

Anti-epidermal growth factor receptor monoclonal antibody-based therapy for metastatic colorectal cancer: a meta-analysis of the effect of *PIK3CA* mutations in *KRAS* wild-type patients

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Abstract

Introduction: We conducted a meta-analysis to dissect the association between *PIK3CA* mutations (exon 9 and exon 20) and resistance to anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (MoAbs) in *KRAS* wild-type metastatic colorectal cancer (mCRC) patients.

Material and methods: In 11 previously published studies, 864 cancer patients were treated with cetuximab or panitumumab-based therapy. Primary outcomes included objective response (complete response + partial response vs. stable disease + progressive disease), progression-free survival (PFS), and overall survival (OS). We calculated the odds ratio (OR) or hazard ratio (HR) with 95% confidence intervals (CIs) to estimate the risk or hazard. We found consistent and clinically substantial risk or hazard for objective response, PFS, and OS in the cetuximab or panitumumab-treated mCRC patients.

Results: *PIK3CA* mutations as a whole were associated with reduced response and poor PFS and OS in *KRAS* wild-type mCRC patients (objective response: OR = 0.42 and 95% CI 0.23–0.75; PFS: HR = 1.54 and 95% CI 1.13–2.09; and OS: HR = 1.4 and 95% CI 1.02–1.91). *PIK3CA* exon 9 mutations had no effect, whereas exon 20 mutations were associated with a worse outcome compared with wild types, with an OR of 0.21 (95% CI 0.05–0.93).

Conclusions: *PIK3CA* mutations as a whole might be useful prognostic factors for assessing clinical outcomes of anti-EGFR MoAb-based chemotherapies in *KRAS* wild-type mCRC patients. In particular, *PIK3CA* exon 20 mutations were significantly associated with lack of response.

Key words: colorectal cancer, *PIK3CA*, monoclonal antibodies, meta-analysis.

Introduction

Colorectal cancer is one of the most common cancers worldwide and one of the leading causes of cancer mortality. There has been an increase in the incidence of colorectal cancer in Poland [1]. Despite ad-

vances in chemotherapy, the 5-year relative survival remains poor at just 11% for patients with metastatic colorectal cancer (mCRC) [2]. Currently, two monoclonal antibodies (MoAbs) targeted at epidermal growth factor receptor (EGFR), the chimeric IgG1 MoAb cetuximab and the fully humanized IgG2 MoAb panitumumab, have shown a relevant clinical effect in treatment of patients with chemotherapy-refractory mCRC [3–6]. Because of common resistance to anti-EGFR MoAbs, recent guideline recommendations suggest that anti-EGFR MoAbs be given only to patients with *KRAS* wild-type mCRC [7, 8]. However, even for non-carriers of *KRAS* mutations, the response rate to anti-EGFR MoAbs is not high, ranging from 17% to 60%, and only a subset of patients benefit from this treatment [9–18]. This heterogeneity suggests that there may be other predictive variables, besides *KRAS*, that determine responsiveness to anti-EGFR. Thus, the predictive value of additional mutations and deregulations of signaling pathways downstream of EGFR such as *BRAF*, *PIK3CA* or *PTEN* is currently under intensive investigation.

Specifically, what is highlighted in the explorations is *PIK3CA*. *PIK3CA* encodes the p110 α catalytic subunit of the class IA phosphatidylinositol 3-kinases (PI3Ks). Tumor-derived *PIK3CA* mutations lead to constitutive activation of p110 α enzymatic activity, stimulate the AKT pathway, and promote cell growth [19]. *PIK3CA* is frequently mutated in several malignancies such as colon, breast, brain, ovarian, liver, and lung cancers [20]. Mutation frequencies in CRC vary from 10% to 30% [17, 21]. The vast majority of activating *PIK3CA* mutations map to 3 sites: exon 9, codons 542 and 545 in the helical domain, and exon 20, codon 1047 in the kinase domain.

