REVIEW

BJC

British Journal of Cancer (2014) 111, 1122–1131 | doi: 10.1038/bjc.2014.404

Keywords: colorectal; biological; meta-analysis

The role of biological therapy in metastatic colorectal cancer after first-line treatment: a meta-analysis of randomised trials

E Segelov[1], D Chan*[,2], J Shapiro[3], T J Price[4], C S Karapetis[5], N C Tebbutt[6] and N Pavlakis[2]

[1]St Vincent's Clinical School, University of New South Wales, Sydney, NSW 2052, Australia; [2]Royal North Shore Hospital, St Leonards, Sydney, NSW 2065, Australia; [3]Monash University and Cabrini Hospital, Melbourne, VIC 3800, Australia; [4]The Queen Elizabeth Hospital and University of Adelaide, Woodville South, SA 5011, Australia; [5]Flinders University and Flinders Medical Centre, Flinders Centre for Innovation in Cancer, Bedford Park, SA, 5042, Australia and [6]Austin Health, VIC 3084, Australia

Purpose: Biologic agents have achieved variable results in relapsed metastatic colorectal cancer (mCRC). Systematic meta-analysis was undertaken to determine the efficacy of biological therapy.

Methods: Major databases were searched for randomised studies of mCRC after first-line treatment comparing (1) standard treatment plus biologic agent with standard treatment or (2) standard treatment with biologic agent with the same treatment with different biologic agent(s). Data were extracted on study design, participants, interventions and outcomes. Study quality was assessed using the MERGE criteria. Comparable data were pooled for meta-analysis.

Results: Twenty eligible studies with 8225 patients were identified. The use of any biologic therapy improved overall survival with hazard ratio (HR) 0.87 (95% confidence interval (CI) 0.82–0.91, $P<0.00001$), progression-free survival (PFS) with HR 0.71 (95% CI 0.67–0.74, $P<0.0001$) and overall response rate (ORR) with odds ratio (OR) 2.38 (95% CI 2.03–2.78, $P<0.00001$). Grade 3/4 toxicity was increased with OR 2.34. Considering by subgroups, EGFR inhibitors (EGFR-I) in the second-line setting and anti-angiogenic therapies (both in second-line and third-line and beyond settings) all improved overall survival, PFS and ORR. EGFR-I in third-line settings improved PFS and ORR but not OS.

Conclusions: The use of biologic agents in mCRC after first-line treatment is associated with improved outcomes but increased toxicity.

The efficacy of biologic agents or 'molecular targeted therapy' in first-line treatment of metastatic colorectal cancer (mCRC) has been extensively investigated. Their role in second-line therapy and beyond, however, has not been systematically examined. For this review, biological agents were defined as drugs targeting specific cancer cell growth factors, receptors and molecular pathways including the epidermal growth factor receptor (EGFR) and angiogenesis pathways.

The objective of this systematic review and meta-analysis was to identify, describe and summarise the benefits of biologic therapies in addition to standard care in patients with mCRC following progression during or after first-line systemic therapy. Two clinical scenarios were addressed: biologic therapy added to standard treatment compared with standard treatment alone (chemotherapy in second line, best supportive care (BSC) in third line and beyond); and biologic therapy added to standard treatment compared with a different biologic therapy added to same standard. The systematic review examined the effect of biologic agents on overall survival (OS), progression-free survival (PFS), and overall response rate (ORR) and documented toxicity and quality of life (QOL).

*Correspondence: Dr D Chan; E-mail: dlhchan1@gmail.com

Received 18 March 2014; revised 7 June 2014; accepted 16 June 2014; published online 29 July 2014

MATERIALS AND METHODS

Search strategy. The authors agreed on study protocol prior to literature search and analysis although this was not centrally registered. The search strategy comprised analysis of: *MEDLINE* 1946-May 2012, *EMBASE* 1974–April 2012, *BIOSIS* 1992–May 2012 and *COCHRANE reviews* until May 2012 (for details, see Supplementary Methods) to identify relevant published randomised Phase II and III studies.

Proceedings of the American Society of Clinical Oncology Annual Scientific Meeting 2009–2013, ASCO Gastrointestinal Cancers Symposium 2010–2013 and the European Society for Medical Oncology Annual Scientific Meeting 2009–2012 were searched by hand. For studies available in abstract only, investigators were contacted for required information.

Patient characteristics. Studies involved patients with histologically confirmed mCRC who had received at least one prior line of chemotherapy for advanced disease. The trials investigated the addition of biological agent to chemotherapy, compared with either chemotherapy alone (Group 1) or the addition of a second biological agent to the same chemotherapy (Group 2).

Study review and inclusion. Two authors (ES/NP) independently reviewed titles and abstracts and agreed on articles to be retrieved. Studies included were registered RCTs evaluating second- or third-line (or beyond) therapy for mCRC, which reported at least one of the following: OS, PFS, ORR and toxicity.

Given the demonstrated efficacy of EGFR inhibitors (EGFR-I) to *KRAS* wild-type (WT) patients, only analysis of this population within EGFR-I trials was included. Potential studies were assessed independently by two reviewers (JS/ES) blinded to authors, journal, sponsor and results. Disagreement was resolved by a third reviewer (NP). Bias was assessed using the MERGE criteria. (Liddle *et al*, 1996) Two reviewers (NP/ES) independently extracted the most recent data from identified trials.

In order to perform a systematic review, categorisation of similar trials was required. Agents were categorised according to mode of action into the following subgroups:

- The monoclonal antibody EGFR-I: Cetuximab (Cunningham *et al*, 2004; Karapetis *et al*, 2008) and panitumumab, (Amado *et al*, 2008; Peeters *et al*, 2010; Hecht *et al*, 2012; Seymour *et al*, 2013). Anti-angiogenesis agents directed against vascular endothelial growth factor (VEGF): The monoclonal antibody bevacizumab (Giantonio *et al*, 2007; Hecht *et al*, 2012), the VEGF-trap agent aflibercept (Van Cutsem *et al*, 2012), and the VEGF receptor tyrosine kinase inhibitors (VEGFR TKIs) regorafenib (Grothey *et al*, 2013), brivanib (Siu *et al*, 2013) and vatalanib (PTK/ZK) (Van Cutsem *et al*, 2011).
- Newer targeted agents with mixed or novel mechanisms of action (target): Conatumumab (Death Receptor 5), ganitumumab and dalotuzumab (IGF-1 R), rilotumumab (HGF), tivantinib (MET), sorafenib (VEGFR/PDGFR/RAF) and vandetanib (VEGF/EGF/RET).

