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Effect of *RAS* status on anti-EGFR monoclonal antibodies + 5-FU infusion-based chemotherapy in first-line treatment of metastatic colorectal cancer: A meta-analysis

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ABSTRACT

Purpose: To investigate the effect of *RAS* on anti-EGFR moAb + 5-FU infusion based chemotherapy in first-line treatment of mCRC.
Methods: The MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, Cochrane databases and ClinicalTrials.gov databases were independently reviewed. Primary end points included overall response rate (ORR), progression-free survival (PFS), overall survival (OS) and toxicities. Correlation between *RAS* status and PFS, OS, ORR or toxicities was expressed as a hazard ratio (HR) or relative risk (RR).
Results: KRAS exon 2 wild-type (-wt) mCRC benefited from adding anti-EGFR moAb (compared with chemotherapy alone: OS: HR 0.88, $P = 0.008$; PFS: HR 0.74, $P < 0.001$; ORR: RR 1.34, $P = 0.003$. Compared with Bevacizumab: OS: HR 0.83, $P = 0.003$). *KRAS* exon 2-wt but other *RAS* mutations mCRC did not benefit from adding anti-EGFR moAb. *RAS*-wt mCRC benefited from adding anti-EGFR moAb (compared with chemotherapy alone: OS: HR: 0.75, $P < 0.001$; PFS: HR 0.65, $P < 0.001$; ORR: RR 1.51, $P = 0.020$. Compared with Bevacizumab: OS: HR 0.79, $P = 0.002$). *KRAS* exon 2-wt but *BRAF* mutation mCRC did not benefit from adding anti-EGFR moAb. Subgroup analysis suggested that anti-EGFR moAb prolonged PFS for male, liver metastasis-only, ECOG 0–1, and colon primary site groups. Anti-EGFR moAb increased controllable grade 3–4 toxicities including rash, diarrhea, and anemia.
Conclusions: Adding anti-EGFR moAb as first-line treatment in *RAS*-wt mCRC prolonged OS. Whether *BRAF* mutation is a predictive marker to anti-EGFR moAb is not clear.

1. Introduction

The 5-year survival rate for metastatic colorectal cancer (mCRC) remains below 10% (Siegel et al., 2012). A first-line regimen of anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (moAb; cetuximab or panitumumab) with 5-FU infusion based chemotherapy by *KRAS* exon 2 wild-type (-wt) status in mCRC, has increased median progression-free survival (PFS) to 8.3–10.9 months, and overall survival (OS) to 17.0–34.2 months. However, not all reported PFS or OS improvements were significant (Maughan et al., 2011; Bokemeyer et al., 2009; Bokemeyer et al., 2015; Van Cutsem et al., 2011; Van Cutsem et al., 2015; Douillard et al., 2013; Schwartzberg et al., n.d.; Stintzing et al., 2012; Heinemann et al., 2014; Venook et al., 2014).

KRAS and *NRAS* are closely related *RAS* oncogene family members (Karnoub and Weinberg, 2008; Fernandez-Medarde and Santos, 2011). Mutations in *KRAS* and *NRAS* codons increase guanosine triphosphate-bound *RAS* proteins, which promote tumor proliferation, invasion, metastasis and drug resistance (Haigis et al., 2008; Diaz et al., 2012; Misale et al., 2012). Besides *KRAS* exon 2 (codons 12 and 13), oncogenic mutations in the *RAS* family have been found in *KRAS* exon 3 (59 and 61), exon 4 (117 and 146); and *NRAS* exon 2 (12 and 13), exon 3 (59 and 61), and exon 4 (117 and 146) (De Roock et al., n.d.). Clinical trials have shown some patients with *KRAS* exon 2-wt mCRC to have no response to anti-EGFR moAb, which suggests that *KRAS* exon 2 mutations are not the only negative predictive markers for anti-EGFR moAb treatment (Allegra et al., 2009).

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Seven randomized controlled trials (RCTs) evaluated the efficacy of anti-EGFR moAb + 5-FU infusion based chemotherapy in first-line treatment of mCRC by *RAS* status, but their results were inconsistent (Maughan et al., 2011; Bokemeyer et al., 2009; Bokemeyer et al., 2015; Van Cutsem et al., 2011; Van Cutsem et al., 2015; Douillard et al., 2013; Schwartzberg et al., n.d.; Stintzing et al., 2012; Heinemann et al., 2014; Venook et al., 2014). Did mCRC patients with *RAS*-wt benefit from adding anti-EGFR moAb? And how did patients with *RAS* mutations other than *KRAS* exon 2 respond to anti-EGFR moAb? Which is better for first-line treatment of mCRC with chemotherapy, anti-EGFR moAb or Bev? Our meta-analysis aimed to evaluate the efficacy and safety of anti-EGFR moAb + 5-FU infusion based chemotherapy in first-line treatment of mCRC according to *RAS* status.

2. Materials and methods

2.1. Literature search

The MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, Cochrane databases and ClinicalTrials.gov databases were independently reviewed from their dates of inception to July 2015. The following search terms were used: "colorectal neoplasms" and "mutation" and "antibodies, monoclonal" and "ras Proteins". Only human studies and RCTs published in English were eligible. Abstracts and information from conferences were also collected independently. Fig. 1 shows a flow chart of the literature search and study selection and results in each step.

2.2. Inclusion criteria

Studies that met the following criteria were included: (1) randomized trials of patients with no prior chemotherapy for mCRC and available *RAS* status; (2) treatment with 5-FU infusion based chemotherapy, with or without anti-EGFR moAb (cetuximab or panitumumab); (3) use of overall response rate (ORR), PFS, OS and/or toxicities as outcomes to assess tumor response and prognosis. Quality assessment of papers was independently performed using the seven-point Jadad ranking system (Jadad et al., 1996).

2.3. Data collection

Data collection was carried out independently by two reviewers. Disagreements were resolved by discussion between the two or by consulting a third reviewer. The following data was collected from each study: name of study, year of publication, total number of patients included in the study, trial phase, intervention, response criteria, ORR,

