Phase I Expansion and Pharmacodynamic Study of the Oral MEK Inhibitor RO4987655 (CH4987655) in Selected Patients with Advanced Cancer with RAS–RAF Mutations

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Abstract

Purpose: This phase I expansion study assessed safety, pharmacodynamic effects, and antitumor activity of RO4987655, a pure MEK inhibitor, in selected patients with advanced solid tumor.

Experimental Design: We undertook a multicenter phase I two-part study (dose escalation and cohort expansion). Here, we present the part 2 expansion that included melanoma, non–small cell lung cancer (NSCLC), and colorectal cancer with oral RO4987655 administered continuously at recommended doses of 8.5 mg twice daily until progressive disease (PD). Sequential tumor sampling investigated multiple markers of pathway activation/tumor effects, including ERK phosphorylation and Ki-67 expression. BRAF and KRAS testing were implemented as selection criteria and broader tumor mutational analysis added.

Results: Ninety-five patients received RO4987655, including 18 BRAF-mutant melanoma, 23 BRAF wild-type melanoma, 24 KRAS-mutant NSCLC, and 30 KRAS-mutant colorectal cancer. Most frequent adverse events were rash, acneiform dermatitis, and gastrointestinal disorders, mostly grade 1/2. Four (24%) of 17 BRAF-mutated melanoma had partial response as did four (20%) of 20 BRAF wild-type melanoma and two (11%) of 18 KRAS-mutant NSCLC. All KRAS-mutant colorectal cancer developed PD. Paired tumor biopsies demonstrated reduced ERK phosphorylation among all cohorts but significant differences among cohorts in Ki-67 modulation. Sixty-nine percent showed a decrease in fluorodeoxyglucose uptake between baseline and day 15. Detailed mutational profiling confirmed RAS/RAF screening and identified additional aberrations (NRAS/non-BRAF melanomas; PIK3CA/KRAS colorectal cancer) without therapeutic implications.

Conclusions: Safety profile of RO4987655 was comparable with other MEK inhibitors. Single-agent activity was observed in all entities except colorectal cancer. Evidence of target modulation and early biologic activity was shown among all indications independent of mutational status. Clin Cancer Res; 20(16); 1–11. ©2014 AACR.

Introduction

The RAS/RAF/MEK/ERK pathway (MAPK pathway) plays a central role in regulating proliferation, differentiation, and survival. MAPK pathway activation can occur through several mechanisms, including mutations in RAS or BRAF (1, 2). Mutated, oncogenic forms of RAS are found in 30% of all human cancers, 45% of colorectal cancer, 20% of non–small cell lung cancer (NSCLC), 15% to 20% of melanomas, and 90% of pancreatic cancers (3). BRAF mutations are reported in approximately 50% of cutaneous melanomas; non-BRAF melanomas; PIK3CA/KRAS colorectal cancer) without therapeutic implications.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Prior presentation: This study has been presented at the 24th EORTC–NCI–AACR Symposium on molecular targets and cancer therapeutics.

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**Translational Relevance**

This multicenter phase I expansion (part 2) study has demonstrated that the MEK inhibitor RO4987655 has clinical activity in patients with BRAF V600-mutated melanoma, BRAF wild-type melanoma, and KRAS-mutated non–small cell lung cancer (NSCLC), but not in KRAS-mutated colorectal cancer. The safety profile of RO4987655 was at the level predicted from the dose escalation in phase I, with no new safety signals being identified. Evidence of target modulation and early biologic activity was shown among all indications independent of mutational status. Further development of RO4987655 based on its toxicity/efficacy profile and early clinical results in this expansion phase may potentially be pursued in BRAF wild-type melanoma in combination with other emerging signal transduction inhibitors after immunotherapy failure. Initial observations of clinical activity in NRAS-mutated melanoma need further confirmation. In KRAS-mutated NSCLC, combinations with taxanes, other cytostatics or PI3K inhibitors and/or mTOR inhibitors may be rational approaches for the future.

Melanomas, in 40% to 70% of papillary thyroid carcinomas, in 5% to 20% of colorectal cancer, in 10% to 20% of cholangiocarcinoma, 1% to 5% of NSCLC, and in the vast majority of hairy cell leukemia (4–6).

