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The challenge of molecular selection in liver-limited metastatic colorectal cancer for surgical resection: a systematic review and meta-analysis in the context of current and future approaches

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Abstract: Objectives: Treatment of metastatic colorectal cancer (mCRC) includes resection of liver metastases (LM), however, no validated biomarker identifies patients most likely to benefit from this procedure. This meta-analysis aimed to assess the impact of the most relevant molecular alterations in cancer-related genes of CRC (i.e., RAS, BRAF, SMAD4, PIK3CA) as prognostic markers of survival and disease recurrence in patients with mCRC surgically treated by LM resection. **Methods:** A systematic literature review was performed to identify studies reporting data regarding survival and/or recurrence in patients that underwent complete liver resection for CRC LM, stratified according to RAS, BRAF, PIK3CA, and SMAD4 mutational status. Hazard ratios (HRs) from multivariate analyses were pooled in the meta-analysis and various adjustment strategies for confounding factors were combined. The search was conducted in numerous databases, including MEDLINE (PubMed), Embase, Cumulative Index to Nursing and Allied Health Literature (CINAHL) (EBSCO host), and WHO Global Index Medicus, through March 18th, 2022. Meta-analyses, editorials, letters to the editor, case reports, studies on other primary cancers, studies with primary metastatic sites other than the liver, studies lacking specific oncological outcome variables or genetic data, non-English language studies, and studies omitting residual disease data from liver metastasectomy were excluded. The remaining 47 studies were summarized in a descriptive table which outlines the key characteristics of each study and final results were graphically presented. **Results:** RAS mutation status was negatively associated with overall survival (OS) (HR, 1.68; 95% CI, 1.54–1.84) and recurrence free survival (RFS) (HR, 1.46; 95% CI, 1.33–1.61). A negative association was also found for BRAF regarding OS (HR, 2.64; 95% CI, 2.15–3.24) and RFS (HR, 1.89; 95% CI, 1.32–2.73) and SMAD4 regarding OS (HR, 1.93; 95% CI, 1.56–2.38) and RFS (HR, 1.95; 95% CI, 1.31–2.91). For PIK3CA only three studies were eligible and no significant association with either OS or RFS could be highlighted. **Conclusion:** RAS, BRAF, and

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SMAD4 are negatively associated with OS and RFS in patients undergoing curative liver metastasectomy from colorectal cancer. No conclusion can be drawn for PIK3CA due to the limited literature availability. These data support the integration of RAS, BRAF, and SMAD4 mutational status in the surgical decision-making for colorectal liver metastasis. Nevertheless, we have to consider several limitations, the major ones being the pooling of results from studies that evaluated patient outcomes as either disease-free survival (DFS) or RFS; the inclusion of patients with minimal residual disease and unconsidered potential confounding factors, such as variability in resectability definitions, chemotherapy use, and a potential interaction between biological markers and pre- and post-resection pharmacological treatments.

Contents:

- Only a small percentage of colorectal cancer patients benefits from surgical liver metastasis resection.
- This meta-analysis was performed with the aim to elucidate the role of previously investigated molecular prognostic factors that may help the surgical decision making for colorectal liver metastasis.
- The association between the mutational status of RAS, BRAF, SMAD4 and PIK3CA and overall survival (OS) and recurrence free survival (RFS) was evaluated in the 47 studies deemed as eligible.

Introduction

Colorectal cancer (CRC) representing 9.7% of global cancer incidence, stands as the third most prevalent cancer type. Often diagnosed in advanced stages, approximately 75% of colon cancers are initially identified as local disease, amenable to surgical intervention [1].

Adjuvant chemotherapy in stage II and III tumors has shown an absolute survival benefit of up to 5% and 20%, respectively [2]. The liver is the primary site for metastases, with about 30% of CRC patients either presenting with or developing liver metastases (LM) during their disease course. The advent of novel targeted agents, a deeper understanding of molecular tumor biology, and the development of precision medicine have markedly improved the life expectancy of patients with advanced CRC, now averaging 40 months in certain subgroups [3–5]. Despite these advances, distant metastases remain the leading cause of mortality in CRC.

The most effective strategy to enhance prognosis in these cases is surgical resection of liver metastases, which can yield a 5-year overall survival (OS) rate of up to 55% when performed radically. Criteria for considering a patient for metastasectomy include the feasibility of complete metastasis removal with clear margins and sufficient residual liver volume [5]. However, only about 20% of LMs are deemed resectable at diagnosis.

Pre-operative or 'conversion' chemotherapy, employing cytotoxic agents like fluoropyrimidines with oxaliplatin and/or irinotecan, alone or in combination with targeted agents such as bevacizumab or cetuximab or panitumumab, serve varied purposes. Perioperative treatment assesses tumor response and conserves hepatic tissue for curative resection, while conversion therapy aims to render initially unresectable or borderline resectable LM surgically treatable. Advances in chemotherapy and perioperative care have broadened the patient pool for hepatic resection [6,7]. Nevertheless, post-resection disease recurrence, impacting 50%–75% of patients within five years, significantly affects survival [7].

Traditionally, clinical criteria like Fong's clinical score and various nomograms have been used to predict outcomes post-LM resection. These consider factors like tumor size, lymph node invasion, number of metastases, and patient demographics [8]. Recently, molecular data, especially with pre-operative targeted therapy based on tumor mutational status, are being increasingly considered for 'precision surgery' in CRC LM [9].

Over the past decade, biomarkers such as *KRAS, NRAS* and *BRAF* have become crucial for the management of metastatic CRC. Notably, about 40% of patients diagnosed with metastatic CRC harbor mutations in exon 2 of *KRAS* codons 12 and 13 [10,11]. Additionally, approximately 5% have mutations in exons 3 or 4 of *KRAS* codons 61 or 146, and another 5% exhibit mutations in exons 2, 3, or 4 of *NRAS* [10,11]. Significantly, mutations affecting *BRAF* codon 600 are found in 10% of patients, with the V600E mutation specifically identified as a marker of poor prognosis [12,13].