Emerging data have suggested that *PIK3CA* mutation is likely to be predictive of a lack of benefit from anti-EGFR therapy in mCRC [22–24], but the results are still inconclusive, partially due to the inclusion of molecularly unselected populations. For example, Prenen *et al.* suggested that *PIK3CA* mutations were not a major determinant of resistance to the anti-EGFR MoAbs in unselected mCRC patients [25], whereas De Roock *et al.* found that patients with *PIK3CA* mutations had a significantly lower response rate in *KRAS* wild-type patients [26]. Besides, the relatively small sample size of each study may have an effect. For example, Sartore-Bianchi *et al.* suggested that *PIK3CA* mutations were significantly associated with clinical resistance to anti-EGFR MoAbs [22] while Moroni *et al.* showed that *PIK3CA* alterations did not correlate with response [21]. Therefore, it is necessary to conduct a new meta-analysis to derive a more precise estimation of predictive value of *PIK3CA* mutations in *KRAS* wild-type mCRC patients treated with anti-EGFR MoAbs.

Material and methods

Study selection

Systematic computerized searches of the PubMed and HuGENet databases (up to 25th February 2013) were performed. The following search terms were used: ‘cetuximab’, ‘panitumumab’, ‘colon cancer’, ‘rectal cancer’, ‘colorectal cancer’, ‘colorectal neoplasm’, ‘CRC’, ‘*PIK3CA*’, ‘phosphoinositide-3-kinase catalytic’, ‘ α polypeptide’, ‘PI 3-kinases’. References of the retrieved articles were further screened for earlier original studies. The inclusion criteria were as follows: (i) the studies focused on mCRC patients; (ii) those exploring the relation between *PIK3CA* mutations and clinical outcomes of *KRAS* wild-type mCRC patients treated with anti-EGFR MoAbs; (iii) those using one or more of the following as outcomes to assess tumor response and survival: objective response, PFS, and OS.

Statistical analysis

For each outcome measure, we estimated effects separately for patients with wild-type *PIK3CA* and *PIK3CA* mutations. For overall response rate, an odds ratio (OR) was calculated from the reported number of objective response (complete response (CR) + (partial response PR)) and no response (progressive disease (PD) + stable disease (SD)) in each arm, using the WHO criteria or RECIST (Response Evaluation Criteria in Solid Tumors) criteria [27]. In order to dissect the complicated relation between *PIK3CA* status and prognosis in relation to the treatment, we did stratified analyses and estimated the pooled OR according to *KRAS* mutation status. As different biological effects have been suggested for *PIK3CA* exon 9 (helical domain) and exon 20 (kinase domain) mutations [28–30], the OR or hazard ratio (HR) was also estimated for each type of mutation as well as for any *PIK3CA* mutations. Progression-free survival (PFS) and overall survival (OS) were evaluated by pooled Cox proportional HRs and 95% CIs by published methods. The between-study heterogeneity was evaluated with I^2 and 50% in I^2 was regarded as the threshold. We carried out initial analyses with a fixed effect model and confirmatory analyses with a random effect model, if there was significant heterogeneity. We used inverted funnel plots and the Egger test to examine the effect of publication bias. All analyses were carried out using the Stata 9.2 and RevMan 5.0 software.

Results

The literature search (as of February 25, 2013) yielded 95 potentially assessable publications. Of these, 74 were excluded for the following reasons: not original studies, not human studies, not cohort

studies and irrelevant studies. After reviewing the remaining 21 studies, we excluded 1 study which was not a retrospective cohort study [31], 1 study [22] that used the same data in another included study [9], 1 study in which no mutation in *PIK3CA* was found in any patient [32] and 7 studies which did not provide data in *KRAS* wild-type patients [14, 17, 33–37]. Finally, 11 relevant studies, comprising a total of 864 patients, were included. The flowchart of the reviews showed the detailed process of selection (Figure 1). The main characteristics of included studies are shown in Table I. Of the 11 studies, sample sizes ranged from 18 to 339. All of these studies were of retrospective design. Anti-EGFR MoAbs were given as second line or more in all the studies. The patients all received anti-EGFR MoAb-based treatment.