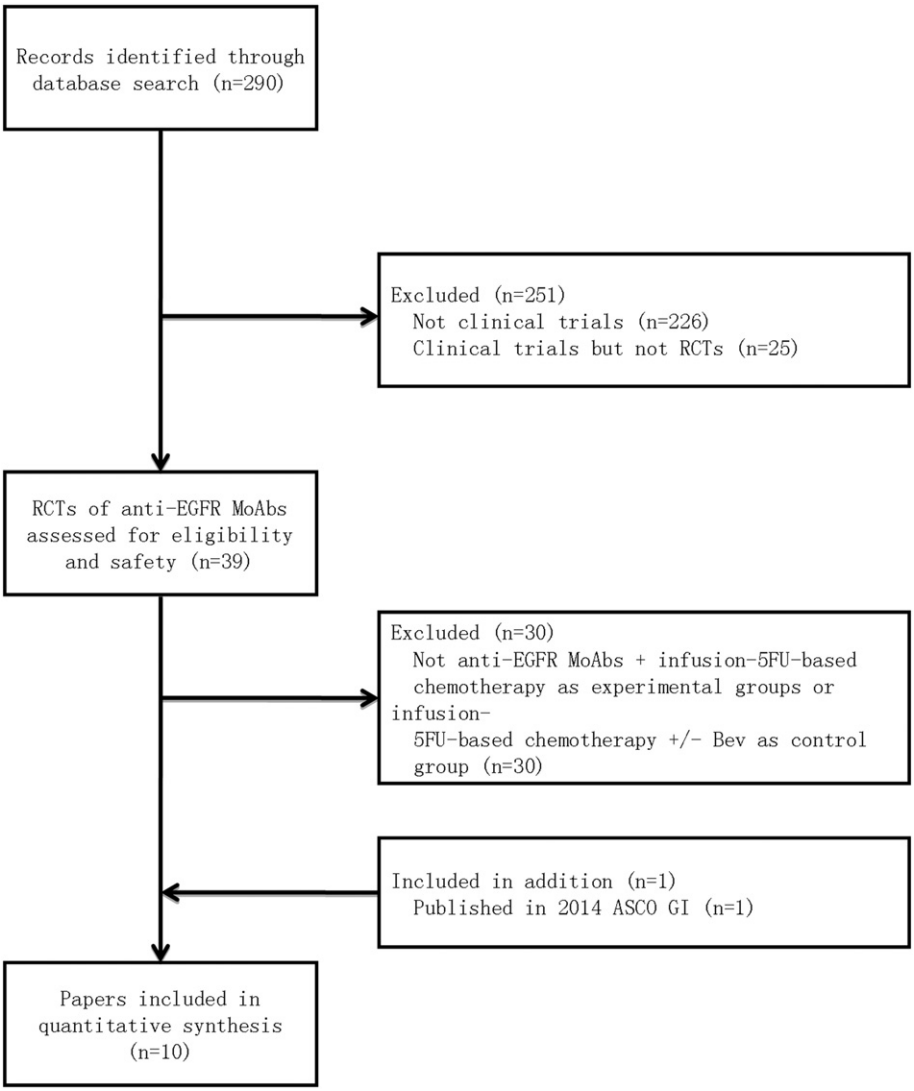

Fig. 1. Selection process for randomized controlled trials included in the meta-analysis.

PFS, and OS. To assess responses, studies of patients with measurable diseases were evaluated by central radiology review. Toxicities were graded according to the Common Terminology Criteria for Adverse Events (version 2.0 or 3.0).

2.4. Statistical analysis

Primary end points included ORR, PFS, OS and toxicities. Association between *RAS* status and ORR or toxicities was expressed as

Table 1
Characteristic of 7 RCTs.

