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A systematic review and meta-analysis of *KRAS* status as the determinant of response to anti-EGFR antibodies and the impact of partner chemotherapy in metastatic colorectal cancer

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ABSTRACT

Background: In the setting of metastatic colorectal cancer (CRC), anti-EGFR antibodies are not currently recommended for individuals with *KRAS* mutant tumours. This is based on subgroup analyses of individual clinical trials rather than a formal synthesis of evidence for *KRAS* status as a predictive biomarker, while newer trials report no benefit for anti-EGFR antibodies irrespective of *KRAS* status. This study systematically reviewed the evidence for *KRAS* mutation status as a treatment effect modifier of response to anti-EGFR antibodies and the influence of partner chemotherapy.

Methods: Medline (1966–2010), EMBASE and American and European oncology meeting abstracts were searched for randomised controlled trials reporting the influence of *KRAS* status on effectiveness of anti-EGFR antibodies in metastatic CRC. The treatment efficacy was summarised by *KRAS* status using hazard ratios (HR) for progression-free survival (PFS) and risk differences (RD) for objective response. For each study, a measure of effect modification was calculated, and aggregated using random effects meta-analysis to assess the interaction between *KRAS* and treatment effect.

Findings: Eleven studies (8924 patients) were selected from 198 reports. Two studies assessed anti-EGFR antibodies as monotherapy and nine their use with chemotherapy. *KRAS* status was reported in 7555 cases. In subgroup analysis, the progression HR for *KRAS* wild patients assigned to anti-EGFR antibodies was 0.80 (4436 patients 95%CI: 0.64, 0.99) and for mutant cases 1.11 (3119 patients, 95%CI: 0.97, 1.27). A significant treatment effect interaction between *KRAS* status and addition of anti-EGFR antibodies to standard treatment was found for PFS (ratio of HRs 0.71, 95%CI: 0.57, 0.90 $p = 0.005$) and response rate difference (difference in RDs 15%, 95%CI: 8, 22%, $p < 0.001$). There was no evidence that the extent of effect modification differed between chemotherapeutic partners for both PFS ($p = 0.3$) and response rate ($p = 0.6$).

Interpretation: *KRAS* mutation status is a treatment effect modifier for anti-EGFR antibodies in metastatic CRC. Further evidence is needed to determine whether this is true for all chemotherapy partners and all clinical circumstances.

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1. Introduction

Over the last decade, the median survival of patients with metastatic colorectal cancer has improved because of the availability of combination chemotherapy (fluoropyrimidines with irinotecan or oxaliplatin) and biological agents. The biological agents have a more favourable toxicity profile than conventional chemotherapy and as such they support a treatment paradigm of continuous therapy consisting of multiple 'lines' of drugs used as single agents or in combination. The biological agents most relevant to colorectal cancer include bevacizumab which targets vascular endothelial growth factor (VEGF) and cetuximab and panitumumab which bind the epidermal growth factor receptor (EGFR).

EGFR was the first growth factor receptor recognised as a target for cancer therapy and anti-EGFR antibodies were developed in the late 1980s. However, it was not until this decade that cetuximab emerged from considerable controversy to become a credible therapy for colorectal and other cancers. A number of clinical trials have demonstrated that cetuximab and panitumumab, used alone or in combination with chemotherapy, have activity against colorectal cancer.[1,2] Less certain has been the magnitude of the clinical benefit of these agents and their place in the clinical treatment algorithm.

Both prognostic and predictive markers may assist in the selection of patients who are most likely to benefit from treatment. A prognostic marker provides information on outcome independent of treatment. In the presence of a fixed relative benefit from a new therapy, prognostic markers can be used to identify those with the greatest opportunity for absolute benefit. A predictive biomarker or treatment effect modifier, prospectively selects individuals with a differential benefit to a specific treatment and as such is of great relevance to targeted therapies. In the case of high cost therapies such as anti-EGFR antibodies, a predictive biomarker represents an opportunity to limit therapy to the subgroup of patients who are likely to benefit, thus improving the cost effectiveness of the drug. In this regard, the status of the EGFR gene and protein were initially proposed as potential treatment effect modifiers. However, EGFR expression as determined by immunohistochemistry failed to show any relationship with the efficacy of the anti-EGFR drugs, with patients lacking EGFR expression responding to cetuximab and panitumumab.[3,4] Similarly unconvincing findings were noted when the predictive significance of somatic *EGFR* mutations or gene amplification were assessed. The breakthrough in this area came with the recognition that patients with activating mutations in the *KRAS* gene did not appear to benefit from anti-EGFR antibodies.[5,6]

Activating mutations in codons 12, 13 (and rarely 61 and 146), of the *KRAS* oncogene are found in approximately 40% of colorectal cancers. These mutations result in EGFR independent constitutive activation of the mitogen-activated protein kinase pathway. On this basis, it was biologically plausible that tumours with *KRAS* mutations would demonstrate resistance to anti-EGFR antibodies. Indeed this hypothesis appeared to be supported by a number of retrospective studies which demonstrated that patients with *KRAS* mutant tumours did not derive a benefit from the anti-EGFR antibodies cetuximab or panitumumab. In 2009, the American Society of Clinical Oncology guidelines concluded that individuals with a *KRAS* mutant colorectal cancer should not receive therapy with anti-EGFR antibodies.[7] This conclusion was based on the observation of differences in efficacy in *KRAS* wild and mutant subgroups in five randomised trials and five single arm studies; no test for effect modification by *KRAS* status was undertaken. Over the last few years, many jurisdictions have provided subsidised access to the expensive anti-EGFR antibodies for patients who have *KRAS* wild tumours. Given the prevailing surety that *KRAS* status was a predictive marker it came as some surprise that a number of recent randomised studies have failed to demonstrate a benefit for anti-EGFR antibodies in patients with *KRAS* wild or mutant tumours.[8,9] Furthermore the addition of anti-EGFR antibodies to bevacizumab has had an adverse effect on treatment outcomes in both *KRAS* wild and mutant subgroups.[10]

It has long been understood that treatment effect modification cannot be established on the basis of separate analyses of treatment effects in different subgroups of patients. Subgroup analyses have a high risk of identifying statistically significant effects when none exist and of missing true treatment effects within subgroups. More than two decades ago the misinterpretation of subgroup analysis in trials of thrombolytic therapy after myocardial infarct potentially led to the unwarranted withholding of effective therapy.[11–13] Although it is now widely known that a conclusion of effect modification requires, at a minimum, a significant test of heterogeneity of treatment effects between subgroups,[14–16] only a minority of clinical trials[17–20] and no systematic reviews have included tests for interaction in the assessment of the predictive value of *KRAS* status. Most studies and guidelines have based their conclusions on subgroup analyses. These limitations, together with the wide range of conclusions drawn from different clinical trials, creates a need to combine multiple data sources in order to determine the role of *KRAS* status as a predictor of response to anti-EGFR antibodies.