The presence of RAS mutations, particularly in the metastatic context, is a well-established negative predictive biomarker for the efficacy of anti-EGFR drug [14,15]. Similarly, *BRAF* mutation, especially the V600E variant, has been shown to diminish the efficacy of anti-EGFR agents [16]. Moreover, both *BRAF* and *RAS* mutations are associated with a poorer prognosis in advanced disease [17,18].

Emerging evidence underscores the importance of pre-treatment identification of patients who are likely to benefit from liver metastasectomy. Studies have linked *KRAS* and *NRAS* mutations with reduced OS and recurrence-free survival (RFS) following hepatic metastasis resection. *BRAF* mutations, which occur in about 3% of patients with resected LM, demonstrate a similar impact. Additionally, somatic mutations in other relevant cancer genes, such as *TP53* and *SMADs*, have been explored, either individually or in combination, for their potential to stratify patients' prognoses post-LM resection in CRC [7,19,20].

The aims of this review are to: 1) systematically analyze current literature and perform a meta-analysis of the available data on the impact of key genetic variants in CRC-related genes (i.e., *KRAS, NRAS, BRAF, SMAD4, PIK3CA*) as prognostic markers in surgically treated metastatic CRC patients; and 2) explore the potential role of emerging biomarkers like HER2 and microsatellite instability (MSI) status in current and novel therapeutic strategies, including liver transplantation for LM.

Material and Methods

Search strategy

A systematic review was conducted by searching databases including MEDLINE (Pubmed), Embase, Cumulative Index to Nursing and Allied Health Literature (CINAHL) (EBSCO host), and WHO Global Index Medicus, through March 18th, 2022. The search algorithm used was: "('BRAF' OR 'RAS' OR 'HER2' 'SMAD4' OR 'TP53' OR 'P53' OR 'APC' OR 'PI3K' OR 'EGFR' OR 'MACC1') and ('colon' or 'colorectal' or 'rectal' or 'rectum') and ('metastasis' or 'metastatic' or 'metastases' or 'mets' or 'metastasectomy') and ('hepatic' or 'liver')". Titles and abstracts of identified records were screened for relevance. Since MEDLINE included all the articles returned by the other two databases, we referred only to MEDLINE in Fig. 1.

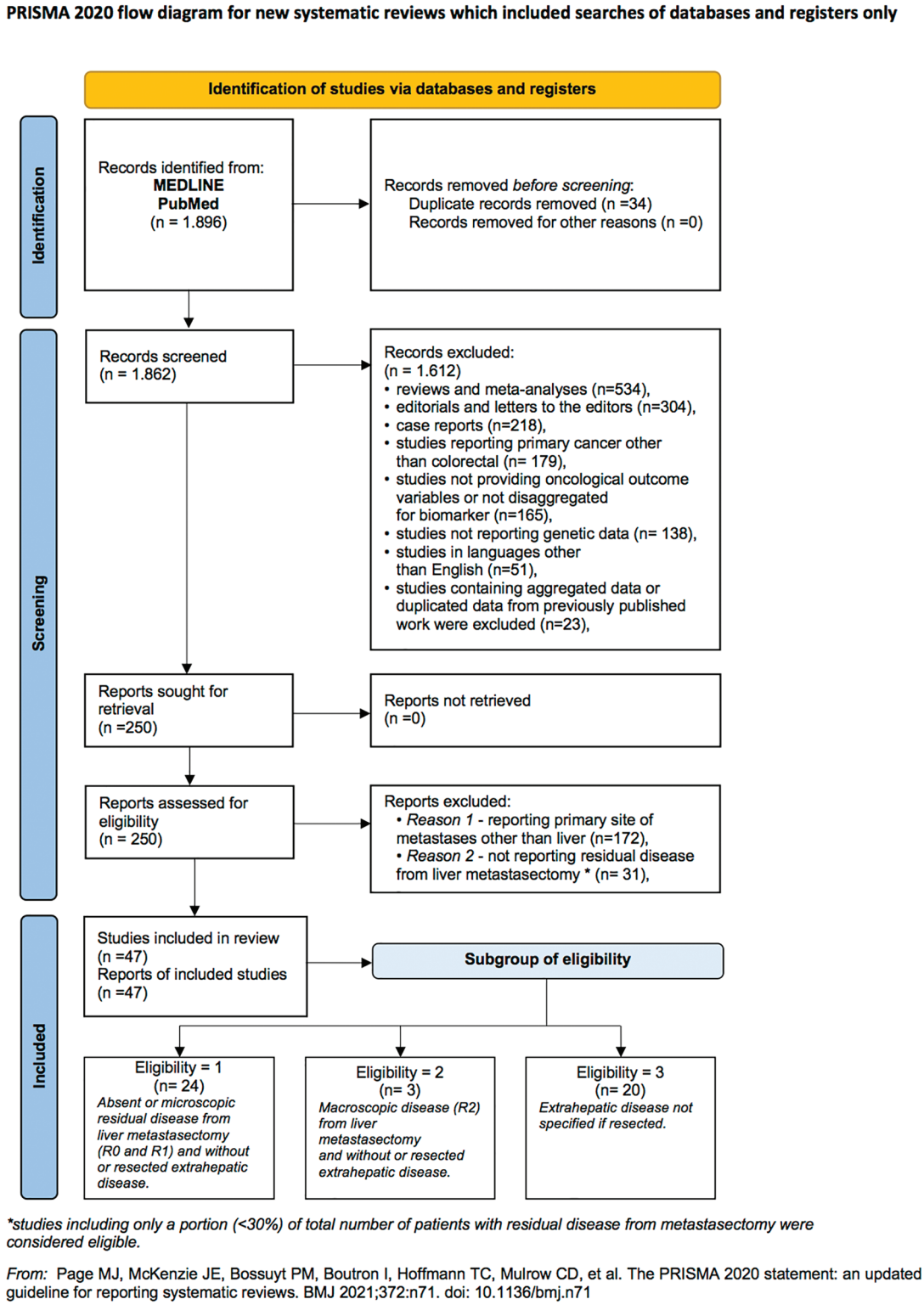

FIGURE 1. Article screening and selection according to preferred reporting items for systematic reviews and meta-analyses 2020 criteria flow diagram.