Study	Study design (number of patients)	Treatment schedule	KRAS test	NRAS test	BRAF test
COIN 2011 (Maughan et al., 2011)	FOLFOX + Cetux. (*KRAS* exon 2-wt: 117) XELOX + Cetux. (*KRAS* exon 2-wt: 245)	Cetux.: initial dose 400 mg/m^2 and 250 mg/m^2/week thereafter, Q2W. FOLFOX: oxaliplatin 85 mg/m^2 over 2 h; fluorouracil 400 mg/m^2 IV bolus and 2400 mg/m^2 infusion over 46 h; L-folinic acid 175 mg or D,L-folinic acid 350 mg over 2 h, Q2W.	Codons 12, 13, 61	Codons 12, 61	Codons 594, 600
	FOLFOX (*KRAS* exon 2-wt: 127) XELOX (*KRAS* exon 2-wt: 240)	FOLFOX: oxaliplatin 85 mg/m^2 over 2 h; fluorouracil 400 mg/m^2 IV bolus and 2400 mg/m^2 infusion over 46 h; L-folinic acid 175 mg or D,L-folinic acid 350 mg over 2 h, Q2W.	Codons 12, 13, 61	Codons 12, 61	Codons 594, 600
OPUS 2011 (Bokemeyer et al. 2009), 2015 (Bokemeyer et al., 2015)	FOLFOX4 + Cetux. (*KRAS* exon 2-wt: 82; *RAS*-wt: 38; *KRAS* exon 2-wt but other *RAS*-mt: 15)	Cetux.: initial dose 400 mg/m^2 and 250 mg/m^2/week thereafter, Q2W. FOLFOX4: oxaliplatin 85 mg/m^2; folinic acid 200 mg/m^2; 5-FU 400 mg/m^2 IV bolus and 600 mg/m^2 22-hour continuous infusion on days 1 and 2, Q2W.	Codons 12, 13, 59, 61, 117, 146	–	–
	FOLFOX4 (*KRAS* exon 2-wt: 97; *RAS*-wt: 49; *KRAS* exon 2-wt but other *RAS*-mt: 16)	FOLFOX4: oxaliplatin 85 mg/m^2; folinic acid 200 mg/m^2; 5-FU 400 mg/m^2 IV bolus and 600 mg/m^2 22-hour continuous infusion on days 1 and 2, Q2W.	Codons 12, 13, 59, 61, 117, 146	–	–
CRYSTAL 2011 (Van Cutsem et al., 2011), 2015 (Van Cutsem et al., 2015)	FOLFIRI + Cetux. (*KRAS* exon 2-wt: 316; *RAS*-wt: 178; *KRAS* exon 2-wt but other *RAS*-mt: 32)	Cetux.: initial dose 400 mg/m^2 and 250 mg/m^2/week thereafter, followed after 1 h by FOLFIRI, Q2W. FOLFIRI: irinotecan 180 mg/m^2, day 1, infused over 30 to 90 min; leucovorin 200 mg/m^2 L-form, or 400 mg/m^2 racemic, infused over 2 h; fluorouracil 400 mg/m^2 IV bolus and 2400 mg/m^2 46-hour continuous infusion, Q2W.	Codons 12, 13, 59, 61, 117, 146	–	Codon 600
	FOLFIRI (*KRAS* exon 2-wt: 350; *RAS*-wt: 189; *KRAS* exon 2-wt but other *RAS*-mt: 31)	FOLFIRI: irinotecan 180 mg/m^2, day 1, infused over 30 to 90 min; leucovorin 200 mg/m^2 L-form, or 400 mg/m^2 racemic, infused over 2 h; fluorouracil 400 mg/m^2 IV bolus and 2400 mg/m^2 46-hour continuous infusion, Q2W.	Codons 12, 13, 59, 61, 117, 146	–	Codon 600
PRIME 2013 (Douillard et al., 2013)	FOLFOX4 + Panit. (*KRAS* exon 2-wt: 325; *RAS*-wt: 259; *KRAS* exon 2-wt but other *RAS*-mt: 51)	Panit.: IV over 1 h, 6 mg/kg on day 1 before FOLFOX4, Q2W FOLFOX4: oxaliplatin 85 mg/m^2 IV infusion on day 1; leucovorin 200 mg/m^2 IV infusion; fluorouracil 400 mg/m^2 IV bolus and 600 mg/m^2 22-hour continuous infusion on days 1 and 2, Q2W.	Codons 12, 13, 61, 117, 146	Codons 12, 13, 61, 117, 146	Codon 600
	FOLFOX4 (*KRAS* exon 2-wt: 331; *RAS*-wt: 253; *KRAS* exon 2-wt but other *RAS*-mt: 57)	FOLFOX4: oxaliplatin 85 mg/m^2 IV infusion on day 1; leucovorin 200 mg/m^2 IV infusion; fluorouracil 400 mg/m^2 IV bolus and 600 mg/m^2 22-hour continuous infusion on days 1 and 2, Q2W.	Codons 12, 13, 61, 117, 146	Codons 12, 13, 61, 117, 146	Codon 600
PEAK 2014 (Schwartzberg et al., n.d.)	FOLFOX6 + Panit (*KRAS* exon 2-wt: 142; *RAS*-wt: 88; *KRAS* exon 2-wt but other *RAS*-mt: 24)	Cetux.: initial dose 400 mg/m^2 and 250 mg/m^2/week thereafter, Q2W. FOLFOX4: oxaliplatin 85 mg/m^2 IV infusion on day 1; leucovorin 200 mg/m^2 IV infusion; fluorouracil 400 mg/m^2 IV bolus and 600 mg/m^2 22-hour continuous infusion on days 1 and 2, Q2W.	Codons 12, 13, 61, 146	Codons 12, 13, 61, 146	Codon 600
	FOLFOX6 + Bev. (*KRAS* exon 2-wt: 143; *RAS*-wt: 82; *KRAS* exon 2-wt but other *RAS*-mt: 27)	Bev.: 5 mg/kg, Q2W. FOLFOX4: oxaliplatin 85 mg/m^2 IV infusion on day 1; leucovorin 200 mg/m^2 IV infusion; fluorouracil 400 mg/m^2 IV bolus and 600 mg/m^2 22-hour continuous infusion on days 1 and 2, Q2W.	Codons 12, 13, 61, 146	Codons 12, 13, 61, 146	Codon 600
FIRE3 2013 (Stintzing et al., 2012), 2014 (Heinemann et al., 2014)	FOLFIRI + Cetux. (*KRAS* exon 2-wt: 297; *RAS*-wt: 171; *KRAS* exon 2-wt but other *RAS*-mt: 34)	Cetux.: initial dose 400 mg/m^2 and 250 mg/m^2/week thereafter, Q2W. FOLFIRI: irinotecan 180 mg/m^2, day 1, infused over 30 to 90 min; leucovorin 200 mg/m^2 L-form, or 400 mg/m^2 racemic, infused over 2 h; fluorouracil 400 mg/m^2 IV bolus and 2400 mg/m^2 46-hour continuous infusion, Q2W.	Codons 12, 13, 61	Codons 12, 13, 61, 146	Codon 600
	FOLFIRI + Bev. (*KRAS* exon 2-wt: 295; *RAS*-wt: 171; *KRAS* exon 2-wt but other *RAS*-mt: 31)	Bev.: 5 mg/kg, Q2W. FOLFIRI: irinotecan 180 mg/m^2, day 1, infused over 30 to 90 min; leucovorin 200 mg/m^2 L-form, or 400 mg/m^2 racemic, infused over 2 h; fluorouracil 400 mg/m^2 IV bolus and 2400 mg/m^2 46-hour continuous infusion, Q2W.	Codons 12, 13, 61	Codons 12, 13, 61, 146	Codon 600
CALGB/SWOG 80405 2014 (Venook et al., 2014)	mFOLFOX6/FOLFIRI + Cetux. (*KRAS* exon 2-wt: 578; *RAS*-wt: 270)	Cetux.: initial dose 400 mg/m^2 and 250 mg/m^2/week. mFOLFOX6: oxaliplatin 85 mg/m^2 IV infused over 2 h followed by leucovorin 400 mg/m^2 IV over 2 h followed by 5-FU 400 mg/m^2 IV bolus, then 2400 mg/m^2 continuous IV infusion over 46–48 h. FOLFIRI: irinotecan 180 mg/m^2 IV infused over 90 min followed by leucovorin 400 mg/m^2 IV over 2 h followed by 5-FU 400 mg/m^2 IV bolus following leucovorin then 2400 mg/m^2 continuous IV infusion over 46–48 h.	–	–	–
	mFOLFOX6/FOLFIRI + Bev. (*KRAS* exon 2-wt: 559; *RAS*-wt: 256)	Bev.: 5 mg/kg, Q2W. mFOLFOX6: oxaliplatin 85 mg/m^2 IV infused over 2 h followed by leucovorin 400 mg/m^2 IV over 2 h followed by 5-FU 400 mg/m^2 IV bolus, then 2400 mg/m^2 continuous IV infusion over 46–48 h. FOLFIRI: irinotecan 180 mg/m^2 IV infused over 90 min followed by leucovorin 400 mg/m^2 IV over 2 h followed by 5-FU 400 mg/m^2 IV bolus following leucovorin then 2400 mg/m^2 continuous IV infusion over 46–48 h.	–	–	–

Abbreviations: -wt = wild-type; -mt = mutations; Bev. = Bevacizumab; Panit. = panitumumab; Cetux. = cetuximab; IV = intravenous.

