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Concordant analysis of *KRAS*, *BRAF*, *PIK3CA* mutations, and PTEN expression between primary colorectal cancer and matched metastases

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Current data on the concordance of *KRAS*, *BRAF*, *PIK3CA* mutation status or PTEN expression status between primary tumors and metastases in colorectal cancer (CRC) are conflicting. We conducted a systematic review and meta-analysis to examine concordance and discordance of the status of these four biomarkers between primary tumors and corresponding metastases in CRC patients. The biomarker status in primary tumors was used as the reference standard. Concordance data for *KRAS*, *BRAF*, *PIK3CA* and PTEN were provided by 43, 16, 9 and 7 studies, respectively. The pooled concordance rate was 92.0% (95% CI: 89.7%–93.9%) for *KRAS*, 96.8% (95% CI: 94.8%–98.0%) for *BRAF*, 93.9% (95% CI: 89.7%–96.5%) for *PIK3CA* and 71.7% (95% CI: 57.6%–82.5%) for PTEN. The pooled false positive and false negative rates for *KRAS* were 9.0% (95% CI: 6.5%–12.4%) and 11.3% (95% CI: 8.0%–15.8%), respectively. *KRAS*, *BRAF* and *PIK3CA* mutations are highly concordant between primary tumors and corresponding metastases in CRC, but PTEN loss is not. Nine percent of patients with wild-type *KRAS* in primary tumors who received anti-EGFR treatment had mutant *KRAS* in metastases, while 11.3% patients with mutant *KRAS* primary tumors had wild-type *KRAS* in the metastases. These 11.3% patients currently do not receive potentially beneficial anti-EGFR treatment.

The Epidermal Growth Factor Receptor (EGFR) is a cell transmembrane tyrosine kinase receptor that has a role in cancer cell proliferation and survival. Monoclonal antibodies (MoAbs) that target and inhibit EGFR function are commonly used in colorectal cancer treatment[1]. Two such MoAbs that target the extracellular domain of EGFR are cetuximab and panitumumab and these have proved effective in combination with chemotherapy or as single agents against metastatic colorectal cancer (mCRC)[1]. Unfortunately, resistance to MoAb treatment is common and in a recent study only 10–20% of the unselected mCRC patients benefitted from the treatment[1]. The resistance is partly ascribed to oncogenic activations of intracellular signaling pathways downstream of EGFR, including the RAS/RAF/MAPK and PI3K/PTEN/AKT pathways[1]. In the RAS/RAF/MAPK pathway, *KRAS* or *BRAF* mutations are present in 35–45% and in 4–15% of mCRC, respectively[2]. In the PI3K/PTEN/AKT pathway, *PIK3CA* mutations and loss of PTEN expression occur in 10–18% and 19–42% of mCRC, respectively[2]. *PIK3CA* mutations may coexist with either *KRAS* or *BRAF* mutations within the same tumor[2], whereas mutations in *KRAS* and *BRAF* appear to be mutually exclusive[3].

To date, *KRAS* codon 12 or 13 mutations in exon 2 have been widely demonstrated as a major predictive biomarker for resistance to the anti-EGFR MoAb treatment in patients with mCRC. Patients with mutant *KRAS* mCRC demonstrate lower objective response rates, decreased progression-free survival and worse overall survival compared with patients with wild-type *KRAS* mCRC[4]. With reference to these findings, the European Medicines Agency and subsequently the US Food and Drug Administration have restricted the use of anti-EGFR MoAbs to patients with wild-type *KRAS* mCRC. However, the occurrence of *KRAS* mutations only accounts for approximately 30–40% of nonresponsive patients[4]. In patients with wild-type *KRAS* mCRC, it remains unclear why a large number of patients are still not responsive to the treatment. The study by Douillard et al[5] suggested that *RAS*