Selection criteria

Eligible studies included those detailing tumor mutation status and oncological outcomes in CRC patients who underwent liver metastasectomy. The inclusion criteria comprised studies performed in metastatic CRC patients undergoing liver metastases resection; reporting genetic test results for *RAS*, *BRAF*, *SMAD4*, or *PIK3CA* mutation status from resected colorectal liver metastasis (CRLM) specimens; providing outcomes of RFS and/or OS based on mutational status; and offering RFS and OS hazard ratios (HR) from multivariate analyses.

Exclusion criteria included reviews, meta-analyses, editorials, letters to the editor, case reports, studies on primary cancers other than colorectal, studies with primary metastatic sites other than liver, studies lacking specific oncological outcome variables or genetic data, non-English language studies, and studies omitting residual disease data from liver metastasectomy.

Three subgroups of eligibility were identified: "group 1" included studies with no or microscopic post-metastasectomy residual disease (R0 and R1) and without or resected extrahepatic disease; "group 2" encompassed studies with macroscopic post-metastasectomy residual disease (R2) and without or resected extrahepatic disease; "group 3" comprised studies where the status of extrahepatic disease was unspecified (either resected or not).

Data extraction and statistical analysis

A predefined protocol was employed for data extraction. Extracted variables included: first author's name, publication year, journal name, enrollment years, country of enrollment, sample size, study design, demographic profile, genes analyzed, genotyping method, tumor mutational status, chemotherapy scheme and schedule, percentage of patients with residual disease post-metastasectomy, percentage with extra-hepatic disease and oncological outcomes (OS, overall response rate (ORR), disease-free-survival (DFS), or RFS) + including main findings and 95% confidence interval (CIs). Four authors (MF, FDG, CLP, EC) independently verified inclusion criteria and executed data extraction. Adjusted estimates from original studies for relevant confounding factors were utilized.

The standard error of the log HR was deduced from the log CIs. Pooled HR and corresponding 95% CI were calculated using the DerSimonian and Laird random-effects models, accounting for within-study and between-study variabilities. In cases of a low number of studies, pooled HR was also estimated via the Hartung-Knapp-Sidik-Jonkman (HKSJ) method for a conservative approach. Statistical heterogeneity among studies was assessed using the I^2 and Q statistics. Influence analysis was conducted, recalculating pooled HR by sequentially omitting each study. Publication bias was evaluated through funnel plot.

Meta-analysis results were graphically presented, depicting HRs as black squares (size inversely proportional to standard error) and 95% CIs. In some studies, 95% CIs were slightly modified from those in original papers due to estimated study variances. Pooled HRs for all studies and various study designs were represented by diamonds, indicating the HR at the center and 95% CIs at the extremes. Statistical significance was set at $p < 0.05$ (two-sided).

Results

We included 47 studies that evaluated the predictive value of the somatic genetic profile for outcomes following liver metastasectomy in CRC patients (Fig. 1) [8,20,21–65]. Our analysis was limited to genetic testing data for *RAS* or *BRAF* or *SMAD4* or *PI3KA* mutations due to insufficient data for other biomarkers. Insufficient study numbers precluded meta-analysis for HER2 and MSI status; however, we described the main evidence available. A descriptive table outlines the key characteristics of studies included in the meta-analysis (Table 1).

Effect of RAS mutation on OS and RFS

Out of 47 studies eligible for the meta-analysis, 43 reported on the association between *RAS* mutation status and patients' OS and RFS and included a total of 24,121 patients, of whom 8,258 had *RAS* mutations. The pooled *KRAS* mutation rate was 34.2%, which is consistent with previous reports in the literature of resected CRC LM. Specifically, 36 studies reported the association with OS and 20 studies with RFS.

The meta-analysis of OS included 36 studies with 15,766 patients, of whom 5,361 had RAS mutations.

The cumulative HR for OS was 1.68 (95% CI, 1.54–1.84) (Fig. 2A), indicating that *RAS* mutation is an independent prognostic factor associated with poorer OS in patients with metastatic CRC undergoing liver resection.

Regarding RFS, 20 studies involving 8,355 patients were analyzed, with 2,897 patients having RAS mutations. The cumulative HR was 1.46 (95% CI, 1.33–1.61) (Fig. 2B) demonstrating that *RAS* mutations are associated with a higher risk of relapse and a negative impact on prognosis compared to patients with wild-type genes.

An analysis of the forest plot illustrating the effect of *RAS* mutations on RFS in Fig. 2B reveals a notable increase in result homogeneity across different studies beginning in 2019. This uniformity is likely attributable to standardized methodologies in patient enrollment and evaluation across studies, enhancing the reliability of meta-analytic findings.

Of the 36 studies included in the meta-analysis on OS, 21 specifically investigated the association between *KRAS* mutation alone and patient OS. The cumulative HR for these studies was 1.63 (95% CI, 1.42–1.86) (Fig. 3A). In contrast, the remaining 15 studies that examined the association between all-*RAS* mutations and patients' OS, reported a cumulative HR of 1.74 (95% CI, 1.56–1.94) (Fig. 3A). The pooled analysis indicates that the impact on OS is similar whether studies considered only *KRAS* mutations or all *RAS* mutations.

For RFS, the findings were similarly consistent. Twelve studies that focused on KRAS mutations alone reported a cumulative HR of 1.36 (95% CI, 1.24–1.49), while eight studies that evaluated the association between all-RAS mutations and RFS presented a cumulative HR of 1.54 (95% CI, 1.30–1.82) (Fig. 3B). This comparison suggests that the prognostic impact of *KRAS* mutations is comparable to that of all-*RAS* mutations on RFS.

