How to integrate biologicals in the continuum of care

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Introduction

Colorectal cancer is the second most common cancer in Europe and has a worldwide annual incidence of 945,000 [1,2]. The landscape for the management of metastatic colorectal cancer (mCRC) is rapidly changing with development of more effective cytotoxic agents, in tandem with clinical development of biological agents. Progress in the multidisciplinary management of resectable and initially non-resectable mCRC and the combination of cytotoxics (oxaliplatin, irinotecan, capecitabine) with biological agents (bevacizumab, cetuximab and panitumumab) have increased the therapeutic armamentarium for patients with mCRC. Bevacizumab, a humanised monoclonal antibody against vascular endothelial growth factor (VEGF), in combination with fluoropyrimidine-based chemotherapy has been accepted as one of the standard first-line treatments for mCRC. Cetuximab, a chimeric IgG_1 monoclonal antibody, and panitumumab, a fully human monoclonal IgG_2 antibody targeting epidermal growth factor receptor (EGFR), have shown activity as single agents in chemo refractory patients and in combination, particularly with irinotecan. In light of emerging data, clinicians are faced with major challenges of choosing the best combination of cytotoxics and biologicals, as well as the best sequence and duration of treatment. Better understanding of the molecular mechanisms of resistance to treatment, risks of adverse events and added costs will enable us to individualise and optimise the treatment for these patients.

Predictive factors for response

Many questions remain to be answered in relation to the use of anti-EGFR and anti-VEGF antibodies as only a subset of patients with mCRC derive clinical benefit. More than ever, it is important to identify patients who are likely to respond to bevacizumab and anti-EGFR monoclonal antibodies (moAbs).

Pre-clinical markers of response

Anti-EGFR monoclonal antibodies

Retrospective evidence from various studies evaluating anti-EGFR moAbs have identified potential predictive biomarkers, namely EGFR expression status in immunohistochemistry (IHC), expression of EGFR ligands, EGFR gene copy number (GCN), EGFR mutations and polymorphism, and alterations in Ras/Raf/MAPK and PI3K/Akt signalling pathways, in particular KRAS mutation status.

Emerging data have highlighted that KRAS mutation status is an acceptable predictive marker. KRAS (human analogue of the Kirsten rat sarcoma 2 virus oncogene) encodes a small guanine triphosphate (GTP)-binding protein that acts as a signal transducer following ligand activation of cell surface receptor EGFR and in the mutated state the KRAS protein remains constitutively active independent of EGFR activation [3]. Several small retrospective single arm studies have shown that KRAS is mutated in 30–40% of mCRC patients and these patients do not respond to anti-EGFR moAbs [4–7]. This was confirmed in well designed randomised trials which have been summarised in Table 1. Amado and colleagues detected KRAS mutation in 43% of 427 KRAS status evaluable mCRC patients in a randomised phase III mCRC trial comparing panitumumab to best supportive care (BSC) in patients who have been exposed to oxaliplatin and irinotecan and, in conclusion, reported that none of the patients with mutant KRAS mCRC responded to panitumumab. Progression-free survival (PFS) was also significantly less for mutant KRAS mCRC patients when compared with KRAS wild type (WT) patients (7.3 weeks versus 12.3 weeks; $P < 0.001$) [9]. Similar results were demonstrated in the CO-17 trial which established a survival advantage for cetuximab when compared to BSC in chemotherapy-refractory mCRC patients, with mutant KRAS mCRC patients having an inferior overall survival (OS), PFS and overall response rate (ORR) when compared to KRAS WT mCRC patients (4.8 months, 1.9 months and 0% versus 9.5 months, 3.7 months and 12.8% respectively) [8]. The predictive value of KRAS mutation was also apparent in combinational studies of chemotherapy

Table 1
Evidence from randomised trials analysing the correlation of efficacy of anti-EGFR moAbs and KRAS mutation in mCRC

Study	Treatment arms	Outcome	KRAS wild type	KRAS mutant
Karapetis et al. [8]	Cetuximab versus(vs)	ORR	12.8% vs 0%	1.2% vs 0%
$n = 394$	BSC	PFS	3.7 vs 1.9 (months)	1.8 vs 1.8 (months)
(Chemo-refractory)				$P = 0.96$
Amado et al. [9]	Panitumumab vs BSC	ORR	17% vs 0%	0% vs 0%
$n = 427$			$P < 0.001$	$P < 0.001$
(Chemo-refractory)		PFS	12.3 vs 7.3 (weeks)	7.4 vs 7.3 (weeks)
			HR: 0.45	HR: 0.99
Bokemayer et al. [10]	FOLFOX + cet vs	ORR	61% vs 37%	33 % vs 49%
$n = 233$	FOLFOX		$P = 0.011$	$P = 0.106$
(First-line)		PFS	7.7 vs 7.2 (months)	5.5 vs 8.6 (months)
			$P = 0.016$	$P = 0.0192$
Van Cutsem et al. [11]	FOLFIRI + cet vs	ORR	59% vs 43%	40% vs 36%
$n = 540$	FOLFIRI		$P = 0.002$	$P = 0.46$
(First-line)		PFS	9.9 vs 8.7 (months)	7.6 vs 8.1 (months)
			$P = 0.017$	$P = 0.75$
Tol et al. [12]	Cap/oxali/bev/cet vs	ORR	61.4% vs 50%	45.9% vs 59.2%
$n = 520$	Cap/oxali/bev		$P = 0.06$	$P = 0.03$
(First-line)		PFS	10.5 vs 10.7 (months)	8.6 vs 12.5 (months)
			$P = 0.10$	$P = 0.043$
Hecht et al. [13]	Ox-CT/bev/pani vs	ORR	50% vs 56%	47% vs 44%
$n = 201$ (Ox-CT)	Ox-CT/bev	PFS	9.8 vs 11.5 (months)	10.4 vs 11.0 (months)
(First-line)				
$n = 664$ (Iri-CT)	Iri-CT/bev/pani vs	ORR	54% vs 48%	30% vs 38%
(First-line)	Iri-CT/bev	PFS	10 vs 12.5 (months)	8.3 vs 11.9 (months)

