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Extended *RAS* mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized controlled trials

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Abstract

Background: Monoclonal antibodies (mAbs) targeting the epidermal growth factor receptor (EGFR) prolong survival in metastatic colorectal cancer *KRAS* exon 2 wild-type tumors. Recent evidence has suggested that other *RAS* mutations (in exons 3 and 4 of *KRAS* and exons 2, 3 and 4 of a related gene, *NRAS*) may also be predictive of resistance.

Methods: Systematic review and meta-analysis of randomized controlled trials (RCTs) evaluating anti-EGFR mAbs that have assessed tumors for new *RAS* mutations. Tumors with the new *RAS* mutations were compared to both tumors without any *RAS* mutations and tumors with *KRAS* exon 2 mutations with respect to anti-EGFR treatment progression-free survival (PFS) and overall survival (OS) benefit.

Results: Nine RCTs comprising a total of 5948 participants evaluated for both *KRAS* exon 2 and new *RAS* mutations met the inclusion criteria. Approximately 20% of *KRAS* exon 2 wild-type tumors harbored one of the new *RAS* mutations. Tumors without any *RAS* mutations (either *KRAS* exon 2 or new *RAS* mutations) were found to have significantly superior anti-EGFR mAb PFS ($P<0.001$) and OS ($P=0.008$) treatment effect compared to tumors with any of the new *RAS* mutations. No difference in PFS or OS benefit was evident between tumors with *KRAS* exon 2 mutations and tumors with the new *RAS* mutations. Results were consistent between different anti-EGFR agents, lines of therapy and chemotherapy partners. Anti-EGFR mAb therapy significantly improved both PFS (hazard ratio 0.62 [95% CI; 0.50 to 0.76]) and OS (hazard ratio 0.87 [95% CI; 0.77 to 0.99]) for tumors without any *RAS* mutations. No PFS or OS benefit was evident with use of anti-EGFR mAbs for tumors harboring any *RAS* mutation ($P>0.05$).

Conclusion: Tumors harboring one of the new *RAS* mutations are unlikely to significantly benefit from anti-EGFR mAb therapy in metastatic colorectal cancer.

Keywords: RAS mutation, pharmacogenomics, cetuximab, panitumumab, predictive biomarker, meta-analysis

Key Message

Not all *KRAS* exon 2 wild-type tumors are likely to benefit from anti-EGFR mAbs in metastatic colorectal cancer. Tumors that harbor one of the new *RAS* mutations (i.e. *KRAS* exon 3 and 4 and *NRAS* exon 2, 3 and 4) have significantly inferior survival benefit from anti-EGFR mAb therapy compared to tumors without any *RAS* mutations. Prior to treatment with anti-EGFR mAbs, tumors should be screened for mutations in exon 2, 3 and 4 of both *KRAS* and *NRAS* genes.

Introduction

Cetuximab and panitumumab are monoclonal antibodies (mAbs) that target the extracellular domain of the epidermal growth factor receptor (EGFR) and are important treatment options in patients with metastatic colorectal cancer (mCRC). RAS proteins are important downstream effectors within the mitogen-activated protein kinase (MAPK) pathway that couples EGFR with intracellular signaling cascades. The RAS gene is often mutated in mCRC, and the most common of these is Kirsten rat sarcoma viral oncogene (*KRAS*). Somatic single nucleotide point mutations in codons 12 and 13 of exon 2 of the KRAS gene lead to constitutive activation of the MAPK pathway, and it is well established from randomized controlled trials (RCTs) that these mutations are predictive of treatment resistance to anti-EGFR mAbs in mCRC. For this reason, only patients with *KRAS* exon 2 wild-type tumors were initially approved for treatment with this class of agents [1], which minimizes unnecessary patient toxicity and improves cost-effectiveness of treatment [2].

Recent studies, in particular the retrospective analysis of the PRIME trial [3], suggest that other mutations in genes of the RAS family (*NRAS* mutations and *KRAS* mutations outside exon 2) are also associated with reduced response to anti-EGFR mAbs. This study aims to quantitatively synthesize the evidence from RCTs that evaluate whether extended *RAS* mutations are negative predictive biomarkers for anti-EGFR mAb therapy in mCRC. Specifically, the primary aim was to evaluate whether the efficacy of anti-EGFR mAb treatment for tumors with one of the new RAS mutations is most similar to that of tumors with *KRAS* exon 2 mutations (not eligible for anti-EGFR mAb treatment) or tumors with no RAS mutations (good responders to anti-EGFR mAb treatment). The secondary aim was to quantify the survival benefit of anti-EGFR mAb therapy in patients with tumors that do not harbor any *RAS* mutations.

Method

Inclusion criteria

Inclusion criteria for the systematic review included: a randomized phase II or III study design involving the comparison of an anti-EGFR mAb (either as monotherapy or in combination with chemotherapy) to an alternative therapy in mCRC; study participants genotyped for at least one of the following in addition to *KRAS* exon 2: *KRAS* mutations in exon 3 (codon 59, 61) or exon 4 (codons 117, 146), or *NRAS* mutations in exon 2, 3 or 4; and follow up for progression free survival (PFS) and/or overall survival (OS).