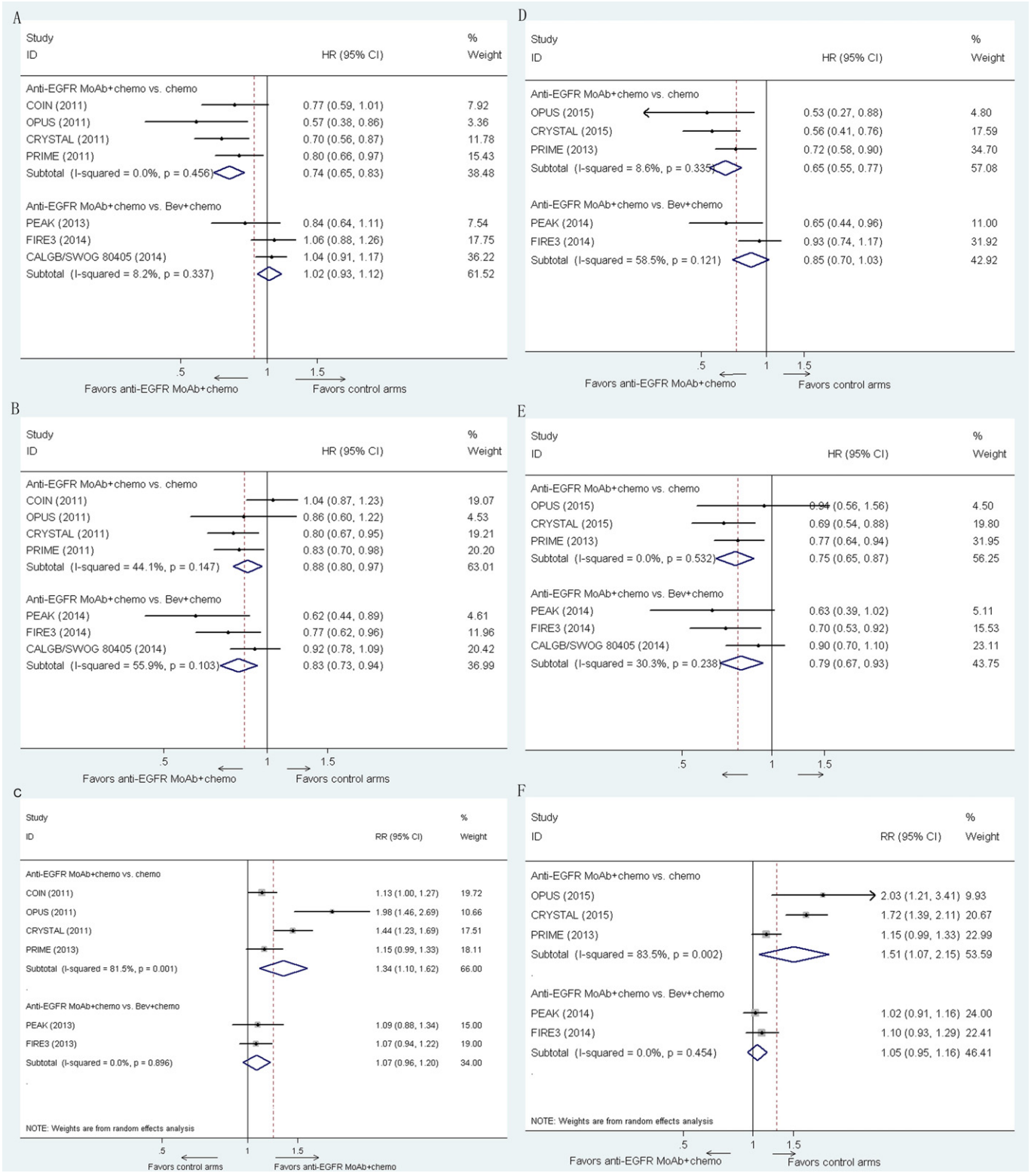

Fig. 2. Efficacy according to tumor *RAS* status. a PFS in patients with *KRAS* exon 2-wt mCRC; b OS in patients with *KRAS* exon 2-wt mCRC; c ORR in patients with *KRAS* exon 2-wt mCRC; d PFS in patients with *RAS*-wt mCRC; e OS in patients with *RAS*-wt mCRC; f ORR in patients with *RAS*-wt mCRC.

relative risk (RR). Association between *RAS* status and PFS or OS was expressed as a hazard ratio (HR). We also investigated whether efficacy of anti-EGFR moAb + 5-FU infusion based chemotherapy in patients with *KRAS* exon 2-wt was affected by different prognostic factors, such as sex, age, liver metastasis only, ECOG score, primary lesion and WBC count. Regrettably, only stratified PFS were performed by *KRAS* exon 2-wt, as stratified HRs of OS were not published until now.

Heterogeneity among trials was assessed with Cochrane's *Q* statistic. Inconsistency was quantified with the I^2 statistic [$100\% \times (Q - df) / Q$]

(Cochran, 1954). $P > 0.05$ was considered to indicate homogeneity. To pool the HRs and RRs, a fixed-effect model was used for homogeneity, and a random-effect model for heterogeneity.

Begg's funnel plots and Egger's linear regression test were used to assess publication bias (Sterne et al., 2001). All the statistical analyses were performed with STATA 11.0 software.

3. Results

3.1. Study characteristics

Fig. 1 showed the process of literature search and selection. First, 290 papers were found in the databases. Second, 39 RCTs about the efficacy of anti-EGFR moAb in mCRC were screened. Third, 6 RCTs that fit our criteria were included. Fourth, one abstract published in the 2014 ASCO GI annual meetings were included. Because the included RCTs had updated papers, finally, 10 papers that described 7 RCTs of the efficacy and safety of anti-EGFR moAb + 5-FU infusion based chemotherapy by *RAS* status in first-line treatment of mCRC were included.

The 7 RCTs included 10 papers are randomized, multicenter, controlled trials (Maughan et al., 2011; Bokemeyer et al., 2009; Bokemeyer et al., 2015; Van Cutsem et al., 2011; Van Cutsem et al., 2015; Douillard et al., 2013; Schwartzberg et al., n.d.; Stintzing et al., 2012; Heinemann et al., 2014; Venook et al., 2014). The OPUS (Bokemeyer et al., 2009; Bokemeyer et al., 2015), CRYSTAL (Van Cutsem et al., 2011; Van Cutsem et al., 2015), PRIME (Douillard et al., 2013), PEAK (Schwartzberg et al., n.d.) and FIRE3 (Stintzing et al., 2012; Heinemann et al., 2014), studies provided data of patients with *RAS*-wt mCRC, whereas the COIN (Maughan et al., 2011) and CALGB/SWOG 80405 (Venook et al., 2014) studies provided data of patients with *KRAS* exon 2-wt mCRC. In the COIN (Maughan et al., 2011) study, for the OS HR of patients with *KRAS* exon 2-wt, only data calculated after pooling both the OxCap and OxFU arms together were available (Table 1). A total of 4166 patients with *KRAS* exon 2-wt mCRC were considered in the meta-analysis, of whom 2102 were in anti-EGFR moAb + 5-FU infusion based chemotherapy groups, and 2064 were in control groups. Of 2004 patients with *RAS*-wt mCRC, 1004 were in anti-EGFR moAb + 5-FU infusion based chemotherapy groups, and 1000 in control groups. Of 318 patients with *KRAS* exon 2-wt but other *RAS* mutations, 156 were in anti-EGFR moAb + 5-FU infusion based chemotherapy groups, and 162 were in control groups. Jadad scores of the 10 papers were 6–7, which meant they were papers with high quality. Details are shown in Table 1.

3.2. Efficacy according to tumor RAS status

3.2.1. KRAS exon 2-wt and PFS, OS and ORR

Patients with *KRAS* exon 2-wt mCRC benefited from anti-EGFR moAb + 5-FU infusion based chemotherapy. Compared with