For the clinical scenario of the addition of one biologic agent compared with another, only three trials were identified, investigating these agents and targets: axitinib (VEGF 1/2/3), cediranib (VEGFR TKI), and panitumumab.

Outcome measures and data analysis. The outcomes of OS, PFS, ORR, toxicity and QoL were analysed based on trial-level data. Details of prior therapy could not be extracted from trial publications. Given possible data pointing toward differing efficacy by line of chemotherapy, analysis was stratified into second-line trials and trials investigating third-line (or later) setting. For the newer targeted agents, given most data were in predominantly second-line or mixed settings, a decision was made to analyse results as a single group. Results were reported according to PRISMA guidelines.

Data analysis was performed using REVMAN 5 for Windows. Individual RRs were pooled and 95% confidence interval (CI) was generated using the Mantel–Haenszel fixed-effects method. Unfortunately, analysis by prior therapy was not possible given usage of trial-level rather than individual patient data.

Meta-analysis of the log hazard ratio (HR) and log upper and lower CIs was performed. The outcome was considered statistically significant if 95% CI for relative risk did not cross 1.

OS and PFS. For each trial, a HR and corresponding standard error was calculated. This was computed by the software in all cases except for Yang *et al* (2009) where PFS was derived by hand from the 80% CI.

ORR. This was calculated as the proportion of patients who achieved partial or complete response. Odds ratios (OR) for response were generated and the individual ratios pooled to give a clinically useful measure of effect.

Toxicity. Data were extracted on incidence of Grade 3 and 4 toxicity combined and Grade 5 toxicity separately with OR and pooled difference in toxicity calculated as for ORR. Detailed statistical analysis for risk of toxicity is presented for the combined cohort. Subgroup analyses are presented in Supplementary data.

Where there were >2 arms in a study, the study was entered twice in the data set (i.e. treated as two separate trials) with the number in the control group divided such that the total number added up to the original group size (as recommended by Cochrane Collaboration; The Cochrane Collaboration).

Heterogeneity was assessed using χ^2 and I^2 tests, with values of $P<0.10$ and $I^2>50\%$ indicating substantial heterogeneity. Where this was identified, the reasons were explored and DerSimonian-Laird random-effects model performed for that outcome. This accounts imperfectly for heterogeneity, and must be taken with caution when the number of trials is less than five (Kontopantelis *et al*, 2013). Sensitivity analyses and funnel plots were undertaken to investigate possible bias.

Quality of life. Where available, QoL data were abstracted and the instrument noted. As there was insufficient data for quantitative analysis, QOL end points were reported descriptively.

RESULTS

Study selection. The literature search identified 218 citations; conference abstracts yielded an additional 331 references. Thirty-four papers representing 20 studies comprising 8225 patients (Table 1, Supplementary Figure 1) were eligible for inclusion in the meta-analysis. Three pivotal studies were excluded: BOND (Cunningham *et al*, 2004), as cetuximab was administered in both arms; BOND2, as cetuximab and bevacizumab were administered in both arms; and EPIC, as analysis by *KRAS* status was available for only 300/1298 patients, with incomplete OS and PFS data (HRs only without CIs).

Risk of bias. The overall quality of the studies was good. Funnel plots (Supplementary Figures) show relative symmetry, arguing against significant publication bias, for all parameters except ORR. Here, the imbalance is not considered biologically plausible (i.e. significant worsening of ORR with addition of biologic), hence likely represents chance.

Table 1. Study Characteristics

Trial name	Line of therapy	Author, year	Experimental arm(s)	Standard arm	# pts.	MERGE Quality
Studies evaluating the addition of biological therapy to standard treatment						
EGFR-I second line						
Study 181	2	Peeters *et al*, 2010	Panitumumab + FOLFIRI	FOLFIRI	597	A
PICCOLO	2	Seymour *et al*, 2013	Panitumumab + Irinotecan	Irinotecan	460	A
EGFR-I third line+						
CO.17	3+	Karapetis *et al*, 2008	Cetuximab	BSC	243 (KRAS WT)	A
	3+	Amado *et al*, 2008	Panitumumab	BSC	230 (KRAS WT)	A
Anti-VEGF second line						
E3200	2	Giantonio *et al*, 2007	Bevacizumab +FOLFOX	FOLFOX	577	A
TML	2	Arnold, 2012	Bevacizumab + FOLFOX/FOLFIRI	FOLFOX/FOLFIRI	820	A
VELOUR	2	Van Cutsem *et al*, 2012	Aflibercept+FOLFIRI	FOLFIRI	1226	A
BEBYP	2	Masi *et al*, 2013	Bevacizumab + FOLFOX/FOLFIRI	FOLFOX/FOLFIRI	184	B2
CONFIRM2	2	Van Cutsem *et al*, 2011	Vatalanib (PTK/ZK) + FOLFOX	FOLFOX	855	A
Anti-VEGF third line+						
CO.20	3+	Siu *et al*, 2013	Brivanib + Cetuximab	Cetuximab	750	A
CORRECT	3+	Grothey *et al*, 2013	Regorafenib	BSC	760	A
Agents against multiple/ novel targets						
	2+	Yang *et al*, 2009	Vandetanib 300 + FOLFOX, Vandetanib 100 +FOLFOX	FOLFOX6	104	B1
	2+	Eng *et al*, 2011	Ganitumumab + Panitumumab, Rilotumumab + Panitumumab,	Panitumumab	142	B2
	3+	Watkins *et al*, 2011	Dalotuzumab 15 + Cetuximab + Irinotecan, Dalotuzumab 10 + Cetuximab + Irinotecan,	Cetuximab+ Irinotecan	345	B1
	2+	Cohn *et al*, 2013	Conatumumab + FOLFIRI, Ganitumab + FOLFIRI	FOLFIRI	155	A
	2	Eng *et al*, 2013	Tivatinib + Cetuximab + Irinotecan	Cetuximab + Irinotecan	117	B1
	2	Hoehler *et al*, 2013	Sorafenib	Placebo	97	B1
Studies comparing combination of chemotherapy with one targeted agent to combination with another targeted agent						
	2	Bendell, 2011	Axitinib + FOLFOX/FOLFIRI	Bevacizumab + FOLFOX/FOLFIRI	171	B1
HORIZONI	2	Cunningham *et al*, 2004	Cediranib 20 + FOLFOX, Cediranib 30 + FOLFOX	Bevacizumab + FOLFOX	210	A
SPIRITT	2	Hecht *et al*, 2012	Panitumumab + FOLFIRI	Bevacizumab + FOLFIRI	182	B1

