Atypical, Non-V600 BRAF Mutations as a Potential Mechanism of Resistance to EGFR Inhibition in Metastatic Colorectal Cancer

Benny Johnson, DO[1]; Jonathan M. Loree, MD, MS[2]; Alexandre A. Jacome, MD, PhD[1]; Shehara Mendis, MBBS[2]; Muddassir Syed, MS[1]; Van K. Morris II, MD[1]; Christine M. Parseghian, MD[1]; Arvind Dasari, MD[1]; Shubham Pant, MD[1]; Victoria M. Raymond, MS[3]; Eduardo Vilar, MD, PhD[1]; Michael Overman, MD[1]; Bryan Kee, MD[1]; Cathy Eng, MD[1]; Kanwal Raghav, MD[1]; and Scott Kopetz, MD, PhD[1]

PURPOSE Atypical, non-V600 BRAF (*aBRAF*) mutations represent a rare molecular subtype of metastatic colorectal cancer (mCRC). Preclinical data are used to categorize *aBRAF* mutations into class II (intermediate to high levels of kinase activity, *RAS* independent) and III (low kinase activity level, *RAS* dependent). The clinical impact of these mutations on anti-EGFR treatment efficacy is unknown.

PATIENTS AND METHODS Data from 2,084 patients with mCRC at a single institution and from an external cohort of 5,257 circulating tumor DNA (ctDNA) samples were retrospectively analyzed. Overall survival (OS) was calculated using Kaplan-Meier and log-rank tests. Statistical tests were two-sided.

RESULTS BRAF mutations were harbored by 257 patients, including 36 with *aBRAF* mutations: 22 class III, 10 class II, four unclassified. For patients with *aBRAF* mCRC, median OS was 36.1 months, without a difference between classes, and median OS was 21.0 months for patients with *BRAF^V600E* mCRC. In contrast to right-sided predominance of tumors with *BRAF^V600E* mutation, 53% of patients with *aBRAF* mCRC had left-sided primary tumors. Concurrent *RAS* mutations were noted in 33% of patients with *aBRAF* mCRC, and 67% of patients had microsatellite stable disease. Among patients with *aBRAF RAS* wild-type mCRC who received anti-EGFR antibodies (monotherapy, n = 1; combination therapy, n = 10), no responses to anti-EGFR therapy were reported, and six patients (four with class III *aBRAF* mutations, one with class II, and one unclassified) achieved stable disease as best response. Median time receiving therapy was 4 months (range, 1 to 16). In the ctDNA cohort, there was an increased prevalence of *aBRAF* mutations and subclonal *aBRAF* mutations (*P* < .001 for both) among predicted anti-EGFR exposed compared with nonexposed patients.

CONCLUSION Efficacy of anti-EGFR therapy is limited in class II and III *aBRAF* mCRC. Detection of *aBRAF* mutations in ctDNA after EGFR inhibition may represent a novel mechanism of resistance.

INTRODUCTION

BRAF^V600E missense mutations are present in 6% to 10% of patients with metastatic colorectal cancer (mCRC).[1,2] Within the BRAF kinase domain, substitution of a valine to glutamic acid at position 600 manifests as constitutive activation and oncogenic signaling along the mitogen-activated protein kinase (MAPK) pathway.[1] *BRAF^V600E* mCRC represents an aggressive molecular subtype of colorectal cancer inherently refractory to standard chemotherapy; thus, tremendous research focus has been directed toward novel therapeutic development.[2] Because of increased use of next-generation sequencing (NGS) in the management of mCRC, various mutational hotspots of clinical significance have emerged within genes of interest, such as expanded *RAS* testing. However, with such broad testing including circulating tumor DNA (ctDNA), alterations of evolving significance without clear predictive, prognostic, or therapeutic implications have also been identified. Atypical, non-V600

BRAF (*aBRAF*) mutations that occur outside of codon 600 are one example of an emerging molecular subtype in colorectal cancer.

aBRAF mutations were retrospectively studied at two large centers comprehensively describing the clinical, pathologic, and survival implications of these mutations in patients with mCRC.[3] A total of 9,643 patients with mCRC underwent NGS testing and 208 patients with *aBRAF* mutations were identified, representing 2.2% of all patients tested. Interestingly, these patients had distinct clinical features from those with traditional *BRAF^V600E* mCRC, with median overall survival (mOS) significantly longer (60.7 months) than that of patients with *BRAF^V600E* (11.4 months) mCRC or wild-type BRAF mCRC (43.0 months).[3] Although this represents an excellent prognosis comparatively, these patients still ultimately succumb to the disease. In addition, the chronicity of their disease suggests a need for improved, novel, targeted therapeutics that

CONTEXT

Key Objective

To determine if atypical, non-V600 BRAF (*aBRAF*) mutations, stratified by class, affect outcomes with anti-EGFR therapy and, secondarily, if acquisition of *aBRAF* mutations imparts resistance to EGFR inhibition.

Knowledge Generated

Clinical outcomes on the basis of functional class and their effect on anti-EGFR efficacy for *aBRAF* metastatic colorectal cancer (mCRC) have not been defined. In patients with *aBRAF* wild-type *RAS* mCRC who received anti-EGFR antibodies, there were no responses among class II or III *aBRAF* mCRC, with stable disease as best response in our internal cohort. Evaluation of a large external cohort of patients with circulating tumor DNA interrogated by an anti-EGFR exposure score revealed increased prevalence of *aBRAF* and subclonal *aBRAF* mutations among predicted anti-EGFR exposed compared with nonexposed patients, suggesting an acquired mechanism of resistance to EGFR inhibition.

