the receptor and the molecule is phosphorylated it can switch on several pathways including the RAS-RAF-MAPK, JAK-STAT and the PIK3-AKT pathways. The signalling pathways activated by different EGF ligands drive various transcription factors to the nucleus that result in different cellular responses such as proliferation, migration, differentiation or apoptosis.

There are four different receptors in the ErbB family named ErbB1 (EGFR; HER or c-erbB the first to be described), ErbB2 (HER2/neu), ErbB3 (HER3) and ErbB4 (HER4). In the active conformation, the protein forms homodimers or heterodimers that are stabilised by the ligand binding. HER2/neu cannot (due to a genetic mutation) bind to EGF-like ligands and ErbB3 does not have a functional tyrosine kinase.

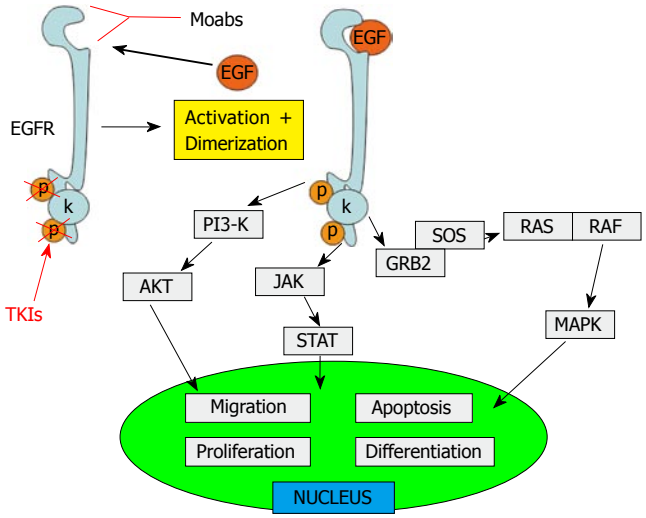

Figure 1 EGFR and its pathways.

Targeting the ErbB network may be achieved by inhibiting the tyrosine kinase (catalytic domain) with small molecules (TKIs) or by inhibiting the extracellular domain with monoclonal antibodies (Moabs) as shown in Figure 1. The moabs block the interaction between natural ligands and the EGF receptor in the extracellular space. The receptor is internalized and that can affect the network, as the timing of this process in the physiological state of the receptor also has its molecular implications[4,5]. Certain antibody isotypes such as IgG1 (cetuximab) have the potential for mediating antibody-dependent cell-mediated cytotoxicity (ADCC) and complement fixation[6], improving thus their antitumor activity. The TKIs compete with the ATP in their binding sites on the catalytic domain of the receptor and so act inside the cell.

CLINICAL APPLICATION

Monoclonal antibodies

Cetuximab: Cetuximab is an IgG1 monoclonal antibody targeting EGFR. Since preclinical data suggested that cetuximab might revert irinotecan resistance *in vitro*[7,8] and *in vivo*[9], a phase Ⅱ study[10] with 121 EGFR expressing mCRC patients refractory to irinotecan was started. A 17% overall response rate (ORR) was documented at an expense of acceptable toxicity grade 3-4. Cetuximab monotherapy has also proved activity in irinotecan refractory patients[11]. A phase Ⅱ open-label clinical trial with 57 EGFR positive mCRC patients was treated and an ORR of 9% was observed. The acne-like skin rash was the main described toxicity related to the drug. Two patients experienced grade 3 allergic reaction and discontinued the study. The study CO.17 that compared cetuximab and best supportive care (BSC) against BSC alone showed that cetuximab provides palliation in pretreated patients with advanced CRC, delaying deterioration in quality of life as well as improving survival[12] (Table 1).

These data led to the design of a study with 329 patients (pts) refractory to irinotecan who were randomized to cetuximab (111 pts) or irinotecan plus cetuximab (CI)

Table 1 Cetuximab in Irinotecan refractory mCRC

	Pts (*n*)	RR (%)	PFS (mo)	OS (mo)
C225 + Innotecan[10]	121	17	-	-
C225[11]	57	9	-	6.4
C225 + Innotecan[13]	329	23	-	8.6

Pts: Patients; mCRC: Metastatic colorectal cancer; RR: Response rate; PFS: Progression free survival; OS: Overall survival; mo: Months; C225: Cetuximab.