GROUP 1: THE EFFECT OF ANY BIOLOGIC AGENT ADDED TO STANDARD THERAPY

Overall survival. Fifteen studies, involving 17 comparisons, reported OS HRs. Using fixed-effects meta-analysis the OS HR was 0.87 (95% CI 0.82–0.91, $P<0.00001$, Figure 1). As expected, there was significant heterogeneity given variation in type of agent and clinical settings (second and third-line therapy). Random-effects analysis was, therefore, performed confirming OS benefit with HR 0.88 (95% CI 0.81–0.97, $P=0.008$).

Progression-free survival. Seventeen studies involving 21 comparisons demonstrated PFS benefit for use of biological agents with fixed-effects HR 0.71 (95% CI 0.67–0.74, $P<0.0001$, Figure 2) and random-effects analysis (given heterogeneity) HR 0.75 (95% CI 0.67–0.85, $P<0.0001$).

Overall response rate. Fourteen studies involving 17 comparisons allowed fixed-effects meta-analysis, which demonstrated pooled RR benefit of +8.6% (OR 2.38; 95% CI 2.03–2.78, $P<0.00001$, Figure 3). Despite significant heterogeneity, benefit was preserved on random-effects modelling with OR 2.04 (95% CI 1.48–2.86, $P<0.0001$).

Toxicity. Fifteen studies involving 19 comparisons were analysed and demonstrated, significantly, increased risk of Grade 3/4 toxicity with OR 2.34 (95% CI 2.12–2.59, $P<0.00001$, Figure 4) on fixed-effects modelling and OR 2.14 (95% CI 1.70–2.69, $P<0.00001$) on random-effects modelling.

Fourteen studies involving 18 comparisons reported Grade 5 (fatal) toxicity. The addition of a targeted agent did not significantly increase the risk of Grade 5 toxicity with pooled rate +0.6% and OR 1.14 (95% CI 0.72–1.81, $P=0.58$), with minimal heterogeneity.

SUBGROUP ANALYSIS BY TARGETED THERAPY CLASS

EGFR inhibitors. Considered as a group, the use of EGFR-I for *KRAS* WT patients in any setting was associated with a benefit to OS with HR 0.87 (95% CI 0.77–0.97, $P=0.01$), PFS with HR 0.62 (95% CI 0.55–0.70, $P<0.00001$) and ORR with pooled benefit +21.6% and OR 5.30 (95% CI 3.85–7.30, $P<0.00001$). Significant heterogeneity was present, and on random-effects modelling, OS benefit was no longer apparent with HR 0.84 (95% CI 0.66–1.06, $P=0.15$) but PFS benefit was preserved with HR 0.57 (95% CI 0.42–0.79, $P=0.0007$).