TABLE 1

Medical treatment administered in the studies included in the meta-analysis

First author	Year	Region	No. of patients	*KRAS*	*BRAF*	*PIK3CA*	*SMAD4*	Endpoint	CT before resection only	CT After resection only	CT before and after resection	Regimen
Kawaguchi et al. [32]	2021a	USA	561	*RAS (KRAS/NRAS)*	*BRAF*	*PIK3CA*	*SMAD4*	OS	90,3%	NA	NA	NA
Kawaguchi et al. [33]	2021b	USA	790	*RAS (KRAS/NRAS)*				OS	88,2%	NA	NA	NA
Hoppener et al. [29]	2021	Netherlands and USA	780	*KRAS*	*BRAF*			OS, RFS	0,0%	0,0%	0,0%	NA
Liu et al. [40]	2021	China	769	*RAS (KRAS/NRAS)*				RFS	61,0%	79,2%	NA	5-FU with OXA/IRI with or without anti-EGFR or anti-VEGF
Nishioka et al. [47]	2021	USA	552		*BRAF*		*SMAD4*	RFS	87,0%	74,0%	NA	5-FU with OXA/IRI with or without anti-EGFR or anti-VEGF
Takeda et al. [62]	2021a	Japan	409	*KRAS*				OS	44,5%	0,0%	0,0%	NA
Takeda et al. [63]	2021b	Japan	341	*RAS (KRAS/NRAS)*				OS	52,0%	56,0%	ND	NA
Margonis et al. [45]	2021	USA (Baltimore)	718	*KRAS*				OS	65,1%	57,0%	NA	NA
Kawaguchi et al. [35]	2020a	USA	476	*RAS (KRAS/NRAS)*	*BRAF*		*SMAD4*	OS, RFS	90.5%	NA	NA	NA
Kawaguchi et al. [34]	2020b	USA	416	*KRAS*				RFS	76,0%	NA	NA	NA
Baldin et al. [24]	2020	Belgium	221	*RAS (KRAS/NRAS)*				OS, RFS	84,6%	NA	NA	5-FU with OXA/IRI with or without anti-EGFR or anti-VEGF
Brunsell et al. [27]	2020	Norway	106			*PIK3CA*		OS	82,0%	NA	NA	5-FU with OXA and/or IRI with anti-EGFR and/or anti-VEGF
Gholami et al. [28]	2020	USA	487	*RAS (KRAS/NRAS)*				OS, RFS	42,4%	100,0%	42,4%	Adjuvant HAI pump with 5-FU (56%) and/or systemic 5-FU
Jàcome et al. [31]	2020	USA (Texas)	573	*RAS (KRAS/NRAS)*	*BRAF*			OS	87,0%	69,0%	NA	NA (oxaliplatin-based regimen plus or minus BEVA)
Saadat et al. [51]	2020	USA	938	*RAS (KRAS/NRAS)*				OS, RFS	64,1%	20,4%	NA	NA

(Continued)

Table 1 (continued)

First author	Year	Region	No. of patients	*KRAS*	*BRAF*	*PIK3CA*	*SMAD4*	Endpoint	CT before resection only	CT After resection only	CT before and after resection	Regimen
Allievi et al. [21]	2019	Italy and USA	806	*KRAS*				OS	NA	NA	NA	NA
Bachet et al. [23]	2019	France	249		*BRAF*			OS, RFS	82,7%	74,7%	NA	5-FU with OXA and targeted agent
Brudvik et al. [25]	2019	USA (Texas)	564	*RAS (KRAS/ NRAS)*				OS	87,2%	NA	NA	5-FU with OXA and/or IRI with BEVA or anti-EGFR
Kawaguchi et al. [34]	2019	USA	507				*SMAD4*	OS, RFS	89,7%	NA	NA	NA
Lang et al. [37]	2019	Germany	822	*KRAS*	*BRAF*	*PIK3CA*	*SMAD3, SMAD2, SMAD4*	OS	51,8%	NA	NA	NA
Liu et al. [8]	2019	China	564	*RAS (KRAS/ NRAS)*				RFS	100,0%	NA	NA	5-FU with OXA/IRI with or without anti-EGFR or anti-VEGF
Margonis et al. [41]	2019	USA and Europe	1099	*KRAS*	*BRAF*			OS	70,0%	62,6%	NA	NA
O'Connor et al. [48]	2019	Argentina	662	*KRAS*				OS, RFS	28,3%	45,2%	NA	5-FU with OXA/IRI with or without anti-EGFR or anti-VEGF
Ruzzenente et al. [50]	2019	USA and Italy	784	*RAS (KRAS/ NRAS)*	*BRAF*			OS	100,0%	48,9%	NA	5-FU with OXA/IRI with or without anti-EGFR or anti-VEGF
Lin et al. [39]	2018	China	139	*KRAS*	*BRAF*			OS, RFS	23,0%	100,0%	23,0%	NA
Margonis et al. [42]	2018	USA and Europe	849	*KRAS*	*BRAF*			OS, RFS	67,3%	50,7%	NA	NA
Mizuno et al. [57]	2018	USA	515	*RAS (KRAS/ NRAS)*			*SMAD4*	OS	91,3%	73,0%	NA	5-FU with OXA/IRI with or without anti-EGFR or anti-VEGF
Amikura et al. [22]	2017	Japan	342	*RAS (KRAS/ NRAS)*				OS	NA	NA	NA	NA
Serenari et al. [54]	2017	Argentina	26	*KRAS*				OS	96,1%	69,2%	NA	NA
Wang et al. [64]	2017	China	300	*KRAS*				OS	100,0%	100,0%	100,0%	NA
Margonis et al. [46]	2016a	USA (Baltimore)	430	*KRAS*				OS	60,0%	67,8%	43,9%	5-FU with or without OXA/IRI with or without anti-EGFR or anti-VEGF

(Continued)

Table 1 (continued)