PIK3CA mutations and objective response in *KRAS* wild-type patients

Nine studies including 693 patients were eligible for the final analysis. *PIK3CA* mutations as a whole were associated with reduced objective response in *KRAS* wild-type mCRC patients (OR = 0.42;

95% CI 0.23–0.75; $p = 0.003$; Figure 2). In stratified analyses, the association remained significant in subgroups of exon 20 mutations (OR = 0.21; 95% CI 0.05–0.93; $p = 0.04$), whereas exon 9 mutations had no effect (OR = 0.54; 95% CI 0.26–1.12; $p = 0.10$). Given that all the ORs for the exon 9 subgroup were less than 1 except the study of Moroni *et al.*, we performed an influence analysis by excluding the study. The result was similar to that of the former (OR = 0.46; 95% CI 0.21–1.01; $p = 0.05$; data not shown). No publication bias was detected by either the funnel plot or the Egger test ($p = 0.065$, $p = 0.060$, $p = 0.185$ for all exons, exon 20 and exon 9, respectively).

PIK3CA mutations and progression-free survival and overall survival in *KRAS* wild-type patients

Four studies including 545 patients were eligible for the final analysis. The *PIK3CA* mutations were associated with a significant increase of hazard for PFS in all patients (mutant vs. wild-type: HR = 1.54; 95% CI 1.13–2.09; $p = 0.006$; Figure 3). No significant between-study heterogeneity was

