

***BRAF* V600E mutation and resistance to anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer: a meta-analysis**

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Abstract Epidemiologic studies have evaluated the association between *BRAF* mutations and resistance to the treatment of anti-EGFR monoclonal antibodies (MoAb) in patients with metastatic colorectal cancer (mCRC). However, the results are still inconclusive. To derive a more precise estimation of the relationship, we performed this meta-analysis. A total of 11 studies were included in the final meta-analysis. There were seven studies for unselected mCRC patients and four studies for patients with wild type *KRAS* mCRC. Among unselected mCRC patients, *BRAF* V600E mutation was detected in 48 of 546 primary tumors (8.8%). The objective response rate (ORR) of patients with mutant *BRAF* was 29.2% (14/48), whereas the ORR of patients with wild-type *BRAF* was 33.5% (158/472). The overall RR for ORR of mutant *BRAF* patients over wild-type *BRAF* patients was 0.86 (95% CI = 0.57–1.30; $P = 0.48$). For patients with *KRAS* wild-type mCRC, *BRAF* V600E mutation was detected in 40 of 376 primary tumors (10.6%). The ORR of patients with mutant

BRAF was 0.0% (0/40), whereas the ORR of patients with wild-type *BRAF* was 36.3% (122/336). The pooled RR of mutant *BRAF* patients over wild-type *BRAF* patients was 0.14 (95% CI = 0.04–0.53; $P = 0.004$). In conclusion, this meta-analysis provides evidence that *BRAF* V600E mutation is associated with lack of response in wild-type *KRAS* mCRC treated with anti-EGFR MoAbs. *BRAF* mutation may be used as an additional biomarker for the selection of mCRC patients who might benefit from anti-EGFR MoAbs therapy.

Keywords *BRAF* · Metastatic colorectal cancer · Monoclonal antibodies · Mutation · Meta-analysis

Introduction

Metastatic colorectal cancer (mCRC) is one of the most common human malignant disease and one of the leading causes of cancer-related death worldwide. Despite of recent advances in chemotherapeutic treatment, the 5-year overall survival (OS) is relatively poor, with a median survival of 18–21 months [1]. There is a continuous need for more effective therapies. Most recently, two monoclonal antibodies (MoAb) targeting epidermal growth factor receptor (EGFR), cetuximab and panitumumab, have been developed and approved for the treatment of chemotherapy-resistant mCRC [2, 3]. However, resistance to anti-EGFR MoAbs was common. Among unselected mCRC patients, only 10–20% of patients can truly benefit from the treatment [4]. Therefore, there is a clear need for predictive biomarkers to maximize likelihood of response while minimizing toxicities and cost. To date, *KRAS* mutations have already been identified as clinical predictors of resistance to anti-EGFR MoAb in mCRC [5–7]. However,

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the occurrence of *KRAS* mutations only accounts for approximately 30–40% of nonresponsive patients [8–12]. Other mechanisms of resistance to anti-EGFR MoAb thus exist. Mutations in one other key mediator of EGFR signaling, *BRAF*, have also been shown to be associated with poor clinical outcomes [8, 13–22]. However, the results are still inconclusive. In this article, we performed a meta-analysis of the published studies to summarize the scientific evidence for the association between *BRAF* mutations and tumor response in mCRC patients treated with anti-EGFR MoAbs.

Materials and methods

Publication search

Systematic computerized searches of the PubMed, EMBase, BIOSIS, and SCOPUS (up to September 30, 2009) were performed. Following search terms were used: “metastatic colon cancer”, “metastatic rectal cancer”, “metastatic colorectal cancer”, “mCRC”, “*BRAF*”, “monoclonal antibodies”, “MoAb”, “cetuximab”, “panitumumab”. The search was limited to human studies. Eligible studies reported complete response (CR) and partial response (PR) stratified by *BRAF* mutation status. All eligible studies were retrieved, and their bibliographies were checked for other relevant publications. When the same patient population was used in several publications, only the most recent, largest or complete study was included in the meta-analysis.

Inclusion criteria

The included studies have to meet the following criteria: (1) evaluating the *BRAF* mutation status and response to anti-EGFR MoAbs in mCRC, (2) studies with full text articles, and (3) sufficient data on CR and PR.