Relevance

Anti-EGFR therapy is limited in *aBRAF* mCRC, with class II *aBRAF* mutations emerging as a negative predictive biomarker. Detection of *aBRAF* mutations in ctDNA may represent a novel mechanism of resistance warranting additional investigation.

can be used sequentially throughout the course of the disease.

Although *BRAF^V600E^* mCRC is known to be predictive of poor response to anti-EGFR therapy, the clinical utility of EGFR inhibition in *aBRAF* mCRC remains unclear.[4,5] Of note, previous retrospective work investigating a cohort of 150 patients with refractory mCRC identified seven patients with *aBRAF* mutations and reported poor progression-free survival when they were exposed to anti-EGFR therapy in comparison with a cohort with *RAS*/RAF wild-type (*RAS/RAF^WT^*) mCRC.[6] Preclinical work has highlighted unique biology among traditional *BRAF^V600E^* mutations (class I) and *aBRAF* mutations (classes II and III).[7,8] *BRAF^V600E^* mutations, designated as class I, result in feedback inhibition of GTP-bound *RAS*, are *RAS* independent, and signal as active monomers. In contrast, class II *aBRAF* mutations have intermediate to high levels of kinase activity, are *RAS* independent, signal as constitutive dimers, and are resistant to vemurafenib, whereas class III *aBRAF* mutations have low or absent kinase activity and are primarily *RAS* dependent and sensitive to ERK-dependent feedback of *RAS*.[7,8] These tumors bind more tightly to *RAS*-GTP than do wild-type BRAF tumors, and binding to wild-type CRAF is enhanced, resulting in increased ERK signaling.[8] These stark differences in underlying tumor biology may explain the varying response to therapy, specifically the potential limitations to anti-EGFR inhibition in *aBRAF* mCRC. To date, clinical outcomes on the basis of functional class designation and the potential effect on anti-EGFR efficacy for *aBRAF* mCRC have not been well defined.

In this study, we aimed to describe the prevalence of *aBRAF* mutations in a large data set of patients who underwent NGS and disease treatment at a single institution; highlight the landscape of *aBRAF* by reviewing the clinical, pathologic, and molecular characteristics of tumors with these mutations; and analyze the survival outcomes of patients with *aBRAF* mCRC exposed to anti-EGFR therapy to define the efficacy of EGFR inhibition in this rare subset and define the frequency of *aBRAF* mutations in patients who underwent ctDNA testing to elucidate the potential role of the mutations in EGFR inhibition resistance.

PATIENTS AND METHODS

This study used an internal cohort created retrospectively with institutional review board approval at the University of Texas MD Anderson Cancer Center (MDACC), and a waiver of written consent was obtained. All databases used were deidentified, and the study adhered to MDACC institutional review board guidelines for deidentified databases. Specific clinico-pathologic, mutational, molecular, and outcomes data for patients with *aBRAF* mCRC were reviewed and collected from this cohort. Cohort 1 included patients with stage IV colorectal cancer who were seen at MDACC between March 1, 2012, and June 27, 2017, and underwent targeted exome sequencing. Biopsy samples for sequencing were obtained from primary tumor (n = 18 patients) and metastatic sites (n = 18 patients). In regard to timing of the samples, 26 patients underwent molecular profiling at initial stage IV diagnosis (before therapy) and 10 patients were profiled subsequently. All clinical and outcomes data were derived from this cohort.

Cohort 2 included patients with mCRC who underwent a targeted NGS ctDNA assay (Guardant360; Guardant Health, Redwood City, CA) as part of their care between June 1, 2014, and December 26, 2017, at multiple institutions. Details of the assay, including detection limit of sensitivity and coverage (covers all exons of *BRAF*), have been published.[9,10] Treatment histories were not known for this cohort; therefore, we used a previously validated and highly specific score to define EGFR-exposed patients.[11] We calculated the prevalence and relative mutational allele

frequencies for both cohorts of *aBRAF* mutations: predicted anti-EGFR exposed and nonexposed.

Statistical Analysis

Clinicopathologic features were compared by χ^2 test or Fisher's exact test for categorical variables and assessment of odds ratios for associations as appropriate, as well as by *t* test to compare continuous variables. Overall survival (OS) was calculated using Kaplan-Meier method and log-rank tests. OS was defined as the time from diagnosis with stage IV disease until death. Matched analysis adjusted by age and sidedness was undertaken to compare OS among all molecular classes. χ^2 or Fisher's exact tests, as well as *t* test, were used to evaluate the adequacy of the matching. Statistical analyses were performed using GraphPad Prism, version 6.07 (GraphPad Software, La Jolla, CA), and SPSS, version 23.0 (IBM, Armonk, NY). All statistical tests were two sided, and $P < .05$ was considered statistically significant.

RESULTS

In cohort 1 (n = 2,084), *aBRAF* mutations occurred in 36 patients with mCRC (prevalence, 1.7%; 95% CI, 1.2% to 2.4%) compared with 221 patients with V600E mutations (prevalence 10.6%; 95% CI, 9.3% to 12.0%; Fig 1; Table 1). In terms of functional class designation, there were 22 patients with class III *aBRAF* mCRC, 10 with class II, and four unclassified patients. The most common class II and class III mutated BRAF codons were 469 (60%) and 594 (59%), respectively (Appendix Fig A1). mOS for *aBRAF* mCRC was 36.1 months (95% CI, 19.9 to 52.3), without difference between class III and II BRAF mCRC (Fig 2). Patients with *BRAF^V600E* mCRC had an mOS of 21.0 months (95% CI, 18.6 to 22.9) and patients with *RAS/RAF^WT* mCRC (n = 438) had a mOS of 42.3 months (95% CI, 36.9 to 47.8; Fig 2). Statistical significance was noted when *aBRAF* mCRC was compared with *BRAF^V600E* mCRC ($P < .001$) and when *RAS/RAF^WT* mCRC was compared with *BRAF^V600E* mCRC ($P < .001$). Among 36 patients with *aBRAF* mCRC, 19 (53%) had left-sided primary tumors and 24 (67%) had microsatellite stable (MSS) disease and were a median age of 56 years (Table 1). Of note, in contrast to *BRAF^V600E* mCRC, which is mutually exclusive with *RAS* mutations, 12 patients with *aBRAF* mCRC had *RAS* mutations (class III, n = 7 of 21 [33%]; class II, n = 5 of 10 [50%]). Among these, one patient with class II *aBRAF* mCRC also had microsatellite instability–high (MSI-H) disease.