Search strategy and data extraction

Embase, Medline and Web of Science (up to 1 April 2014) were searched for the following terms: (colon cancer or colorectal cancer or metastatic colorectal cancer) and (K-RAS or KRAS or mutant KRAS or mutant RAS) and (panitumumab or cetuximab or anti-EGFR) and (N-RAS or NRAS or exon 3 or exon 4 or codon 59 or codon 61 or codon 117 or codon 146). No restrictions were placed on the search, and relevant MeSH (Medline) or Emtree (Embase) terms were utilized where possible. Additionally, abstracts from the 2014 ASCO meetings were hand searched to scan for updated data and to identify any new studies. Initially, duplicate titles were removed and then the title of the articles was scanned to determine if the article was irrelevant. The abstract of all remaining articles were then retrieved and reviewed, and irrelevant articles were discarded. Of the remaining articles, full-text manuscripts and/or conference posters/presentations were obtained, and those that fulfilled the inclusion criteria were documented. After identification of relevant articles, reference lists of the articles that were identified as relevant were hand-searched to identify any additional articles that were missed with the search strategy. Studies reported in conference abstracts and published in languages other than English were included if sufficient information was available in the (English) abstract and the associated posters or presentations. Data was extracted using a data template. If multiple versions of the data were presented, the largest and most recently updated data was preferentially used in the

meta-analysis, and data was cross-checked against other publications were possible. The search strategy and data extraction were undertaken independently by two investigators with any discrepancies resolved by another investigator.

RAS subgroups compared

Three mutually exclusive *RAS* subgroups were primarily evaluated (Figure 1). The '*KRAS* exon 2 mutant' subgroup represents tumors with a *KRAS* exon 2 mutation which have previously been shown not to benefit from anti-EGFR mAbs [4, 5]. In contrast, individuals that are wild-type for *KRAS* exon 2 have been thought of as likely to benefit from anti-EGFR therapy [4, 5]. For the purpose of this study, these individuals with wild-type *KRAS* exon 2 were divided into two subgroups; the 'new *RAS* mutant' subgroup (wild-type for *KRAS* exon 2, but with a *KRAS* mutation in exons 3 or 4 and/or a *NRAS* mutation in exons 2, 3 or 4) representing an additional group of patients that potentially do not benefit, and the 'all *RAS* wild-type' subgroup (no mutations in exons 2, 3 and 4 for either *KRAS* or *NRAS*), representing patients that are expected to respond best to anti-EGFR therapy.

Should the 'new *RAS* mutant' subgroup be found to have an anti-EGFR mAb treatment effect that is more similar to the '*KRAS* exon 2 mutant' subgroup than the 'all *RAS* wild-type' subgroup, the 'new *RAS* mutant' and '*KRAS* exon 2 mutant' subgroups would to be merged into an 'any *RAS* mutant' group for further evaluation.

Statistical Analysis

Since some anti-EGFR mAb RCTs have significant cross-over following progression, the pre-specified primary outcome was PFS, and the secondary outcome was OS. The hazard ratio was used to represent the comparative treatment effect on survival outcomes for anti-EGFR mAb therapy compared to either no anti-EGFR mAb therapy or alternative therapy. Studies generally reported hazard ratios derived using Cox proportional-hazards models stratified according to randomization factors (e.g. ECOG performance status). If the hazard

ratio for a *RAS* subgroup was not reported, the value was estimated where possible by combining smaller subgroups with a fixed-effect meta-analysis.

Evaluation of the primary aim was undertaken based on the comparison of the anti-EGFR mAb treatment effect (i.e. hazard ratio for survival outcomes) between pairs of *RAS* subgroups (i.e. 'all *RAS* wild-type' vs 'new *RAS* mutant' and '*KRAS* exon 2 mutant' vs 'new *RAS* mutant'). The interaction hazard ratio (relative size of the treatment effect between subgroups) was calculated by dividing the hazard ratio for the 'all *RAS* wild-type' or '*KRAS* exon 2 mutant' subgroup by the hazard ratio for the 'new *RAS* mutant' subgroup. A value less than one indicates that the 'new *RAS* mutant' subgroup has an inferior treatment effect to the other *RAS* subgroup (and vice-versa). The log of the interaction hazard ratios estimated for each study were pooled using a random-effects model and the inverse variance method.

For aim 2, subgroup-specific summary estimates of the treatment effect hazard ratios were pooled using a random-effects model based on the inverse variance method. The treatment effect of the 'all *RAS* wild-type' and 'new *RAS* mutant' subgroups were evaluated along with the 'any *RAS* mutant' group – the merged '*KRAS* exon 2 mutant' and 'new *RAS* mutant' subgroups (Figure 1). Analysis was limited to RCTs that assessed the addition of anti-EGFR mAbs to background therapy (i.e. cytotoxic or best-supportive care) as this was the largest group of studies with sufficiently similar treatment comparison. Hazard ratios for the *KRAS* exon 2 wild-type subgroup was estimated for reference.

Objective response rate was based on the Response Evaluation Criteria in Solid Tumors (RECIST), and response was defined as either a complete or partial response. The effect of treatment on response was measured as an odds ratio (i.e. odds of response with anti-EGFR mAb therapy vs odds of response without anti-EGFR mAb therapy).

Heterogeneity between studies was assessed using the Cochrane's Q statistic and I^2 statistic. Small-study effects (and risk of publication bias) were assessed by visual inspection of funnel plots and Egger's linear regression test. Pre-specified analyses were undertaken by grouping trials according to the anti-EGFR mAb evaluated (cetuximab or panitumumab), the line of therapy and the background chemotherapy regimen (oxaliplatin-based vs irinotecan based). A sensitivity analysis was undertaken that excluded studies with fluropyrimidine backbone therapy other than infusional 5FU, on the basis of a potential interaction between fluropyrimidine backbone and anti-EGFR mAb efficacy [6].

