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Prognostic Role of *BRAF* Mutations in Colorectal Cancer Liver Metastases

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Abstract. *Background/Aims: The impact of tumor biology on prognosis in patients with colorectal liver metastasis (CRLM) has been the topic of intense research. Specifically, the presence of BRAF mutations has been recently associated with adverse long-term outcomes. We examined the existing literature on the prognostic implications of BRAF mutations in patients with CRLM. Materials and Methods: A structured review of the literature was performed between 5/1/2016 and 6/1/2016 using the PubMed database. Original research articles published between 1/1/2010 and 4/01/2016 were considered eligible. The primary end-points were overall survival (OS)/disease-specific survival (DSS) and recurrence-free survival (RFS) among patients with BRAF mutated CRLM who underwent resection. Results: Eight studies were included. All studies reported on OS/DSS, while 6 reported on RFS. BRAF mutant status was a strong independent predictor of both worse OS/DSS and RFS in 7 and 4 studies, respectively. Conclusion: BRAF-mutant lesions are consistently associated with poor prognosis. Consequently, the indications of CRLM resection in this patient group should be reconsidered.*

Colorectal cancer (CRC) is simultaneously the third most common cancer and third most common cause of cancer-related death worldwide (1). Disseminated disease is not uncommon in such patients and serves as a main driver of morbidity and mortality. In fact, two recent population-based studies from the Netherlands suggest that up to 20% of CRC patients harbor metastatic disease at presentation, with an additional 20% developing metachronous lesions at a later time, predominantly in the liver (2). Although the therapeutic landscape in the management of colorectal liver metastasis (CRLM) has undergone dramatic shifts in recent years with expansion of operative indications, substantial technical advances and introduction of novel chemotherapeutic regimens, 5-year overall survival (OS) still does not exceed 62.25% under the best of circumstances (3-5).

As such, the development of novel targeted therapies and the optimal allocation of currently available treatments remain important areas of research in CRLM. Molecular biology is of paramount importance in addressing these questions as an intimate understanding of aberrant genetic expression may hold the key to anticipating, rather than merely reacting to, neoplastic behavior. In particular, the RAS-ERK (rat sarcoma and extracellular signal regulated kinases, respectively) signaling pathway has attracted significant scientific attention for its role in carcinogenesis, especially with respect to colorectal cancer (6). One of its components, the RAF (rapidly accelerated fibrosarcoma) family of kinases is central to the signaling pathway derangements observed in many cancers, including those of the colon and rectum, a role currently thought to be mediated by its more active, as well as more extensively studied member, BRAF (7).

BRAF is a serine/threonine protein kinase downstream of RAS in the RAS-ERK kinase pathway, which mediates cellular response to growth signals (7). Dimerization after interaction with RAS is central to BRAF catalytic activation and function

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Table I. *Individual study characteristics.*

Reference	Study type	Year	Study origin	Study interval	Number of patients
Teng *et al.*	Retrospective cohort	2012	Taipei Veterans General Hospital, Taipei, Taiwan	2000-2010	292
Umeda *et al.*	Retrospective cohort	2012	Okayama University Hospital, Okayama, Japan	1997-2009	100
Huang *et al.*	Retrospective cohort	2013	Taipei Veterans General Hospital, Taipei, Taiwan	2000-2010	228
Karagkounis *et al.*	Retrospective cohort	2013	Johns Hopkins Hospital, Baltimore, MD, USA	2003-2008	202
Yaeger *et al.*	Retrospective cohort	2014	Memorial Sloan- Kettering Cancer Center, New York, NY, USA	2009-2012	515
Lin *et al.*	Retrospective cohort	2014	Zhongshan Hospital, Fudan University, Shanghai, China	2003-2013	154
Schirripa *et al.*	Retrospective cohort	2015	3 Italian Oncology Units (Pisa, Padova, Udine), Italy	1995-2012	309
Loes *et al.*	Retrospective cohort	2016	Haukeland University Hospital, Bergen, Norway	2006-2013	164

(6). Mutations in the *BRAF* gene, serving to 'lock' the resulting protein product in a constitutively activated state, have been described as an alternative carcinogenic mechanism to *KRAS* mutation in the neoplastic pathway of CRC (8). Alterations in the *BRAF* gene are also thought to be associated with the microsatellite instability (MSI) pathway of colorectal tumorigenesis (9). The V599E mutation, which is located on exon 15 of the *BRAF* gene, is the most frequently identified mutation in CRC tumors with mismatch-repair (MMR) gene defects (10, 11). In contrast, the V600E mutation is overwhelmingly the most commonly identified *BRAF* mutation in metastatic CRC with a reported incidence of 95% (12, 13).

