VOLUME 33 · NUMBER 34 · DECEMBER 1 2015

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Combined BRAF and MEK Inhibition With Dabrafenib and Trametinib in *BRAF* V600–Mutant Colorectal Cancer

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Published online ahead of print at www.jco.org on September 21, 2015.

Supported by GlaxoSmithKline and in part by a Damon Runyon Clinical Investigator Award and Awards No. P50CA127003 and K08CA166510 from the National Cancer Institute (NCI), National Institute of Health (NIH; R.B.C.); by Award No. K08CA175143 from the NCI, NIH (C.E.A.); by NIH Clinical and Translational Science Award No. UL1 RR024148 and NIH Cancer Center Support Grant No. CA016672 to MD Anderson Cancer Center (G.F.); and by Awards No. R01CA172670 and R01CA184843 from the NCI (S.K.).

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

Presented in part at the 49th Annual Meeting of the American Society of Clinical Oncology (ASCO), Chicago, IL, May 31-June 4, 2013, and 50th ASCO Annual Meeting, Chicago, IL, May 30-June 3, 2014.

Authors' disclosures of potential conflicts of interest are found in the article online at www.jco.org. Author contributions are found at the end of this article.

Clinical trial information: NCT01072175.

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0732-183X/15/3334w-4023w/$20.00

DOI: 10.1200/JCO.2015.63.2471

ABSTRACT

Purpose
To evaluate dabrafenib, a selective BRAF inhibitor, combined with trametinib, a selective MEK inhibitor, in patients with *BRAF* V600–mutant metastatic colorectal cancer (mCRC).

Patients and Methods
A total of 43 patients with *BRAF* V600–mutant mCRC were treated with dabrafenib (150 mg twice daily) plus trametinib (2 mg daily), 17 of whom were enrolled onto a pharmacodynamic cohort undergoing mandatory biopsies before and during treatment. Archival tissues were analyzed for microsatellite instability, PTEN status, and 487-gene sequencing. Patient-derived xenografts were established from core biopsy samples.

Results
Of 43 patients, five (12%) achieved a partial response or better, including one (2%) complete response, with duration of response > 36 months; 24 patients (56%) achieved stable disease as best confirmed response. Ten patients (23%) remained in the study > 6 months. All nine evaluable during-treatment biopsies had reduced levels of phosphorylated ERK relative to pretreatment biopsies (average decrease ± standard deviation, 47% ± 24%). Mutational analysis revealed that the patient achieving a complete response and two of three evaluable patients achieving a partial response had *PIK3CA* mutations. Neither PTEN loss nor microsatellite instability correlated with efficacy. Responses to dabrafenib plus trametinib were comparable in patient-derived xenograft–bearing mice and the biopsied lesions from each corresponding patient.

Conclusion
The combination of dabrafenib plus trametinib has activity in a subset of patients with *BRAF* V600–mutant mCRC. Mitogen-activated protein kinase signaling was inhibited in all patients evaluated, but to a lesser degree than observed in *BRAF*-mutant melanoma with dabrafenib alone. *PIK3CA* mutations were identified in responding patients and thus do not preclude response to this regimen. Additional studies targeting the mitogen-activated protein kinase pathway in this disease are warranted.

J Clin Oncol 33:4023-4031. © 2015 by American Society of Clinical Oncology

INTRODUCTION

Missense mutation of the v-raf murine sarcoma viral oncogene homolog B (*BRAF*) gene is present in 5% to 10% of metastatic colorectal cancers (mCRCs).[1,2] *BRAF* encodes a protein kinase in the mitogen-activated protein kinase (MAPK) pathway that may be rendered constitutively active by substitution at valine 600 (V600), most commonly to glutamic acid (V600E). Patients with mCRC whose tumors harbor *BRAF* V600 mutations generally respond poorly to standard therapies,[3-8] with a median progression-free survival (PFS) of 2.5 months after second-line chemotherapy[8] and median overall survival (OS) < 1 year, versus > 2 years for patients with *BRAF* wild-type mCRC.[9] New therapeutic approaches for patients with *BRAF*-mutated mCRC are critically needed.

Patients with *BRAF*-mutated mCRC are more likely to be female and older, with right-sided primary tumors and an unusual pattern of metastatic spread, including frequent peritoneal and distant lymph node involvement.[2,9] *BRAF* mutation defines a unique molecular subtype of CRC, commonly

originating from serrated adenomas with low rates of chromosomal instability and high rates of hypermethylation and microsatellite instability (MSI).[10-12] MSI is a favorable prognostic marker in early-stage CRC, but may not improve outcomes in the context of *BRAF* V600E mCRC.[9,11,13]

BRAF inhibition with vemurafenib or dabrafenib has resulted in significantly prolonged PFS and OS in patients with *BRAF*-mutated metastatic melanoma.[14-19] Trametinib, targeting mitogen-activated extracellular signal–related kinase kinase (MEK), downstream of BRAF in the MAPK pathway, has similarly been associated with improved PFS and OS compared with conventional chemotherapy in *BRAF*-mutated metastatic melanoma.[20,21] These pivotal studies led to the approval of vemurafenib, dabrafenib, and trametinib by the US Food and Drug Administration, revolutionizing treatment of *BRAF* V600–mutated melanoma.