(218 pts). The ORR was 22.9% (95% CI: 17.5% to 29.1%) in the CI arm as opposed to 10.8% (95% CI: 5.7% to 18.1%) in the cetuximab arm. OS (8.6 mo *vs* 6.9 mo) and time to progression (TTP) (4.1 mo *vs* 1.5 mo) also favoured the CI arm. The toxicity presented in the CI group was very similar to that of patients treated with irinotecan alone[13] (Table 1).

More mature data regarding the role of CPT-11 and cetuximab in irinotecan refractory patients have been recently reported in the MABEL trial[14]. A multicenter study with 1461 CPT-11 refractory mCRC EGFR positive patients, 64% of whom had received two or more chemotherapy lines; 1123 patients are currently evaluable and a 12-week overall progression free survival (PFS) rate is 61% (58%-64%), and 34% (31%-37%) at 24 wk. The current estimate of median survival is 9.2 mo (8.7-9.9) with grade 3/4 adverse events being diarrhea (20%), skin toxicity (including acne-like rash) (19%), neutropenia (9%) and asthenia (8%). Hypersensitivity reactions occurred in 1.5% of the patients.

The above mentioned results provided the rationale for the BOND2 study that compared the combination of irinotecan, bevacizumab and cetuximab against bevacizumab plus cetuximab in CPT-11 refractory mCRC patients. A 43% ORR as opposed to 27% in favour of the irinotecan arm was presented. The median time to progression was 7.1 mo *vs* 4.6 mo and the median survival was 18.0 mo *vs* 10.3 mo for the irinotecan group[15,16]. The toxicity observed was the expected for each agent alone.

A variety of preclinical data have suggested activity of cetuximab in oxaliplatin resistant tumors[17]. Thus, a phase Ⅱ trial that combined CAPOX (oxaliplatin 85 mg/m^2, d 1, and capecitabine 2000 mg/m^2, d 1-7, every 2 wk) plus Cetuximab in patients who had progressed to oxaliplatin-based regimens has recently been presented[18]. Eighty percent of the 40 patients had also progressed on prior irinotecan-based chemotherapy. The study achieved 1 complete response (CR) (2.5%) and 7 partial responses (PR) (17.5%) with a 20% ORR and a 47.5% disease control rate (DC). The median TTP was 3 mo and the median survival 10.7 mo. Toxicity included grade 3-4 neutropenia (12.5%) and diarrhea (7.5%) and grade 2-3 neurotoxicity (22.5%). The second trial named EPIC is a phase Ⅲ study comparing cetuximab plus irinotecan and irinotecan as a second line in EGFR positive patients who received oxaliplatin plus fluoropyrimidines as a first line therapy. The primary endpoint was overall survival and quality of life being one of the secondary endpoints. Cetuximab plus irinotecan (n = 648) was superior to irinotecan alone (n = 650)

regarding progression-free survival and response rate (16.4% *vs* 4.2%, $P < 0.0001$). OS was comparable between both arms, but it may have been influenced by crossover. Health related quality of life was better preserved on the combination arm with less deterioration in symptom scores (pain, nausea, insomnia) and better health status scores[19]. Main toxicity (> 10%) grade 3-4 were neutropenia (30%) and diarrhea (21%). There is also a study by Lenz *et al*[20] analyzing with 346 refractory to irinotecan, fluoropyrimidines or oxaliplatin EGFR positive patients that achieved a RR of 12% with cetuximab monotherapy in patients.