All reported *P*-values are two-sided. Analyses were carried out R 3.0.0 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Overview of studies included

Nine RCTs comprising a total of 5948 participants evaluated for both established and new *RAS* mutations met the inclusion criteria (Table 1, Figure S1) [3, 7-18]. Seven studies evaluated the addition of an anti-EGFR mAb to background therapy (FOLFOX, FOLFIRI, irinotecan, oxaliplatin and fluoropyrimidine chemotherapy, or best supportive care), and two studies compared the addition of anti-EGFR mAb or bevacizumab to cytotoxic therapy (FOLFOX or FOLFIRI). Five RCTs studied panitumumab and three studied cetuximab. All studies other than the COIN study reported data sufficient to estimate PFS and OS treatment effects for the three RAS subgroups. The COIN study only reported sufficient data for the OS treatment hazard ratio for the 'all *RAS* wild-type' subgroup. Details of ascertainment, specific mutations evaluated, and proportions of study participants evaluated to be *KRAS* exon 2 wild-type and *RAS* wild-type are summarized in Table 1. The risk of bias of the trial intention-to-treat (ITT) populations was generally similar between studies with respect to inclusion criteria, randomization, allocation concealment, blinding, outcome reporting and loss to follow-up [4, 5]. *KRAS* exon 2 mutation status was evaluable in 79% to

100% of the ITT populations, and new *RAS* mutations were evaluable in 65% to 100% of *KRAS* exon 2 wild-type tumor populations. Other than the COIN study (which genotyped only *KRAS* codon 61, and *NRAS* codons 12 and 61), all studies genotyped the majority of the new *RAS* codons (Table 1, Figure 2). The methods used to detect *KRAS* and *NRAS* mutations varied between studies, and included bidirectional Sanger sequencing, pyrosequencing, MALDI-ToF analysis and WAVE-based SURVEYOR analysis (Table S1). Based on random-effects meta-analysis of 5 studies that had genotyped for new *RAS* mutations in all 10 codons (*N*=1911) it was estimated that 19.9% (95% CI; 16.7% to 23.4%) of *KRAS* exon 2 wild-type tumors harbored at least one of the new *RAS* mutations. Significant heterogeneity between study estimates of the prevalence was identified (*P*=0.016, I^2=67%). The estimated prevalence of new *RAS* mutations in each exon is summarized in Figure 2.

Comparison of anti-EGFR mAb treatment effect size between RAS subgroups

Eight studies reported sufficient data to evaluate whether anti-EGFR mAb efficacy differed between the three *RAS* subgroups. Anti-EGFR mAb efficacy was found to be significantly superior for tumors in the 'all *RAS* wild-type' subgroup compared to tumors in the 'new *RAS* mutant' subgroup with respect to both PFS (interaction test *P*<0.001, Figure 3a) and OS (interaction test *P* =0.008, Figure 3a). However, no significant difference in anti-EGFR mAb treatment effect with respect to either PFS (interaction test *P* =0.88) or OS (interaction test *P* =0.35) was apparent between tumors in the 'new *RAS* mutant' and '*KRAS* exon 2 mutant' subgroups (Figure 3b).

No or minimal heterogeneity was evident between studies for the test of interaction between *RAS* subgroups, and there was no indication that the differences in treatment effect between *RAS* subgroups differed significantly on the basis of the anti-EGFR mAb studied, line of therapy, or chemotherapy partner (Table S2). Visual inspection and regression tests did not indicate significant funnel plot asymmetry or small study bias.

Analysis based on response rates indicated similar findings to those for the survival outcomes. Specifically, the size of the anti-EGFR mAb treatment effect on response rates was significantly greater for all *RAS* wild-type tumors than for new *RAS* mutant tumors (interaction test *P*=0.001) Additionally, no significant difference in treatment effect, as measured by response rates, was apparent between *KRAS* exon 2 mutant tumors and the new *RAS* mutant tumors (interaction test *P*=0.32).

Anti-EGFR mAb treatment effect size for the 'all RAS wild-type' subgroup

A significant PFS benefit of anti-EGFR mAb therapy was evident for tumors without any *RAS* mutations (hazard ratio 0.62 [95% CI; 0.50 to 0.76], Figure 4a). By way of comparison, based on the same set of studies, the PFS hazard ratio for tumors without any *KRAS* exon 2 mutations (i.e. before exclusion of tumors with the new *RAS* mutations) was estimated to be 0.68 [0.58 to 0.80]. Similarly, tumors without any *RAS* mutations gained significant OS benefit with anti-EGFR mAb therapy (hazard ratio 0.87 [0.77 to 0.99], Figure 4b). In comparison, tumors without any *KRAS* exon 2 mutations were estimated to have a hazard ratio of 0.90 [0.83 to 0.98]. A sensitivity analysis excluding studies with fluropyrimidine backbone therapy other than infusional 5FU, resulted in

For the PFS outcome, significant heterogeneity in anti-EGFR treatment effect estimates was apparent between studies (I^2=67%, *P* =0.009). No significant small study effects were apparent for either PFS or OS outcomes. No significant differences in the anti-EGFR mAb PFS and OS treatment effect for *RAS* wild-type tumors were evident between cetuximab and panitumumab, or between irinotecan-based and oxaliplatin-based background chemotherapy (Table S3). Anti-EGFR mAb third-line monotherapy (based on a single study [10, 19]) was estimated to have significantly greater benefit than first/second line anti-EGFR mAb therapy in combination with chemotherapy with respect to PFS (*P*=0.002), but not OS (*P*=0.66). Specifically, the PFS hazard ratio was 0.36 [0.25 to 0.52] for 'all *RAS* wild-type'

tumors (compared to PFS hazard ratio of 0.45 [0.34 to 0.59] for *KRAS* exon 2 wild-type tumors) [10, 19].

A significant increase in response rate (odds ratio of 3.71 [2.16 to 6.36]) was observed via addition of anti-EGFR mAb therapy for 'all *RAS* wild-type tumors' (Figure S2).

Anti-EGFR mAb treatment effect size for mutant RAS tumors

Based on 6 studies, no improvement in PFS (hazard ratio 0.99 [0.77 to 1.27) or OS (hazard ratio 1.16 [0.91 to 1.47]) from anti-EGFR mAb therapy was apparent for tumors with the new *RAS* mutations. For tumors with any *RAS* mutation (*KRAS* exon 2 mutation or new *RAS* mutation) there was also no improvement in PFS (hazard ratio 1.12 [0.94 to 1.34) or OS (hazard ratio 1.08 [0.97 to 1.21]) with anti-EGFR mAb therapy (Figure 5). For tumors with any *RAS* mutation, significant heterogeneity in anti-EGFR treatment effect was apparent between studies with respect to PFS (I^2=63%, P =0.02), but not OS (I^2=19%, P =0.29).