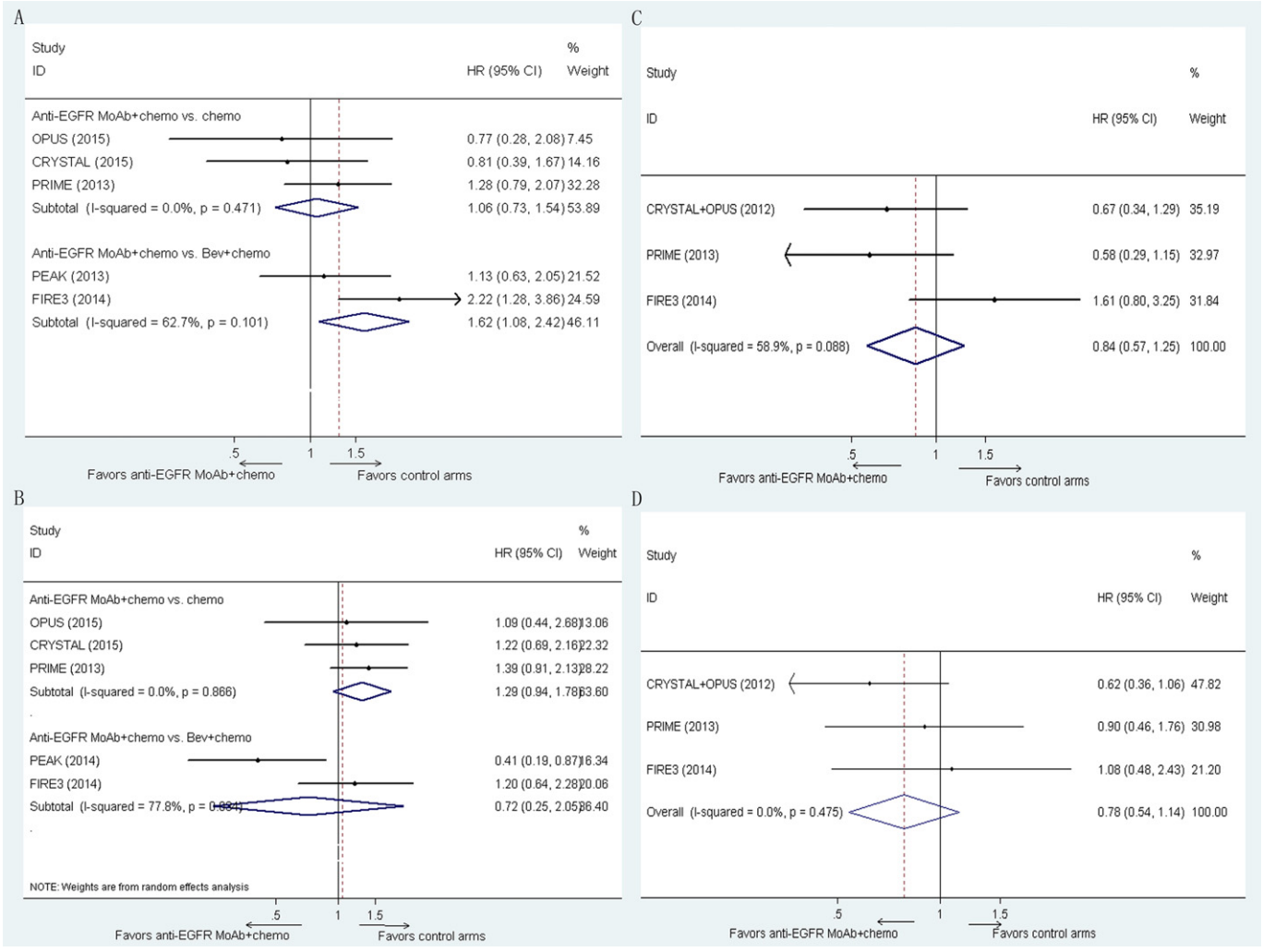

Fig. 3. Efficacy according to tumor *RAS* and *BRAF* status. a PFS in patients with *KRAS* exon 2-wt but other *RAS* mutations mCRC; b OS in patients with *KRAS* exon 2-wt but other *RAS* mutations mCRC; c PFS in patients with *RAS*-wt but *BRAF* mutations mCRC; d OS in patients with *RAS*-wt but *BRAF* mutations mCRC.

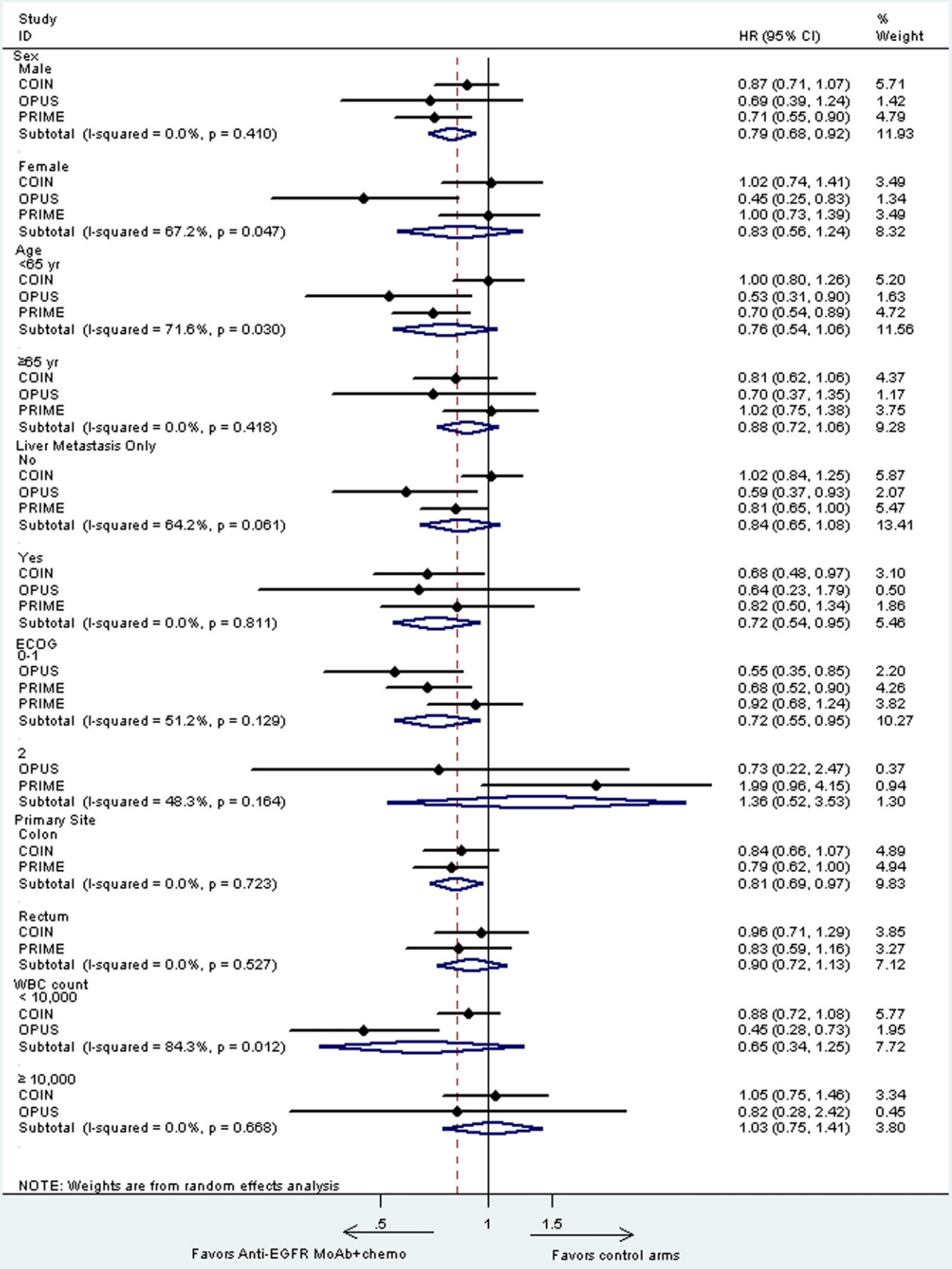

Fig. 4. PFS by baseline risk factor.