For a more accurate comparison of survival outcomes among respective molecular classes, we performed a matched analysis by age and sidedness among the 36 patients with *aBRAF* mCRC compared with 36 patients with *BRAF^V600E* mCRC and 36 with *RAS/RAF^WT* disease (Fig 3; Appendix Table A1). OS was 36.1 months for patients with *aBRAF* mCRC (95% CI, 19.9 to 52.3 months), 18.9 months for those with *BRAF^V600E* mCRC (95% CI, 15.4 to 22.5 months), and 48.0 months for those with *RAS/RAF^WT* mCRC (95% CI, 15.4 to 80.6 months). Statistical significance was noted when *aBRAF* mCRC was compared with *BRAF^V600E* mCRC ($P = .003$) and when *RAS/RAF^WT* mCRC was compared with *BRAF^V600E* mCRC ($P < .001$).

Clinical Efficacy of Anti-EGFR Monoclonal Antibodies in Patients With *aBRAF* mCRC

To describe the clinical efficacy of EGFR inhibition in *aBRAF* mCRC, we assessed survival outcomes for patients
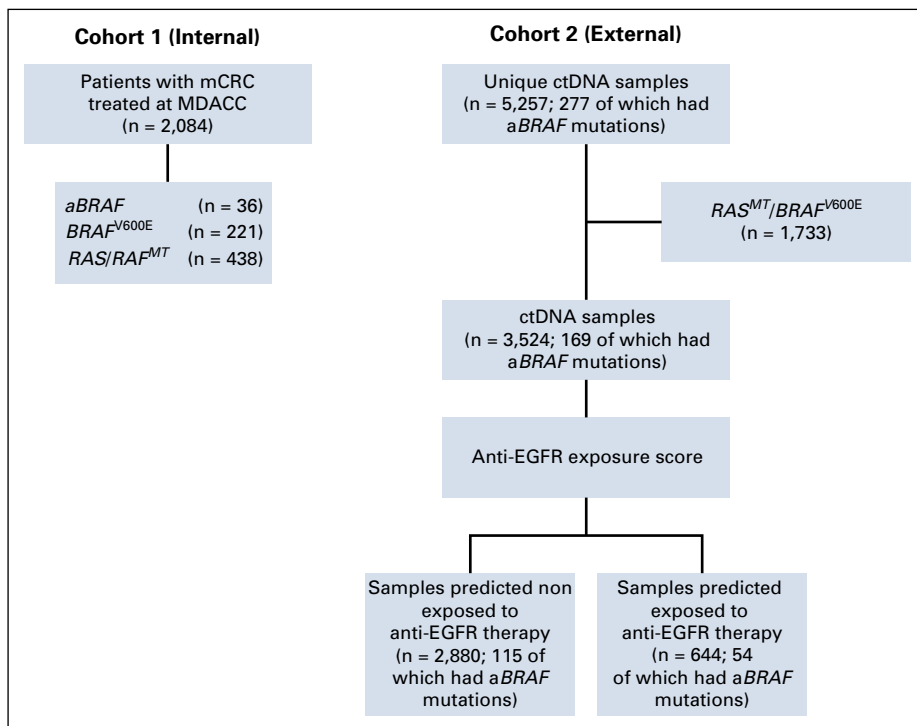

FIG 1. Study CONSORT diagram. *aBRAF*, atypical, non-V600 BRAF; ctDNA, circulating tumor DNA; mCRC, metastatic colorectal cancer; MDACC, MD Anderson Cancer Center; MT, mutant type.

TABLE 1. Baseline Characteristics of Cohort 1

Characteristic	aBRAF (n = 36)	BRAF^{V600E} (n = 221)	RAS/RAF^{WT} (n = 438)
Age, years, median (range)	56 (36-80)	61 (51-68)	54 (19-82)
Sex			
Male	20 (55)	113 (51)	265 (61)
Female	16 (45)	108 (49)	173 (39)
Sidedness			
Right	14 (39)	144 (65)	88 (20)
Left	22 (61)	77 (35)	350 (80)
RAS			
Wild type	22 (61)	217 (98)	438 (100)
Mutated	12 (33)	4 (2)	–
Microsatellite instability			
MSI-H	3 (8)	23 (10)	11 (3)
MSS	25 (69)	136 (62)	342 (78)
Unknown	8 (22)	62 (28)	85 (19)
BRAF class			
II	10 (28)	–	–
III	22 (61)	–	–
Unclassified	4 (11)	–	–
Anti-EGFR exposure			
Yes	11 (31)	10 (5)	–
No	25 (69)	211 (95)	–

Data reported as No. (%) unless otherwise indicated.