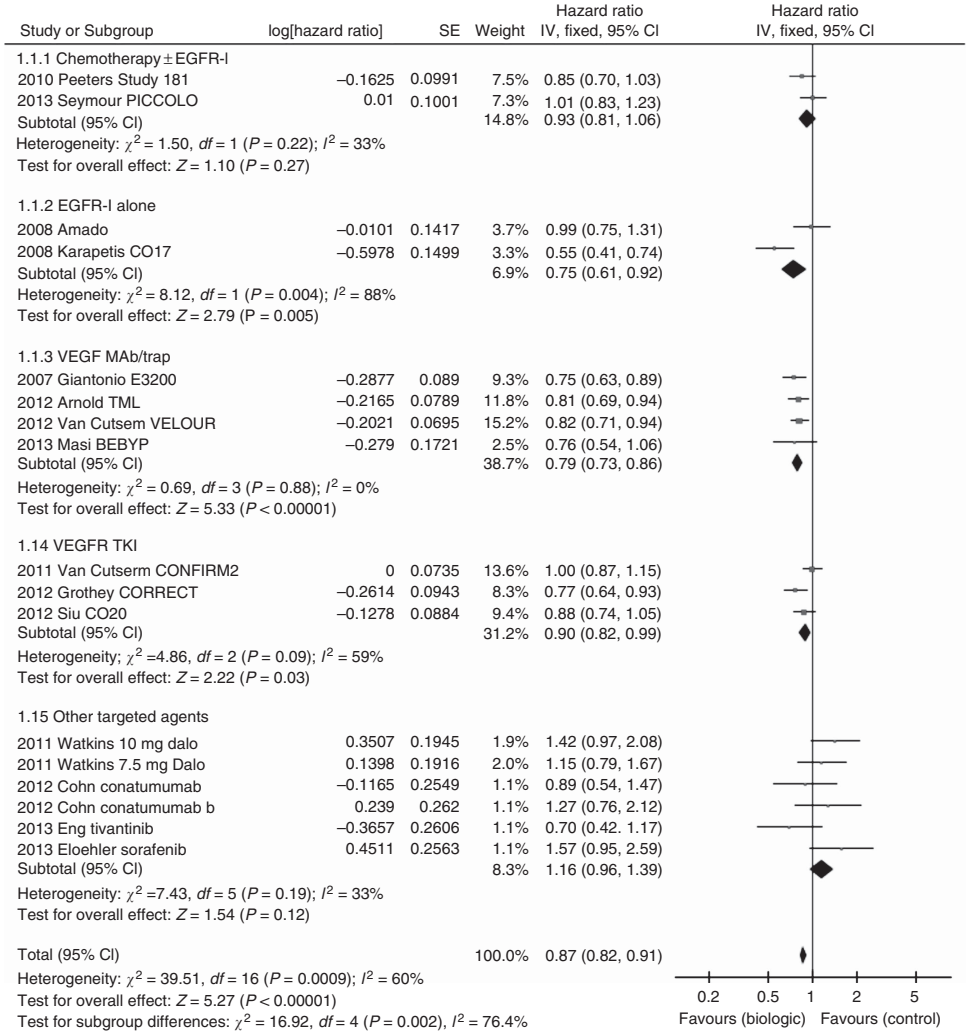

Figure 1. Forest plot for OS.

EGFR-I in second-line setting. Two trials – Study 181 (Peeters *et al*, 2010) and PICCOLO (Seymour *et al*, 2013) – investigated EGFR-I in the second-line setting, both in combination with (irinotecan-based) chemotherapy. Meta-analysis in 1057 *KRAS* WT patients demonstrated no improvement in OS with HR 0.93 (95% CI 0.81–1.06, $P=0.27$), however, significant improvement in PFS with HR 0.76 (95% CI 0.65–0.87, $P=0.0002$) and ORR with pooled RR +24.0% and OR 4.44 (95% CI 3.20–6.18, $P<0.00001$). No significant heterogeneity was present.

EGFR-I in third-line setting and beyond. Two trials (CO.17 (Karapetis *et al*, 2008) and Amado 2008 (Amado *et al*, 2008)) were identified involving 473 *KRAS* WT patients; both used EGFR-I as monotherapy. Benefit was demonstrated for OS with HR 0.75 (95% CI 0.61–0.92, $P=0.005$), PFS with HR 0.42 (95% CI 0.35–0.52, $P<0.0001$), and ORR with pooled RR +16% and OR 42.29 (95% CI 5.76–310.58, $P=0.0002$).

Significant heterogeneity was noted for OS, likely attributable to crossover in the Amado study. Using a random-effects model, the OS benefit was no longer significant with HR 0.74 (95% CI 0.42–1.32, $P=0.30$).

Anti-angiogenic agents. The use of any anti-angiogenic agent in any setting was associated with OS benefit with HR 0.84 (95% CI 0.79–0.89, $P<0.00001$), PFS benefit with HR 0.68 (95% CI 0.64–0.72, $P<0.00001$) and ORR with pooled RR +4.8% and OR 2.01 (95% CI 1.62–2.49, $P<0.00001$). There was considerable statistical heterogeneity for PFS, attributable to differences in class of drug, line of therapy and prior anti-angiogenic treatment. Random-effects meta-analysis showed preservation of benefit, with PFS HR of 0.67 (95% CI 0.59–0.77, $P<0.00001$).

Anti-angiogenic agents in second-line setting. Five trials involving 3662 patients E3200 (Giantonio *et al*, 2007), TML (Bennouna *et al*, 2013), VELOUR (Van Cutsem *et al*, 2012), BEBYP (Masi *et al*, 2013), CONFIRM2 (Van Cutsem *et al*, 2011)) were identified investigating the addition of bevacizumab, aflibercept or vatalanib in the second-line setting. Fixed-effects meta-analysis demonstrated benefit for OS with HR 0.84 (95% CI 0.78–0.91, $P<0.00001$), PFS with HR 0.72 (95% CI 0.67–0.77, $P<0.00001$), and ORR with pooled RR +7.2% and OR 2.00 (95% CI 1.57–2.54, $P<0.00001$). Heterogeneity in ORR was noted but benefit was preserved using random-effects modelling with OR 1.89 (95% CI 1.28–2.80, $P=0.001$). Sensitivity analysis with exclusion of BEBYP(Masi *et al*, 2013) study (judged as lesser quality owing to poor accrual) did not alter results, retaining benefit to OS (HR 0.85), PFS (HR 0.72) and ORR (OR 2.11).

Anti-angiogenic agents in third-line setting and beyond. Two trials – CORRECT (Grothey *et al*, 2013) and CO.20 (Siu *et al*, 2013) – were analysed investigating regorafenib and brivanib, respectively, in a total of 1510 patients. Benefit was shown for OS with HR 0.83 (95% CI 0.73–0.94, $P=0.003$), PFS with HR 0.60 (95% CI 0.54–0.67, $P<0.00001$) and ORR with pooled RR +0.9%