The preliminary promising efficacy seen with C225 in refractory mCRC has prompted its use as front line therapy. In the ACROBAT study 43 EGFR positive mCRC patients were treated with cetuximab plus FOLFOX with a 77% RR, a median survival of 30 mo and a median PFS of 12.3 mo[21]. The study presented by Rosemberg *et al*[22] in 2002 was designed as a phase Ⅱ study with 27 EGFR positive patients that were treated with irinotecan, 5-fluorouracil/leucovorin (IFL) and cetuximab as frontline. They showed a 44% PR rate with another 20% of patients showing minor responses. Twenty-six out of 27 patients presented with rash, but only 19% were grade 3. Another study with a similar chemotherapeutic scheme was presented by Folprecht *et al*[23] in 2005 with a 67% RR and 29% stable disease rate in 20% of whom their liver metastases were resected after treatment. They used high and normal doses of 5-fluorouracil/leucovorin, three out of fifteen patients presented dose limiting toxicity (DLT) in the group of high dose (2000 mg/m^2). A phase Ⅱ study with 23 EGFR positive mCRC patients of whom 22 were assessable for response were treated with FOLFIRI and cetuximab in first line therapy. It showed a 46% PR rate and a 41% SD rate with a median TTP of 10.9 mo. Most common grade 3/4 toxicities were diarrhea, neutropenia and rash[24]. Seven patients underwent secondary surgery of metastases. Another study with FOLFOX-6 plus cetuximab in chemo-naive patients showed a preliminary 53% ORR with 3 CR[25]. It was a phase Ⅱ study with 82 mCRC patients showing positive or undetectable EGFR expression. 14 patients discontinued the study due to toxicity and 10% of the patients had grade 4 neutropenia and 2% grade 4 sepsis (Table 2).

More recently, results of the CRYSTAL study, a phase Ⅲ clinical trial that compares FOLFIRI plus cetuximab (arm A) *versus* FOLFIRI alone (arm B) in 1217 mCRC have been presented. The median PFS was significantly longer for arm A compared to arm B [8.9 mo (CI: 8-9.5) for group A *versus* 8 mo (CI: 7.6-9) for group B, $P = 0.036$]. RR was also significantly increased by cetuximab (46.9% *vs* 38.7%, $P = 0.005$). 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)[26]. The OPUS study is a phase Ⅲ clinical trial[27] that randomized patients to FOLFOX or FOLFOX plus cetuximab in chemo-naive patients. Their primary objective was response rate and secondary objectives 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 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 COIN study is a phase Ⅲ trial[28] (804 pts) comparing either continuous chemotherapy plus cetuximab or intermittent chemotherapy with the standard palliative combination. The addition of cetuximab to oxaliplatin-fluoropyrimidine combinations results in increased grade 3/4 toxicities overall and specifically to the gastrointestinal (GI), skin rash and lethargy. Capecitabine combination is associated with more GI toxicity but less neutropenia. Unexpectedly, no hypersensitivity reactions have been seen yet on FOLFOX (with or without cetuximab) (Table 3).

Table 2 Cetuximab as frontline, Phase Ⅱ studies

C225 plus:	Pts (*n*)	RR (%)	PFS (mo)	OS (mo)
FOLFIRI[25]	22	80	10.9	-
FOLFOX-4[22]	43	77	12.3	30
FOLFOX-6[26]	82	53	-	-

Pts: Patients; RR: Response rate; PFS: Progression free survival; OS: Overall survival; C225: Cetuximab.

Table 3 Cetuximab as frontline, Phase Ⅲ studies

C225 plus:	Pts (*n*)	RR (%)	PFS (mo)	OS (mo)
FOLFOX Cetuximab *vs* FOLFOX[28]	337	46.6% *vs* 35.5%	-	-
FOLFIRI Cetuximab *vs* FOLFIRI[27]	1217	46.9% *vs* 38.7%	8.9 *vs* 8.0	-

Pts: Patients; RR: Response rate; PFS: Progression free survival; OS: Overall survival.

Panitumumab: Panitumumab is a fully human IgG2 monoclonal antibody directed against the epidermal growth factor receptor. Several trials have tested its role in pretreated mCRC. The study with 148 mCRC refractory to FOLFOX/FOLFIRI EGFR positive patients treated with panitumumab alone showed a 10% RR with 36% of SD. 90% of the patients appeared with skin rash but only 4% G3[29]. Another study with panitumumab in refractory patients to FOLFOX/FOLFIRI[30] showed benefit for treating those patients with Panitumumab *vs* BSC. They were 463 EGFR positive patients who were assigned to panitumumab or BSC alone. The median progression free survival was 8 wk in the Panitumumab group *vs* 7.3 wk in the BSC group and the mean PFS 13.8 wk *vs* 8.5 wk. The RR was 10% in the Panitumumab group and 0% in the BSC group. The main toxicities were rash, diarrhea and hypomagnesemia. They did not find any advantage in overall survival due to the crossover but it resulted in a 46% reduction in the risk of tumor progression. Another study with 91 mCRC pretreated patients with negative or low EGFR by immunohistochemistry (IHC) showed a 7%-9% PR rate with 36%-42% of DC presenting skin and hypomagnesemia as main toxicities[31] (Table 4).