Abbreviations: –, not applicable; aBRAF, atypical, non-V600 BRAF; MSI-H, microsatellite instability–high; MSS, microsatellite stable; WT, wild type.

exposed to anti-EGFR therapy in their treatment course. Among patients with aBRAF RAS^{WT} mCRC, 11 (50%) received anti-EGFR monoclonal antibodies (mAbs; monotherapy, n = 1, combination therapy, n = 10; class III, n = 7 of 14 [50%], class II, n = 3 of 5 [60%], unclassified, n = 1). There were no responses reported, and six patients (class III, n = 4; class II, n = 1; unclassified, n = 1) achieved stable

FIG 2. Overall survival according to BRAF mutation status. aBRAF, atypical, non-V600 BRAF.

disease as best response. Median time receiving anti-EGFR therapy was 4 months (range, 1 to 16). Patients with class II RAS^{WT} mCRC treated with anti-EGFR mAbs had an mOS of 31.7 months versus 46.8 months for those not exposed (hazard ratio, 2.0; 95% CI, 0.3 to 15.9). Median time receiving therapy for patients with class II aBRAF mCRC was 3 months. Patients with class III RAS^{WT} mCRC treated with anti-EGFR mAbs had an mOS of 44.2 months versus 45.7 months for those not treated (hazard ratio, 0.80; 95% CI, 0.2 to 2.6). Median time receiving therapy for patients with class III aBRAF mCRC was 5 months. In line with previous reports, patients with class I or BRAF^{V600E} mCRC had worse survival outcomes when exposed to EGFR inhibition (Fig 4).

aBRAF Mutations as a Potential Mechanism of Resistance to Anti-EGFR Therapy

To understand our institutional findings of limited efficacy of anti-EGFR therapy in aBRAF mCRC, we analyzed an external ctDNA cohort of 5,257 samples (cohort 2) from 4,465 patients with mCRC. Of 277 aBRAF mutations identified, 24 were class II, 56 were class III, and 197 unclassified. Of note, 62% of aBRAF mutations were subclonal, compared with only 18% of the BRAF^{V600E} mutations. Considering the lack of treatment history in this
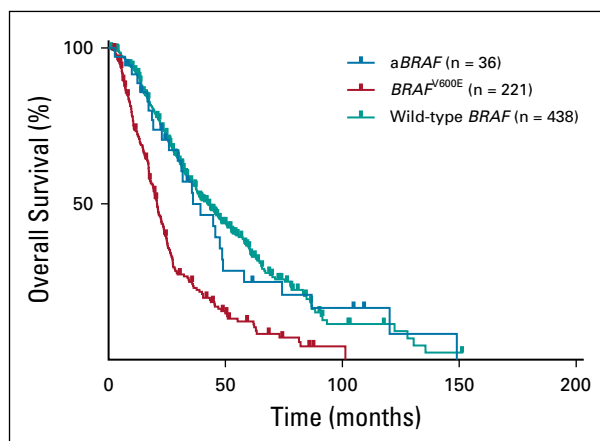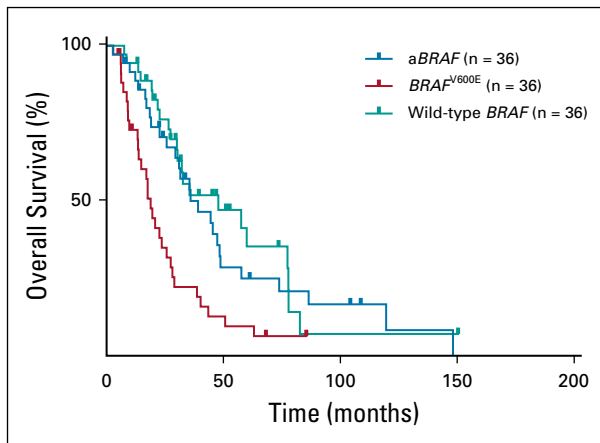
FIG 3. Overall survival with matched analysis. *aBRAF*, atypical, non-V600 BRAF.

cohort, after excluding *RAS* and *BRAF^V600E* mutations, we applied a previously validated anti-EGFR exposure score to divide patients into predicted prior exposure to anti-EGFR (n = 644) versus predicted nonexposed (n = 2,880).[10] This revealed that the prevalence of *aBRAF* mutations increased from 3.99% among patients predicted to have no prior anti-EGFR exposure to 8.38% among those with predicted anti-EGFR exposure (*P* < .001; Fig 5A).

As a sensitivity analysis, we evaluated the prevalence of *aBRAF* mutations among patients with EGFR ectodomain mutations, compared with those without, because EGFR ectodomain mutations are unequivocally present only after exposure to EGFR inhibition. We found that the prevalence of *aBRAF* mutations increased from 6.6% among patients without EGFR ectodomain mutations to 12.8% among those with EGFR ectodomain mutations (*P* = .039).

We then calculated the relative mutational allele frequencies for both cohorts of *aBRAF* mutations (anti-EGFR exposed and nonexposed) and found an increase in subclonal *aBRAF* mutations among patients with predicted anti-EGFR exposure (*P* < .001; Fig 5B). When stratified by class designation, both class II and class III *aBRAF* mutations were found to increase in prevalence among
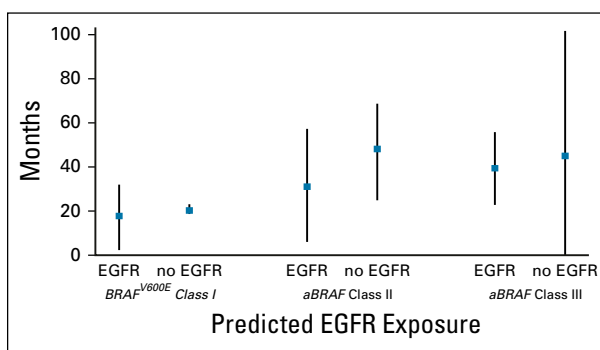
FIG 4. Overall survival among *BRAF* mCRC classes according to anti-EGFR exposure.