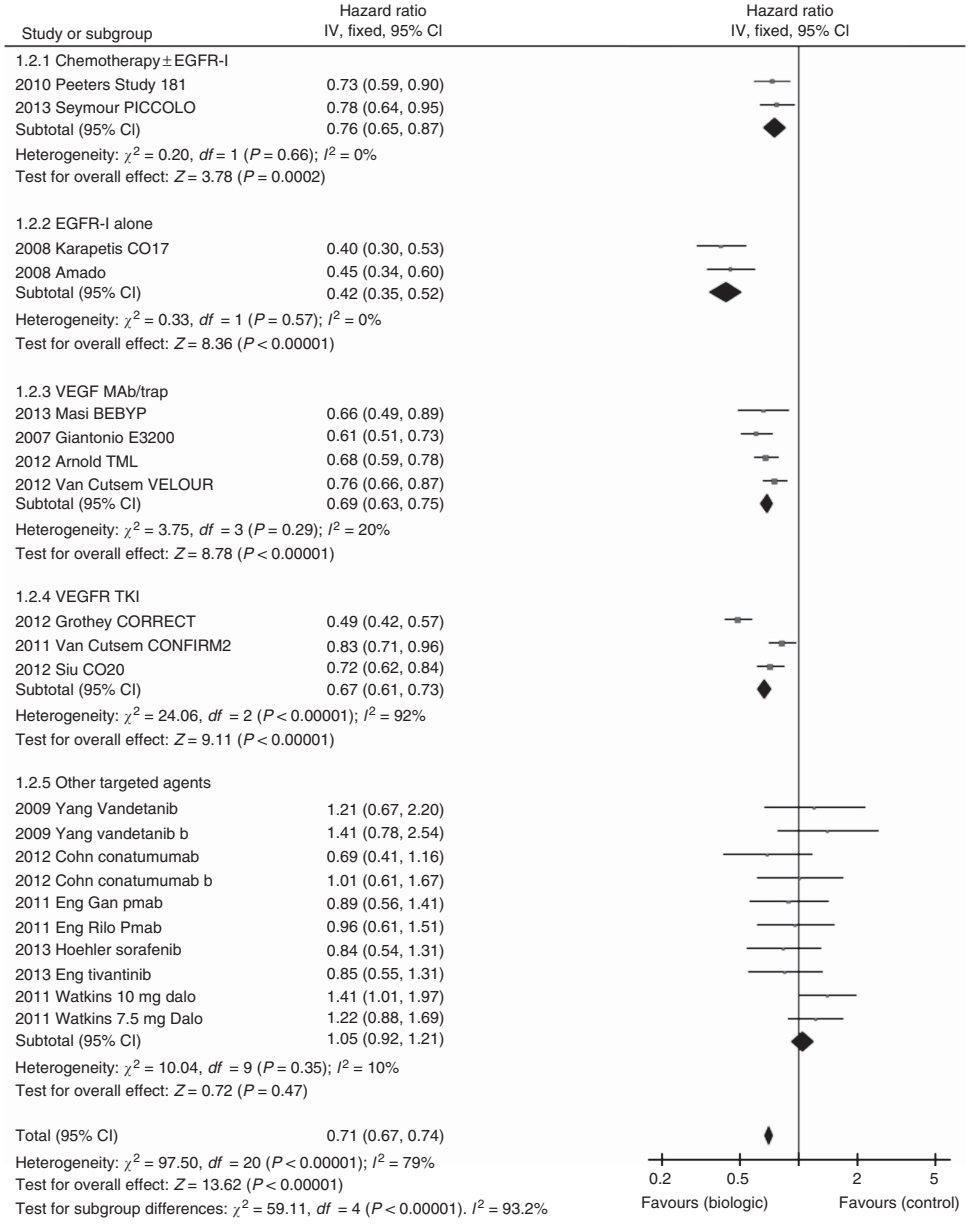

Figure 2. Forest plot for PFS.

and OR 2.05 (95% CI 1.27–3.30, $P = 0.003$). Given considerable heterogeneity in PFS, random-effects modelling was performed, which preserved PFS benefit (HR 0.59, 95% CI 0.41–0.87, $P = 0.007$). The heterogeneity was thought to be predominantly due to the different study settings and populations. The CORRECT study examined the effect of regorafenib in chemotherapy-refractory patients, of which 48% had received four or more prior lines of therapy but all were ECOG 0–1. The CO.20 trial investigated *KRAS* WT patients with both arms receiving cetuximab, of whom 91% had received four or more prior lines of therapy but that also allowed enrolment of ECOG two patients.

Other targeted agents. Six trials involving 960 patients investigated the addition of targeted agents not primarily directed against EGFR or VEGF/VEFGR – namely, conatumumab, ganitumab, dalotuzumab, rilotumumab, tivantinib, sorafenib and vandetanib. Given the varied modes of action of the above agents, meta-analysis was not performed.

Sensitivity analysis. Remodelling of analysis of overall effect to exclude the six trials of 'other targeted agents', as they are not currently used in clinical practice, preserved benefit in OS with HR 0.84 (95% CI 0.80–0.89, $P < 0.00001$), PFS with HR 0.67 (95% CI 0.63–0.70, $P < 0.00001$) and ORR with OR 2.79 (95% CI 2.34–3.33, $P < 0.00001$).

Given the high degree of crossover in the Amado study, the effect of its exclusion from analysis of all EGFR-I (1.1) was investigated. A significant benefit to OS was maintained on fixed-effects modelling (HR 0.84, 95% CI 0.74–0.95) but was again absent on random-effects modelling (HR 0.79, 95% CI 0.58–1.08).

Similarly, analysis to include data from the EPIC trial (entire cohort) did not change results for second-line EGFR-I, with OS HR 0.95 (95% CI 0.86–1.05), PFS HR 0.72 (95% CI 0.65–0.78), ORR OR 4.46 (95% CI 3.43–5.81).

GROUP 2: COMPARING ONE BIOLOGIC THERAPY WITH ANOTHER

There were only three eligible trials, with five comparisons, in a total of 551 patients, where one biologic therapy (axitinib,