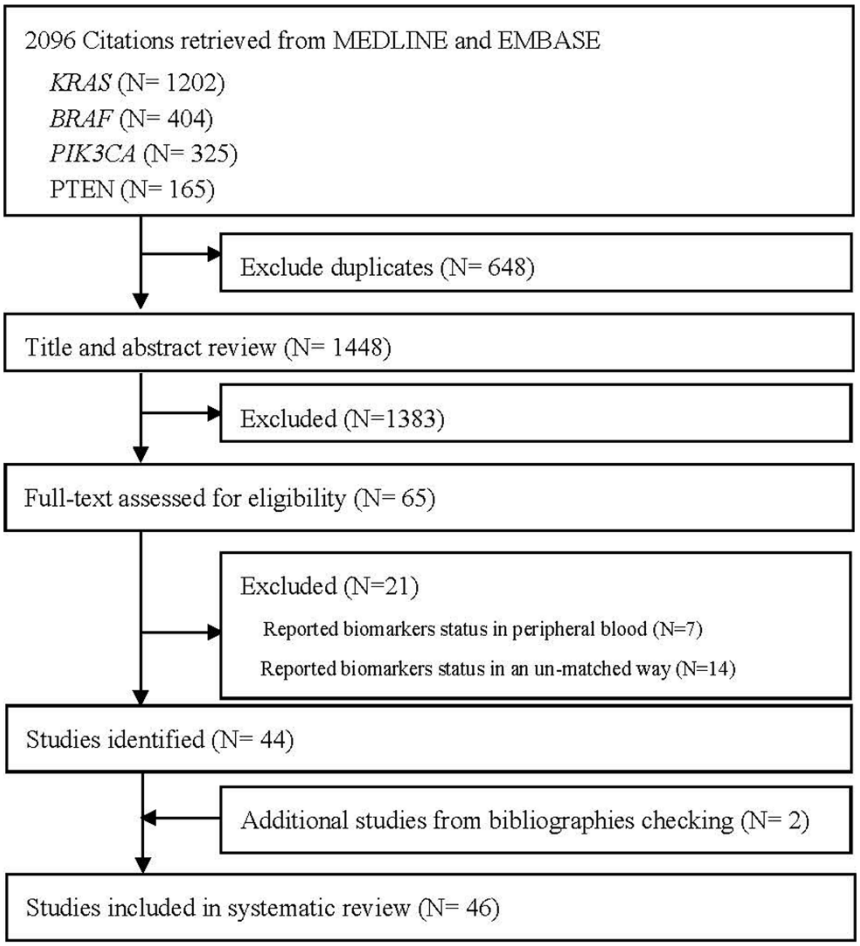

Figure 1 | **Flow chart of study selection.**

mutations (*KRAS* and *NRAS*), in addition to *KRAS* exon 2 mutations, may be a reason why some patients without *KRAS* exon 2 mutations are not responsive to anti-EGFR MoAbs treatment. Recently, other oncogenic mutations, such as *BRAF*[6,7], *PIK3CA* mutations[6] and loss of PTEN expression[7], have been presented as promising predictors for treatment resistance in these patients, although their predictive value has not yet been established. An additional explanation for the resistance to anti-EGFR MoAbs in patients with wild-type *KRAS* mCRC is discordance of *KRAS* mutation status between primary tumors and corresponding metastases. Crucially, this suggests that selecting patients for anti-EGFR MoAb treatment based on the characteristics of the primary tumor and not their metastases may not be optimal.

Current data on the concordance of *KRAS*, *BRAF*, *PIK3CA* mutation status and PTEN expression status between primary tumors and metastases are conflicting. Take *KRAS* mutations as an example, some studies[8–10] showed 100% concordance between primary CRC tumors and corresponding metastases. In contrast to these data, others have reported 4–30% discordance[11–14]. These inconsistent results between studies probably reflect the heterogeneity in methods, sample sizes, technical skills, the wide variety of metastatic sites or tumor biology (i.e., the genetic heterogeneity of the tumor cell population in the primary tumor, or changes in mutation status during progression of CRC). Therefore, it is still uncertain whether *KRAS* mutation status in primary tumor correctly reflects the *KRAS* mutation status of corresponding metastases. It also raises the question of whether mutation status of the primary tumor is sufficient to predict the response to anti-EGFR MoAbs.

In the present study, we performed a systematic review and meta-analysis to examine the overall concordance and discordance rates of the *KRAS*, *BRAF*, *PIK3CA* mutations status and PTEN expression status between primary CRC tumors and corresponding metastases.

Results

Literature search results. A total of 2096 records were retrieved from MEDLINE and EMBASE databases. After excluding duplicates and screening of titles and abstracts, 65 citations were left for full text screening. Among these 21 were excluded according to inclusion criteria, leaving 44 relevant articles. Searching of ASCO did not identify any further eligible studies, while reference list checking of reviews and included studies identified 2 additional studies. In total, 46 relevant studies[4,7–51] were identified. Details are presented in figure 1.

Mutation status concordance was reported in 43 studies for *KRAS*[4,7–30,34–51], in 16 studies for *BRAF*[7,13–18,24–27,33,34,41,43,48], in 9 studies for *PIK3CA*[13–15,18,25,27,34,48,49] and in 8 studies for PTEN[7,13,17,31,32,34,47,48]. The majority of studies (40/43) tested the *KRAS* mutations on codons 12 and 13[4,7–25,27,29,30,34–39,41–51]. Eight of 16 studies tested the *BRAF* mutations on exon 15[7,13,14,17,18,33,34,41] and 5 of 9 studies tested the *PIK3CA* mutations on exon 9 and/or exon 20[13,14,18,25,34]. Seven studies reported concordance information for lymph node metastases[7,14,26,28,36,37,49] and 11 studies for liver metastases[9–12,27,29,37,38,42,48,49]. Details are shown in Table 1 (Appendix 1).

Methodological quality of included studies. The reporting quality score of included studies varied considerably, from 6 to 21 of a