Discussion

The current meta-analysis establishes that individuals with tumors that are *KRAS* exon 2 wild-type (which includes both the 'all *RAS* wild-type' and 'new *RAS* mutant' subgroups) should not be considered to be a single homogenous group with respect to anti-EGFR mAb efficacy. The 'all *RAS* wild-type' subgroup had a significantly superior anti-EGFR mAb efficacy compared to the 'new *RAS* mutant' subgroup, whereas the anti-EGFR mAb efficacy of the '*KRAS* exon 2 mutant' and 'new *RAS* mutant' subgroups where not distinguishable. This indicates that tumors with one of the new *RAS* mutations are more appropriately grouped with the tumors with a *KRAS* exon 2 mutation (forming the 'any *RAS* mutant' group), rather than with tumors that do not have any *RAS* mutations.

A significant PFS and OS benefit was observed with addition of anti-EGFR mAb therapy for tumors without any *RAS* mutations, whereas no PFS or OS benefit was observed for tumors that harbored any *RAS* mutation. Hence, approximately 53% of mCRC tumors (~42% with

KRAS exon 2 mutations and ~11% with *KRAS* exon 3 or 4 or *NRAS* exons 2, 3, or 4 mutations) are likely resistant to anti-EGFR mAbs. Some studies, most notably the PRIME trial [3] have indicated that anti-EGFR mAb treatment for tumors with *RAS* mutations may lead to a detrimental effect on PFS and OS. Although the detrimental effect of anti-EGFR mAb therapy for *RAS* mutant tumors was not statistically significant in the current analysis, significant heterogeneity between studies was apparent. It is currently uncertain why some, but not all studies report a detrimental effect of anti-EGFR mAbs when used for *RAS* mutant tumors.

To the authors' knowledge, this is the first meta-analysis to systematically and quantitatively summarize the evidence from RCTs with respect to the predictive value of the new *RAS* mutations. The meta-analysis includes panitumumab and cetuximab, different lines of therapy and a range of background chemotherapy. There was little evidence to suggest that the effects of *RAS* mutations differ between mAbs, line of therapy, or background chemotherapy, although the power to detect important heterogeneity was relatively limited due to the relatively small number of studies included in the analysis. There was significant heterogeneity in the frequency of new *RAS* mutations between studies, which could be explained, at least in part, by the different methods used to detect *RAS* mutations.

We acknowledge that the trial results included in this meta-analysis were extracted from published data rather than being based on an individual patient data meta-analysis. An additional potential limitation of the study is that some of the studies included in the analysis have only been reported in conference presentations rather than full published manuscripts. Although it is possible that the results may differ modestly between the conference presentations and future full publication due to updated data, such differences are likely to be relatively modest. Due to the clear differences observed in this study, modest updating prior to publication is unlikely to substantively alter the results reported here. In addition, for

most studies results were confirmed through second sources such as the European Medicines Agency assessment documents [8, 9]. Although results of nine RCTs are included in this analysis, there are a small number of additional trials (NCIC CTG CO.17 [20], CALGB/SWOG 80405 [21], NORDIC VII [22], EPIC [23]) that could potentially be retrospectively analyzed for the additional *RAS* mutations in the future. New information from these trials could potentially alter the results of the current analysis.

A potential direction of future research is the evaluation of individual *RAS* mutations to understand whether the magnitude of effect on anti-EGFR mAb efficacy varies from mutation to mutation, including if some *RAS* mutations but not others are associated with worse PFS and OS when treated with anti-EGFR mAb therapy. Data was not reported in a manner to enable such an analysis to be performed in this study and a collaborative meta-analysis based on patient-level data will likely be required. As the prevalence of individual mutations is low, the power to detect differences between individual mutations may be limiting. An additional direction for future research is the further evaluation of mutations and expression of genes within the MAPK pathway but outside the RAS family such as PTEN, PIK3CA and EREG [24-26]. Moreover, in order to confirm that *RAS* subgroup estimates of treatment effect are unbiased, future patient-level analyses of the trial data should aim to further evaluate the balance of prognostic factors within *RAS* subgroups and the impact of excluding trial participants with insufficient tumor sample for *RAS* mutation status ascertainment.

In conclusion, meta-analysis analysis of nine RCTs indicates that not all *KRAS* exon 2 wild-type tumors benefit from anti-EGFR mAb treatment in mCRC. Individuals who are *KRAS* exon 2 wild-type, but have one of the new *RAS* mutations, have distinctly inferior anti-EGFR mAb treatment benefit compared to individuals without any *RAS* mutations. Rather, individuals with one of the new *RAS* mutations appear to be much more similar to individuals with a *KRAS* exon 2 mutation in that both groups have little evidence of a significant survival benefit from anti-EGFR mAbs. These results suggest that extended *RAS* mutation testing

(*KRAS* exon 3 and 4 and *NRAS* exon 2, 3 and 4, in addition to *KRAS* exon 2 currently undertaken) should be undertaken prior to the administration of an anti-EGFR mAb. The weight of current evidence indicates that both cetuximab and panitumumab should only be prescribed for patients with mCRC that are wild-type for all known *RAS* activating mutations.