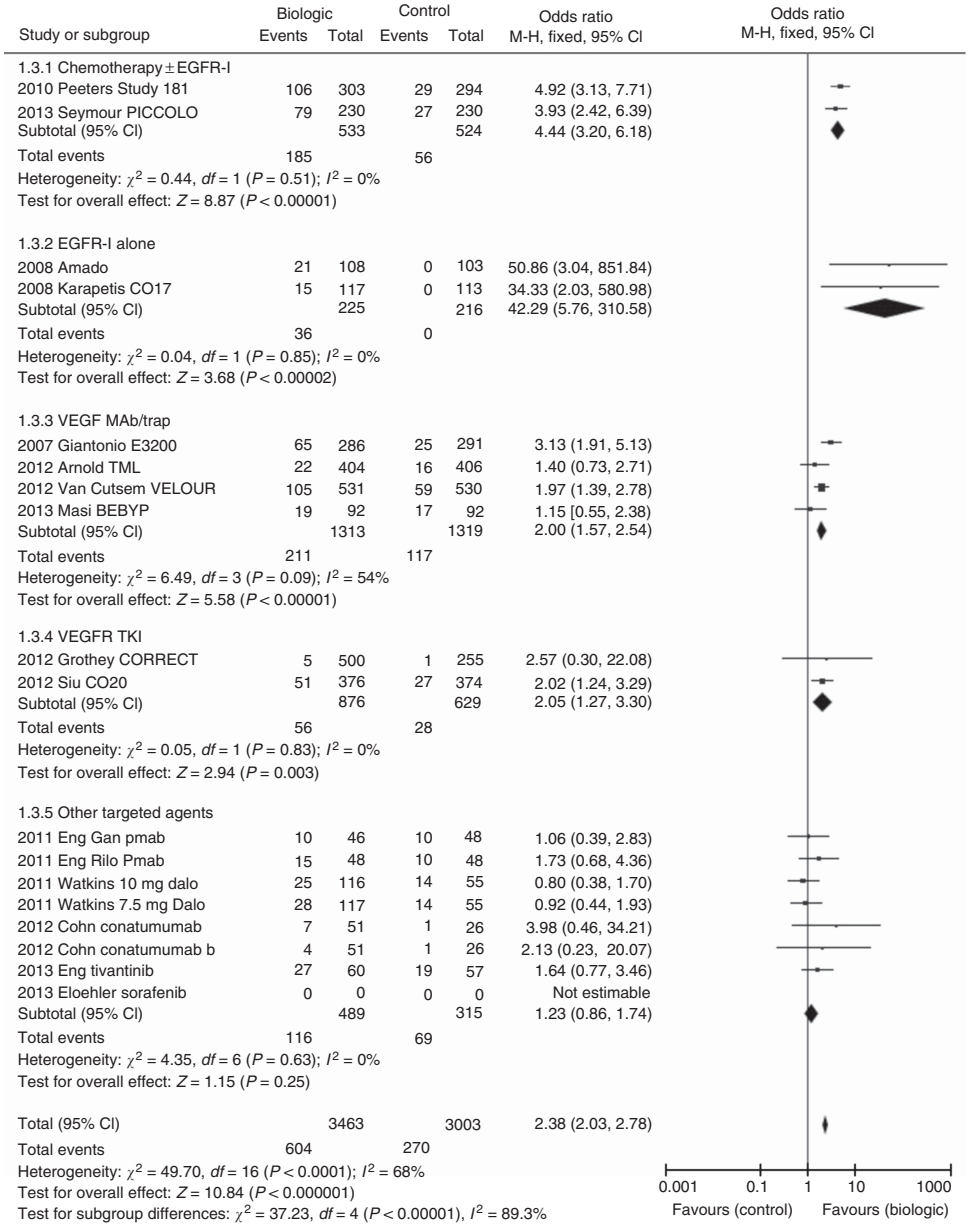

Figure 3. Forest plot for ORR.

cediranib and panitumumab) was compared with different biologic (bevacizumab) added to the same standard treatment, so that the trials were appropriate for comparison. There was no significant overall difference in OS for any of the experimental biological agents with HR 1.07 (95% CI 0.88–1.29, $P = 0.51$, Figure 5), PFS with HR 1.14 (95% CI 0.93–1.40, $P = 0.20$, Supplementary Figure 6) and ORR with OR 0.69 (95% CI 0.46–1.04, $P = 0.07$).

No significant difference in the incidence of overall Grade 3/4 toxicity was present with OR 0.70 (95% CI 0.40–1.20), $P = 0.19$ (Figure 6).

Quality of life. Only 5 of the 20 studies reported QoL data (Table 2). The two studies of EGFR-I reported significant QoL improvement: CO.17 with cetuximab monotherapy *vs* BSC and the PICCOLO study in second-line treatment examining cetuximab with chemotherapy. By contrast, significant deterioration in QoL was recorded with the addition of brivanib to cetuximab in the CO.20 study. Regorafenib and cediranib did not alter QoL.

DISCUSSION

Despite the sometimes nihilistic view of lack of progress in the management of patients with mCRC, when considered as a therapeutic class, the addition of either EGFR or angiogenesis inhibitors to standard therapy for mCRC beyond the first-line setting has impacted positively on overall survival as well as progression-free survival. As a group, these agents also improved overall response rate and did not diminish overall QOL. As expected, the rate of any Grade 3/4 toxicity was increased, but there was no increase in treatment-related mortality. The size of benefit is analysed in detail in this systematic review, to inform discussion regarding best placement of each biological agent in the treatment paradigm, as the optimal sequencing of these drugs has not been clearly defined. Cost-effectiveness, not analysed here, is also important.

There are several strengths to this study. By identifying all relevant trials, it rigorously shows that the addition of biological