In contrast to *BRAF*-mutant melanoma, mCRC with the same *BRAF* V600 mutation has shown a marked lack of sensitivity to BRAF or MEK inhibitor monotherapy in early clinical trials. Whereas approximately 50% of patients with metastatic melanoma responded to vemurafenib, the response rate was only 5% among 19 assessable patients with *BRAF*-mutated mCRC.[16,17,22] One of nine assessable patients with *BRAF*-mutated CRC experienced partial response (PR) after dabrafenib monotherapy.[18] The phase I/II trial of trametinib alone showed no responses in a small number of patients with *BRAF*-mutant mCRC.[23]

This was predicted by preclinical studies, which suggested that BRAF or MEK inhibitors alone do not produce sustained MAPK pathway inhibition in *BRAF*-mutant CRC, likely because of feedback signals that reactivate MAPK signaling.[24-26] This is hypothesized to be a major factor underlying the lack of clinical response to these agents in *BRAF*-mutant CRC. However, laboratory studies have also suggested that combined inhibition of BRAF and MEK can lead to improved suppression of MAPK signaling and increased efficacy.[24,25] Combined inhibition of BRAF and MEK with dabrafenib plus trametinib has been studied extensively in patients with unresectable or metastatic *BRAF* V600–mutated melanoma and was recently granted accelerated US Food and Drug Administration approval after demonstrating an acceptable safety profile with a statistically significant improvement in PFS and response rates compared with dabrafenib alone.[27]

We report here on a 43-patient expansion cohort of the dabrafenib plus trametinib combination study,[27,28] comprising patients with *BRAF* V600–mutated mCRC, to test the hypothesis that the MAPK pathway is a valid therapeutic target in this population. Conducting clinical trials in the patient population with *BRAF*-mutated mCRC presents several challenges, including the relative rarity of *BRAF* mutations, the failure to routinely test for the mutation, and the aggressive nature of the disease. Thus, a strong emphasis was placed on correlative science to guide subsequent investigations aimed at personalizing therapy to improve clinical outcomes for patients with *BRAF*-mutated mCRC.

PATIENTS AND METHODS

Patient Selection

Patients with histologically confirmed mCRC with either a *BRAF* V600E or V600K mutation were eligible to enroll. Eligibility criteria also included measurable disease according to RECIST (version 1.1) and Eastern Cooperative Oncology Group performance status of 0 or 1. Exclusion criteria included history of central serous retinopathy, retinal vein occlusion, and serious cardiac comorbidities. The study was approved by the institutional review board at each site. All patients provided written informed consent before any study procedures.

Table 1. Baseline Patient Demographic and Clinical Characteristics (N = 43)

Characteristic	No. (%)
Age, years	
Mean	55
SD	13
Female sex	34 (79)
ECOG performance status	
0	24 (56)
1	19 (44)
BRAF V600E mutation	43 (100)
No. of disease sites at screening	
< 3	22 (51)
≥ 3	21 (49)
No. of lines of prior systemic anticancer therapy*	
0	1 (2)
1	6 (14)
2	14 (33)
≥ 3	22 (51)
Prior EGFR inhibitor	20 (47)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; SD, standard deviation.
*Prior chemotherapy, immunotherapy, hormonal, biologic, or small-molecule targeted therapy regimens.

Study Design

This open-label phase I/II study assessed the safety, pharmacokinetics, pharmacodynamics, and clinical activity of combination therapy with dabrafenib plus trametinib.[27,28] Here we report results for a portion of part B: the mCRC cohort of patients with *BRAF* V600–mutated tumors, including a pharmacodynamic expansion cohort (Data Supplement).

Table 2. Adverse Events

Adverse Event	No. (%)	
	Grade 3	Any Grade
Any	25 (58)	42 (98)
Nausea	2 (5)	27 (63)
Pyrexia	5 (12)	27 (63)
Fatigue	3 (7)	23 (53)
Chills	1 (2)	21 (49)
Vomiting	3 (7)	20 (47)
Diarrhea	1 (2)	15 (35)
Headache	0	15 (35)
Anemia	7 (16)	12 (28)
Constipation	0	11 (26)
Decreased appetite	2 (5)	11 (26)
Peripheral edema	0	11 (26)

NOTE. Listed are all adverse events reported in ≥ 25% of patients, irrespective of whether causal relationship was likely. Six grade 4 events independent of attribution were reported: dyspnea, thrombocytopenia, hypotension, large intestine obstruction, pulmonary embolism, and sepsis syndrome.

Pharmacodynamic and Exploratory Biomarker Assessments

Patients in the pharmacodynamic cohort consented to mandatory tumor biopsies before treatment (within 21 days before starting study treatment) and on day 15 of study treatment (± 7 days). Formalin-fixed paraffin-embedded (FFPE) and flash-frozen core biopsy samples were collected. Pathway inhibition was assessed in the paired tumor tissue for change in expression levels of phosphorylated ERK (P-ERK) and AKT (P-AKT) by immunohistochemistry (IHC).[29] The frozen paired tumor tissue was used for reverse-phase protein array (RPPA) analysis (George Mason University, Manassas, VA).[30]

Targeted next-generation sequencing (NGS) was performed for a 487-candidate cancer gene panel (Data Supplement). PTEN and epidermal growth factor receptor (EGFR) IHC were conducted at Ventana Medical Systems (Tucson, AZ) using rabbit monoclonal antibody D4.3 or 5B7, respectively. MSI status was evaluated using Promega MSI Analysis System (version 1.2 [product No. MD1641]; Madison, WI). DNA was extracted using Qiagen QIAamp DNA FFPE Tissue Kit (product No. 56404; Valencia, CA) Details on IHC, RPPA, and NGS are provided in the Data Supplement.

