Combined BRAF, EGFR, and MEK Inhibition in Patients with $BRAF^{V600E}$-Mutant Colorectal Cancer

Ryan B. Corcoran1, Thierry André2, Chloe E. Atreya3, Jan H.M. Schellens4, Takayuki Yoshino5, Johanna C. Bendell6, Antoine Hollebecque7, Autumn J. McRee8, Salvatore Siena9, Gary Middleton10, Kei Muro11, Michael S. Gordon12, Josep Tabernero13, Rona Yaeger14, Peter J. O’Dwyer15, Yves Humblet16, Filip De Vos17, A. Scott Jung18, Jan C. Brase19, Savina Jaeger20, Severine Bettinger19, Bijoyesh Mookerjee21, Fatima Rangwala21, and Eric Van Cutsem22

ABSTRACT

Although BRAF inhibitor monotherapy yields response rates >50% in $BRAF^{V600E}$-mutant melanoma, only approximately 5% of patients with $BRAF^{V600E}$ colorectal cancer respond. Preclinical studies suggest that the lack of efficacy in $BRAF^{V600E}$ colorectal cancer is due to adaptive feedback reactivation of MAPK signaling, often mediated by EGFR. This clinical trial evaluated BRAF and EGFR inhibition with dabrafenib (D) + panitumumab (P) ± MEK inhibition with trametinib (T) to achieve greater MAPK suppression and improved efficacy in 142 patients with $BRAF^{V600E}$ colorectal cancer. Confirmed response rates for D+P, D+T+P, and T+P were 10%, 21%, and 0%, respectively. Pharmacodynamic analysis of paired pretreatment and on-treatment biopsies found that efficacy of D+T+P correlated with increased MAPK suppression. Serial cell-free DNA analysis revealed additional correlates of response and emergence of KRAS and NRAS mutations on disease progression. Thus, targeting adaptive feedback pathways in $BRAF^{V600E}$ colorectal cancer can improve efficacy, but MAPK reactivation remains an important primary and acquired resistance mechanism.

SIGNIFICANCE: This trial demonstrates that combined BRAF + EGFR + MEK inhibition is tolerable, with promising activity in patients with $BRAF^{V600E}$ colorectal cancer. Our findings highlight the MAPK pathway as a critical target in $BRAF^{V600E}$ colorectal cancer and the need to optimize strategies inhibiting this pathway to overcome both primary and acquired resistance. Cancer Discov; 8(4); 428–43. © 2018 AACR.

See related commentary by Janku, p. 389.
See related article by Hazar-Rethinam et al., p. 417.
INTRODUCTION

Activating gene mutations in the MAPK pathway are frequently observed in cancer and promote tumor cell migration, proliferation, and survival (1, 2). The serine/threonine protein kinase BRAF belongs to the RAF family of kinases [including ARAF and CRAF (RAF1); refs. 1, 2], which are normally activated by RAS family members (KRAS, NRAS, and HRAS), typically in response to signals from receptor tyrosine kinases (RTK; refs. 2, 3). BRAFV600E mutations lead to constitutive, RAS-independent activation of BRAF kinase activity and MAPK pathway signaling through downstream activation of MEK (MEK1 and MEK2) and ERK (ERK1 and ERK2) kinases (2, 3).

Oncogenic BRAFV600E mutations are present in approximately 10% of colorectal cancers (2, 4) and approximately 50% of melanomas (5). In colorectal cancer, BRAFV600E mutations confer a poor prognosis, resulting in nearly a 2-fold increase in mortality relative to wild-type BRAF in the metastatic setting (1, 6, 7). BRAFV600E mutation in colorectal cancer is associated with a right-sided primary site, advanced age, female sex, high tumor grade, and precursor sessile serrated adenomas (8). BRAFV600E colorectal cancer is also associated with the CpG island methylator phenotype (i.e., hypermethylated phenotype), which may result in the epigenetic inactivation of MLH1, inducing a mismatch repair (MMR) deficiency and consequently a microsatellite instability (MSI) phenotype (9). Among patients harboring BRAFV600E metastatic colorectal cancer, approximately 20% exhibit deficient MMR deficiency (8). BRAF inhibitors, such as vemurafenib and dabrafenib, selectively inhibit RAF monomers and have produced dramatic response rates >50% in metastatic melanoma, leading to their FDA approval for this indication (10, 11). However, single-agent BRAF inhibitors have demonstrated a surprising and striking lack of efficacy in patients with colorectal cancer harboring the same BRAFV600E mutation (12–16). Indeed, an initial study of vemurafenib in patients with the BRAFV600E mutation had a response rate of only 5% (16).

Preclinical studies have suggested that a primary reason for the differential sensitivities of BRAFV600E melanoma and colorectal cancer is that colorectal cancers harbor robust adaptive feedback signaling networks that lead to reactivation of MAPK signaling following BRAF inhibitor treatment (12, 15). In this proposed model, inhibition of BRAFV600E leads to an initial reduction in MAPK signaling, causing a loss of expression of ERK-dependent negative feedback mediators that act to constrain MAPK pathway activation (Fig. 1A; ref. 12). Loss of negative feedback leads to an induction of RAS activity and activation of other RAF kinases (such as
CRAF), which bypass the effects of the BRAF inhibitor by generating BRAF inhibitor-resistant RAF dimers and restore MAPK pathway signaling (12). Increased RAS activity following BRAF inhibition is thought to be driven primarily by RTK signaling, which is present to a greater degree in colorectal cancer than in melanoma, and preclinical studies have suggested that one RTK in particular—the EGFR—may play a dominant role in mediating MAPK reactivation in many BRAFV600E colorectal cancers (12, 15). Indeed, the combination of BRAF and EGFR inhibition was found to produce improved MAPK suppression and lead to tumor regression in BRAFV600E colorectal cancer xenografts (12, 15).

