Non-V600 *BRAF* Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer

Jeremy C. Jones, Lindsay A. Renfro, Humaid O. Al-Shamsi, Alexa B. Schrock, Andrew Rankin, Ben Y. Zhang, Pashtoon M. Kasi, Jesse S. Voss, Alexis D. Leal, James Sun, Jeffrey Ross, Siraj M. Ali, Joleen M. Hubbard, Benjamin R. Kipp, Robert R. McWilliams, Scott Kopetz, Robert A. Wolff, and Axel Grothey

Author affiliations and support information (if applicable) appear at the end of this article.

Published at jco.org on May 9, 2017.

The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Corresponding author: Axel Grothey, MD, Mayo Clinic, 200 First St SW, Rochester, MN 55905; e-mail: grothey.axel@mayo.edu.

© 2017 by American Society of Clinical Oncology

0732-183X/17/3523w-2624w/\$20.00

ABSTRACT

Purpose

Molecular diagnostic testing has become an integral part of the evaluation of patients with metastatic colorectal cancer (CRC). Expanded mutational testing, such as next-generation sequencing (NGS), often identifies mutations with unclear clinical or prognostic implications. One such example is *BRAF* mutations that occur outside of codon 600 (non-V600 *BRAF* mutations).

Methods

We conducted this multicenter, retrospective cohort study to characterize the clinical, pathologic, and survival implications of non-V600 *BRAF* mutations in metastatic CRC. We pooled patients in whom non-V600 *BRAF* mutations were identified from NGS databases at three large molecular genetics reference laboratories.

Results

A total of 9,643 patients with metastatic CRC underwent NGS testing. We identified 208 patients with non-V600 *BRAF* mutations, which occurred in 2.2% of all patients tested and accounted for 22% of all *BRAF* mutations identified. Cancers with non-V600 *BRAF* mutations, compared with cancers with V600E *BRAF* (V600E *BRAF*) mutations, were found in patients who were significantly younger (58 v 68 years, respectively), fewer female patients (46% v 65%, respectively), and patients who had fewer high-grade tumors (13% v 64%, respectively) or right-sided primary tumors (36% v 81%, respectively). Median overall survival was significantly longer in patients with non-V600 *BRAF*-mutant metastatic CRC compared with those with both V600E *BRAF*-mutant and wild-type *BRAF* metastatic CRC (60.7 v 11.4 v 43.0 months, respectively; $P < .001$). In multivariable analysis, non-V600 *BRAF* mutation was independently associated with improved overall survival (hazard ratio, 0.18; $P < .001$).

Conclusion

Non-V600 *BRAF* mutations occur in approximately 2.2% of patients with metastatic CRC and define a clinically distinct subtype of CRC with an excellent prognosis.

J Clin Oncol 35:2624-2630. © 2017 by American Society of Clinical Oncology

INTRODUCTION

Molecular diagnostics, including testing for extended *RAS* (rat sarcoma) and *RAF* (rapid accelerated fibrosarcoma) mutations, have become a standard component of the initial work-up of patients with metastatic colorectal cancer (CRC). The role of *KRAS*, *NRAS*, and, to a lesser extent, *HRAS* mutations (collectively referred to as *RAS* mutations) in development, pathogenesis, and resistance to epidermal growth factor receptor (EGFR) antagonists has been well defined previously.¹ There is also emerging evidence of a similar resistance to EGFR inhibition in patients with V600E *BRAF* (V600E *BRAF*) mutations.² The

prognostic impact of V600E *BRAF* mutations in metastatic CRC is also clear and quite dramatic. Patients with V600E *BRAF*-mutant CRC survive, on average, less than half as long as patients with *BRAF* wild-type (WT *BRAF*) metastatic CRC.³⁻⁵

Advances in next-generation sequencing (NGS) have allowed for more comprehensive testing of multiple mutational hotspots within various genes of interest. These expanded mutational analyses helped to define the nearly 20% of patients who were originally classified as having *KRAS* wild-type (WT) metastatic CRC but were subsequently found to have exon 3 or 4 *KRAS* or *NRAS* mutations.⁶ In the case of these novel *RAS* mutations, the clinical phenotype was similar to those originally described in exon 2 *KRAS* mutations. It is clear that expanded

ASSOCIATED CONTENT

See accompanying Editorial on page 2598

DOI: <https://doi.org/10.1200/JCO.2016.71.4394>

mutational information can and should influence clinical practice; however, for the majority of identified mutations, the clinical, therapeutic, and prognostic implications remain unclear. One such example is mutations within the *BRAF* gene that occur outside of the 600th codon (non-*V600* *BRAF* mutations).