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References

1. Dahabreh IJ, Terasawa T, Castaldi PJ, Trikalinos TA. Systematic review: Anti–epidermal growth factor receptor treatment effect modification by KRAS mutations. Ann Intern Med 2011; 154: 37-49.
2. Behl AS, Goddard KAB, Flottemesch TJ et al. Cost-effectiveness analysis of screening for KRAS and BRAF mutations in metastatic colorectal cancer. J Natl Cancer Inst 2012; 104: 1785-1795.
3. Douillard JY, Oliner KS, Siena S et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med 2013; 369: 1023-1034.
4. Adelstein B-A, Dobbins TA, Harris CA et al. 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. Eur J Cancer 2011; 47: 1343-1354.
5. Vale CL, Tierney JF, Fisher D et al. Does anti-EGFR therapy improve outcome in advanced colorectal cancer? A systematic review and meta-analysis. Cancer Treat Rev 2012; 38: 618-625.
6. Grothey A, Lenz H-J. Explaining the Unexplainable: EGFR Antibodies in Colorectal Cancer. J Clin Oncol 2012; 30: 1735-1737.
7. Maughan TS, Adams RA, Smith CG et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. The Lancet 377: 2103-2114.
8. European Medicines Agency, Committee for Medicinal Products for Human Use. Erbitux Assessment report EMA/CHMP/701107/2013. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000558/WC500160158.pdf (accessed 8 April 2014).
9. European Medicines Agency, Committee for Medicinal Products for Human Use. Vectibix Assessment report EMA/413562/2013. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000741/WC500148667.pdf (accessed 8 April 2014).
10. Patterson S, D., Peeters M, Siena S et al. Comprehensive analysis of KRAS and NRAS mutations as predictive biomarkers for single agent panitumumab (pmab) response in a randomized, phase III metastatic colorectal cancer (mCRC) study (20020408). J Clin Oncol 2013; 31: abstr 3617.
11. Peeters M, Oliner KS, Price TJ et al. Analysis of KRAS/NRAS mutations in phase 3 study 20050181 of panitumumab (pmab) plus FOLFIRI versus FOLFIRI for second-line treatment (tx) of metastatic colorectal cancer (mCRC). J Clin Oncol 2014; 32: abstr LBA387.
12. Peeters M, Douillard JY, Van Cutsem E et al. Mutant KRAS Codon 12 and 13 Alleles in Patients With mCRC: assessment as biomarkers of Response to Panitumumab. J Clin Oncol 2013; 31: 759-765.
13. Seymour MT, Brown SR, Middleton G et al. Panitumumab and irinotecan versus irinotecan alone for patients with KRAS wild-type, fluorouracil-resistant advanced colorectal cancer (PICCOLO): A prospectively stratified randomised trial. Lancet Oncol 2013; 14: 749-759.
14. Stintzing S, Jung A, Rossius L et al. Mutations within the EGFR signaling pathway: Influence on efficacy in FIRE-3-A randomized phase III study of FOLFIRI plus cetuximab or bevacizumab as first-line treatment for wild-type (WT) KRAS (exon 2) metastatic colorectal cancer (mCRC) patients. J Clin Oncol 2014; 32: abstr 445.
15. Bokemeyer C, Kohne CH, Ciardiello F et al. Treatment outcome according to tumor RAS mutation status in OPUS study patients with metastatic colorectal cancer (mCRC) randomized to FOLFOX4 with/without cetuximab. J Clin Oncol 2014; 32: abstr 3505.
16. Schwartzberg LS, Rivera F, Karthaus M et al. PEAK: A Randomized, Multicenter Phase II Study of Panitumumab Plus Modified Fluorouracil, Leucovorin, and Oxaliplatin

(mFOLFOX6) or Bevacizumab Plus mFOLFOX6 in Patients With Previously Untreated, Unresectable, Wild-Type KRAS Exon 2 Metastatic Colorectal Cancer. J Clin Oncol 2014.
17. Karthaus M, Schwartzberg L, Rivera F et al. Updated overall survival (OS) analysis of novel predictive KRAS/NRAS mutations beyond KRAS exon 2 in PEAK: A 1st-line phase 2 study of FOLFOX6 plus panitumumab (pmab) or bevacizumab (bev) in metastatic colorectal cancer (mCRC). Eur J Cancer 2013; 49: S516.
18. Ciardiello F, Lenz HJ, Kohne CH et al. Treatment outcome according to tumor RAS mutation status in CRYSTAL study patients with metastatic colorectal cancer (mCRC) randomized to FOLFIRI with/without cetuximab. . J Clin Oncol 2014; 32: abstr 3506.
19. Amado RG, Wolf M, Peeters M et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 2008; 26: 1626-1634.
20. Jonker DJ, O'Callaghan CJ, Karapetis CS et al. Cetuximab for the Treatment of Colorectal Cancer. N Engl J Med 2007; 357: 2040-2048.
21. Venook AP, Niedzwiecki D, Lenz HJ et al. CALGB/SWOG 80405: Phase III trial of irinotecan/5-FU/leucovorin (FOLFIRI) or oxaliplatin/5-FU/leucovorin (mFOLFOX6) with bevacizumab (BV) or cetuximab (CET) for patients (pts) with KRAS wild-type (wt) untreated metastatic adenocarcinoma of the colon or rectum (MCRC). J Clin Oncol 2014; 32: abstr LBA3.
22. Tveit K GT, Glimelius B et al. . Randomized phase III study of 5-fluorouracil/folinate/oxaliplatin given continuously or intermittently with or without cetuximab, as first-line treatment of metastatic colorectal cancer: The NORDIC VII study (NCT00145314), by the Nordic Colorectal Cancer Biomodulation Group. Ann Oncol 2010; viii9.
23. Sobrero AF, Maurel J, Fehrenbacher L et al. EPIC: Phase III Trial of Cetuximab Plus Irinotecan After Fluoropyrimidine and Oxaliplatin Failure in Patients With Metastatic Colorectal Cancer. J Clin Oncol 2008; 26: 2311-2319.
24. Jonker DJ, Karapetis C, Harbison CT. Epiregulin gene expression as a biomarker of benefit from cetuximab in the treatment of advanced colorectal cancer. Br J Cancer 2014; 110: 648-655.
25. Sartore-Bianchi A, Martini M, Molinari F et al. PIK3CA Mutations in Colorectal Cancer Are Associated with Clinical Resistance to EGFR-Targeted antibodies. Cancer Res 2009; 69: 1851-1857.
26. De Roock W, De Vriendt V, Normanno N et al. KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. Lancet Oncol 2010; 12: 594-603.