Data extraction

Information was carefully extracted from all eligible studies. The following data were collected from each study: first author’s name, year of publication, study designs, number of patients screened, number of patients with *BRAF* mutations, line of treatment, study treatment protocols, response criteria, CR and PR stratified by *BRAF* mutation status. Data extraction was done independently by two of the authors. Disagreement was resolved by discussion between the two authors. If these two authors could not reach a consensus, another author was consulted to resolve the dispute and a final decision was made by the majority of the votes.

BRAF mutations and clinical endpoints

The primary endpoint was objective response rate (ORR). The ORR was defined as the sum of CR and PR. The correlation between *BRAF* mutations and ORR was expressed as a relative ratio (RR) for ORR of gene mutant patients over wild-type patients. Thus, a RR equal to 1 indicates a lack of association between *BRAF* mutations and tumor response to anti-EGFR MoAbs therapy; a RR more than 1 corresponds to a direct correlation between higher ORR and *BRAF* mutations, and a tendency of mutant *BRAF* patients to have non-responsiveness is indicated by a RR less than 1.

Statistical methods

The association between *BRAF* mutations and efficacy of anti-EGFR MoAb therapy was measured by RR with 95% CI. Heterogeneity was checked by a *Q*-test with degree of freedom equal to the number of analyzed studies minus 1 [23]. A *P* value of more than 0.10 for the *Q*-test indicates a lack of heterogeneity across studies, so the pooled RR was calculated by the fixed-effects model [24]. Otherwise, the random-effects model was used [25]. Sensitivity analyses were carried out to check if modification of the inclusion criteria of the meta-analysis affected the final results. Begg’s funnel plots and Egger’s linear regression test were used to assess publication bias. Funnel plot asymmetry was assessed by the method of Egger’s linear regression test, a linear regression approach to measure funnel plot asymmetry on the natural logarithm scale of the RR. The significance of the intercept was determined by the *t*-test as suggested by Egger (*P* < 0.05 was considered representative of statistically significant publication bias) [26]. All the statistical tests used in our meta-analysis were performed with STATA version 10.0 (Stata Corporation, College Station, TX).

Results

Studies characteristics

Based on our search criteria, a total of 11 studies [8, 13–22] were included in the final meta-analysis. Table 1 lists the studies identified and their main characteristics. Of the 11 studies, sample sizes ranged from 31 to 259. All of these studies were retrospective design. Anti-EGFR MoAb was given as first-line treatment in one studies, as second line or more in ten studies. The patients of two studies received anti-EGFR MoAb monotherapy, while the patients of nine studies received anti-EGFR MoAb-based therapy.

Table 1 Main characteristics of studies included in the meta-analysis

First author	Year	Study design	Patients assessed	Mutant BRAF (%)	Line of treatment	Study treatment protocols	Response criteria
Moroni [8]	2005	Retrospective	31	1/30 (3.3)	≥1st	C alone; or P alone; or C + I based	RECIST
Benvenuti [13]	2007	Retrospective	48	6/48 (12.5)	≥1st	C alone; or P alone; or C + I based	RECIST
Cappuzzo [14]	2008	Retrospective	79	4/79 (5.0)	>1st	C alone	RECIST
Di Nicolantonio [15]*	2008	Retrospective	110	11/110 (10.0)	≥1st	C alone; or P alone; or C + Chem	RECIST
Freeman [16]	2008	Retrospective	62	4/62 (6.5)	≥1st	P alone	RECIST; or WHO
Laurent-Puig [17]*	2009	Retrospective	171	5/171 (2.9)	>1st	C + I; or C + F	RECIST
Loupakis [18]*	2009	Retrospective	87	13/87 (14.9)	>1st	C + I	RECIST
Molinari [19]	2009	Retrospective	36	2/36 (5.6)	≥1st	C-based; or P-based	RECIST
Perrone [20]	2009	Retrospective	31	3/31 (9.7)	>1st	C + I	RECIST
Sartore-Bianchi [21]*	2009	Retrospective	132	11/132 (8.3)	≥1st	C alone; P alone; or C + I based	RECIST
Tol [22]	2009	Retrospective	259	28/259 (10.8)	1st	CBC	RECIST

