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Association of *BRAF* Mutations With Survival and Recurrence in Surgically Treated Patients With Metastatic Colorectal Liver Cancer

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IMPORTANCE *BRAF* mutations are reportedly associated with aggressive tumor biology. However, in contrast with primary colorectal cancer, the association of V600E and non-V600E *BRAF* mutations with survival and recurrence after resection of colorectal liver metastases (CRLM) has not been well studied.

OBJECTIVE To investigate the prognostic association of *BRAF* mutations with survival and recurrence independently and compared with other prognostic determinants, such as *KRAS* mutations.

DESIGN, SETTING, AND PARTICIPANTS In this cohort study, all patients who underwent resection for CRLM with curative intent from January 1, 2000, through December 31, 2016, at the institutions participating in the International Genetic Consortium for Colorectal Liver Metastasis and had data on *BRAF* and *KRAS* mutational status were retrospectively identified. Multivariate Cox proportional hazards regression models were used to assess long-term outcomes.

INTERVENTIONS Hepatectomy in patients with CRLM.

MAIN OUTCOMES AND MEASURES The association of V600E and non-V600E *BRAF* mutations with disease-free survival (DFS) and overall survival (OS).

RESULTS Of 853 patients who met inclusion criteria (510 men [59.8%] and 343 women [40.2%]; mean [SD] age, 60.2 [12.4] years), 849 were included in the study analyses. Forty-three (5.1%) had a mutated (mut) *BRAF*/wild-type (wt) *KRAS* (V600E and non-V600E) genotype; 480 (56.5%), a wt*BRAF*/wt*KRAS* genotype; and 326 (38.4%), a wt*BRAF*/mut*KRAS* genotype. Compared with the wt*BRAF*/wt*KRAS* genotype group, patients with a mut*BRAF*/wt*KRAS* genotype more frequently were female (27 [62.8%] vs 169 [35.2%]) and 65 years or older (22 [51.2%] vs 176 [36.9%]), had right-sided primary tumors (27 [62.8%] vs 83 [17.4%]), and presented with a metachronous liver metastasis (28 [64.3%] vs 229 [46.8%]). On multivariable analysis, V600E but not non-V600E *BRAF* mutation was associated with worse OS (hazard ratio [HR], 2.76; 95% CI, 1.74-4.37; $P < .001$) and DFS (HR, 2.04; 95% CI, 1.30-3.20; $P = .002$). The V600E *BRAF* mutation had a stronger association with OS and DFS than the *KRAS* mutations (β for OS, 10.15 vs 2.94; β for DFS, 7.14 vs 2.27).

CONCLUSIONS AND RELEVANCE The presence of the V600E *BRAF* mutation was associated with worse prognosis and increased risk of recurrence. The V600E mutation was not only a stronger prognostic factor than *KRAS* but also was the strongest prognostic determinant in the overall cohort.

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During the past 2 decades, genetic predictors of prognosis have been used with increasing frequency for patients with colorectal liver metastasis (CRLM).[1] Several investigators have reported on the prognostic value of somatic mutations in the *KRAS* (OMIM 190070) in patients with resectable CRLM.[2-6] Although only 2 studies had assessed *KRAS* mutational status as a prognostic factor in the 1990s, at least 14 additional studies have been published since 2000.[7,8] A 2015 meta-analysis demonstrated the adverse effect of *KRAS* mutations on prognosis by pooling survival data from 326 patients with *KRAS*-mutated tumors.[9]

BRAF (OMIM 164757) mutations affect the same signaling pathway as *KRAS* mutations, the most important difference being that the genetic product of a *BRAF*-mutated gene exerts its influence downstream from *KRAS*.[10] Although these similarities suggest a possible role for *BRAF* as a prognostic indicator of CRLM, this hypothesis has not been well studied because of the low (2%-4%) incidence of *BRAF* mutations in resected CRLM compared with the 30% to 40% incidence of *KRAS* mutations. The largest relevant study conducted to date (a multi-institutional study from Italy)[11] identified only 12 *BRAF* mutations in a total population of 309 patients who underwent surgical resection of CRLM. According to the most recent meta-analysis,[7] only 4 studies have reported overall survival according to *BRAF* mutational status, and disease-free survival has been examined by only a single study. Of importance, all these studies combined included only 22 patients with *BRAF* mutations. Although *BRAF* mutations were associated with adverse prognosis, the sample was too small to allow for a thorough statistical analysis.