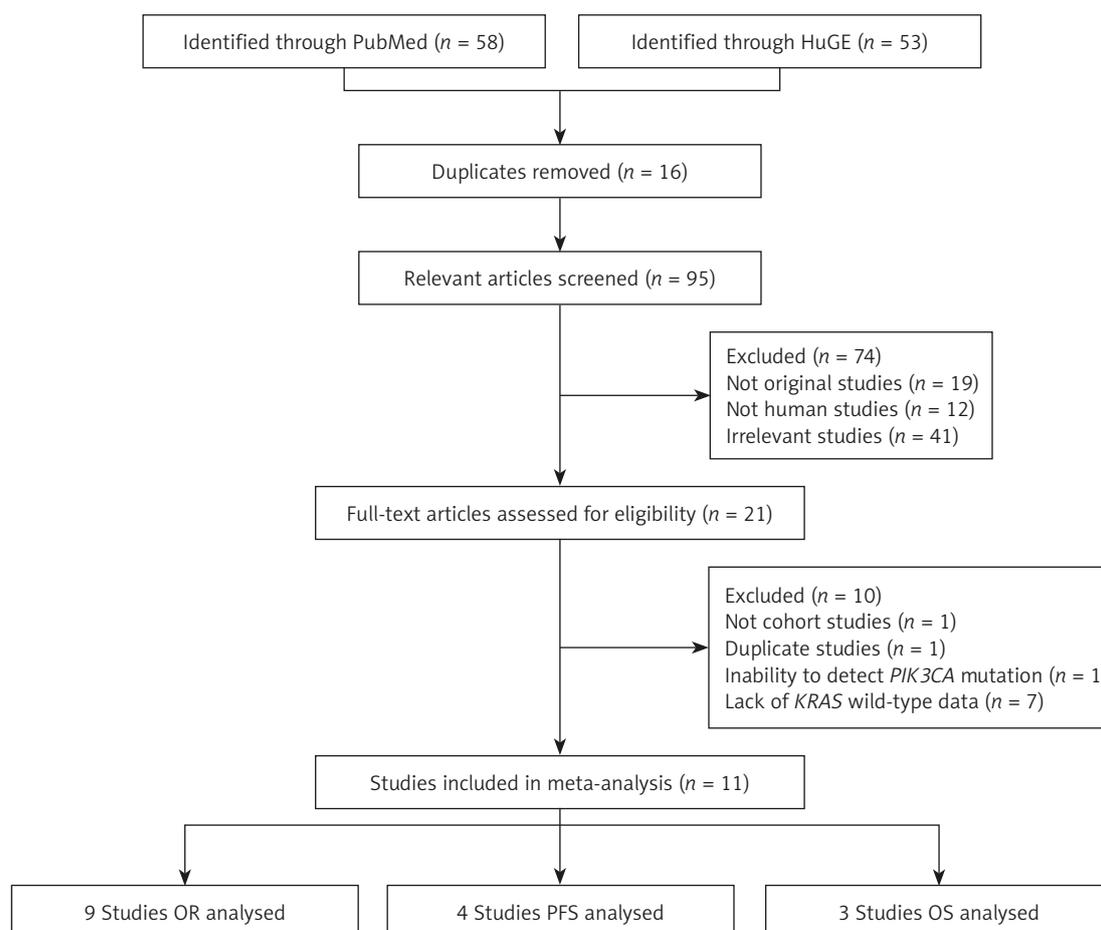
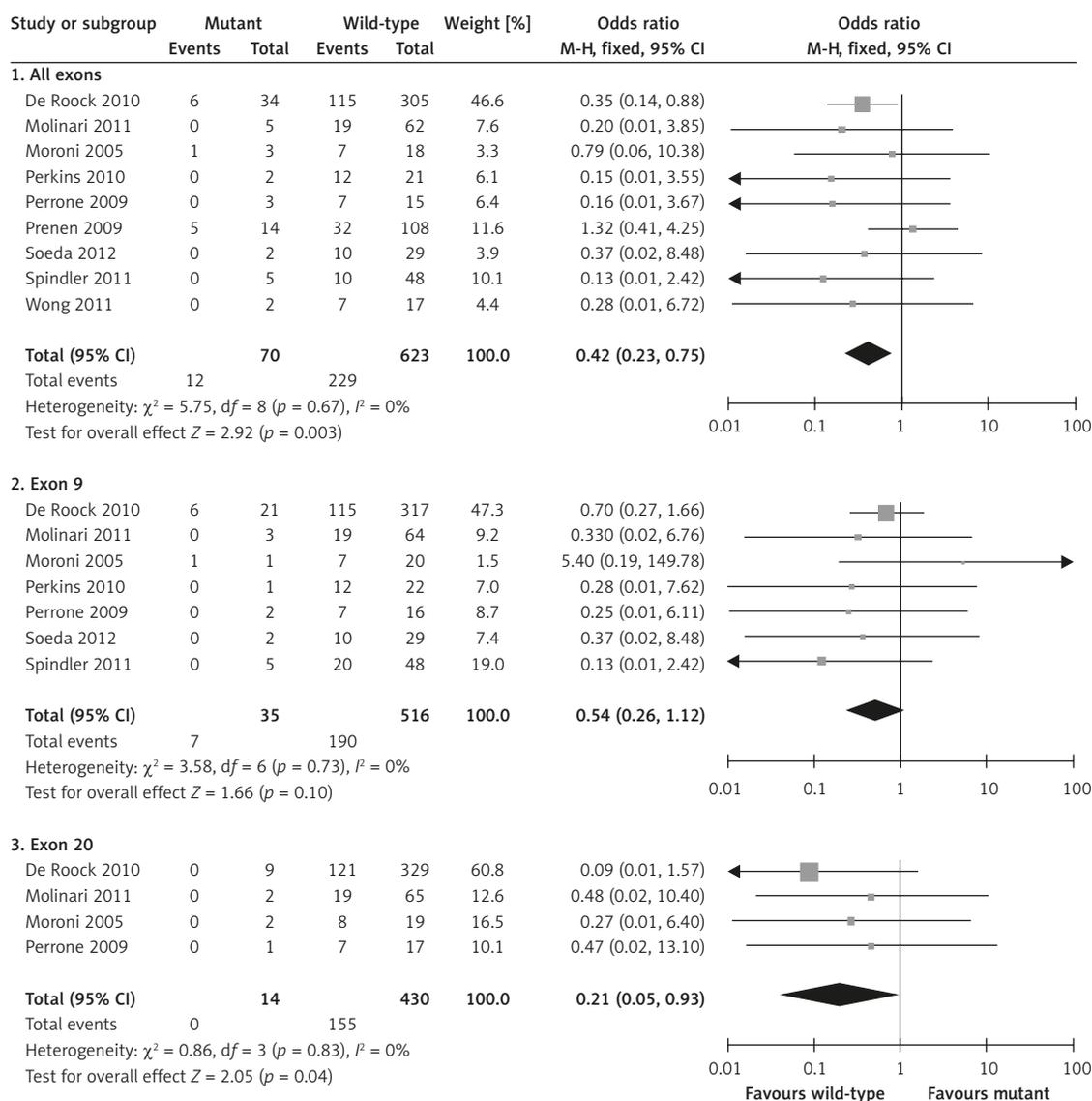