C cetuximab, CBC capecitabine, oxaliplatin, and bevacizumab plus cetuximab, Chem chemotherapy, F folfiri, I irinotecan

*The study performed in patients with wild-type KRAS metastatic colorectal cancer

BRAF mutations and ORR in unselected mCRC patients

Totally, seven studies [8, 13, 14, 16, 19, 20, 22] were performed in unselected mCRC patients. BRAF mutational analysis was performed successfully on 546 mCRC. BRAF V600E mutation was detected in 48 (8.8%) primary tumors. Among 546 analyzed patients, 520 patients were assessable for tumor response. The ORR of mCRC patients with mutant BRAF was 29.2% (14/48), whereas the ORR of mCRC patients with wild-type BRAF was 33.5% (158/472). The overall RR for ORR of mutant BRAF patients over wild-type BRAF patients was 0.86 (95% CI = 0.57–1.30; $P = 0.48$), with no heterogeneity between studies ($P = 0.27$; $I^2 = 21.1\%$; Table 2; Fig. 1). No publication bias was found by Egger's test ($t = 0.18$; $P = 0.86$).

BRAF mutations and ORR in patients with wild-type KRAS mCRC

Totally, four studies [15, 17, 18, 21] were performed in patients with wild-type KRAS mCRC. BRAF mutational analysis was performed successfully on 376 KRAS wild-

type mCRC. BRAF V600E mutation was detected in 40 (10.6%) primary tumors. Among 376 analyzed patients, all of patients were assessable for tumor response. The ORR of patients with mutant BRAF was 0.0% (0/40), whereas the ORR of patients with wild-type BRAF was 36.3% (122/336). BRAF V600E mutation had negative effect on tumor response to anti-EGFR MoAbs (pooled RR = 0.14; 95% CI = 0.04–0.53; $P = 0.004$) in patients with KRAS wild-type mCRC, with no heterogeneity between studies ($P = 1.00$; $I^2 = 0.0\%$; Table 2; Fig. 1). No publication bias was found by Egger's test ($t = 2.10$; $P = 0.17$).

Discussion

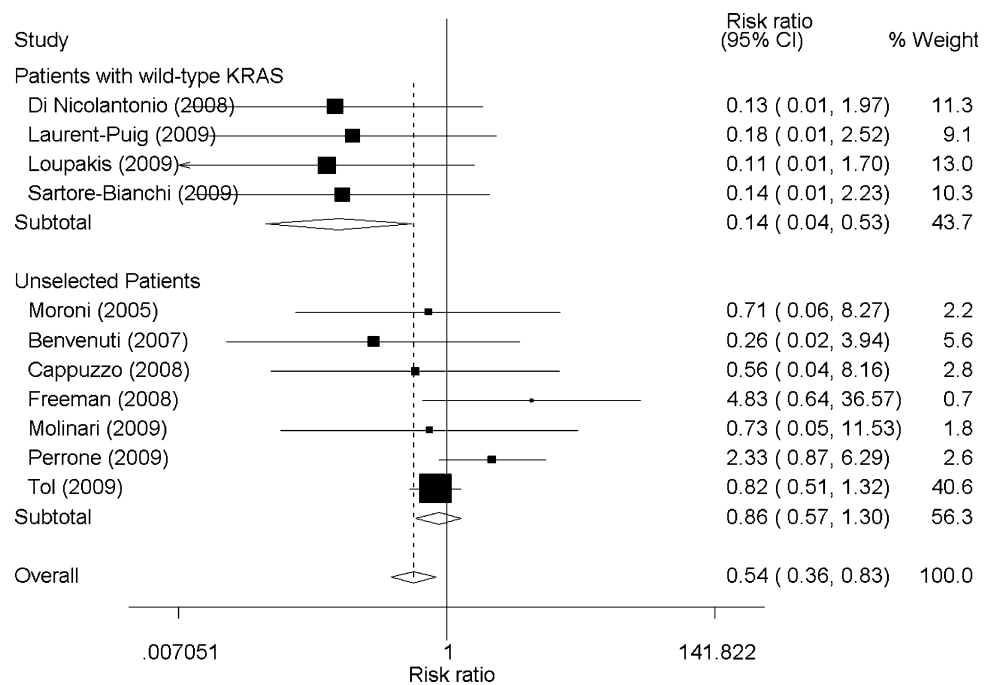
The RAS/RAF/MAPK pathway were major downstream signaling proteins of EGFR, which regulates biological responses such as cell proliferation, cell survival and cell differentiation [27]. Mutations in downstream signaling effectors of EGFR could result in resistance to EGFR inhibitors [28]. In mCRC, KRAS mutations have already been identified as clinical predictors of resistance to anti-EGFR MoAbs [5–7]. However, the occurrence of KRAS