Patient-Derived Xenografts

A companion protocol with a separate consent form permitted collection of additional tissue at the time of tumor biopsies in the pharmacodynamic cohort for patient-derived xenograft (PDX) development at the Jackson Laboratory (Sacramento, CA). Mice bearing subcutaneous PDXs (seven to 10 per group) were treated with vehicle (0.1% Tween-20 or 0.5% hydroxypropyl methylcellulose and 0.2% Tween-80), dabrafenib (30 mg/kg per day), and trametinib (1 or 0.6 mg/kg per day) by oral gavage for 21 days at University of California San Francisco (San Francisco, CA) or MD Anderson Cancer Center (Houston, TX; details provided in Data Supplement).

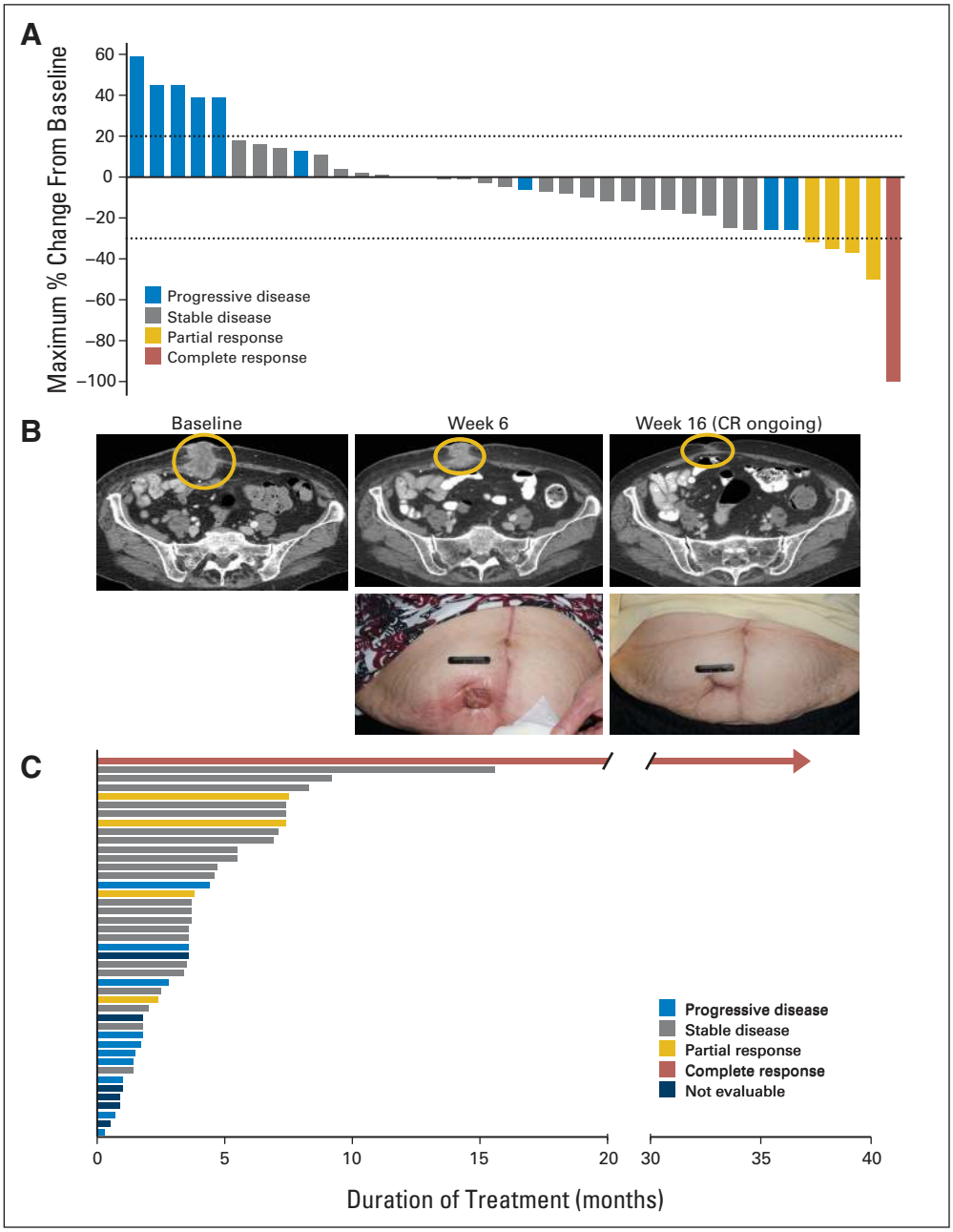

Fig 1. (A) Waterfall plot of maximum percent reduction in target lesion size by RECIST. Horizontal lines at + 20% and − 30% denote boundaries of stable disease. (B) Computed tomography images and photographs from patient achieving complete response. (C) Time receiving study treatment plot.

Statistical Methods

The objectives of enrolling the part B colorectal cohort were to collect safety, clinical activity, and pharmacodynamic response in paired biopsies among patients with *BRAF*-mutant mCRC. PFS was summarized with Kaplan-Meier methodology using medians and 95% CIs (estimated using Brookmeyer-Crowley method).