Figure 1. Flow chart for the process of selecting eligible publications

Table 1. Characteristics of studies identified for the meta-analysis

References	Country of origin	Year	Mutation analysis methods	No. of KRAS wild-type patients	Age	Previous treatment protocols	Study treatment protocols	Outcomes	Criteria
Soeda <i>et al.</i> [40]	Japan	2012	DS	31	57	≥ 1 Chemotherapy	C alone; or C + I	CR, PR, SD, PD	RECIST
Molinari <i>et al.</i> [41]	Italy, Switzerland	2011	DS	67	60	I and/or O	C alone, P alone, or C + Chem	PR, SD, PD	RECIST
Spindler <i>et al.</i> [39]	Denmark	2011	DxS	53	62	≥ 1 Chemotherapy	C + I	PFS, OS, CR, PR, SD, PD	RECIST
Wong <i>et al.</i> [24]	USA	2011	DxS	19	56	≥ 1 Chemotherapy	C + capecitabine, C + O, C + B	CR, PR, SD, PD	RECIST
Saridaki <i>et al.</i> [18]	Greece	2011	Sanger sequencing	75	66	≥ 1 Chemotherapy	C + I based, or C + O based	TTP, OS	NR
De Roock <i>et al.</i> [26]	7 countries	2010	DxS	339	61	≥ 1 Chemotherapy	C + Chem	PFS, OS, CR, PR, SD, PD	RECIST or WHO
Perkins <i>et al.</i> [16]	France	2010	DS	23	61.8	I based	C alone, P alone, C + I, or C + FOLFIRI	CR, PR, SD, PD	RECIST
Prenen <i>et al.</i> [25]	Belgium	2009	AD + DS	122	61	I based	C + I, or C alone	PFS, OS, CR, PR, SD, PD	RECIST
Sartore-Bianchi <i>et al.</i> [9]	Italy	2009	DS	96	64	≥ 1 Chemotherapy	C alone, P alone, or C + I based	PFS, OS, CR, PR, PD, SD	RECIST
Perrone <i>et al.</i> [23]	Italy	2009	DS	18	57.3	I based	C + I	PFS, PR, SD, PD	RECIST
Moroni <i>et al.</i> [21]	Italy	2005	DS	21	65.6	≥ 1 Chemotherapy	C alone, P alone, or C + I based	PR, SD, PD	RECIST

AD – allelic discrimination, C – cetuximab, Chem – chemotherapy, DS – direct sequencing, DxS – DxS PI3K Mutation Test Kit, FOLFIRI – fluorouracil, folinic acid, and irinotecan; I – irinotecan, NR – not reported, C – cetuximab, P – panitumumab, B – bevacizumab, O – oxaliplatin, FOLFIRI – fluorouracil, folinic acid, and irinotecan; OS – overall survival, PFS – progression-free survival, DS – direct sequencing, DxS – DxS PI3K Mutation Test Kit, FOLFIRI – fluorouracil, folinic acid, and irinotecan; response, PR – partial response, SD – stable disease, PD – progressive disease



CI – confidence interval, M-H – Mantel-Haenszel, Mutant – *PIK3CA* mutant, Wild-type – *PIK3CA* wild-type

Figure 2. Forest plot of the analysis of the relative risk according to the *PIK3CA* exon of the mutation in *KRAS* wild-type patients

found in the initial fixed model, so a random model did not need to be performed ($I^2 = 36.6\%$). Additionally, Egger’s test did not show publication bias ($p = 0.894$), and no significant outcome of influence analysis was observed (data are not shown).

Three studies including 527 patients were eligible for the final analysis. There was a substantial effect of *PIK3CA* mutations on death hazard in all patients (mutant vs. wild-type: HR = 1.40; 95% CI 1.02–1.91; $p = 0.036$; Figure 4). No significant between-study heterogeneity was observed in the initial fixed model ($I^2 = 0.0\%$). Moreover, no publication bias was detected with a p value of 0.552 in Egger’s test, and no significant outcome of influence analysis was observed (data are not shown).

Discussion

This meta-analysis used objective response, PFS, and OS as primary parameters to assess the influence of *PIK3CA* mutations on clinical outcomes of anti-EGFR MoAb chemotherapy in mCRC with *KRAS* wild-type. Our results imply that *PIK3CA* mutations were a biomarker of low objective response as well as short PFS and OS in *KRAS* wild-type patients with anti-EGFR MoAb chemotherapy.

