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Role of anti-EGFR target therapy in colorectal carcinoma

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TABLE OF CONTENTS

1. ABSTRACT

The epidermal growth factor receptor (EGFR) has become an important target in cancer treatment. In consequence, drugs directed at this and other molecular targets are an increasingly important part of the treatment of numerous tumours. Cetuximab and panitumumab, two monoclonal antibodies that target EGFR, have proved to be effective in metastatic colorectal cancer treatment. However, some patients do not respond to treatment with EGFR inhibitors and, for this reason, interest in the identification of patients most likely to benefit from treatment with these agents has grown considerably. K-Ras, a member of the RAS family of signalling proteins plays an important role in EGFR- mediated regulation of cellular proliferation and survival. Patients with wild-type K-Ras were found to have significantly greater overall survival, progression-free survival and/or response rate compared with patients harbouring K-Ras mutations.

2. INTRODUCTION

Colorectal cancer continues to be the fourth most common cause of cancer in the world and the third cause of death from cancer in the U.S.A (1).

In recent years, a greater knowledge of the molecular bases of colorectal cancer, together with the development of new antineoplasic agents, has considerably changed the therapeutic management of this disease. Thus, the field of specifically targeted therapies is beginning to use known molecular mechanisms to act more selectively on the tumour cell and new agents against these specific targets are being developed.

In colorectal cancer monoclonal antibodies have been developed against two specific target proteins, the epidermal growth factor receptor (EGFR) and the vascular endolethial growth factor (VEGF). However, at present, the

Table 1. Influence of KRAS mutation status and efficacy of cetuximab in mCCR

Authors	Anti-EGFR	KRAS mutant	ORR[1] %	
			Mutant	Wild-type
A				
Lièvre (12)	**Cetuximab**	27	40	0
Benvenuti(13)	**Panitumumab or cetuximab**	33	31	6
De Roock(9)	**Cetuximab or Panitumumab**	39	41	0
Di Fiore(10)	**Cetuximab**	27	28	0
Khambata-Ford(11)	**Cetuximab**	38	10	0

[1].ORR: Overall response rate

molecular mechanisms underlying clinical response to these drugs are not fully understood.

This study will review the anti-EFGR agents used at present in clinical practice in treatment of colorectal cancer: cetuximab and panitumumab.

3.EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)

The EGFR is a transmembrane receptor tyrosine kinase that belongs to the erbB family, which also includes erbB2 (HER2/neu), erbB3 (HER3) and erbB4 (HER4). EGFR, like the other erbB receptors, is a glycoprotein comprising three components: an extracellular ligand binding domain, a hydrophobic transmembrane domain and an intracellular tyrosine-kinase domain (2).

Similarly to erbB2, there are specific ligands for each of the erbB receptors: among them the epidermal growth factor (EGF) and the transforming growth factor alpha (TGFα), bind selectively to EGFR.

In the absence of ligand, EGFR remains in a state of autoinhibition. However, binding alters the extracellular domain conformation, which exposes the dimerization loop. In this state, EGFR complexes with other EGFR receptors or a member of the erbB family to form homo or heterodimers respectively. The dimer activates the phosphorylation of tyrosine kinases in the EGFR intracellular domain, triggering downstream signalling pathways (in which K-Ras plays an important role) that, finally, regulate cellular proliferation, migration, adhesion, differentiation and survival (3).

3.1. Ras

Ras constitutes a family of proto-oncogenes with three different members, HRas, KRas and NRas, which codify membrane proteins with guanosine triphosphate activity. These proteins bind to GDP (inactive protein) or GTP (active protein) and are active in signal transduction induced by various extracellular signals.

Among the Ras oncogenes, K-Ras is known to play an important role in the EGFR function. It is found in the internal cellular membrane and, as has been mentioned before, has GTPase activity (4). The binding of extracellular ligands to transmembrane receptors such as EGFR triggers the activation of a signal transduction cascade to the nucleus. Initially, the intracellular tyrosine kinase domain is phosphorylated and then it triggers a transitory activation of the RAS protein; in an inactive state this protein is bound to guanosine diphosphate (GDP) and activation is produced by the conversion of GDP to guanosine triphosphate (GTP). Moreover, the signal transduction is linked to RAF, MEK, and ERK, which leads to gene transcription in the cellular nucleus originating tumour progression.

KRAS gene mutation has been described in 40% (20-50%) of sporadic colon tumours (5, 6) and, approximately, 98,4% of the oncogenic mutations in the K-Ras gene are found in codons 12 y 13. The presence of mutations in these proteins activates them in constitutive form making the Ras-GTP conformation permanent and insensitive to the GTPase action. Mutations in codons 61 are more rare (7).

Knowledge of the erbB family stimulated the interest in developing agents that inhibited this signalling pathway. Two classes of EGFR targeting agents have been trialled in different tumours: monoclonal antibodies that bind to the EGFR extracellular domain, blocking the tyrosine kinase activation and also, small molecules that inhibit tyrosine-kinase competing reversibly with ATP in the EGFR intracellular domain.