First author	Year	Region	No. of patients	*KRAS*	*BRAF*	*PIK3CA*	*SMAD4*	Endpoint	CT before resection only	CT After resection only	CT before and after resection	Regimen
Margonis et al. [44]	2016b	USA (Baltimore)	512	*KRAS*				RFS	59,3%	69,4%	NA	NA
Brudvik et al. [26]	2016	USA	633	*RAS (KRAS/ NRAS)*				OS	86,1%	NA	NA	5-FU with OXA/IRI with BEVA
Sasaki et al. [52]	2016	USA	485	*KRAS*				OS	79,0%	NA	NA	5-FU with OXA/IRI with anti-VEGF or anti-EGFR
Shindoh et al. [55]	2016	Japan	163	*KRAS*				OS, RFS	49,3%	62,2%	NA	NA
Margonis et al. [43]	2015	USA (Baltimore)	334	*KRAS*				OS, RFS	67.7%	62,9%	NA	NA
Zimmitti et al. [60]	2015	USA	184	*RAS (KRAS/ NRAS)*				OS	100,0%	NA	NA	5-FU with OXA/IRI with anti-VEGF
Schirripa et al. [53]	2015	Italy	309	*RAS (KRAS/ NRAS)*	*BRAF*			OS, RFS	50,0%	21,0%	36,0%	5-FU with OXA/IRI with anti-VEGF or anti-EGFR
Kemeny et al. [36]	2014	USA (New York)	169	*KRAS*				OS, RFS	84,0%	100,0%	NA	5-FU with or without OXA/IRI with HAI
Lin et al. [38]	2014	China	154	*KRAS*	*BRAF*			OS, RFS	24,7%	100,0%	24,7%	NA
Shoji et al. [61]	2014	Japan	108	*KRAS*	*BRAF*	*PIK3CA*		RFS	NA	NA	NA	NA
Isella et al. [30]	2013	Italy	64	*KRAS*		*PIK3CA*		RFS	56,3%	67,2%	20,3%	5-FU with or without OXA/IRI with or without anti-VEGF
Karagkounis et al. [20]	2013	USA (Maryland)	202	*KRAS*				OS, RFS	81,0%	65,0%	NA	NA
Vauthey et al. [59]	2013	USA	193	*RAS (KRAS/ NRAS)*		*PIK3CA*		OS, RFS	100,0%	100,0%	100,0%	5-FU with OXA/IRI and anti-VEGF
Stremitzer et al. [56]	2012	Austria	60	*KRAS*				OS	100,0%	100,0%	100,0%	5-FU with OXA and anti-VEGF
Teng et al. [58]	2012	Japan	292	*KRAS*	*BRAF*			OS	22,6%	83,9%	11,3%	5-FU with or without OXA/IRI with or without anti-EGFR
Petrowsky et al. [49]	2001	Germany	41	*KRAS*				OS	NA	NA	NA	5-FU

Note: Abbreviations: BEVA, bevacizumab; CT, chemotherapy; EGFR, epidermal growth factor receptor; 5-FU, 5-fluorouracil; HAI, hepatic arterial infusion; IRI, irinotecan; NA, not available; OS, overall survival; OXA, oxaliplatin, RFS, recurrence-free survival; VEGF, vascular endothelial growth factor.

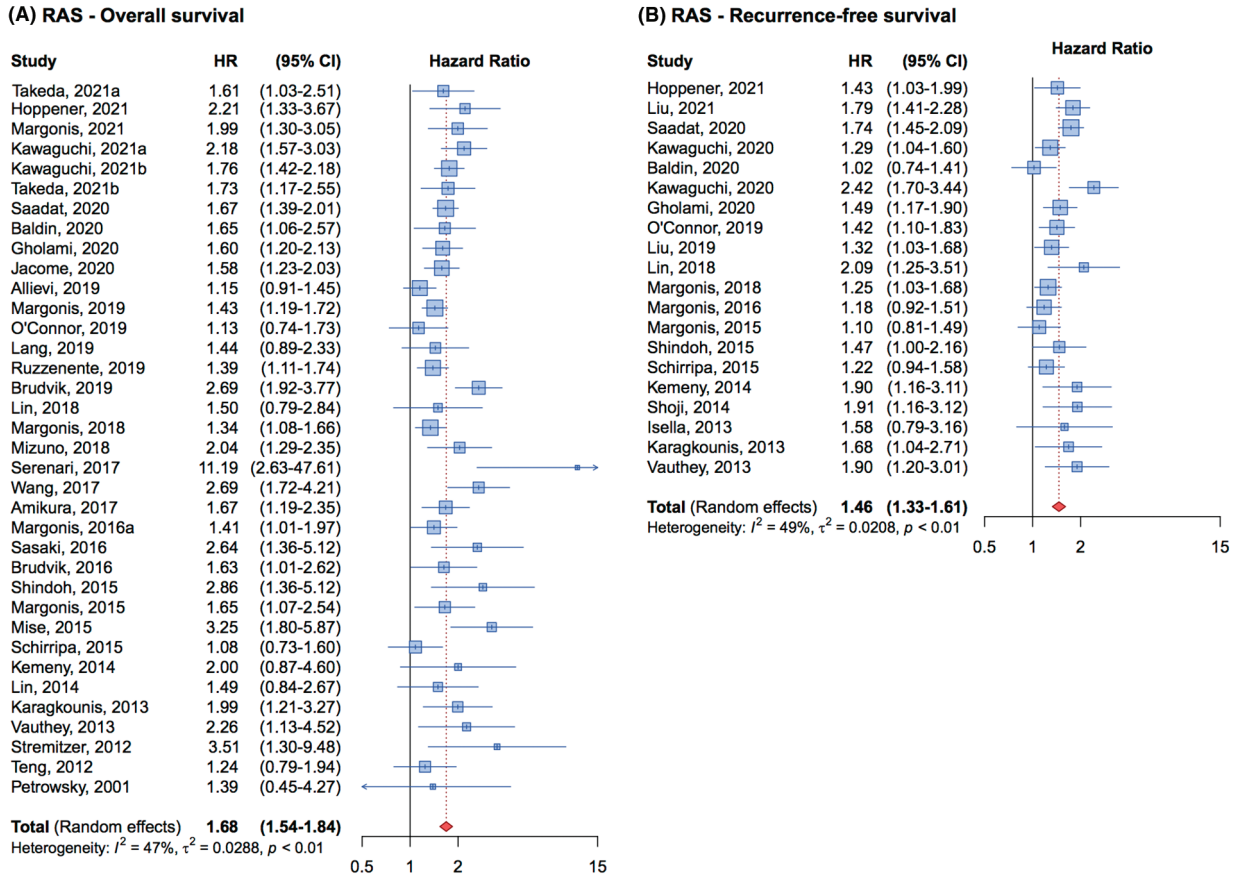

FIGURE 2. Forrest plot of association between RAS mutation status and overall survival (OS) (A) and recurrence free survival (RFS) (B).