Consequently, limited information exists regarding the association of *BRAF* mutations with the prognosis of resectable CRLM. To increase sample size and mitigate the limitations of previous analyses, an international, multi-institutional consortium was organized to explore the effects of *BRAF* in patients with CRLM (International Genetic Consortium for Colorectal Liver Metastasis [IGCLM]). Based on this collaborative effort, we aimed to investigate the clinical profile of patients with tumors with *BRAF* mutations and assess the prognostic association of *BRAF* mutations with survival and recurrence independently and compared with *KRAS* mutations. Last, in line with previous work on metastatic colorectal cancer (mCRC), we investigated whether different *BRAF* mutations (V600E vs non-V600E) may also have a distinct association with prognosis.

Key Points

Questions What is the prognostic association of *BRAF* mutations with survival and recurrence in patients with metastatic colorectal liver cancer, and how does it compare with *KRAS* mutations?

Findings In this study of 853 patients with colorectal liver metastases, those with a mutant *BRAF*/wild-type *KRAS* genotype more commonly were female and 65 years or older, had right-sided primary tumors, and presented with metachronous liver metastasis. V600E but not non-V600E *BRAF* mutation was associated with worse overall and disease-free survival, and V600E *BRAF* mutations had a stronger association with overall and disease-free survival than *KRAS* mutations.

Meaning The presence of the *BRAF* V600E mutation was associated with worse prognosis and increased risk of recurrence and was the strongest prognostic determinant in the overall cohort.

Methods

Patient Selection

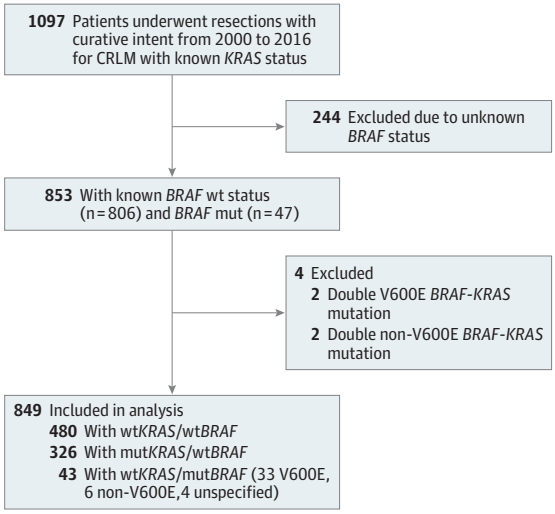
Owing to the multi-institutional nature of this study, all examined hypotheses and variables of interest were determined in advance. All patients who underwent curative-intent surgery for CRLM from January 1, 2000, through December 31, 2016, at 7 academic institutions participating in the IGCLM (Johns Hopkins University, Baltimore, Maryland; Stanford University School of Medicine, Stanford, California; Digestive Disease Institute, Cleveland Clinic, Cleveland, Ohio; University of Berlin-Charité, Berlin, Germany; Medical University of Vienna, Vienna, Austria; Medical University of Graz, Graz, Austria; and Haukeland University Hospital, Bergen, Norway) and had available data on *BRAF* and *KRAS* mutation status were identified. The IGCLM was officially established when the institutional review board of Johns Hopkins University approved the current study. The institutional review boards of the 7 participating centers approved the study. Owing to the retrospective nature of the study, all institutional review boards waived the requirement for obtaining informed consent.

We collected standard demographic, clinicopathologic, and genetic variables. These variables included age, sex, and characteristics of the primary colorectal tumor, including the American Joint Committee on Cancer T stage, primary tumor location (left vs right colon), and the presence or absence of lymph node metastasis. Information on the following preoperative factors was also recorded and analyzed: receipt of preoperative chemotherapy, preoperative carcinoembryonic antigen levels, and synchronous (<6 months) vs metachronous presentation of liver disease. In addition, we collected data on CRLM tumor size, number of tumors, presence of extrahepatic disease, margin status (R1 was defined as microscopically positive resection margins), data on somatic mutations (*KRAS* and *BRAF* mutation status assessed in the primary tumor or the corresponding liver lesions), and the administration of postoperative therapy. Overall survival (OS) was defined as the interval from curative-intent liver resection until death or last follow-up. Similarly, disease-free survival (DFS) was calculated from the date of resection until the first radiologic or pathologic evidence of recurrence or, in the case of no recurrence, until the date of the last follow-up.