Thus, these data suggest that therapies capable of blocking feedback reactivation may produce more robust inhibition of MAPK signaling, resulting in improved efficacy in BRAFV600E colorectal cancer. As an initial test of this hypothesis in BRAFV600E colorectal cancer, we previously performed a clinical trial of combined BRAF and MEK inhibition with dabrafenib and trametinib that demonstrated improved pathway suppression in preclinical models of
BRAF\textsuperscript{V600E} colorectal cancer (17). Indeed, this strategy has been successful in BRAF\textsuperscript{V600E/K} melanoma and BRAF\textsuperscript{V600E} non–small cell lung cancer, improving outcomes in patients who received the combination of dabrafenib and trametinib versus dabrafenib alone, leading to FDA approval for this combination in these indications (18–21). Combined BRAF and MEK inhibition led to a modestly improved response rate of 12% in 43 patients with BRAF\textsuperscript{V600E}-metastatic colorectal cancer, but analysis of paired pretreatment and on-treatment biopsy specimens suggested that MAPK pathway suppression remained suboptimal (17). Therefore, we hypothesized that targeting EGFR as a key mediator of feedback signaling in combination with a BRAF inhibitor, with or without a MEK inhibitor, may optimize MAPK pathway suppression and lead to improved efficacy in BRAF\textsuperscript{V600E} colorectal cancer (17).

Here, we report the results of a clinical trial of combined BRAF and EGFR inhibition, combined MEK and EGFR inhibition, and combined BRAF, EGFR, and MEK inhibition in patients with metastatic BRAF\textsuperscript{V600E} colorectal cancer. Paired pretreatment and on-treatment biopsy specimens were collected and analyzed to assess the pharmacodynamic effects of each therapy. Serial plasma specimens were obtained, and cell-free DNA (cfDNA) was analyzed to provide correlates of response and to identify mechanisms of acquired resistance.

**RESULTS**

**Patient Characteristics**

Between December 2012 and the time of data cutoff for this interim analysis (May 6, 2016), 142 patients with metastatic BRAF\textsuperscript{V600E} colorectal cancer were enrolled in 1 of 3 treatment arms, as outlined in Fig. 1B: Arm 1, combined BRAF and EGFR inhibition with dabrafenib and panitumumab (D+P, \(n = 20\)); Arm 2, the “triplet” combination of BRAF, MEK, and EGFR inhibition with dabrafenib, trametinib, and panitumumab (D+T+P, \(n = 91\)); and Arm 3, combined MEK and EGFR inhibition with trametinib and panitumumab (T+P, \(n = 31\)). Patient characteristics are shown in Table 1. In general, patient characteristics were well-balanced across groups.

### Dose Determination and Safety

The initial dose assessment began with the evaluation of D+P at their full labeled doses [dabrafenib 150 mg orally twice a day (b.i.d.) and panitumumab 6 mg/kg i.v. every 2 weeks (Q2W)]. No dose-limiting toxicities (DLT) were observed, and a total of 20 patients were treated at this dose level. D+P was well tolerated, and the majority of events were grade 1 or 2; 45% of patients had a grade 3/4 event. The most common adverse events (AE) of all grades were dermatitis acneiform (60%), nausea (50%), fatigue (50%), and diarrhea (45%); none were grade 3/4 (Table 2). Only one grade 3/4 AE [hypophosphatemia: \(n = 2 (10\%\)] occurred in >1 patient in the D+P group.

Dose escalation to the full label doses of each of the triplet agents, D+T+P, was completed (dabrafenib 150 mg orally b.i.d., trametinib 2 mg orally daily, and panitumumab 6 mg/kg i.v. Q2W). A total of 48 patients were enrolled at the highest dose, and the spectrum of AEs was similar to that with D+P. Diarrhea (65% all grades, 7% grade 3/4), nausea (56% all grades, 2% grade 3/4), and dermatitis acneiform (59% all grades, 1% grade 3/4) were the most common AEs.
months (1.5–47.2 months), and for patients with a BRAF V600E mutation treated with T+P was 6.4 months (0.4–18.6 months).

Two fatal SAEs occurred in patients enrolled in the D+T+P arm. One event was due to hemorrhage, and the other was death due to an unknown cause; however, neither event was considered to be related to the study drugs (Supplementary Table S1).

### Efficacy

Efficacy measures for the 3 treatment arms are also based on a data cutoff date of May 6, 2016 (Fig. 2A–C). Two patients (10%) in the D+P arm had a confirmed complete response (CR) or partial response (PR), and 16 patients (80%) had stable disease; disease control was 90% overall. In the T+P arm, no patients achieved CR/PR, and 17 patients (55%) had stable disease. The D+T+P arm resulted in a confirmed CR/PR in 19 patients (21%), stable disease in 59 patients (65%), and an overall disease control rate of 86%. Duration of response (DOR) in the D+T+P arm was estimable but not mature, with a median of 7.6 months [95% confidence interval (CI), 2.9–not evaluable months; Table 3].

The median progression-free survival (PFS) was 3.5 months (95% CI, 2.8–5.8 months) in the D+P arm, 2.6 months (95% CI, 1.4–2.8 months) in the T+P arm, and 4.2 months (95% CI, 4.0–5.6 months) in the D+T+P arm (Fig. 2D). Median overall survival (OS) was 13.2 months (95% CI, 6.7–22.0 months) in the D+P arm, 8.2 months (95% CI, 6.5–9.4 months) in the T+P arm, and 9.1 months (95% CI, 7.6–20.0 months) in the D+T+P arm (estimable but not mature; Supplementary Fig. S1).