The primary aim of this study is to use a systematic review and meta-analytical strategy to determine if *KRAS* mutation status is a treatment effect modifier for response to anti-EGFR antibodies (cetuximab or panitumumab) in patients with metastatic colorectal cancer. A further aim is to explore the possibility that treatment effect modification by *KRAS* status is influenced by the partner chemotherapy used with anti-EGFR antibodies.

2. Methods

2.1. Search strategy and study identification

We conducted a comprehensive search of the medical literature for all studies evaluating the effect of *KRAS* mutation status on treatment outcomes for patients with metastatic colorectal cancer treated with cetuximab or panitumumab. We searched Ovid MEDLINE (1966–2010) and EMBASE, using terms that combined the drugs with *KRAS* and colorectal cancer (full search methodology is shown in webappendix p 1). The final search was undertaken in October 2010. We also searched the proceedings (abstracts, poster and video

presentations) of the American Society of Clinical Oncology (ASCO) and the European Society for Medical Oncology (ESMO) from 2006–2010.

The literature selection was based initially on the paper title, and if thought relevant, followed by the abstract or full paper. If there was still uncertainty about inclusion, we reviewed the entire paper. We did not restrict the language in our search. We checked the citations for each relevant paper identified using the cited reference component of the Web of Science database. Further, we assessed all the references in all selected papers, as well as review articles, for possible inclusion in the systematic review.

Randomised controlled studies which reported the influence of tumour *KRAS* mutation status on the effectiveness of cetuximab or panitumumab in the treatment of metastatic colorectal cancer were eligible for inclusion in this meta-analysis. Studies were included on the proviso that the difference in objective response or hazard ratios for progression free survival (PFS) could be determined for the *KRAS* wild and mutant subgroups. Where possible, we combined information from the full published paper and conference abstracts to ensure the most up to date study results were included.

2.2. Data extraction and assessment of study quality

Two reviewers (BA and CH) independently extracted data from the papers. Agreement on all items was achieved by consensus. For each study, data about methodology, quality, patient characteristics and drug treatments were extracted. Where the *KRAS* analysis had been published subsequent to the publication of the original randomised trial evaluating the drug efficacy, we used both data sources. Data items extracted included patient numbers, patient performance status group (Eastern Cooperative Oncology Group (ECOG) or WHO), gender, age, previous treatment, and disease site (colon or rectum) and number of metastatic sites. For each study, information for the total *KRAS* group as well as for the wild and mutant groups was collected. For objective response, we extracted data about the number of patients who responded (complete response or partial response) for each treatment group (anti-EGFR antibody or no anti-EGFR antibody), stratified by *KRAS* status (wild or mutant). For progression-free survival, we extracted the hazard ratios for each *KRAS* group.

We were unsuccessful in our attempts to obtain missing data from the authors of the published papers. For this reason, in one study[10] we estimated log hazard ratios and variances from published survival curves and numbers at risk.[21] For another study[9] which reported only study totals, we assumed approximately equal treatment groups and then generated a range of possible treatment arm totals and numbers of responders and non-responders. The scenarios that yielded summary statistics closest to the published ones were used in our analyses.

2.3. Statistical analysis

We began by summarising treatment efficacy separately for the *KRAS* wild and mutant subgroups using log hazard ratios for progression-free survival and risk differences for treatment response. Risk differences were used in preference to relative risks or odds ratios as exploratory binomial regression analyses comparing logistic, log-linear and linear models which showed that the linear model provided a substantially better fit to the data. Confidence intervals for risk differences were estimated using exact methods when response rates were equal to zero.[22] Results were pooled using random effects meta-analysis, with heterogeneity assessed using the I^2 statistic, which expresses the proportion of heterogeneity that cannot be explained by chance.[23] Pooled risk differences were also calculated using an exact meta-analysis[24] to check that there was no sensitivity to the presence of zero response rates.

Since effect modification cannot be assessed solely on the basis of separate analyses within the two *KRAS* status subgroups,[15,16] we also conducted a meta-analysis of the interaction between *KRAS* status and treatment effect. For each trial we first calculated a measure of the treatment effect modification associated with *KRAS* status. This was the difference between the log hazard ratios or risk differences in the *KRAS* wild and mutant subgroups. These study-specific measures of effect modification represent the difference in treatment effects between the wild and mutant subgroups. Confidence intervals were calculated[25] and the study-specific measures of effect modification were then pooled using random effects meta-analysis. Although the meta-analyses of progression-free survival were conducted using log hazard ratios, the results were presented on the hazard ratio scale. The robustness of the results to the effects of single studies was assessed using 'leave-one-out' cross-validation analyses (total 11 analyses). Differences in the extent of effect modification between treatment partners were assessed using an analysis of variance approach performed with meta-regression.[26]

3. Results

3.1. Number of studies

Of 198 papers, 36 were selected for full review and eight full papers met our inclusion criteria (Fig. 1).[10,17–20,27–29] One paper described 2 distinct clinical trials, involving different patient groups and drug combinations.[28] For this reason, this paper was analysed as two separate studies. Two studies[17,19] presented an updated analysis by *KRAS* group of previously published randomised controlled trials.[30,31] Four relevant abstracts from conferences were identified, of which two provided updated outcome data based on *KRAS* mutation results on additional cases to those presented in the full papers identified[32,33] and two described new studies.[8,21] On this basis a total of 11 open label randomised studies met the inclusion criteria for this meta-analysis (Table 1). These studies were categorised into four groups according to chemotherapy partner. Monotherapy was used for studies where patients were randomised to anti-EGFR antibodies and best supportive care (BSC) or BSC only. For the remaining three groups, anti-EGFR antibodies were evaluated as an addition to regimens containing fluoropyrimidines and one or more of irinotecan, oxaliplatin or bevacizumab. These three groups were labelled according to their predominant chemotherapy partner (Table 1).