Effect of BRAF mutation on OS and RFS

Among the 47 studies included in our meta-analysis, 13 reported on OS after CRC LM resection, stratified by *BRAF* mutation status, while 8 studies reported on RFS. The studies included data on 3- or 5-years OS or both, and RFS; 3- or 5-years DFS data were also considered as part of RFS. A total of 8,969 patients were analyzed for *BRAF*-related OS and RFS outcomes, with 433 patients harboring BRAF mutations. The pooled *BRAF* mutation rate was 4.83%, consistent with previous literature on resected CRC LM.

For the OS, the analysis of 13 studies included 5,831 patients, 270 of whom had *BRAF* mutations. The cumulative HR was 2.62 (95% CI, 2.14–3.20) (Fig. 4A), indicating that the *BRAF* mutation is an independent prognostic factor negatively associated with OS in metastatic CRC patients undergoing complete liver resection.

In terms of RFS, 8 studies with 3,138 patients were analyzed, 163 of whom had BRAF mutations. The cumulative HR was 1.89 (95% CI, 1.32–2.73) (Fig. 4B) demonstrating that *BRAF* mutations are associated with a worse prognosis and a higher risk of relapse compared to wild-type patients.

Only 6 studies provided data on OS and/or RFS for CRC LM patients with *SMAD4* mutations. These studies, which focused on *SMAD4* 3-year OS or RFS or DFS, included a total of 3,020 patients in the analysis for both *SMAD4*-related OS and RFS, with 347 harboring a *SMAD4* mutation. Of these, 222 patients were analyzed for OS, and 125 for RFS. The cumulative HR for OS was 1.93 (95% CI, 1.56–2.38) (Fig. 5A), and for RFS was 1.95 (95% CI, 1.31–2.91) (Fig. 5B), confirming *SMAD4* mutations as a detrimental prognostic factor in CRC patients undergoing liver metastasectomy.

Effect of PIK3CA mutation on OS and RFS

For *PIK3CA* mutations, only 3 studies were deemed eligible for the evaluation of OS and RFS: two addressing RFS and one addressing OS. No significant association with either OS or RFS was found.

Effect of RAS, BRAF, SMAD4 mutation on OS and RFS according to subgroup of eligibility

No significant differences were observed in the analyses of OS and RFS for studies evaluating *RAS*, *BRAF*, and *SMAD4* mutations, even when data were subdivided according to the subgroup of eligibility (Suppl. Figs. 1–3).

Other exploratory biomarkers in CRC patients treated with liver metastasectomy: MSI/MMR

Microsatellites, repeated sequences of 1 to 6 base pairs in length composed mostly of non-coding DNA [66,67], are primarily located at chromosome ends and contribute to the individual genetic fingerprint [68].

Microsatellite instability (MSI) results from accumulated errors at microsatellite sites due to a deficient DNA mismatch repair (dMMR) system. This deficiency may arise sporadically, such as through CpG island methylator phenotype (CIMP) due to oxidative stress in *MLH1*