### Table 2. AEs occurring in >30% of patients in any treatment arm

<table>
<thead>
<tr>
<th>AE, n (%)</th>
<th>Total</th>
<th>Grade 3/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any event</td>
<td>20 (100)</td>
<td>9 (45)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>9 (45)</td>
<td>0</td>
</tr>
<tr>
<td>Dermatitis acneiform</td>
<td>12 (60)</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>10 (50)</td>
<td>0</td>
</tr>
<tr>
<td>Dry skin</td>
<td>7 (35)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>10 (50)</td>
<td>0</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>7 (35)</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>6 (30)</td>
<td>0</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>5 (25)</td>
<td>0</td>
</tr>
<tr>
<td>Rash</td>
<td>3 (15)</td>
<td>0</td>
</tr>
<tr>
<td>Hypomagnesemia</td>
<td>8 (40)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Constipation</td>
<td>7 (35)</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

*Safety data for the T+P arm are for all patients, including those with BRAF wild-type (n = 20) and BRAF V600E (n = 31).
**Figure 2.** Efficacy of D+P, T+P, and D+T+P in patients with BRAF<sup>V600E</sup> colorectal cancer. **A-C,** Waterfall plots showing best response by RECIST in the D+P (A), T+P (B), and D+T+P (C) cohorts. Dotted lines represent the 30% threshold for PR. Bar color represents the best confirmed response by RECIST. **D,** PFS for the D+P, T+P, and D+T+P cohorts. Median PFS with 95% CIs are shown for each treatment arm.
with paired biopsy specimens from patients with BRAF pretreatment biopsy, was evaluated. Values were compared from the day 15 on-treatment biopsy specimen, relative to the phosphorylated ERK (pERK) levels by immunohistochemistry therapy on MAPK signaling output [assessed as the change in + not observed. The D cant correlation between pERK inhibition and response was falls short of the nation for why even the D + those patients treated with D+T (ref. 17) are shown for comparison.

**Table 3. Summary of efficacy by treatment cohort (investigator review)**

<table>
<thead>
<tr>
<th>Assessment</th>
<th>D+T-P (n = 91)</th>
<th>T-P (n = 31)</th>
<th>D+P (n = 20)</th>
<th>D+T (n = 43)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best confirmed response, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>1 (1)</td>
<td>0</td>
<td>1 (5)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>PR</td>
<td>18 (20)</td>
<td>0</td>
<td>1 (5)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>SD</td>
<td>59 (65)</td>
<td>17 (55)</td>
<td>16 (80)</td>
<td>24 (56)</td>
</tr>
<tr>
<td>PD</td>
<td>8 (9)</td>
<td>12 (39)</td>
<td>2 (10)</td>
<td>10 (23)</td>
</tr>
<tr>
<td>NE</td>
<td>5 (5)</td>
<td>2 (6)</td>
<td>0</td>
<td>6 (14)</td>
</tr>
<tr>
<td>ORR (CR + PR), n (%) (95% CI)</td>
<td>19 (21) (13.1–30.7)</td>
<td>0 (0–11.2)</td>
<td>2 (10) (1.2–31.7)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>DOR (95% CI), months</td>
<td>7.6 (2.9–NR)</td>
<td>0</td>
<td>6.9 (5.9–8.0)</td>
<td>-</td>
</tr>
<tr>
<td>DCR (CR + PR + SD), %</td>
<td>86</td>
<td>55</td>
<td>90</td>
<td>68</td>
</tr>
<tr>
<td>Median PFS, months</td>
<td>4.2</td>
<td>2.6</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Unconfirmed CR + PR, n (%)</td>
<td>29 (32)</td>
<td>1 (3)</td>
<td>3 (15)</td>
<td>5 (12)</td>
</tr>
</tbody>
</table>

Abbreviations: DCR, disease control rate; NE, not evaluable; NR, not reached; ORR, overall response rate; PD, progressive disease; SD, stable disease.

*Key efficacy measures are shown across treatment arms.

**Target Engagement: Pharmacodynamic Analysis of Paired Tumor Biopsy Specimens**

Per the protocol, paired fresh tumor biopsy specimens obtained before treatment (within 3 weeks of treatment start) and on day 15 of treatment were required for all patients enrolled. Pharmacodynamic markers were analyzed in 10, 21, and 26 paired biopsy specimens collected from patients in the D+P, T+P, and D+T+P arms, respectively. The effect of each therapy on MAPK signaling output [assessed as the change in phosphorylated ERK (pERK) levels by immunohistochemistry from the day 15 on-treatment biopsy specimen], relative to the pretreatment biopsy, was evaluated. Values were compared with paired biopsy specimens from patients with BRAFV600E colorectal cancer treated in our previous trial of BRAF+MEK inhibition with dabrafenib and trametinib (17) and with patients with BRAFV600E-mutant melanoma treated with BRAF inhibition (dabrafenib) alone (ref. 22; Fig. 3). A significant reduction in pERK levels was seen between the baseline and on-treatment biopsy specimens with the T+P doublet and D+T+P triplet (P = 0.002 for both), but not with the D+P doublet (P = 0.5; Fig. 3A). The D+T+P triplet, which demonstrated the greatest efficacy, also resulted in the greatest amount of pERK inhibition (60%) compared with T+P (41%), D+T (37%; ref. 17), and D+P (23%; Fig. 3B); however, a statistically significant correlation between pERK inhibition and response was not observed. The D+T+P triplet also produced the greatest suppression of phosphorylated ribosomal protein S6 (pS6), which is regulated by ERK activity in BRAF-mutant cancers, and represents a potential mechanistic/pharmacodynamic marker of responsiveness (ref. 23; Supplementary Fig. S2). However, none of the therapies produced as robust a degree of pERK inhibition as did the previously published data for dabrafenib monotherapy in melanoma samples (84%; ref. 22; Fig. 3B). Taken together, these findings provide a likely explanation for why even the D+T+P triplet in colorectal cancer still falls short of the >50% response rate observed with the single-agent BRAF inhibitor in BRAFV600E-mutant melanoma and supports the hypothesis that inadequate MAPK suppression due to robust and complex adaptive feedback in BRAFV600E colorectal cancer limits clinical benefit.