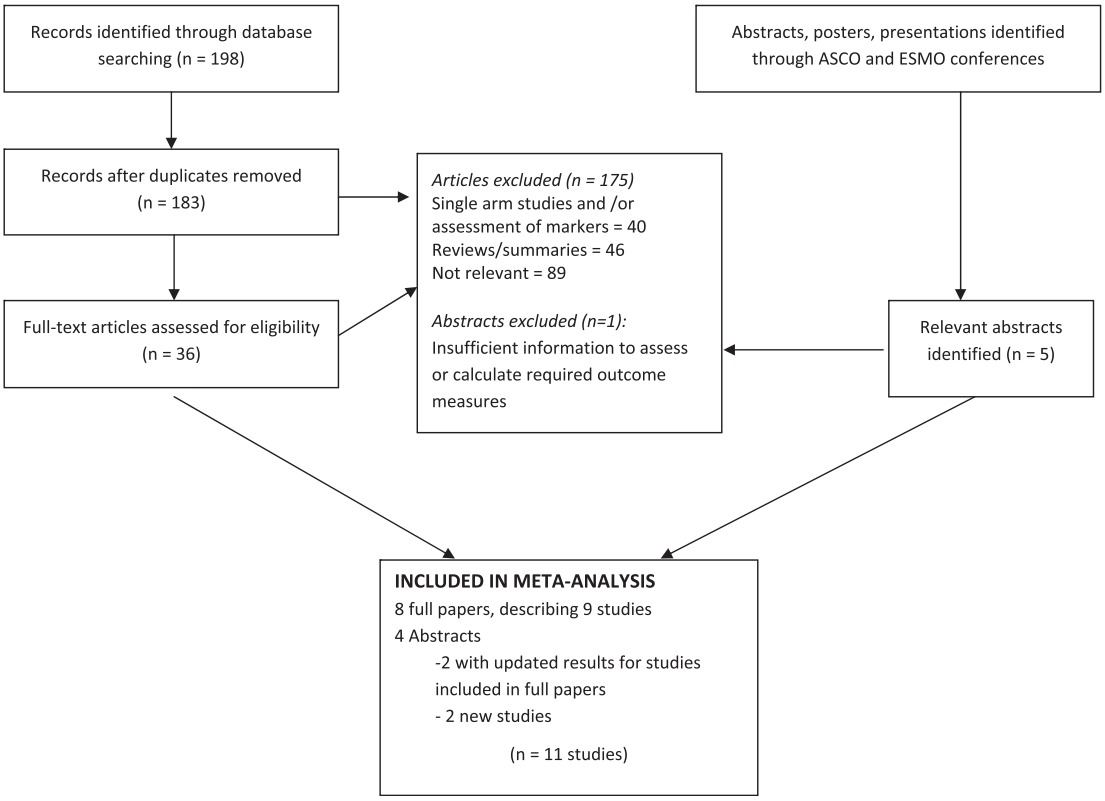

Fig. 1 – Flow diagram for selection of studies.

3.2. *Quality of studies*

There was little to distinguish the studies in terms of risk of bias. Inclusion criteria and randomisation techniques and stratification (based predominantly on ECOG status and region), were similar in all the studies. All studies required a performance status of two or better for entry and five studies[10,17–20] required tumour overexpression of EGFR. All studies, except Tol[10] involved at least two multinational recruitment sites. For all studies, withdrawals and loss to follow up numbers, albeit probably small, were difficult to assess. Two studies[28] discontinued early after an interim analysis showed worse outcomes in the experimental treatment arms. In four of the studies[10,17–19] it was clear that the analysis was undertaken by 'intention to treat.' All papers provided information about response rates, with six of the 11 studies using modified RECIST criteria, and two[18,20] using modified WHO criteria. There was stated blinding of the assessment of progression in three of the studies; one study used both local and central review, one used only local review and one paper provided no information about this.

3.3. *KRAS mutation status*

In nine studies included in this analysis, outcome assessment according to *KRAS* mutation status was conducted retrospectively on a subgroup of the trial population, while the other two studies[27,29] prospectively specified their intention to analyse the effect of anti-EGFR antibodies in *KRAS* wild tumours. In the full papers, *KRAS* status was available on 45–92% of tumours; however this number increased to 71–94%, when updated data from abstracts were included (Table 1). *KRAS* mutations were present in 36–43% of tumours. The frequency of *KRAS* mutations was constant across clinicopathological variables including ECOG status, previous adjuvant treatment and disease site (Table 1: webappendix). Of the seven studies which reported that *KRAS* status was a predictive marker, only four studies provided the results of a test for treatment effect interaction.[17–20]

3.4. *Treatment regimens*

Two of the studies used cetuximab or panitumumab alone as third line treatment, comparing the drug to best supportive care only.[17,19] Cetuximab or panitumumab was used as first or second line treatment in the nine other studies, in combination with fluoropyrimidines (fluorouracil or capecitabine), with either oxaliplatin[8–10,18,27,28] or irinotecan.[20,28,29] Three of the studies also added bevacizumab.[10,28] For all studies, the doses of cetuximab and panitumumab complied with the product information for these drugs.

3.5. *Efficacy of anti-EGFR antibodies in KRAS wild and mutant subgroups*

This meta-analysis demonstrated a 20% reduction in the hazard of progression with the addition of anti-EGFR antibodies to standard treatment (11 trials, 4436 patients, HR 0.80; 95% CI 0.64, 0.99) in the subgroup of patients with *KRAS* wild tumours (Fig. 2). In contrast, the same treatment combinations

Table 1 – Design characteristics of studies used in the meta-analysis (grouped by partner chemotherapy).