From a clinical perspective, nearly all of our current understanding of *BRAF*-mutant CRC has been derived from patients with the *V600E* *BRAF* mutation. The only report including clinical characteristics of patients with non-*V600* *BRAF*-mutant CRC described only 10 patients with codon 594 and 596 *BRAF* mutations.⁷ These patients had markedly different molecular, pathologic, and clinical features compared with patients who harbored *V600E* *BRAF* mutations. Given the limited sample size and exclusivity to mutations at codons 594 and 596, it is difficult to draw definitive conclusions with regard to the clinical phenotype of all non-*V600* *BRAF* mutations from this report alone. However, the implication that not all *BRAF* mutations have the same dismal prognosis is of great clinical and biologic interest. In this multicenter, retrospective cohort study, we aim to further characterize the clinical characteristics of all non-*V600* *BRAF* mutations by pooling NGS data from three large US reference laboratories.

METHODS

Study Design

After approval by the respective institutional review boards, patients were identified from three separate NGS databases from the Mayo Clinic (MC), The University of Texas MD Anderson Cancer Center (MDACC), and Foundation Medicine (FM). Each database contains mutational and basic demographic information from all patients with CRC who underwent

NGS at the three reference laboratories between the years of 2013 and 2016. Patients with non-*V600* *BRAF* mutations from all three institutions were identified and pooled for the primary analysis. Concurrently treated samples from 133 patients with *V600E* *BRAF*-mutant and 249 patients with *WT* *BRAF* metastatic CRC from MC were identified from the same NGS database and included for comparative analysis. Therefore, the study population consisted of 208 patients with non-*V600* *BRAF*-mutant, 133 patients with *V600E* *BRAF*-mutant, and 249 patients with *WT* *BRAF* metastatic CRC (Fig 1). Baseline demographic information was available for all patients. Clinical follow-up, histopathologic characteristics, and date of death information were collected from review of electronic medical records at both academic institutions. In the case of FM samples, histopathologic and demographic information was collected from the referral pathology reports. Clinical follow-up and death data were obtained from referring physicians and cross-referenced with Social Security Death Index searches for all patients.

NGS Testing Platforms

Pathologic specimens from all patients were submitted for clinically driven mutation profile testing at the three reference laboratories. DNA was extracted, purified, and quantified from formalin-fixed paraffin-embedded solid tumor specimens in a standard, clinically validated fashion at all laboratories. In the case of MC and MDACC samples, the Ampliseq Cancer Hotspot Panel v2 (Life Technologies, Carlsbad, CA) was used to perform multiplex polymerase chain reaction analysis containing 205 amplification reactions from genomic DNA for regions covering the mutation hotspots in 50 cancer-related genes. FM samples were tested in a Clinical Laboratory Improvement Amendments–certified laboratory performing hybrid-capture–based comprehensive genomic profiling of at least 3,769 exons of at least 236 cancer-related genes plus 47 introns from 19 genes frequently rearranged in cancer, as described previously.⁸

Statistical Analysis

Demographic, clinical, and molecular features were summarized for all patients and tested for differences according to *BRAF* status

Fig 1. Patient flow diagram. FM, Foundation Medicine; MC, Mayo Clinic; MDACC, The University of Texas MD Anderson Cancer Center; NGS, next-generation sequencing; WT, wild type.

(^{V600E}*BRAF*-mutant CRC ν non-^{V600}*BRAF*-mutant CRC ν ^{WT}*BRAF* CRC) and *BRAF* mutation type (^{V600E}*BRAF*-mutant CRC ν non-^{V600}*BRAF*-mutant CRC) using Fisher's exact tests for categorical variables and analysis of variance (three groups) or two-sample *t* tests (two groups) for continuous variables where approximate normality was satisfied. Otherwise, non-parametric Kruskal-Wallis tests (three groups) and Wilcoxon rank sum tests (two groups) were used.