The first studies that evaluated the KRAS mutation status suggested that it was unlikely to be predictive of response to standard chemotherapy regimens in colorectal cancer. Among patients receiving exclusively fluoropyrimide therapy for metastatic colorectal cancer, response rate and survival did not seem to be influenced by tumor KRAS mutation status (8).

Moreover, the importance of wild-type KRAS as a mediator of EGFR signaling and the high frequency of KRAS mutation in colorectal cancer created with arose the hypothesis that tumors harboring KRAS mutation could be resistant to EGFR inhibitors. All retrospective analysis of studies, concerning standard treatments in chemorefractory colorectal cancer, showed response to anti-EGFR target therapy occurred in patients with tumors with K-RAS wild-type status (9-13), as reported in table1. These data, together with the earlier one by Lièvre and colleagues (14), clearly evidence that KRAS mutation status may be predictive of response to cetuximab in colorectal cancer patient (Table 1).

Two monoclonal antibodies targeting EGFR have been approved for colorectal cancer treatment in clinical practice: cetuximab y panitumumab.

4.CETUXIMAB

Cetuximab (IMC-225, C225, Erbitux, ImClone Systems, Branchburg, NJ, and Bristol-Myers-Squibb,

Table 2. CRYSTAL trial

Efficacy measure	**Intention-to-treat**		**KRAS wild-type**		**KRAS mutant**	
	***Cetuximab+ FOLFIRI*[1]**	***FOLFIRI*[1]**	***Cetuximab+ FOLFIRI*[1]**	***FOLFIRI*[1]**	***Cetuximab+ FOLFIRI*[1]**	***FOLFIRI*[1]**
N[2]	599	599	172	176	105	87
mPFS[3] (months)	8,9	8	9,9	8,7	7,6	8,1
	p: 0.048		p: 0,17		p: 0,47	
ORR[4] (%)	46,9	38,7	59,3	43,2	36,2	40,2
	p:0,004		p: 0,0025		p: 0,46	
HR[5]	0,85		0,68		1,07	

[1].FOLFIRI: Leucovorin/fluorouracil/irinotecan;[2].N: total number of patients in each arm; [3].mPFS: median progression-free survival [4].ORR: overall response rate; 5.HR: hazard ratio

Princeton, NJ) is a chimeric monoclonal antibody (IgG1) that recognises and binds specifically to the EGFR extracellular domain with higher affinity than endogenous ligands. As a result, cetuximab blocks the activation of the EGFR by preventing tyrosine kinase mediated phosphorylation and the subsequent signal transduction. Its mechanism of action impairs the cell cycle, promotes apoptosis, inhibits angiogenesis, tumour cell invasion and metastases and enhances the anti-tumoral effects of chemotherapy and radiotherapy. Moreover, cetuximab potentially induces an immunological response via antibody dependent cellular cytotoxicity (2).

4.1. The role of cetuximab in refractory advanced colorectal cancer.

The first clinical trials with cetuximab (Phase I clinical trials) showed the activity of the new agent in epithelial tumours, offered information about tolerance and established the dose to be used in following trials: 400 mg/m² as loading dose followed by 250 mg/ m² weekly (15).

Later, in 2001, Saltz (16) *et al* presented the first Phase II trial that showed the efficacy of cetuximab in patients with advanced colorectal cancer who had progressed to chemotherapy based on irinotecan. A total of 121 patients were included who received cetuximab and irinotecan, obtaining a response rate of 17% and 48% disease control. The toxicity attributed to cetuximab showed that 3% of patients developed allergy or anaphylactic reactions causing interruption of the treatment and 75% of patients developed a skin rash (12% Grade 3).

After these promising results, Saltz carried out another study to explore the activity of cetuximab as a single agent. Fifty-seven patients with advanced colorectal cancer, refractory to irinotecan treatment, received cetuximab in monotherapy. The results showed a response rate of 11% and 46% disease control (17).

However, it was the data of the BOND (18) clinical trial that conferred approval for cetuximab use in advanced colorectal cancer patients refractory to treatment with irinotecan. This study compared the efficacy of cetuximab plus irinotecan with cetuximab monotherapy. The 329 patients included were randomized to receive cetuximab in monotherapy (111 patients) or cetuximab plus irinotecan (218 patients). The results showed a higher response rate (22.9% vs 10,8%; p: 0,007), greater disease control (56% vs 32%) and longer time to progression (4,1 m vs 1,5 m p: 0,001) in the combined treatment arm respect to the treatment with cetuximab as single agent.

4.2. The role of cetuximab in first and second line treatment

Phase II clinical trials have evaluated the role of cetuximab in combination with standard chemotherapy (treatment schedules based on oxaliplatin or irinotecan), obtaining a response rate between 44-72% (19-21).

