Systematic review

Meta-analysis of *KRAS* mutations and survival after resection of colorectal liver metastases

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Background: **In patients with advanced colorectal cancer, *KRAS* mutation status predicts response to treatment with monoclonal antibody targeting the epithelial growth factor receptor (EGFR). Recent reports have provided evidence that *KRAS* mutation status has prognostic value in patients with resectable colorectal liver metastases (CLM) irrespective of treatment with chemotherapy or anti-EGFR therapy. A meta-analysis was undertaken to clarify the impact of *KRAS* mutation on outcomes in patients with resectable CLM.**

Methods: **PubMed, Embase and Cochrane Library databases were searched systematically to identify full-text articles reporting *KRAS*-stratified overall (OS) or recurrence-free (RFS) survival after resection of CLM. Hazard ratios (HRs) and 95 per cent c.i. from multivariable analyses were pooled in meta-analyses, and a random-effects model was used to calculate weight and overall results.**

Results: **The search returned 355 articles, of which 14, including 1809 patients, met the inclusion criteria. Eight studies reported OS after resection of CLM in 1181 patients. The mutation rate was 27·6 per cent, and *KRAS* mutation was negatively associated with OS (HR 2·24, 95 per cent c.i. 1·76 to 2·85). Seven studies reported RFS after resection of CLM in 906 patients. The mutation rate was 28·0 per cent, and *KRAS* mutation was negatively associated with RFS (HR 1·89, 1·54 to 2·32).**

Conclusion: ***KRAS* mutation status is a prognostic factor in patients undergoing resection of colorectal liver metastases and should be considered in the evaluation of patients having liver resection.**

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Introduction

In the evaluation of patients for resection of colorectal liver metastases (CLM), extent of disease is the primary consideration. Resection of all viable disease with acceptable postoperative morbidity is crucial to maximize the survival benefit and achieve cure. Improvements in surgical and non-surgical techniques have increased the proportion of patients eligible for curative resection of CLM[1]. However, for a number of patients rapid recurrence or postoperative complications offset the benefit associated with surgery.

In the 1990s, several groups[2–4] published scoring systems to predict recurrence after resection of CLM on the basis of clinical parameters such as sex, age, tumour location, size and number, disease-free interval and disease stage. However, risk factors determined before the era of modern chemotherapy have been shown[5–7] to perform less well in recent series of patients with CLM, in whom liver resection was performed in combination with modern chemotherapy. Compared with clinical parameters that serve as surrogate markers for tumour biology, direct indicators of tumour biology may explain the diverse outcomes after resection of CLM and provide useful information to guide treatment of patients with CLM.

Currently, medical oncologists are using the mutation status of the Kirsten rat sarcoma viral oncogene homologue (*KRAS*) gene to select patients with advanced-stage colorectal cancer with wild-type *KRAS* for treatment with monoclonal antibodies that target the epithelial growth factor receptor (EGFR); the antibodies used are panitumumab and cetuximab[8–11]. Recently, mutations in the *KRAS* gene have received much attention as the most promising mutations for prognostication in patients undergoing resection of CLM[12–14], indicating that knowledge of *KRAS* mutation status may also be valuable for evaluation of patients for possible resection of CLM. To clarify the value of *KRAS* mutation status in predicting outcome after resection of CLM, a systematic literature review and meta-analysis was performed of studies reporting overall (OS) and recurrence-free (RFS) survival stratified by *KRAS* mutation status (irrespective of chemotherapy and anti-EGFR treatment) in patients undergoing CLM resection.

Methods

Data sources and search strategy

A systematic literature review was performed in April 2014 using the US National Library of Medicine PubMed database, Embase and the Cochrane Library. A detailed search string was constructed to return full-text articles that reported studies with patients who had resection of CLM and that provided information regarding outcome and *KRAS* mutation status: 'colorectal AND cancer AND (liver OR hepatic) AND (metastasis OR metastases) AND (resection OR surgery OR hepatectomy) AND (mutation OR mutations) AND (*KRAS* OR *K*-RAS OR *NRAS* OR *N*-RAS OR *RAS*)'. The review adhered to the guidelines outlined in the PRISMA statement[15].