RESULTS

Patient Population

Between January 3, 2011, and April 25, 2013, 43 patients with *BRAF* V600–mutated mCRC were enrolled at eight centers: 26 patients in the original efficacy cohort, and 17 patients in the pharmacodynamic expansion cohort with mandatory tumor biopsies before treatment and on day 15 of treatment. All patients initiated treatment with dabrafenib 150 mg twice daily and trametinib 2 mg daily. Baseline characteristics of patients with mCRC are listed in Table 1.

Safety

Adverse events (AEs) were consistent with those reported in patients with metastatic melanoma treated with dabrafenib 150 mg twice daily plus trametinib 2 mg daily[27] (Table 2). The most frequent AEs were nausea, pyrexia, and fatigue. Pyrexia was the most common reason for dose interruption, occurring in 13 patients (30%), and dose reduction, occurring in 12 patients (28%). One patient (2%) discontinued treatment because of pyrexia. Left ventricular ejection fraction (LVEF) reduction occurred in eight patients (19%), including two

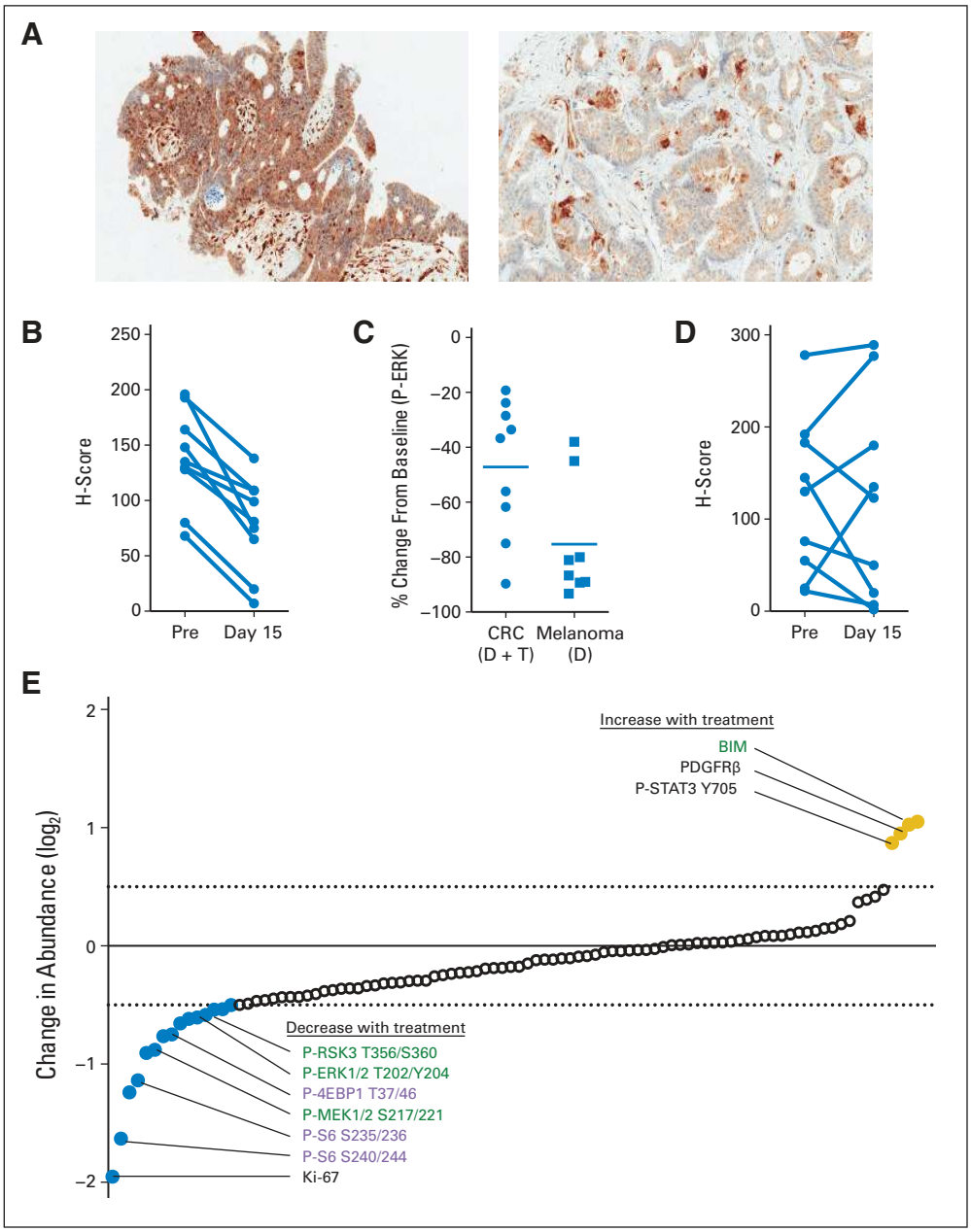

Fig 2. Pharmacodynamic biomarkers from nine evaluable paired pre- and during-treatment (day 15, 2 to 4 hours after dabrafenib [D] plus trametinib [T] dosing) tumor biopsies. (A) Representative images of phosphorylated ERK (P-ERK) immunohistochemistry staining in pre- (left) and during-treatment (right) biopsies. (B) H scores for P-ERK and (C) percent change in P-ERK H score in patients with *BRAF* V600–mutant colorectal cancer (CRC) treated with dabrafenib (150 mg twice daily) and trametinib (2 mg daily) as compared with patients with *BRAF*-mutant melanoma treated with dabrafenib only (70 to 200 mg twice daily; $P < .001$ by paired t test).[18] (D) Phosphorylated AKT H scores before and during treatment. (E) Change in abundance of specific proteins or phosphoproteins in during-treatment biopsies relative to paired pretreatment biopsies was analyzed by reverse-phase protein array . Targets showing greatest average increase (yellow) or decrease (blue) after treatment are shown. Specific targets of interest are labeled, with mitogen-activated protein kinase pathway targets shown in green and mammalian target of rapamycin pathway targets shown in purple. PDGFRβ, platelet-derived growth factor receptor–β.