Table 1 | Characteristics of studies included in this systematic review

Study	Site of metastasis	Studied biomarkers	Methods for detection of biomarkers status	STROBE score	QUADAS-2 score
Oudejans, 1991	Liver and lung	*KRAS*, exon 2, codons 12, 13 and 61	Hybridization	NA	3
Losi, 1992	Liver, 33%; others, 67%	*KRAS*, exon 2, codons 12 and 13	AS-PCR	NA	4
Suchy, 1992	NR	*KRAS*, exon 2, codon 12	ASO	NA	3
Finkelstein, 1993	NR	*KRAS*, exon 2, codons 12 and 13	Sequencing	13	4
Al-Mulla, 1998	Liver, 46%; lymph nodes, 54%	*KRAS*, exon 2, codons 12 and 13	ASO and sequencing	19	7
Schimanski, 1999	Liver	*KRAS*, exon 2, codons 12 and 13	PCR-RFLP	15	5
Thebo, 2000	Lymph nodes	*KRAS*, exon 2, codons 12 and 13	AS-PCR	16	6
Zauber, 2003	Liver, 5%; lymph nodes, 93%;others, 2%	*KRAS*, exon 2, codons 12 and 13	SSCP	17	6
Albanese, 2004	Liver	*KRAS*, exon 2, codons 12 and 13	SSCP	16	7
Weber, 2007	Liver	*KRAS*, exon 2, codons 12 and 13	Sequencing	18	6
Oliveira, 2007	Lymph nodes	*KRAS*; BRAF	NR	7	4
Artale, 2008	Liver, 81%; lymph nodes, 2%; others, 17%	*KRAS*, exon 2, codons 12 and 13; *BRAF*, exon 15	Sequencing	8	5
Etienne-Grimaldi, 2008	Liver	*KRAS*, exon 2, codons 12 and 13	PCR-RFLP	18	7
Santini, 2008	Liver, 81%; lymph nodes, 1%; lung, 7%; others,11%	*KRAS*, exon 2, codons 12 and 13	Sequencing	17	4
Molinari, 2009	Liver, 74%; lung, 8%; others, 18%	*KRAS*, exon 2, codons 12 and 13; *BRAF*, exon 15; PTEN	KRAS and BRAF: Sequencing; PTEN: IHC	20	7
Cejas, 2009	Liver, 83.3%; lung,16.7%	*KRAS*, exon 2, codons 12 and 13	Sequencing	NA	5
Cejas, 2009(2)	Liver, 85%; lung, 15%	*KRAS*, exon 2, codons 12 and 13	Sequencing	18	6
Loupakis, 2009	Liver and others	*KRAS*, exon 2, codons 12 and13; PTEN	KRAS, Sequencing; PTEN: IHC	20	4
Perrone, 2009	Liver, 85%; others, 15%	*KRAS*, exon 2, codons 12 and 13; *BRAF*, exons 11 and 15; *PIK3CA*, exons 9 and 20; PTEN	KRAS, BRAF and PIK3CA: Sequencing; PTEN: IHC	18	6
GarmSpindler, 2009	NR	*KRAS*, exon 2, codons 12 and 13	Sequencing	17	5
Sood, 2010	NR	PTEN	IHC	NA	5
Baldus, 2010	Lymph nodes, 73%; distance metastasis, 27%	*KRAS*, exon 2; *BRAF*, exon 15; *PIK3CA*, exons 9 and 20	Sequencing	17	6
Italiano, 2010	NR	*KRAS*, exon 2, codons 12 and 13; *BRAF*, exon 15	Sequencing	18	6
Santini, 2010	Liver, Majority; others	*BRAF*, exon 15, codon 600	NR	6	4
Mariani, 2010	Liver,80%; others, 20%	*KRAS*, exon 2, codons 12 and 13; *BRAF*, exon 15, codon 600	Sequencing, SNAPShot multiplex PCR and Scorpion Taqman PCR analysis.	19	7
Negri, 2010	NR	PTEN	IFI	11	5
Xian, 2010	Liver	*KRAS*, exon 2,codons 12 and 13	Sequencing	15	6
Shen, 2010	Lymph nodes and others	*KRAS*	Sequencing	14	5
Melucci, 2010	NR	*KRAS*	Sequencing	NA	3
Watanabe, 2011	Liver, 64%; lung, 21%; others, 15%.	*KRAS*, exon 2, codons 12 and 13	Real-time TaqMan PNA clamp PCR and sequencing	17	6
Tie, 2011	Liver, 37%; lung, 27%; others, 36%	*KRAS*, exon 2, codons 1–37; *BRAF*, exon 15, codons 582–620; *PIK3CA*, exon 20, codons 1016–1067	KRAS and BRAF: Sequencing; PIK3CA: F-SSCP	18	7
Knijn, 2011	Liver	*KRAS*, exon 2, codons 12 and 13	Sequencing	18	7
Park, 2011	NR	*KRAS*, exon 2, codons 12 and 13; *BRAF*, exon 15; PTEN	KRAS and BRAF: Sequencing; PTEN: IHC	20	6
Cejas, 2012	Liver,85%; lung, 15%	*KRAS*, exon 2, codons 12 and 13; *BRAF*, exon 15; *PIK3CA*, exons 9 and 20; PTEN	KRAS, BRAF and PIK3CA: Sequencing; PTEN:IHC	21	6
Vermaat, 2012	Liver	*KRAS*, exon 2, codons 12 and 13; *BRAF*, exon 15, codon 600; *PIK3CA*, codons 542, 545 and 1047	Sequencing	15	7
Vakiani, 2012	Liver, 91%; others, 9%	*KRAS*, codons 12, 13, 22, 61, 117and 146; *BRAF*, exon 15, codon 600; *PIK3CA*, codons 345, 420, 542, 545, 546, 1043 and 1047	Sequencing	15	5

Table 1 | Continued

Study	Site of metastasis	Studied biomarkers	Methods for detection of biomarkers status	STROBE score	QUADAS-2 score
Kim, 2012	Liver, 33%; lymph nodes,12%; lung, 26%; others, 29%	*KRAS*, exon 2, codons 12, 13 and 61	Sequencing	20	5
Bossard, 2012	Liver, 78%; others, 22%	*KRAS*, exon 2, codons 12 and 13	Sequencing,	16	7
Kawamoto, 2012	Liver, 61%; lung, 28%; others, 11%	*KRAS*, exon 2, codons 12 and 13	ARMS/Scorpions technology-based *KRAS* PCR Kit	17	6
Miglio, 2013	Lymph nodes, 18%; others, 82%	*KRAS*, exon 2, codons 12 and 13	FAST Real Time PCR	18	7
Voutsina, 2013	Liver, 86%; lung, 7%; others, 7%	*KRAS*, exon 2, codons 12 and 13; *PIK3CA*, exons 9 and 20; *BRAF*, exon 15, codon 600	Sequencing	17	6
Mostert, 2013	Liver, 84%; others,16%	*KRAS*, exon 2, codons 12, 13 and 61; *BRAF*, exon 15, codon 600	COLD PCR and ASB-PCR	14	5
Atreya, 2013	Liver	*KRAS* (G12 and G13), *PIK3CA* (E542, E545, and H1047), and *BRAF* (V600), PTEN	*KRAS*, *BRAF* and *PIK3CA*, sequencing; PTEN, IHC	17	8
Murata, 2013	Liver or lymph nodes	*KRAS* (codon 12); *PIK3CA* (NR)	Pyrosequencing	15	8
Kaneko, 2014	Liver	*KRAS* codons 12, 13	Direct sequence	18	9
Paliogiannis, 2014	NR	*KRAS* codons 12, 13, 15, 61	Sequencing	16	9