**Clinical Factors, MSI Status, and Response to D+T+P**

The relationship between response rate and several clinical factors (including prior anti-EGFR therapy and panitumumab dose) was evaluated in patients treated with D+T+P (Supplementary Fig. S3).

MSI is frequently associated with BRAFV600E mutation in colorectal cancer (24), with MSI/MMR status previously reported to affect prognosis in patients with BRAFV600E colorectal cancer (8, 25). MSI/MMR status was available for 78 patients (86%) treated with D+T+P and who had evaluable best clinical response and PFS data (Supplementary Fig. S4A). In the 11 of 78 patients (14%) whose tumors were MSI-high/MMR-deficient (dMMR), the response rate was 46% (5 of 11; 95% CI, 17%–77%) compared with 27% (18 of 67; 95% CI, 17%–39%) in patients whose tumors were microsatellite stable (MSS/pMMR), which was not statistically significant (Supplementary Fig. S4B). However, a trend toward a statistically significant increase in PFS (HR, 2.624; 95% CI: 1.060–6.508; log-rank test, P = 0.0449) was noted in patients with MSI receiving D+T+P, although it is not possible to determine whether this effect is predictive or prognostic (Supplementary Fig. S4C). None (0/67) of the MSS/pMMR patients with colorectal cancer remained on study for >1 year, whereas 3 of 11 (27%) of the MSI-high/dMMR patients with colorectal cancer remained on study for >1 year. Of these 3 patients, 1 achieved a PR lasting >24 months, and another patient demonstrated a CR lasting >26 months. Of note, the 1 patient treated with D+P who achieved CR was MSS/pMMR.

**Analysis of cfDNA and Response to D+T+P**

We used a highly sensitive method for the detection of tumor-derived mutations in cfDNA termed BEAMing (Beads,
Figure 3. Pharmacodynamic analysis of paired tumor biopsy specimens. **A**, H-scores for pERK in paired baseline and day 15 on-treatment tumor biopsy specimens from patients treated with D+P, T+P, and D+T+P. P values represent the paired t test. **B**, The percentage change in pERK H-score in the on-treatment tumor biopsy specimen relative to the baseline biopsy specimen in individual patients according to treatment. The percentage change in pERK H-score in paired on-treatment biopsy specimens for patients with BRAF<sup>V600E</sup> colorectal cancer treated with D+T and BRAF<sup>V600E</sup>-mutant melanoma treated with dabrafenib alone are shown for comparison. Horizontal bars represent the median.

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*Data from Falchook GS, et al. (ref. 22)*

*Data from Corcoran RB, et al. (ref. 17)*

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BRAF/EGFR/MEK Inhibition in BRAF<sup>V600E</sup> Colorectal Cancer
BRAF V600E levels in cfDNA, the change in CEA levels by 6 weeks of treatment was not statistically significant between patients who achieved CR/PR and those with stable or progressive disease (Fig. 4A). In serial blood collections obtained throughout therapy, a consistent rebound in BRAF V600E levels was observed in cfDNA at the time of disease progression, whereas a consistent pattern was not observed with CEA levels (Fig. 4C). Taken together, these data suggest that monitoring BRAF V600E levels in cfDNA during therapy correlates well with response and disease trajectory in patients with BRAF V600E-mutant colorectal cancer, and that cfDNA was more informative than CEA—the standard clinical tumor marker for colorectal cancer.

cfDNA analysis can also be an effective tool for identifying and detecting mechanisms of acquired resistance to therapy (27–31). Prior studies have revealed that acquired resistance to BRAF-directed therapy in patients with BRAF V600E colorectal cancer is frequently driven by genomic alterations (e.g., RAS mutations), which lead to reactivation of MAPK signaling (28, 32, 33). We used a BEAMing panel to detect the presence of 11 common hot-spot mutations in KRAS and NRAS
(see Methods for further details) in cfDNA before treatment, during treatment, and at disease progression. We observed that, of the 29 evaluable patients who achieved a response (CR or PR) or stable disease with D+T+P and had cfDNA data available at the time of progression, 14 patients (48%) developed ≥1 detectable KRAS or NRAS mutation in cfDNA at the time of disease progression, which was not detectable at baseline. As shown in Fig. 4D, the initial decrease in BRAFV600E mutation levels after initiation of therapy in these patients was followed by an eventual rebound in BRAFV600E levels on disease progression, accompanied by the emergence of ≥1 KRAS or NRAS mutation. In 6 of 29 patients (21%), >1 subclonal RAS mutation was observed on disease progression, suggesting the potential for tumor heterogeneity in the context of acquired resistance to therapy.

**DISCUSSION**

We present the results of a clinical trial of combined BRAF and EGFR inhibition with or without MEK inhibition in
**BRAF**

colorectal cancer. The trial was designed to target the key adaptive feedback pathways driving primary resistance to BRAF inhibition alone. Both combined BRAF and EGFR inhibition (with D+P) and combined BRAF, EGFR, and MEK inhibition (with D+T+P) were tolerated at the full label doses of all agents. However, the frequency and severity of AEs were greater in the D+T+P arm than in the D+P arm, most notably in terms of dermatologic toxicity. Remarkably, although all three agents were tolerated together at full dose, combined EGFR and MEK inhibition only (T+P) was not tolerated at full dose, due to dermatologic toxicity. Although this may be considered counterintuitive, it highlights the unique biology of the MAPK pathway and its key implications for therapy. Although BRAF inhibitors effectively suppress MAPK signaling by mutant **BRAF**