Table 1. Summary of studies included in the meta-analysis

Figure 1. Grouping of tumors by *KRAS* exon 2 mutations and extended *RAS* mutations.

Figure 2. Prevalence of new *RAS* mutations across studies

NA; not applicable, NE; not evaluated, NR; evaluated but not reported

[a]New *RAS* mutations are reported as a proportion of the *KRAS* exon 2 wild-type group.

[b]*KRAS* and *NRAS* codon 59 mutation not evaluated.

[c]*KRAS* codon 117 mutation not evaluated

[d]includes exon 3 codon 61 mutations in addition to the exon 2 mutations

[e]only *NRAS* mutation G12C evaluated

[f]Random-effects meta-analysis summary estimates (95% confidence interval) based on studies that have evaluated all relevant codons

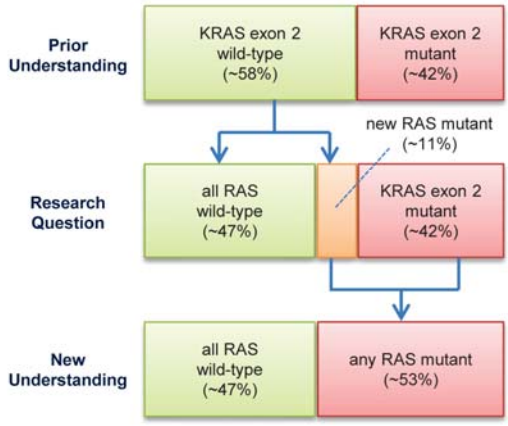
Figure 3. The relative size of the anti-EGFR treatment effect for tumors with one of the new *RAS* mutations compared to (A) tumors without any *RAS* mutations, and (B) tumors with any *KRAS* exon 2 mutations.

Figure 4. Anti-EGFR treatment benefit for tumors without any *RAS* mutations (all RAS wild-type) and tumors without any *KRAS* exon 2 mutations (KRAS exon 2 wild-type) with respect to (A) progression-free survival, and (B) overall survival.

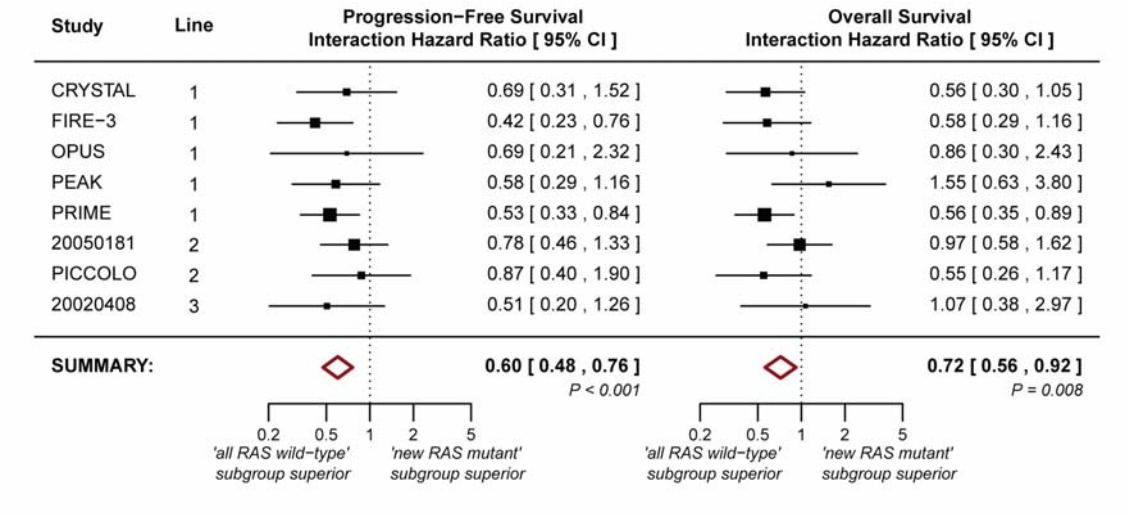
cmab; cetuximab, OS; overall survival, PFS; progression-free survival, pmab; panitumumab

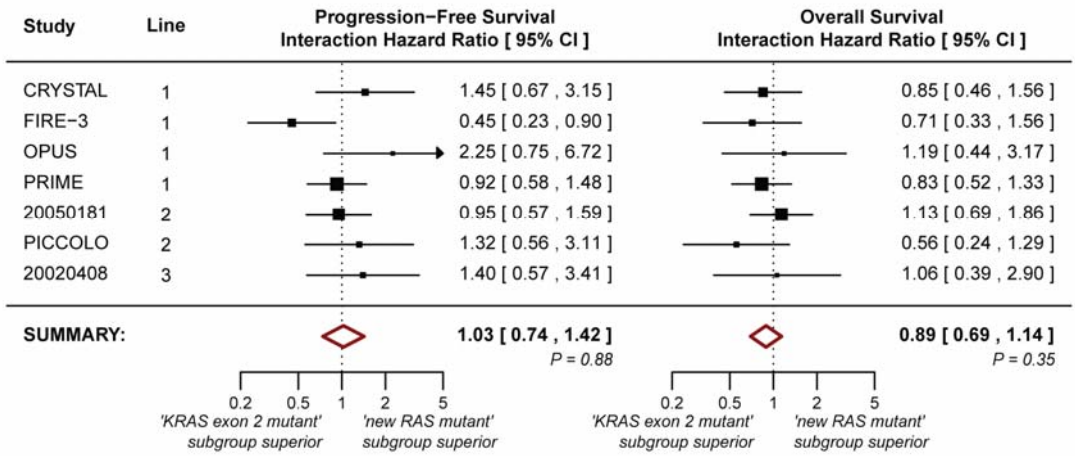
Figure 5. Anti-EGFR treatment benefit for tumors with any *RAS* mutations and tumors with any *KRAS* exon 2 mutations with respect to (A) progression-free survival, and (B) overall survival.