Kaplan-Meier curves for overall survival (OS), defined as the time from metastatic diagnosis until death as a result of any cause, were plotted with 95% CIs according to *BRAF* mutation status, both overall and for MC patients only. Survival by *BRAF* status (overall and MC population) was statistically compared using a log-rank test, with $P < .05$ indicating statistical significance. To further quantify the prognostic effects, a Cox proportional hazards regression model was constructed from all patients with available outcome and *BRAF* status, where *BRAF* status served as a key covariate. The assumption of proportional hazards was tested using the method of Grambsch and Therneau.⁹ Subsequently, an adjusted multi-variable Cox regression model was constructed, controlling for age, sex, tumor grade (low-intermediate ν high), side of primary tumor (right ν left), *RAS* mutational status, and presence versus absence of liver, lung, peritoneal, lymph node, and other metastases. In the adjusted model, any secondary variables missing for less than 50% of patients were imputed using multiple imputation with predictive mean matching; variables with high missing data (eg, microsatellite instability [MSI] status) were not considered for imputation.¹⁰ Also, within the adjusted model, continuous variables such as patient age were modeled as potentially nonlinear on the log relative hazard scale using restricted cubic splines.^{11,12} To investigate whether the effect of *BRAF* status on clinical outcome differed by any other patient features, tests for two-way interactions between *BRAF* status and each of the other patient or disease variables were also performed, where an interaction $P < .01$ was deemed to indicate statistical significance.

Within the group of patients with non-^{V600}*BRAF* mutations, survival differences between those with codon 594 involvement versus involvement of other codons as well as differences between patients with activated versus impaired kinase activity were tested using a log-rank test, and where statistical significance was indicated, Cox proportional hazards modeling was used to report relative effects. All statistical analyses were performed using the R statistical programming language (version 3.2.2) from The R Project for Statistical Computing, with packages "rms" (version 3.3.2) for imputation and Cox modeling and "survminer" (version 3.3.2) for survival plotting.¹³

RESULTS

Within the pooled cohort, we identified a total of 208 patients with non-^{V600}*BRAF* mutations. The specific mutations, as well as their respective frequencies, are presented in Figure 2. The estimated overall prevalence of non-^{V600}*BRAF* mutations in patients with metastatic CRC who underwent NGS was approximately 2.2%, which accounted for 21.6% of all *BRAF* mutations (Table 1).

Clinical Characteristics

As summarized in Table 2, several demographic, clinical, and pathologic characteristics differed significantly by *BRAF* status, and specifically, these differences were most pronounced between patients with ^{V600E}*BRAF*-mutant versus non-^{V600}*BRAF*-mutant CRC. In terms of demographics, patients harboring a non-^{V600}*BRAF* mutation, compared with those with ^{V600E}*BRAF* mutations, were significantly younger (median age, 58 ν 68 years, respectively; $P < .001$) and less likely to be female (46% ν 65%, respectively; $P < .001$). In terms of clinical and pathologic characteristics,

Fig 2. Non-V600 *BRAF* codon mutation distribution.

patients with non-^{V600}*BRAF* mutations, compared with patients with ^{V600E}*BRAF* mutations, were significantly less likely to have high-grade tumors (13% ν 64%, respectively; $P < .001$) or right-sided primary tumors (37% ν 81%, respectively; $P < .001$). When comparing sites of metastatic disease, patients with non-^{V600}*BRAF* mutations were less likely than patients with ^{V600E}*BRAF* mutations to have peritoneal metastases (15% ν 59%, respectively; $P < .001$); no significant differences in the rates of liver, lung, or nodal metastases were noted.

Concomitant RAS Mutations and MSI

Patients with non-^{V600}*BRAF* mutations were more likely to have concomitant *RAS* mutations than patients with ^{V600E}*BRAF* mutations (26% ν 2%, respectively; $P < .001$). Patients with non-^{V600}*BRAF*-mutant CRC with concomitant *RAS* mutations were similar to patients without concomitant *RAS* mutations for most demographic, clinical, and pathologic features. Only right-sided primary tumors were more common in patients with concomitant *RAS* mutations compared with those without concomitant *RAS* mutations (54% ν 31%, respectively; $P = .02$). Patients with non-^{V600}*BRAF* mutations were less likely to have MSI than patients with ^{V600E}*BRAF* mutations (6% ν 30%, respectively; $P < .001$).

Survival Analysis

Complete follow-up and survival data were available for 101 patients with non-^{V600}*BRAF* mutations, 99 patients with ^{V600E}*BRAF* mutations, and 249 patients with ^{WT}*BRAF* tumors. Across all patients considered, *BRAF* status was significantly associated with OS (log-rank $P < .001$; Fig 3A); significance was maintained within the set of MC-only patients ($P < .001$; Fig 3B). Among all patients with ^{V600E}*BRAF* mutations, median OS time was 11.4 months (95% CI, 9.4 to 13.5 months). In contrast, patients with non-^{V600}*BRAF* mutations had a significantly longer median OS time of 60.7 months (95% CI, 36.5 to 81.2 months), exceeding the median OS of 43.0 months among patients with ^{WT}*BRAF* status (95% CI, 30.7 to 62.0 months; Fig 3). In single-variable Cox regression modeling, *BRAF* was significantly prognostic for OS ($P < .001$; hazard ratio [HR] for non-V600/^{V600E}, 0.14; 95% CI, 0.09 to 0.22; HR for non-V600/^{WT}, 0.62; 95% CI, 0.40 to 0.95;