Table 4 Panitumumab, Phase Ⅱ and Ⅲ studies

	Pts (*n*)	RR (%)	PFS	Naive	Phase
Alone[30]	148	10	-	No	Ⅱ
Alone *vs* BSC[31]	463	10	8 wk	No	Ⅲ
Alone[32]	91	8	8 wk	No	Ⅱ
IFL + Panitumumab *vs* FOLFIRI + Panitumumab[33]	19	46	5.6 mo	Yes	Ⅱ
	24	42	10.9 mo		

Pts: Patients; RR: Response rate; PFS: Progression free survival; OS: Overall survival; mo: months; BSC: Best supportive care.

Panitumumab showed better tolerability combined with FOLFIRI than with IFL[32]. In a pooled analysis of several trials[33] the skin toxicity in panitumumab patients was 90%-95% but only in 3%-5% was grade 3 and treatment limiting. The other relevant toxicities were gastrointestinal (nausea, diarrhea and anorexia) which accounts for 25%-30% of all grades (2% grade 3) and hypomagnesemia (41%; 7% grade 3). The severity of skin rash was correlated with increased efficacy in terms of ORR, PFS, and OS[34,35]. A recent study with panitumumab has correlated skin toxicity with increased efficacy and better health-related quality of life[34]. In this phase Ⅲ study patients were randomized to panitumumab plus BSC (231 patients) or BSC alone (232 patients) and the skin toxicity was analyzed in relation to PFS and OS. The incidence of grade 2-4 skin toxicity was higher in the panitumumab arm. OS was significantly prolonged in patients with more severe skin toxicity (gr 2-4 *vs* gr 1; HR = 0.67; $P = 0.0235$) (Table 4).

Tyrosine kinase inhibitors

Gefitinib: Gefitinib is a potent, specific EGFR tyrosine kinase activity inhibitor. Phase Ⅰ/Ⅱ trials in patients with mCRC showed little activity[36,37] but preclinical studies *in vitro* and *in vivo* suggested a supra-additive growth inhibitory effect of gefitinib when combined with different cytotoxic drugs[38] which gave support to several clinical trials of gefitinib combined with chemotherapy in mCRC patients.

The study by Magné *et al*[39] support studies that combined gefitinib with fluoropirimidines[40]. The study was designed in two parts with 23 patients overall. One part with intermittent dose-escalated gefitinib plus 5-fluorouracil (370 mg/m^2 Ⅳ)/LV (20 mg/m^2 Ⅳ) and the other with continuous gefitinib at the safest dose assigned by part one. The safest dose assessed was 500 mg/d achieving a 23% OS with skin rash and diarrhea as main toxicities. Preliminary results from a small phase Ⅰ/Ⅱ trial combining gefitinib 250 mg/d plus capecitabine 1000-1250 mg bid. after failure to first line therapy also suggests some evidence of activity[41].

A dose-finding trial was performed with irinotecan plus gefitinib in 18 patients with advanced CRC refractory to fluoropyrimidine-based chemotherapy. It defined irinotecan given at a dose of 225 mg/m^2 as a single agent every 3 wk plus gefitinib at a dose of 250 mg/d as the maximum tolerated dose (MTD) of this regimen[42]. Dose-limiting toxicities, such as neutropenia and diarrhea, occurred at unexpectedly low doses of irinotecan. Disease stabilization was achieved in 21% (4 out of 18 patients). Once they achieved the recommended dose level (RDL) they expanded the study to a multicenter one with a total of 27 patients at the RDL with an objective tumor response rate of 11% and median survival 9.3 mo[43]. The toxicity grades 3-4 included diarrhea (35.9%), lethargy (15.4%), neutropenia (15.4% with 10.3% febrile neutropenia) and skin rash (7.7%).