patients with predicted anti-EGFR exposure compared with patients with predicted nonexposure. Patients with class II *aBRAF* mCRC had a prevalence of 0.34% among patients predicted to not have anti-EGFR therapy exposure versus 1.24% among those with predicted anti-EGFR exposure (*P* = .009). The prevalence of patients with class III *aBRAF* mCRC increased from 0.94% among nonexposed patients to 2.48% among those predicted to have anti-EGFR exposure (*P* = .004). Class II activating G469A mutations (*P* < .001) and class III D594N (*P* < .001) were the most common alterations among patients with predicted anti-EGFR exposure (Fig 5C). Furthermore, we observed multiple *aBRAF* mutations present in ctDNA samples of individual patients, with the prevalence of multiple *aBRAF* mutations increasing from 0.5% among nonexposed patients to 3.5% among those with predicted anti-EGFR exposure (*P* < .001).

DISCUSSION

Patients with *aBRAF* mCRC have class-specific biochemical signaling mechanisms with a unique clinical phenotype.[3,7,8] In fact, unlike *BRAF^V600E* mCRC, patients with *aBRAF* mutations tend to be younger, male, and more often have left-sided colon tumors without MSI-H.[3,12-15] These distinct differences highlight the importance of understanding tumor biology in *aBRAF* mCRC to provide a more personalized therapeutic approach moving forward.

Although activation of the MAPK pathway is critical for both *BRAF^V600E* and *aBRAF* mCRC, preclinical work suggests these mutations differ in their signaling mechanisms, so much so that *aBRAF* tumors can be categorized into respective classes.[8] For instance, *aBRAF* tumors can have impaired kinase activity but still activate the signaling pathway through dimerization, whereas *BRAF^V600E* tumors activate as monomers. As a result, inhibiting BRAF is not as effective for *aBRAF* tumors. Agents such as vemurafenib are not active, because the drug is designed to target the shape of the V600E-mutated protein and is more than 10-fold less active against nonmutated forms. This fundamental difference highlights why traditional BRAF monomer inhibitors are ineffective in the management of *aBRAF* mCRC. Dimerization sets *aBRAF* classes II and III apart from traditional *BRAF^V600E* (class I) mutations and is a critical distinction that must be appreciated to best target these alterations in the future.

Aligning with earlier reports, we found that *aBRAF* mCRC had an underwhelming response to anti-EGFR therapy.[6,16-18] Specifically, class II *RAS^WT* *aBRAF* mCRC seems to have a *BRAF^V600E*-like phenotype in its response to anti-EGFR exposure, which may be explained by the similarity of *RAS*-independent MAPK signaling between these subtypes. Our cohort highlights that those patients with class II *aBRAF* mCRC exposed to anti-EGFR therapy may have inferior outcomes than those who are nonexposed. This is suggestive of class II *aBRAF* mutations
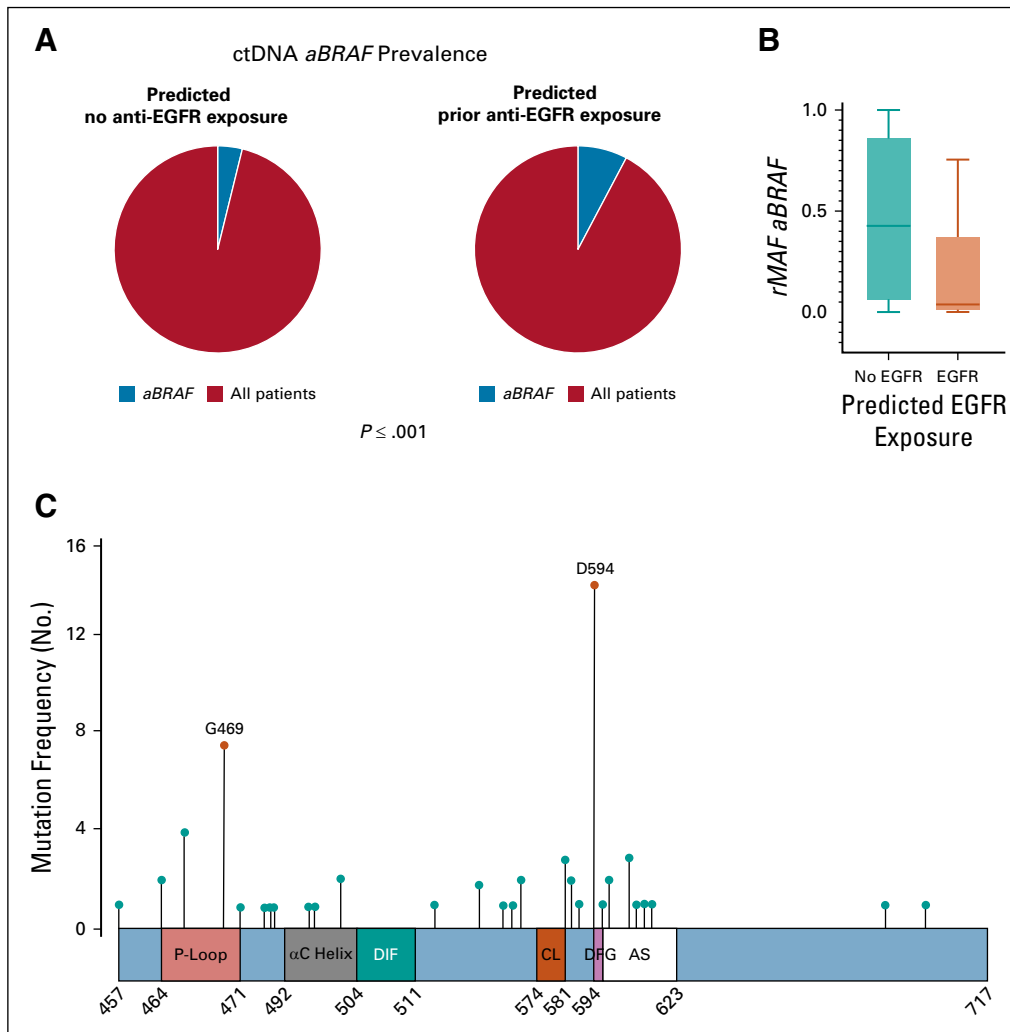
FIG 5. Circulating tumor DNA (ctDNA) analysis of atypical, non-V600 BRAF (aBRAF) in cohort 2. (A) ctDNA prevalence of aBRAF (3.99%) among non-exposed patients (n = 2,880) v ctDNA prevalence of aBRAF (8.38%) among predicted anti-EGFR exposed (n = 644). (B) Relative mutational allele frequency (rMAF) of aBRAF among predicted anti-EGFR exposed v nonexposed patients, $P < .001$. (C) Frequency and location of mutations in the BRAF protein in mCRC patients predicted to be exposed to anti-EGFR antibodies. Schematic figure of the conserved region-3 (CR3) of the BRAF protein, also known as the BRAF kinase domain (amino acids 457 to 717), and the location of the class II and III hotspot mutations. The kinase domain contains the P-loop (amino acids 464-471), the αC helix (amino acids 492-504), the dimerization interface (DIF, amino acids 504-511), the catalytic loop (CL, amino acids 574-581), the DFG motif (amino acids 594-596), and the activation segment (AS, amino acids 594-623). class II, activating G469A mutations ($P < .001$) and class III, D594N ($P < .001$) were the most common, respectively, among predicted anti-EGFR exposed patients. Figure adapted.[12] rMAF, relative mutational allele frequency.