Determination of *KRAS* and *BRAF* Mutation Status

Genomic DNA was isolated from primary colorectal cancer (CRC) or CRLM tissue specimens and was used as a template for sequencing the *BRAF* gene locus (V600E and non-V600E mutations) and *KRAS* codons 12, 13, and 61 using standard techniques previously described.[3] Patients from Haukeland University Hospital only underwent sequencing of *KRAS* exon 2 (harboring codons 12 and 13) and *BRAF*. Of note, the data on

Figure 1. Study Flowchart



CRLM indicates colorectal liver metastases; mut, mutated genotype; and wt, wild-type genotype.

BRAF mutations from Haukeland University Hospital have been previously reported.[12] We elected to use primary CRC or CRLM tissue to determine *KRAS* and *BRAF* mutational status; this decision was based on a number of studies that have consistently demonstrated a high concordance rate for *KRAS* and *BRAF* mutational status (>90%) between primary and metastatic lesions.[13-16]

Statistical Analysis

We estimated differences between categorical values using the χ^2 test or Fisher exact tests, whereas differences between continuous values were assessed using the Mann-Whitney or Kruskal-Wallis test, as appropriate. Survival estimates for the study population were generated using the Kaplan-Meier method. A Cox proportional hazards regression model was used to assess the association of several variables with OS and DFS. Variables that were statistically significant in univariable analysis ($P < .2$) were retained in the multivariable model. For the multivariable analysis, multiple imputations were performed using the *mice* package for R software (version 3.3.1; https://cran.r-project.org/). The prognostic power of independent factors was assessed by calculating the β coefficient as previously described.[17] All analyses were performed using SPSS (version 22.0; IBM Corp) and R (version 3.3.1). All tests were 2-sided, and $P < .05$ defined statistical significance.

Results

Study Cohort Characteristics

Of 853 patients who met inclusion criteria (510 men [59.8%] and 343 women [40.2%]; mean [SD] age, 60.2 [12.4] years), 4 patients with double *BRAF-KRAS* mutations were excluded, resulting in a final study population of 849 adult patients in the study cohort (**Figure 1**). A total of 326 patients demonstrated a wild-type (wt) *BRAF*/mutated (mut) *KRAS* genotype; 480, wt-*BRAF*/wt*KRAS* genotype; and 43, mut*BRAF*/wt*KRAS* genotype. Among the patients with the mut*BRAF*/wt*KRAS* genotype, 33 had a V600E substitution mutation, 6 had a non-V600E mutation, and 4 had a nonspecified variant of *BRAF* mutation. Clinicopathologic, genetic, and treatment-related characteristics of the entire cohort are summarized in eTable 1 in the Supplement.

The baseline characteristics of patients with wt*BRAF*/wt*KRAS* and mut*BRAF*/wt*KRAS* genotypes are presented in **Table 1.** Among those with available data, patients with a *BRAF* mutation were significantly more likely to be 65 years or older (22 [51.2%] vs 176 [36.9%]) and female (27 [62.8%] vs 169 [35.2%]). Primary CRC tumors in patients with the mut*BRAF*/wt*KRAS* genotype were also significantly more likely to be right sided (27 [62.8%] vs 83 [17.4%]) and of a more advanced T stage (41 [95.3%] vs 381 [81.9%]); metachronous liver metastases were significantly more common in this patient group (28 [64.3%] vs 229 [46.8%]).