Table 1. Patients Included in Three Databases

Measure	MC Database	MDACC Database	FM Database	All Databases
Patients with metastatic CRC included in database, No.	1,014	2,276	6,353	9,643
Patients with any <i>BRAF</i> mutation, No.	162	334	469	965
Patients with non-V600 <i>BRAF</i> mutations, No.	29	53	126	208
Prevalence of any <i>BRAF</i> mutation within tested population, % (95% CI)	16.0 (13.8 to 18.4)	14.7 (13.3 to 16.2)	7.4 (6.8 to 8.1)	10.0 (9.4 to 10.6)
Prevalence of non-V600 <i>BRAF</i> mutations within tested population, % (95% CI)	2.9 (2.0 to 4.1)	2.3 (1.8 to 3.1)	2.0 (1.7 to 2.4)	2.2 (1.9 to 2.5)

Abbreviations: CRC, colorectal cancer; FM, Foundation Medicine; MC, Mayo Clinic; MDACC, The University of Texas MD Anderson Cancer Center.

HR for V600E/WT, 4.30; 95% CI, 3.10 to 5.98). In multivariable analysis adjusting for other covariates potentially associated with OS (age at diagnosis, sex, histologic grade, primary tumor site, *RAS* mutational status, and presence ν absence of liver, lung, nodal, and peritoneal metastases), non-V600 *BRAF* mutations remained significantly associated with improved OS (HR, 0.18; 95% CI, 0.10 to 0.32; $P < .001$). No significant two-way interactions involving *BRAF* status were found; that is, the effect of *BRAF* status on OS was not mediated by any other patient, disease, or mutational characteristics. Median follow-up by *BRAF* cohort was as follows: 20.3 months for patients with WT *BRAF*, 25.9 months for patients with non-V600 *BRAF* mutations, and 22.8 months for patients with V600E *BRAF* mutations. In the lattermost group, the 27 patients without known death dates were right-censored (lost to follow-up) before 33.4 months (Fig 3). In the Cox regression models, the assumption of proportional hazards was found to be satisfied.

To confirm that OS was not solely influenced by the most common mutation in our cohort (codon 594, representing 45% of all non-V600 *BRAF* mutations), we compared survival for patients with non-V600 *BRAF* mutations at codon 594 with survival for patients with all other non-V600 *BRAF* mutations and found no significant difference in OS between the two groups, conceivably as a result of low sample size, although OS trended shorter among patients with non-V600 *BRAF* mutations involving codon 594 (38.5 months for codon 594 ν 61.9 months for other non-V600 codons; $P = .404$).

Finally, we compared OS times for patients with non-V600 *BRAF* mutations that are known to have increased in vitro basal kinase activity to those with known impaired kinase activity and those with unknown kinase activity, as described in Table 3.¹⁴⁻¹⁶ There was no significant difference in OS for patients with activated versus impaired kinase mutations (median OS, 37.5 ν 38.5 months, respectively; $P = .544$).

DISCUSSION

In this collaborative effort, we have defined and comprehensively characterized non-V600 *BRAF*-mutant CRC as a distinct molecular subset of CRC that has little to no clinical similarity to V600E *BRAF*-mutant CRC. By pooling sequencing data from nearly 10,000 patients, we are able to estimate the overall prevalence of non-V600 *BRAF* mutations among patients with metastatic CRC in the US population at approximately 2.2%.

We confirmed a number of previously described clinical and pathologic characteristics of V600E *BRAF*-mutant CRC such as female predominance, older age, right-sided primary tumors, preponderance of high-grade histology and MSI, and frequent peritoneal spread. All of these findings are in contrast to our findings in patients with non-V600 *BRAF* mutations.

In terms of survival analyses, we confirmed the poor prognostic impact of V600E *BRAF* mutations, with these patients having a median OS of only 11.4 months in our cohort, which is in keeping with previous studies in this population.^{3,5,17} However, we found a substantially longer median OS of 60.7 months in patients with non-V600 *BRAF* mutations. These survival estimates exceed not only the median OS of 43.0 months for WT *BRAF* patients in our cohort but also most other contemporary estimates of survival for patients with metastatic CRC in general.^{17,18} Of note, Cox proportional hazards models stratified by institution (ie, allowing for institution-specific baseline hazard functions) were additionally considered and were found to produce *BRAF* effects nearly identical to the pooled (unstratified) models. For this reason and because MDACC and FM only contributed survival data for patients with non-V600 *BRAF* mutations, we present only the pooled analyses for brevity.