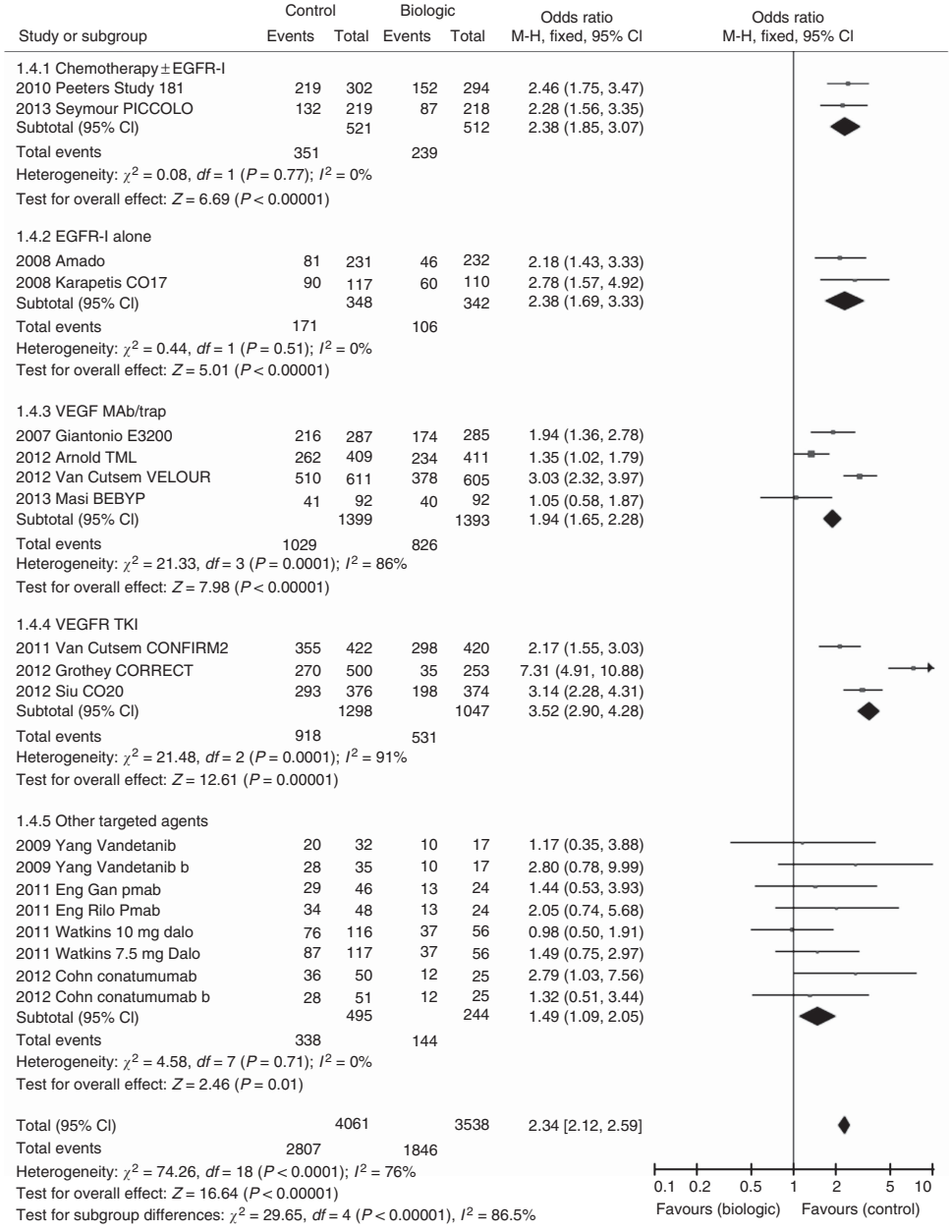

Figure 4. Forest plot for Grade 3/4 toxicity.

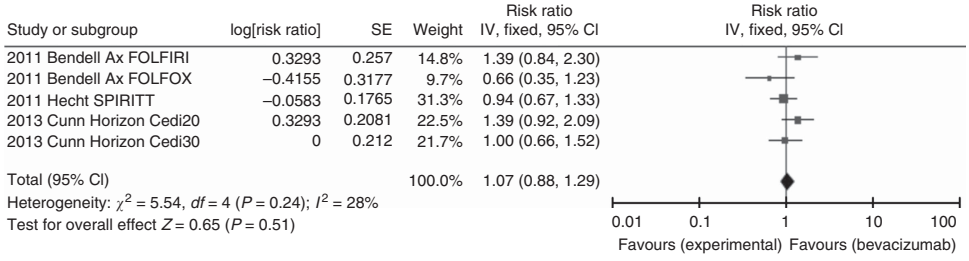

Figure 5. Forest plot for OS-chemotherapy + targeted agent 1 *vs* chemotherapy + bevacizumab.

therapy improves outcomes. The large number of trials and patients lent itself to a meta-analytic approach.

There are also limitations to the current study. The meta-analysis of anti-angiogenic agents as a group provides a broad answer to the questions posed as it pools both bevacizumab and aflibercept data together in analysis, despite slightly differing modes of action. The number of trials in some subgroups is small. This makes random-effects modelling less certain. It also highlights the difficulty in performing subsequent trials with new agents once proof of efficacy is established. The use of individual patient data would have been ideal, and work is underway to obtain this information to enable further analysis.

With respect to agents targeting EGFR, the monoclonal antibodies cetuximab and panitumumab overall added OS and

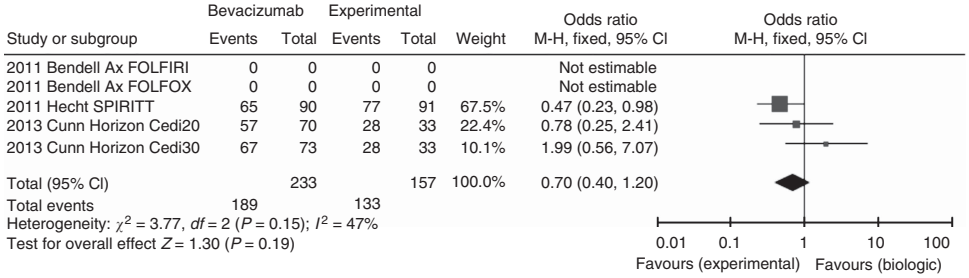

Figure 6. Forest plot for Grade 3/4 toxicity-chemotherapy + targeted agent 1 *vs* chemotherapy + bevacizumab.