Subsequently, we compared the baseline characteristics of the wt*BRAF*/wt*KRAS* group with each of the 2 major subgroups of the mut*BRAF*/wt*KRAS* group (eTable 2 in the Supplement). Patients with non-V600E mutations were more similar to patients with wt*BRAF* genotypes compared with patients who had the V600E *BRAF* mutation. Specifically, all 6 patients with a non-V600E mutation were younger than 65 years, and 3 (50.0%) were female, with a similar pattern being observed in patients with wt*BRAF* genotypes. The location of primary CRC was evenly distributed among patients with non-V600E mutations (2 of 6 rectal, 2 of 6 right sided, and 2 of 6 left sided). Synchronous liver metastases were significantly more common in patients with non-V600E mutations (4 of 6 [66.7%]) compared with patients with the V600E *BRAF* mutation (10 of 33 [30.3%]).

OS Analysis

At a median follow-up of 28.3 months (interquartile range [IQR], 13.5-50.7 months), 377 of 853 patients (44.2%) had died. The 1-year OS rate was 87.5%; 3-year OS rate, 61.6%; and 5-year OS rate, 43.2%. On univariable analysis, the presence of a *BRAF* mutation (all subtypes included) was associated with significantly worse OS (eFigure, A, in the Supplement) ($P = .003$). However, although the presence of a V600E mutation was associated with significantly worse OS (hazard ratio [HR], 2.39; 95% CI, 1.53-3.72; $P < .001$), survival among patients with non-V600E mutations did not differ from that among patients with wt*BRAF* genotypes (eFigure, B, in the Supplement) (HR, 1.34; 95% CI, 0.43-4.19; $P = .61$).

The univariable and multivariable analyses of OS are summarized in **Table 2**. The following factors were independently associated with OS on multivariable analysis: 65 years or older, primary tumor lymph node metastasis, prehepatectomy chemotherapy, carcinoembryonic antigen level of more than 8.5 ng/mL (to convert to micrograms per liter, multiply by 1.0), resection of extrahepatic disease, synchronous liver metastasis, tumor size, presence of multiple CRLMs, positive surgical margin, presence of *KRAS* mutation, postoperative chemotherapy, and presence of *BRAF* mutation.

Table 1. Baseline Differences Between wt*BRAF*/wt*KRAS* vs mut*BRAF*/wt*KRAS* Genotype Groups

Characteristic	Tumor Genotype[a]		*P* Value
	wt*BRAF*/wt*KRAS* (n = 480)	mut*BRAF*/wt*KRAS* (n = 43)	
Age, y			
<65	301 (63.1)	21 (48.8)	.06
≥65	176 (36.9)	22 (51.2)	
Female	169 (35.2)	27 (62.8)	<.001
Primary T stage			
0-2	84 (18.1)	2 (4.7)	.02
3-4	381 (81.9)	41 (95.3)	
Lymph node metastases	307 (65.3)	31 (72.1)	.37
Tumor location			
Right side	83 (17.4)	27 (62.8)	<.001
Left side	202 (42.4)	7 (16.3)	
Transverse	17 (3.6)	3 (7.0)	
Rectum	174 (36.6)	6 (14.0)	
Prehepatectomy chemotherapy	331 (69.5)	21 (48.8)	.005
CEA level >8.5 ng/mL	186 (48.8)	15 (53.6)	.63
Extrahepatic disease	39 (8.2)	6 (14.0)	.20
Synchronous liver metastases	251 (53.2)	15 (35.7)	.03
Size, mean (range), cm	2.3 (1.4-3.5)	1.5 (1.1-3.0)	.09
Multiple CRLMs	255 (53.6)	19 (44.2)	.24
R1 resection	48 (12.7)	4 (12.1)	.93
Posthepatectomy chemotherapy	245 (63.8)	20 (57.1)	.43

Abbreviations: CRLMs, colorectal liver metastases; mut, mutated genotype; wt, wild-type genotype.

SI conversion factor: To convert CEA to micrograms per liter, multiply by 1.0.

[a] Owing to missing data, totals may not sum to numbers in column headings. Unless otherwise indicated, data are expressed as number (percentage) of patients.

The presence of non-V600E *BRAF* mutations was not associated with OS in the univariable (HR, 1.34; 95% CI, 0.43-4.19; *P* = .61) or multivariable (HR, 1.75; 95% CI, 0.54-5.60; *P* = .35) analysis. However, the presence of the V600E *BRAF* mutation (HR, 2.76; 95% CI, 1.74-4.37; *P* < .001) was the strongest prognostic factor for OS in the multivariable analysis.