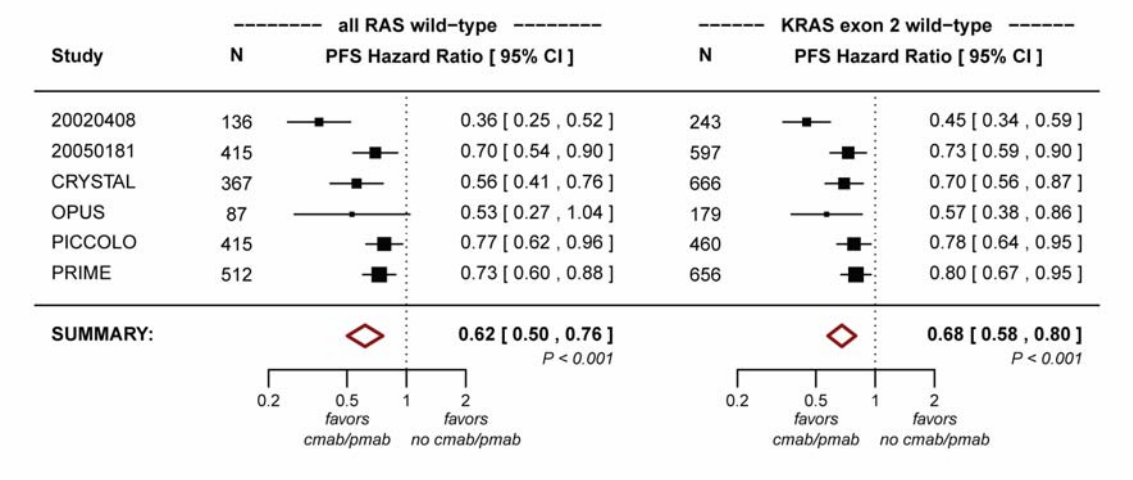
cmab; cetuximab, OS; overall survival, PFS; progression-free survival, pmab; panitumumab