There are a number of immediate clinical implications that can be drawn from our findings. First, we show that NGS-based testing identifies more than 20% of patients with *BRAF* mutations that would otherwise go undiagnosed using V600E *BRAF*-directed polymerase chain reaction tests. Thus, we characterized a subgroup of patients with metastatic CRC who are significantly more common than previously described. Second, we demonstrated that non-V600 *BRAF* mutations are distinctly different from V600E *BRAF* mutations in terms of clinical characteristics or prognostic impact. Patients with non-V600 *BRAF* mutations present with a less aggressive clinical phenotype and are likely to have significantly longer OS. It is conceivable that patients with non-V600 *BRAF* mutations will not require the aggressive chemotherapeutic regimens that have emerged as beneficial in V600E *BRAF*-mutant tumors, but might actually require a more measured approach to therapy given their overall excellent prognosis. Similarly, it is unclear whether CRCs with non-V600 *BRAF* mutations harbor the same resistance to anti-EGFR therapy as cancers with V600E *BRAF* mutations. However, concomitant *RAS* mutations, which occur more frequently in non-V600 *BRAF* mutants, will have to be considered in this scenario.

Although the immediate clinical implications of our findings are numerous, perhaps the most powerful insights come from understanding non-V600 *BRAF* mutations as naturally occurring models of *BRAF* signaling modulation that seem to escape the

Table 2. Baseline Demographic and Clinical Characteristics According to *BRAF* Mutation Status

Characteristic	No. of Patients (%)				Test 1 <i>P</i> *	Test 2 <i>P</i> †
	All Patients	<i>BRAF</i> V600E	<i>BRAF</i> Non-V600	<i>BRAF</i> WT		
Total	590 (100)	133 (23)	208 (35)	249 (42)		
Mean age, years (range)	61 (25-91)	68 (33-88)	58 (26-83)	60 (25-91)	< .001	< .001
Sex					< .001	< .001
Male	306 (52)	46 (35)	112 (54)	148 (59)		
Female	284 (48)	87 (65)	96 (46)	101 (41)		
Tumor grade					< .001	< .001
Low-Intermediate	363 (75)	35 (36)	117 (87)	211 (85)		
High	118 (25)	62 (64)	18 (13)	38 (15)		
Missing	102	36	73	0		
Lung metastases					< .001	.1333
Yes	133 (27)	11 (12)	29 (20)	86 (35)		
No	355 (73)	83 (88)	116 (80)	163 (65)		
Missing	102	39	63	0		
Liver metastases					.0337	.5475
Yes	281 (58)	46 (49)	78 (54)	157 (63)		
No	207 (42)	48 (51)	67 (46)	92 (37)		
Missing	102	39	63	0		
Peritoneal metastases					< .001	< .001
Yes	133 (27)	55 (59)	22 (15)	56 (22)		
No	355 (73)	39 (41)	123 (85)	193 (78)		
Missing	102	39	63	0		
LN metastases					.3102	1.000
Yes	116 (24)	25 (27)	39 (27)	52 (21)		
No	372 (76)	69 (73)	106 (73)	197 (79)		
Missing	102	39	63	0		
Other metastases					.2287	.2523
Yes	85 (17)	22 (23)	24 (17)	39 (16)		
No	403 (83)	72 (77)	121 (83)	210 (84)		
Missing	102	39	63	0		
Tumor side					< .001	< .001
Left	295 (60)	19 (19)	92 (63)	184 (74)		
Right	198 (40)	80 (81)	53 (37)	65 (26)		
Missing	97	34	63	0		
<i>RAS</i> status					< .001	< .001
MT	179 (31)	3 (2)	51 (26)	125 (50)		
WT	396 (69)	130 (98)	142 (74)	124 (50)		
Missing	15	0	15	0		
MSI status					< .001	< .001
Yes	33 (15)	13 (30)	6 (6)	14 (22)		
No	181 (85)	31 (70)	99 (94)	51 (78)		
Missing	376	89	103	184		
Codon					NA	NA
594			93 (45)			
Other			115 (55)			
Missing			0			
Kinase					NA	NA
Activated			44 (28)			
Impaired			112 (72)			
Missing			52			

Abbreviations: LN, lymph node; MSI, microsatellite instability; MT, mutant; NA, not available because only collected for non-V600 *BRAF* MT patients; WT, wild type.