Selection criteria

To be included in the review, an article had to: report on a study that included and reported patients who underwent resection of CLM; include results of genetic testing for *KRAS* mutations; and include outcomes for survival or recurrence assessed against the mutations. Publication date was not an inclusion or exclusion criterion. Duplicate articles were removed and the title of the remaining articles was reviewed; if the title did not reveal a reason for exclusion, the abstract was used to determine whether the article or study met any of the following exclusion criteria: language other than English; primary cancer other than colorectal cancer; article type other than report of original research (review, editorial, letter, comment, case report, or abstract); only the primary tumour treated with surgery; oncological but not surgical outcome reported; survival or recurrence not a primary or secondary outcome; basic science report; or study in cell lines or animals. The reference lists of the remaining articles were assessed for missed studies meeting the inclusion criteria. A qualitative systematic literature review and critical evaluation of the evidence were performed. Articles reporting *KRAS* mutation effect estimates from multivariable analyses for OS and articles reporting RFS after resection of CLM were pooled in separate meta-analyses.

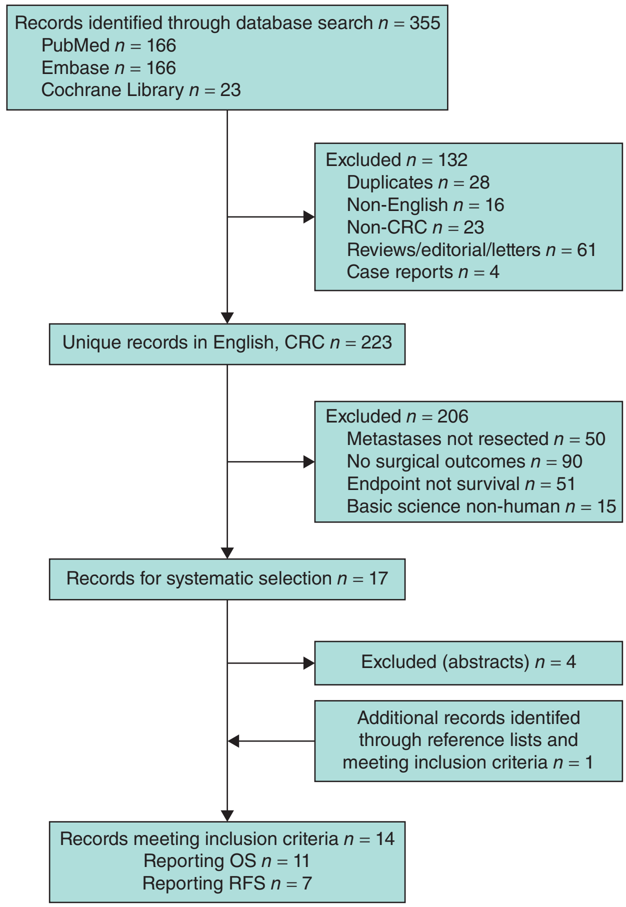

Fig. 1 PRISMA flow chart showing the article selection process. The search string was built to identify studies of patients undergoing resection of colorectal liver metastases (CLM) in whom survival was assessed according to *KRAS* mutation status. CRC, colorectal cancer; OS, overall survival; RFS, recurrence-free survival

Data extraction and outcome measures

The following data were extracted from the included articles: first author, study origin, year of publication, study period, sample size, metastatic site, rate of *KRAS* mutations, *KRAS* codons included in the mutational analysis, use of preoperative chemotherapy (regimen and number of patients), use of adjuvant chemotherapy (regimen and number of patients), summary of findings regarding survival, and multivariable effect estimates for OS and RFS (hazard ratio (HR), 95 per cent c.i., *P* value). The individual studies were graded into low or high risk of bias based on the Grading of Recommendations, Assessment Development and Evaluation (GRADE) Working Group criteria: quality of evidence, uncertainty about the balance between desirable and undesirable effects, uncertainty of

Table 1 Characteristics of the included studies

Reference	Year	Study origin	Study interval	*n**	Metastatic site
Kastrinakis *et al.*[26]	1995	Boston, USA	1982–1992	19	Liver
Russo *et al.*[17]	1998	Palermo, Italy	1988–1992	35†	Liver
Petrowsky *et al.*[19]	2001	Frankfurt, Germany	1985–1995	41	Liver
Cejas *et al.*[18]	2009	Madrid, Spain	1997–2007	110	Liver and lung
Nash *et al.*[16]	2010	New York, USA	1991–1997	188‡	Liver
Teng *et al.*[22]	2012	Taipei, Taiwan	2000–2010	292§	Liver
Stremitzer *et al.*[13]	2012	Vienna, Austria	2005–2010	60	Liver
Huang *et al.*[20]	2013	Taipei, Taiwan	2000–2010	228§	Liver
Umeda *et al.*[23]	2013	Okayama, Japan	1997–2009	100	Liver
Isella *et al.*[21]	2013	Torino, Italy	2008–2010	64	Liver
Vauthey *et al.*[14]	2013	Houston, USA	1997–2011	193	Liver
Karagkounis *et al.*[12]	2013	Baltimore, USA	2003–2008	202	Liver
Shoji *et al.*[25]	2014	Tokyo, Japan	2004–2009	108	Liver
Kemeny *et al.*[24]	2014	New York, USA	2003–2013	169	Liver