Paper	Trial phase	Treatment comparisons	Line of therapy	Number of study participants				KRAS analysis	
				Overall	KRAS subgroup (% of total)	KRAS wild (% of KRAS)	KRAS mutant (% of KRAS)	Blinded	Codon
Monotherapy									
Amado[17]	3	BSC versus BSC + Pmab	3rd	463	427 (92.2)	243 (56.9)	184 (43.1)	Yes	12,13
Karapetis[19]	3	BSC versus BSC + Cmab	3rd	572	394 (68.9)	230 (58.3)	164 (41.6)	Yes	12,13
Irinotecan									
Van Cutsem[20]	3	FOLFIRI versus FOLFIRI + Cmab	1st	1198	540 (45.1)	348 (64.4)	192 (35.6)	ns	12,13
Van Cutsem[32]	Update				1063 (88.7)	666 (62.7)	397 (37.3)		
Peeters[29]	3	FOLFIRI versus FOLFIRI + Pmab	2nd	1186	1083 (91.3)	597 (55.1)	486 (44.9)	Yes	ns
Oxaliplatin									
Bokemeyer[18]	2	FOLFOX-4 versus FOLFOX-4 + Cmab	1st	337	233 (69.1)	134 (57.5)	99 (42.5)	ns	12,13
Bokemeyer[33]	Update				315 (93.4)	179 (56.8)	136 (43.2)		
Maughan[8]	3	Ox, 5FU versus Ox, 5FU + Cmab[b]	1st	1630	1316 (80.7)	729 (55.4)	565 (42.9)	ns	12,13,61
Douillard[27]	3	FOLFOX4 versus FOLFOX4 + Pmab	1st	1183	1096 (92.6)	656 (59.9)	440 (40.1)	Yes	ns
Tveit[9]	3	FLOX versus FLOX + Cmab[b]	1st	566	498 (87.9)	303 (60.8)	195 (39.2)	ns	ns
Oxaliplatin/Irinotecan + Bevacizumab									
Hecht[a28](Ox)	3B	Ox-CT/Bev versus Ox-Ct/Bev + Pmab	1st	823	664 (80.7)	404 (60.8)	260 (39.1)	ns	ns
Hecht[a28] (Iri)	3B	Iri-CT/Bev versus Iri-CT/Bev + Pmab	1st	230	201 (87.3)	115 (57.2)	86 (43.0)	ns	ns
Tol[10]	3	Cap, Ox, Bev versus Cap, Ox, Bev + Cmab	1st	736	520 (70.6)	314 (60.3)	206 (39.6)	ns	12,13

BSC, Best supportive care; Cmab, cetuximab; Pmab, panitumumab; Iri, Irinotecan; Ox, oxaliplatin; Ox-CT = oxaliplatin based chemotherapy; Iri-CT, irinotecan based chemotherapy; Bev, bevacizumab; Cap, capecitebine; FOLFOX, fluorouracil + leucovorin + oxaliplatin; FOLFIRI, fluorouracil + leucovorin + irinotecan; FU, 5fluoruracil. ns, not stated in paper.

[a] Differing drug regimens described in one paper.

[b] Additional randomisation undertaken but not reported in this analysis.