*Test 1 *P* value: two-sided *P* value for test of differences in factor means across three levels of *BRAF*: WT v MT V600E v MT non-V600.

†Test 2 *P* value: two-sided *P* value for test of differences in factor means between *BRAF* V600E MT v *BRAF* non-V600 MT only.

paradoxical ERK reactivation that inevitably occurs with exogenous *BRAF* inhibitors. More than 1000 unique *BRAF* mutations have been previously described in patients with various malignancies.^{19,20} Although these reports have included little clinical information, attempts have been made to further classify the in vitro kinase activity and signaling properties of these mutations. One such effort was reported by Wan et al,¹⁴ who classified 17 non-V600 *BRAF* mutations according to their basal kinase activity compared with ^{WT}*BRAF*. Surprisingly, they found that even mutations with impaired kinase activity were able to phosphorylate

ERK nearly two-fold more than ^{WT}*BRAF*, but only in the presence of endogenous CRAF. This finding led to the discovery that impaired *BRAF* mutants, such as those that occur at codon 594, phosphorylate ERK by a mechanism involving *BRAF*-CRAF dimerization.^{21,22} The structural mechanism of this interaction was later found to be mediated by a dimerization interface (DIF), which is required for downstream signaling in both normal *RAF* and kinase-impaired *RAF* mutants.²¹

Given the reliance on *BRAF*-CRAF heterodimerization, for impaired mutants, it seems unlikely that the therapeutic use of

Fig 3. Kaplan-Meier estimates of overall survival (OS) according to *BRAF* mutation status, with simultaneous 95% CIs displayed as shaded regions, for (A) all institutions and (B) Mayo Clinic only.

selective BRAF inhibitors will offer any significant clinical benefit. In fact, these inhibitors stabilize a structural confirmation that induces dimerization similar to *BRAF* codon 594 mutants.²³ An ideal therapy in this case is one that disrupts the BRAF-CRAF DIF. Indeed, when impaired mutants are cultured with a peptide inhibitor of DIF, BRAF-CRAF dimerization is halted and RAF-induced ERK signaling ceases.²⁴ Although this same peptide inhibitor has little effect on

V600E *BRAF*-mutant cells, perhaps if used in combination with a potent BRAF kinase inhibitor, one could eliminate paradoxical ERK activation that inevitably leads to resistance.

A major strength of our study is that we pooled patient data from three large molecular genetics reference laboratories. Although rare at any single institution, non-V600 *BRAF* mutations make up more than 20% of all *BRAF* mutations identified in patients with metastatic CRC. Pooling data in this manner allowed for more robust clinical description and survival analyses.

There are a number of limitations of our study. First, our analysis is limited by the retrospective nature of our cohort. Given the relative scarcity of non-V600 *BRAF* mutations, it would be difficult to assemble a cohort of this size in a prospective fashion; nonetheless, there is risk for an inherent selection bias in retrospective studies such as this study. In defining non-V600 *BRAF*-mutant CRC as a distinct clinical entity, we hope to inform future clinical trial development in these patients, similar to that which has been undertaken for patients with ALK- or ROS1-positive, non-small-cell lung cancers, which are equally rare. Second, we were limited by the fact that we did not have complete clinical follow-up and treatment information available for all patients sampled. In attempts to reduce selection bias toward patients with more follow-up data, we included all patients with mutational and demographic data and limited individual analyses to patients with available data for the characteristic in question, with the exception of adjusted Cox regression modeling, where imputation was used to address missing data of secondary variables.

In summary, non-V600 *BRAF*-mutant metastatic CRC represents a molecular subtype of CRC with excellent prognosis and distinct clinical and pathologic characteristics. These findings not only have immediate clinical implications, but also provide valuable insights into a model of *BRAF* modulation that could potentially be exploited to better understand and treat *BRAF*-mutant cancers.

Table 3. In Vitro Kinase Activity by Specific Mutation

Mutation	No. of Patients
Kinase activity activated	44
K601E	9
G469A	7
G469V	6
L597R	6
K601N	4
G464V	3
N581S	2
L597Q	2
A598V	2
G464E	1
G466A	1
G469E	1
Kinase activity impaired	112
D594G	69
D594N	16
G466V	7
G466R	6
G466E	5
D594E	3
D594H	3
D594V	1
G596R	1
D594Y	1

NOTE. Data adapted.¹⁴⁻¹⁶

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Jeremy C. Jones, Lindsay A. Renfro, Humaid O. Al-Shamsi, Ben Y. Zhang, Jesse S. Voss, Jeffrey Ross, Joleen M. Hubbard, Benjamin R. Kipp, Robert R. McWilliams, Scott Kopetz, Robert A. Wolff, Axel Grothey