*Number of patients included with known *KRAS* mutation status. †Thirteen of 35 and ‡126 of 188 patients underwent resection. §These two publications were based on the same patient cohort.

variability in values and preference, and uncertainty about whether the intervention represents a wise use of resources (www.gradeworkinggroup.org). The following study characteristics were assessed in light of the GRADE criteria and a concern regarding individual study bias: sample size, heterogeneity of selection to chemotherapy and resection, unresectability, study interval, lung and not liver resections, *KRAS* codons tested, other included genes, heterogeneity regarding variables included in multivariable analysis, and consistency of findings.

Statistical analysis

The primary outcome of the study was OS after resection of CLM; the secondary outcome was RFS after resection of CLM. Meta-analyses were performed using HRs with 95 per cent c.i.; the chosen effect measure was dichotomous data, which were available in 11 articles (8 of which reported OS and 7 RFS). The effect measures were converted into logarithmic values and a random-effects model with inverse-variance method was used to calculate weight and overall results of the meta-analyses. Interstudy statistical heterogeneity was assessed with I^2 statistics, and moderate to high degree was assumed when the value was more than 30 per cent. Data were presented in Forest plots in which a HR of less than 1 represents better outcome and a HR of more than 1 represents worse outcome in patients harbouring a *KRAS* mutation. Funnel plot analyses were performed to evaluate the presence of publication bias. Where some summary statistics for OS were reported for patients with *KRAS* and wild-type mutations, but the HR and 95 per cent c.i. were not made available in the article, these studies were assessed individually and not included in the main meta-analysis. Stata/SE™ version 11·0 (StataCorp LP, College Station, Texas, USA) was used for the meta-analyses.

Results

Literature search result

The initial search returned 355 records (*Fig. 1*); after exclusions, 223 articles remained for assessment of eligibility. After application of the other exclusion criteria and review of reference lists for missed articles, 14 unique articles remained[12–14,16–26] (*Tables 1* and *2*). These 14 articles reported on 1809 patients, 1725 of whom had resection of CLM. The *KRAS* mutation rate among all patients was 30·6 per cent.

Assessment of the study characteristics based on the GRADE criteria found that two studies[16,17] ($n = 84$) included patients with unresectable CLM, one study[18] ($n = 17$) included patients with lung metastasis, one study[19] tested *KRAS* codons 12 only, and one study[20] ($n = 6$) included *BRAF* mutations in the survival analysis. The risk of individual study bias was assessed as low[12–14,16,18,20–25] and high[17,19,26] in the included articles.

KRAS mutation and overall survival

Eight[12–14,16,19,20,23,24] of the 14 studies reported OS after resection of CLM stratified by *KRAS* mutation status and were pooled in a meta-analysis (*Fig. 2*). Three[17,18,26] of the 14 studies did not perform multivariable analyses for OS, two[21,25] reported RFS only, and one[22] reported results from the same patient cohort as another study[20] that was included in the meta-analysis. The study by

Table 2 *KRAS* mutations, use of chemotherapy and survival findings in the included studies