The prognostic association of the V600E mutation, as modeled by the β coefficient, was almost twice as large as that of the second strongest prognostic factor and more than 3 times greater than that of the presence of a *KRAS* mutation (β for V600E vs *KRAS*, 10.15×10^{-1} vs 2.94×10^{-1}). Kaplan-Meier OS curves according to *KRAS* and *BRAF* mutation status (wt*BRAF*/wt*KRAS* vs wt*BRAF*/mut*KRAS* vs V600E *BRAF* mutation) are displayed in **Figure 2**.

DFS Analysis

During the study period, 473 of 849 patients (55.7%) developed recurrence or metastasis. The univariable and multivariable analyses of DFS are summarized in **Table 3**. The following variables were independently associated with DFS on multivariable analysis (Table 3): primary tumor lymph node metastasis, primary rectal cancer, prehepatectomy chemotherapy, resection of extrahepatic disease, presence of multiple CRLMs, positive surgical margin, presence of *KRAS* mutation, and postoperative chemotherapy.

Similar to the OS analysis, *BRAF* mutation (all subtypes included) was associated with an increased risk of recurrence in the multivariable analysis (HR, 1.62; 95% CI, 1.07-2.47; *P* = .02). When the molecular subtypes of *BRAF* mutation were examined separately, the presence of the V600E *BRAF* mutation (HR, 2.04; 95% CI, 1.30-3.20; *P* = .002) was the strongest prognostic factor of DFS identified in the multivariable analysis. This was not the case for non-V600E mutations (HR, 0.67; 95% CI, 0.22-2.06; *P* = .48). Similar to OS, the prognostic association of the V600E mutation, as modeled by the β coefficient, was almost twice as large as that of the second strongest prognostic factor and more than 3 times greater than that of a *KRAS* mutation (β for V600E vs *KRAS*, 7.14×10^{-1} vs 2.27×10^{-1}).

Discussion

In this international collaborative effort, we assembled the largest cohort, to our knowledge, of surgically treated patients with CRLM and *BRAF* mutations to be reported in the literature to date. In fact, the number of patients with *BRAF* mutations in the present study (n = 47) exceeded the cumulative population of the most recent meta-analysis (n = 22) by a factor greater than 2.[7] The presence of a *BRAF* mutation was a negative independent prognostic factor for survival and recurrence, thus confirming earlier, preliminary reports[18] as well as the largest study to date conducted by Schirripa et al.[11] Although the number of patients with non-V600E *BRAF* mutations can be considered to be too small to draw definitive conclusions, our results indicate that different *BRAF* mutations may have a distinct association with survival. Specifically, the V600E mutation was associated with worse prognosis, whereas the presence of non-V600E mutations was not associated with significantly different outcomes compared with tumors with wt*BRAF* genotypes (for OS and DFS). Nonetheless, the latter finding should be interpreted with caution because of the small