Abbreviations: AS-PCR, allele-specific polymerase chain reaction; ASO, allele-specific oligonucleotide hybridization; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; NR, not reported; SSCP, single-strand conformation polymorphism; IHC, immunohistochemical analyses; IFI, Immunofluorescence; F-SSCP, fluorescence single-strand conformation polymorphism analysis; ASB-PCR, combines Allele-Specific PCR with a Blocking reagent.

maximum score of 22 using STROBE criteria. Considering methodological quality, all the included studies were identified as being of good quality in terms of the reference standard that was selected; and in terms of keeping all the patients received the same standard. Case-control study design was avoided in 42 studies[4,7–34,36–39,41–43,46,47–51] while the study design was unclear or not reported in the remaining 4 studies[35,40,44,45]. Only 2 studies[7,37] (2/46) reported assessor blinding, whereas the rest[4,8–36,38–51] did not explicitly reported whether test readers were blinded or not. One study[12] (1/46) had pre-specified threshold for *KRAS* mutations. In total, 27 out of the 46 included studies fulfilled 6 or more of the 11 methodological quality items[7–14,16–21,23,25,27,29,34,36,37,41,42,48–51].

Concordance and discordance between primary tumors and corresponding metastases. The pooled rates of mutation presence did not differ significantly between primary tumors and corresponding metastases for *KRAS* mutations (40.3%, 95% *CI*: 37.0%–43.8% vs. 39.9%, 95% *CI*: 36.5%–43.4%; $p=0.330$), *BRAF* mutations (6.1%, 95% *CI*: 4.0%–9.4% vs. 5.7%, 95% *CI*: 3.4%–9.3%; $p=0.362$), *PIK3CA* mutations (13.5%, 95% CI: 9.3%–19.2% vs. 13.8%, 95% *CI*: 9.8%–19.1%; $p=0.392$) and loss of PTEN expression (41.0%, 95% *CI*: 26.7%–61.3% vs. 57.0%, 95% *CI*: 33.6%–66.8%; $p=0.373$).

The pooled concordance rate was 92.0% (95% *CI*: 89.7%–93.9%) for *KRAS* (Appendix 2), 96.8% (95% *CI*: 94.8%–98.0%) for *BRAF* (Appendix 3), 93.9% (95% *CI*: 89.7%–96.5%) for *PIK3CA* (Appendix 4) and 71.7% (95% *CI*: 57.6%–82.5%) for PTEN (Appendix 5) (Table 2). When we just focused on *KRAS* codon 12 and 13 mutation status, the pooled concordance was 92.0% (30 studies, 1760 pairs; 95% *CI*: 88.5%–94.5%; $I^2 = 41.9\%$).

The pooled false positive and false negative rates were 9.0% (95% *CI*: 6.5%–12.4%) and 11.3% (95% *CI*: 8.0%–15.8%) for *KRAS* (Figure 2), 5.9% (95% *CI*: 3.5%–9.8%) and 34.0% (95% *CI*: 17.5%–55.7%) for *BRAF*, 9.1% (95% *CI*: 5.4%–16.8%) and 25.2% (95% *CI*: 13.5%–42.0%) for *PIK3CA* and 26.0% (95% *CI*: 4.2%–73.9%) and 16.3% (95% *CI*: 8.4%–29.4%) for PTEN (Table 2).

For *KRAS*, we further explored the mutational details in individual cases of discordance, where this had been reported. Ten studies[7,12,13,15,16,20,24–27,48,51] provided *KRAS* mutational information on a total of 57 discordant cases including 19 with wild-type *KRAS* in primary tumor but mutant *KRAS* in their metastases, 31 cases with mutant *KRAS* in primary tumors but wild-type *KRAS* in their metastases, and the rest (7 cases) showed a different mutation sub-type between the primary tumor and metastases. The most common mutation in primary tumor and metastases were G13D (7/38) and G12D (9/26), respectively. Details are presented in Figure 3.

Subgroup analyses according to metastases sites and testing methods. Subgroup analyses according to the site of metastases or testing methods were performed for concordance of *KRAS*, *BRAF* and *PIK3CA* status (Table 3). The pooled concordance of the genetic mutation or expression status in liver or lymph node metastases and primary CRC was 93.0% (95% *CI*: 87.4%–96.3%) and 73.4% (95% *CI*: 65.1%–80.3%) for *KRAS*, 98.6% (95% *CI*: 91.0%–99.8%) and 93.6% (95% *CI*: 86.0%–97.2%) for *BRAF*, 97.7% (95% *CI*: 72.3%–99.9%) and 85.5% (95% *CI*: 73.5%–92.6%) for *PIK3CA*.

The pooled concordance of *KRAS* status was 81.1% (95% *CI*: 69.3%–89.1%) for AS-PCR, 91.6% (95% *CI*: 89.0%–93.5%) for sequencing, 91.6% (95% *CI*: 25.0%–99.7%) for SSCP, 92.5% (95% *CI*: 72.0%–98.3%) for ASO and 97.3% (95% *CI*: 87.4%–99.4%) for PCR-RFLP. The pooled concordance of *BRAF* status was 97.4% (95% *CI*: 95.8%–98.3%) for sequencing and 93.0% (95% *CI*: 80.5%–97.7%) for AS-PCR (Table 3). The pooled concordance of *PIK3CA* status was 94.0% (95% *CI*: 86.3%–97.5%) for sequencing and 95.9% (95% *CI*: 89.5%–98.4%) for SSCP.