BRAF mutations are becoming increasingly relevant in clinical practice. They are present in approximately 10% of CRC (3), are considered negative prognostic factors in advanced disease (14) and may be significant predictors of resistance to epidermal growth factor receptor (EGFR)-targeted treatments, such as cetuximab and panitumumab (14). Moreover, interest in the apparently negative prognostic and clinical implications of mutant *BRAF* status in patients with CRLM, irrespective of biologic agent administration, has increased in recent years and the published literature on the subject has grown accordingly (15, 16).

The aim of this study was to review the current literature on the impact of *BRAF* mutations on overall survival (OS)/disease-specific survival (DSS) and recurrence-free survival (RFS) in patients with CRLM. In addition, as *BRAF* mutations are but one step in the long and perplexing pathways resulting in neoplastic transformation, we also aimed to examine the interplay and relative significance of *BRAF* mutant status *vis-à-vis* other mutations commonly encountered in CRLM, whenever relevant data were available.

Materials and Methods

A review of the literature was performed using the PubMed database to identify original research articles assessing the impact of *BRAF* mutation on OS/DSS and RFS among patients undergoing surgical resection for CRLM. Specifically, articles published between 01/01/2010 and 04/01/2016 were identified using the search strings (BRAF OR RAS) AND (colorectal OR CRC) AND (liver OR hepatic) AND (metastasis OR metastatic) or, alternatively, (BRAF OR RAS) AND (CLM OR CRLM). Additionally, the references of all selected articles were reviewed to identify any additional, potentially eligible studies. The results were cross checked to exclude overlapping series or double entries. In such cases, only the most recent relevant study or the one with the largest study population was considered eligible for inclusion. When the degree of overlapping could not be ascertained or study outcomes were different, both studies were included. Only publications in the English language were reviewed. Conference abstracts that did not proceed to publication in peer-reviewed journals, studies with primary outcomes other than OS/DSS and RFS, as well as studies limited to cell lines or animal models, were excluded from this review.

The initial search identified 700 articles; of these, 468 were rejected outright due to their failure to meet the stated inclusion criteria. The remaining 232 studies underwent more detailed evaluation. Ultimately, a total of 8 eligible publications were identified (Table I). Data pertaining to patient demographics, *BRAF* mutational status, type of surgery and the number of patients included were collected for each research article. Additionally, data on OS/DSS, RFS and their association with *BRAF* mutational status were recorded for each research article included. Secondary outcomes of each study were also discussed, based on our stated objectives and their respective clinical significance.

Results

One of the first studies to assess the association of *BRAF* mutation status with prognosis in patients with CRLM was a retrospective cohort study by Teng *et al.* The study population consisted of 292 patients who underwent curative-intent resection for CRLM at a single institution. Five-year OS of the entire cohort was 55.5%. *KRAS* and *BRAF* mutation status were determined by genetic analysis of metastatic lesions. The impact of both *KRAS* and *BRAF* mutation status on OS was examined. Specifically, *BRAF* was shown to be an independent predictor of worse OS (hazard ratio (HR)=5.181, 95% confidence interval (CI)=1.859-14.437, p=0.002) on multivariable analysis. This result served to underline the prognostic power of *BRAF*

status as only 2.1% of the cohort actually harbored *BRAF* mutant lesions. On the contrary, *KRAS* was not shown to be an independent predictor of OS (p=0.349) (17).