Table 2 Meta-analysis on the association between BRAF mutation and overall response rate

	ORR(%)	Test of association		Test of heterogeneity	
		RR (95% CI)	P	P	I ² (%)
Unselected patients					
Patients with mutant BRAF	14/48 (29.2)	0.86 (0.57-1.30)	0.48	0.27	21.1
Patients with wild-type BRAF	158/472 (33.5)				
Patients with wild-type KRAS					
Patients with mutant BRAF	0/40 (0.0)	0.14 (0.04-0.53)	0.004	1.00	0.0
Patients with wild-type BRAF	122/336 (36.3)				

ORR objective response rate, RR relative ratio

Fig. 1 The forest plot of relative ratios for objective response rate according to *BRAF* mutational status



mutations only accounts for approximately 30–40% of nonresponsive patients [8–12]. Other mechanisms of resistance to anti-EGFR MoAb thus exist. Recently, emerging data indicate that *BRAF* mutations were associated with poor clinical outcomes [8, 13–22]. Unfortunately, the results remain conflicting rather than conclusive. To derive a more precise estimation of the predictive significance of *BRAF* mutations in mCRC patients treated with anti-EGFR MoAbs, we performed this meta-analysis.

Among unselected mCRC patients, data for *BRAF* mutations and tumor response were available in seven studies, with 520 patients included. The ORR of mCRC patients with mutant *BRAF* was 29.2% (14/48), whereas the ORR of mCRC patients with wild-type *BRAF* was 33.5% (158/472). The overall RR indicated that *BRAF* mutation was not associated with lack of response in unselected mCRC patients treated with anti-EGFR MoAbs.

For patients with *KRAS* wild-type mCRC, data concerning *BRAF* mutations and tumor response were available in four studies, with 376 patients included. *BRAF* V600E mutation was detected in 40 (10.6%) primary tumors. The ORR of patients with mutant *BRAF* was 0.0% (0/40), whereas the ORR of patients with wild-type *BRAF* was 36.3% (122/336). The overall RR indicated that *BRAF* V600E mutation had negative effect on tumor response to anti-EGFR MoAbs in patients with *KRAS* wild-type mCRC.

Heterogeneity is a potential problem that may affect the interpretation of the results of meta-analyses. Although some diversity in the studies about study designs, inclusion

criteria, treatment protocols and response criteria, there was no statistically significant heterogeneity among the studies. This indicated that it may be appropriate to use an overall estimation of the predictive value of *BRAF* mutations.

Our studies had several limitations that need to be taken into consideration when interpreting the findings. First, inadequate reporting of survival data precludes any meaningful assessment of the effect of *BRAF* status on survival in mCRC patients treated with anti-EGFR MoAbs. Only three studies presented data on hazard ratio (HR) with 95% CI for progression-free survival (PFS) and overall survival (OS). Therefore, we did not perform pooled analysis for HR. Second, our result was based on unadjusted estimates, while a more precise analysis should be conducted if more detailed individual data were available, which would allow for an adjusted estimate by other prognostic factors such as sex, age, tumor location and previous chemotherapy lines.

Despite of some limitations, this meta-analysis provided evidence that *BRAF* V600E mutation is associated with lack of response in wild-type *KRAS* mCRC treated with anti-EGFR MoAbs. The results indicated that *BRAF* mutation may be used as an additional biomarker for the selection of mCRC patients who might benefit from anti-EGFR MoAbs therapy. However, the number of studies and the number of subjects included in the meta-analysis are relatively small. Large prospective studies using standardized unbiased methods are needed to confirm our results, using homogeneous mCRC patients, with assessors blinded to the clinical data.

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