Discordance of *KRAS* status was also explored through subgroup analysis of metastasis location. The pooled false positive and false negative rates were 8.0% (9 studies, 408 pairs; 95% *CI*: 3.2%–18.4%;

Table 2 | Pooled concordance and discordance between primary tumors and corresponding metastases

	Concordance			Discordance					
Biomarkers	No. of studies (no. of pairs)	Concordance rate (95% CI)	I^2, %	No. of studies (no. of pairs)	False positive rate (95% CI)	I^2, %	No. of studies (no. of pairs)	False negative rate (95% CI)	I^2, %
KRAS	43 (2774)	92.0 (89.7–93.9)	40.1	41 (1441)	9.0 (6.5–12.4)	35.1	41 (892)	11.3 (8.0–15.8)	37.7
BRAF	16 (962)	96.8 (94.8–98.0)	15.7	5 (367)	5.9 (3.5, 9.8)	39.9	5 (26)	34.0 (17.5–55.7)	0.0
PIK3CA	9 (534)	93.9 (89.7–96.5)	28.7	4 (163)	9.1 (5.4–16.8)	0.0	5 (51)	25.2 (13.5–42.0)	11.9
PTEN	8 (320)	71.7 (57.6–82.5)	44.1	3 (34)	26.0 (4.2–73.9)	43.6	2 (50)	16.3 (8.4–29.4)	0.0

Abbreviation: CI, confidence interval.

I^2 = 40.3%) and 9.7% (95% *CI*: 4.5%–19.5%; I^2 = 35.0%) for liver metastases and 24.6% (6 studies, 78 pairs; 95% *CI*: 10.1%–48.7%; I^2 = 38.2%) and 25.5% (6 studies, 91 pairs; 95% *CI*: 10.8%–49.4%; I^2 = 40.7%) for lymph node metastases.

Sensitivity analysis and publication bias. Robust pooled results for *KRAS*, *BRAF*, *PIK3CA* and PTEN concordance (Table 4) were shown in sensitivity analyses when excluding studies that collected tissue after the initiation of chemo-therapy, studies fulfilling less than 6 of the 11 methodological quality criteria, studies with sample-size less than 50 and studies reporting concomitant *KRAS* and *BRAF* mutations. Significant publication bias was observed among studies for *KRAS* concordance ($z = -9.64$, $p < 0.001$), while no publication bias was observed among studies for *BRAF* ($z = -3.53$, $p > 0.1$), *PIK3CA* ($z = -1.55$, $p > 0.1$) or loss of PTEN expression ($z = -1.43$, $p > 0.1$).

Discussion

In clinical practice, analysis of *KRAS* mutations are usually performed on the primary tumor to determine patient eligibility for anti-EGFR MoAbs treatment, often because tissue samples from