Provision of study materials or patients: Humaid O. Al-Shamsi, Pashtoon M. Kasi

Collection and assembly of data: Jeremy C. Jones, Lindsay A. Renfro, Humaid O. Al-Shamsi, Alexa B. Schrock, Andrew Rankin, Ben Y. Zhang, Pashtoon M. Kasi, Jesse S. Voss, Jeffrey Ross, Joleen M. Hubbard, Benjamin R. Kipp, Robert R. McWilliams, Scott Kopetz, Robert A. Wolff, Axel Grothey

Data analysis and interpretation: Jeremy C. Jones, Lindsay A. Renfro, Humaid O. Al-Shamsi, Ben Y. Zhang, Pashtoon M. Kasi, Alexis D. Leal, James Sun, Jeffrey Ross, Siraj M. Ali, Joleen M. Hubbard, Benjamin R. Kipp, Robert R. McWilliams, Robert A. Wolff, Axel Grothey

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

REFERENCES

- Douillard JY, Oliner KS, Siena S, et al: Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 369: 1023-1034, 2013
- Pietrantonio F, Petrelli F, Coinu A, et al: Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: A meta-analysis. *Eur J Cancer* 51: 587-594, 2015
- Richman SD, Seymour MT, Chambers P, et al: KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: Results from the MRC FOCUS trial. *J Clin Oncol* 27: 5931-5937, 2009
- Tran B, Kopetz S, Tie J, et al: Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. *Cancer* 117:4623-4632, 2011
- Van Cutsem E, Köhne C-H, Láng I, et al: Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: Updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol* 29:2011-2019, 2011
- Allegra CJ, Rumble RB, Hamilton SR, et al: Extended RAS gene mutation testing in metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy: American Society of Clinical Oncology Provisional Clinical Opinion Update 2015. *J Clin Oncol* 34:179-185, 2016
- Cremolini C, Di Bartolomeo M, Amatu A, et al: BRAF codons 594 and 596 mutations identify a new molecular subtype of metastatic colorectal cancer at favorable prognosis. *Ann Oncol* 26:2092-2097, 2015
- Frampton GM, Fichtenholtz A, Otto GA, et al: Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol* 31:1023-1031, 2013
- Grambsch PM, Therneau TM: Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika* 81:515-526, 1994
- Little RJA, Rubin DB: *Statistical Analysis With Missing Data* (ed 2). Hoboken, NJ, Wiley, 2002
- Harrell FE: *Regression Modeling Strategies: With Applications to Linear Models, Logistic Regression, and Survival Analysis*. New York, NY, Springer, 2001
- Steyerberg EW: *Clinical Prediction Models: A Practical Approach to Development, Validation, and Updating*. New York, NY, Springer, 2009
- The R Core Team: *R: A Language and Environment for Statistical Computing*. Vienna, Austria, R Foundation for Statistical Computing, 2008
- Wan PT, Garnett MJ, Roe SM, et al: Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* 116:855-867, 2004
- Zheng G, Tseng LH, Chen G, et al: Clinical detection and categorization of uncommon and concomitant mutations involving BRAF. *BMC Cancer* 15:779, 2015
- Noeparast A, Teugels E, Giron P, et al: Non-V600 BRAF mutations recurrently found in lung cancer predict sensitivity to the combination of trametinib and dabrafenib. *Oncotarget* 10.18632/oncotarget.11635 [epub ahead of print on August 26, 2016]
- Loupakis F, Cremolini C, Masi G, et al: Initial therapy with FOLFIRI and bevacizumab for metastatic colorectal cancer. *N Engl J Med* 371: 1609-1618, 2014
- Heinemann V, von Weikersthal LF, Decker T, et al: FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): A randomised, open-label, phase 3 trial. *Lancet Oncol* 15:1065-1075, 2014
- Davies H, Bignell GR, Cox C, et al: Mutations of the BRAF gene in human cancer. *Nature* 417: 949-954, 2002
- Forbes SA, Beare D, Gunasekaran P, et al: COSMIC: Exploring the world's knowledge of somatic mutations in human cancer. *Nucleic Acids Res* 43:D805-D811, 2015
- Rajakulendran T, Sahmi M, Lefrançois M, et al: A dimerization-dependent mechanism drives RAF catalytic activation. *Nature* 461:542-545, 2009
- Heidorn SJ, Milagre C, Whittaker S, et al: Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. *Cell* 140: 209-221, 2010
- Lavoie H, Thevakumaran N, Gavory G, et al: Inhibitors that stabilize a closed RAF kinase domain conformation induce dimerization. *Nat Chem Biol* 9: 428-436, 2013
- Freeman AK, Ritt DA, Morrison DK: Effects of Raf dimerization and its inhibition on normal and disease-associated Raf signaling. *Mol Cell* 49: 751-758, 2013