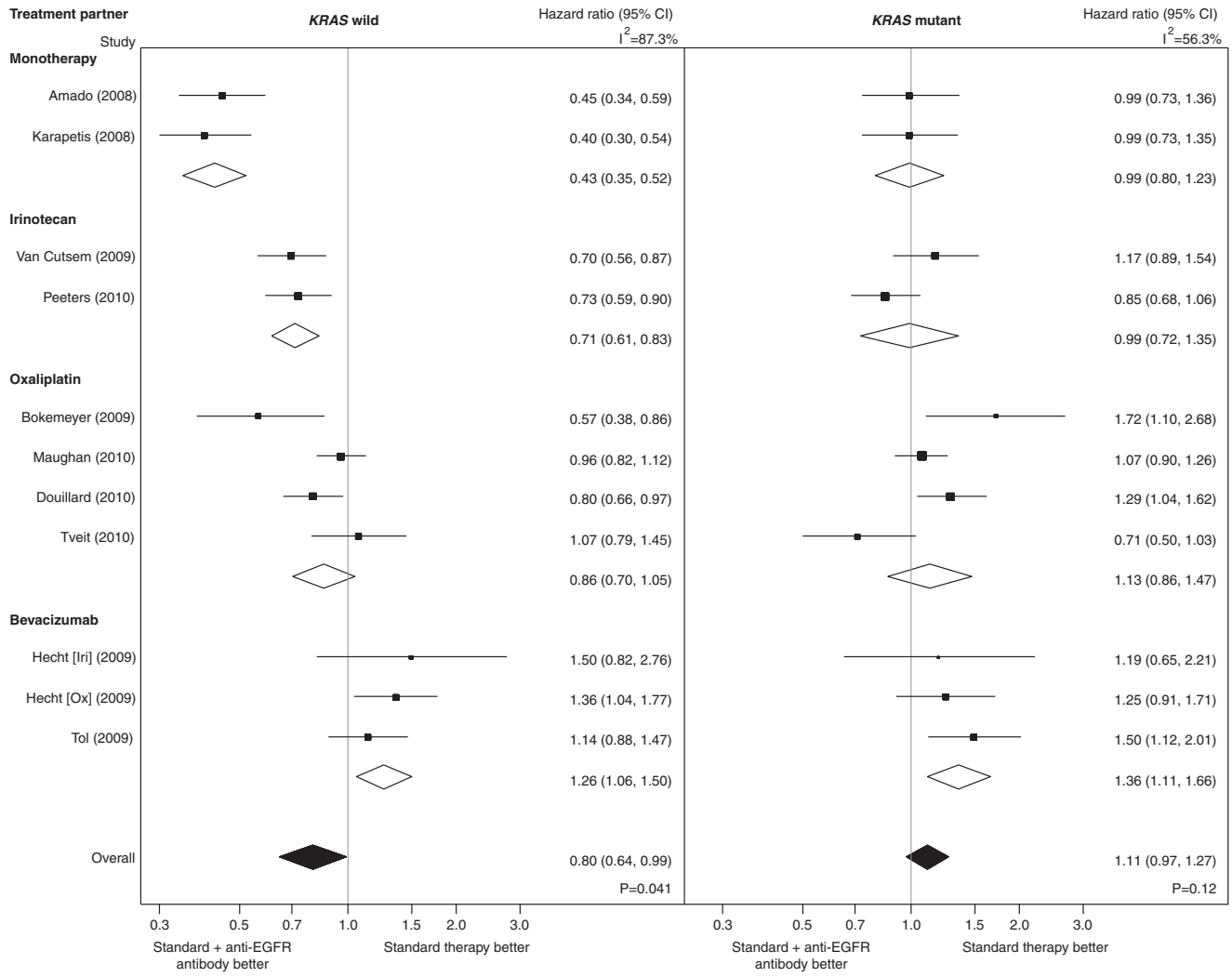

Fig. 2 – Progression-free survival in *KRAS* wild and mutant groups in trials of anti-EGFR antibodies. HR for Tol estimated from published survival curves and numbers at risk. Treatment partner = the chemotherapy with which the anti-EGFR antibody therapy was combined: Monotherapy = anti-EGFR antibody therapy alone; Irinotecan = anti-EGFR antibody therapy + fluoropyrimidine + leucovorin + irinotecan; Oxaliplatin = anti-EGFR antibody therapy + fluoropyrimidine + leucovorin + oxaliplatin; Bevacizumab = anti-EGFR antibody therapy + fluoropyrimidine + leucovorin + irinotecan or oxaliplatin + bevacizumab.

showed no beneficial effect on progression in the *KRAS* mutant subgroup (3119 patients, HR 1.11, 95% CI 0.97, 1.27). In the *KRAS* wild subgroup the largest absolute improvement in progression-free survival (2 months) was reported in a study comparing FOLFIRI with FOLFIRI and panitumumab in the second line setting[29] (Table 2). However in four studies, the median progression-free survival with anti-EGFR antibodies was less than the standard therapy.[9,10,28] In the *KRAS* mutant group, in all but one study[9] those treated with anti-EGFR antibodies had a shorter progression-free survival than the comparator arm.

The benefit of anti-EGRF antibodies in the *KRAS* wild subgroup was also reflected in an 11% higher response rate than that observed with standard therapy (95% CI: 5%, 17%), while there was no evidence of a response benefit in the *KRAS* mutant group (Fig. 3).

3.6. *Treatment effect modification on the basis of KRAS status*

Meta-analyses of the measures of treatment effect modification found that there was a significant interaction between *KRAS* status and the addition of anti-EGFR antibodies to standard treatment, for both progression ($p = 0.005$; Fig. 4) and response ($p < 0.001$; Fig. 5). The ratio of the progression hazard ratio in the *KRAS* wild subgroup to the progression hazard ratio in the *KRAS* mutant subgroup was 0.71 (95% CI: 0.57, 0.90), reflecting a greater benefit of anti-EGFR antibodies in the *KRAS* wild subgroup (Fig. 4). Compared to the *KRAS* mutant subgroup, the *KRAS* wild subgroup had a 15% (95% CI: 8, 22%) greater difference in response rates between the two treatment groups, again reflecting a greater benefit of anti-EGFR antibodies in the *KRAS* wild subgroup (Fig. 5). A

Table 2 – Change in median progression-free survival in KRAS wild and mutant groups.

	Median PFS (months) in KRAS wild group			Median PFS (months) in KRAS mutant group		
	With anti-EGFR antibody therapy	No anti-EGFR antibody therapy	Difference	With anti-EGFR antibody therapy	No anti-EGFR antibody therapy	Difference
Monotherapy						
Armado[17]	2.8	1.7	1.1	1.7	1.7	0.0
Karapetis[19]	3.7	1.9	1.8	1.8	1.8	0.0
Irinotecan						
Van Cutsem[32a]	9.9	8.4	1.5	7.4	7.7	−0.3
Peeters[29]	5.9	3.9	2.0	5.0	4.9	0.1
Oxaliplatin						
Bokemeyer[33a]	8.3	7.2	1.1	5.5	8.6	−3.1
Maughan[8]	8.6	8.6	0.0	NR	NR	NR
Douillard[27]	9.6	8.0	1.6	7.3	8.8	−1.5
Tveit[9]	7.9	8.7	−0.8	9.2	7.8	1.4
Bevacizumab						
Hecht: Iri[28]	10.0	12.5	−2.5	8.3	11.9	−3.6
Hecht: Ox[28]	9.8	11.5	−1.7	10.4	11.0	−0.6
Tol[10]	10.5	10.6	−0.1	8.1	12.5	−4.4

[a] Updated (abstract) results used, NR = not reported in paper.

validation exercise, conducted by removing one study at a time and repeating the analysis, demonstrated that the interaction effects remained significant for both progression-free survival (ratio of HRs varied between 0.67 and 0.76) and objective response (difference in risk difference varied between 13% and 17%).

3.7. *Effect of chemotherapy partners on the efficacy of anti-EGFR antibodies*

An exploratory analysis was conducted to determine whether the extent of treatment effect modification differed by chemotherapeutic partner. Non-significant results were found for both progression-free survival ($p = 0.3$) and response rate ($p = 0.6$). However, this analysis was underpowered to detect such a difference. For progression-free survival, the upper bound of the confidence intervals for three (irinotecan, oxaliplatin and bevacizumab) of the four groups cross 1 meaning it remains possible that effect modification does not occur with all partner drugs (Fig. 4). For the response rate end-point the summary estimate of treatment effect interaction was similar in all groups except bevacizumab.

4. Discussion

In the context of a systematic review of treatment effect modification, we have shown for the first time that KRAS mutation status predicts response to anti-EGFR antibodies in metastatic colorectal cancer. Importantly our observation of significant interaction effects was made for both progression-free survival and response rate differences, thus strengthening the conclusion that KRAS mutation status is indeed a true predictive marker in this treatment setting. Two previous meta-analyses of anti-EGFR antibodies did not assess the role of KRAS[1,2] at all and the only meta-analysis to consider KRAS status did not study its role as a treatment effect modifier.[34] This latter study included single arm studies and only four of the randomised trials that were included in our analysis.

Our results did not provide evidence that the extent of treatment effect modification varied according to chemotherapy partner; however this analysis was underpowered to detect such a difference. Indeed two of the randomised controlled studies using bevacizumab in combination with anti-EGFR antibodies were prematurely terminated because of an inferior outcome overall, irrespective of KRAS status.[28] The summary estimates of interaction by drug group for progression-free survival (Fig. 4) and response rate difference (Fig. 5) demonstrated the lack of treatment effect modification for bevacizumab. For the more clinically relevant end-point of progression-free survival (Fig. 4) suggests that other chemotherapy partners such as oxaliplatin may influence the extent of treatment effect interaction. The issue of whether treatment interaction occurs with each and every background chemotherapy combination is clearly of great interest[35] however our study has shown that this cannot be definitively resolved with the currently available evidence.