Abbreviations. n, KRAS evaluable population; cet, cetuximab; bev, bevacizumab; oxali, oxaliplatin; cap, capecitabine; pani, panitumumab; Ox-CT, Oxaliplatin-based chemotherapy; Iri-CT, Irinotecan-based chemotherapy.

and EGFR moAbs in first-line treatment of mCRC. In the randomised phase III study comparing cetuximab plus FOLFIRI to FOLFIRI alone as first-line treatment of mCRC (CRYSTAL), with PFS as the primary endpoint, the median PFS favoured the combination arm [14,15]. Retrospective subset analyses revealed a substantially enhanced treatment effect in WT KRAS mCRC. In WT KRAS mCRC, the PFS was 9.9 months for FOLFIRI/cetuximab versus 8.7 months in the FOLFIRI alone arm (hazard ratio (HR)0.68, 95% confidence interval [CI] 0.501–0.934; $P = 0.017$) and KRAS mutant mCRC patients did not benefit from cetuximab [11]. The above trend was also seen in the OPUS trial of folinic acid, fluorouracil and oxaliplatin (FOLFOX) with or without cetuximab in the first-line treatment of mCRC, which demonstrated that the ORR for FOLFOX plus cetuximab was higher than FOLFOX alone (46% versus 36%; odds ratio (OR) 1.52, 95%CI 0.975–2.355; $P = 0.064$) but no difference in PFS. However, on retrospective review of KRAS status, both PFS (7.7 versus 7.2 months, $P = 0.02$) and ORR (61% versus 37%; $P = 0.011$) were significantly better in KRAS WT than mutant KRAS mCRC [10]. This study, notably, raised the possibility of a negative interaction between oxaliplatin and cetuximab, as mutant KRAS mCRC patients had worse outcomes both in terms of PFS and ORR. Recent data from trials combining dual antibodies with chemotherapy have also strengthened the case for KRAS mutation status as a predictor of response to anti-EGFR therapy (Table 1).

Ongoing prospective trials (CALBG C80405), which have been amended to recruit patients with WT KRAS mCRC only, could confirm the above findings. Nevertheless, based on the extensive data from these randomised trials, ASCO has given a provisional clinical opinion that all patients with mCRC should have the KRAS status checked and anti-EGFR moAbs therapy should be restricted to patients with WT KRAS mCRC only [16]. This is also reflected in the licence granted to panitumumab in Europe.

Additional data suggests alteration in other EGFR downstream pathways could also confer resistance to anti-EGFR moAbs therapy. Loss of PTEN protein

expression may also be a predictor of response as demonstrated by Frattini and colleagues who concluded that none of the patients ($n = 10$) with loss of PTEN expression responded to cetuximab therapy, while 10 of 16 patients with intact PTEN responded to cetuximab therapy [17]. Consistent with the above results were the findings from Perrone and colleagues who also concluded that deregulation in the PI3KCA/PTEN pathway significantly correlated with lack of response to cetuximab [18]. The pivotal BOND (bowel oncology using erbutix antibody) trial [19], which established cetuximab as a treatment option for patients with chemo-refractory mCRC, excluded patients with absent EGFR expression evaluated by IHC but did not reveal any correlation between degree of EGFR expression and clinical response. A subsequent study by Chung and colleagues [20] concluded that EGFR analysis by IHC does not predict response to treatment with cetuximab. Similarly, antitumour activity was noted in mCRC patients whose tumour had no EGFR immunostaining [21]. Variation in IHC methodology and tissue handling may be a plausible explanation for this hypothesis. Khambata-Ford and colleagues analysed 110 patients with mCRC enrolled into a cetuximab monotherapy trial and postulated that expression of high levels of EGFR ligands, epiregulin (EREG) and amphiregulin (AREG), are more likely to achieve disease control with cetuximab with PFS of 103 days in EREG high expressors and 57 days in EREG low expressors. The results were similar for AREG expression (115.5 days versus 57 days; $P < 0.001$) [6].

Increased EGFR GCN has also been investigated as a potential predictive biomarker of responses to anti ECFR moAbs, with discrepant results. At least two studies concluded that high GCN by fluorescence *in situ* hybridisation (FISH) analysis may predict the response to anti-EGFR moAbs [22,23]. However, other studies employing different techniques of assessing GCN (quantitative polymerase chain reaction, chromosomal *in situ* hybridisation) did not strengthen the case for GCN as a predictive marker [6,8].

The other potential biomarker is EGFR mutation, but in contrast to non-small cell lung cancer, where EGFR mutations have been associated with treatment outcome, currently there is no evidence that EGFR mutations are linked to response to cetuximab therapy in mCRC [24].

Bevacizumab

Currently there are no validated preclinical biomarkers of response to bevacizumab. Mutations of KRAS, b-raf and p53 could not predict for prolonged survival on bevacizumab plus irinotecan and bolus 5FU/folinic acid [25]. The response to bevacizumab treatment is independent of KRAS status though there was a trend towards better outcome in patients with WT KRAS mCRC [26]. Jubb and colleagues performed an exploratory subset analyses on the samples available from 813 mCRC patients from the landmark trial (AVF 2107g) to explore the impact of VEGF, thrombospondin expression and microvascular density (MVD) on response and concluded that there is no significant correlation between VEGF or thrombospondin expression and response to bevacizumab [27]. Discordance in the VEGF expression in primary and metastatic tumour also questions the value of archived primary tumour samples for assessing potential predictive and prognostic markers for metastatic disease. Elucidating the precise mechanism of action of bevacizumab may help in identification of predictive markers.