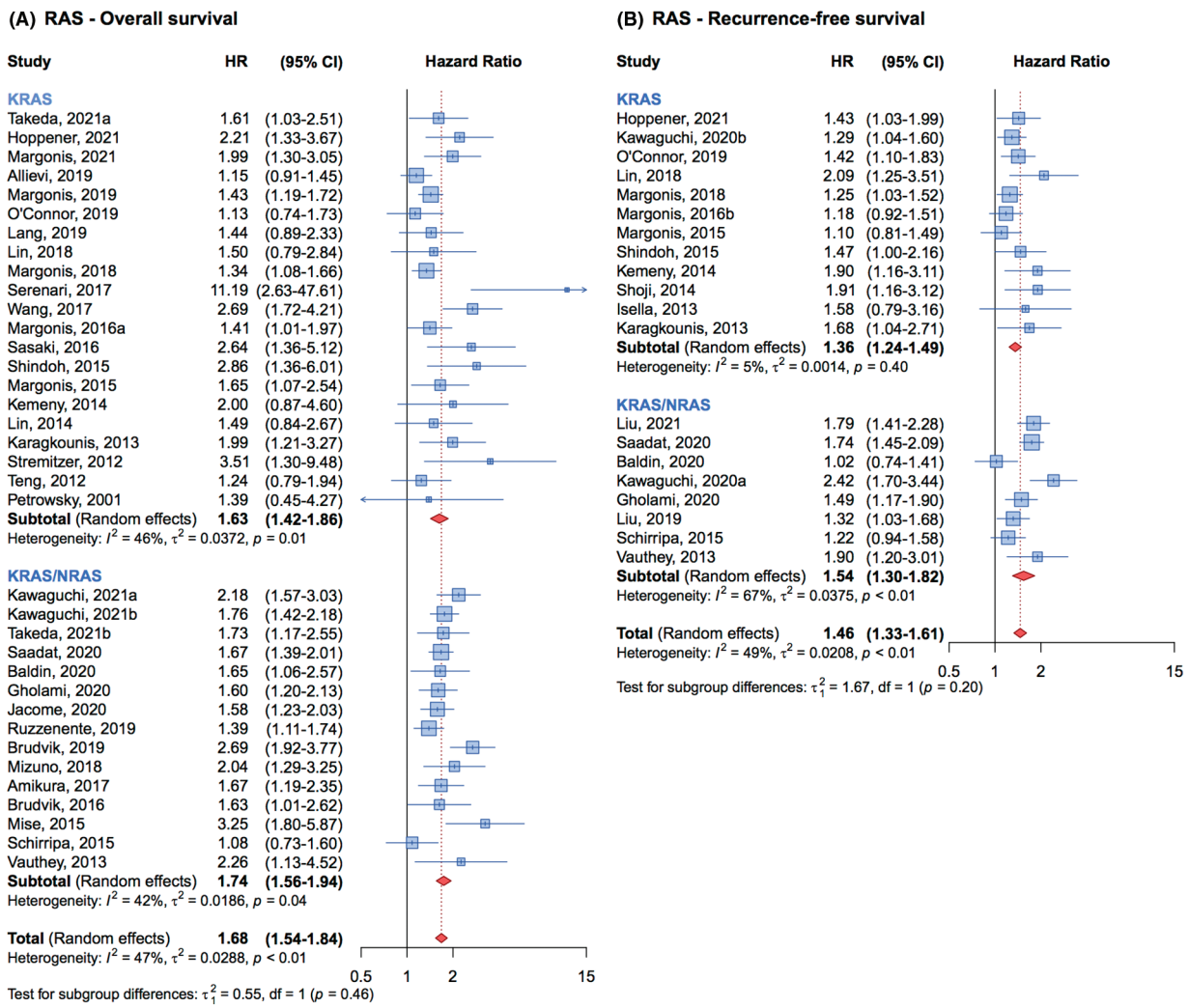

FIGURE 3. Forrest plot of association between KRAS mutation status *vs.* all-RAS mutation status and overall survival (OS) (A) and recurrence free survival (RFS) (B).

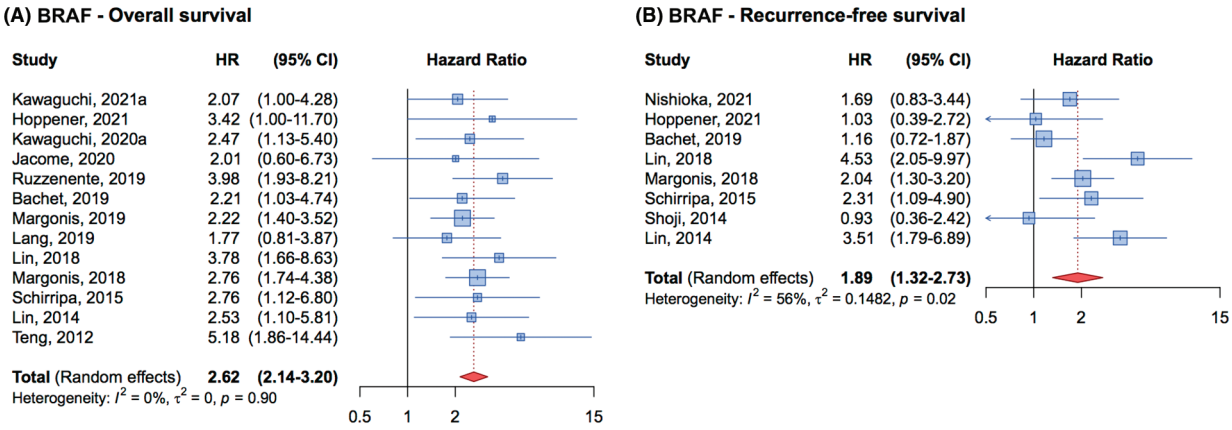

FIGURE 4. Forrest plot of association between BRAF mutation status and overall survival (OS) (A) and recurrence free survival (RFS) (B).

promoter hypermethylation, or through inherited conditions like Lynch syndrome or Muir-Torre syndrome [66,69–73]. The MMR system, involving four main genes and their encoded proteins, plays a critical role in DNA replication accuracy by correcting erroneous insertions, deletions, or mis-incorporated bases, thus preventing mismatches between DNA strands [71–74]. Abnormal MMR function leads to frameshift mutations, contributing to a high tumor mutational burden (TMB) and an increase in tumor infiltrating lymphocytes (TILs) [71,74–76].

These factors are pivotal for the effectiveness of immune checkpoint inhibitors in treating unresectable or metastatic tumors with such pathogenesis [77–83]. The meticulous characterization of immune infiltration has become essential

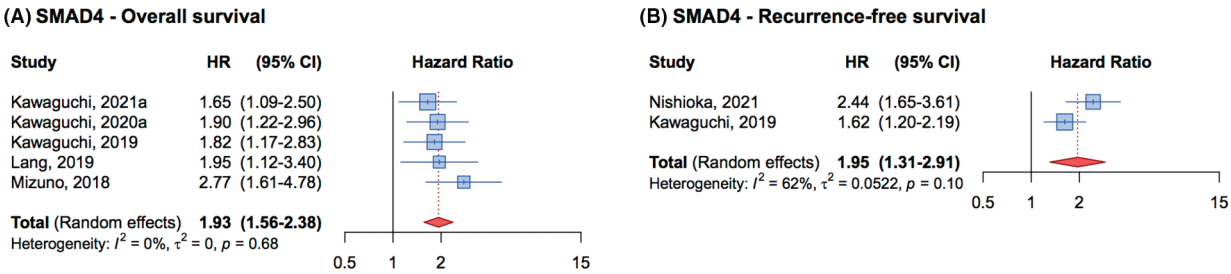

FIGURE 5. Forrest plot of association between SMAD4 mutation status and overall survival (OS) (A) and recurrence free survival (RFS) (B).

for the development of the Immunoscore, a prognostic tool that enhances the TNM classification for high microsatellite instability (MSI-H) CRC [84–87]. Ongoing trials aim to refine survival predictions by introducing new biomarkers [84,88,89].

MSI is associated with various neoplasms, particularly CRC, where it is found in up to 15% of cases, but only 5% in metastatic settings [71,90–93].