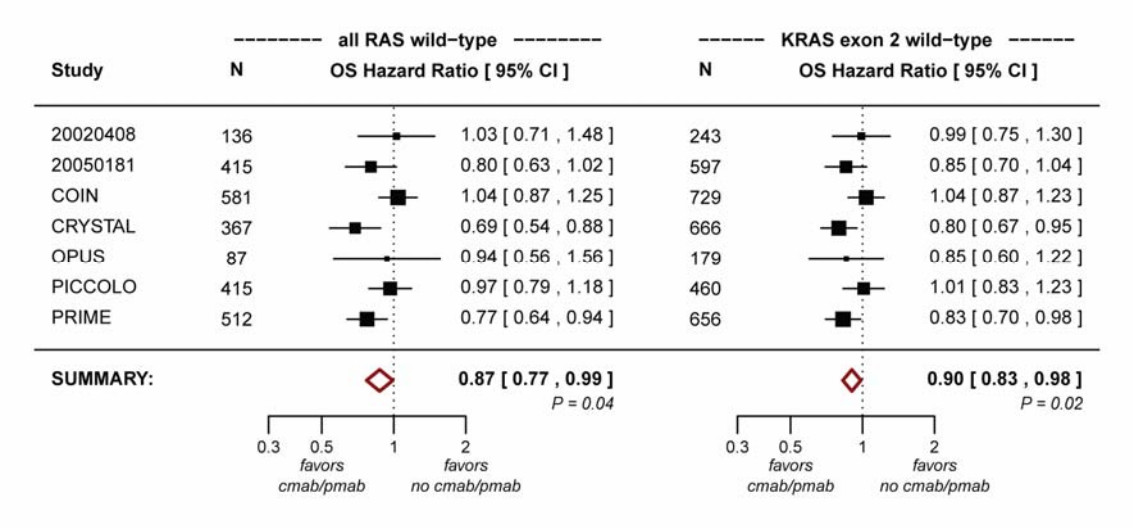


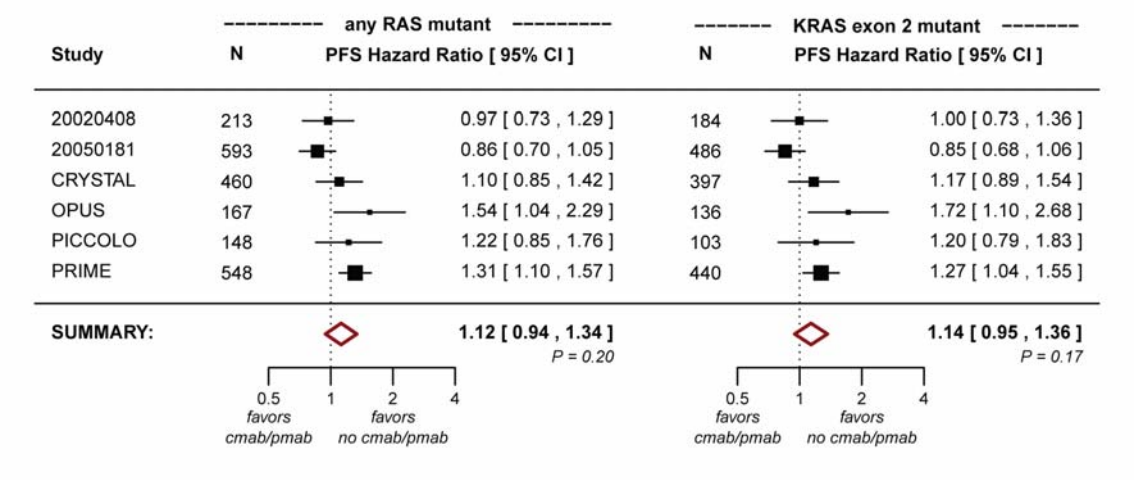
	New RAS Total[a]	KRAS Exon 3[a] (59, 61)	KRAS Exon 4[a] (117, 146)	NRAS Exon 2[a] (12, 13)	NRAS Exon 3[a] (59, 61)	NRAS Exon 4[a] (117, 146)
OPUS	26.3%	5.9%	9.3%	6.8%	5.1%	0.8%
PICCOLO	9.8%	NR[b]	3.7%[c]	6.3%[d]	NR[b]	NE
20020408	17.6%	4.8%[b]	5.0%	4.2%	3.0%[b]	1.1%
20050181	20.5%	4.6%	7.9%	2.3%	5.8%	0.0%
PRIME	17.4%	3.7%[b]	5.6%	3.4%	4.1%[b]	0.0%
FIRE-3	16.0%	4.3%[b]	4.9%[c]	3.8%	2.0%[b]	0.0%
PEAK	20.1%	4.1%	7.7%	5.4%	5.9%	0.0%
COIN	8.4%	2.1%[b]	NE	0.9%[e]	3.0%[b]	NE
CRYSTAL	14.7%	3.3%	5.6%	3.5%	2.8%	0.9%
SUMMARY[f]	19.9% (16.7%,23.4%)	4.3% (3.3%, 5.5%)	6.7% (5.7%, 7.9%)	3.8% (3.0%, 4.8%)	4.8% (3.4%, 6.8%)	0.5% (0.2%, 1.2%)











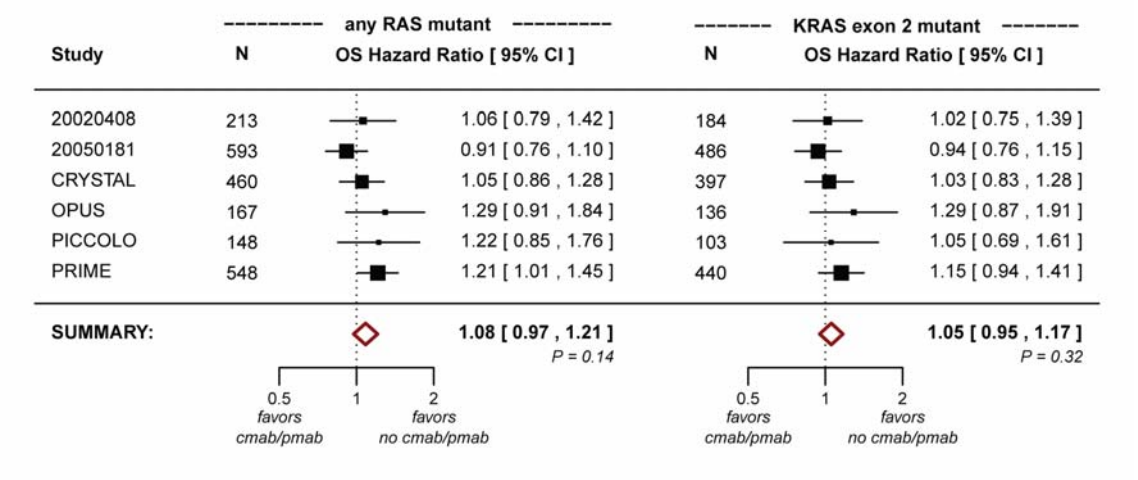


Table 1. Summary of studies included in the meta-analysis

Anti-EGFR Agent Vs. Comparator	Trial Name	Line, Background Treatment	*N* (ITT)	Exons Evaluated[a]						Ascertainment *KRAS* exon 2 / New *RAS*[b] exons	Wild-type Proportion[c] *KRAS* exon 2 / All *RAS* exons
				KRAS			*NRAS*				
				2	3	4	2	3	4		
Cetuximab Vs. No Cetuximab	OPUS	1st line, FOLFOX	337	■	■	■	■	■	■	94% / 66%	57% / 42%
	COIN	1st line, OxFp	1630	■	[d]					79% / 100%	56% / 53%
	CRYSTAL	1st line, FOLFIRI	1198	■	■	■	■	■	■	89% / 65%	63% / 54%
Panitumumab Vs. No Panitumumab	PRIME	1st line, FOLFOX	1183	■		■	■		■	93% / 95%	60% / 51%
	20050181	2nd line, FOLFIRI	1186	■	■	■	■	■	■	91% / 87%	55% / 44%
	PICCOLO	2nd line, Irinotecan	615	■	[d]		■			92% / ~81%	NA
	20020408	3rd line, BSC	463	■		■	■		■	92% / 68%	57% / 47%
Cetuximab Vs. Bevacizumab	FIRE-3	1st line, FOLFIRI	735	■			■	■	■	96% / 69%	NA
Panitumumab Vs. Bevacizumab	PEAK	1st line, FOLFOX	285	■	■	■	■	■	■	100% / 79%	NA

Notes:

BSC: best supportive care, FOLFIRI: folinic acid, fluorouracil and irinotecan, FOLFOX: folinic acid, fluorouracil and oxaliplatin, ITT: overall intention-to-treat population, NA: not applicable, OxFp; oxaliplatin and fluoropyrimidine chemotherapy

[a]dark grey shading indicates that all codons within the specified exon were assessed, and light grey shading indicates some but not all codons were assessed.

[b]Ascertainment of the new RAS mutations refers to the proportion of *KRAS* exon 2 wild-type participants that were evaluable for the new *RAS* mutations