Table 2. Quality of life data for included trials

Study title	Treatment	Control	*N*	QoL instrument	QoL effect	*P*-value	Details
PICCOLO	Irinotecan + Panitumumab (IrPan)	Irinotecan	597	EORTC QLQ-C30, EQ-5D, dermatology life quality index	Significantly better No data	0.032 N/A	QLQ-C30 global scores favoured IrPan group (56.4 *vs.* 49.5), but QoL symptom scores worse with IrPan
CO.17	Cetuximab	BSC	243 (*KRAS* WT)	EORTC QLQ-C30	Significantly better	0.0002	Mean diff at 8wk 10.9 (95% CI 4.2–17.6, $P = 0.0002$), 16 wk 17.9 (95% CI 7.6–28.2, $P < 0.0001$)
CO.20	Brivanib + Cetuximab	Cetuximab	750	EORTC QLQ-C30	Significantly worse	0.02	Global and physical QoL scores deteriorated significantly faster in brivanib arm compared with placebo
CORRECT	Regorafenib	BSC	760	EORTC QLQ-C30, EQ-5D index, EQ-5D VAS	Not significantly different	N/A	
Cediranib	Cediranib 20 + FOLFOX, Cediranib 30 + FOLFOX	Bevacizumab + FOLFOX	210	FACT-C symptom index (FCSI) subscale; trial outcome index (TOI), total FACT score	Not significantly different	0.15 (FCSI Cedi 20), 0.22 (FCSI Cedi 30)	Time to worsening of TOI, Total FACT score also non-significant

PFS benefit to standard therapy. However, no OS advantage was seen when used in the second-line setting (in combination with chemotherapy), although there was benefit for PFS and ORR. The lack of OS benefit is most likely attributable to the crossover to later receive anti-EGFR therapy, which occurred in both trials. Subsequent EGFR-I use, allowed by protocol, was reported in 31% of patients in the FOLFIRI-only arm of Study, 181 compared with 10% in the FOLFIRI-panitumumab arm, although only in 6% of the chemotherapy alone arm in the PICCOLO study, compared with $<0.5\%$ in the irinotecan-panitumumab arm.

The impact of crossover is strikingly demonstrated in the third-line setting. In the CO.17 study, which demonstrated significant OS benefit, crossover was not allowed from BSC to cetuximab, with only 13/285 patients later receiving EGFR-I. By contrast, in the Amado trial, which allowed crossover, this occurred in 90/119 *KRAS* WT patients resulting in no OS benefit being demonstrated. Even with the exclusion of the Amado study from analysis, however, no OS benefit was demonstrated on random-effects modelling.

Another explanation for the lack of OS benefit of the EGFR-I in the second-line setting is the issue of patient selection. New data strongly support tumour testing for additional RAS mutations, which have also been shown to be robust negative predictive factors for EGFR-I response. Extended *KRAS* testing beyond the traditional examination of exon 2 to include mutational hotspots in exons 3 and 4, as well as the *NRAS* gene (exons 2, 3 and 4) and (in some series) *PI3KCA* exon 20, has been established from the analysis of several first-line EGFR-I trials: PRIME (Douillard *et al*, 2013) and FIRE-3 (Stintzing *et al*, 2012). Extended mutation analysis in the EFGR-I studies in second-line therapy may alter the OS data in the 'pure' WT subgroups and, these data are eagerly awaited. As both studies in the second-line EGFR-I analysis used irinotecan backbones, concerns regarding possible negative interactions between oxaliplatin and EGFR-I are not relevant.

Current opinion is divided on best placement of EGFR-I use in the treatment pathway of mCRC. In first-line therapy of patients with *KRAS* WT mCRC, the use of an EGFR-I with chemotherapy has to be considered against an anti-VEGF agent. There are many countries where first-line EGFR-I is not freely available; additional concerns were recently raised by the New EPOC study (Primrose *et al*, 2013) for a possible detrimental effect, at least in the trial population of resectable liver metastases. In contrast, data from the FIRE-3 study (Stintzing *et al*, 2012), directly comparing cetuximab with bevacizumab in the first-line setting and available as abstract only, showed a significant OS advantage for first- line cetuximab. However this was a secondary end point and there was no difference in the primary end point of RR nor in PFS or downstaging to resection. The late separation of survival curves does point to possible ongoing influence of first-line biologic choice, which appears more exaggerated with selection by extended *RAS* testing (Stintzing *et al*, 2012).

Regarding anti-angiogenesis agents as a group, their addition to chemotherapy backbones was associated with improved OS, PFS and ORR. This benefit was present whether they were used in second-line settings or third-line settings and beyond.

The VEGF antibody bevacizumab and the VEGFtrap aflibercept had remarkably similar efficacy (1.4 months median OS benefit), despite different patient populations (30% prior exposure to bevacizumab in VELOUR study; 100% in TML study). This may be due to continuous VEGF inhibition, which may be important throughout the refractory setting, as a proportion of patients in the CORRECT trial population had been bevacizumab pre-treated. Vatalanib failed to enter clinical practice because it did not meet its primary end point of OS despite demonstration of PFS benefit. The lack of robust predictive biomarkers in anti-angiogenic therapy remains a major challenge.

Anti-angiogenesis trials in the third-line and beyond setting investigated the VEGFR TKIs regorafenib and brivanib. Considered together, benefit was shown for OS, PFS and ORR. Only regorafenib has moved into standard of care; however, predictive markers remain elusive. Brivanib has not entered routine clinical practice, despite significant benefit in ECOG 0-1 patients (compared with ECOG 2) in the CO.20 trial, primarily due to adverse effects on QoL.

Many studies of targeted agents did not include formal QoL measurements. This is a lost opportunity. Despite some of these trials achieving the gold standard of OS improvement, the drugs remain non-curative and it is always necessary to consider the impact of treatment-related side effects on global and specific functioning. The CO.20 trial illustrates the importance of measuring QoL, as brivanib may otherwise have been taken forward in the good performance status group.

CONCLUSIONS

This systematic review has provided evidence for a class effect with the addition of targeted therapies, when considered together, improving OS, PFS and ORR for patients with mCRC. When analysed separately by mechanism of action and by line of therapy, results demonstrate that progress has been made in the extension of life of patients with mCRC.

ACKNOWLEDGEMENTS

We acknowledge the authors who provided additional data: B Giantonio, J Tabernero, C O'Callaghan and D Jonker. Sanofi-Aventis provided financial support to WriteSource Medical Pty Ltd to undertake the literature search and paper retrieval. Sanofi-Aventis had no role in data analysis/interpretation and were not involved in writing the manuscript.

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Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)