Reference	*KRAS* mutation rate (%)	Codons	Chemotherapy for CLM: Preop. therapy	Chemotherapy for CLM: Adjuvant therapy	Chemotherapy for CLM: Regimen	Findings regarding survival
Kastrinakis *et al.*[26]	37	12, 13	0	0		*KRAS* mutation rate similar in long- and short-term survivors
Russo *et al.*[17]	43	12, 13	0	0	5-FU*	*KRAS* codon 13 mutation negatively associated with OS (but not codon 12 and not both)
Petrowsky *et al.*[19]	15	12	n.a.	n.a.	5-FU†	*KRAS* mutation not associated with OS
Cejas *et al.*[18]	32·0#	12, 13	0	110	5-FU ± OXA/IRI	*KRAS* mutation negatively associated with RFS, not OS
Nash *et al.*[16]	27·0	12, 13	16	n.a.	5-FU‡	*KRAS* mutation negatively associated with OS
Teng *et al.*[22]	38·0	12, 13	44	145	n.a.	*KRAS* mutation not associated with OS
Stremitzer *et al.*[13]	25	12, 13, 61	60	60	5-FU + OXA + BEV	*KRAS* mutation negatively associated with OS and RFS
Huang *et al.*[20]	36·7	12, 13, 14	52	193	n.a.	*KRAS*/*BRAF* mutations negatively associated with OS
Umeda *et al.*[23]	27·0	12, 13	33	85	5-FU ± OXA/IRI ± BEV**	*KRAS* mutation negatively associated with OS
Isella *et al.*[21]	33	12, 13, 61, 146	36	43	5-FU + OXA/IRI ± BEV††	*KRAS* mutation negatively associated with RFS (not in multivariable analysis)
Vauthey *et al.*[14]	17·6‡‡	12, 13, 61, 146	193	193	5-FU + OXA/IRI + BEV	*RAS* mutation negatively associated with OS, RFS (any site) and lung RFS, but not liver RFS
Karagkounis *et al.*[12]	29·0	12, 13	162	130	n.a.	*KRAS* mutation negatively associated with OS and RFS
Shoji *et al.*[25]	36·1	12, 13	n.a.	n.a.	n.a.§	*KRAS* mutation negatively associated with RFS
Kemeny *et al.*[24]	30·2	12, 13	n.a.	169	5-FU ± OXA/IRI + HAI¶	*KRAS* mutation negatively associated with RFS, not OS, in multivariable analysis

*Chemotherapy used in study, but only in patients with unresectable disease. Chemotherapy used at some point in †29, ‡161, §14 and ¶142 patients, but adjuvant for primary *versus* preoperative/adjuvant for liver frequencies not available (n.a.). #Liver *KRAS* mutation rate. **One patient received cetuximab before resection of colorectal liver metastases (CLM), and three patients received cetuximab/panitumumab after resection of CLM. ††Chemotherapy was given only to patients with initially unresectable CLM. ‡‡Includes *NRAS* mutations. 5-FU, 5-fluorouracil-based chemotherapy; OS, overall survival; OXA, oxaliplatin; IRI, irinotecan; RFS, recurrence-free survival; BEV, bevacizumab; HAI, hepatic arterial infusion.

Huang and colleagues[20] was included in the meta-analysis because these authors included *KRAS* mutations in the multivariable analysis. The eight studies included in the meta-analysis represented 1181 patients who underwent resection of CLM; in these patients the *KRAS* mutation rate was 27·6 per cent. The results from these eight studies were generally consistent and *KRAS* mutation was negatively associated with OS (HR 2·24, 95 per cent c.i. 1·76 to 2·85). The funnel plot did not suggest notable publication bias (data not shown).

Three early studies reported overall survival in 19[26], 13[17] and 110[18] patients who underwent resection of CLM without providing HR and 95 per cent c.i. for *KRAS* mutations from multivariable analysis. These studies were assessed individually; no significant association between *KRAS* mutation and OS was found.

KRAS mutation and recurrence-free survival

Seven[12–14,18,21,24,25] of the 14 studies included in the systematic literature review reported RFS. Multivariable Cox regression analysis was performed for all seven studies, and the HR data were pooled in a separate meta-analysis (*Fig. 3*). The seven studies reported a total of 906 patients undergoing resection of CLM; in these patients the *KRAS*

Reference	*n*	*KRAS* mutation (%)	Hazard ratio	Weight (%)
Petrowsky *et al.*[19]	41	15	1·39 (0·45, 4·27)	4·6
Nash *et al.*[16]	188*	27·0	2·40 (1·40, 4·00)	21·2
Stremitzer *et al.*[13]	60	25	3·51 (1·30, 9·45)	5·9
Huang *et al.*[20]	228	36·7	2·38 (1·29, 4·37)	15·7
Umeda *et al.*[23]	100	27·0	2·38 (1·02, 5·40)	8·4
Vauthey *et al.*[14]	193	17·6	2·30 (1·10, 4·50)	11·7
Karagkounis *et al.*[12]	202	29·0	1·99 (1·21, 3·26)	23·7
Kemeny *et al.*[24]	169	30·2	2·00 (0·87, 4·46)	8·7
Overall (I^2 = 0·0%, P = 0·965)	1181	27·6	2·24 (1·76, 2·85)	100·0