These results were confirmed by a Phase III randomized trial, CRYSTAL (22), which evaluated the efficacy of cetuximab plus chemotherapy based on irinotecan in first line advanced colorectal cancer treatment. A total of 1217 patients were randomized to treatment with FOLFIRI (Irinotecan 180 mg/m², 5-fluoracil 400mg/m² bolus, followed by 2400mg/m² in 46h continuous infusion plus leucovorin) and cetuximab (400mg/m² day 1, and, then, 250mg/m²/weekly) versus FOLFIRI every 2 weeks. The primary end-point was progression free survival (PFS).

The results showed a significant increase in (PFS) (8,9m vs 8,0 m HR: 0,85; p: 0,048), a higher response rate (46,9% vs 38,7%; p: 0,004) with an Odds Ratio of 1,40 (IC of 95%, 1,12 to 1,77; p= 0,004), an increase in the metastases surgery rate (7% versus 3,7%) and resection R0 (4,8% vs 1,7%) in the cetuximab + FOLFIRI treatment arm respect to FOLFIRI alone.

A retrospective analysis of this study evaluated the efficacy data according to KRAS status. Tumour samples from 540 patients were assessed, of which 348(64%) were wild-type KRAS and 192 (36%) showed mutated KRAS in the codons 12 or 13. The patient sample was analysed by intent to treat when the primary endpoint (PFS) was evaluated.

There was a significant statistical difference in favour of patients with wild-type KRAS who received cetuximab in contrast with those who did not receive it (HR: 0,68 (95% CI 0,50-0,94); p: 0,02) with a PFS of (9,9m vs 8,7m) respectively. However, significant differences in PFS were not found in patients with mutated KRAS. The Odds Ratio was 1,91 (95% CI 1,24-2,93) in patients with wild-type KRAS and 0,80 (95% CI 0,44-1,45) in patients with mutated KRAS (Table 2).

These data show the predictive value of the KRAS mutation for treatment with cetuximab plus FOLFIRI in first line chemotherapy in metastatic colorectal cancer.

The safety profile was similar in both treatment arms, irrespective of KRAS status.

Table 3. OPUS trial(19) Results

Efficacy measure	**Intention-to-treat**	**KRAS wild-type**	**KRAS mutant**			
	Cetuximab+ FOLFOX-4[1]	***FOLFOX-4[1]***	***Cetuximab+ FOLFOX-4[1]***	***FOLFOX-4[1]***	***Cetuximab+ FOLFOLX-4[1]***	***FOLFOX-4[1]***
N[2]	169	168	61	73	52	47
mPFS (months)[3]	7,2	7,2	7,7	7,2	5,5	8,6
	p: 0,617		p: 0,016		p: 0,019	
ORR [4](%)	46	36	60,7	37	32,7	48,9
	p:0,064		p: 0,011		p:0,106	
HR[5]	0,931		0,57		1,83	

[1]FOLFOX-4: Leucovorin/fluorouracil/oxaliplatin; [2]N: total number of patients in each arm ; [3]mPFS: median progression-free survival; [4] ORR: overall response rate;5. HR: hazard ratio

The incidence of Grade 3 or 4 adverse events was 79.3% in the cetuximab + FOLFIRI arm and 61% in the FOLFIRI alone arm (p<0,001). Moreover, a significant increase of cutaneous reactions was observed (9,7% versus 0,2% p<0,001), acniform rash (16,2% versus 0,0% p: 0,001), Grade 3/4 diarrhea (15,7% versus 10,5% p: 0,008) and infusion reactions (2,5% versus 0,0 p: <0,001) in the cetuximab treatment arm respect to the control arm.

The European randomized Phase II trial OPUS (19) randomly assigned 337 patients in first-line treatment with EGFR expression to receive FOLFOX-4 (oxaliplatin 85 mg/m², 5FU 400mg/m² bolus, followed by 2400mg/m² during 46 hours in continuous infusion, folinic acid 200mg/m2 Day 1 and 2 every 2 weeks) and cetuximab (400mg/m2 initial dose followed by 250mg/m2 weekly) versus FOLFOX 4. The primary endpoint was the response rate and the mutation status of KRAS was assessed. The primary analysis did not show a significant increase between both treatment arms. However, a high response rate was observed in those patients with good performance status (PS or ECOG 0-1).

KRAS status was analysed retrospectively in 233 patients of whom 134 patients (58%) were wild-type KRAS and 99 (42%) mutated KRAS. Patients with wild-type K-RAS presented an increase in progression free survival (7,7 versus 7,2; p: 0,016), significant increase in response rate (65% Odds Ratio 2,54; p: 0,011) and a decrease in progression risk (43% HR 0,57; p: 0,016) with cetuximab combined treatment opposed to FOLFOX alone. The resection rate R0 was higher (9,8%) in those patients who received cetuximab plus FOLFOX 4 compared to those who received FOLFOX alone (4,1%). In contrast, no differences in response rate were observed between the two treatment arms in patients with K-RAS mutated tumours (Odds Ratio 0,51, p: 0,11) with similar resection rates in both groups (1,9% versus 2,1%) (Table 3).