**V600E** monomers in tumor cells, they do not inhibit the MAPK pathway in normal cells, where RAF signals as a RAS-dependent dimer and paradoxically activates MAPK signaling (34–36). This activation underlies the frequent development of MAPK-driven tumors (e.g., proliferative skin lesions and secondary cutaneous malignancies) in patients receiving BRAF inhibitor monotherapy (37). Thus, BRAF inhibitors exhibit greater selectivity than other MAPK pathway inhibitors, allowing a greater degree of specific tumor MAPK suppression with less systemic toxicity; conversely, agents that inhibit MAPK signaling in all cells (such as MEK inhibitors) have greater systemic toxicity, limiting the achievable dose in patients and resulting in suboptimal MAPK inhibition in tumor cells. Moreover, the potential opposing effects of BRAF and MEK or EGFR inhibitors in normal cells likely counteract the effects on the MAPK pathway, providing a mechanistic explanation for the decreased toxicity seen with the triplet regimen in this trial. Taken together, these data illustrate how the therapeutic window advantages offered by BRAF inhibitors make them key components of therapeutic combinations for **BRAF**

**V600E** cancers.

Modest clinical activity was seen in the D+P arm, compared with reported response rates with BRAF inhibitor monotherapy; the confirmed response rate was 10%, whereas 15% were unconfirmed. These data are consistent with the efficacy reported for similar BRAF/EGFR inhibitor combinations (13, 38–40). Notably, a recent update of a study evaluating cetuximab + irinotecan with or without the BRAF inhibitor vemurafenib demonstrated that in patients treated with the triple combination, response rate was 16% (n = 44 evaluable patients), with a median PFS of 4.3 months among all patients in this arm (n = 49; ref. 40). Despite preclinical studies supporting EGFR as the primary driver of MAPK reactivation in **BRAF**

**V600E** colorectal cancer (12, 15), these data suggest that EGFR may be a critical mediator of resistance but that many patients may harbor other redundant mechanisms of adaptive MAPK reactivation. Consistent with this hypothesis, we observed that D+P led to MAPK suppression in on-treatment tumor biopsy specimens in only a subset of patients, suggesting that EGFR-independent mechanisms of MAPK reactivation play an important role in this disease. In support of this, some **BRAF**

**V600E** colorectal cancers do not express elevated levels of EGFR, and **BRAF**

**V600E** colorectal cancer cell lines have been identified in which MAPK reactivation and resistance are driven by RTKs other than EGFR, such as MET (12, 41). Collectively, these data support the need to inhibit both EGFR-dependent and EGFR-independent feedback signals in **BRAF**

**V600E** colorectal cancer.

Combined BRAF, MEK, and EGFR inhibition with D+T+P demonstrated increased efficacy, with confirmed and unconfirmed response rates of 21% and 32%, respectively—these figures being one of the highest response rates observed with any regimen to date in **BRAF**

**V600E**-mutant colorectal cancer (16, 17). Consistent with the potential importance of inhibiting EGFR-dependent and EGFR-independent feedback signals, D+T+P produced the greatest degree of MAPK pathway suppression in on-treatment biopsy specimens. However, D+T+P still produced suboptimal MAPK suppression when compared with dabrafenib alone in **BRAF**

**V600E**-mutant melanoma, providing a possible explanation for why the efficacy of this triplet in colorectal cancer still falls short of BRAF inhibitors alone in melanoma. This observation may also support the existence of adaptive feedback signals capable of overcoming the D+T+P triplet to drive MAPK reactivation and primary resistance to therapy. Therefore, developing therapeutic strategies that can overcome these signals and optimize MAPK pathway inhibition will be key.

In addition to driving primary resistance, our data also suggest that MAPK reactivation is a key mechanism of secondary or acquired resistance to therapy in **BRAF**

**V600E** colorectal cancer. We and others have reported that acquired resistance to BRAF inhibitor combinations in **BRAF**

**V600E** colorectal cancer can be driven by an array of alterations in MAPK pathway components and lead to pathway reactivation, including **RTK**

amplification, **RAS**

mutation or amplification, **BRAF**

**V600E** amplification, and **MEK**

mutations. This finding also highlights the critical importance of MAPK signaling in these cancers (28, 32, 33, 42). Here, in a larger cohort of patients, we observed that almost half of patients (48%) demonstrated emergence of **KRAS**

or **NRAS**

mutations in cfDNA at the time of disease progression. MAPK pathway alterations may be present in an even larger percentage of patients, because the cfDNA panel used detects only a limited number of mutations in **KRAS**

and **NRAS**

; therefore, other MAPK pathway alterations known to drive resistance, such as other **KRAS**

or **NRAS**

mutations, **RAS**

or **BRAF**

amplifications, and **MEK**

mutations, would not be detected. Furthermore, many (21%) of these patients exhibited emergence of multiple subclonal **RAS**

mutations at progression, suggesting the potential for tumor heterogeneity in the context of acquired resistance to therapy. Indeed, a previous study by Kopez and colleagues suggested that many **BRAF**

**V600E** colorectal cancers may harbor preexisting tumor subclones with 1 or more **RAS**

mutations prior to therapy, leading to the potential for rapid emergence of heterogeneous resistant subclones (16).

Collectively, these observations raise an important conceptual issue: Even though the D+T+P combination contains a MEK inhibitor, many of the resistance signals driving resistance occur upstream of MEK, including RTK-driven feedback in primary resistance and MAPK pathway alterations upstream of MEK in acquired resistance. Theoretically, these signals should still be intercepted by the MEK inhibitor and should not lead to MAPK reactivation. In
targeted therapy paradigms, resistance alterations almost always occur at the level of or downstream of the drug target, not upstream. This finding highlights a key vulnerability of MEK inhibitors, i.e., increased upstream pathway flux can lead to MEK hyperactivation and a reduced ability of MEK inhibitors to maintain pathway suppression, which has been demonstrated in preclinical studies (28, 43). This also suggests that alternative strategies or agents capable of maintaining profound blockade of MAPK signaling may be key to enhancing activity in BRAF\textsuperscript{V600E} colorectal cancer. We reported that ERK inhibitors, which act immediately downstream of MEK, can more effectively maintain MAPK suppression and can overcome many of the upstream resistance mechanisms to which MEK inhibitors are vulnerable (28, 32, 42). Thus, investigating ERK inhibitors or other agents that might achieve more robust and complete MAPK blockade may be key future strategies for BRAF\textsuperscript{V600E} colorectal cancer.