grade 3 events. LVEF reduction led to dose reduction in five patients (12%) and treatment discontinuation in one patient (2%). Grade 1 ocular events (eg, photophobia, blurred vision, and visual impairment) occurred in three patients (7%). No grade 4 pyrexia, LVEF reduction, or ocular events were reported. No grade 5 AEs were reported. Overall, 17 AEs (40%) led to dose reduction, 25 AEs (58%) led to dose interruption, and four patients (9%) discontinued treatment because of an AE.

Efficacy

Of 43 patients enrolled, five (12%) achieved a PR or better (Fig 1A), including one patient (2%) with a complete response (CR), with duration of response > 36 months (ongoing; last data cut, January 15, 2015). This patient achieved a CR by week 32 of study treatment, with complete resolution of a large tumor mass invading through the abdominal wall (Fig 1B). Among the 43 patients, only the patient achieving the CR had not received prior systemic therapy. Two of four patients achieving a PR and the patient achieving a CR had confirmed responses. A total of 24 patients (56%) achieved stable disease as their best confirmed response. Overall, 16 patients (37%) experienced a reduction in target lesion size by RECIST of ≥ 10%. Median PFS was 3.5 months (95% CI, 3.4 to 4.0 months), and median duration of study treatment was 3.6 months (range, 0.3 to 36.8 months). Ten patients (23%) remained in study treatment > 6 months (Fig 1C).

Pharmacodynamic Analysis of Paired Biopsy Specimens

Of 17 patients enrolled onto the pharmacodynamic cohort, paired pretreatment and day-15 treatment biopsies with evaluable tumor content were available for nine patients. Biopsies from the other eight patients were not evaluable, either because the pre- or during-treatment samples lacked tumor cells or because during-treatment samples were not collected. All nine during-treatment biopsies showed reduced levels of P-ERK relative to pretreatment biopsies (paired *t* test $P < .001$; Figs 2A and 2B). However, the mean decrease (± standard deviation) in P-ERK in these patients was 47% ± 24% (median, 37%), which was significantly less than the mean decrease of 75% ± 21% (median, 84%) in P-ERK observed among patients with *BRAF*-mutated melanoma treated with dabrafenib alone (Fig 2C).[31] No consistent changes in the levels of P-AKT after treatment were observed (Fig 2D). Within this limited patient sample, no clear correlations between changes in pharmacodynamic markers after treatment and response could be determined.

Analysis of paired biopsies by RPPA showed modulation of MAPK targets, including decreased levels of phosphorylated MAPK signaling components after treatment and increased levels of BIM, a key proapoptotic protein known to be induced on MAPK inhibition (Fig 2E).[32] Decreases in mammalian target of rapamycin (mTOR) pathway targets were also observed, consistent with previous studies suggesting that mTOR activity is predominantly regulated by MAPK signaling in *BRAF*-mutant cancers.[33] We also observed a marked decrease in the proliferation marker Ki67. Increases in the levels of platelet-derived growth factor receptor–β and STAT3 phosphorylation on tyrosine 705 were also seen, both of which have been implicated in resistance to BRAF inhibition.[34,35] Our analysis did not detect a clear increase in phosphorylation at any of the five EGFR phosphorylation sites analyzed after therapy with dabrafenib plus trametinib (Data Supplement).

Molecular Analyses on Archived Tissues

Mutational analysis was performed on FFPE primary tumor samples available from 15 patients (Fig 3A). As expected, the majority of tumors harbored mutations in the Wnt/β-catenin and p53 pathways.[10] No clear correlation between the presence of these alterations and clinical response was evident.

Interestingly, *PIK3CA* mutations at known hotspots in exons 9 and 20 were identified in five of 15 evaluable patients, including in three of five patients with a PR or CR. All but one patient with a *PIK3CA* mutation achieved a reduction in target lesion size by RECIST. A separate analysis for PTEN loss by IHC was performed in 20 patients. PTEN loss was identified in four of the 19 patients with interpretable PTEN status, all of whom achieved a reduction in target lesion size by RECIST. There was no difference in PFS by PTEN status (Appendix Fig A1A, online only). In addition, all patients with tumors harboring transforming growth factor beta (TGF-β) pathway alterations (six of 15) had a reduction in target lesion size by RECIST, although again, definitive correlations cannot be established because of the limited sample size.