The combination of gefitinib plus FOLFIRI in both chemo-naive mCRC patients[44] and as salvage therapy[45] was considered too toxic despite dose reduction in 5-fluorouracil, leucovorin and irinotecan. Toxicity was also the main issue when combining gefitinib with capecitabine in patients who had previously received one or two chemotherapy lines being diarrhea and neutropenia, the principal related DLTs[46].

In a study by Kuo *et al*[47] with 27 patients who had previously received at least one regimen (oxaliplatin based mainly) they employed FOLFOX-4 and gefitinib at a dose of 500 mg/d. 33% of the patients achieved objective responses and 48% showed stable disease. Median OS was 12.0 mo, while median event-free survival was 5.4 mo. For first-line treatment, a 74% RR with a clinical benefit rate of 98% and a median TTP of 9.5 mo. was reported by Zampino *et al*[48] with the FOLFOX-6 regimen plus gefitinib at a dose of 250 mg/daily.

The study by Zeuli *et al*[49] assessed the doses of gefitinib (250 mg/d) plus capecitabine (2000 mg/m^2 per day, d 1-15) and oxaliplatin (120 mg/m^2 d 1) every 3 wk for six courses as first-line treatment in patients with metastatic disease. The most common grade 3 adverse events were diarrhea and neutropenia. A 50% response rate (6 out of 12 patients; 5 PRs, 1 CR) and a clinical benefit rate of 58% (7 out of 12 patients) were communicated.

In an *in vitro* study working with cetuximab-resistant cell lines, authors observed that gefitinib or erlotinib retained the capacity to inhibit growth of tumor cells that were highly resistant to cetuximab[50]. These data suggest that tyrosine kinase inhibitors may further modulate intracellular signalling that is not fully blocked by extracellular anti-EGFR antibody treatment. A phase Ⅰ/Ⅱ study that combined cetuximab and gefitinib[51] presented 56% of PR in mCRC patients. This observation deserves further evaluation.

Erlotinib: Erlotinib is a small molecule that competes with ATP for the intracellular tyrosine kinase domain of EGFR, thereby inhibiting receptor autophosphorylation and blocking downstream signal transduction (Figure 1). Evidence of single agent erlotinib activity *in vitro* and in mCRC patients, derived from disease specific phase Ⅱ studies[52,53], led to the design of several trials in combination with chemotherapy. One phase Ⅱ study presented a PR rate of 4% in 51 mCRC patients. 46 of them were assessed for response. Skin rash was observed in 62% of the patients (13% G3) and grade 3 diarrhea and nausea were also observed after erlotinib monotherapy. Another phase Ⅱ study on 38 mCRC patients treated with 150 mg of erlotinib in a continuous daily schedule presented a 39% SD rate, as the best response, with rash and diarrhea as the main toxicity events[53]. Additive activity of erlotinib when combined with

capecitabine in preclinical studies with human xenografts[54] supported a phase Ⅱ study with 10 pts evaluating the combination of erlotinib 150 g daily with capecitabine 1000 mg/m^2 bid. for 14 d in chemotherapy-naive metastatic CRC patients. Grade 3 diarrhea (30%), grade 3 renal insufficiency (10%) and grade 3 hyperbilirubinemia (10%) were the most troublesome toxicities. Regarding efficacy, no complete responses were achieved whereas disease control rate (PR + SD) was 34%[55].

In the study by Meyenhart *et al*[56] when combining oxaliplatin, capecitabine and erlotinib patients started receiving 1000 mg/m^2 bid. of capecitabine that was reduced to 750 mg/m^2 bid for 14 d after the first 13 patients experienced excess of grade 3/4 toxicities. Thus, the final doses were capecitabine 750 mg/m^2 bid. for 14 d, oxaliplatin at 130 mg/m^2 on d 1, and erlotinib 150 mg daily. The ORR was 20%. In addition, the group of Delord *et al*[57]presented a dose-finding study establishing erlotinib 100 mg/d, capecitabine 1650 mg/m^2 qd (d 1-14), and oxaliplatin130 mg/m^2 every 3 wk as the MTD for this regimen.

Erlotinib (50-150 mg/d) is also being investigated in combination with FOLFOX-4 for untreated or minimally pretreated patients with CRC, with a preliminary reported 43% response rate. The most commonly communicated grade 3 or 4 toxicities were diarrhea and neutropenia[58].