Clinical markers of response

Skin reactions

Acne-form or maculopapular rash is a class effect of drugs inhibiting the EGFR pathway, due to alteration of the mediation of epidermal basal keratinocytes, probably highlighting the role of EGFR in maintaining the integrity of the skin. In the BOND trial, the incidence of acne-like rash was 9.4% in the irinotecan plus cetuximab combinational arm and 5.2% in the cetuximab monotherapy arm. Subgroup analyses revealed significantly improved ORR for patients with skin reaction after cetuximab treatment both in the combination treatment arm and monotherapy arm (25.8% versus 13%) when compared to patients without skin reaction (6.3 % versus 0 %) [19]. Lenz and colleagues also noted that the severity of the rash correlated strongly with response. In their phase II trial of cetuximab in chemo-refractory mCRC patients, 82% (311 of 346 patients) experienced skin rash and the response rate was 17% (95%CI 11.4–23.9%) in patients with grade 2 skin rash compared to 7.2%(95%CI 3.5–12.9%) in patients with grade 1 skin rash [21]. Consistent with the above findings were the results from the phase III trial of panitumumab plus BSC compared with BSC alone in patients with chemo-refractory mCRC, which demonstrated better PFS for patients with grade 2 or more skin toxicity due to panitumumab when compared to those patients with grade 1 skin toxicity (HR 0.62, 95%CI 0.44–0.88) [28]. A similar trend was also noted in the trial with cetuximab [29]. The severity of cetuximab-related skin reactions correlated significantly with PFS

of mCRC patients receiving cetuximab in the CAIRO2 trial with median PFS of 7.8, 10.2 and 11.4 months ($P < 0.001$) in patients with grade 0/1, grade 2 and grade 3 skin reactions, respectively [12]. However, the difference was not statistically significant ($P = 0.72$) in comparison with the non-cetuximab arm. The occurrence of skin reaction to anti-EGFR therapy has been correlated with increased response rate and improved survival in other tumour sites as well [30,31].

The above data suggests that skin rash could be a potential predictive marker but this has not been validated. The EVEREST trial was designed to prospectively show that cetuximab dose escalation in patients with no or mild skin reaction to standard treatment dose of cetuximab may improve ORR in this group of patients [32]. EVEREST was a phase I/II randomised study investigating the effect of cetuximab dose escalation in EGFR-expressing mCRC patients who had failed on irinotecan therapy. One hundred and sixty six patients were treated with standard dose of cetuximab (initial dose 400mg/m^2, followed by 250mg/m^2/week) plus irinotecan (2-weekly regimen of 180mg/m^2). On day 22, patients who tolerated irinotecan and cetuximab were then randomised to dose escalation ($n = 44$) or standard dose arm ($n = 45$) based on the absence of $\geqslant$grade 2 skin reactions (randomised group). The dose of cetuximab was increased by 50 mg/m^2 every 2 weeks until $\geqslant$grade 2 skin toxicity, tumour response or a maximum dose of 500 mg/m^2 was achieved. Patients with $\geqslant$grade 2 skin reaction ($n = 77$) continued to receive the standard dose treatment (non-randomised group). Efficacy analyses in the randomised group showed that patients in the dose escalation arm achieved higher ORR and PFS (30% and 4.8 months) when compared to the standard dose arm (13% and 3.9 months). As expected, patients in the dose escalation arm had a higher incidence of other grade 3/4 toxicities. On testing the predictive value of KRAS in the dose escalation group, none of the 19 patients with mutant KRAS mCRC responded to cetuximab and irinotecan combination and dose escalation did not have any impact on response in this group of patients with mutant KRAS mCRC [33]. These data suggest that dose escalation of cetuximab may improve response rates in patients with WT KRAS mCRC and absence of <grade 2 skin reactions.

Hypertension

Arterial hypertension is a prominent side effect of angiogenesis inhibitors. The incidence of hypertension in the phase III trial by Hurwitz and colleagues was 22.4% (as compared with 8.3% in the control arm), with 11% experiencing grade 3 hypertension [34]. A similar incidence of grade 3 hypertension was seen in other mCRC trials with bevacizumab [35,36]. The pathogeneses of anti-VEGF inhibitors-induced hypertension is poorly understood. VEGF plays a important role in maintaining blood pressure and administration of VEGF results in a dose-dependent decrease in mean arterial pressure in rats (probably mediated by nitric oxide and prostacyclin synthesis) [37]. Reduced formation of nitric oxide due to VEGF inhibition could cause hypertension. Another potential hypothesis is hypertension secondary to vascular rarefaction (reduction of micro-vessels) leading to increased peripheral vascular resistance. Bevacizumab therapy has also been shown to cause hypertension by endothelial dysfunction and capillary rarefaction [38,39]. Scartozzi and colleagues conducted a retrospective analysis on 39 mCRC patients receiving bevacizumab in combination with 5-FU and irinotecan and found that a partial response was obtained in 75% of patients with grade 2/3 hypertension versus 32% in patients without hypertension [40]. Though the authors suggest that bevacizumab induced hypertension could be a biomarker of efficacy, given the retrospective nature of the study and the small number of patients involved, this could only be considered as hypothesis generating and validation in prospective trials is warranted.

Use of biologicals in the neoadjuvant setting

Neoadjuvant chemotherapy to downsize liver metastases in patients with resectable and potentially resectable mCRC has been accepted as a standard of care in the management of mCRC, thereby potentially "curing" a selected group of patients. Tumour shrinkage is a key determinant for liver resection and there is a close correlation between response rates and metastasectomies [41] underscoring the importance of exposing the patients to the most active regimens. The addition of biologicals to chemotherapy has increased the response rate in the first-line setting by at least 10% [15,34]. There is now an accumulating body of evidence highlighting the efficacy and safety of addition of moAbs to neoadjuvant chemotherapy.