reflecting a potential negative predictive biomarker for EGFR inhibition. Although previous reports have suggested class III aBRAF mCRC may achieve responses with anti-EGFR therapy owing to RAS dependency, our cohort, albeit small, only revealed stable disease as best response.[6,8,12-15,19] These results support the findings of the BREAC study, which revealed no responses in six patients with aBRAF mCRC (n = 2 each with class II, class III, and unclassified).[6]

Of note, the finding of concomitant RAS mutations with aBRAF mCRC in tissue, confirmed in our external ctDNA cohort, clearly precludes the use of EGFR inhibition for all patients,

highlighting the need for novel combination strategies that consider this distinct signaling biology.[20,21] In our internal and external cohorts, we did not find a difference between patients with class II and class III aBRAF mCRC in regard to the presence of concurrent RAS mutations. Therefore, continued investigation is warranted to understand the physiologic role of concomitant RAS mutations in patients with aBRAF mutations. Also, whether the actionability of aBRAF mutations in patients with MSI-H disease would be less than in those with MSS disease warrants additional investigation; conclusions could not be made due to the sample size of our internal cohort.

In our MDACC internal cohort, four patients had unclassified disease (*Y656D, A415V, K752R, F109I*) and had poor survival outcomes. These mutations have not yet been functionally annotated; therefore, understanding their role in tumorigenesis and survival outcomes is critical. Furthermore, co-mutation status in unclassified patients should be investigated because it is possible these may represent codriver events. In the external ctDNA cohort, we found numerous unclassified *aBRAF* mutations that are yet to be functionally described; most were commonly co-mutated with *RAS, PIK3CA,* and *EGFR.*

Development of acquired *RAS* and subclonal *EGFR* ectodomain mutations are known mechanisms of resistance to anti-EGFR therapy in RAS^{WT} mCRC.[22-26] Considering the limitation of EGFR inhibition noted in our internal cohort, whether the emergence of *aBRAF* potentially reflects a novel mechanism of acquired resistance to EGFR inhibition was investigated. Analysis of our ctDNA cohort showed statistically significant increases in the prevalence of *aBRAF* mutations, subclonal *aBRAF* mutations, and multiple *aBRAF* mutations in individual ctDNA samples among patients predicted to be exposed to EGFR inhibition versus those predicted to be nonexposed. It is important to note that the rate of *aBRAF* mutation in our ctDNA cohort was higher than in our internal cohort, as well as higher than what has been published in tissue-based testing.[3] These findings highlight the potential of *aBRAF* mutations as a novel acquired resistance mechanism to EGFR inhibition and warrant additional prospective investigation. Of note, in patients with EGFR-mutant non–small-cell lung cancer, *aBRAF* mutations have been reported as a mechanism of acquired resistance to EGFR tyrosine kinase inhibition.[27]

Our results underscore the need for innovative combinatorial targeted approaches for *aBRAF* mutations exploiting class biology. Data suggest that downstream signaling with novel inhibitors of MEK or ERK may be useful, because the pathway is still active.[20] Furthermore, because both class II and class III mutations signal through BRAF and/or CRAF dimers, next-generation RAF inhibitors that target dimerization is of interest in combination with these agents as a rationale next step for therapeutic intervention.[21] Of note, safety lead-in data for a novel triplet regimen using encorafenib, binimetinib, and cetuximab for refractory $BRAF^{V600E}$ mCRC have been published showing impressive response rates and significant improvement in survival outcomes.[28] Subsequent studies will need to investigate whether this novel triplet combination strategy can also enhance optimal MAPK inhibition and provide durable benefit in patients with *aBRAF* mCRC.