Affiliations

Jeremy C. Jones, Lindsay A. Renfro, Ben Y. Zhang, Pashtoon M. Kasi, Jesse S. Voss, Alexis D. Leal, Joleen M. Hubbard, Benjamin R. Kipp, Robert R. McWilliams, and Axel Grothey, Mayo Clinic, Rochester, MN; Humaid O. Al-Shamsi, Scott Kopetz, and Robert A. Wolff, The University of Texas MD Anderson Cancer Center, Houston, TX; and Alexa B. Schrock, Andrew Rankin, James Sun, Jeffrey Ross, and Siraj M. Ali, Foundation Medicine, Cambridge, MA.

Support

Supported by Clinical and Translational Science Award Grant No. KL2 TR000136 from the National Center for Advancing Translational Science.

Prior Presentation

Presented, in part, at the 52nd Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, June 3-7, 2016.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTERESTNon-V600 **BRAF** Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/jco/site/ifc.

Jeremy C. Jones

No relationship to disclose

Lindsay A. Renfro

No relationship to disclose

Humaid O. Al-Shamsi

No relationship to disclose

Alexa B. Schrock

Employment: Foundation Medicine

Stock or Other Ownership: Foundation Medicine

Andrew Rankin

Employment: Foundation Medicine

Leadership: Foundation Medicine

Stock or Other Ownership: Foundation Medicine

Research Funding: Foundation Medicine

Travel, Accommodations, Expenses: Foundation Medicine

Ben Y. Zhang

No relationship to disclose

Pashtoon M. Kasi

No relationship to disclose

Jesse S. Voss

No relationship to disclose

Alexis D. Leal

No relationship to disclose

James Sun

Employment: Foundation Medicine

Stock or Other Ownership: Foundation Medicine

Jeffrey Ross

Employment: Foundation Medicine

Leadership: Foundation Medicine

Stock or Other Ownership: Foundation Medicine

Research Funding: Foundation Medicine (Inst)

Siraj M. Ali

No relationship to disclose

Joleen M. Hubbard

Consulting or Advisory Role: Genentech (Inst), Boehringer Ingelheim (Inst)

Research Funding: Genentech (Inst), Boston Biomedical (Inst), Senhwa Biosciences (Inst), Bayer (Inst), Merck (Inst)

Benjamin R. Kipp

Research Funding: Abbott Molecular (Inst)

Patents, Royalties, Other Intellectual Property: Abbott Molecular

Robert R. McWilliams

Consulting or Advisory Role: Merrimack (Inst)

Research Funding: PRISM BioLab (Inst), Genentech (Inst), Novartis (Inst), Newlink Genetics (Inst), Eli Lilly (Inst), Aduro Biotech (Inst), Pfizer (Inst), Sanofi (Inst)

Travel, Accommodations, Expenses: AstraZeneca

Scott Kopetz

Stock or Other Ownership: MolecularMatch

Consulting or Advisory Role: Amgen, Roche, Merrimack, Bayer, Taiho Pharmaceutical, Sanofi, Array BioPharma, Genentech, MolecularMatch

Research Funding: Roche, Amgen, GlaxoSmithKline, Sanofi, Sysmex, Biocartis, Guardant Health, Agendia

Robert A. Wolff

Honoraria: Precision Medicine Research Associates, The Queens Health System

Consulting or Advisory Role: Precision Medicine Research Associates, Clovis Oncology

Patents, Royalties, Other Intellectual Property: Royalties from McGraw-Hill: Editor: *The MD Anderson Manual of Medical Oncology*, 3rd edition

Axel Grothey

Consulting or Advisory Role: Genentech (Inst), Bayer (Inst), Sanofi (Inst), Bristol-Myers Squibb (Inst), Eli Lilly (Inst), Boston Biomedical (Inst), Amgen (Inst)

Research Funding: Genentech (Inst), Bayer (Inst), Pfizer (Inst), Eisai (Inst), Sanofi (Inst), Eli Lilly (Inst), Boston Biomedical (Inst), Boehringer Ingelheim (Inst)

Travel, Accommodations, Expenses: Genentech, Bayer, Bristol-Myers Squibb, Boston Biomedical, Amgen, Boehringer Ingelheim