A novel aspect of our study is the fact that it is a systematic review of treatment effect modification. According to widely accepted subgroup analysis principles, effect modification cannot be assessed using separate tests of treatment effect in subgroup-specific analyses.[15,16] Thus, it is not possible to assess whether KRAS mutation status is a treatment effect modifier using separate meta-analyses of the wild and mutant subgroups. Such an assessment requires a test of interaction or heterogeneity, in which the difference between the two subgroup-specific treatment effects is assessed for statistical significance. This is not commonly undertaken in meta-analyses, and we have achieved it here by extracting study-specific measures of effect modification, and applying meta-analytical principles to aggregate these study-specific measures into a single measure of effect modification for each end-point. This provides a meta-analytic strategy that is consistent with the broader principles of subgroup analysis.

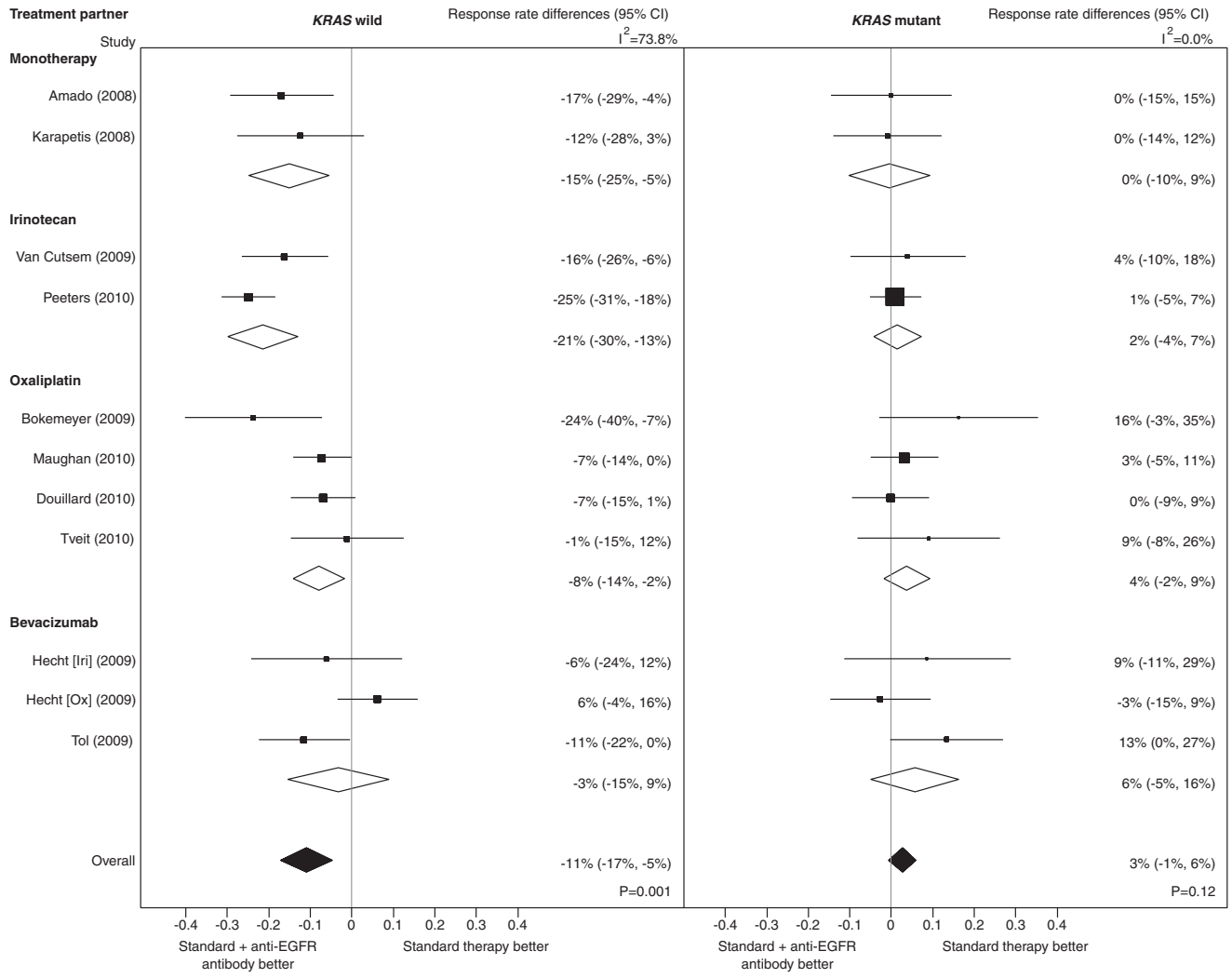

Fig. 3 – Response rate in KRAS wild and mutant groups in trials of anti-EGFR antibodies. Treatment partner = the chemotherapy with which the anti-EGFR antibody therapy was combined: Monotherapy = anti-EGFR antibody therapy alone; Irinotecan = anti-EGFR antibody therapy + fluoropyrimidine + leucovorin + irinotecan; Oxaliplatin = anti-EGFR antibody therapy + fluoropyrimidine + leucovorin + oxaliplatin; Bevacizumab = anti-EGFR antibody therapy + fluoropyrimidine + leucovorin + irinotecan or oxaliplatin + bevacizumab.

Our observations concerning interaction effects thus provide solid evidence that *KRAS* mutation status is a treatment effect modifier.

Having established that *KRAS* mutation status is a treatment effect modifier, it is then worth noting that in the subgroup with *KRAS* wild tumours, there was a modest improvement in response rate (11%) and reduction in the rate of progression (20%) in patients exposed to anti-EGFR antibodies compared with standard treatment. For disease progression, there was a suggestion of greatest benefit for anti-EGFR antibodies with monotherapy, less benefit with irinotecan combinations and even lesser benefit when combined with oxaliplatin. Despite these observations the absolute difference in progression-free survival between anti-EGFR antibody arms and standard therapy in the *KRAS* wild group was all less than 2 months. For all analyses, the trend was for either no effect or an adverse outcome in the *KRAS* mutant group.