Table 2. Overall Survival Univariable and Multivariable Analyses

	Univariable Analysis		Multivariable Analysis		
Characteristic	HR (95% CI)	*P* Value	β Coefficient	HR (95% CI)	*P* Value
Age, y					
<65	1 [Reference]	NA	NA	NA	NA
≥65	1.36 (1.11-1.68)	.003	3.47	1.41 (1.14-1.76)	.002
Female	1.05 (0.85-1.29)	.66	NA	NA	NA
Primary T stage					
0-2	1 [Reference]	NA	NA	NA	NA
3-4	1.19 (0.89-1.60)	.24	NA	NA	NA
Lymph node metastases	1.42 (1.14-1.78)	.002	3.20	1.38 (1.09-1.74)	.007
Tumor location					
Right side	1 [Reference]	NA	NA	NA	NA
Left side	0.79 (0.62-1.02)	.07	NA	NA	NA
Transverse	1.17 (0.68-2.01)	.56	NA	NA	NA
Rectum	0.91 (0.70-1.18)	.48	NA	NA	NA
Prehepatectomy chemotherapy	1.20 (0.96-1.50)	.11	3.45	1.41 (1.11-1.80)	.006
CEA level >8.5 ng/mL	1.69 (1.35-2.13)	<.001	4.17	1.52 (1.20-1.92)	<.001
Extrahepatic disease	2.02 (1.43-2.84)	<.001	6.55	1.92 (1.34-2.76)	<.001
Synchronous liver metastases	1.18 (0.96-1.44)	.12	2.45	1.28 (1.04-1.58)	.02
Size, cm	1.07 (1.03-1.12)	.003	0.60	1.06 (1.01-1.12)	.02
Multiple CRLM	1.49 (1.21-1.83)	<.001	3.32	1.39 (1.13-1.73)	.003
BRAF mutation					
Wild-type	1 [Reference]	NA	NA	1 [Reference]	NA
V600E	2.39 (1.53-3.72)	<.001	10.15	2.76 (1.74-4.37)	<.001
Non-V600E	1.34 (0.43-4.19)	.61	5.57	1.75 (0.54-5.60)	.35
KRAS mutation	1.34 (1.09-1.65)	.005	2.94	1.34 (1.08-1.67)	.008
R1 resection	2.09 (1.56-2.82)	<.001	5.74	1.77 (1.30-2.43)	<.001
Posthepatectomy chemotherapy	0.66 (0.54-0.82)	<.001	−3.97	0.67 (0.53-0.85)	<.001

Abbreviations: CEA, carcinoembryonic antigen; CRLM, colorectal liver metastases; HR, hazard ratio; mut, mutated genotype; NA, not applicable.

SI conversion factor: To convert CEA to micrograms per liter, multiply by 1.0.

number of patients in the non-V600E subgroup. Of importance, our analysis indicated that the *BRAF* V600E mutation may be not only a stronger prognostic factor than *KRAS*, thus confirming the distinct biological features of the 2 mutations, but also the strongest determinant of prognosis in patients with resectable CRLM. To our knowledge, this is the first time that such results are reported in an exclusively surgical cohort.

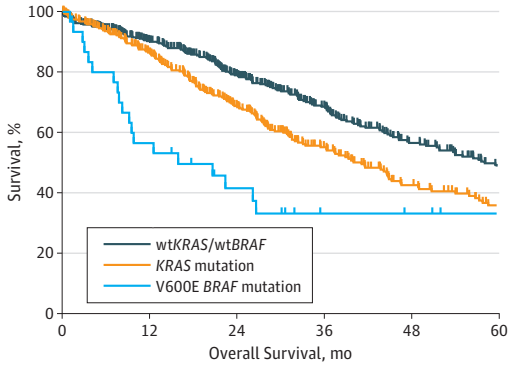
The incidence of *BRAF* mutation in our study population was 5.5%, in line with previous reports on resected CRLM.[11,18,19] Of interest, *BRAF* mutations are reported to occur more frequently in mixed mCRC cohorts (5%-11%) that include patients with resectable and unresectable metastatic disease.[20-22] This disparity may reflect the inherent biological aggressiveness of *BRAF* mutations, which leads to a decreased incidence of liver-limited disease and a higher likelihood of multiorgan involvement, thus precluding curative resection for many patients.[23] In our cohort, patients with a *BRAF* mutation more frequently were female and 65 years or older, had right-sided primary tumors, and were more likely to present with metachronous liver metastases. The latter finding is interesting because the reverse association has been observed in cohorts that include patients who are not candidates for resection.[22]

Patients with mut*BRAF*/wt*KRAS* genotypes had a median overall survival of only 26 months compared with 60 months for patients with wt*BRAF*/wt*KRAS* tumors. Of importance, the study cohort had sufficient size to allow us to control for pertinent prognostic factors with the aid of multivariable analysis. These findings contribute significantly to the literature because the previous 4 studies on the implications of *BRAF* mutations in CRLM[11,18,19,24] generated conflicting results. Our findings are in line with the study by Schirripa et al[11] and with reports from even larger patient populations with unresectable mCRC.[20-22,25,26] Furthermore, to our knowledge, this study is only the second to date to investigate the association of *BRAF* mutations with recurrence after CRLM resection.[11] The median time to recurrence was only 9.9 months, consistent with mCRC studies that reported a similar, poor median DFS of 5.7 months.