CLINICAL AND MOLECULAR MARKERS OF RESISTANCE AND RESPONSE TO EGFR INHIBITORS

A peculiar toxic effect of cetuximab is a papulopustular skin rash, generally on the face and upper torso, which is thought to be mechanism- and dose-related[59]. Findings suggest that there is a correlation between intensity of skin rash and response and survival[13]. 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 mo, whereas those who experienced grade 3 skin rash had a 20% RR and a median survival of almost 1 year[60]. 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.

The EVEREST study was designed as a phase Ⅲ trial with cetuximab escalated-doses. They started with standard dose and increased dose every 2 wk until skin toxicity grade 2 or 500 mg/m^2 of cetuximab were achieved. The dose-escalation of up to 500 mg/w indicated improvement of RR in pts with no or slight skin reactions on standard dose treatment[61] with 166 patients included in the study. The mechanism underlying the correlation between skin toxicity and tumour response is currently unclear, however, some research groups hypothesized that the rash is a surrogate indicator of an adequate degree of receptor saturation by cetuximab. If this is the case, targeting doses to achieve a desired level of cutaneous toxicity may further increase the efficacy of this agent. While this is an appealing prospect from a potential efficacy point of view, it would suggest, if true, that there might be a narrow therapeutic window when working with this drug[59].

In early clinical trials, EGFR positivity on tumor specimen by IHC was mandatory for the use of cetuximab. However today, EGFR expression status is known not to be a predictive factor of response to cetuximab since major responses in patients with EGFR negative tumors are expected after cetuximab treatment. In fact, responses have been reported by some authors[62] and nowadays EGFR status is not mandatory for the management of CRC patients[63]. Several factors might explain this apparent discrepancy, such as low sensitivity of IHC, cytological heterogeneity of CRC and differential EGRF expression in primary and metastatic tumor niches[64,65]. There are other reasons that might explain these striking data. Two distinct EGFRs have been identified in A431 cells by epidermal growth factor-binding studies. These are a major class of low-affinity EGFR (representing approximately 95% of the receptors) and a minor class of high-affinity EGFR (representing approximately 5% of the receptors), with binding affinities differing by an order of magnitude[66-68]. The current EGFR IHC detection systems used today derived from A431 cells do not distinguish between these two distinct EGFRs. It is known that high-affinity EGFRs are the biologically active receptors that switch the ErbB pathway whereas low-affinity receptors do not contribute significantly[66,69]. Another possible explanation is related to the ADCC capacity of cetuximab antibodies and two polymorphisms related to fragment C of the immunoglobulin G that are related to progression and survival[70].

In order to assess response to EGFR inhibitors in the clinical practice different molecular approaches are being evaluated. There are some studies where they try to find a correlation between some germinal polymorphisms involved in angiogenesis, the EGFR pathway, DNA repair and drug metabolism[15,71]. In a recent study they found a correlation, in patients treated only with cetuximab, between a Cyclin D1 polymorphism (A870G) and overall survival[72]. The Cyclin D1 is a protein related to p27^{KIP1} which is involved in the G1 phase arrest produced by EGFR inhibitors and that is correlated to apoptosis in tumor biopsies of patients treated with gefitinib[73]. The heterozygous AG genotype was significantly related to higher overall survival. Patients with AA homozygous genotype survived a median time of 2.3 mo (95% CI 2.1, 5.7) compared to those having homozygous GG genotype that survived a median of 4.4 mo (95% CI 1.8, 9.8). Even patients with a heterozygous AG genotype presented in comparison, a median survival of 8.5 mo (95% CI 5.5, 11.7), ($P < 0.05$)[72]. Another study showed similar results finding a correlation between EGFR (G497C GA), Cox-2 (G-765C CC) and EGF (A61G GG) polymorphisms and PFS[74].