Our study has some strengths although a similar meta-analysis was conducted before. The previous meta-analysis suggested that *PIK3CA* exon 20 mutations might be a potential biomarker for resistance to anti-EGFR MoAbs in *KRAS* wild-type mCRC [38], even though it failed to reach stati-

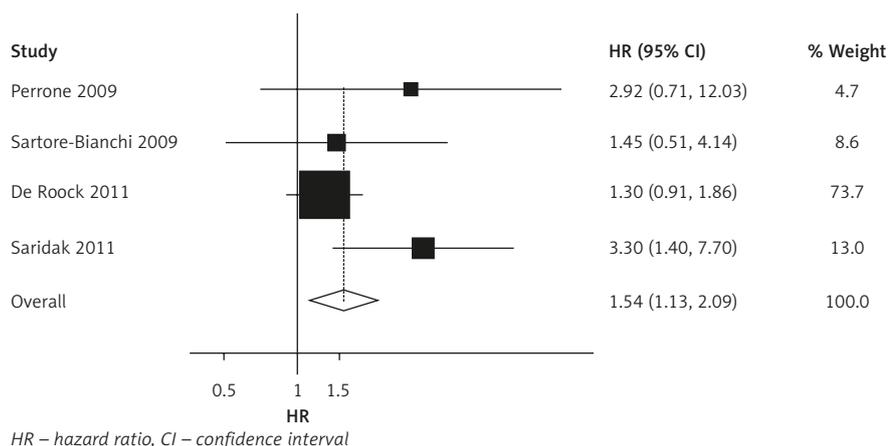


Figure 3. Forest plot of PFS according to the *PIK3CA* mutation in *KRAS* wild-type patients (mut vs. wt, reference group = wt)

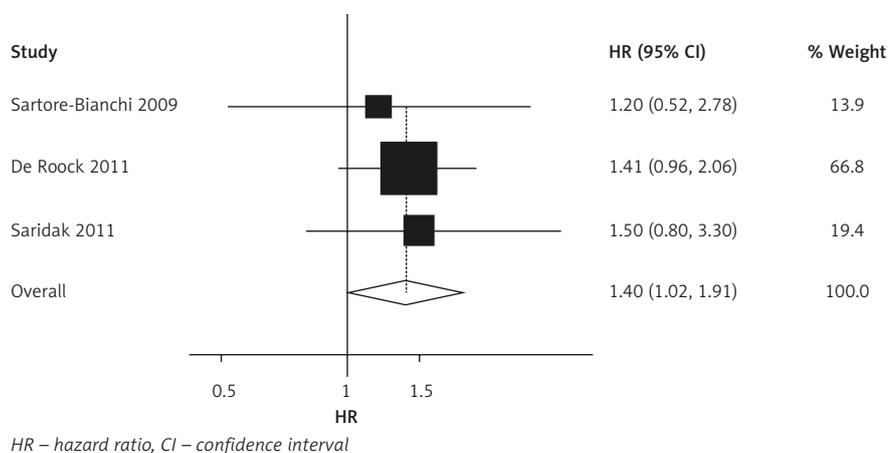


Figure 4. Forest plot of OS according to the *PIK3CA* mutation in *KRAS* wild-type patients (mut vs. wt, reference group = wt)

istical significance, most likely due to the small sample size. Moreover, flaws in the previous meta-analysis were that PFS and OS data were not sensibly combined. Because of the different ways and the incompleteness of reported data, the previous authors stated that the results could not be combined. But we believe that it is possible to combine the survival data if they were made full use of. For example, there were two duplicate studies, and we included a study [9] which provided survival data in wild-type *KRAS* patients, while the previous authors excluded it. In addition, a study was available because original data information was provided by the authors [23]. Recently, 6 studies with a larger sample size have been reported [32, 36, 37, 39–41]. Several studies have suggested that *PIK3CA* mutation was likely to be predictive of a lack of benefit from anti-EGFR therapy in mCRC. Among them, two studies [39, 40] provided data in *KRAS* wild-type patients and two studies [36, 37] were in patients unselected by *KRAS* mutation status. However, one study suggested that *PIK3CA* alterations were not associated with response [41]. Considering the

inconsistent conclusions, we updated the previous meta-analysis and confirmed its finding that *PIK3CA* exon 20 mutations were a potential biomarker for a low objective response in *KRAS* wild-type patients treated with anti-EGFR MoAbs.