Although the presence of the V600E *BRAF* mutation was strongly associated with inferior prognosis, non-V600E mutations demonstrated no significant association with prognosis. Although these results have not been reported previously in a surgically treated CRLM cohort, they are consistent with those of 2 recent studies in unresectable mCRC.[27,28] Cremolini et al[27] demonstrated that patients with mCRC harboring *BRAF* codon 594- and 596-mutated tumors (n = 10) had better OS than did patients with the V600E *BRAF* mutation (n = 77). A subsequent collaborative study from the Mayo Clinic and MD Anderson Cancer Center[28] confirmed these results in the largest cohort of patients with mCRC and non-V600E *BRAF* mutations reported

to date. Reports from basic and translational science[29-34] provide a possible explanation for these findings by suggesting that non-V600E *BRAF* mutations may confer only intermediate (at best) kinase activity compared with an increase in kinase activity as high as 700-fold in the presence of V600E mutations.

Figure 2. Kaplan-Meier Estimates of Overall Survival



No. at risk

wt*KRAS*/wt*BRAF*	480	357	357	175	119	89
KRAS mut	326	254	174	105	65	45
V600E *BRAF*	33	17	10	4	3	1

For wt*KRAS*/wt*BRAF* vs mut*KRAS*/wt*BRAF*, *P* = .002; mut*KRAS* /wt*BRAF* vs V600E *BRAF*, *P* = .008. mut indicates mutated genotype; wt, wild-type genotype.

After characterizing the relative prognostic association of different mutations in the *BRAF* gene, we used a β coefficient analysis to compare the relative prognostic weight of the most potent *BRAF* mutation, namely, V600E, with that of *KRAS* mutations. V600E had more than 3 times the prognostic weight of a *KRAS* mutation. This finding contradicts previous reports that mutations in *KRAS* and *BRAF* may have similar phenotypic implications.[35] Our results suggest that *BRAF* and *KRAS* mutations should not be used interchangeably as markers of aggressive tumor biology. Furthermore, our study suggests that the presence of the V600E *BRAF* mutation is associated with poor prognosis and may serve as a more useful tool for preoperative patient selection than *KRAS* mutation status. Although this clinical study is the first, to our knowledge, to compare *KRAS* with V600E *BRAF* mutations directly, the results are consistent with those of preclinical studies and can be readily interpreted.[36] Both of these somatic mutations constitutively activate the extracellular signal-regulated kinase pathway that in turn phosphorylates and regulates the functions of numerous cellular components. However, V600E *BRAF*–mutated cells show a 138-fold increase in oncogenic activity compared with wt*BRAF*, thus suggesting that the oncogenic activity of the V600E *BRAF* mutation is greater than that

Table 3. Disease-Free Survival Univariable and Multivariable Analyses

	Univariable Analysis		Multivariable Analysis		
Characteristic	HR (95% CI)	*P* Value	β Coefficient	HR (95% CI)	*P* Value
Age, y					
<65	1 [Reference]	NA	NA	NA	NA
≥65	1.06 (0.88-1.28)	.56	NA	NA	NA
Female	1.14 (0.95-1.37)	.172	1.13	1.14 (0.95-1.38)	.17
Primary T stage					
0-2	1 [Reference]	NA	NA	NA	NA
3-4	1.22 (0.93-1.61)	.147	−0.02	1.00 (0.75-1.32)	.99
Lymph node metastases	1.48 (1.21-1.81)	<.001	3.25	1.38 (1.12-1.71)	.002
Tumor location					
Right side	1 [Reference]	NA	NA	1 [Reference]	NA
Left side	1.05 (0.83-1.32)	.71	2.05	1.23 (0.96-1.57)	.10
Transverse	1.24 (0.68-1.87)	.65	2.52	1.29 (0.77-2.16)	.34
Rectum	1.31 (1.03-1.66)	.06	3.62	1.44 (1.12-1.85)	.005
Prehepatectomy chemotherapy	1.44 (1.18-1.77)	<.001	3.40	1.40 (1.13-1.74)	.002
CEA level >8.5 ng/mL	1.22 (1.00-1.50)	.05	1.17	1.12 (0.91-1.38)	.27
Extrahepatic disease	1.71 (1.28-2.28)	<.001	5.03	1.65 (1.23-2.23)	.001
Synchronous liver metastases	1.05 (0.88-1.27)	.58	NA	NA	NA
Size, cm	1.00 (0.95-1.04)	.83	NA	NA	NA
Multiple CRLM	1.68 (1.39-2.04)	<.001	4.57	1.58 (1.30-1.91)	<.001
BRAF mutation					
Wild-type	1 [Reference]	NA	NA	1 [Reference]	NA
V600E	1.60 (1.02-2.51)	.04	7.14	2.04 (1.30-3.20)	.002
Non-V600E	0.67 (0.21-2.07)	.48	−4.04	0.67 (0.22-2.06)	.48
KRAS mutation	1.22 (1.01-1.47)	.03	2.27	1.25 (1.03-1.53)	.02
R1 resection	1.67 (1.26-2.20)	<.001	3.54	1.42 (1.08-1.88)	.01
Posthepatectomy chemotherapy	0.68 (0.56-0.82)	<.001	−4.64	0.63 (0.51-0.77)	<.001