These results suggest that the benefit from the addition of cetuximab to standard chemotherapy treatments is restricted to patients with wild-type KRAS tumours.

Recently, at ASCO 2010 (unpublished data) Bokemeyer *et al* evaluated overall survival (OS), progression-free survival (PFS), and best overall response (OR) of the CRYSTAL and OPUS patients. Data confirms that the addition of cetuximab to chemotherapy first line in patients with *KRAS* wild-type tumors achieves a statistically significant improvement in OR rate, PFS, and OS compared with chemotherapy alone. The best outcome was observed in patients with *KRAS* wildtype/*BRAF* wild-type tumors (90% of *KRAS* wild-type patients).

COIN trial was presented at ASCO 2010(unpublished data). This study randomized continuous oxaliplatin (Ox) and fluoropyrimidine (Fp) chemotherapy (CT) and the same chemotherapy plus cetuximab (C) in first line treatment. The choice of Fp, capecitabine (Cap) or infusional 5-fluouroracil plus leucovorin (FU), was decided prior to randomization. The primary outcome was overall survival (OS) in KRAS wild-type patients. Further analysis of NRAS, BRAF, MSI and EGFR status has been performed. 1630 patients were randomized between (A) continuous OxFp (OxFU or OxCap and (B) OxFp + weekly C. Tumor samples from 1316 (81%) patients were available for KRAS analysis. 729 patients (55%) were KRAS wild-type. The results showed no evidence of a difference in either OS or progression-free survival (PFS) from addition of cetuximab to Oxaliplatine based chemotherapy (OS: HR = 1.04, 95% CI 0.87-1.23, p = 0.67; PFS: HR = 0.96, 95%CI 0.82-1.12, p = 0.60). A small difference in best overall response (CR/PR at any time on treatment) was observed (57% with OxFp, 64% with OxFp + C, p = 0.049). From pre-specified exploratory analyses of 15 potential predictive covariates, they observed a suggestion of an interaction between the choice of chemotherapy (OxFU versus. OxCap) and the effect of adding cetuximab on PFS (p=0.07). Data suggesting a benefit from cetuximab in OxFU treated patients, but no evidence of a benefit in OxCap treated patients. Although more research is needed ,this study shows that the addition of cetuximab to chemotherapy did not give any benefit in wild-type KRAS patients.

The Phase II trial EPIC(23) assessed cetuximab activity in second-line treatment. A total of 1298 patients were included who had progressed to oxaliplatin based treatment and they were randomized to two arms that included irinotecan combined or not with cetuximab. A significant increase in progression free survival was observed (4 m vs 2,6 m; p: 0,0001) and an increase in response rate (16,4 vs 4,2 % respectively p: 0,0001) in favour of the cetuximab combined treatment arm. Although the increase in overall survival was not statistically significant, this could be influenced by 150 patients randomized to irinotecan monotherapy receiving cetuximab on progression of the disease.

4.3. Cetuximab toxicity

The use of new antineoplastic agents has introduced new secondary effects unknown till now. The most frequent cetuximab adverse effects are the acniform rash, asthenia, general discomfort, infusion reactions and hypomagnesaemia.

Clinical observations when this drug was first used offered the interesting suggestion that the degree of cutaneous rash could be connected to response to the anti-EGFR monoclonal antibodies. With this in mind, the EVEREST (24) Phase II trial led by Teipar and co-authors aimed to determine the association between cetuximab, cutaneous rash and response to treatment. Patients with Grade 0/1 cutaneous rash after 22 days of treatment with cetuximab at standard doses combined with irinotecan were randomized to receive the standard dose of cetuximab (250mg/m2) and escalated dose of cetuximab (500mg/m2). Of the 86 patients analysed 62% were wild-type KRAS and 37.2% were mutated KRAS. As demonstrated in the previously mentioned studies, treatment with irinotecan and escalated dose cetuximab benefited wild-type KRAS patients. Moreover, they showed a higher objective response rate when compared to patients with mutated KRAS tumours. Mutated KRAS patients showed no improvement with standard dose or escalated dose, with a shorter PFS (2,8 m) than wild-type KRAS patients (5,8 m). These results reconfirm that patients with mutated KRAS do not derive any clinical benefit with the addition of cetuximab.