Firstly, the BEAT observational study was opened to evaluate the safety profile and efficacy of bevacizumab in a broader patient population using a variety of chemotherapy regimens and enrolled 1965 patients from 41 countries: 225 patients (12%) underwent curative surgery and R0 resection was reported in 173 patients (77%). Patients receiving

bevacizumab plus oxaliplatin-based chemotherapy appeared to have favourable resection rates. In the 704 patients with metastases confined exclusively to the liver, hepatic metastasectomy was performed in 107 patients (15.2%) and 79% of these patients ($n = 85$) achieved R0 resection. The 2-year OS rate was 86% in patients undergoing hepatic metastasectomy and 89% in patients achieving R0 resection [42]. The NO16966 trial evaluated bevacizumab plus fluoropyrimidine/oxaliplatin chemotherapy in 1401 patients and 59 patients (8.4%) in the bevacizumab arm underwent curative metastasectomy compared with 43 patients (6.1%) in the placebo arm. Postoperative evaluation revealed R0 resection in 44 patients (75% R0 resection rate) in the bevacizumab arm and 34 patients (79% R0 resection rate) in the placebo arm [42,43].

In the CRYSTAL trial, the number of mCRC patients who underwent hepatic resection was higher in the cetuximab plus FOLFIRI arm when compared to the FOLFIRI alone arm (6% versus 2.5%) and a significantly higher numbers of patients achieved R0 resection (4.3% versus 1.5%; $P = 0.0034$). In patients with liver metastases alone, 9.8% in the cetuximab arm and 4.5% in the control arm underwent an R0 resection [11].

The benefit of pre-operative cetuximab was also confirmed when given in combination with oxaliplatin-based chemotherapy in the OPUS trial in which 4.7% (8 of 169 patients) in the cetuximab plus FOLFOX arm had an R0 resection in contrast to 2.4% (4 of 168 patients) in the FOLFOX only arm. These benefits were more pronounced on analysing the KRAS mutation status, as in WT KRAS mCRC; patients receiving cetuximab/FOLFOX achieved double the R0 resection rate when compared with patients receiving FOLFOX alone (9.8% versus 4.1%) [10]. The doubling of R0 resection rate with pre-operative cetuximab in KRAS WT mCRC is promising but given the small patient numbers, no firm conclusion should be derived. Hepatic resection after rescue cetuximab treatment in heavily pre-treated patients has also been reported to be feasible. Adam and colleagues [44] have reported the outcome from 115 patients who had progressive disease after conventional treatment and 27 of these 115 patients underwent surgery with curative intent following treatment with a combination of cetuximab plus irinotecan and/or oxaliplatin. Postoperative mortality and complication rate was reported to be 3.7% (1 of 27) and 50%, respectively. The median PFS and OS were 12 and 20 months respectively. These figures are commendable as this is a group of patients who did not respond to their conventional chemotherapy.

Postoperative complications with biologicals

VEGF plays a critical role in liver regeneration after hepatectomy [45–47] and, in animal models, the inhibition of angiogenesis has resulted in impaired wound healing [48,49]. Cytotoxic agents used in mCRC have been associated with steatohepatitis and vascular changes which can increase post-operative morbidity [49]. There are concerns that biologicals may augment the chemotherapy-induced hepatotoxicity and VEGF inhibition could diminish hepatic regeneration after resection besides the concerns regarding wound healing. Results from registries and single/multi-institutional studies suggest that pre-operative administration of the combination of biologicals and chemotherapy can be safe and effective. In the first BEAT trial it was recommended that bevacizumab be stopped 6–8 weeks prior to surgery due to the long half-life and potential interference with wound healing. The median time between the last dose of bevacizumab and surgery was 64 days. Of the 225 patients undergoing curative surgery, serious adverse events (SAE) were recorded in 30% and the most common grade 3/4 adverse events or SAE were neutropaenia (19%), neuropathy (10%), diarrhoea (8%) and fatigue (5%). This side effect profile is similar to that noted in other pre-operative chemotherapy trials [50]. The grade 3/4 wound healing complication was only 1.3% and 0.4% of patients had grade 3/4 bleeding events [42].

Scappaticci and colleagues pooled the data from two randomised trials of bevacizumab [34,51,52] to evaluate the effect of bevacizumab on wound healing. Seventy five of 616 patients in the bevacizumab plus chemotherapy arm and 29 of 516 patients in the chemotherapy alone arm underwent major surgeries including procedures for intestinal obstruction, bowel perforation and liver metastases. More patients in the bevacizumab arm experienced grade 3/4 wound complications, but this difference was not statistically significant (13% versus 3.4%; $P = 0.28$) and most of these surgeries were emergencies or urgent [51]. In the matched case control study it was shown that peri-operative bevacizumab does not increase the perioperative morbidity or long-term complications. The overall postoperative complication rate was 40.6% in the bevacizumab group and 37.5% in the control arm ($P = 1.0$) [53]. Reddy and colleagues also showed no increase in the perioperative morbidity of hepatic resection in patients receiving bevacizumab and chemotherapy on comparison with chemotherapy alone in their retrospective analyses of 96 patients undergoing hepatic resections (43.6% versus 38.6%).

However, there was a non- statistically significant difference in the complication rate (62.5% versus 30.4%; $P = 0.06$) when surgery was done within 8 weeks after the last administration of bevacizumab [54].

Pharmacokinetic studies demonstrate that plasma half-life of bevacizumab is approximately 21 days, (range 11–50 days). Even at a relatively low dose of 0.3 mg/kg, bevacizumab can lead to undetectable levels of free VEGF in systemic circulation [55]. Waiting for 6 weeks (two half-lives) would still leave a significant amount of bevacizumab to exert its anti-VEGF action. Although circulating VEGF has not been established as a reliable predictor of the biological effect, it was proposed that there should be an 8-week bevacizumab-free interval prior to hepatic resection [56]. Of interest, Kesmodel and colleagues [57] reported no significant difference in the complication rate in mCRC patients undergoing hepatic resection within 60 days ($n = 40$) of administration of bevacizumab compared to >60 days ($n = 36$), though only 13 patients received bevacizumab 31–45 days before surgery. Notably, Gruenberger and colleagues concluded that there was no increase in morbidity even when bevacizumab was given till 5 weeks prior to surgery, there was no perioperative mortality [58]. The above data suggest that safety and feasibility of resection of metastatic lesions with curative intent does not appear to be compromised by the addition of bevacizumab.