There are limitations to this study. First, this is a nonrandomized, retrospective cohort analysis at a single institution. Second, considering the rarity of *aBRAF* mCRC, we were only able to identify 36 patients in our internal cohort, of whom 30% received EGFR inhibition therapy. Third, detailed clinical annotation is not available for the external ctDNA cohort, and we were unable to confirm that these patients had received prior anti-EGFR therapy. Therefore, we applied a previously validated and published anti-EGFR exposure score to identify patients predicted to have anti-EGFR exposure versus patients predicted to be nonexposed to conduct our ctDNA analysis.

Our results suggest selection criteria for the use of anti-EGFR therapy in a*BRAF*-mutated mCRC should be based not only on RAS^{WT} status but also the functional class of the atypical variant. In our cohort, although limited by sample size, findings continue to generate more hypotheses in that class II *aBRAF* mutations may represent a negative predictive biomarker of EGFR inhibition, and exposure to anti-EGFR mAbs may adversely affect survival outcomes, whereas efficacy of EGFR inhibition was limited in patients with class III *aBRAF* mCRC. Increased subclonal *aBRAF* mutations and prevalence of class II and class III variants in ctDNA among patients with predicted anti-EGFR exposure suggest a novel mechanism of resistance warranting additional prospective investigation. Also, our data support the clinical utility of comprehensive genomic profiling (both tissue and ctDNA) in the management of advanced mCRC, because standard hotspot polymerase chain reaction testing would not capture emerging clinically significant alterations. Of note, many *aBRAF* alterations remain unclassified to date, highlighting the need for continued functional annotation. Efforts are needed to establish the predictive impact of functional classes on anti-EGFR efficacy and to design novel therapeutic strategies for *aBRAF* mCRC.

AFFILIATIONS
[1]The University of Texas MD Anderson Cancer Center, Houston, TX
[2]BC Cancer, Vancouver, British Columbia, Canada
[3]Guardant Health, Redwood City, CA

CORRESPONDING AUTHOR
Scott Kopetz, MD, PhD, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 426, Houston TX 77030; Twitter: @benjohnson1112; e-mail: skopetz@mdanderson.org.

REFERENCES

1. Tie J, Gibbs P, Lipton L, et al: Optimizing targeted therapeutic development: Analysis of a colorectal cancer patient population with the BRAF(V600E) mutation. Int J Cancer 128:2075-2084, 2011

2. Morris V, Overman MJ, Jiang ZQ, et al: Progression-free survival remains poor over sequential lines of systemic therapy in patients with BRAF-mutated colorectal cancer. Clin Colorectal Cancer 13:164-171, 2014

3. Jones JC, Renfro LA, Al-Shamsi HO, et al: Non-V600 BRAF mutations define a clinically distinct molecular subtype of metastatic colorectal cancer. J Clin Oncol 35:2624-2630, 2017

4. 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

5. 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

6. Shinozaki E, Yoshino T, Yamazaki K, et al: Clinical significance of BRAF non-V600E mutations on the therapeutic effects of anti-EGFR monoclonal antibody treatment in patients with pretreated metastatic colorectal cancer: The Biomarker Research for Anti-EGFR Monoclonal Antibodies by Comprehensive Cancer Genomics (BREAC) study. Br J Cancer 117:1450-1458, 2017

7. Yao Z, Torres NM, Tao A, et al: BRAF mutants evade ERK-dependent feedback by different mechanisms that determine their sensitivity to pharmacologic inhibition. Cancer Cell 28:370-383, 2015

8. Yao Z, Yaeger R, Rodrik-Outmezguine VS, et al: Tumours with class 3 BRAF mutants are sensitive to the inhibition of activated RAS. Nature 548:234-238, 2017

9. Strickler JH, Loree JM, Ahronian LG, et al: Genomic landscape of cell-free DNA in patients with colorectal cancer. Cancer Discov 8:164-173, 2018

10. Lanman RB, Mortimer SA, Zill OA, et al: Analytical and clinical validation of a digital sequencing panel for quantitative, highly accurate evaluation of cell-free circulating tumor DNA. PLoS One 10:e0140712, 2015

11. Parseghian CM, Loree JM, Morris VK, et al: Anti-EGFR resistant clones decay exponentially after progression: Implications for anti-EGFR re-challenge. Ann Oncol 30:243-249, 2019

12. Dankner M, Rose AAN, Rajkumar S, et al: Classifying BRAF alterations in cancer: New rational therapeutic strategies for actionable mutations. Oncogene 37:3183-3199, 2018

13. De Roock W, De Vriendt V, Normanno N, et al: KRAS, BRAF, PIK3CA, and PTEN mutations: Implications for targeted therapies in metastatic colorectal cancer. Lancet Oncol 12:594-603, 2011

14. Wang Y, Jones JC, Kipp BR, et al: Activity of EGFR antibody in non-V600 BRAF mutant metastatic colorectal cancer. Ann Oncol 30:147-149, 2019

15. 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

16. Sullivan RJ, Infante JR, Janku F, et al: First-in-class ERK1/2 inhibitor ulixertinib (BVD-523) in patients with MAPK mutant advanced solid tumors: Results of a phase I dose-escalation and expansion study. Cancer Discov 8:184-195, 2018

17. Yaeger R, Chatila WK, Lipsyc MD, et al: Clinical sequencing defines the genomic landscape of metastatic colorectal cancer. Cancer Cell 33:125-136.e3, 2018