Table 2
Selected adverse events in 4 RCTs: COIN, OPUS, CRYSTAL and PRIME.

Clinical trial	Interventions	Size of sample	Neutropenia		Rash		Diarrhea		Neurologic toxicities		Infusion-related reaction		Anemia		Leukopenia	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
COIN 2011 (Maughan et al., 2011)	FOLFOX/XELOX	279	86	31	0	0	31	11	63	23	NR	NR	6	2	28	10
	FOLFOX/XELOX + Cetux.	281	88	31	56	20	55	20	38	14	NR	NR	21	7	33	12
OPUS 2011 (Bokemeyer et al., 2009)	FOLFOX4	97	31	32	0	0	5	5	8	8	2	2	2	2	5	5
	FOLFOX4 + Cetux.	82	29	35	9	11	7	9	3	4	1	1	3	4	6	7
CRYSTAL 2011 (Van Cutsem et al., 2011)	FOLFIRI	350	83	23.7	0	0	35	10	NR	NR	0	0	NR	NR	17	4.9
	FOLFIRI + Cetux.	317	97	30.6	28	8.8	52	16.4	NR	NR	5	1.6	NR	NR	25	7.9
PRIME 2013 (Douillard et al., 2013)	FOLFOX4	327	134	41	7	2	29	9	51	16	NR	NR	NR	NR	NR	NR
	FOLFOX4 + Panit.	322	136	42	116	36	59	18	52	16	2	0.006	NR	NR	NR	NR
I^2 (%)			0.0		0.0		0.0		61.4		62.6		0.0		0.0	
RR			1.09		20.71		1.80		0.74		2.41		3.03		1.34	
(95% CI)			(0.97,1.23)		(10.51,40.80)		(1.43,2.27)		(0.46,1.19)		(0.11,51.05)		(0.37,6.72)		(1.34,0.94)	
P			0.167		0.000		0.000		0.215		0.573		0.006		0.108	

Abbreviations: Panit. = panitumumab; Cetux. = cetuximab; RR = relative risk; NR = not recorded.

chemotherapy alone, adding anti-EGFR moAb significantly improved PFS (HR: 0.74, CI: 0.65–0.83, $P < 0.001$, fixed-effect model; 4 studies, 1745 patients; $I^2 = 0.0\%$, $P = 0.456$; Fig. 2a), OS (HR: 0.88, CI: 0.80–0.97, $P = 0.008$, fixed-effect model; 4 studies, 2230 patients; $I^2 = 44.1\%$, $P = 0.147$; Fig. 2b) and ORR (RR: 1.34, CI: 1.10–1.62, $P = 0.003$; 4 studies, 2230 patients; $I^2 = 81.5\%$, $P = 0.001$; Fig. 2c). Compared with Bevacizumab (Bev) + 5-FU infusion based chemotherapy, adding anti-EGFR moAb did not prolong PFS (HR: 1.02, CI: 0.93–1.12, $P = 0.706$, fixed-effect model; 3 studies, 2014 patients; $I^2 = 8.2\%$, $P = 0.337$; Fig. 2a). But adding anti-EGFR moAb significantly prolonged OS (HR: 0.83, CI: 0.73–0.94, $P = 0.003$, fixed-effect model; 3 studies, 2014 patients; $I^2 = 55.9\%$, $P = 0.103$; Fig. 2b). and adding anti-EGFR moAb did not improve ORR (RR: 1.07, CI: 0.96–1.20, $P = 0.21$; 2 studies, 2014 patients; $I^2 = 0.0\%$, $P = 0.896$; Fig. 2c).

3.2.2. KRAS exon 2-wt with other RAS mutations; PFS and OS

PFS was shorter in anti-EGFR moAb + 5-FU infusion based chemotherapy arms compared with Bev + 5-FU infusion based chemotherapy arms (HR: 1.62, CI: 1.08–2.42, $P = 0.019$, fixed-effect model; 2 studies, 116 patients; $I^2 = 62.7\%$, $P = 0.101$; Fig. 3a). No difference of OS was observed between adding anti-EGFR moAb and Bev (HR: 0.72, CI: 0.25–2.05, $P = 0.534$, random-effect model; 2 studies, 116 patients; $I^2 = 77.8\%$, $P = 0.034$; Fig. 3b). No difference of PFS and OS was observed between adding anti-EGFR moAb and chemotherapy alone (PFS: HR: 1.06, CI: 0.73–1.54, $P = 0.767$, fixed-effect model; 3 studies, 202 patients; $I^2 = 0.0\%$, $P = 0.471$; Fig. 3a. OS: HR: 1.29, CI: 0.94–1.78, $P = 0.113$; 3 studies, 202 patients; $I^2 = 0.0\%$, $P = 0.866$; Fig. 3b). The effect of anti-EGFR moAb on *KRAS* exon 2-wt but other *RAS* mutations mCRC patients was consistent with *KRAS* exon 2 mutations (Douillard et al., 2013).

3.2.3. RAS-wt but BRAF mutation and PFS and OS

Further retrospective analysis of CRYSTAL and OPUS enlarged the sample and provided more evidence about the relationship between efficacy of anti-EGFR moAb + 5-FU infusion based chemotherapy and *BRAF* status (Bokemeyer et al., 2012). Totally, three papers included four RCTs performed *BRAF* testing (n = 182) (Schwartzberg et al., n.d.; Heinemann et al., 2014; Bokemeyer et al., 2012). Because *NRAS* and *BRAF* were mutually exclusive (De Roock et al., n.d.), we pooled the HRs of four studies. Conclusively, *RAS*-wt but *BRAF* mutation mCRC patients did not benefit from adding anti-EGFR moAb (PFS: HR 0.84, CI 0.57–1.28, $P = 0.403$, fixed-effect model; 4 studies, 182 patients; $I^2 = 58.9\%$, $P = 0.088$, Fig. 3c; OS: HR 0.78, CI 0.54–1.14, $P = 0.199$, fixed-effect model; 4 studies, 182 patients; $I^2 = 0.0\%$, $P = 0.475$; Fig. 3d).

3.3. Subgroup analysis

Estimation of the effect of anti-EGFR moAb (vs. chemotherapy alone) on PFS was stratified by various prognostic factors (Fig. 4). Only HRs of PFS in patients with *KRAS* exon 2-wt mCRC were available in 3 RCTs (Maughan et al., 2011; Bokemeyer et al., 2009; Bokemeyer et al., 2015; Douillard et al., 2013). A random-effect model was used to perform the meta-analysis due to the heterogeneity in some subgroups.