To date, there is a paucity of data regarding the safety of pre-operative anti-EGFR moAbs, though limited preclinical and retrospective data suggests it is safely tolerated [44,59]. Moreover, given the shorter half-life of cetuximab, a longer preoperative treatment-free interval may not be needed.

Clearly, the jury is out on the optimal duration of cessation of bevacizumab before surgery and the time period after which it could be restarted post-resection. Based on these retrospective evidences, experts recommend withholding bevacizumab for 6–8 weeks prior to surgery. The planned National Cancer Institute (NCI) phase III trial to evaluate neoadjuvant versus adjuvant bevacizumab plus FOLFOX in patients with potentially resectable hepatic colorectal metastases could address most of the outstanding issues.

Use of a single agent biological plus chemotherapy

Bevacizumab

The addition of bevacizumab to fluoropyrimidine-based combination chemotherapy has been shown to be advantageous both in the first- and second-line treatment of mCRC [34,43,60]. Two large phase III trials have investigated the role of bevacizumab in newly diagnosed mCRC patients. Following the promising results from the phase II trial of the addition of bevacizumab to 5-FU/leucovorin (LV) in the first-line treatment of mCRC, Hurwitz and colleagues conducted the pivotal phase III trial (AVF 2107g) of bevacizumab plus irinotecan, 5-FU and leucovorin (IFL), wherein 813 previously untreated mCRC patients were randomised to receive IFL with bevacizumab or placebo. Addition of bevacizumab led to a 37% reduction in the risk of death (HR 0.67; $P < 0.001$) when compared to placebo. Median OS was 20.3 months in the bevacizumab/IFL arm versus 15.6 months in the IFL alone arm. PFS (10.4 months versus 6.2 months,; HR 0.56; $P < 0.001$) and ORR (44.8% versus 34.8%; $P = 0.004$) were also in favour of the bevacizumab arm [34]. Based on these convincing results, bevacizumab was approved as a standard first-line treatment for mCRC in combination with irinotecan-based chemotherapy. Saltz and colleagues evaluated the efficacy of addition of bevacizumab to a first-line fluoropyrimidine/oxaliplatin doublet in a randomised phase III mCRC trial (NO16966) and PFS was the primary endpoint. 1401 patients were randomised in 2×2 factorial design to XELOX (capecitabine plus oxaliplatin) versus FOLFOX (5FU/folinic acid plus oxaliplatin) and then to bevacizumab versus placebo. This study met its primary endpoint by demonstrating a statistically significant difference in median PFS of 1.4 months in favour of the bevacizumab arm (9.4 months versus 8.0 months; HR 0.83, 97.5%CI 0.72–0.95; $P = 0.0023$) [43]. There was a trend towards improved median OS with bevacizumab with a median OS of 21.3 months in the bevacizumab plus chemotherapy arm and 19.9 months in the chemotherapy alone arm but this was not statistically significant (HR 0.89, 97.5%CI 0.76–1.03; $P = 0.077$). There was no difference in response rate. The smaller than expected benefit with bevacizumab could be explained by the observation that the treatment duration in the bevacizumab arm was not longer than in the placebo arm despite longer duration of PFS in the bevacizumab arm. The study protocol allowed treatment till progression. However, only 29% of patients in the bevacizumab arm continued treatment until disease progression (47% in the placebo arm), despite the protocol stipulating that treatment could be continued with remaining components should any one of the components be discontinued due to toxicity. For example, if oxaliplatin was discontinued (due to neurotoxicity) the protocol permitted continuation of

bevacizumab and fluoropyrimidine but this was not done. Indeed, the treatment was often not continued despite the absence of toxicity or progression of disease. This also raises questions regarding the optimal treatment duration for bevacizumab (discussed below).

The addition of bevacizumab in second-line treatment of mCRC was investigated in the ECOG 3200 phase III trial where 829 mCRC patients previously treated with irinotecan were randomly assigned to treatment with one of three arms, namely bevacizumab plus FOLFOX, FOLFOX alone or bevacizumab alone. The bevacizumab alone arm was closed early as the interim analysis showed inferior PFS and RR when compared to the chemotherapy arms. The results demonstrated superiority for the bevacizumab plus FOLFOX arm when compared with FOLFOX alone in median OS (12.9 months versus 10.8 months; HR 0.75; $P = 0.0011$), median PFS (7.3 months versus 4.7 months; HR 0.61; $P < 0.001$) and ORR (22.7% versus 8.6%; $P < 0.0001$) [60]. The most common grade 3/4 toxicities related to bevacizumab in these trials were hypertension (4–11%), thromboembolic events (3–4%), bleeding (2–3.4%), gastrointestinal perforation (0–1.5%) and proteinuria (<1%). The safety of bevacizumab with different regimens of fluoropyrimidine (infusional 5-FU,bolus 5-FU or capecitabine) and oxaliplatin was the primary endpoint of the TREE study, which concluded that bevacizumab is well tolerated with no overall increase in toxicity when given in addition to combinations of oxaliplatin and fluoropyrimidine regimens. The median OS was significantly longer in the bevacizumab plus chemotherapy group in comparison with the chemotherapy alone group (23.7 months versus 18.2 months) [36]. Fuchs and colleagues published a similar study (BICC-C) comparing the addition of bevacizumab to three different fluoropyrimidine regimens and showed that FOLFIRI plus bevacizumab was superior to IFL plus bevacizumab (OS 28 months versus 19.2 months; HR 0.79; $P = 0.037$) [61]. Toxicities were higher in the capecitabine plus irinotecan arm [62]. The result from these trials was also reflected in community practice as evident from the results of the First BEAT study. At median follow-up of 21 months, patients receiving bevacizumab plus combination chemotherapy had PFS and OS of 10.8 and 22.7 months, respectively [63].