Notably, the result is different if mutations on exon 9 and exon 20 are considered separately, as confirmation of the fact that the two exons have different mechanisms of action and related effects [42]. To induce transformation exon 20 mutants depend on binding with the regulatory subunit p85 α , whereas exon 9 mutants circumvent p85 α binding but depend on RAS binding instead [43, 44]. Regarding objective response, exon 20 mutations alone significantly decrease the chance of achieving an objective response, whereas exon 9 mutations are unable to reproduce these data (Figure 2). Unfortunately, the survival results could not be divided into exon 9 and exon 20 mutations in a meta-analysis so far. Only one study by De Roock *et al.* [26] provided survival data according to the *PIK3CA* exon of the mutations. It reported that *PIK3CA* exon 20 mutations were statistically significantly associated with shorter PFS and OS

in *KRAS* wild-type patients. It seems that the predictive power of exon 20 mutation is greater than that of any exon mutations and exon 9 mutations. A recent study in which stage χ CRC patients were present in 151 (13%) cases found that coexistence of *PIK3CA* exon 9 and 20 mutations, but not *PIK3CA* mutation in either exon 9 or 20 alone, was associated with poor prognosis of CRC (not mCRC) patients [45]. So far, it is not certain whether exon 20 mutation could be a potential biomarker for resistance to anti-EGFR MoAbs in *KRAS* wild-type mCRC. Taking lessons from studies in which the distinctive prognostic role of exon 9 and exon 20 mutations has been described, additional larger studies are still warranted to investigate the possibilities since the ultimate aim of the treatment of mCRC is to prolong the survival without affecting health-related quality of life parameters.

Despite our efforts to make an accurate and comprehensive analysis, limitations of our meta-analysis need to be addressed. First, most of the included studies were retrospective and not all the methods of *PIK3CA* mutation determination were consistent. This may have caused heterogeneity between studies. Second, not all treatment arms used anti-EGFR MoAbs as a single compound but in combination with irinotecan, oxaliplatin or 5-Fu, which could cause some bias in our estimates but was unlikely to change our major conclusions, because no studies showed that chemotherapy correlated with *PIK3CA*. Third, only 4 studies presented data on HR with 95% CI for PFS and only three studies presented data on HR with 95% CI for OS. The relatively small sample size might not have enough statistical power to detect the real association. Fourth, our analysis largely used unadjusted estimates, because not all published studies presented adjusted estimates, or when they did, the estimates were not adjusted by the same potential confounders. Fifth, we were unable to analyze the association between *PIK3CA* mutations and cetuximab or panitumumab toxicities, because few studies provided this information or used different toxicity profiles. Finally, our study was restricted to mCRC patients with *KRAS* wild-type, so we failed to assess the influence of other mutations. *BRAF* mutations have been associated with a poor prognosis in colorectal cancer [46] and might therefore confound analyses of *PIK3CA* mutations and survival in colorectal cancer. In contrast, Ulivi *et al.* concluded that *BRAF* and *PIK3CA* mutations would seem to be independent predictors of anti-EGFR therapy effectiveness [37]. *PIK3CA* exon 9 mutations in primary tumors and loss of PTEN nuclear expression in metastases correlated with *KRAS* mutations [47]. We were unable to investigate potential interactions between *PIK3CA* mutations on exon 9 and exon 20 due to

the limited publications available on this topic in which only one sample carried a mutation in both exon 9 and exon 20 [25].

Overall, our meta-analysis showed that *PIK3CA* mutations as a whole might be useful prognostic factors for assessing clinical outcomes and further confirmed that *PIK3CA* mutation on exon 20 decreases the response rate of anti-EGFR MoAb-based chemotherapies in wild-type *KRAS* mCRC patients. But we could not exclude the potential confounding by the interaction effect of other mutations which frequently associated with *PIK3CA* exon 20 mutations. We also strongly recommend that exon 9 and 20 mutations be studied separately. And future prospective studies with a larger sample size are required to confirm our findings.

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