Subsequently, Umeda *et al*. reported on the association of *BRAF* and *KRAS* mutations with prognosis in a retrospective single-institution cohort of 100 patients who underwent curative resection for CRLM. Median survival and mean follow-up period were 36 and 41.1 months, respectively, for the entire cohort. RFS at 1, 3 and 5 years was 63.7%, 31.3% and 21.7%, respectively. *KRAS* and *BRAF* mutation status were determined by genetic analysis of metastatic lesions. Patients with *BRAF* mutant lesions (n=3) were demonstrated to have significantly worse OS and RFS compared to both *KRAS* mutant and wild-type patients (all p<0.005). On the other hand, *KRAS* mutant status was associated with worse OS (p=0.034), but was not an independent predictor of RFS (18).

An additional study by Huang *et al*. also served to confirm the prognostic role of *BRAF* in patients with CRLM. Originally designed to assess the impact of C-reactive protein polymorphisms on cancer-specific survival (CSS), the study also examined the role of *BRAF* and *KRAS* mutations as a secondary outcome. The study cohort consisted of 228 patients who underwent surgery at a single-institution. *KRAS* and *BRAF* mutation status were determined by genetic analysis of metastatic lesions. The incidence of BRAF mutations was estimated at 2.8%. When combined as a single variable, *KRAS* and *BRAF* mutations were shown to be independent negative predictors of CSS in multivariate analysis (HR=2.377, 95% CI=1.293-4.368, p=0.005). Interestingly, however, this association was only valid for patients with synchronous liver metastasis. It should also be noted that the present study likely had a degree of overlap with the previously reported study by Teng *et al*. as both recruited patients from the same institution during the same time period. As the degree of overlap could not be ascertained and the two studies had different outcomes, we chose to include both in the present review (19).

In a subsequent publication, Karagkounis *et al*. investigated the genetic profile of 202 patients with CRLM who underwent hepatectomy with or without ablation in a retrospective single-institution cohort. A minority of patients (n=20) had synchronous extrahepatic disease that was resected with curative-intent. Genetic data were prospectively collected and information on *KRAS* and *BRAF* mutation status was obtained by analyzing tissue from the metastatic liver lesions. Median and 5-year OS for the entire cohort was 70.7 months and 55.1%, respectively. On the other hand, median and 3-year RFS for the entire cohort was 18.9 months and 32.2%, respectively. Contrary to previous reports, *BRAF* mutant status was not significantly associated with OS or RFS but the very low frequency of *BRAF* mutations in the cohort (n=4) may have served to limit the power of statistical analysis. Nonetheless, a trend towards worse prognosis was evident in patients harboring *BRAF* mutant tumors. On the other hand, *KRAS* mutant status was shown to be an independent predictor of both RFS (HR=1.68, 95% CI=1.04-2.7, p=0.034) and OS (HR=1.99, 95% CI=1.21-3.26, p=0.007) in multivariable analysis (20).

In one of the largest studies up to that time, Yaeger *et al*. collected data from 1,941 consecutive patients with mCRC who underwent *KRAS/BRAF* mutation testing between 2009 and 2012 at a single institution. *BRAF* mutation was identified in 92 mCRC cases by genetic analysis of metastatic lesions (5%). The prognosis of these patients was then compared with the prognosis of 423 *BRAF* wild-type controls derived from the same patient population. Median OS for the controls was 47 months *versus* 20 months for *BRAF* mutant mCRC (p<0.01). Consistent with previous studies, Yaeger *et al*. reported that *BRAF* mutant status was associated with poor OS in the overall cohort, after adjusting for clinicopathological factors and the occurrence of metastasectomy in multivariate analysis (HR=2.95% CI=1.4-2.8, p<0.01). In the *BRAF*-mutant subgroup that underwent metastasectomy (n=23) a significant association (p=0.003) with worse OS was noted compared to *BRAF*-wild-type patients who also underwent metastasectomy (n=178). A trend towards inferior RFS in the *BRAF*-mutant subgroup was also noted, although it did not reach statistical significance (p=0.084). Interestingly, of the 15 patients with *BRAF*-mutant tumors who underwent curative-intent hepatectomy for CRLM, 13 developed recurrence. Eight patients developed intrahepatic recurrence, while the remainder presented with extrahepatic disease. Interestingly, the prognostic impact of *BRAF* mutation was evident even among patients considered low-risk by the traditionally employed Clinical Risk Score, suggesting the superiority of tumor biology over clinicopathological predictive factors (21, 22).