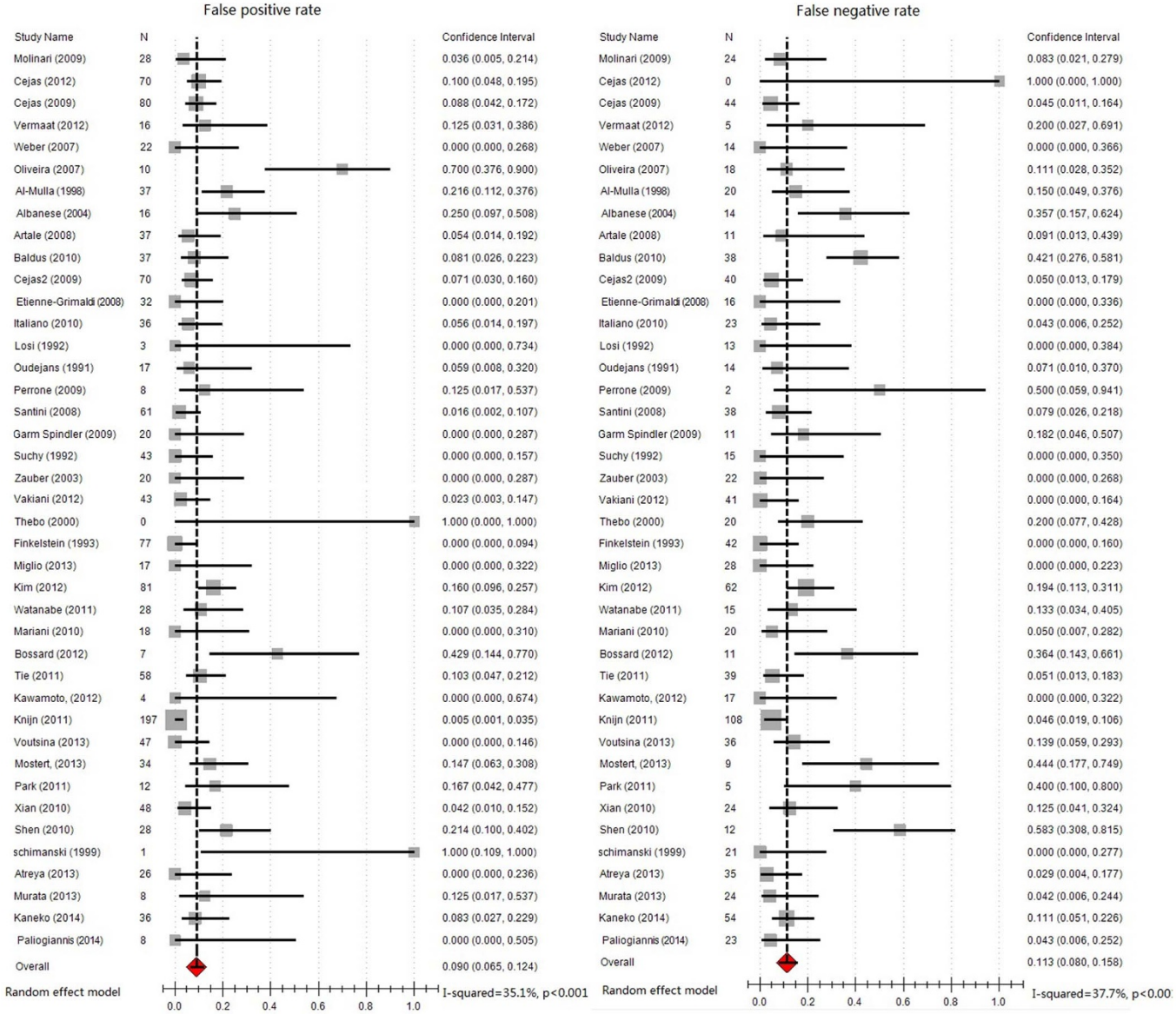

Figure 2 | Pooled false positive rate and false negative rate for *KRAS* mutations in patients with colorectal cancer.

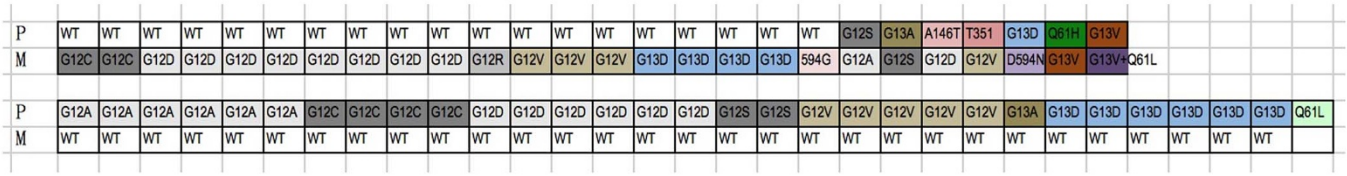

Figure 3 | Discordance on mutation status of *KRAS* between primary tumor and metastases tissue. Abbreviations: P, primary tumor; M, metastases.

the metastasis is not available. However, current data on the concordance of *KRAS* mutation status between primary tumors and corresponding metastases are conflicting. Therefore, the question of whether *KRAS* mutation status of the primary tumor is sufficient to predict the response to anti-EGFR MoAbs has been a controversial issue to date. In the present study, we performed a systematic review and meta-analysis to examine the overall concordance and discordance rates of *KRAS* and three other promising predictive biomarkers (*BRAF*, *PIK3CA* mutations and PTEN expression) between primary tumors and corresponding metastases. We found that the frequency of *KRAS*, *BRAF*, *PIK3CA* mutations and loss of PTEN expression did not differ significantly between primary tumors and corresponding metastases. Additionally, analysis of matched primary tumors and metastases showed a high concordance rate for *KRAS* (92.0%), *BRAF* (96.8%) and *PIK3CA* mutations (93.9%) but not for loss of PTEN expression (71.7%).

Although high concordance of *KRAS* status was observed between primary tumors and their metastases, discordance is still a concern, occurring in 9.0% of wild -type primary tumors and 11.3% in mutant primary tumors. Essentially, 9.0% of patients with wild-type *KRAS* primary tumors who receive the anti-EGFR MoAbs treatment actually have mutated *KRAS* in the metastases and 11.3% of patients with mutant *KRAS* primary tumors who have wild-type *KRAS* in the metastases will not receive potentially beneficial treatment. Most previous studies examined *KRAS* mutational status in only a single corresponding metastatic site. The representativeness of *KRAS* mutational status detected in only a single corresponding metastasis in the discordance cases is questionable[52]. Insufficient published data made it impossible to explore this question here.

In addition to *KRAS*, discordance rates of *BRAF*, *PIK3CA* mutation status and PTEN expression status were also analyzed. The status of these three biomarkers is not currently considered in clinical practice but given the higher concordance rate and lower rate of false positives for *BRAF* compared to *KRAS*, even though the rate of false negatives was higher, perhaps it is worth considering whether other biomarkers can be incorporated into the clinical decision making process.

Data for *KRAS*, *BRAF* and *PIK3CA* concordance were available for us to perform subgroup analyses according to metastases sites (liver or lymph nodes). A high concordance rate was observed between primary tumors and liver metastases for these three biomarkers. However, the concordance rate in lymph node metastases was not-

Table 3 | Subgroup analyses for concordance of *KRAS*, *BRAF* and *PIK3CA* status

	Concordance		
Biomarkers	No. of studies (no. of pairs)	Concordance% (95% CI)	I^2, %
KRAS			
Metastases sites			
Liver	11 (830)	93.0 (87.4–96.3)	42.5
Lymph nodes	7 (175)	73.4 (65.1–80.3)	11.2
Testing methods			
AS-PCR	3 (79)	81.1 (69.3–89.1)	6.5
Sequencing	30 (2116)	91.6 (89.0–93.5)	38.3
SSCP	2 (72)	91.6 (25.0–99.7)	45.4
ASO	3 (146)	92.5 (72.0–98.3)	41.6
PCR-RFLP	2 (70)	97.3 (87.4–99.4)	0.0
Other methods	2 (76)	98.4 (89.7–99.8)	0.0
Not reported	1 (28)	67.9 (48.9–82.4)	NA
BRAF			
Metastases sites			
Liver	2 (82)	98.6 (91.0–99.8)	0.0
Lymph nodes	3 (98)	93.6 (86.0–97.2)	0.0
Testing methods			
Sequencing	13 (960)	97.4 (95.8–98.3)	0.0
AS-PCR	1 (43)	93.0 (80.5–97.7)	NA
Not reported	2 (49)	91.9 (76.3–97.6)	9.2
PIK3CA			
Metastases sites			
Liver	1 (21)	97.7 (72.3–99.9)	NA
Lymph nodes	1 (55)	85.5 (73.5–92.6)	NA
Testing methods			
Sequencing	5 (323)	94.0 (86.3–97.5)	34.3
F-SSCP	1 (97)	95.9 (89.5–98.4)	NA
Not reported	1 (21)	97.7 (72.3–99.9)	NA