Overall, our study provides an example of how identifying and targeting key adaptive feedback signals can overcome resistance and improve response in BRAF\textsuperscript{V600E} colorectal cancer, although further optimization is needed. We observed MAPK reactivation as a consistent mechanism of both primary and acquired resistance, underscoring the MAPK pathway as a critical target in this disease. However, despite improvements in the response rate, the DOR is poor and median PFS is only 4.2 months. Our data suggest that rapid emergence of resistant subclones harboring MAPK-activating alterations may be a major driver of treatment failure and that future strategies aimed at suppressing or overcoming these resistance mechanisms may help to sustain clinical benefit. Such strategies might include next-generation targeted combinations or combinations with other classes of agents, such as cytotoxic chemotherapy, as was recently reported (40).

Prior studies, including The Cancer Genome Atlas, have demonstrated frequent associations between BRAF\textsuperscript{V600E} mutation and MSI in colorectal cancer (24), with MSI status reported to affect prognosis in patients with BRAF\textsuperscript{V600E} colorectal cancer (25). In the current study, many of the small group of patients who achieved prolonged benefit for >1 year while on therapy (including 3 patients who had a DOR ≥20 months) were noted to have MSI-high tumors. Similarly, the tumor from the 1 patient from our prior trial of dabrafenib and trametinib in BRAF\textsuperscript{V600E} colorectal cancer who maintained a CR for >4 years was also MSI (17).

Given recent data supporting the increased immunogenicity of MSI colorectal cancer and increased responsiveness to immune checkpoint inhibition (44–46), this observation suggests a potential role for the immune system in promoting durable response. Indeed, as data from melanoma and KRAS-mutant colorectal cancer suggest a potential synergy between MAPK inhibition and immune checkpoint inhibition (47, 48), combining optimal MAPK inhibition with immunotherapy may be a promising future strategy. Collectively, we hope that identifying and targeting key resistance mechanisms in BRAF\textsuperscript{V600E} colorectal cancer will continue to lead to important improvements in clinical outcome for patients with this poor-prognosis molecular subtype of colorectal cancer.

METHODS

Study Design

This trial was an open-label, phase I study to investigate the safety, pharmacokinetics, pharmacodynamics, and clinical activity of trametinib and dabrafenib when administered in combination with the anti-EGFR antibody panitumumab in patients with BRAF\textsuperscript{V600E} mutation–positive metastatic colorectal cancer (NCT01750918). Patients were enrolled to receive D+P, T+P, or D+T+P (Fig. 2) in initial dose-escalation studies to identify the optimal dosing strategy, followed by expansion cohorts to investigate the safety and clinical activity of each of the combination treatments. The appropriate ethics committee or Institutional Review Board at each study center approved the study protocol. The study was conducted in accordance with Guidelines for Good Clinical Practice and the ethical principles described in the Declaration of Helsinki, following all applicable local regulations.

Study Population

Eligible patients were required to have histologically or cytologically confirmed advanced or metastatic BRAF\textsuperscript{V600E} mutation–positive colorectal cancer with measurable disease as per RECIST v1.1. BRAF\textsuperscript{V600E} mutation status was determined by local testing. Patients were required to be aged ≥18 years, have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, have adequate baseline organ function (as determined by laboratory parameters), and be of non-child-bearing potential or agree to use contraception as outlined in the protocol. Key exclusion criteria included history of prior malignancy (other than colorectal cancer), BRAF mutation other than V600E, any serious or unstable preexisting medical condition, active hepatitis B or C infection, and prior exposure to a BRAF or MEK inhibitor. All patients provided written informed consent before enrollment.

Study Treatment

The study began with dose-escalation cohorts for all 3 drug combinations (D+P, D+T+P, and T+P) using a standard 3+3 enrollment scheme. Expansion cohorts were then enrolled to investigate the safety and clinical activity of the combinations. Patients in the D+P doublet arm were started in a dose-escalation cohort at the full monotherapy doses of dabrafenib (150 mg b.i.d.) and panitumumab (6 mg/kg Q2W; Fig. 1B). No dose de-escalations were required. Once the D+P dose was confirmed at the full dose of both agents, another cohort of patients was assigned to the D+T+P triplet arm. In the initial cohort, dabrafenib was started at full dose of 150 mg orally b.i.d., trametinib had a starting dose of 1.5 mg once daily, and panitumumab had a starting dose of 4.8 mg/kg i.v. Q2W. Dose escalation continued until the MTD was determined, and the full dose of all 3 agents was tested in the final cohort: dabrafenib 150 mg b.i.d., trametinib 2 mg orally daily, and panitumumab 6 mg i.v. Q2W. The DLT observation period was 28 days, and no DLTs were identified in the D+T+P cohort; the MTD was declared as the labeled dose of all 3 agents. Patients in the T+P arm, which included patients with BRAF\textsuperscript{V600E} metastatic colorectal cancer and BRAF wild-type metastatic colorectal cancer with anti-EGFR therapy acquired resistance, received a starting dose of trametinib 2 mg once daily and panitumumab 6 mg/kg i.v. Q2W. No DLTs were identified in this cohort, but patients experienced delayed dermatologic toxicity with long-term dosing. Thus, sub-MTD doses were explored: trametinib 1.5 mg once daily and panitumumab 6 mg/kg i.v. Q2W; trametinib 2 mg once daily and panitumumab 4.8 mg/kg i.v. Q2W. Approximately 20 patients were then enrolled into expansion cohorts for each arm (including dose-escalation patients from selected dose groups). To further optimize the dose for the D+T+P arm, the protocol was later amended to explore the additional patients at 2 doses of panitumumab: 4.8 mg/kg i.v.
versus 6 mg i.v. Q2W. At the time of radiologic disease progression, patients in the D+P and T+P arms had the option of crossing over to the D+T+P arm.