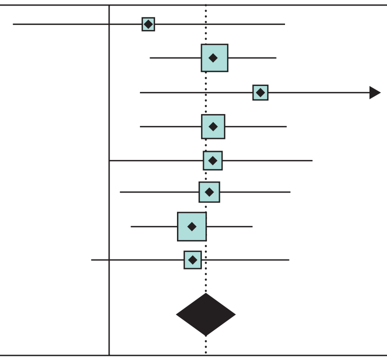

Fig. 2 Forest plot of association between *KRAS* mutation status and overall survival (OS) after resection of colorectal liver metastases in eight studies. *Only 126 of the 188 included patients underwent resection. A random-effects model with inverse-variance method was used for meta-analysis. Hazard ratios are shown with 95 per cent c.i.

Reference	*n*	*KRAS* mutation (%)	Hazard ratio	Weight (%)
Cejas *et al.*[18]	110	32·0	2·07 (1·14, 3·77)	11·6
Stremitzer *et al.*[13]	60	25	2·48 (1·26, 4·89)	9·1
Isella *et al.*[21]	64	33	1·58 (0·79, 3·16)	8·8
Vauthey *et al.*[14]	193	17·6	1·90 (1·20, 3·00)	19·9
Karagkounis *et al.*[12]	202	29·0	1·68 (1·04, 2·70)	18·4
Shoji *et al.*[25]	108	36·1	1·91 (1·16, 3·12)	17·1
Kemeny *et al.*[24]	169	30·2	1·90 (1·16, 3·31)	15·2
Overall (I^2 = 0·0%, P = 0·997)	906	28·0	1·89 (1·54, 2·32)	100·0

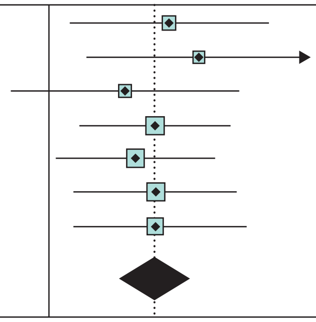

Fig. 3 Forest plot of association between *KRAS* mutation status and recurrence-free survival (RFS) after resection of colorectal liver metastases in seven studies. A random-effects model with inverse-variance method was used for meta-analysis. Hazard ratios are shown with 95 per cent c.i.

mutation rate was 28·0 per cent. The results from these seven studies were consistent and *KRAS* mutation was negatively associated with RFS (HR 1·89, 95 per cent c.i. 1·54 to 2·32).

Discussion

In the meta-analyses reported here, *KRAS* mutations predicted inferior OS and inferior RFS among patients who had resection of CLM. The effect of *KRAS* mutations on survival cannot be attributed to the perioperative use of targeted agents, as perioperative anti-EGFR treatment was used in only four of 100 patients in just one study[23]. Furthermore, the superior RFS in patients with wild-type *KRAS* indicates that the impact of *KRAS* mutation in the present study was not due to treatment of recurrence with anti-EGFR.

KRAS mutations have been associated with migration and invasion through disruption of the actin cytoskeleton and regulation of integrin expression, among other mechanisms[27–29]. These behaviors are mediated via a wider class of effectors beyond the mitogen-activated protein kinase pathway, including Rho guanosine-5′-triphosphatases and Rap1[30,31]. As a result, the prognostic importance of activating *KRAS* mutations extends beyond their ability to predict sensitivity to anti-EGFR monoclonal antibodies, and *KRAS* mutations may reflect a more migratory and invasive tumour biology resulting in a propensity for early and frequent recurrences after resection of metastatic disease.

The pooled *KRAS* mutation rate in the present study, 30·6 per cent, was lower than the 35–45 per cent rates that have been reported in most studies of patients with metastatic colorectal cancer[13,14,19,32–34]. The high concordance of *KRAS* mutation status between primary colorectal tumours and metastatic sites (more than 90 per cent) indicates that mutations are acquired early in tumorigenesis, before metastatic spread[35,36]. As such, the lower rate of *KRAS* mutations in the present study is unlikely to be due to differences in the tissue source for the *KRAS* testing[18,37–39]. Instead, patients who are deemed candidates for surgery are more likely than those not deemed candidates for surgery to have oligometastatic disease, reflecting potential differences in metastatic propensity. The lower *KRAS* mutation rate in the present study is in agreement with recent findings demonstrating significantly higher *KRAS* mutation rates in patients with extrahepatic metastasis from colorectal cancer than in patients with CLM, and unresectable extrahepatic disease is in most patients considered a contraindication for resection of CLM[18,37,40].