Abbreviations: AS-PCR, allele-specific polymerase chain reaction; ASO, allele-specific oligonucleotide hybridization; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SSCP, single-strand conformation polymorphism; Other methods, ARMS/Scorpions technology-based KRAS PCR Kit and FAST Real Time PCR; NA, not applicable; F-SSCP, fluorescence single-strand conformation polymorphism analysis.

Table 4 | Sensitivity analysis for pooled concordance between primary tumors and corresponding metastases

Biomarkers	No. of studies (no. of pairs)	Concordance (95% CI)	I^2, %
KRAS			
Tissue collection before the initiation of chemo-therapy	15 (971)	90.3 (85.7–93.5)	64.5
Fulfilling more than 6 methodological quality items	27 (1596)	90.8 (87.6–93.2)	35.3
Sample size more than 50 pairs	20 (1712)	92.1 (89.3–94.2)	40.4
With no concomitant *KRAS* and *BRAF* mutations	25 (1685)	92.9 (89.5–95.3)	42.6
BRAF			
Tissue collecting before the initiation of chemo-therapy	6 (436)	97.1 (95.0–98.4)	0.0
Fulfilling more than 6 methodological items	9 (534)	98.2 (96.3–99.2)	0.0
Sample size more than 50 pairs	8 (724)	98.0 (96.3–98.9)	0.0
With no concomitant *KRAS* and *BRAF* mutations	7 (414)	98.3 (96.3–99.2)	0.0
PIK3CA			
Tissue collecting before the initiation of chemo-therapy	2 (86)	88.3 (79.7–93.6)	0.0
Fulfilling more than 6 methodological items	8 (450)	93.1 (89.0–95.8)	23.2
Sample size more than 50 pairs	6 (470)	95.1 (90.1–97.6)	35.5
With no concomitant *KRAS* and *BRAF* mutations	4 (205)	97.4 (85.5–99.6)	40.2
PTEN			
Tissue collecting before the initiation of chemo-therapy	1 (11)	81.8 (49.3–95.4)	NA
Fulfilling more than 6 methodological items	5 (204)	80.1 (59.6–91.6)	44.9
Sample size more than 50 pairs	2 (120)	89.3 (26.1–99.5)	47.3
With no concomitant *KRAS* and *BRAF* mutations	2 (123)	78.8 (48.3–93.6)	46.6

Abbreviation: CI, confidence interval; NA, not applicable.

ably lower, especially for *KRAS* or *PIK3CA* mutations, in addition to *BRAF* which had slightly poorer concordance in lymph node compared to liver metastases. This potentially indicates that lymph node metastases are unsuitable for genetic mutation analysis. Our pooled results are in line with Bass and colleagues' narrative review of this topic[52].

Variation of the accuracy among different testing methods may be another reason for the discordance. Subgroup analyses based on testing methods for *KRAS, BRAF* and *PIK3CA* mutations showed that concordance varied among different testing methods. The most widely used method among included studies was sequencing, which is a classic method and has proven to be a reliable method. Results from this study showed that concordance for *KRAS, BRAF* and *PIK3CA* from sequencing were similar to their respective overall concordance, which suggested that sequencing was a stable method in testing mutations status of these three biomarkers or reflects the great contribution that sequencing makes to the total as it was the most frequently used method. Results from *KRAS* and *PIK3CA* suggested that SSCP may also a stable test method. However, AS-PCR showed the lowest concordance for both *KRAS* and *BRAF*. Interestingly, PCR-RFLP assessment showed the highest concordance (97.3%) for *KRAS* status (n=2 studies). Poor quality of testing methods may contribute somewhat to discordance in our results, however they are unlikely to explain all heterogeneity.

In addition to metastasis location or gene mutation testing methods, other potential explanations for discordance exist. Improper tumor sampling may cause a high proportion of normal cells or necrotic tissue to be included[4], tumor cells may have departed from the primary tumor before the acquisition of *KRAS* mutations or heterogeneity of cell type and therefore of biomarker status may exist in the primary tumor. The initiation of anti-EGFR MoAb treatment could also induce novel mutations and cause discordance[53]. As information on these factors were not available, sensitively analysis was performed based on the time of tissue collection (before or after MoAb treatment), study quality, sample size and whether there were concomitant mutations of *KRAS* and *BRAF*. Subgroup results varied little from the main pooled concordance estimates where more than four studies were available to pool. The exception being for PTEN with approximately 10% higher concordance reported in studies of better methodological quality compared to the main pooled result.

A key limitation in this work is the inability to generalize findings to other sites of metastasis because very few studies reported subgroup information on concordance between primary tumor and lung or brain metastases. Another limitation of our findings relates to mutational subgroups of PTEN expression or genetic mutations of *BRAF* or *PIK3CA*. Limited studies, compared *KRAS* mutational status, which prevented subgroup analysis for these. Furthermore, we identified significant publication bias for studies reporting *KRAS* concordance, indicating that the concordance values may not be as high as suggested by the studies here.