Furthermore, a different investigation treated mCRC patients with cetuximab or panitumumab assessing the

EGFR copy number and the mutation profile of the EGFR catalytic domain and of selected exons in KRAS, BRAF, and PIK3CA[75] in the tumor sample. They found that in 8 out of 9 patients with an objective response the EGFR copy number was increased whereas only 1 out of 21 non-responders had an increased EGFR copy number. A retrospective study showed a linkage between EGFR mRNA levels by RT-PCR and TTP but not with survival[76] and found no correlation between any other ErbB receptors or EGFR by IHC and clinical outcome. There are other studies that suggested a correlation of KRAS mutation and poor outcome in terms of response and survival[77-79]. In the study by Finocchiaro *el al*[77] they analyzed tumor blocks from 85 colorectal cancer patients for EGFR expression (IHC and FISH), HER2 (FISH) and KRAS (mutation). EGFR FISH positive patients (41 patients) had a significantly higher RR and TTP than EGFR FISH negative individuals (44 patients). EGFR expression assessed by IHC was not associated with any clinical endpoint. Increased HER2 gene copy number predicts early escape from cetuximab therapy. Compared to patients with wild type KRAS, KRAS mutation carriers (32 patients) had a significantly lower RR (6.3% *vs* 26.5%, $P = 0.02$), shorter TTP (3.7 mo *vs* 6.3 mo, $P = 0.07$) and shorter survival (8.3 mo *vs* 10.8 mo, $P = 0.2$). In 22 patients with available primary and metastatic tumor samples, there was no difference between these sites for EGFR FISH, HER2 FISH and KRAS results. A study of 59 mCRC patients treated with cetuximab plus chemotherapy looked for KRAS mutations using first direct sequencing and two sensitive methods based on SNaPshot and PCR-ligase chain reaction (LCR) assays. They compared clinical response with gene mutations. No KRAS mutation was found in the 12 patients presenting clinical response. On the contrary KRAS mutation was associated with disease progression ($P = 0.0005$) and TTP was significantly decreased in patients with mutated KRAS tumors (3 mo *vs* 5.5 mo, $P = 0.015$)[78].

The other important mutations associated with the activity of EGFR inhibitors that are related to response to TKIs in lung cancer are mutations in exons 18, 19 and 21[80,81]. In mCRC it seems not to be the case. That may be due to the fact that those mutations are not commonly found in mCRC patients[20,82,83]. Because of this issue other predictive factors of response to Gefitinib such as the insulin receptor isoform A are currently under research[84].

FUTURE DIRECTIONS IN EGFR TARGETING

Monoclonal antibodies

EMD 72000: EMD 72000 (Matuzumab) is a humanized IgG1 anti-EGFR MoAb. It has completed phase I clinical testing in EGFR-positive solid tumors. 22 patients of different origin (including colorectal) received EMD 72000 weekly[85] and a 23% RR was demonstrated. EMD 72000 administered to 22 patients with colon (15 patients), gastric, or renal tumors demonstrated PR in 2 patients and a minor response in 1 patient[86] all of them with colon cancer. Another phase I study showed near-complete EGFR signalling suppression at the 1200 mg dose level[87]. A phase I study of matuzumab administered weekly to 26 patients (18 of which had CRC) showed 2 PR, and 10 SD in patients with colon cancer. In addition a preliminary analysis of skin biopsies showed that matuzumab produced inhibition of pEGFR and pMAPK with a decrease in Ki67 expression and an increase in p27[88].

AEE788: AEE788 is an oral inhibitor against EGFR, ErbB2, VEGFR-2 and KDR. A phase I study in these patients with advanced CRC and liver metastases showed the lack of clinical activity of AEE up to 400 mg with an inhibitory effect of 100%, 90% and 39% over pEGFR, pMAPK and Ki67 respectively by IHC in tumor biopsies[89]. Another study that investigated the effects of AEE *in vitro* and in biopsies from 22 advanced colorectal cancer patients did not find any major clinical responses even at the higher dose schedule (400 mg). Laser scanning cytometry quantitative analysis confirmed the target inhibition of AEE *in vitro* and in wound-induced skin pairs[90]. The lack of significant target inhibition in tumors has to do with the lack of clinical activity of AEE in this cohort of patients and is consistent with other studies.

HKI-272: HKI-272 is an irreversible pan-erbB receptor tyrosine kinase inhibitor. It inhibits the growth of tumor cells that express erbB-1 and erbB-2 (HER-2) in culture and in xenografts. HKI-272 also inhibits the growth of cultured cells that contain sensitizing and resistance-associated EGFR mutations[91]. A phase I study with 73 patients is ongoing and the preliminary results for 51 patients (3 of which are mCRC) showed a MTD of 320 mg/d with diarrhea as the DLT. Two breast cancer patients had confirmed partial responses and 2 had unconfirmed PRs[92].