Anti-EGFR monoclonal antibodies

Cetuximab has been shown to be effective in the first-line setting when added to an irinotecan doublet or oxaliplatin doublet. CRYSTAL was a phase III trial of cetuximab plus FOLFIRI compared to FOLFIRI alone in EGFR expressing mCRC patients. PFS was the primary endpoint. 1217 patients were enrolled and both PFS (8.9 months versus 8.0 months; HR 0.85; $P = 0.0479$) and ORR (47% versus 37%; $P = 0.0038$) were significantly higher in the cetuximab plus FOLFIRI arm [11,14,15]. OPUS, a large randomised phase II trial ($n = 344$), assessed the influence of cetuximab on ORR when added to FOLFOX-4 in a similar group of patients to CRYSTAL. The ORR (primary endpoint) was higher in the cetuximab plus FOLFOX arm (46% versus 36%) but a statistically significant increase in the odds ratio for a response was not established (OR 1.52; $P = 0.064$) [10]. As discussed earlier, patients with KRAS WT mCRC had better outcomes than mutant KRAS mCRC in both of these trials. Various phase II trials have studied the impact of the addition of cetuximab to oxaliplatin- or irinotecan-based chemotherapy and concluded that there was no significant difference in efficacy when cetuximab is added to fluoropyrimidine (5-FU or capecitabine) and oxaliplatin or irinotecan [64,65]. The results of these trials also raise questions regarding the best chemotherapy partner, which is discussed below. Cetuximab was also investigated as a second-line treatment in combination with irinotecan in patients who did not respond to oxaliplatin. In the EPIC trial, 1298 patients with EGFR-positive mCRC randomly received cetuximab plus irinotecan or irinotecan alone. Median OS, the primary endpoint, was similar: 10.7 months in the cetuximab plus irinotecan group and 10.0 months in the irinotecan only group (HR 0.975; $P = 0.71$). The PFS, however, was improved by the addition of cetuximab (4.0 months versus 2.6 months; HR 0.692; $P < 0.001$) as was the response rate (16.4% versus 4.2%; $P < 0.001$) [66]. 47% of patients in the irinotecan arm went on to receive cetuximab as post-trial therapy and this could well have confounded the OS benefit. Cetuximab was initially investigated as a treatment option for chemotherapy-refractory mCRC and in the pivotal BOND trial, cetuximab showed significant activity alone and in combination with irinotecan in this group of patients. The ORR was significantly higher than in the monotherapy group (22.9% versus 10.8%; $P = 0.007$). This increased ORR also translated to an increment of 1.6 months in PFS and a trend towards increased OS favouring the cetuximab and chemotherapy group [19]. The toxicities were manageable and were predominantly skin reactions, diarrhoea, infections and hypomagnesaemia.

Unlike bevacizumab, both the anti-EGFR moAbs have shown ORRs of around 10% when administered

as a monotherapy in chemotherapy-refractory mCRC. In comparison with BSC, cetuximab was associated with improvement in OS (6.1 months versus 4.6 months; HR 0.77, 95%CI 0.64–0.92; $P = 0.005$) and ORR (8.0% versus 0%; $P < 0.001$) in the CO.17 trial [29]. Consistent with the above finding, Van Cutsem and colleagues published the results of a phase III trial comparing panitumumab with BSC in a similar group of patients and demonstrated significantly prolonged PFS of 8.0 weeks and an ORR of 10% with panitumumab in comparison with 7.3 weeks and 0% with BSC. Nearly three quarters of patients in the BSC arm crossed over to receive panitumumab which probably explains the lack of OS advantage [28].

Use of combinations of biologicals plus chemotherapy

Based on the intriguing pre-clinical data indicating synergistic activity of anti-VEGF and anti-EGFR moAbs [67,68], trials have been conducted combining both bevacizumab and cetuximab or panitumumab along with chemotherapy. The proof of concept for dual inhibition of VEGF and EGF pathways came from the BOND-2 trial where cetuximab and bevacizumab were combined with irinotecan (CBI arm, $n = 43$) to evaluate the safety and feasibility of this combination in comparison with cetuximab and bevacizumab (CB arm, $n = 40$) in patients with irinotecan refractory mCRC. There was a convincing superiority for the CBI arm over the CB arm in all efficacy parameters: OS (14.5 months versus 11.4 months), median time-to-progression (7.3 months versus 4.9 months) and response rate (37% versus 20%), and there were no significant safety issues [69].