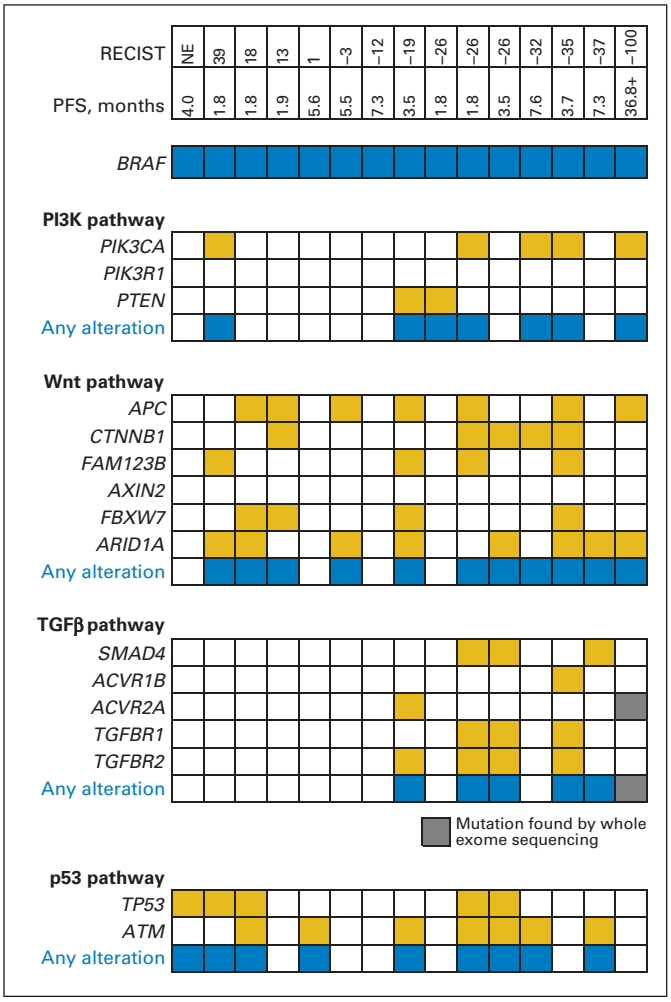

Fig 3. 487-gene mutational analysis of archival tumor specimens available from 15 patients. RECIST value is maximum percent reduction in measurement of target lesions from baseline. Whole-exome sequencing was performed on tumor from patient achieving complete response. PDGFRβ, platelet-derived growth factor receptor–β; PFS, progression-free survival; PI3K, phosphatidylinositol 3-kinase.

MSI analysis using a polymerase chain reaction–based assay was performed on tumor specimens from 29 patients, with 24 specimens yielding interpretable results; eight (33%) of these 24 tumors were microsatellite unstable. No statistically significant difference in PFS was observed between the MSI and MSS subpopulations (Appendix Fig A1B, online only). Total EGFR, evaluable in archival tumor specimens from 22 patients, did not correlate with PFS (median EGFR membrane H score, 85).

PDXs

We undertook an exploratory effort to generate PDX models from pretreatment biopsies obtained from five patients enrolled onto this study, because these may represent valuable tools for future correlative studies. PDX models were successfully generated from four of five patients and were successfully expanded for drug testing (Fig 4A). Response in PDX tumors after 21 days of treatment with dabrafenib plus trametinib mirrored the response in the patients' biopsied lesions from which they were derived, ranging from partial regression to progressive disease (Fig 4B).

DISCUSSION

Our study suggests that dual MAPK pathway blockade with the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib can lead to meaningful clinical benefit in a subset of patients with *BRAF*-mutant mCRC. This heavily pretreated population of patients with a poor prognosis mutational subtype of mCRC achieved several PRs and a durable CR ongoing for > 3 years. In addition, 56% of patients achieved stable disease as their best confirmed response, and 23% of patients remained in the study > 6 months. We believe this study represents an important therapeutic step forward for patients with *BRAF*-mutant mCRC. However, the median PFS for all patients was only 3.5 months. Although this is greater than the median PFS of 2.5 months observed with standard chemotherapy,[8] it is substantially less than the median PFS of 9.4 months observed with dabrafenib plus trametinib in patients with *BRAF*-mutant melanoma.[27]

Our data suggest that suboptimal MAPK pathway inhibition by dabrafenib plus trametinib in *BRAF*-mutant mCRC may be a major factor underlying the more limited efficacy observed in these patients. Indeed, our pharmacodynamic analyses of paired pretreatment and during-treatment biopsies showed that although the combination of inhibitors suppressed MAPK signaling, the degree of inhibition was significantly less than what has been achieved in *BRAF*- mutant melanoma with dabrafenib alone. This finding is critical, because studies have suggested that robust MAPK pathway suppression is required for response in *BRAF*-mutant cancers.[14] The importance of MAPK in suppression in *BRAF*-mutant mCRC is also supported by a recent study demonstrating that the first mechanisms of acquired resistance identified in patients experiencing initial clinical benefit from BRAF inhibitor combinations (including dabrafenib and trametinib) all involve components of the MAPK pathway and lead to MAPK reactivation.[36] Although it is possible that other signaling pathways play an important role in this disease, these data suggest that effective suppression of MAPK signaling is paramount in *BRAF*-mutant CRC and that