**Study Assessments**

The primary endpoint was the safety of each of the drug combinations. Secondary endpoints included investigator-assessed overall response rate, DOR, PFS, OS, and the pharmacokinetics and pharmacodynamics of the drug combinations. All patients treated with the T+P combination (n = 51) were evaluated for safety, and the full safety data set for these patients was derived from this population. However, only 31 patients treated with T+P were BRAF mutant, and efficacy is reported only for this subset.

Patients received study therapy until disease progression, unacceptable toxicity, death, or discontinuation for any other reason. Patients were assessed weekly for the first 28 days of dosing and then every 4 weeks throughout the continuation period. Follow-up visits were conducted at 14 days, 4 weeks, and 8 weeks after study drug discontinuation and then subsequently every 8 weeks for survival follow-up. Safety was monitored throughout the study for all patients across cohorts via physical examinations, laboratory evaluations, vital sign and weight measurements, performance status evaluations, ocular and dermatologic examinations, concomitant medication monitoring, electrocardiograms, echocardiograms, and AE monitoring (characterized and graded per Common Terminology Criteria for Adverse Events, v4.0). AEs were recorded using standard Medical Dictionary for Regulatory Activities coding. Dose interruptions, reductions, and discontinuations for all of the study drugs were monitored.

Tumors were assessed using investigator-read CT or MRI at baseline, every 6 weeks until week 24, and then every 8 weeks until progression or death. Response determination was based on RECIST v1.1. In addition to imaging, the CEA tumor marker was collected. For the subset of patients who showed a confirmed CR or PR, DOR was defined as the time in weeks from the first documented evidence of CR or PR (the first response prior to confirmation) until the time of documented disease progression or death due to any cause, whichever was first. PFS was defined as the time in weeks between the first dose and the date of disease progression or death due to any cause. Finally, OS was defined as the time in weeks from the first dose of study drug until death due to any cause.

Serial blood samples for assessment of pharmacokinetic parameters were collected before dose and after dose on days 1 and 15 and before dose on day 21 in the first 28 days of dosing. In the continuation period, blood samples were collected every 4 weeks up to and including week 20 on study.

**Statistical Methods**

The all-treated population was used for analysis of clinical activity, which included all patients who received ≥1 dose of study medication. Patients evaluable for efficacy were defined as those who had ≥1 adequate post-baseline radiologic disease assessment. The pharmacokinetics population included all treated patients for whom a blood sample for pharmacokinetics analysis was available. The biomarker population was defined as the participants in the all-treated population for whom a tumor biopsy/tissue sample was obtained and analyzed. Analysis of patients who received an intratumor dose escalation or who transferred from doublet to triplet therapy was included in the crossover population.

Dose-escalation phases of the study followed a 3 + 3 dose-escalation procedure. Evaluation of safety data from ≥3 patients who had completed ≥28 days of dosing on study was required prior to defining a new dose and starting the next cohort. To facilitate dose-escalation/de-escalation decisions, an adaptive Bayesian logistic regression model (BLRM) was used to predict the probability of DLTs at the dose levels yet to be tested. Specifically, an 8-parameter BLRM for combination treatment was fitted on the DLT data (i.e., absence or presence of DLT) accumulated throughout the dose-escalation phase to model the dose-toxicity relationship of D+T+P when given in combination (49).

Prior distributions for trametinib were calculated based on the toxicity data observed in the first-time-in-human study MEK111054, in which trametinib was administered alone. Similarly, prior distributions for dabrafenib were determined based on data observed in the first-time-in-human study BRF112680, in which dabrafenib was administered alone. Prior distributions of the parameter trametinib–dabrafenib interaction were based on data observed in study BRF113220, in which trametinib and dabrafenib were administered in combination. A noninformative prior was assumed for the other combination of the 2 or 3 compounds with panitumumab. The model was used only as a guide for what further doses to study in the presence of DLTs along with the 3 + 3 results.

The expansion phases of the study used a Bayesian predictive adaptive design that allowed the trial to be monitored more frequently at multiple stages (49). The criterion was based on a historically unimportant response rate of 15% versus a response rate of interest of 30%.

**Biomarker Analyses**

**Pharmacodynamic Analyses.** Fresh predose (baseline) and paired on-treatment (day 15) tumor biopsy specimens were collected and analyzed to assess the pharmacodynamic effects of each therapy. The MAPK pathway activation status was determined via immunohistochemistry assessment of pERK levels (Cell Signaling Technology; M0S075, clone 20G11). In addition, pS6 (Cell Signaling Technology; M0S341, clone D68F8) was also analyzed in a subset of the available fresh biopsy specimens at a sponsor-designated laboratory. For pERK and pS6, the H-score was derived as follows: [1 × (% cells 1+)] + 2 × (% cells 2+) + 3 × (% cells 3+)]. Nonparametric P values for the median differences between pretreatment and day 15 (±3) H-scores were derived for comparisons within and across arms.

**MSI Analyses.** Genomic DNA was isolated from tumor and non-tumor regions of tissue, and paired normal and tumor DNA were analyzed for MSI with 5 markers: BAT-25, BAT-26, NR-21, NR-24, and MONO-27. DNA was amplified by PCR. Fragment size distribution analysis was performed using high-resolution capillary electrophoresis with fluorescence detection. Fragment size distributions from tumor and nontumor tissue for each of the 5 markers were compared, and the stability or instability in size distribution patterns was determined. Significant changes in a marker indicate instability and imply a phenotypic decrease in tumor MMR activity. MSI status was reported as stable or high. In positive cases, 2 of 5 loci need to show instability. Instability was defined as variation of ≥3 bp PCR product size at the specific locus between nontumor and tumor samples. In a subset of samples, no sufficient normal DNA was available; MLH1, MSH2, MSH6, and PMS2 were analyzed immunohistochemically. If all markers stained positive, the tumor was considered to be MSS. If one of the markers was negative, the tumor was considered to be MSI.