18. Rankin A, Klempner SJ, Erlich R, et al: Broad detection of alterations predicted to confer lack of benefit from EGFR antibodies or sensitivity to targeted therapy in advanced colorectal cancer. Oncologist 21:1306-1314, 2016

19. Schirripa M, Biason P, Cortiula F, et al: Clinico-pathological and molecular characterization of BRAF mutant metastatic colorectal cancer (mCRC): Are all mutations created equal? J Clin Oncol 36, 2018 (suppl; 3590)

20. 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

21. Karoulia Z, Gavathiotis E, Poulikakos PI: New perspectives for targeting RAF kinase in human cancer. Nat Rev Cancer 17:676-691, 2017

22. Thierry AR, Pastor B, Jiang ZQ, et al: Circulating DNA demonstrates convergent evolution and common resistance mechanisms during treatment of colorectal cancer. Clin Cancer Res 23:4578-4591, 2017

23. Pietrantonio F, Vernieri C, Siravegna G, et al: Heterogeneity of acquired resistance to anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer. Clin Cancer Res 23:2414-2422, 2017

24. Arena S, Bellosillo B, Siravegna G, et al: Emergence of multiple EGFR extracellular mutations during cetuximab treatment in colorectal cancer. Clin Cancer Res 21:2157-2166, 2015

25. Siravegna G, Mussolin B, Buscarino M, et al: Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. Nat Med 21:827, 2015 [Erratum: Nat Med doi:10.1038/nm.3870, 2015]

26. Van Emburgh BO, Arena S, Siravegna G, et al: Acquired RAS or EGFR mutations and duration of response to EGFR blockade in colorectal cancer. Nat Commun 7:13665, 2016

27. Yu HA, Suzawa K, Jordan E, et al: Concurrent alterations in EGFR-mutant lung cancers associated with resistance to EGFR kinase inhibitors and characterization of MTOR as a mediator of resistance. Clin Cancer Res 24:3108-3118, 2018

28. Van Cutsem E, Huijberts S, Grothey A, et al: Binimetinib, encorafenib, and cetuximab triplet therapy for patients with *BRAF* V600E-mutant metastatic colorectal cancer: Safety lead-in results from the phase III BEACON Colorectal Cancer Study. J Clin Oncol 37:1460-1469, 2019
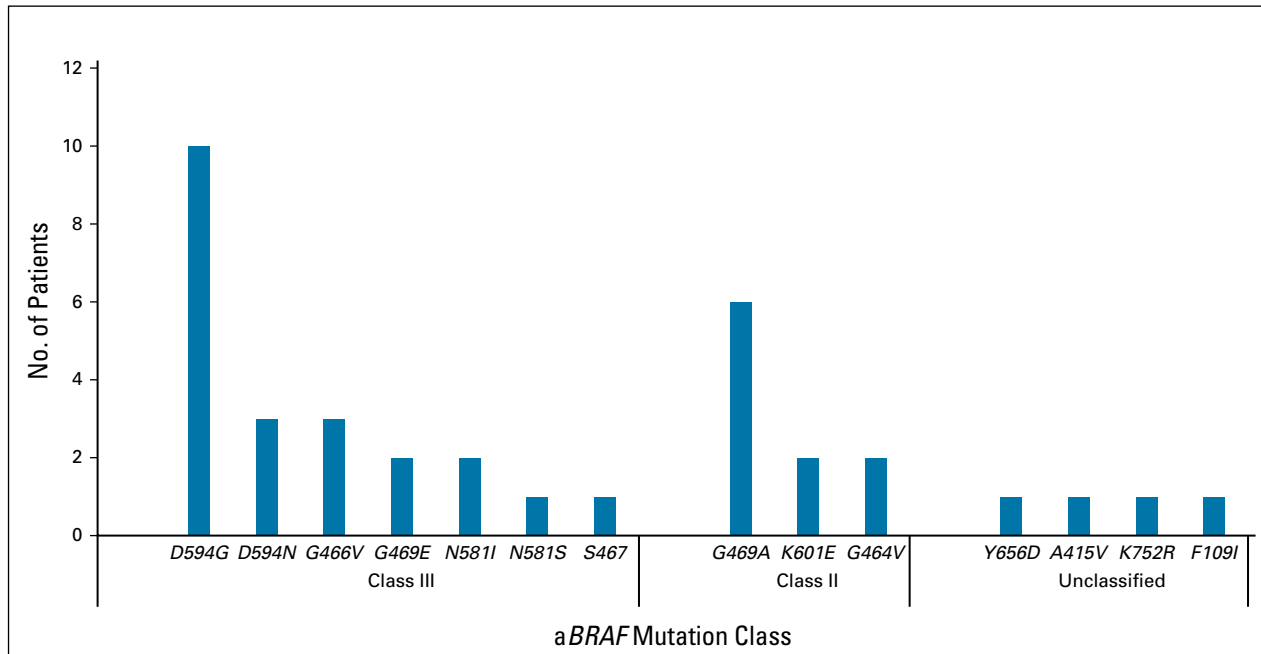
- - -

APPENDIX

FIG A1. Distribution of *aBRAF* (atypical, non-V600 BRAF) mutations in cohort 1 (n = 36).

TABLE A1. Matched Analysis of Patients with *aBRAF*, *BRAF*^V600E, and *RAS/RAF*^WT Metastatic Colorectal Cancer Tumors

Characteristic	aBRAF (n = 36)	BRAF^V600E (n = 36)	RAS/ RAF^WT (n = 36)	P	Population
Median age, years	56.0	57.2	56.0	0.84	56.0
Sidedness, right, %	38.9	41.7	41.7	0.96	40.7

Abbreviations: *aBRAF*, atypical, non-V600 *BRAF*; WT, wild type.