3.3.1. Sex

PFS was improved by anti-EGFR moAb + 5-FU infusion based chemotherapy, significantly for male mCRC patients (HR: 0.79, CI: 0.68–0.92, $P = 0.003$, 926 patients), but not significantly for female patients (HR: 0.83, CI: 0.56–1.24; $P = 0.373$, 490 patients).

3.3.2. Age

Anti-EGFR moAb + 5-FU infusion based chemotherapy improved PFS for mCRC patients both younger and older than 65 years; differences were not significant (<65 years: HR: 0.76, CI: 0.54–1.06, $P = 0.105$, 842 patients; ≥65 years: HR: 0.88; CI: 0.72–1.06, $P = 0.179$, 574 patients).

3.3.3. Liver metastasis only

Anti-EGFR moAb + 5-FU infusion based chemotherapy improved PFS significantly for patients with liver metastasis alone (HR: 0.72, CI: 0.54–0.95, $P = 0.018$, 317 patients), but not significantly for patients with other metastasis (HR: 0.84, CI: 0.65–1.08; $P = 0.171$, 1099 patients).

3.3.4. ECOG

Anti-EGFR moAb + 5-FU infusion based chemotherapy improved PFS for patients with ECOG 0–1 (HR: 0.72, CI: 0.55–0.95, $P = 0.019$, 779 patients). However, improvement was not significant for patients with ECOG 2 (HR: 1.36, CI: 0.52–3.53; $P = 0.528$, 55 patients).

3.3.5. Primary site

Anti-EGFR moAb + 5-FU infusion based chemotherapy improved PFS for patients with colon primary sites (HR: 0.81, CI: 0.69–0.97, $P = 0.018$, 731 patients). However, improvement was not significant for patients with rectal primary sites (HR: 0.90, CI: 0.72–1.13; $P = 0.360$, 422 patients).

3.3.6. WBC count

Anti-EGFR moAb + 5-FU infusion based chemotherapy improved PFS in patients with WBC count both below and above or equal to) 10,000, but not significantly (<10,000: HR: 0.65, CI: 0.34–1.25, $P =$

0.201, 568 patients; ≥10,000: HR: 1.03; CI: 0.75–1.41, $P = 0.867$, 187 patients).

3.4. Toxicities

Grade 3–4 toxicities from the 4 RCTs (Maughan et al., 2011; Bokemeyer et al., 2009; Bokemeyer et al., 2015; Van Cutsem et al., 2011; Van Cutsem et al., 2015; Douillard et al., 2013) are detailed in Table 2. A random-effect model was used for the meta-analysis due to heterogeneity of some subgroups. Our meta-analysis found adding anti-EGFR moAb increased risk of grade 3–4 toxicities, including rash (RR, 20.71; CI: 10.51–40.80; $P < 0.001$), diarrhea (RR, 1.80; CI: 1.43–2.27; $P < 0.001$), and anemia (RR, 3.03; CI: 1.37–6.72; $P = 0.006$). The two arms showed no significant differences in rates of other toxicities, including neutropenia, neurologic toxicities, infusion-related reaction, and leukopenia (Fig. 5).

3.5. Publication bias

No evidence for publication bias was shown in PFS (Begg's test: $z = 0.75$, $P = 0.452$; Egger's test: $\tau = -1.31$, $P = 0.261$, Fig. 6) and OS

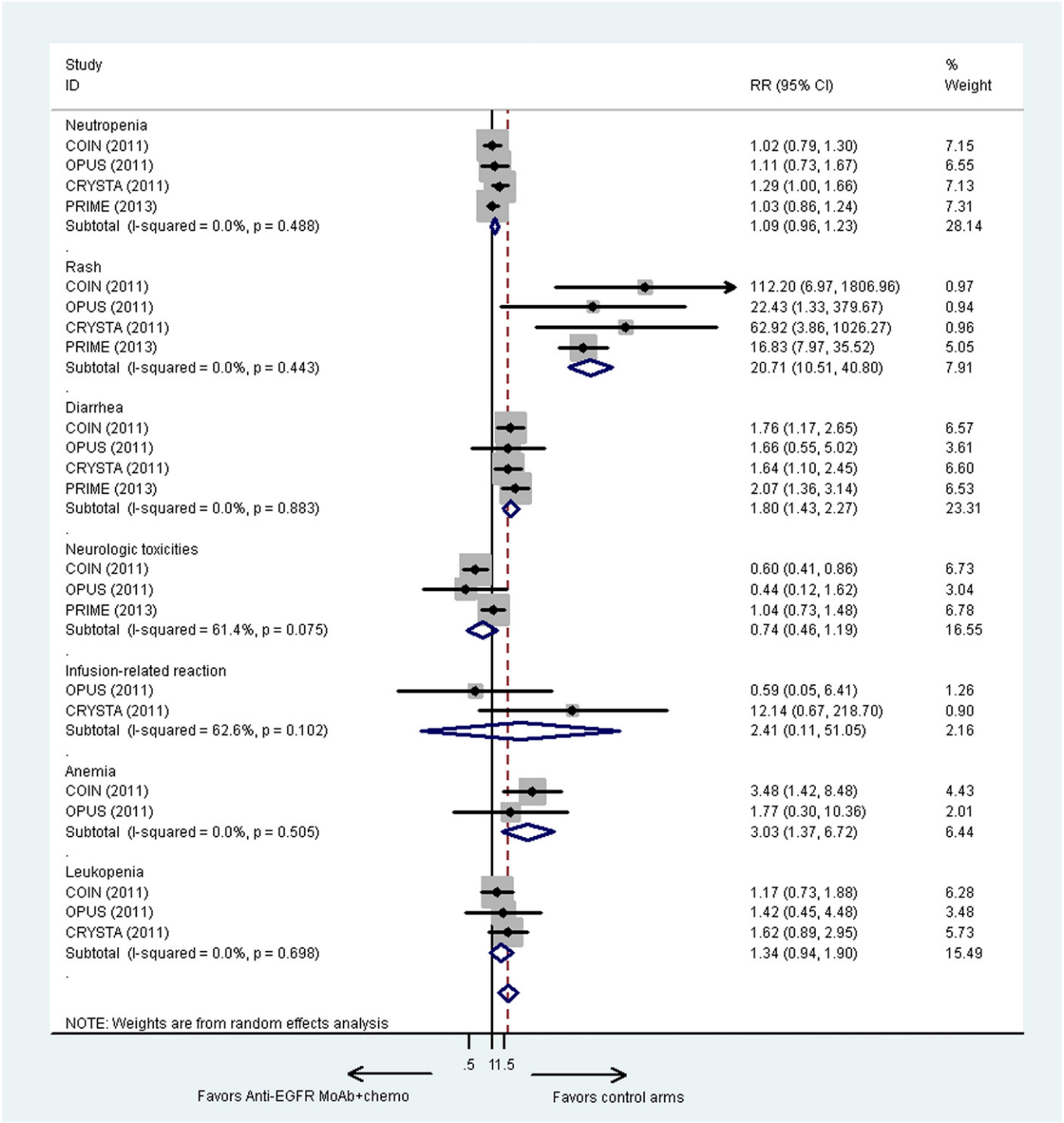

Fig. 5. Grade 3–4 toxicities.