The PACCE (Panitumumab advanced colon cancer evaluation) trial evaluated the addition of panitumumab to bevacizumab plus chemotherapy which was either oxaliplatin (Ox-CT cohort, $n = 823$) or irinotecan based (Iri-CT cohort, $n = 230$). The Iri-CT cohort was used for safety analysis. The trial was discontinued when a planned interim analysis revealed increased toxicity and decreased PFS in the panitumumab arm. In the final analysis, when compared with the control arm, mCRC patients in the panitumumab arm had decreased PFS (10.4 months versus 11.4 months; HR 1.27, 95%CI 1.06–1.52), median survival (19.4 months versus 24.5 months) and RR (46% versus 48%) along with an increased incidence of toxicities. Panitumumab was associated with a worse outcome irrespective of KRAS mutation status [13]. Similarly, CAIRO2 randomly assigned mCRC patients to capecitabine, oxaliplatin and bevacizumab (CB arm, $n = 378$) or CB plus weekly cetuximab (CBC arm, $n = 377$) with PFS as primary endpoint. Addition of cetuximab resulted in an inferior PFS of 10.7 months in the CB arm and 9.4 months in the CBC arm ($P = 0.01$) with no significant difference in ORR or OS between the two groups [12]. These results suggest that combinations of dual biologicals can be detrimental and besides answering some important questions they have still left us with many unanswered questions. In the PACCE study, nearly two thirds discontinued treatment, without treatment failure, mostly due to adverse events. Relative dose intensity for chemotherapy was lower in the panitumumab arm (33% versus 42%). This could have adversely affected the efficacy of chemotherapy. Addition of cetuximab to oxaliplatin led to inferior outcomes in mutant KRAS mCRC both in OPUS and CAIRO2 raising the possibility of a negative interaction between cetuximab and oxaliplatin in this group. Unlike PACCE, there were no major unexpected adverse events in CAIRO2 and, again, there remains a possibility of a negative interaction between the antibodies. In light of the above results, the SWOG/CALBG 80405 intergroup trial [70], comparing FOLFOX- or FOLFIRI-based chemotherapy (investigators' choice) with either bevacizumab and/or cetuximab, has been amended to recruit patients with WT KRAS mCRC only. The oncology community will await the results before making firm conclusions against treatment with dual biologicals. The data from pivotal randomised studies of combination of biologicals and chemotherapy are summarised in Table 2.

Which is the best chemotherapy/biological partner?

A few years ago the most important challenge for oncologists was to find the right sequence in which to use oxaliplatin- and irinotecan-based chemotherapy. It is now evident that there is no advantage for any specific sequence and patients get better outcomes if all three active cytotoxics are used [71,72]. We are now in a similar situation with regards to finding the best partner and sequence with regards to using the three biologicals and cytotoxics.

Bevacizumab was investigated as a first treatment option and cetuximab primarily in chemotherapy-refractory mCRC. However, the results of trials (discussed above) have blurred the distinction between cetuximab and bevacizumab. In the context of neo-adjuvant therapy where tumour shrinkage is vital,

Table 2
Randomised studies of biological agents and chemotherapy regimens

Study	Setting	Treatment arms	PFS/TTP (mo)		OS (mo)		ORR%	
			Bio	Control	Bio	Control	Bio	Control
Bevacizumab								
AVF 2107g [34]	First-line	IFL ± bev	10.6	6.2	20.3	15.6	45%	35%
$n = 813$			$P < 0.001$		$P < 0.001$		$P = 0.004$	
ECOG 3200 [60]	2nd line	FOLFOX ± bev	7.3	4.7	12.9	10.8	22.7%	8.6%
$n = 829$			$P < 0.001$		$P = 0.0011$		$P < 0.001$	
NO16966 [43]	First-line	FOLFOX/XELOX ± bev	9.4	8.0	21.3	19.9	47%	49%
$n = 1401$			$P = 0.0023$		$P = 0.007$		$P = 0.31$	
Cetuximab								
BOND [19]	2nd/3rd line	Iri ± cet	4.1	1.5	8.6	6.9	23%	11%
$n = 329$			$P < 0.001$		$P < 0.001$		$P = 0.007$	
EPIC [66]	2nd/3rd line	Iri ± cet	4.0	2.6	10.7	10	16.4%	4.2%
$n = 1298$			$P < 0.0001$		$P = 0.007$		$P < 0.001$	
CRYSTAL [11]	First-line	FOLFIRI ± cet	8.9	8.0	19.9	18.6	46.9%	38.7%
$n = 1217$			$P = 0.036$		$P = 0.0011$		$P = 0.005$	
Dual biologicals plus chemotherapy								
CAIRO 2 [12]	First-line	cap/oxali/ bev± cet	9.8	10.7	20.3	20.4	43.9%	40.6%
$n = 755$			$P = 0.019$		$P = 0.21$		$P = 0.44$	
PACCE [13]	First-line	5FU/oxali/bev± pani	10.0	11.4	19.4	24.5	46%	48%
$n = 1053$			HR:1.27		HR:1.43			
		5FU/iri/bev ± pani	10.1	11.7	20.7	20.5	43%	40%
			HR:1.19		HR:1.42			

Abbreviations.PFS, progression-free survival; TTP, time to progression; OS, overall survival; mo, months; n, number of patients; bio, biologicals; bev, bevacizumab; cet, cetuximab; pani, panitumumab; oxali, oxaliplatin; iri, irinotecan; HR, hazard ratio.

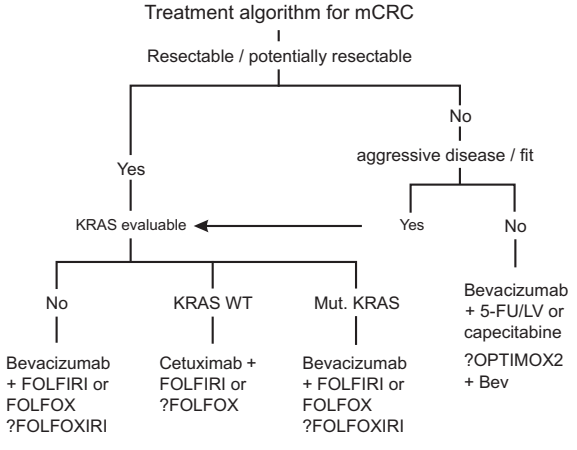
the optimal combination to downsize the metastatic disease needs to be defined and cetuximab appears to be associated with an increased response rate (first-line setting) and slightly better R0 resection rates but only in WT KRAS mCRC [10,11]. Bevacizumab, on the other hand, is beneficial in combination with active cytotoxic agents and induces response irrespective of the KRAS mutational status, but is not active as a single agent or in the chemotherapy-refractory setting [60]. To date, in the absence of head to head comparative data, both FOLFOX and FOLFIRI seem to be equal partners for bevacizumab. The case is similar for cetuximab but the available data suggests that cetuximab and oxaliplatin might possibly have a negative interaction in mutant KRAS mCRC. The CALGB 80203 study of FOLFIRI or FOLFOX ± cetuximab in the first-line treatment of mCRC might have given us an answer. However, the trial, initiated in 2004 with an accrual goal of 2200 patients, had to be stopped due to slow recruitment (owing to acceptance of bevacizumab as the standard first-line treatment) and analysis of data from 238 recruited mCRC patients was not sufficiently powered to analyse PFS and OS [73]. With the above information in mind it is reasonable to conclude that bevacizumab can be given in addition to FOLFOX or FOLFIRI in the first-line setting irrespective of KRAS status, and in WT KRAS mCRC, cetuximab can be added to FOLFIRI and probably FOLFOX to treat mCRC patients with no other contraindications, whilst we await the results of SWOG/CALGB 80405.