We combined the confident calls that passed the quality-control criteria for MSI/MSS from both of the platforms. The box-plot comparisons across MSI/MSS were statistically assessed using the nonparametric Kruskal–Wallis P values. Time-to-event models stratifying based on MSI status were built, and Kaplan–Meier survival plots were assessed between MSI/MSS status using HR and 95% CIs and log-rank P values.

**ctDNA Analyses.** Plasma samples were collected at baseline, at week 4, and at progression. Baseline ctDNA and serial ctDNA collections were analyzed for the presence of mutations to provide correlates of response and to identify mechanisms of acquired resistance. Mutations were assessed in plasma ctDNA using BEAMing technology
(Sysmex Inostics) and a predefined targeted hot-spot mutation panel: BRAF<sup>V600E</sup> KRAS (G12S, G12R, G12C, G12D, G12A, G12V, and G13D), NRAS (Q61K, Q61R, Q61L, and Q61H), and PIK3CA (E542K, E545K, H1047R, and H1047L). The BEAMing assay uses emulsion PCR on magnetic beads and flow cytometry to quantify the fraction of mutation-positive DNA to wild-type DNA. The mutant fraction (MF)—defined by the ratio of the mutant beads to the sum of wild-type, mixed, and mutant beads—was used to compare mutation hot-spot levels in cfDNA.

The BRAF<sup>V600E</sup> MF ratio between week 4 and baseline was defined as follows:

$$\log_{10}(MF \text{ at week } 4 + 1 \times 10^{-5}) - \log_{10}(MF \text{ at baseline } + 1 \times 10^{-5}).$$

The BRAF<sup>V600E</sup> MF ratio between “progression” and baseline was defined as follows:

$$\log_{10}(MF \text{ at progression } + 1 \times 10^{-5}) - \log_{10}(MF \text{ at baseline } + 1 \times 10^{-5}).$$

Nonparametric Kruskal–Wallis P values were derived to compare the BRAF<sup>V600E</sup> MF ratios between week 4 and baseline across response groups. Pearson correlation was used to measure the linear correlation between SI level and the best percentage tumor change.

**CEA Analyses.** Serum intensity (SI) levels of CEA (or CEACAM5), which is commonly used as a blood-based tumor marker in patients with colorectal cancer as part of standard clinical practice, were used to profile the patients from this trial. We limited our CEA-related analyses to only patients’ samples with baseline SI levels above the upper normal range as derived per the clinical protocol. The changes in SI level between week 6 and baseline were calculated as the log ratio

$$\log_{10}(SI \text{ at week } 6) - \log_{10}(SI \text{ at baseline}).$$

Nonparametric Kruskal–Wallis P values were derived to compare SI ratios between week 6 and baseline across response groups.

**Study Oversight**

This study was designed, conducted, and analyzed by the funder (Novartis) in conjunction with the authors. All authors had full access to the study data and share final responsibility for the content of the manuscript and the decision to submit for publication.

**Disclosure of Potential Conflicts of Interest**

R.B. Corcoran reports receiving commercial research grants from AstraZeneca and Sanofi and is a consultant/advisory board member for Amgen, Astex, Avidity Biosciences, BMS, FOG Pharma, LOXO Oncology, Merrimack, N-of-One, Roche, Shire, and Taiho. T. André has received honoraria from the speakers bureaus of Amgen, Bristol Myers-Squibb, Bayer, Lilly, MSD Oncology, Novartis, Roche, Servier, and Sanofi, and is a consultant/advisory board member for Amgen, Bayer, Bristol Myers-Squibb, MSD Oncology, and Roche. C.E. Atreya reports receiving commercial research support from Guardant Health and Novartis, and is a consultant/advisory board member for Genentech. J.H.M. Schellens is an employee of Modra Pharmaceuticals. T. Yoshino reports receiving commercial research grants from GlaxoSmithKline K.K. and Boehringer Ingelheim GmbH. S. Siena is a consultant/advisory board member for Novartis. M.S. Gordon is a consultant/advisory board member for Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Celgene, Chugai, F. Hoffmann-La Roche Ltd, Genentech, Inc., Lilly, MSD, Merck Serono, Novartis, Pfizer, Sanofi, Symphogen, Taiho, and Takeda. R. Yaeger is a consultant/advisory board member for GlaxoSmithKline. P.J. O’Dwyer reports receiving a commercial research grant from GSK. A.S. Jung has ownership interest (including patents) in Amgen. B. Mookerjee is Program Physician Lead at GSK. No potential conflicts of interest were disclosed by the other authors.

**Authors’ Contributions**

**Conception and design:** R.B. Corcoran, J.H.M. Schellens, J. Tabernero, P.J. O’Dwyer, J.C. Brase, B. Mookerjee, F. Rangwala

**Development of methodology:** R.B. Corcoran, J.H.M. Schellens, J. Tabernero, B. Mookerjee, F. Rangwala


**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** T. André, T. Yoshino, F. Rangwala

**Study supervision:** R.B. Corcoran, J.H.M. Schellens, M.S. Gordon, J. Tabernero, P.J. O’Dwyer, F. De Vos, S. Bettringer, B. Mookerjee, F. Rangwala

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Combined BRAF, EGFR, and MEK Inhibition in Patients with BRAF V600E-Mutant Colorectal Cancer

Ryan B. Corcoran, Thierry André, Chloe E. Atreya, et al.

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