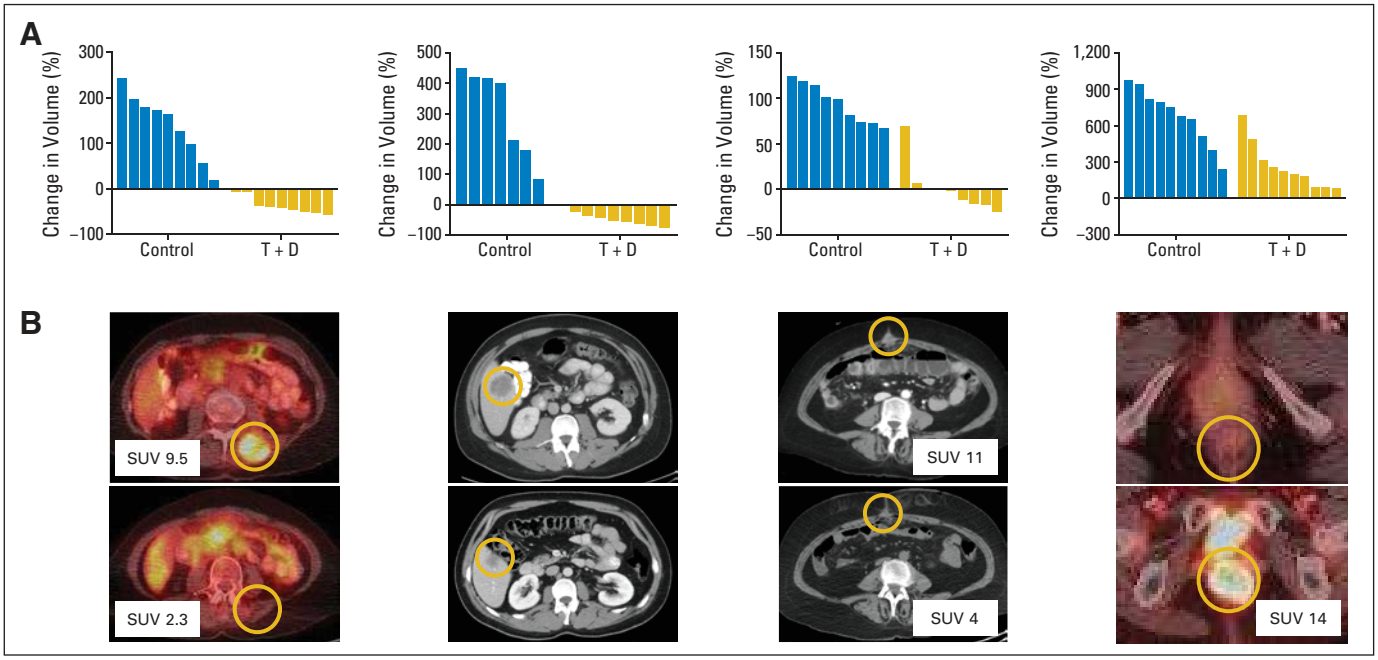

Fig 4. Patient-derived xenograft (PDX) –bearing mice (seven to 10 per group) treated with vehicle or dabrafenib (D) plus trametinib (T) daily for 21 days. (A) Plotted is change in PDX tumor volume after treatment, relative to initial tumor volume (all $P < .001$ by unpaired t test). (B) Computed tomography images showing lesion in patient who was biopsied to generate PDX pretreatment and at maximal response (weeks 32, 8, 3, and 8, respectively). First patient had spinal intramuscular mass biopsied, which regressed by 23% after 8 weeks of treatment and was not measurable by week 16 (overall best response in patient, confirmed partial response). Second patient had liver lesion biopsied, which regressed by 41% after 8 weeks of treatment (overall best response in patient, stable disease). Third patient had superficial paraumbilical nodule biopsied. This patient was not evaluable (ended study treatment because of toxicity), but imaging at week 3 showed early response in biopsied lesion. Fourth patient had posterior vaginal mass resected pretreatment, with anterior rectal recurrence by first restaging (overall best response in patient, progressive disease). If positron emission tomography was performed, maximum standardized uptake value (SUV) is noted for biopsied lesion.

therapeutic strategies capable of achieving improved MAPK suppression are needed.

It is likely that we achieved suboptimal MAPK pathway inhibition despite dual MAPK pathway blockade with dabrafenib and trametinib. This suggests that feedback reactivation of MAPK signaling may be limiting the effectiveness of the regimen. Recent preclinical studies have suggested that EGFR may drive resistance to BRAF inhibitors in many (but perhaps not all) *BRAF*-mutant CRCs, likely by leading to feedback activation of RAS, which can reactivate the MAPK pathway and other important signaling pathways.[25,37] Although some studies have proposed that the mechanism involves increases in EGFR phosphorylation on MAPK inhibition, other studies have observed increased signaling downstream of EGFR with no increase in EGFR phosphorylation. We did not observe a clear increase in EGFR phosphorylation in paired during-treatment biopsies by RPPA, which is more consistent with the latter model. Several clinical trials are evaluating combinations of EGFR antibodies and BRAF inhibitors.[38-42] Initial results of these studies suggest that combination of an EGFR antibody and a BRAF inhibitor together with a MEK inhibitor, phosphatidylinositol 3-kinase (PI3K) inhibitor, or irinotecan may be more effective than two-drug strategies.[40-42] Given its comparable tolerability profile relative to BRAF inhibitors alone, the combination of dabrafenib plus trametinib represents a promising backbone for therapeutic combinations that provide some degree of MAPK pathway suppression, regardless of whether MAPK activity is driven by EGFR-dependent or -independent resistant signals. The combination of dabrafenib, trametinib, and the EGFR antibody panitumumab is being evaluated in an ongoing clinical trial.[40]

In an effort to identify the subset of patients with *BRAF*-mutant CRC most likely to derive benefit from the combination of dabrafenib plus trametinib, we performed several exploratory biomarker analyses. Although small numbers of patients limited the power of these analyses, we found that mutations in the p53 and Wnt/β-catenin pathways and MSI status did not clearly predict response to or outcome with this therapy. The potential association of PI3K pathway alterations and improved response is surprising, given that PI3K pathway activation has previously been proposed as a mechanism of resistance to BRAF and MAPK pathway inhibition based on differential in vitro sensitivity in a panel of *BRAF*-mutated CRC cell lines.[43] Two of our four PDX models had activating *PIK3CA* mutations and demonstrated regression with the combination, supporting the utility of in vivo models for biomarker discovery in *BRAF*-mutant CRC.[44] Additional biomarker studies will be required to better define the subpopulation of patients with *BRAF*-mutant mCRC most likely to respond to this therapeutic strategy, including investigation of a potential association between response and TGF-β pathway alterations.