In a subsequent study by Lin *et al*., the prognostic impact of *BRAF* mutant status was examined in the context of the long-term outcomes of patients undergoing resection of synchronous CRLM. The study cohort consisted of 154 patients who underwent curative-intent resection for synchronous CRLM at a single-institution. Mutations in *BRAF* and *KRAS* genes were detected with the use of genetic analysis of tissue derived from metastatic lesions. Fourteen patients harbored *BRAF* mutations (9.1%). Median and 5-year OS of the entire cohort was 49 months and 46%, respectively. In addition, 5-year RFS for the entire cohort was 35%. Median follow-up was 36 months. The presence of *BRAF* mutation was an independent predictor of both worse OS (HR=2.531, 95% CI=1.102-5.811, p=0.029) and shorter RFS (HR=3.514, 95% CI=1.791-6.896, p<0.0001), while *KRAS* mutant status was not prognostic in either case. These results further confirmed the predictive role of *BRAF* mutant status in the context of synchronous CRLM, in line with the findings reported by Huang *et al*. (19, 23).

In a later study by Schirripa *et al*., the distinct effects of *BRAF* and *RAS* mutations on long-term outcomes were further compared in a multi-institutional retrospective cohort of 309 patients who underwent CRLM resection. *BRAF* and *RAS* mutant status were determined by genetic analysis of either primary CRC or metastatic lesions, depending on specimen availability. The frequency of *BRAF* mutations in the study population was 4%. Median follow-up of the entire cohort was 45.6 months. In spite of the relative low frequency of *BRAF* mutations, their strong negative impact on prognosis was confirmed. Specifically, *BRAF* was shown to be an independent predictor of RFS in multivariate analysis when compared to both *RAS*/*BRAF* wild-type patients (HR=2.31, 95% CI=1.09-4.87, p=0.029) and *RAS* mutant patients (HR=2.06, 95% CI=1.02-4.14, p=0.044). A similar prognostic association between *BRAF* mutant status and OS was demonstrated, with *BRAF* mutant patients having worse OS than both *RAS*/*BRAF* wild-type patients (HR=3.07, 95% CI=2.12-22.94, p=0.002) and RAS mutant patients (HR=2.09, 95% CI=1.05-7.87, p=0.041) in multivariate analysis. On the other hand, *RAS* mutant status did not appear to have an independent impact on long-term outcomes in multivariate analysis. Patterns of recurrence were also examined but no statistically significant association between *BRAF* mutant status and site-specific recurrence was noted. Interestingly, *RAS* mutant status was associated with lung-specific recurrence (p=0.008, p=0.027 and p=0.03 when compared to all wild-type patients and *BRAF* mutant patients respectively). This study served to demonstrate the stronger prognostic effect of *BRAF* mutant status when compared with other known indicators of unfavorable biology such as *KRAS* (24-26).

More recently, Loes *et al*. analyzed the interplay of chemotherapy, genetic markers (*KRAS, BRAF, PIK3CA, TP53*) and mutation heterogeneity (defined as different mutation status between metastatic lesions resected at the same time) on time to recurrence (TTR) and disease-specific survival (DSS) in patients with CRLM. The study population consisted of 164 patients who underwent resection for CRLM. Mutation status was identified after genetic analysis of the resected liver specimens. *BRAF* mutation frequency in this cohort was 6.1%. *BRAF* mutations demonstrated a statistically significant association with prognosis, resulting in worse TTR (p=0.002) and reduced median DSS (p<0.001). Similar results were observed for *KRAS* mutant status for both TTR (p<0.001) and DSS (p=0.008). Of the other mutations studied, only *PIK3CA* was associated with reduced TTR (p=0.023). It should be noted that these results were obtained with the use of univariate analysis and no multivariate analysis was performed. In addition, possible interactions between mutation status and chemotherapy administration were examined. Interestingly, *KRAS* mutant status was associated with improved TTR after pre-operative chemotherapy (p=0.018). No association between chemotherapy response or long-term outcomes after chemotherapy administration and *BRAF*/*PIK3CA*/*TP53* mutations was noted. Furthermore, the impact of mutation heterogeneity on long-term outcomes was examined. Interestingly, a significant difference in TTR (p<0.001) and DSS (p<0.001) was detected, with patients harboring heterogeneous lesions having worse prognosis in univariate analysis. This effect was independent of mutation type. These findings serve to validate the poor prognostic outcome of patients with *BRAF* mutations in the setting of CRLM (27).