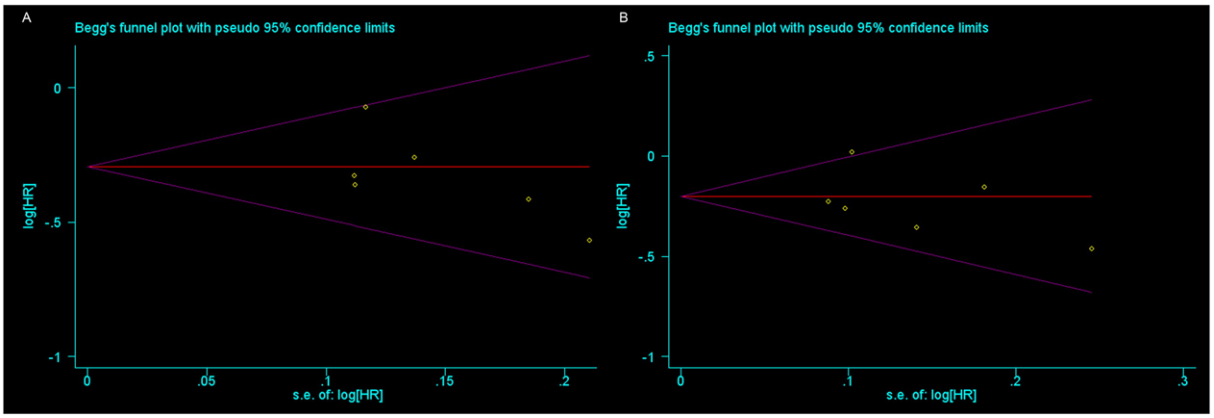

Fig. 6. Publication bias.

(Begg's test: $z = 0.38$, $P = 0.707$; Egger's test: $\tau = -0.75$, $P = 0.493$, Fig. 6).

4. Discussion

Anti-EGFR moAb + 5-FU infusion based chemotherapy as first-line treatment in *RAS*-wt mCRC patients significantly improved efficacy than *KRAS* exon 2-wt. *KRAS* exon 2-wt but other *RAS* mutations mCRC patients did not benefit from adding anti-EGFR moAb. Patients with *KRAS* exon 2-wt but with *BRAF* mutation did not benefit from adding anti-EGFR moAb. Whether *BRAF* mutation is a predictive marker to anti-EGFR moAb needs more data to answer.

For *KRAS* exon 2-wt mCRC patients, adding anti-EGFR moAb significantly improved PFS, OS and ORR compared with chemotherapy alone (COIN, OPUS, CRYSTAL and PRIME studies). Compared with adding Bev to 5-FU infusion based chemotherapy, adding anti-EGFR moAb significantly prolonged OS, but did not improve PFS and ORR (PEAK and FIRE3 studies). *KRAS* exon 2-wt but other *RAS* mutations mCRC patients did not benefit from adding anti-EGFR moAb. *RAS*-wt mCRC patients derived greater reduction of death and progression risk from adding anti-EGFR moAb. *BRAF* is tested as a prognostic factor to treat mCRC, but the current indications for anti-EGFR moAb still do not include *BRAF* testing. Our meta-analysis suggests that patients with *KRAS* exon 2-wt but with *BRAF* mutations benefit from adding anti-EGFR moAb but the difference is not significant. Another meta-analysis according to predictive role of *BRAF* mutations in mCRC was published in February 2015, in which, BRAF mutation assessment was suggested before initiation treatment of anti-EGFR moAb. But unfortunately, first- and second-line treatments of anti-EGFR moAb were pooled together in this meta-analysis. Our meta-analysis focused the predictive role of *BRAF* mutations in the first-line treatment of mCRC. Besides, an examination of 2530 individual patient data was presented in ASCO meeting this year. It suggested that *BRAF* mutations conferred worse OS in the first-line treatment of chemotherapy rather than the second-line treatment (Seligmann et al., 2015). Whether *BRAF* mutations predict response to anti-EGFR moAb need to be answered by clinical trials with more patients in the future.

Our analyses also included subgroups for patients with *KRAS* exon 2-wt mCRC according to prognostic factors. We found that in male, liver metastasis-only, ECOG 0–1, and colon primary site groups, anti-EGFR moAb + 5-FU infusion based chemotherapy significantly improved PFS, but made marginal, non-significant improvements in PFS in most other subgroups.

Addition of anti-EGFR moAb was associated with higher incidence of grade 3–4 toxicities including rash, diarrhea, and anemia; no additional toxicities were reported in the more recent RCTs. This suggests that use of anti-EGFR moAb + 5-FU infusion based chemotherapy as first-line treatment for mCRC would not be influenced by toxicities. Furthermore, selecting patients with *RAS*-wt tumors could improve response to anti-EGFR moAb and reduce unnecessary toxicities.

A meta-analysis according to *KRAS* exon 2-wt mCRC was published in November 2012, in which, oxaliplatin-based chemotherapy was control treatment. No survival benefit in the addition of cetuximab or panitumumab to oxaliplatin-based chemotherapy was observed in this meta-analysis. Firstly, oxaliplatin-based chemotherapy included oxaliplatin, FU (infusion or bolus) or capecitabine. Secondly, dose reduction or discontinuation of treatment due to increased toxic effects weakened the efficacy (Maughan et al., 2011; Tveit et al., 2012; Haller et al., 2008). Our meta-analysis focused on *RAS* status and only patients given 5-FU infusion based chemotherapy as backbone treatment were included. These differences explained why significance was observed in our meta-analysis rather than the former one.

Our meta-analysis had some limitations. Some data from PEAK and FIRE3 studies were not included because the two papers had not been published. Individual participant data were not available when we performed the meta-analysis. For the HRs of OS in COIN, only data from patients who received both OxCap and OxFU were available. Comparisons between patients with *KRAS* exon 2-wt but other *RAS* mutations mCRC and *KRAS* exon 2 mutations mCRC need more individual participant data in RCTs.

In conclusion, our meta-analysis suggests that patients with *RAS*-wt mCRC benefit more from anti-EGFR moAb + 5-FU infusion based chemotherapy than do those with *KRAS* exon 2-wt mCRC; and patients with *KRAS* exon 2-wt but other *RAS* mutations did not benefit from adding anti-EGFR moAb—similar to patients with *KRAS* exon 2 mutated tumors. *BRAF* mutation is still not the predictive marker to anti-EGFR moAb. Whether *BRAF* mutation is a predictive marker to anti-EGFR moAb needs more data to answer. Which is the better choice for backbone chemotherapy in first-line treatment and which is the optimal sequence for addition of target drugs will become clearer. With development of molecular studies, more really beneficial patients to anti-EGFR moAb will be enriched.

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Conflict of interest

The authors declare that they have no conflict of interest.

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