Although the survival impact of *KRAS* mutations has been demonstrated across multiple studies utilizing the most common codons in *KRAS* (12 and 13)[32], recent data on resistance to anti-EGFR monoclonal antibodies have suggested that the current standard-of-care panel should be expanded to include codons in exons 3 (codon 61) and exon 4 (codon 146) of *KRAS*[41]. These *KRAS* alleles were included in several studies[13,14,21] in the present meta-analysis. The rarity of mutations in these additional alleles precludes testing of their individual prognostic impact, but their mutual exclusivity in patients[37] and ability to transform cells *in vitro* suggest that their oncogenic function is preserved and that these alleles should therefore be included in testing panels for assessment of *RAS* mutation status[9,42,43]. A recent analysis[9] indicated that *NRAS* mutations should also be considered in the determination of *RAS* mutation status. In one study[14] included in the present meta-analyses, the investigators analysed all *RAS* mutations (*NRAS* and extended *KRAS* to include codon 61 and 146); the addition of *NRAS* increased the yield of *RAS* mutations by 20 per cent and likely strengthened the impact of mutations on prognosis.

The main challenge in patients with CLM is to identify those who can derive a survival benefit from resection. Historically, predictors of survival were based on morphological characteristics of the primary tumour and metastases (including primary tumour location and TNM stage; number and size of liver metastases), carcinoembryonic antigen level, and the disease-free interval between detection of the primary tumour and metastasis. Various scores with combinations of these factors have been proposed to predict prognosis after resection of CLM[2–4,44–48]. However, in recent years, large single-institution studies have questioned the validity and clinical usefulness of risk scores[5–7]. Zakaria and colleagues[7] found that risk scoring systems had limited clinical value, and Kattan and co-workers[49] created a nomogram with better discriminatory ability to improve scoring in resectable CLM. The studies used in the present meta-analyses assessed many of these factors in multivariable analysis, and *KRAS* mutations consistently indicated an independent twofold increase in the risk of death (8 studies) or recurrence (7 studies). In recent years, investigators have proposed pathological and radiological responses to chemotherapy as alternative outcome endpoints for predicting survival after resection of CLM[50–52]. However, pathological response can be assessed only after surgery, and the survival association with radiological response was found to be present mostly in patients receiving preoperative antivascular endothelial growth factor therapy[50–53]. In this context, *KRAS* mutation stands out as a new predictor of prognosis in patients with resectable CLM. It has many advantages over tumour characteristics and response to chemotherapy, as *KRAS* mutation is an early event in carcinogenesis that appears to be unaffected by chemotherapy[54].

This study has several limitations. First, there may be heterogeneity between the studies regarding the definition of resectability (between centres and surgeons), the use of chemotherapy, and the factors analysed in multivariable analysis. Despite this, the effect of *KRAS* on survival was consistent in almost all of the included studies, and no studies were identified reporting a favourable outcome in patients harbouring *KRAS* mutations. Such findings would have been published by now, and the funnel plot analyses of the included studies did not suggest publication bias. Second, some patients with wild-type *KRAS* may have received anti-EGFR treatment at the time of recurrence after liver resection, which could explain the OS benefit in these patients. However, this concern would have applied only to OS and not to RFS, and the majority of the study intervals predated the approval of anti-EGFR treatment for colorectal cancer. Third, a recent editorial[55] raised the possibility that *KRAS* mutation may be a ‘byproduct of patient selection’ that would explain the association with inferior survival. However, up to now, surgeons have determined patient resectability based on tumour and biological characteristics irrespective of *KRAS* mutation status. Furthermore, *KRAS* mutation testing may have been utilized in the patients with the most extensive disease, but this selection could not explain the OS and RFS differences between the included mutants and wild-types.

This meta-analysis indicates that *KRAS* mutation status is a prognostic factor in patients undergoing resection of CLM irrespective of chemotherapy regimen and should be considered in the evaluation of patients undergoing liver resection for CLM. In practice, the use of *KRAS* mutation status alone cannot be recommended as grounds for excluding patients from surgery, but the finding of wild-type *KRAS* may encourage the use of more aggressive treatment in patients with borderline resectable disease. *KRAS* mutation status is clearly useful, together with other clinicopathological predictors, both in the preoperative assessment of patients with CLM and at follow-up to assess the risk of recurrence and death.

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