Other MoAbs directed against EGFR have recently undergone clinical testing e.g., hR3[93] and ICR62[94].

NEW GENERATION OF TYROSINE KINASE INHIBITORS

Additional oral TKIs currently under clinical evaluation, include the reversible dual EGFR/Her-2 TKI lapatinib and the irreversible EGFR TKI EKB-569.

Lapatinib: Lapatinib is a reversible inhibitor of ErbB1/ErbB2 tyrosine kinases. 64 patients (22 with colon cancer) were included in a phase I study. One CR and 22 SD were achieved. Most of the patients with SD overexpressed either ErbB1 or ErbB2. The most frequent toxicities presented were rash, diarrhea, nausea/vomiting, fatigue, and anorexia. Serum VEGF may be a potential biomarker for lapatinib activity[95]. A study in combination with FOLFOX-4 to assess the safety included 13 patients (2 colon). The dose of lapatinib 1500 mg/d with FOLFOX-4 was well tolerated although 2 patients had grade $\geqslant$ 3 hematological toxicities, which resolved after delay of the next cycle. Seven patients were evaluable for response and 2 PR, 2 SD and 3 PD were confirmed[96]. A phase Ⅱ study with lapatinib as the single-agent in 86 mCRC patients who progressed to prior therapy showed 5 patients who experienced clinical benefit with stable disease

for ⩾ 20 wk[97]. The median TTP and overall survival were 8 and 42.9 wk respectively. The most commonly encountered adverse events were diarrhea (45% grade 1-2, 5% grade 3), rash (33% grade 1-2, 2% grade 3), fatigue (27% grade 1-2, 2% grade 3), nausea (20% grade 1-2, 1% grade 3), anorexia (16% grade 1-2, 2% grade 3), and vomiting (14% grade 1-2).

EKB-569: EKB-569 is a selective, irreversible inhibitor of the EGFR, was well tolerated in patients with advanced solid tumors of the colon, lung, breast, head and neck. A phase I study with 30 patients with advanced tumors of different origins established the MTD at 75 mg EKB-569 per day for both cohorts, intermittent-dose schedule (14 d of a 28-d cycle) and continuous-dose schedule (each day of a 28-d cycle) being the DLT grade 3 diarrhea[98]. In a phase Ⅰ/Ⅱa study of EKB-569 in combination with FOLFOX-4 (29 patients), 4 out of 11 patients who completed 4 cycles achieved a PR, 6 patients had stable disease, and 1 patient had progressive disease[99]. Grade 3/4 Toxicity included neutropenia and diarrhea. Moreover, a phase Ⅰ/Ⅱa study of EKB-569 in combination with FOLFIRI (39 evaluable patients out of 47) showed a 38% of RR[100].

CONCLUSIONS

When administered alone new targeted therapies have demonstrated activity in different *in vitro* and *in vivo* studies. However, the clinical use in patients when administered as a single agent is not so brilliant. On the other hand the combination of these drugs with classical chemotherapies has shown better clinical profiles reflected in an improvement in OS and PFS. The FDA approved Cetuximab as a second line therapy in combination and Panitumumab has also been approved as a second and third line therapy for advanced CRC patients. An important number of clinical trials with second or first generation of TKIs is ongoing. Perhaps the role of TKIs in mCRC patients is maintenance treatment in individuals with objective response or stabilisation of their tumor.

There is also the challenging possibility of combining different targeted therapies in order to overpass tumor resistance. Combining targeted therapies against different pathways is also a possibility. The cross-talk at a molecular level of the different networks implicated in cell biology is almost unknown. However there are more data that implicate different molecular networks when studying resistance to targeted therapies against one pathway.

All these data must encourage clinicians and basic researches to hold on in their efforts of untangling the network behind EGFR trying to transform all that effort in improving patients quality of life as well as improving survival There are different clinical scenarios in our patients and each of them should have its own solution. In some cases the approach will be combining chemotherapy with targeted therapy, targeted therapy with radiotherapy or even targeted therapy alone. In anyway we have still a lot of clinical trials to start and new drugs to be tested in order to find the adequate solution for each of our patients.

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