Abbreviations: CEA, carcinoembryonic antigen; CRLM, colorectal liver metastases; HR, hazard ratio; mut, mutated genotype; NA, not applicable; wt, wild-type genotype.

SI conversion factor: To convert CEA to micrograms per liter, multiply by 1.0.

of the *KRAS* G12V point mutation.[37,38] Because the G12V mutation is the most prognostic among all point mutations in CRLM, the V600E mutation would likely have a stronger association with survival than other *KRAS* mutations.[2]

Limitations

Our analysis is limited by the retrospective nature of the study and its exclusive focus on patients with surgically resectable disease. As such, a degree of selection bias was largely unavoidable. However, because of the scarcity of *BRAF* mutations, assembling a prospective cohort of this size would be difficult. Second, although our study was comparable in terms of cohort size to a 2015 study,[27] in general, the number of patients with non-V600E *BRAF* mutations is relatively small. Although a type II error is a strong possibility given the small size of the non-V600E subgroup, results similar to ours have been reported by 2 adequately powered studies in BRAF-mutated, unresectable mCRC.[27,28] With respect to follow-up, serum carcinoembryonic antigen measurements and radiologic imaging were performed in all participating centers every 3 to 6 months during the first 2 to 3 years after surgery and every 6 months or annually thereafter. However, because the timing and duration of surveillance may also be influenced by patient risk profiles, some heterogeneity in posthepatectomy surveillance was likely present in our multi-institutional cohort. Another limitation of the study is the lack of data on the mismatch repair system. As shown in studies on primary CRC, the mismatch repair system is important in the interpretation of *BRAF* mutations.[39] In addition, detailed information on systemic therapy, especially with respect to chemotherapy regimens and treatment cycles, were not available. Of interest, we found that prehepatectomy chemotherapy was independently associated with worse OS. Although previous studies from Memorial Sloan-Kettering Cancer Center[40] and MD Anderson Cancer Center[41] have reported similar findings, the retrospective design of the study, the heterogeneity of the chemotherapeutic protocols used, and the lack of randomization preclude any reliable interpretation.

Conclusions

The size of this study population with *BRAF*-mutated tumors exceeded that of previous studies, and the study was adequately powered to indicate that patients with *BRAF* mutations may be at an increased risk of recurrence and death. A novel (in surgical CRLM cohorts) finding is that the V600E mutation alone (rather than V600E and non-V600E mutations together) may confer a distinctly aggressive clinical phenotype, thus driving the adverse outcomes associated with *BRAF* mutation. However, this finding needs to be interpreted with great caution because of the small number of patients with non-V600E mutations in the cohort. Although 2 previous studies of unresectable mCRC[27,28] reported similar results, additional confirmation from larger cohorts is needed. No recommendations can be made regarding the selection of surgical or medical treatment for patients with *BRAF*-mutated CRLM based on our findings. Future cohort studies free of selection bias that will incorporate a complete denominator of patients with CRLM (namely, those with resected, unresected, and unresectable disease) and appropriately designed clinical trials are warranted to address this issue.

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