Treatment algorithm – aggressive and palliative context

There are different groups of mCRC patients based on their clinical presentation and an over simplified

classification could be: patients at risk of rapid disease progression, with good performance status (PS); patients with resectable or potentially resectable metastatic disease; and elderly patients with co-morbidities/young patients with borderline PS. The latter group is under-represented in clinical trials and robust evidence to guide treatment decisions is limited. The former groups require aggressive treatment with quick results in view of the narrow window of opportunity for intervention. There is evidence that bevacizumab is beneficial in the last group of mCRC from Kabbinavar and colleagues, where bevacizumab plus 5-FU/LV was superior to 5-FU/LV alone in terms of PFS (9.2 months versus 5.5 months; $P = 0.002$), OS (16.6 months versus 12.9 months; HR 0.79; $P = 0.19$) and RR (26.0% versus 15.5%; $P = 0.055$) [52]. Similarly, the pooled analysis of cohorts of older patients with mCRC also confirmed that bevacizumab is safely tolerated in combination with 5-FU/LV in elderly (age $\geqslant$65 years) patients [74]. There is no published data to support cetuximab monotherapy in this group of patients, though cetuximab has single agent activity.

On careful consideration of the available data regarding the KRAS mutational status, clinical benefits of combining biologicals with chemotherapy, potential negative interaction between oxaliplatin and cetuximab in KRAS WT mCRC, treatment options in an aggressive and palliative setting, we propose the following treatment algorithm:



Duration of therapy

The other challenge in integrating the biologicals with cytotoxics is to define the optimal duration of therapy. Despite participation in a predefined protocol stipulating continuation of treatment until progression, patients in most trials experience treatment discontinuation for reasons other than progression, though most of the discontinuations are due to toxicities. Efforts have been made to continue the effective therapy by using the "stop and go" strategy, as demonstrated in OPTIMOX1. The CONcePT (combined neurotoxicity prevention) trial explored the option of optimising FOLFOX plus bevacizumab by reducing the neurotoxicity of oxaliplatin by using intermittent oxaliplatin (IO) as per OPTIMOX strategy (patients alternated with and without oxaliplatin every eight cycles) and adding calcium/magnesium (Ca/Mg) as neuroprotectant and comparing in a 2×2 factorial design with continuous oxaliplatin (CO) and placebo. The trial was closed following review by the independent data monitoring committee, as patients receiving Ca/Mg had an inferior response rate compared to the control arm. However, on later independent radiology review, the RRs were not inferior in the Ca/Mg arm. Final analysis on the 139 patients showed that patients receiving intermittent oxaliplatin had a longer time-to-progression (25 weeks versus 18 weeks; HR 0.58, 95%CI 0.41–0.83; $P = 0.0025$) than CO [75]. Despite the small size of the trial, due to early discontinuation, this "stop and go" strategy seems to be a promising approach in maintaining patients on effective treatment for longer duration. The recently completed COIN trial using cetuximab and oxaliplatin/fluoropyrimidine as continuous and intermittent treatment could add further evidence [76].

Treatment beyond progression

The exact mechanism of action of bevacizumab is not clearly understood and inhibition of neovascularisation, vascular regression, vascular normalisation, direct anti-tumour activity, offsetting the effect of chemotherapy induction of VEGF levels and inhibition of VEGF repression of dendritic cell function have all been proposed to be the possible mechanism of action [77]. There is a concern of rapid tumour regrowth and rebound increase in VEGF levels following withdrawal of VEGF inhibitors. Based on the above concerns there might be a case for continuation of bevacizumab after treatment progression. Data from the US BRiTE registry supports this concept. In this large observational cohort study, previously untreated mCRC patients ($n = 1445$) who had disease progression were given bevacizumab beyond first progression (BBP), post-progressive disease treatment without bevacizumab and no post-progressive disease treatment. OS and PFS for the overall study population were 25.1 and 10.0 months respectively. Patients in the BBP group had an OS of 31.8 months compared with 19.9 and 12.6 months in the post-progressive disease

treatment group and no post-progressive disease treatment group, respectively [78]. Notwithstanding the limitations of an observational study, this suggests that continued VEGF inhibition plays a role in controlling the disease. This hypothesis is prospectively tested in the SWOG 0600/iBET (irinotecan Bevacizumab continuation trial) [79]. Patients who have progressed after oxaliplatin and bevacizumab are randomised to irinotecan plus cetuximab ± bevacizumab. This trial can answer the BBP question and together with SWOG/CALGB 80405 can throw more light on the use of dual biologicals with chemotherapy.

Conclusion

Biologicals have clearly increased the therapeutic armamentarium for patients with mCRC and never before have physicians been posed with such challenging questions on how to maximise patient benefit while taking into account toxicity and expense. Better understanding of molecular pathways and mechanisms of resistance along with collaborative efforts between clinicians and scientists should, in the foreseeable future, allow us to tailor therapy to the individual patient.

Conflict of interest statement

None declared.

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