4.4. The role of cetuximab in the adjuvant setting of colorectal cancer

In recent years studies have been designed to assess the impact of the addition of cetuximab to standard chemotherapy treatments in an adjuvant setting. At ASCO 2010, data of a Phase III trial were presented in Abstrac form comparing modified FOLFOX-6 vs modified FOLFOX-6 plus cetuximab in wild-type KRAS patients with resected stage III colon cancer(unpublished data) A total of 1769 patients were included and the primary endpoint was progression free survival at 3 years with overall survival and toxicity as secondary endpoints. The study was closed early following an interim analysis (included in the study design) after 50% of expected events had been produced without demonstrating that the addition of cetuximab to the chemotherapy was beneficial. Indeed, the chemotherapy alone arm showed better results in progression free survival at 3 years (HR 1,18, IC 95% 0,92-1.52 p 0,33). No benefit from the addition of cetuximab was observed in the analysis of sub-groups. In the sub-group of patients over 70 years of age greater differences were observed in favour of the chemotherapy alone arm respect to progression free survival at 3 years and Grade 3/4 toxicities.

The European randomized Phase III study (PETACC-8) is at present aiming to assess the role of cetuximab in an adjuvant setting for wild-type K-Ras patients and results are awaited(unpublished data)

In ASCO 2010 another Abstract presented the results of the Phase III trial previously mentioned (modified FOLFOX-6 versus modified FOLFOX-6 plus cetuximab, with resected stage III colon cancer) for mutated KRAS patients without reporting benefit in the cetuximab arm or in the sub-groups analysis(unpublished data). Taking into account the results currently available and awaiting the results of on-going studies, the use of cetuximab in an adjuvant setting must be reserved to the clinical trial field.

4.5 The role of cetuximab in rectal cancer neoadjuvant setting

No Phase III trials are available at present that assess the association of cetuximab with pre-operative chemoradiotherapy in rectal cancer. There are, however, several Phase II trials. The study by McCollum, presented at ASCO 2010(unpublished data), selected patients with stage II and III rectal cancer (according to AJCC staging) who were randomized to two arms: pelvic radiotherapy (4,500 cGy) plus continuous infusion of 5-FU (225 mg/m^2/day) during radiotherapy versus radiotherapy plus 5-FU with the same schedule as the first arm with the addition of cetuximab (400 mg/m^2 the first week and afterwards 250 mg/m^2). Patients underwent radical surgery 6-8 weeks after terminating treatment. The primary endpoint was the pathological response rate. The pathological response rate was similar in the cetuximab arm and the chemoradiotherapy alone arm and matched results in other Phase II trials. Results in function of mutated KRAS status are awaited.

In ASCO 2010, Kim *et al.* presented the results of the analysis of two Phase II trials (IRIX: irinotecan plus capecitabine and ERBIRIX: irinotecan plus capecitabine plus cetuximab) correlating the results of neoadjuvant treatment with cetuximab plus chemoradiotherapy in rectal cancer in function of K-Ras, N-raf and P13KCA status(unpublished data) . It was concluded that in wild-type KRAS patients the addition of cetuximab to chemoradiotherapy based on irinotecan plus capecitabine does not improve results (disease free survival) compared with chemoradiotherapy alone.

At present, therefore, the use of cetuximab in rectal cancer adjuvant treatment in association with chemoradiotherapy must be reserved to clinical trials and should not be considered part of normal clinical practice.

5.PANITUMUMAB

Panitumumab (ABX-EGF, Vectibix, Amgem, Thousand, Oaks, CA) is a fully human anti-EGFR monoclonal antibody with high affinity to the EGFR, blocking ligand binding and preventing receptor internalization, but it does not induce antibody-dependent cellular cytotoxicity.

Like cetuximab, this new therapeutic agent has demonstrated antitumoral activity in advanced colorectal cancer in pre-clinical models.

5.1. The role of panitumumab in refractory advanced colorectal cancer

Following the Phase I trials a Phase II trial was carried out on 148 patients with advanced colorectal cancer refractory to standard chemotherapy, obtaining 29% stable disease and 9% partial responses(25).

Table 4. Best support care with/Without Panitumumab(26)

Efficacy measure	Intention-to-treat		KRAS wild-type		KRAS mutant	
	BSC [1]	***BSC*** [1] **+ *PAN*** [6]	***BSC*** [1]	***BSC*** [1] **+ *PAN*** [6]	***BSC*** [1]	***BSC*** [1] **+ *PAN*** [6]
N[2]	232	231	119	124	100	84
mPFS (months)[3]	7,3	8	7,3	12,3	7,3	7,4
	HR[4]: 0,54 (p<0,0001)		HR[4] : 0.00 (p<0,0001)		HR [4] : 0,99	
mOS[7] (months)	HR: 1,00 (95 CI 0,88-1,22); p: 0,81		7,6	8,1	4,4	4,9
			NR[5]		NR[5]	

[1]BSC: best supportive care; [2]N: total number of patients in each arm ; [3].PFS: progression-free survival. [4].HR: hazard ratio; [5]NR: not reported; [6]PAN: panitumumab; [7] OS: overall survival

A pivotal Phase III trial (26) was then carried out, which compared panitumumab associated with the best supportive treatment (BSC) versus BSC alone, in advanced colorectal cancer patients who had progressed to standard chemotherapy. A total of 463 patients were randomized to receive panitumumab (6mg /m² every 2 weeks) + BSC (N: 231) versus BSC alone (N: 232). Patients who progressed could receive panitumumab. There was a significant increase in mean progression free survival (mPFS) (13,8w vs 8,5w) and response rate (10% versus 0,0% p: <0,001) in the panitumumab treatment arm (Table 4)

Amado *et al* (27) studied the effect of panitumumab on progression free survival according to KRAS status in 427 patients (208 patients in the panitumumab arm and 219 in the best supportive treatment arm), observing mutated KRAS in 43% of patients.

In the panitumumab treatment group responses were observed only in wild-type KRAS patients (p<0,0001). On analysing only patients from the panitumumab group together with patients who received panitumumab after progression, greater overall survival was observed in wild-type KRAS tumours respect to mutated KRAS tumours (HR: 0,67 95% CI 0,55-0,82).

On the basis of these results both the FDA and the EMEA approved the use of panitumumab for the treatment of advanced colorectal cancer refractory to other treatments.

Another study by Hecht and co-authors investigated the interaction of KRAS status and the efficacy of panitumumab in advanced colorectal cancer patients refractory to standard treatment (28). The patients received panitumumab (6 mg/kg every 2 weeks) until disease progressed or they developed unacceptable toxicity. A total of 171 patients were evaluated: 55% had wild-type K-RAS and 45% had mutated KRAS. Once again the results agreed with previous published studies with a median PFS (15m vs 7m), a response rate (12% versus 0,0%) and median survival (54 months versus 29 months) superior in patients with wild-type KRAS.

The toxicity profile with panitumumab is similar to cetuximab with skin reactions, gastrointestinal toxicity and hypomagnesaemia. However, infusion reactions are rare.

The results of all these studies stress the need for patients with colorectal cancer to be assessed for KRAS mutation prior to starting anti-EGFR treatment.

Panitumumab is being evaluated for use in first and second-line treatment in combination with standard chemotherapy regimens. The preliminary results have been presented and have shown an improvement in PFS in patients with wild type KRAS.

5.2. The Role of Panitumumab in first line treatment

The use of panitumumab in first-line treatment of metastatic colorectal cancer is being evaluated in combination with standard chemotherapy regimen. At ASCO 2010, data of a Phase III trial were presented in Abstract form comparing FOLFOX versus FOLFOX + panitumumab(unpublished data). Were randomized 1183 patients: 593 in arm "Panitumumab + FOLFOX" and 590 in arm "FOLFOX" . 1183 patients (93%) had KRAS results: 656 (60%) wild-type (WT), 440 (40%) mutated (MT). Results showed that the addition of Panitumumab significantly improved progression free survival (PFS) (HR = 0.80; 95% CI: 0.66-0.97; p = 0.02; median 9.6 vs. 8.0 mo) in patients with wild-type *KRAS.*

The authors concluyed that 97% of patients with wild-type *KRAS* and 95% with mutated *KRAS* tumor status receiving panitumumab plus FOLFOX4 as first-line treatment for metastatic colorectal cancer significantly improved Progression Free Survival in patients with Wild-type *KRAS* and was well tolerated.

Other Phase III clinical trial was presented as abstract form at ASCO 2010 by Hofheinz for to evaluate the resections and curative surgery(unpublished data). This study compared panitumumab (6 mg/kg) and FOLFIRI every 2 weeks. The primary endpoint was objective response rate and secondary endpoints included progression-free survival and safety. A total of 154 patients were enrolled. *KRAS* evaluable samples were available for 94% (n = 145) of patients: 86 (59%) patients had *KRAS* wildtype tumors and 59 (41%) patients had *KRAS* mutated tumors. Response rate in the *KRAS* wildtype group was 56% versus 38% in the mutated group and median progression-free survival was 8.9 months versus 7.2 months. Resection rate was 15% (95% CI 8.3%, 24.5%) and 7% (95% CI 1.9%, 16.5%), and the majority of patients that underwent surgery had liver only metastases 12 (92%) and 2 (50%), in the *KRAS* wildtype and mutated groups respectively. Complete removal was achieved in 8% (95% CI 3.3%, 16.1%) of patients in the *KRAS* WT group and 5% (95% CI 1.1%, 14.2%) in the *KRAS* MT group.

The results of "PRIME" trial were presented at ASCO 2010. This study compared FOLFOX-4 plus panitumumab to FOLFOX alone as first line treatment for

Table 5. PACCE trial(30)

Efficacy measure	**Oxaliplatin based chemotherapy + Bevacizumab**	**Oxaliplatin based chemotherapy + Bevacizumab + Panitumumab**
ORR (%)[1]	46	45
PFS (Months)[2]	11,1	9,6
HR (95%CI)[3]	1,27 (1,05-1,53)	
Efficacy measure	**Irinotecan based chemotherapy + bevacizumab**	**Irinotecan based chemotherapy + bevacizumab + Panitumumab**
ORR(%)[1]	39	43
PFS (months)[2]	11,7	10,1
HR (95% CI)3	1,21 (0,80-1.82)	

1.ORR: overall response rate; 2.PFS: progression-free survival; 3. HR: Hazard ratio

metastatic colorectal cancer(unpublished data). The primary endpoint was progression-free survival (PFS); 1,183 pattients were randomized. 1,096 pts (93%) had *KRAS* results. For wildtype *KRAS*, median Progression Free Survival was 9.6 months for Arm 1 and 8.0 months for Arm 2; median Overall Survival was 23.9 months for Arm 1 and 19.7 months for Arm 2. 813 (69%) patients had EGFR results. Of 479 wildtype *KRAS* patients with EGFR results, 326 (68%) were EGFR+. The authors concluded that panitumumab added to FOLFOX for first-line metastatic colorectal cancer treatment significantly improves progression free survival and is well tolerated in patients with wildtype *KRAS*.

6. DUAL ANTIBODY THERAPY

In the initial phases, preclinical models showed a synergistic antitumour effect by blocking both the EGFR and VEGF. The Phase II BOND–2 (29) trial was the first to analyse this double inhibition by comparing the concurrent administration of cetuximab and bevacizumab with or without irinotecan. The study evaluated a total of 83 patients who had progressed to chemotherapy but were monoclonal antibody naive.

A higher response rate (37% versus 20%), an increased median time to progression (7,3 m vs 4,9 m) and better overall survival (14,5m vs 11,4 m) were observed with the three drug combination.

The Phase III trial PACCE (30) studied the role of panitumumab in combination with standard chemotherapy and bevacizumab in first-line advanced colorectal cancer treatment with two treatment groups.

The first cohort (n: 800) received treatment based on oxaliplatin (FOLFOX) plus bevacizumab with or without panitumumab. The second cohort (n: 200) received treatment based on irinotecan (FOLFIRI) plus bevacizumab with or without panitumumab.

In the first cohort , the median progression free survival and overall response rate were 9,6m y 45% respectively in the bevacizumab alone arm. Toxicity was increased in the panitumumab arm (60% vs 38%) respect to the control arm. In view of these results the addition of panitumumab to FOLFOX in combination with bevacizumab adversely affects progression free survival and increases toxicity.

In the second cohort, median progression free survival and response rate were 10,1 m and 43% respectively in the panitumumab arm and 11,7 m and 39% in the bevacizumab alone arm. The FOLFIRI + bevacizumab/panitumumab combination worsened toxicity considerablyy; 37% of patients developed cutaneous toxicity.

Retrospective analysis of tumour samples showed that 55% of patients were mutant K-Ras (57 samples in 103 patients) in the panitumumab treatment arm and 60% (59 of 97 patients) were wild-type K-Ras in the control arm. The response rate was higher in the panitumumab arm compared to the control arm respect to wild-type K-Ras patients (54% vs 47%) (Table 5)

CAIRO 2 (31), another Phase III trial, studied the efficacy of dual therapy (anti-EGFR and anti-VEGF) in colorectal cancer treatment. A total of 736 patients were randomized to two groups. One arm received capecitabine (1000mg/m²/12 hours from day 1 to day 14 every 3 weeks plus oxaliplatin (130 mg/m2 day 1) plus bevacizumab (7,5 mg/Kg day 1 every 3 weeks) and the other arm received capecitabine (same dose) plus oxaliplatin (same dose) plus bevacizumab (same dose) plus cetuximab (250mg/m2 weekly after an initial dose of 400 mg/m2). Oxaliplatin was suspended after 6 treatment cycles, at the same time modifying the capecitabine dose to 1250mg/m² d1-d14 every 3 weeks. The primary endpoint was progression free survival, which was significantly less in the cetuximab arm (9,4m) compared to the capecitabine plus oxaliplatin plus bevacizumab (10,7m) with a HR of 1,21 (p: 0,01). Neither overall survival (20,3 vs 19,4m p: 0,16) nor response rate (50% vs 52,7% p: 0,49).were affected by the addition of cetuximab

Significantly higher Grade 3/4 toxicity was observed in the treatment arm with cetuximab (72% vs 82% p: 0,0013).

The subgroups were analysed to assess KRAS status. Of the 420 patients evaluated 314 (60.3%) were wild-type KRAS and 206 (39.6%) were mutated KRAS. As in other studies, wild-type KRAS patients showed no differences in response rates between the two treatment arms. However, patients with mutated KRAS who received cetuximab presented shorter progression free survival (8,1m) than those who did not receive cetuximab (12,5m; p:0,003). There were no differences in overall survival (Table 6).

In short, CAIRO 2 did not observe any benefit in the addition of cetuximab to the oxaliplatin-capecitabine plus bevacizumab treatment schedule. These results are

Table 6. CAIRO 2 trial(31)

Efficacy measure	All Patients		KRAS wild-type		KRAS mutant	
	Capecitabine+ Oxaliplatine+ Bevacizumab	***Capecitabine+ Oxaliplatine+ Bevacizumab+Cetuximab***	***Capecitabine+O xaliplatine+ Bevacizumab***	***Capecitabine+ Oxaliplatine+ Bevacizumab+C etuximab***	***Capecitabine+Oxaliplatine+ Bevacizumab***	***Capecitabine+ Oxaliplatine+ Bevacizumab+Cetuximab***
N[1]	368	368	156	158	108	98
mPFS (months)[2]	10,7	9,4	10,6	10,5	12,5	8,1
	p:0,01		p:0,30		p:0,003	
mOS (months)[3]	20,3	19,4	22,4	21,8	24,9	17,2
	p:0,16		p:0,69		p:0,03	

[1].N ; total number of patients in each arm ; [2]PFS: Progression-free survival. [3]OS: overal survival

consistent with those of the PACCE clinical trial and, therefore, dual anti-EGFR y anti-VEGF therapy is not justified in normal clinical practice of colorectal cancer treatment.

7. PERSPECTIVES

In the last decade, cancer treatment has experienced a dramatic revolution due to the developement of new specific target-oriented drugs. These new agents have been investigated in the context of an individualized medicine with the aim to develop effective treatments with the least possible toxicity. Thus the use of chemotherapy combined with agents against specific molecular targets has improved previous therapeutical results. In colorectal cancer, new drugs anti-EGFR, cetuximab and panitumumab, have demostrated better results in patients with wild-type KRAS tumors, as it has been mentioned previously. KRAS mutation status has become an important predictor of response to both agents and, nowadays, it is an useful tool in our daily clinical practice. However, in addition to the KRAS gene mutations, there are other alternative mechanisms of activation of signaling pathways. They could explain the lack of effectiveness of these new agents in patients with advanced colorectal cancer with wild-type KRAS. For this reason, different research lines have been prometed in order to establish other molecular signaling cascades such as Ras-Raf-MAPK and PI3K/AKT pathways.

Activating mutations of BRAF serine/threonine-protein kinase were first described in melanomas and have been subsequently detected in colorectal cancers. These mutations, which are exclusive with respect to the KRAS mutations, have a deep impact on tumour biology. Retrospective studies have shown that 10-15% of patients whose tumors are KRAS wildtype, have also BRAF mutation (31, 32). These patients with BRAF mutation seems to be resistant to anti-EGFR monoclonal antibodies therapy. Recently, at the American Society of Clinical Oncology (ASCO 2010), Van Cutsem and collegues showed the results of BRAF mutations in metastatic colorectal cancer of the CRYSTAL trial. In this study, patients were randomized to recive Cetuximab plus FOLFIRI or FOLFIRI alone. Their data confirms that KRAS still being a predictive factor of response in patients treated with cetuximab. These authors have also state that BRAF may be a marker of poor prognosis and it does not seem to be a predictive biomarker for the addition of cetuximab to FOLFIRI(unpublished data). Future investigations are needed to clarify these results.

PI3K-AKT appears to have a role in the pathogenesis of colorectal cancers. Phosphatidylinositol 3-Kinase (PI3K) is a major component of the PI3K-AKT pathway. Mutated PI3K may promote growth and invasion of cancer cells (33). PIK3 is activated by recruitment to the cell surface by activated receptor tyrosine kinases, as well as by binding to activated RAS. Between 8-10% of colorectal tumors have mutations in the tyrosine kinase activating PIK3. These mutations appear to be associated with PTEN (methylation of phosphatase homologue to tensin). PTEN is a tumor suppressor protein that regulates the PI3K/AKT signal transduction. Its loss is associated with an intrinsic activation of the AKT pathway and is known to confer resistance to inhibitors of the EGFR *in vitro* (34) and *in vivo* (35-37).

At this moment, other new drugs are currently being developed as zalutumumab (Genmab), BMI (ImClone) or nimotuzumab (YM Biosciencies). Small molecules tyrosine kinase inhibitors have been tested with poor results.

Additional reliable and prospective randomized trials are needed to identify new pronostic markers and other predictive factors different than KRAS in order to optimize the response to anti-EGFR target therapy in colorectal cancer.

8. ACKNOWLEDGEMENT

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Abbreviations: EGFR: epidermal growth factor receptor; VEGF: vascular endolethial growth factor; EGF: epidermal growth factor; TGFα; transforming growth factor alpha; GDP: guanosine diphosphate; GTP: guanosine

triphosphate; Ox: oxaliplatin (Ox); Fp:fluoropyrimidine ; CT: chemotherapy; C: cetuximab; Cap: capecitabine; 5FU: infusional 5-fluouroracil plus leucovorin;

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