Discussion

The incidence of *BRAF* mutations in the 8 studies that met inclusion criteria ranged between 2-9%. This is considerably lower than the reported incidence in primary CRC (10%) (28). However, this disparity is justifiable, as *BRAF* mutant metastatic CRC is known to commonly present with diffuse metastatic spread that is less likely to be amenable to surgical resection (29-31). As such, it is likely that patients with *BRAF* mutant metastatic CRC are underrepresented in surgical cohorts. Furthermore, important differences in baseline clinicopathological features were noted among patients depending on their *BRAF* mutational status. For example, when Yaeger *et al*. examined a cohort of patients with metastatic CRC lesions, they found that *BRAF* mutant tumors were significantly more common in older and female patients, as well as more likely to originate from the right colon (21). This finding is in line with the finding from Shirripa and coworkers that *BRAF* mutations were more common among lesions corresponding to right-sided primary CRC, whereas wild-type lesions were more frequent among patients with primary left -sided or rectal tumors (32). Furthermore, in terms of pathologic and molecular features, *BRAF*-mutant tumors were more likely to display poor differentiation, mucinous histology and microsatellite instability. Teng *et al*. corroborated these findings by demonstrating that patients with CRLM from a primary right-sided tumor had an increased likelihood of harboring *BRAF* mutations compared to patients with left-sided primary tumors (17). Karagkounis *et al*. also confirmed that *BRAF* mutations were more likely to occur in right-sided tumors and in older patients (20). These findings are in line with previous reports on the clinicopathological implications of *BRAF* status in primary CRC lesions, thus underlining the phenotypic and genetic continuity between primary and metastatic tumors (33, 34).

Regarding long-term outcomes, the prognostic role of *BRAF*-activating mutations in CRLM has not been studied extensively. In fact, while *KRAS* mutations have attracted much attention in the literature, we identified only 8 original studies that explicitly examined the prognostic implications

of *BRAF* mutational status in the specific context of CRLM. Nonetheless, the findings reported in theses 8 studies are remarkably consistent and allow for the formulation of more general conclusions. Specifically, based on the contemporary literature, *BRAF* mutations were negatively associated with both RFS (in 4/6 studies) and OS (7/8 studies) in patients undergoing surgery for CRLM. The statistical significance of these findings is further underlined by the relative rarity of *BRAF* mutant status (2%-9%) in the included studies. Furthermore, 4 out of 8 studies demonstrated a stronger prognostic effect of *BRAF* mutant status when compared with other known indicators of unfavorable biology, such as *KRAS* (17, 18, 24-26, 32). Therefore, patients and surgeons can utilize *BRAF* mutational status as a reliable index of prognostic information.

However, the ultimate end-point for patients and surgeons alike is the provision of effective evidenced-based care and not merely the successful 'forecasting' of adverse outcomes. As such, the practical clinical implications of *BRAF* mutational status should be further explored. For example, it has been demonstrated that the presence of *BRAF* mutations in metastatic CRC predicts lack of response to anti-EGFR agent treatment similarly to what occurs in the presence of KRAS pathway mutations (35-37). Nonetheless, no specific therapy for this patient subgroup is currently in widespread use. Fortunately, direct BRAF inhibitors or MEK inhibitors that block the signaling cascade downstream from the BRAF pathway are either under development or already in clinical use for indications, such as melanoma and future clinical trials, examining their clinical safety and efficacy in patients with CRLM, are eagerly anticipated (35, 38, 39).