Our study has several limitations. Firstly, overall survival could not be used as an end-point because for most studies the survival analysis was not reported, and where it was captured this data was confounded by early cross over and the use of the additional lines of chemotherapy. While response rates do not provide an influential end-point for decision making about drug efficacy, regulators and third party payers have accepted progression-free survival as an informative end-point in its own right and to a lesser extent as a predictor of overall survival.[36,37] For these reasons we have placed greater emphasis on our results related to progression-free survival rather than response rates. A second limitation relates to the inevitable inter-study variations such as study design, concomitant therapy and line of therapy (first, second line or last line). These differences do not preclude pooling since in meta-analysis patients are compared with others in the trial not across studies. The validity of our conclusions is also supported by the consistency of our meta-analysis

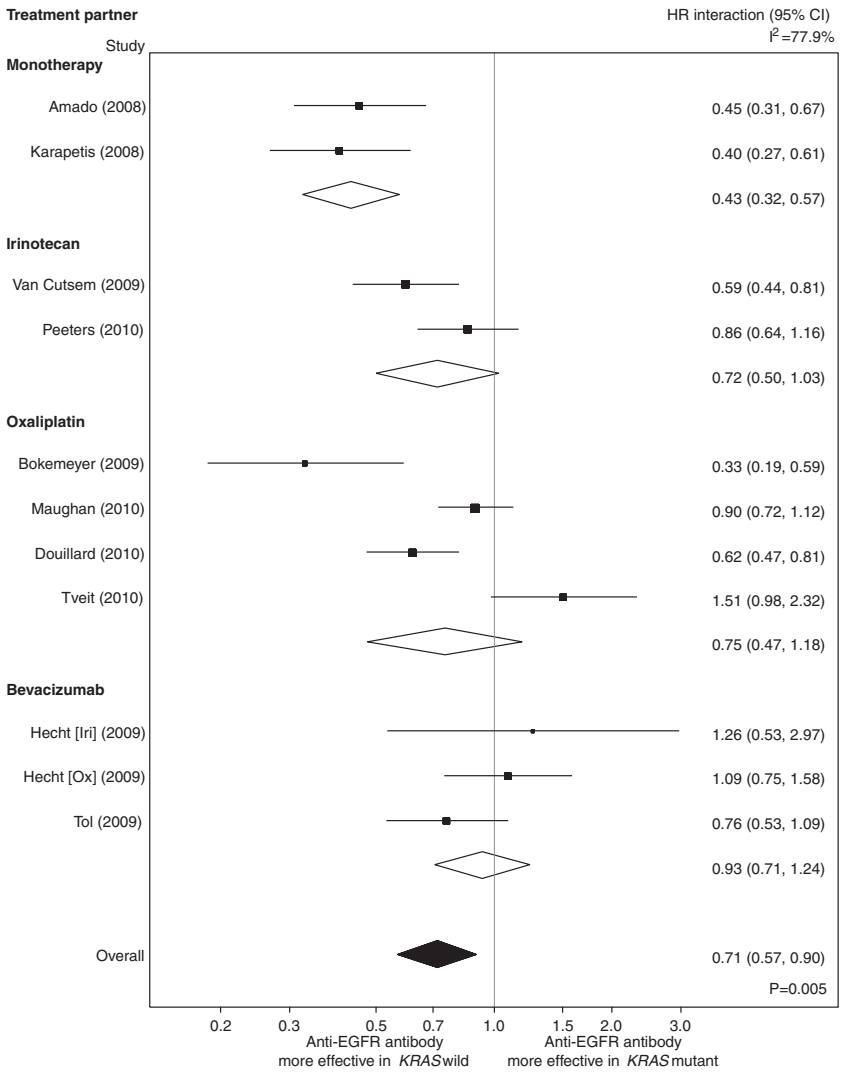

Fig. 4 – Treatment effect modification by KRAS status for progression-free survival. HR for Tol estimated from published survival curves and numbers at risk. Treatment partner = the chemotherapy with which the anti-EGFR antibody therapy was combined: Monotherapy = anti-EGFR antibody therapy alone; Irinotecan = anti-EGFR antibody therapy + fluoropyrimidine + leucovorin + irinotecan; Oxaliplatin = anti-EGFR antibody therapy + fluoropyrimidine + leucovorin + oxaliplatin; Bevacizumab = anti-EGFR antibody therapy + fluoropyrimidine + leucovorin + irinotecan or oxaliplatin + bevacizumab.

with the 4 studies which have assessed *KRAS* as a treatment effect modifier. Meta-analysis is necessarily influenced by publication bias and the limitations of the studies themselves. To address this limitation we searched exhaustively for missing information, and also approached the investigators of published studies. The latter strategy was unsuccessful, so we estimated the log hazard ratio from the survival curve in one study and the numbers in treatment groups in another study. This strategy delivered outcomes which were consistent with the published results so we do not consider that our findings were materially altered.

Finally a most important limitation of our study relates to the *KRAS* data itself. *KRAS* status was reported only for a proportion of patients and although we were able to significantly reduce this data gap by updating data using abstracts, the impact of this missing data on our conclusions cannot be determined. Another source of bias is attributable to the fact that none of the studies incorporated *KRAS* status as part of their stratification procedure and as a consequence the numbers of patients in mutant and wild subgroups were imbalanced. The extent of this problem may fortuitously be partly mitigated by the relatively high frequency of *KRAS* mutations (40%) in patients with metastatic colorectal cancer. Also we were unable to identify an obvious difference in age, performance status or other factors between the mutant and wild *KRAS* subgroups, although clearly this assessment was limited given that only summary data was available to us. In this regard our study highlights the need to ensure that study-quality criteria are updated to include an assessment of the proper collection and reporting of biomarkers. A further confounding factor relates to the possible role of *KRAS* as a prognostic marker. Large cohort studies of patients undergoing curative resection of colorectal cancer have shown that *KRAS* is not a prognostic factor in this setting.[38] Recently however Richman