Although PDXs have been increasingly used in preclinical studies, to our knowledge, this is the first study in patients with mCRC to report prospective PDX testing from during-study biopsies and correlate the findings with clinical response. Encouragingly, the responsiveness of these PDX models to dabrafenib plus trametinib seemed to recapitulate the responsiveness of the individual patient tumor lesions from which they were derived. PDX models may thus be valuable representative models with which to study individual responsiveness or resistance in tumors, overcoming an existing barrier to performing detailed correlative analyses, which are typically limited by the finite amount of tissue obtained through standard biopsies. The routine generation of PDX models in future clinical trials may help to accelerate efforts to develop more effective therapies for *BRAF*-mutant mCRC.

Overall, we believe our study provides proof of concept that the MAPK pathway is a valid therapeutic target in *BRAF*-mutant mCRC and that effective targeting of this pathway has the potential to produce meaningful clinical responses. Even though combined BRAF and MEK inhibition led to a decrease in MAPK pathway activity in all patients, the degree of MAPK inhibition achieved remains suboptimal. Additional studies evaluating therapeutic strategies designed to more effectively target the MAPK signaling pathway in *BRAF*-mutant CRC are in progress.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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GLOSSARY TERMS

***BRAF*:** an isoform of RAF. See *Raf*.

MAPK (mitogen-activated protein kinase): a family of enzymes that form an integrated network influencing cellular functions such as differentiation, proliferation, and cell death. These cytoplasmic proteins modulate the activities of other intracellular proteins by adding phosphate groups to their serine/threonine amino acids.

MEK (MAPK-ERK kinase): a protein kinase, MEK is activated by c-Raf through phosphorylation of specific serine residues. Activation of ERK by activated MEK may lead to translocation of ERK to the nucleus, resulting in activation of specific transcription factors.

microsatellite instability (MSI): an alteration in the length of the microsatellites from cell to cell.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Combined BRAF and MEK Inhibition With Dabrafenib and Trametinib in *BRAF* V600–Mutant 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 jco.ascopubs.org/site/ifc.

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Research Funding: Roche, Amgen, GlaxoSmithKline, sanofi-aventis, Sysmex, Biocartis, Guardant Health, Agendia

Acknowledgment

We thank Emanuel Petricoin and Isela Gallagher for contributing to the reverse-phase protein array analyses; Byron Hann, Julia Malato, Neal Goodwin, Van Morris, Ji Wu, and Feng Tian for contributing to the patient-derived xenograft experiments; and Amy J. Markowitz for editorial assistance.

Appendix

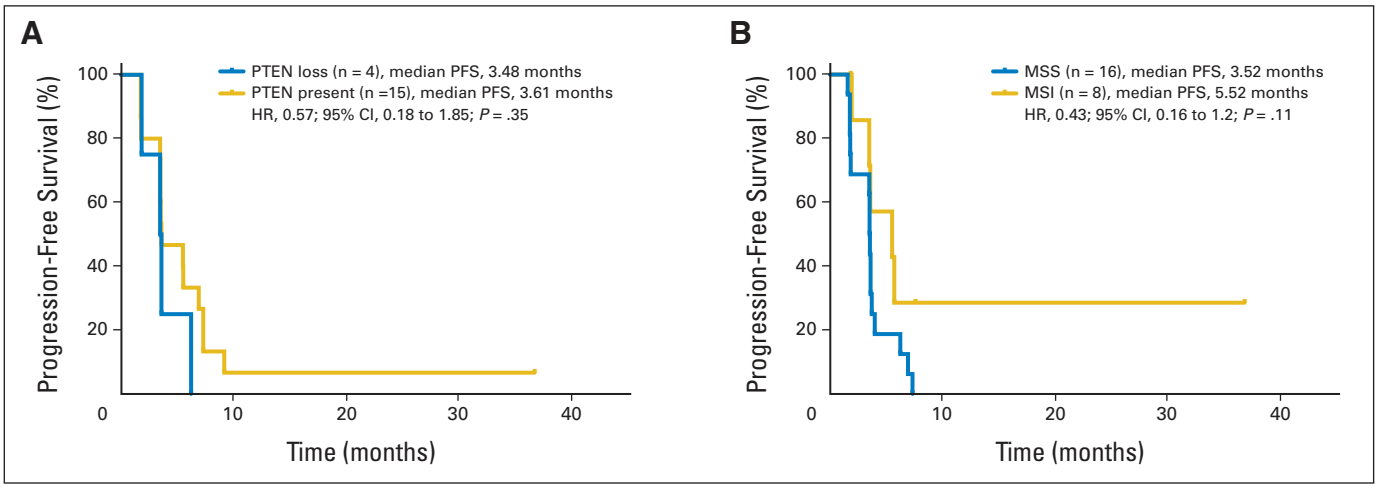

Fig A1. Kaplan-Meier curves for progression-free survival (PFS) by (A) PTEN expression (loss or present) and (B) microsatellite stability (MSS) or instability (MSI). HR, hazard ratio.