At present only the mutational status of *KRAS* is used as a predictive marker for EGFR inhibitor therapy[52]. Our results show good concordance between *KRAS* status in primary tumors and metastases indicating that the majority of patients will receive appropriate treatment. However, cases of discordance do clearly occur and are not uncommon and patients may therefore not be receiving the best or most appropriate treatment. The narrative review by Baas and colleagues suggested that discordance was uncommon and that additional testing is not justified, where no tissue already exists for the metastasis, because of increased risk of infection, increased costs of additional testing and the fact that the mutation status of one metastasis is no guarantee for the status of other tumors[52]. Despite the validity of these comments, the number of patients potentially affected by this issue is not insignificant at approximately 10% of those with CRC metastases. As with many issues in medicine, we must find a balance between the burden on patients or potential risk of further testing and the number of potential false positives.

In conclusion, high concordance rates were observed in *KRAS*, *BRAF* and *PIK3CA* mutation status but not in PTEN expression status between primary tumors and corresponding metastases. Liver metastases had a high concordance with primary tumor for *KRAS*, *BRAF* and *PIK3CA* mutation status while lymph node metastases showed a low concordance rate for these three biomarkers. Mutation concordance values were comparable with sequencing, SSCP, ASO and PCR-RFLP, indicating stability, while AS-PCR may be less reliable in this context.

Despite high concordance, discordance rates were not negligible for the four biomarkers examined. Future clinical decisions will need to consider that 9.0% of patients with wild-type *KRAS* primary tumors who currently receive the anti-EGFR MoAbs treatment have mutated *KRAS* in the metastases and 11.3% patients with mutant

KRAS primary tumors who actually have wild-type *KRAS* in the metastases and currently do not receive anti-EGFR treatment. As yet, it is unclear whether to recommend testing for other mutations or change of policy on treatment allocation. Comprehensive studies are required to address the issue of concordance and weigh up all the potential harms and benefits to patients of additional biopsy testing and anti-EGFR treatment, or not, in respect of metastatic gene mutation status.

Methods

Search strategy and selection criteria. We conducted an electronic literature search of PubMed and EMBASE from their respective inception to October 2014, with different combinations of the following keywords: "colon cancer", "rectal cancer", "colorectal cancer", "CRC", "primary", "*KRAS*", "*BRAF*", "*PIK3CA*" and "PTEN". In addition, we searched the abstract database of American Society of Clinical Oncology (ASCO) by using the previously mentioned terms. We subsequently manually searched the bibliographies of included studies and recent narrative reviews for additional studies. We applied no language restrictions. We considered both published and unpublished studies for inclusion, including those published in abstract form only.

We included all studies that reported concordance of any one of *KRAS*, *BRAF*, *PIK3CA* mutation status or PTEN expression status in primary tumors and corresponding metastases in colorectal cancer. Two reviewers (WXY & YZY) independently reviewed titles, abstracts, and full texts of all citations that were likely to meet the predefined selection criteria. Any discrepancies were resolved by consensus or by consulting with a third reviewer. For multiple publications from the same study, we selected the most recent and complete versions of studies.

Quality assessment and data extraction. Two reviewers (WXY & YJQ) independently extracted data using a predefined data abstraction form and critiqued the quality of the studies, with a third reviewer (MC) consulted in case of disagreement. For each study and for each of the four biomarkers, where reported, we constructed 2×2 tables to display the number of patients with mutations or normal/wild-type tumors present during assessment of primary or metastatic cancer. Thus, we could examine the proportion of cases where the genetic profile had changed from mutated to wild-type or vice-versa. In addition, the following data were extracted from each study: study characteristics (such as first author's name, year of publication, study design and number of patients enrolled), patients' characteristics (such as mean or median age, percent of male participants and histology), sites of metastases for biomarker testing, biomarker testing method and items necessary to assess study quality.

We assessed the methodological and reporting quality of studies by using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool[54] and the criteria for reporting observational studies proposed in the STROBE statement[55], respectively, giving equal weight to all items.

Statistical analysis. We used the biomarker status in primary tumors as the reference standard and calculated agreement rate, false positive rate (wild-type or normal expression in primary tumor but mutant or loss of expression in metastases) and false negative rate (mutant or loss of expression in primary tumor but wild-type or normal expression in metastases) for each study using the above-mentioned 2×2 tables. The concordance was measured by agreement rate and discordance was measured by false positive rate or false negative rate. We combined rates of mutation/loss of expression, concordance and discordance using the fixed-effect model unless there was evidence of heterogeneity ($p \leq 0.1$), in which case a random-effect model was used. Heterogeneity was explored by the Q-test with degree of freedom equal to the number of analyzed studies minus 1. A p value of 0.10 or below in the Q-test indicates the presence of heterogeneity across studies. We performed subgroup analyses to detect potential sources of heterogeneity according to metastases site (liver or lymph nodes) and methods used to test biomarker mutation (sequencing, allele-specific oligonucleotide hybridization [ASO], allele-specific polymerase chain reaction [AS-PCR], single-strand conformation polymorphism [SSCP], PCR-restriction fragment length polymorphism [PCR-RFLP] and other methods). For *KRAS* status, we also pooled the concordance of mutations on codons 12 and 13. We performed sensitivity analyses to assess the robustness of the final results by excluding studies that collected tumor tissue after the initiation of chemo-therapy, studies fulfilling less than 6 of the 11 methodological items, studies with sample-size less than 50, and studies reporting concomitant *KRAS* and *BRAF* mutations in samples of either primary tumors or metastases. We performed Egger's funnel plots to assess the possible presence of publication bias. Egger's test was performed to assess the symmetry of the funnel plot. We used STATA Version 12.0 (STATA Corporation, College Station, TX, USA) and MetaAnalyst Version Beta 3.13 (Tufts Medical Center, Boston, MA, USA) for the analyses, with a two-tailed significance level of 0.05 except for the assessment of heterogeneity ($\alpha = 0.10$).

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