While we await these future developments, substantial clinical benefit may be derived from personalized follow-up strategies according to the prognostic information known to be implicit in *BRAF* mutational status. For example, as patients with *BRAF* mutations appear to have significantly worse RFS, it may be advisable to shorten follow-up intervals in such cases. However, as none of the included studies has reported any impact of *BRAF* mutational status on patterns of recurrence in patients with CRLM, future studies will be necessary before any more specific follow-up or treatment recommendations with respect to recurrence can be formulated.

It is also the Authors' opinion that the rationale underlying surgical intervention in patients with *BRAF* mutated tumors should be revisited. For example, it may be that aggressive curative-intent resection has little to offer these patients in terms of survival benefit. As such, alternative treatments with lower morbidity and economic cost may be more appropriate in selected case. It should be noted, however, that in the absence of randomized trials and empirical data addressing these issues, little more than speculation and suggestions towards future research efforts can be offered. To this end, it might be interesting to examine the association of resection margin status with survival in the *BRAF* mutated group, as has already been done for patients with *KRAS* mutations (40).

Several limitations should be considered when interpreting the presented data. For example, all studies included in the present review were non-randomized and mostly retrospective in nature. As such, a true control group on which to base meaningful comparisons was lacking. Furthermore, due to the inherent methodological weaknesses of the retrospective study design, the possibility of selection bias or confounding should not be dismissed lightly. In addition, it should be noted that a few studies failed to detect prognostic differences in either RFS or OS. As *BRAF* mutated lesions have extremely low frequency in patients with CRLM, it is possible that small sample sizes may have impacted the ability of the included studies to detect a true difference between wild-type and mutated tumors in terms of long-term outcomes (type II error).

Furthermore, additional factors could have contributed to the aforementioned discrepancies in long-term outcomes among the included studies. Specifically, although the V600E mutation is by far the most frequent *BRAF* mutation identified in metastatic CRC, different *BRAF* mutations are known to exist and may well be associated with distinct prognostic implications (12, 13). This hypothesis is similar to the one examined by Margonis *et al*. in a recent paper on the prognostic impact of codon-specific *KRAS* mutations on long-term outcomes in patients with CRLM (25). Indeed, the Authors were able to demonstrate distinct codon-specific effects on survival (25). Specifically, they found that codon 12 mutations, unlike codon 13 mutations, conferred a more aggressive tumor phenotype that, in turn, led to inferior survival. As such, it should be noted that all previous studies investigated only *BRAF* V600E codon mutational status. Of note, only Huang *et al*. and Teng *et al*. reported on *BRAF* mutations except for *BRAF* V600E; however, only 2 patients had a V599E mutation and, therefore, a meaningful sub-analysis could not be performed (17, 19). Nonetheless, published evidence from different patient cohorts supports our hypothesis regarding the possible disparate effect of specific *BRAF* mutations on prognosis. For example, Cremolini and colleagues in a cohort of patients with overall metastatic CRC recently showed that *BRAF* codon 594 or 596 mutated mCRCs significantly differed from tumors with *BRAF* V600E mutations in terms of associated molecular features, pathological characteristics and clinical outcomes (41). Their findings are consistent with preclinical evidence of a kinase inactivating effect of *BRAF* codon 594 or 596 mutations, which may serve to weaken rather than enhance BRAF function. As such, it is possible that the distinct effects of specific mutations may have led to contradictory results when combined under the 'umbrella' of *BRAF* mutant lesions.

Nonetheless, a strong case can be made in favor of *BRAF* status being a true biologic determinant of prognosis. The association of *BRAF* mutation with worse prognosis has been reported with remarkable consistency in different studies that focused on independent patient cohorts and utilized different methodologies. The strength and consistency of the association is all the more remarkable given the extremely small number of patients with *BRAF* mutant lesions in the included studies. Future studies should include sub-analyses pertaining to the individual effects of codon-specific *BRAF* mutations on prognosis and patterns of recurrence in an effort to further delineate the influence of molecular factors on patient outcomes.

Conflicts of Interest

No conflicts of interest disclosures.

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