MEK 1/2 is the only enzyme known to activate ERK 1/2. ERK 1/2 being the only known substrate of MEK; therefore, MEK inhibition represents an attractive mechanism for blocking MAPK pathway activation (7). Recently, the MEK inhibitor trametinib has been shown to improve overall survival in BRAF V600-mutated metastatic melanoma (8) compared with dacarbazine or paclitaxel chemotherapy and has been approved by the Food and Drug Administration. Other small-molecule inhibitors of MEK are currently under clinical investigation (9–20). RO4987655 is a potent, highly selective adenosine triphosphate noncompetitive oral MEK inhibitor with manageable toxicity profile, favorable pharmacokinetics/pharmacodynamics characteristics, and preliminary antitumor activity in a phase I dose escalation (part 1) study in advanced solid cancers (21). Dose-limiting toxicities (DLT) were blurred vision (n = 3) and elevated creatine phosphokinase (CPK; n = 3), all of which were reversible without treatment. The MTD of RO4987655 was 8.5 mg twice daily. At the MTD, high (mean 75%) and sustained (90% of time >IC50) pERK inhibition was observed in peripheral blood mononuclear cells, and plasma drug concentrations were in the range predicted to be efficacious in preclinical models. Therefore, a dose regime of 8.5 mg twice daily was recommended for the phase I expansion (part 2) study. The significant incidence of RAS and/or RAF mutations in colorectal cancer, in NSCLC, and in melanoma (3–5) associated with favorable preclinical data (12, 14, 18, 20, 21) was the rationale to choose these specific tumor types for the expansion study. The relatively low response rate expected in each tumor type based on previous study data (18, 22) was the rationale for the chosen number of patients treated in each cohort.

The objective of this multicenter part 2 expansion study was to investigate the safety, the pharmacokinetics/pharmacodynamic profile, and single-agent antitumor activity of RO4987655 given at defined MTD (21) in diagnostically preselected cohorts with RAS or RAF driver mutations, including BRAF V600 mutation carrying melanoma, melanoma without BRAF V600 mutation (including possible other pathway-specific aberrations, i.e., NRAS), KRAS-mutated NSCLC, and KRAS- and/or BRAF V600-mutated colorectal cancer. The study was supported by extensive translational/imaging investigations to evaluate biologic activity and to identify potential predictive markers for single-agent efficacy of RO4987655 in tumors bearing activating mutations in the MAPK pathway.

**Materials and Methods**

**Patients**

Eligibility criteria included histologic or cytologic evidence of advanced or metastatic melanoma carrying BRAF V600 mutation, melanoma without BRAF V600 mutation, NSCLC with KRAS mutation, colorectal cancer with KRAS, and/or BRAF V600 mutation. Inclusion was restricted to two prior systemic therapies for melanoma and colorectal cancer, and three prior regimens for NSCLC. All prior systemic therapies were permitted. Additional requirements included age ≥18 years, Eastern Cooperative Oncology Group (ECOG) performance status ≤1, life expectancy of ≥12 weeks, measurable disease according to RECIST 1.1 (23), adequate bone marrow, renal, hepatic, and cardiac function. Mandatory tumor biopsies were obtained at baseline and on day (D) 15/cycle (C) 1. Patients with history of retinal vein occlusion, glaucoma, central serous retinopathy, corneal erosion, or risk factors for these ocular disorders were excluded. Patients with asymptomatic, corticosteroid-free, and radiographically stable previously treated brain metastases (≥4 weeks after radiation treatment) were eligible.

**Study design**

We performed this multicenter, open-label, phase I study in two parts. In part 1 (dose escalation), the MTD, DLTs, the pharmacokinetics/pharmacodynamic profile, safety, and preliminary antitumor activity of RO4987655 in patients with solid tumors were identified. The results of part 1 were reported after completion (21). Part 2 (first patient in) started 8 months after completion of part 1. In part 2 (cohort expansion), we assessed the safety, the pharmacokinetics/pharmacodynamic profile, and single-agent antitumor activity of RO4987655 given at defined MTD in patients with RAS/RAF mutations in melanoma, NSCLC, and colorectal cancer. This part 2 expansion study was conducted at 12 European sites, approved by institutional
ethics committees, and conducted in accordance with Declaration of Helsinki/Good Clinical Practice. All patients gave written inform consent. RO4987655 was administered twice daily at 8.5 mg, using a 28-day cycle, until progressive disease (PD), intolerable toxicity, or patient withdrawal. For any given patient, a maximum of 1 dose reduction or interruption was allowed after day 28 of cycle 1. Dose reductions were made for grade 3 or other intolerable drug-related toxicity. Re-escalation was permitted for grade ≥3 skin toxicity and/or isolated CPK elevation, which improved to grade ