EUROPEAN JOURNAL OF CANCER 44 (2008) 652–662

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Review

Where to position monoclonal antibodies in first-line treatment of advanced colorectal cancer

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ARTICLE INFO

Article history:
Received 23 January 2008
Accepted 25 January 2008
Available online 3 March 2008

Keywords:
Colorectal cancer
VEGF
EGFR
Antibody therapy
Targeted agents

ABSTRACT

The treatment of metastatic colorectal cancer with modern cytotoxic agents in combination with monoclonal antibodies against vascular endothelial growth factor (VEGF) and the epidermal growth factor receptor (EGFR) has improved median overall survival from 6 months to almost 2 years. Uncertainty remains over the optimal chemotherapy combination and sequencing, and to which line of treatment monoclonal antibodies should be added. This article reviews the rationale and evidence for the use of monoclonal antibodies in the first-line treatment of metastatic colorectal cancer in both general and specific situations, and provides a perspective on how to position their use in contemporary practice.

1. Introduction

In Europe, colorectal cancer is the second most commonly diagnosed cancer with over 412,000 patients newly diagnosed with the disease and over 207,000 deaths in 2006 alone.[1] Although the incidence over the last 30 years has remained relatively static, the mortality has declined,[2] attributable to advances in awareness, early detection and screening, as well as improvements in surgical technique and post-surgical therapy.[3] Despite these improvements, 30–40% of patients undergoing curative resection develop distant relapse and a further 20% of patients have metastases at the time of presentation.[4,5] Whilst some patients with advanced disease can be cured by surgical metastasectomy,[6] chemotherapy remains the principle treatment modality for most, with the goals of symptom palliation and prolongation of life rather than cure.

2. Primary chemotherapy compared to best supportive care alone

With supportive care alone, median survival from metastatic colorectal cancer is approximately 6 months.[7] The use of multiple cytotoxic agents – 5-fluorouracil, irinotecan and oxaliplatin – either sequentially or in combination has been shown to prolong progression-free and overall survival to 20–24 months.[8–13] The greatest improvements in overall survival times have been seen in treatment programmes where there are high rates of exposure to all three active cytotoxics,[14] and an approach of primary chemotherapy at the time of diagnosis of metastatic disease rather than expectancy has been shown to prolong median survival and time without symptoms.[15] When patients are otherwise fit, chemotherapy for metastatic colorectal cancer is firmly established as standard practice.

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2008.01.021

3. The biology of angiogenesis

At an early stage of growth, a tumour colony relies on simple diffusion of oxygen, nutrients and waste products to meet its metabolic demands. Once it exceeds a critical size (thought to be approximately 1 mm^3), an independent blood supply is required for ongoing cellular replication.[16] At this critical point, tissue oxygen tension is reduced which induces growth factors such as hypoxia inducible factor-1 (HIF-1) leading to the upregulation of genes involved in angiogenesis, including vascular endothelial growth factor (VEGF).[17] Other mediators of increased VEGF expression include TGFα and β, the epidermal growth factor (EGF), IL-1, FGF and PGE_2. VEGF is a potent mitogen for endothelial cells of arteries, veins and lymphatics, resulting in neoangiogenesis and increased vascular density. It also results in endothelial fenestrations causing vascular permeability, resulting in leakage of plasma proteins into the interstitium providing a rich growth media for endothelial and tumour cells.[18]

As well as inducing neoangiogenesis, high circulating levels of VEGF correlate with invasion, recurrence, metastasis, microvascular density and poor prognosis in colorectal cancer,[19–21] making it a rational target for anti-cancer therapy. A murine IgG1 antibody directed against VEGF was shown to inhibit neoangiogenesis and normalise aberrant tumour vasculature resulting in an increased efficiency of cytotoxic drug delivery to tumour cells.[22] The antibody was able to potently inhibit the growth of a variety of tumour cell lines and tumours in nude mice, and these experiments led to the development of a humanised version of this antibody – bevacizumab – which was then taken to clinical trials.[23]

4. Addition of anti-VEGF agents to chemotherapy

In the first study to utilise bevacizumab in patients with colorectal cancer, 104 previously untreated patients with metastatic disease were randomised to receive 5-FU and leucovorin (LV) alone or in combination with one of two doses of bevacizumab. In this small phase 2 study, patients who received bevacizumab at 5 mg/kg every two weeks in combination with chemotherapy experienced a higher objective tumour response rate (40% versus 17%), and longer progression-free survival (9.0 versus 5.2 months, hazard ratio (HR) = 0.46, 95% confidence interval (CI) 5.8–10.9, $p = 0.005$) than those who received chemotherapy alone. Overall survival was prolonged, but not significantly so (median overall survival 21.5 versus 13.8 months, $p = 0.137$). The response rates and median survival were slightly lower in the group that received a higher dose of bevacizumab, and so the 5 mg/kg/dose was selected to go forward for future studies. There was a significant increase in the incidence of grade 3/4 toxicities in the group receiving bevacizumab, including thrombosis, epistaxis, hypertension, and proteinuria.[24] (Table 1). This study demonstrated that the addition of bevacizumab to chemotherapy could enhance response rates and prolong survival in combination with chemotherapy, and thus led to testing in phase 3 trials. A second trial of 209 patients not considered 'ideal' candidates for irinotecan (age >65, PS > 0, albumin <35 g/l, or prior abdominal/pelvic radiotherapy) randomised participants to receive 5FU-LV with either bevacizumab or placebo. The addition of the anti-VEGF antibody did not significantly prolong overall survival (16.6 months versus 12.9 months, HR = 0.79; $p = 0.16$) but progression-free survival was extended (9.2 versus 5.5 months, HR = 0.50; $p = 0.0002$).[25] A combined analysis of the raw data from these two trials with 249 patients receiving 5-FU/LV/Bev and 241 receiving either IFL or 5-FU/LV alone showed a reduction in the hazard ratio for death with the addition of bevacizumab (HR 0.74, $p = 0.008$), as well as improvements in progression-free survival and response rate.[26]

5. Irinotecan–bevacizumab combinations

In a landmark phase 3 trial, 813 patients with previously untreated metastatic colorectal cancer were randomised to receive bolus irinotecan, 5-FU and LV (IFL) with either bevacizumab 5 mg/kg every 2 weeks or placebo.[27] A third arm of 5-FU/LV plus placebo was closed at a protocol-specified analysis by an independent data monitoring committee, as there was no additional toxicity with IFL-bev compared to FU/LV-bev treatment and IFL was superior to FU/LV alone in an earlier trial.[28]

Improvement in overall survival was the primary endpoint, and was met, with a median overall survival of 20.3 versus 15.6 months favouring the investigational arm (HR for death, 0.66, $p < 0.001$). There was a significant increase in

Table 1 – Grade 3/4 toxicities of interest with chemotherapy-bevacizumab from selected studies (%)

Study	AVF2107[27]	Kabbinivar[26] (Combined analysis)	E3200[62]	NO16966[33]	BEAT[35]
Chemotherapy backbone + Bev	IFL	FU/LV	FOLFOX	FOLFOX/CAPOX	Any
N (Bev-arm)	393	244	287	694	1789
Hypertension	11.0	16	6.2	4	0.4
Bleeding	3.1	5	3.4	2	1.3
Perforation	1.5	1	1.0	<1	1.2
DVT/PE/VTE	12.5	10	3.4	8[b]	2.6
Arterial thrombotic event	NR	5	0.9[a]	2	0.7

NR = not reported as a separate group.
DVT/ PE/ VTE: composite of deep venous thrombosis, pulmonary embolus and venous thromboembolism.
a Coded as cardiac or cerebrovascular ischaemia.
b Estimated from graphs.

the rate of grade 3/4 adverse events, particularly hypertension, bowel perforation, leucopaenia and diarrhoea. 50% of patients in each of the arms went on to receive second-line therapy with 25% in each arm receiving subsequent oxaliplatin.

Patients in the bevacizumab-containing arms were permitted to receive greater treatment exposure to this agent than has been seen in many other studies: patients with treatment limiting chemotherapy-related toxicity were allowed to continue on bevacizumab alone, and patients were permitted to receive bevacizumab beyond tumour progression in combination with second-line chemotherapy. Additionally, patients who had not experienced progression at 96 weeks were permitted to continue bevacizumab in a separate extension study. Patients randomised to receive placebo were not allocated to receive cross-over bevacizumab at the time of progression. Similar to the NO16966 study (discussed below), the greater exposure to bevacizumab may have accentuated the positive findings of the study, but the contribution of bevacizumab beyond disease progression has not been evaluated in prospective studies.

As infused 5-FU regimens have been shown to be superior to bolus administration,[29] researchers have attempted to assess whether the schedule of administration of irinotecan and 5-FU has a significant impact on outcome. The BICC-C study had a 3×2 factorial design and aimed to compare 3 regimens of irinotecan–fluorouracil combinations with or without COX-2 inhibition. Four hundred and thirty patients were randomly assigned to one of three arms: FOLFIRI, capecitabine with irinotecan (CapeIRI) or a modified IFL regimen (2 weeks of 3 compared to 4 weeks of 6). After the results of the Hurwitz trial were published, the design was modified to include bevacizumab. A disproportionately high incidence of grade 3/4 diarrhoea was seen in the CapeIRI arm (47.5% of all patients), probably because the dose of capecitabine was too high (2000 mg/m^2/day), so this arm was discontinued. One hundred and seventeen additional patients were randomised to receive FOLFIRI-bevacizumab or mIFL-bevacizumab.

Of the regimens that did not include bevacizumab, FOLFIRI was associated with a significantly longer PFS compared to either mIFL or CapeIRI (7.6 months, 5.9 and 5.8 months, respectively, HR 1.51; 95%CI 1.16–1.97, $p = 0.004$ for the FOLFIRI-mIFL comparison). The overall survival was also longest in the FOLFIRI arm but not significantly so.[30] (Table 2).

The progression-free survival with FOLFIRI-bevacizumab exceeded that of mIFL-bevacizumab (11.2 versus 8.3 months) but this difference did not reach statistical significance. The median overall survival in the FOLFIRI-bev arm had not been reached with a median follow-up of 22.6 months. Importantly, there were no significant differences in the rates of post-study second-line chemotherapy.

The view that IFL is not the optimum regimen of 5-FU and irinotecan was reinforced in this study. The 60-day death rates were 3.6% for FOLFIRI and 5.1% for mIFL, and the rates of diarrhoea, dehydration and febrile neutropaenia were higher in the mIFL arm. Nausea and vomiting were modestly higher in those receiving FOLFIRI. Somewhat incongruously, the rates of grade 3/4 toxicity were higher for FOLFIRI-bev than for mIFL-bev in all domains except diarrhoea. However, numbers were too small to be reliably assessed for statistical validity. The study closed early to recruitment after enrolment slowed when the cardiovascular safety concerns of celecoxib arose, despite this aspect of the study being discontinued.

This study demonstrated superiority of FOLFIRI over mIFL and CapeIRI, but did not clearly establish superiority of one bevacizumab containing regimen over another.

6. Oxaliplatin–bevacizumab combinations

Results from other trials demonstrated that treatment with FOLFOX4 resulted in higher response rates, median and overall survival than with IFL regimens in the first-line setting, with lower rates of severe nausea, vomiting, diarrhoea, febrile neutropaenia and dehydration.[31,32] These findings have led many to argue that FOLFOX should be considered the reference regimen for the first-line treatment of advanced colorectal cancer.

The TREE-1 study (Three Regimens of Eloxatin Evaluation) was designed to compare three oxaliplatin (Eloxatin)-based regimens: mFOLFOX6, bFOL and CapeOx. When the Hurwitz study data became available, a second cohort to receive bevacizumab was recruited (TREE-2). The primary end-point was toxicity, with efficacy being a secondary measure. Two hundred and twenty-three patients were recruited to the second part of the study. Although TREE-1 and TREE-2 are sequential cohorts and therefore comparisons between the groups are indirect, the addition of bevacizumab to either mFOLFOX-6 or CapeOx appeared to increase response rates by 10–13% and prolonged median overall survival from 17–19 months to 26–27 months. Neither hazard ratios nor p values were calculated for this limited comparison, nor was there control for the effect of subsequent chemotherapy.

Table 2 – Efficacy results from BICC-C study[30]

Regimen	n	Response rate (%)	PFS	OS	% with post-study chemotherapy
FOLFIRI	144	47.2	7.6[a]	23.1[b]	77
mIFL	141	43.3	5.9[a]	17.6	75
CapeIRI	145	38.6	5.8	18.9	77
FOLFIRI-Bev	57	57.9	11.2[b]	Not reached	68
mIFL-Bev	60	53.3	8.3	19.2	68

PFS, progression-free survival (months); OS, overall survival (months); mIFL, modified irinotecan 5-fluorouracil, leucovorin; Bev, bevacizumab.
a $p = 0.004$ for this comparison.
b p not significant.

There was a higher incidence of hypertension, bleeding, bowel perforation, impaired wound healing and on-treatment deaths with the addition of bevacizumab, but these did not reach statistical significance given the small number of events that occurred.

An international Roche sponsored study (NO16966/XELOX-1) initially set out to compare the efficacy of CapeOx to FOLFOX in the first-line treatment of metastatic colorectal cancer, but as with the TREE study, the design was amended to a 2×2 factorial design after efficacy data on bevacizumab became available. The trial aimed to establish non-inferiority of CapeOx +/– bevacizumab compared to FOLFOX +/– bevacizumab for progression-free survival, and for superiority of chemotherapy with bevacizumab compared to placebo for PFS. In the most recent analysis of 1400 patients, CapeOx was shown to be non-inferior to FOLFOX, and bevacizumab with chemotherapy was shown to be superior for PFS (median PFS from 8.0 to 9.4 months, HR 0.83, 97.5% CI 0.72–0.95, $p = 0.0023$).[33] No significant difference was seen for the secondary endpoint of overall survival.

The protocol specified that patients who suffered treatment-related toxicity should continue to receive agents not associated with toxicity; for example, patients with oxaliplatin-related neurotoxicity should continue to receive bevacizumab and 5-FU/capecitabine. In practice, many patients who experienced toxicity had all agents discontinued; only 38% of patients receiving bevacizumab and 56% of patients receiving placebo discontinued treatment due to disease progression. In a pre-specified analysis, the PFS in those receiving treatment as per protocol ('on-treatment PFS') was compared to the whole population. The PFS benefit seen in the intent-to-treat analysis was accentuated, with a median PFS of 10.4 versus 7.9 months (HR 0.63, 95% CI 0.52–0.75; $p < 0.0001$). The investigators concluded that it may be necessary to continue bevacizumab until disease progression to optimise the effect of bevacizumab on PFS; an observation that would support the findings of the Hurwitz trial.

Patients in the bevacizumab containing arm were more likely to discontinue treatment for an adverse event (31% versus 21%), including neurotoxicity, GI events, general disorders and haematological events. Of specific grade 3/4 events of interest, there was a less than 1% incidence of perforation, 4% versus 1% hypertension and 4% versus 2% thromboembolic and bleeding events in the bevacizumab and placebo arms, respectively. Most treatment discontinuations were attributed to chemotherapy rather than bevacizumab-related events.

In contrast to results seen in most prior studies, the addition of bevacizumab did not improve objective response rates despite a prolongation of PFS. No specific explanation for this observation was offered; however, it may be that cytostasis is a more important mechanism of action than cytotoxicity with targeted agents.

The phase 3 E3200 study demonstrated the efficacy of bevacizumab in combination with FOLFOX chemotherapy in the second-line setting. Eight hundred and twenty-nine patients with metastatic colorectal cancer who had previously received treatment with a fluoropyrimidine and irinotecan were randomised to one of three arms: FOLFOX 4 alone, FOLFOX 4 with bevacizumab (10 mg/kg/dose), or bevacizumab alone. The bevacizumab-monotherapy arm was closed early by a data monitoring committee for inferior efficacy (response rate, 3.3%; median PFS, 2.7 months). Median survival was prolonged with the addition of bevacizumab to FOLFOX (12.9 months versus 10.8 with FOLFOX alone, HR 0.75, $p = 0.0011$). There was an increase in the incidence of neuropathy, hypertension, bleeding and vomiting with the addition of bevacizumab to chemotherapy, but this did not result in an increase in treatment-related mortality. This study demonstrated a survival benefit with the addition of bevacizumab to chemotherapy in the second-line setting, and demonstrated that bevacizumab monotherapy is relatively inactive.

7. Expanded access programmes

Further data on the safety and efficacy of bevacizumab in combination with chemotherapy is available from the BriTE registry study. In this observational cohort study, patients were treated with investigator-selected chemotherapy in combination with chemotherapy. Because it was a community study, patients enroled were on average older and of worse Eastern Cooperative Oncology Group – (ECOG) performance status than in the pivotal Hurwitz trial. Most patients (55.9%) received FOLFOX, but other regimens frequently chosen were FOLFIRI (14.3%), IFL (9.7%), FU/LV (6.8%) and XELOX (2.4%). The median survival for all patients in this study was 25.1 months – one of the highest seen in any report of patients with metastatic colorectal cancer.[34]

Additionally, the median survival of patients who received bevacizumab beyond progression (i.e. into second and subsequent lines of therapy) compared to patients who received chemotherapy alone or with EGFR inhibitors was 31.8 months compared to 19.9 months. This striking hypothesis-generating observation would require testing in a randomised study before it could be considered a standard strategy, but reinforces the observation of prolongation of survival with increased exposure to bevacizumab, as seen in the NO16966 study.

Further safety data are available from the BEAT study (bevacizumab expanded access trial). In this study of 1927 patients in 41 countries, bevacizumab was the combined investigator-selected chemotherapy. The most commonly used regimens were FOLFOX, FOLFIRI and CapeOx. There were 23 episodes of serious bleeding, and 21 incidents of GI perforation. Perforation occurred not only in patients who had their primary tumour in situ, but also in those with previously resected tumours. There were 13 episodes of severe arterial thromboembolic events, 2 of which were fatal. This trial demonstrated a low incidence of severe or serious toxicity with the addition of bevacizumab to a variety of chemotherapy regimens.[35]

8. Rationale for targeting the EGFR pathway

The epidermal growth factor receptor (EGFR) is a transmembrane protein with an intracellular tyrosine kinase domain whose activation mediates cellular replication in response to growth factor stimulation and its inhibition leads to cytostasis or apoptosis in malignant cell lines.[29,36,37] It is over-expressed in up to 70% of human colorectal cancers[38,39], with such over-expression correlated with an adverse clinical

course.[40] Cetuximab is a chimeric IgG1 monoclonal antibody that binds to the extracellular domain of the EGFR with higher affinity than its endogenous ligand, preventing dimerisation of the receptor and downstream activation of the signalling pathway. Panitumumab is a fully human IgG2 antibody against the EGFR that lacks the immunogenicity caused by the murine component of cetuximab, but does not induce antibody dependant cell-mediated cytoxicity. Laboratory work in nude mice demonstrated that the administration of C225 (Cetuximab) in combination with CPT-11 (irinotecan) had greater efficacy in combination than either as a single agent, and that the addition of C225 to CPT-11 had efficacy in CPT-11 refractory mouse models.[41] This encouraging activity led to studies in humans.

9. EGFR inhibition in the treatment of metastatic colorectal cancer

In a single-arm phase 2 study, 121 patients with metastatic colorectal cancer refractory to irinotecan and fluorouracil were treated with cetuximab in combination with the same irinotecan regimen on which they had progressed. Twenty-one patients (17%) achieved a partial response, and a further 37 patients (31%) achieved disease stabilization.[42]

Regulatory approval for cetuximab was not attained until the results of the BOND study were available. In this study, 329 patients with metastatic colorectal cancer refractory to irinotecan, most of whom had also received oxaliplatin, were randomised 2:1 to receive either cetuximab with irinotecan or cetuximab alone. Cross-over to cetuximab–irinotecan was permitted on disease progression for those that received monotherapy. The primary end-point of superiority for response rate with combination treatment was met, with an objective tumour response rate of 22.9% with combination therapy compared to 10.8% for cetuximab alone ($p = 0.007$). The median time to progression also favoured the combination (4.1 months versus 1.5 months, $p < 0.001$) but there was no significant difference in overall survival possibly due to permitted cross-over to cetuximab for those that received monotherapy.[43] Toxicity attributed to cetuximab was modest, and consisting mainly of the classic acneiform rash.

Because of the effectiveness of cetuximab in combination with irinotecan in irinotecan refractory colorectal cancer, the combination was trialled in the first-line setting. In the CRYSTAL trial, 1217 previously untreated patients with metastatic colorectal cancer were randomly assigned to receive FOLFIRI either alone or with Cetuximab. The primary end-point of a prolongation of progression-free survival was met, with a median PFS of 8.0 months versus 8.9 months with the combination (HR 0.851; 95% CI 0.726–0.998; $p = 0.0479$). Overall survival has not yet been reported. The response rate was higher with the addition of cetuximab (46.9% versus 38.7%, $p = 0.0038$) but the disease control rate was no different. Toxicity was greater in the group who received cetuximab, particularly grade 3/4 neutropaenia, diarrhoea and skin rash. In a pre-planned analysis, the numbers of patients undergoing potentially curative metastasectomy after chemotherapy were compared, and favoured the combination group (2.5 versus 6%), as did the rates of R0 resection (1.5 versus 4.3%). However, the numbers in these subgroups were small and whilst statistically significant were potentially subjected to bias as it is impossible to blind investigators to knowledge of investigational treatment with the presence of cetuximab induced skin rash. This sub-group analysis was also a small aspect of the statistical plan and caution should be taken before over-interpreting these initial results.

The EPIC trial was a randomised phase 3 trial of irinotecan–cetuximab compared to irinotecan alone following oxaliplatin failure and also demonstrated an increase in progression-free survival without prolongation of overall survival, attributed to salvage therapy.[44] The NCIC CO-17 trial also reported in 2007 demonstrated that in patients previously treated with oxaliplatin, irinotecan and 5-FU, cetuximab improved overall survival when compared to best supportive care alone (median OS 6.1 versus 4.6 months, HR 0.77, 95% CI 0.64–0.92; $p = 0.0046$).[45] Quality of life variables favoured cetuximab despite an increase in the number of adverse events attributable to therapy. Panitumumab monotherapy in patients who had failed standard chemotherapy was shown to prolong mean PFS compared to best supportive care alone; however, cross-over at progression was permitted possibly resulting in an absence of a survival benefit.[46]

The results of the CRYSTAL study are preliminary in that although the primary end-point has been reported, overall survival has not. Whilst the FDA accepts PFS as a valid end-point for registration studies in diseases where there is salvage therapy that may influence overall survival data, many clinicians would consider that the appropriate setting to use an agent is one where an improvement in survival or quality of life has been demonstrated. It can be argued that if a survival benefit with an agent is obtained by use in later lines of therapy rather than earlier lines, and that this will lead to less toxicity and lower cost, then a later line of treatment is the appropriate time point to select this agent for use.

The majority of available data with chemotherapy and either panitumumab or cetuximab is in combination with irinotecan-based regimens, and there is only limited data for their combination with oxaliplatin. A US phase 3 study designed to compare FOLFOX to FOLFOX with cetuximab in irinotecan refractory patients aimed to recruit 1100 patients but was closed early due to poor accrual when FDA approval was granted for the use of oxaliplatin in the first-line setting.[47] Results of ongoing trials of oxaliplatin-based regimens with cetuximab and panitumumab are awaited.

10. Dual target inhibition

Preclinical models have demonstrated that EGFR stimulation leads to increased VEGF expression, and that VEGF expression is induced by increased circulating levels of EGF, indicating an interaction between these two important pathways and providing a rationale for the hypothesis that inhibition of both pathways may result in greater tumour inhibition.[48]

The BOND-2 study was a randomised phase 2 study aimed to evaluate the safety and feasibility of the combination of cetuximab and bevacizumab with or without irinotecan in an irinotecan-refractory population, and compare efficacy to historical data in an exploratory fashion. The response rate of the triplet combination was higher than that seen with cetuximab and irinotecan in the BOND study (37% versus

22.9%), and the time to tumour progression also compared favourably (7.3 versus 4.1 months). However the trial did not test specifically for the efficacy of the addition of bevacizumab, but did appear to demonstrate the safety of the combination of bevacizumab to cetuximab, with no supra-additive toxicities experienced to that which were expected with the individual agents.[49] Such cross-trial comparisons should always be interpreted with caution.

A fully human IgG2 monoclonal antibody against the EGFR, panitumumab, has been developed and tested in combination with bevacizumab and chemotherapy. Patients were assigned to receive a backbone of either FOLFOX or FOLFIRI at investigator discretion, in combination with bevacizumab, with or without Panitumumab.

The study was closed by the IDMC at an interim analysis after 800 patients were recruited, when those in the experimental arm were found to have a worse progression-free and overall survival than those in the standard arm.

There was a 14% incidence of grade 3 toxicity in the panitumumab arm compared to 4% in the control, and 2% versus 1% grade 4 toxicity, associated with an incidence of death in the arm receiving the combination of targeted agents. The adverse events reported were predominantly diarrhoea, vomiting, dehydration and infection. Progression free survival was 8.8 months for those receiving dual inhibition compared to 10.5 months for those receiving chemotherapy with bevacizumab only (HR 1.44, 95% CI 1.13–1.85 $p = 0.004$). Median OS was 18.4 months versus not reached. Possible explanations for the worse outcomes include an increase in toxicity resulting in a lower dose intensity of FOLFOX, leading to a worsening of PFS.

The results from this study sound a note of caution for dual target inhibition,[25] as although the BOND-2 data did not highlight the increased toxicity of concern with cetuximab–bevacizumab in combination, the numbers were relatively small. Further data are awaited from the ongoing US Intergroup/SWOG trial 80405, in which 2300 chemotherapy naive patients are randomised to receive chemotherapy and bevacizumab with or without cetuximab. The results of the PACCE trial prompted a review of the 80405 trial by its independent safety and monitoring board trial, which concluded that there were no significant safety concerns to warrant a premature closure.

11. Patients with potentially resectable metastatic disease

Some patients with metastatic colorectal cancer who undergo metastasectomy are cured of their disease.[6] In a randomised trial of 173 patients with completely resected hepatic metastases from colorectal cancer, adjuvant infused 5-FU/LV chemotherapy administered after resection of liver metastases was shown to improve 5 year disease free survival (33.5% versus 26.7%, HR0.66; 95% CI, 0.46 – 0.96; $p = .028$) and saw a trend towards improved overall survival (5 year OS 51.1% versus 41.1%; $p = 0.13$), when compared to observation alone.[50] This regimen would not currently be considered optimal, but was standard at the time of study initiation.

Peri-operative chemotherapy is an alternative strategy to adjuvant treatment, and has the benefit of demonstrating *in vivo* chemo-sensitivity, may result in tumour shrinkage with a higher chance of successful R0 resection, and attends to distant micrometastatic disease at an earlier time in the treatment programme. The EORTC 40983 study compared peri-operative chemotherapy with FOLFOX to surgery alone in patients with resectable liver metastases. Three hundred and sixty four patients were randomised, and 342 were considered eligible. Rates of peri-operative complications were higher in the group who received pre-operative chemotherapy (25.2% versus 15.9%), including higher rates of liver failure, biliary leak and intraabdominal infection. Similar numbers randomised to each arm received surgery. In the intent-to-treat population, the 3-year PFS was higher (28.1–35.4%, HR 0.79, 95%CI 0.62–1.02; $p = 0.058$), but this result did not reach conventional levels of significance.[51]

Chemotherapy in patients with initially unresectable disease may result in some patients undergoing potentially curative resection. The success of this so-called 'conversion' chemotherapy appears to correlate with tumour response rate.[52] In a phase 3 study, 244 chemotherapy-naive patients with unresectable metastatic colorectal cancer were randomised to receive either FOLFIRI or FOLFOXIRI. A higher proportion of all patients treated with the FOLFOXIRI combination experienced a response to treatment (ORR 60% versus 34%, $p < 0.0001$) and were able to undergo R0 resection of their metastases (15% versus 6%; $p = 0.033$).[53]

Although the use of bevacizumab with chemotherapy improves outcomes in patients with metastatic disease, its use in the peri-operative setting has raised concerns regarding potential problems with wound healing and liver regeneration following hepatic resection. The safety of the combination of bevacizumab in combination with XELOX has been assessed in single arm phase 2 studies. In one report of 32 patients receiving neo-adjuvant XELOX with bevacizumab prior to surgical resection of metastatic disease, an objective response rate of 59% was seen, and liver function and regeneration at 3 month post-resection were normal in all patients. Bevacizumab was discontinued 5 weeks pre-operatively, and peri-operative complications were reported to be comparable to historical controls.[54]

As discussed above, the use of cetuximab in combination with FOLFIRI resulted in a higher rate of R0 resections for liver-only metastases. A prospective study of peri-operative FOLFOX with or without Cetuximab in patients with potentially resectable metastatic colorectal cancer is currently recruiting.

The addition of either cetuximab or bevacizumab to chemotherapy increases objective response rates, and appears to have manageable toxicity profiles in patients with potentially resectable disease. FOLFOXIRI also improves response rates compared to FOLFIRI, but this combination has not been tested with EGFR or VEGF antibodies.

12. Patient selection

Given that bevacizumab and cetuximab target specific cellular pathways, it is theoretically possible to identify which patients should derive greater benefit from treatment with these agents. Unfortunately, the clinical reality has been more difficult to implement.

Although high tumour expression of EGFR is correlated with adverse outcome, high EGFR expression has not correlated well with response to EGFR inhibition. Possible explanations for this include a discordance of EGFR expression between primary tumour and metastatic sites, with one report noting a 50% discordance.[55] Alternatively, it may be that the measurement of EGFR expression by IHC is not truly representative of tumour EGFR expression. In one report on the tumours of 85 patients, response rate and TTP were positively correlated with EGFR expression according to FISH analysis but not by IHC, suggesting the importance of the method of assay.[56] Other explanations include mutations in the EGFR transmembrane protein accounting for variability in response and activity despite an increase in receptor number.[57]

Downstream signalling protein mutations may also explain variable response to anti-EGFR agents, particularly as a result of mutations in the *Kirsten-Ras* (*K-ras*) gene. Ligand binding to the EGFR protein results in activation of the G protein K-ras, the protein kinase RAF (Ras/mitogen-activated protein kinase) pathway and phosphoinositide 3-kinase (PI3K/Akt pathway).

In one study, the retrospective analysis of *K-ras* mutation was performed and correlated with response to cetuximab. No patients who responded to cetuximab had a *K-ras* mutation, and conversely no patient with a mutation achieved a partial response. Of those patients that did not respond to cetuximab therapy, 68% carried the mutation ($p = 0.0003$), demonstrating a strong association between *K-ras* mutation status and cetuximab resistance.[58]

A recent study correlated response to panitumumab monotherapy in a chemotherapy-refractory population with *K-ras* mutation status from a paraffin embedded tissue sample from the historically obtained tumour biopsy. Specimens from 427 of 462 randomised patients were available for analysis and were able to be correlated with response. In the group receiving panitumumab therapy, 17% of those with wild-type (WT) *K-ras* responded and 34% had stable disease compared with a 0% response rate and 12% with stable disease in the mutant *K-ras* group. PFS time in those treated with panitumumab was 12.3 weeks in the WT group and 7.4 weeks in the mutant group, compared to 7.3 weeks in the supportive care only arm.[59] This differential response is striking, but is to be validated in prospective studies (Table 3).

Table 3 – K-ras status and response to cetuximab or panitumumab

Treatment	Number of patients	Response rate (%)	
		Wild-type *K-ras*	Mutant *K-ras*
Antibody with chemotherapy			
Cetuximab ± chemotherapy[58]	76	49	0
Cetuximab ± chemotherapy[56]	81	26	6
Cetuximab + chemotherapy[63]	59	28	0
Cetuximab ± irinotecan[64]	113	40	0
Panitumumab or cetuximab ± chemotherapy[65]	48	31	6
Antibody monotherapy			
Cetuximab[37]	80	10	0
Panitumumab[59]	208	17	0
Total	665	27	1.2

Modified from Amado[59].

Circulating levels of VEGF have been correlated with the presence of metastasis as the well as risk of recurrence in resected colon cancer[60] as well as with survival; however, high levels of VEGF were not associated with response to Bevacizumab in a translational sub-study of the AVF2107 trial. Similarly, although the thrombospondin gene family and microvessel density are critically involved in angiogenic pathways and are deranged in response to increased circulating levels of VEGF, they were not correlated with response to bevacizumab therapy.[61]

13. Selection of first-line therapy

In the first-line setting, bevacizumab in combination with IFL has been shown to prolong overall survival compared to IFL, and FOLFOX/CapeOx chemotherapy prolongs PFS compared to chemotherapy alone. Data from the BICC-C study suggests that FOLFIRI with bevacizumab is associated with a longer median survival compared to FOLFIRI alone, but these data are not yet mature and are a comparison of two time-separated cohorts. mIFL is more toxic than FOLFIRI and is no longer recommended. We consider that the prolongation of PFS of bevacizumab in combination with chemotherapy across multiple studies and the positive results of the Hurwitz study for survival justify the use of bevacizumab in the first-line setting. Where patients have not received FOLFOX-bevacizumab in first-line treatment, a survival advantage in second line has been demonstrated (Fig. 1).

Results from NO16966 and the BRiTE study suggest that treatment with bevacizumab until disease progression is associated with a longer progression-free survival than early discontinuation, but continuing treatment beyond progression is not yet validated by prospective randomised studies. Current data suggest that patients treated with FOLFOX/CapeOx and bevacizumab who develop oxaliplatin-related toxicity should continue the fluoropyrimidine and bevacizumab to disease progression. Stop-and-go strategies with reintroduction of agents at progression are under evaluation.

Contraindications to bevacizumab include recent TIA/stroke or unstable vascular disease, uncontrolled hypertension, proteinuria or non-resolved surgical wound. Where contraindications to bevacizumab exist, cetuximab in combination with FOLFIRI is an alternative.

Where a patient has potentially resectable metastatic disease and a neo-adjuvant strategy is selected, either chemotherapy with bevacizumab or FOLFIRI-cetuximab can be utilised. Bevacizumab should be discontinued at least 5 weeks prior to operation.

Selection of the chemotherapy backbone (either FOLFIRI or FOLFOX/CapeOx) to use in combination with bevacizumab should be made according to patient characteristics, such as whether oxaliplatin has been used in the adjuvant

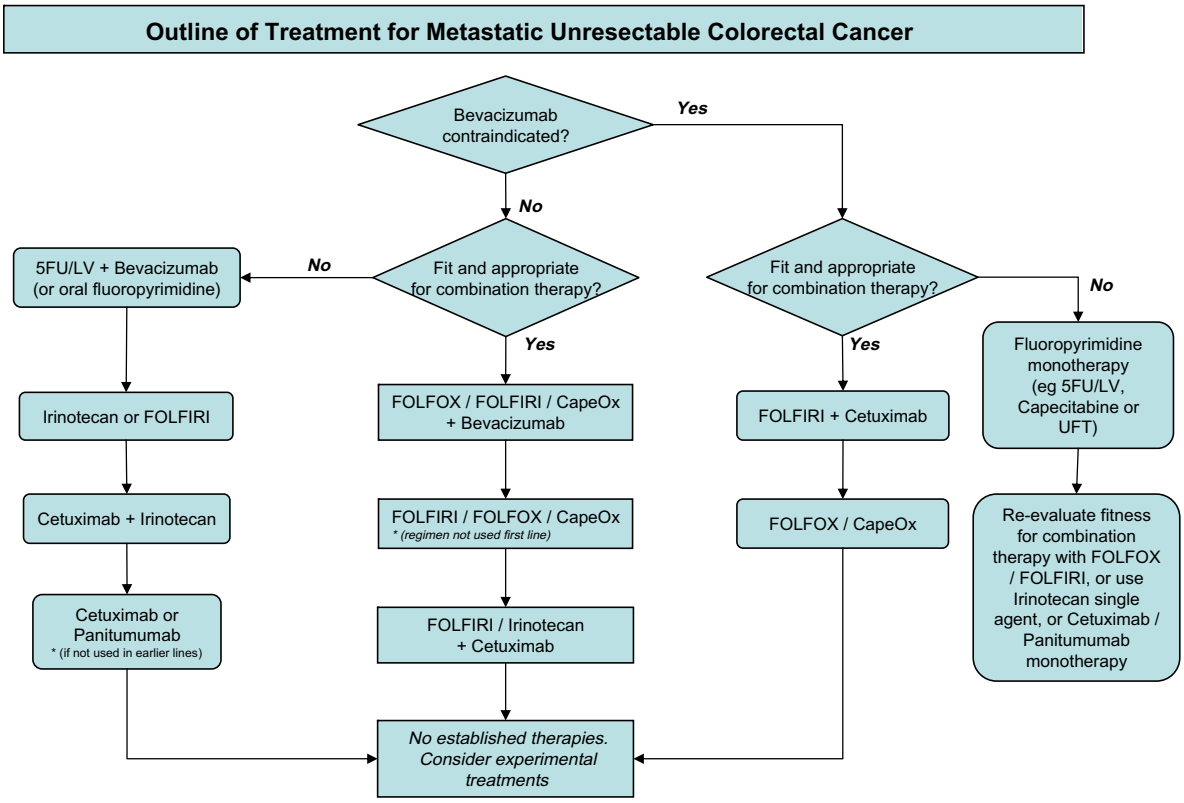

Fig. 1 – Outline of treatment for metastatic unresectable colorectal cancer. This outline is for treatment with chemotherapy only without provision for surgical metastasectomy or ablative therapies, or the use of radiotherapy, which should be considered if appropriate according to individual circumstances.

setting and subsequent disease free interval, resolution of neurotoxicity, presence of comorbidity such as pre-existing neuropathy and patient choice (for example, if neurotoxicity is an unacceptable outcome for patients). The selection of the first-line chemotherapy back-bone seems to be less important than ensuring that patients are exposed to all three active cytotoxics at some stage in their treatment, depending on the individual comorbidities and fitness of the patient. Data on chemotherapy schedules utilising all three cytotoxics (e.g. FOLFOXIRI) in combination with targeted agents are immature.

The combination of bevacizumab and panitumumab with FOLFOX has been shown to worsen the outcome in one study, and results from large studies of chemotherapy-bevacizumab with or without cetuximab are awaited before the combination of two targeted agents can be recommended.

There is good evidence that cetuximab can reverse irinotecan resistance and prolong progression-free survival in combination with chemotherapy in later lines of therapy, and prolong overall survival in the last-line setting; panitumumab has been shown to prolong PFS in this setting. The obtained data which show that cetuximab and panitumumab are inactive in patients with *K-ras* mutations are compelling, but testing is not yet part of routine clinical practice. The European licence for panitumumab has been granted for patients with wild-type *K-ras* only.

14. Summary

The goals of treatment of metastatic colorectal cancer are to prolong survival and maximise quality of life, and to facilitate potentially curative metastasectomy where possible.

Whilst general recommendations for the selection of chemotherapy and antibody can be made, the consideration of the individual patient is paramount to a successful treatment strategy. For example, a diabetic patient with peripheral neuropathy should not receive an oxaliplatin- based regimen for fear of worsening their underlying functional capability. A patient with impaired wound healing after a recent operation should not be treated immediately with bevacizumab. It may be that patients are able to have tumour-specific factors such as *K-ras* mutation status tested and used to predict who will benefit from EGFR inhibition. Access to targeted agents remains restricted for patients in many countries, considerably impacting on the treatment choices that are available.

The median survival of patients with metastatic colorectal cancer has improved from a meagre 6 months with supportive care alone to 20–24 months with modern therapy. The contribution of targeted agents to improvements in overall survival is supported by a large and growing body of literature; however, their cost-effectiveness continues to be debated. New treatment permutations as well as biomarkers, which maximise efficacy and minimise toxicity, continue to

be elaborated. As we learn more, the treatment paradigm continues to evolve.

Conflicts of interest statement

Professor David Cunningham: Research Funding, Merck Serono, Roche, Sanofi-Aventis. Honoraria: Roche, Pfizer, Advisory Board: Roche.

No other relevant conflicts of interest to declare. Dr. Christopher Jackson: Speaking fees from Roche. No other relevant conflicts of interest to declare.

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