Sporadic MSI-H tumors, often identified in older women and associated with the right colon, exhibit distinct features such as poorly differentiated mucinous histology and pronounced lymphocytic infiltration, and a Crohn's-like host response [66,90,94]. While MSI is a positive prognostic factor in early-stage CRC, its benefit does not extend to metastatic disease, where outcomes are similar to microsatellite stable (MSS) tumors [91,95–99] and dMMR. Recent studies have highlighted the complex role of MSI in predicting intrahepatic recurrences post-liver resection [100], underscoring the need for further research into its prognostic significance alongside other genes. This is in contrast with a previous paper by Haddad et al., which challenged the prognostic significance of MSI, suggesting that the survival of patients with stage IV MSI-positive tumor was not influenced by the surgical resectability of liver metastases [99]. This divergence highlights the complex nature of MSI's role in CRC and underscores the necessity for more in-depth analysis. Specifically, the relationship between MSI and other commonly examined genes warrants further investigation to clarify its prognostic implications in the context of CRC liver metastases [91,101–103].

Liver transplantation

The prognostic and predictive value of somatic genetic mutations is under investigation for selecting CRC patients with liver-only metastases for liver transplantation as a curative strategy. Despite the survival benefits observed with surgical treatment, only a minority qualify for resection, and recurrence remains a significant concern. The concept of completely removing the affected liver and performing transplantation emerged in the early eighties but faced challenges due to the high perioperative mortality rate and a significant disease recurrence rate within the first year. However, advancements in the surgical management of CRC, alongside the introduction of fluoropyrimidines-based combination chemotherapy with irinotecan and oxaliplatin (FOLFOX and FOLFIRI achieving response rates around 50%, and the triplet regimen FOLFOXIRI reaching up to 60%) as well as the advent of targeted therapies, have reopened the consideration of liver transplantation as a viable treatment option for patients with metastatic CRC [104].

A landmark in this evolving field was the Secondary Cancer (SECA) I trial, reported in 2013. This trial presented significant survival benefits in a small cohort of stage IV CRC patients with liver-only metastases, documenting 1-, 3-, and 5-year OS rates 95%, 68%, and 60%, respectively, for patients with unresectable liver-only metastases undergoing liver transplantation. The promising results have catalyzed a series of prospective studies aimed at evaluating the feasibility and clinical impact of liver transplantation in patients with surgically inoperable LM from CRC [105].

Particular emphasis has been placed on the molecular criteria for selecting patients for these studies, with the goal of excluding those whose tumors possess characteristics indicating a poorer prognosis. As a result, most ongoing trials require patients to have tumors that are wild-type for both *RAS* and *BRAF* genes.

The results of the Italian COLT trial were recently published [106]. It demonstrated that liver transplantation in metastatic CRC, coupled with improved patients selection strategies including tumor molecular characterization for RAS/RAF can give patients a 5-year OS similar to other indications for liver transplantation and a better outcome than those undergoing chemotherapy alone [106].

Discussion

Significant advancements in survival rates for patients with metastatic CRC through the introduction of new chemotherapy combination regimens and targeted agents such as cetuximab and bevacizumab, coupled with an enhanced molecular understanding of cancer biology. Nonetheless, surgical intervention remains the sole potentially curative option for patients with oligometastases confined to a single organ, such as the liver. Surgical and oncological criteria are employed to properly select patients for whom the liver metastasectomy is feasible and who are likely to derive a sustained benefit from the surgical procedure. The evolving knowledge of CRC's molecular biology and liver metastases has unveiled crucial somatic alterations in specific genes, specifically affecting patients' prognosis and their tumors' response to pharmacological treatments. While these findings are integral to therapy selection, their role in determining suitability for liver metastasectomy continues to be explored.

Our metanalysis reaffirmed that mutations in *RAS*, particularly *BRAF*, serve as independent prognostic factors,

diminishing OS and RFS in patients with CRCLM who undergo resection.

These findings align with previous meta-analyses that advocate for incorporating molecular profiling into patient selection for liver surgery [19,107–109].

In evaluating *RAS* mutations, we also considered the impact of testing solely for *KRAS* mutations versus. assessing the entire *RAS* gene family. Our analysis indicated that patients with any *RAS* mutation exhibited similar OS and RFS outcomes to those with only *KRAS* mutations.

To our knowledge, this meta-analysis is the most up-to-date and comprehensive, extending beyond *RAS* and *BRAF* mutations to include additional biomarkers. We also examined the role of *SMAD4* and *PIK3CA*, two significant factors in CRC carcinogenesis. Although only a few studies addressed this topic, three recent ones highlighted the adverse impact of *SMAD4* mutations on OS following liver metastasectomy. Conversely, data on the effect of *SMAD4* on RFS were unavailable. Regarding *PIK3CA* mutations, only two studies were found, yielding inconclusive results on their prognostic significance after liver metastasectomy.

Our meta-analysis underscores no significant differences in OS and RFS across subgroups of eligibility, supporting our overall findings. However, several limitations are noted: 1) the pooling of results from studies assessing patient outcomes as either DFS or RFS, despite their statistical differences; 2) the inclusion of patients with minimal residual disease, where positive resection margins significantly impact prognosis; 3) potential confounding factors not considered, such as variability in resectability definitions, chemotherapy use, and other variables in multivariable analyses; 4) a lack of specificity in some studies regarding the *BRAF* mutations examined; 5) an unassessed potential interaction between biological markers and pre- and post-resection pharmacological treatments due to treatment heterogeneity. For patients ineligible for primary hepatic metastasectomy due to extensive liver involvement, liver transplantation is being considered in ongoing clinical trials. Here, the tumor's underlying biology, especially *RAF/RAS* status, is crucial for patient selection in experimental liver transplantation protocols. Additionally, other tumor molecular characteristics, such as MMR proficiency status, which has already shown predictive and prognostic value in CRC, could be evaluated as prognostic markers for patients undergoing liver resection. These could eventually serve as additional criteria for patient selection, potentially enhancing the clinical management of metastatic CRC patients.

Conclusions

Mutational status of *BRAF*, *RAS*, and *SMAD4* in tumor tissue from mCRC patients candidate for liver metastasectomy should be considered to select those patients with a higher chance of benefiting from the surgical treatment. This could spare useless procedures and complications in patients with high disease recurrence chances. Tumor mutational status could also inform clinicians of the potential benefit of a liver transplantation procedure. The effect of *PIK3CA* mutations on the outcome of hepatic metastasectomy is still controversial and should be better investigated in future studies.

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