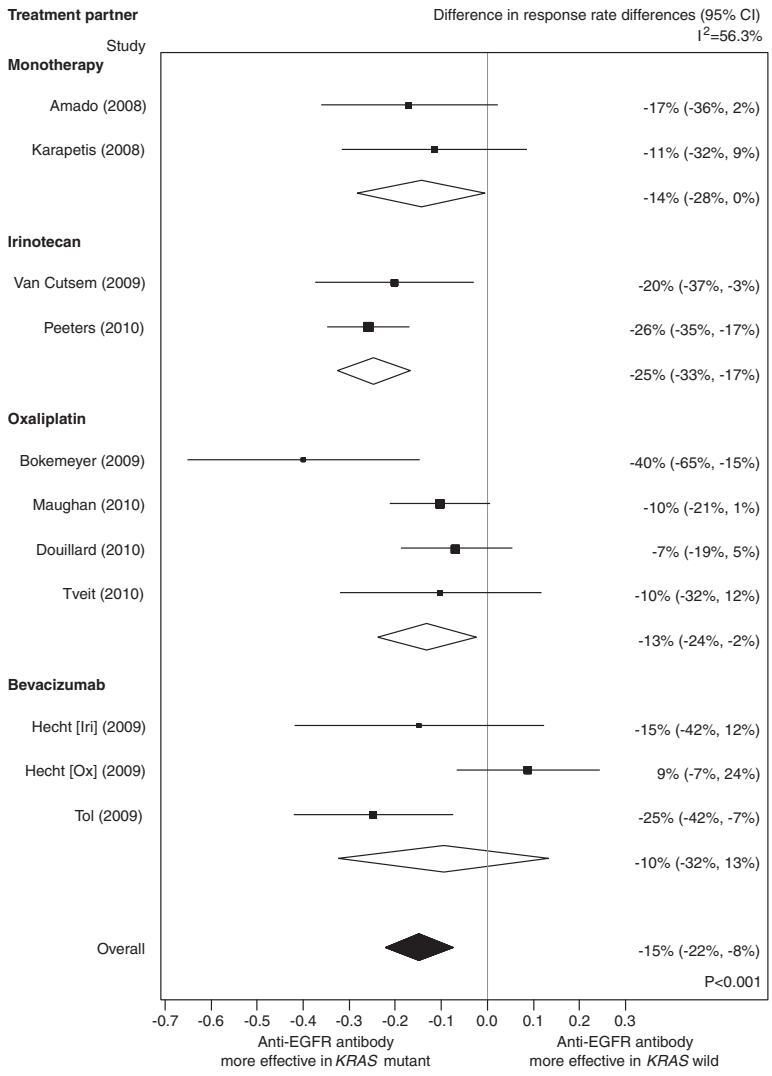

Fig. 5 – Treatment effect modification by *KRAS* status for response rate difference. Treatment partner = the chemotherapy with which the anti-EGFR antibody therapy was combined: Monotherapy = anti-EGFR antibody therapy alone; Irinotecan = anti-EGFR antibody therapy + fluoropyrimidine + leucovorin + irinotecan; Oxaliplatin = anti-EGFR antibody therapy + fluoropyrimidine + leucovorin + oxaliplatin; Bevacizumab = anti-EGFR antibody therapy + fluoropyrimidine + leucovorin + irinotecan or oxaliplatin + bevacizumab.

showed that metastatic colorectal cancer patients with *KRAS*-mutant tumours had worse overall survival but not disease free survival than patients with *KRAS* wild tumours (HR 1.24, 95% CI 1.06–1.46; $p = 0.008$).[39] A final source of uncertainty as it relates to *KRAS* testing concerns the reliability and accuracy of the test itself, the context in which it was applied and the range of genotypes tested. In this respect all studies included in this analysis were found wanting. Only 6 of the 11 studies reported the codon coverage, no studies reported the nature of the mutations identified and none described the analytical performance of their laboratory test. Clearly the lack of transparency in this area limits meaningful comparisons between studies and raises challenges with respect to the extrapolation of findings from these trials to clinical practice. Recently De Roock has shown that not all *KRAS* mutant tumours respond in the same way to cetuximab. Patients with p.G13D-mutated tumours treated with cetuximab had longer overall and progression-free survival than similarly treated patients with another spectrum of mutations in *KRAS*.[40]

This study has a number of strengths which include the use of randomised controlled trials and also the fact that patients were recruited irrespective of *KRAS* status. This is particularly important as approval for the use of anti-EGFR antibodies has now been limited to patients with *KRAS* wild tumours and so the only data concerning outcomes in *KRAS* mutant individuals is contained within the studies included in this meta-analysis. As such our study is likely to be a definitive analysis of the effect of *KRAS* status as a treatment effect modifier for anti-EGFR antibodies in metastatic colorectal cancer.

Given the clinical need of patients with metastatic cancer it is important that new therapies are implemented into clinical practice in a timely manner. In this regard, the rapid

adoption of *KRAS* testing as part of the management guidelines for patients with metastatic colorectal cancer has been heralded as triumph for translational molecular medicine. Our analysis has demonstrated that for this particular gene, the decision to exclude individuals with *KRAS* mutant tumours from exposure to anti-EGFR antibodies was probably correct. We note however that this decision was not based on a comprehensive and robust appraisal of all the evidence. We consider that our study has filled this gap and that the veracity of the claim that *KRAS* is a predictive marker has been substantiated. However, we note that the jury is still out on whether the predictive role of this marker holds true for all chemotherapy partners and in all clinical circumstances. Furthermore our observation concerning the treatment modifying effect of *KRAS* in relation to anti-EGFR antibodies should not be extrapolated to other EGFR inhibitors. Finally we advocate for increased attention to the challenges of incorporating and reporting gene testing in clinical trials.

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This study was funded by grants from Cancer Australia and the National Health and Medical Research Council, neither of which was involved in the study design, collection or interpretation of data, writing the paper or in the decision to submit it for publication. The corresponding author had full access to all the data in the study and final responsibility for the decision to submit for publication.

Authors' contributions

RW and IM conceived the study, BA and CH reviewed the papers and extracted data; TD and IM did the statistical analysis; BA and RW wrote the manuscript; All authors contributed to the interpretation of the data and were responsible for reviewing the manuscript.

Ethics approval

As this is a systematic review of previously published papers and did not use patient level data, no Ethics Committee approval is required.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2011.03.031.

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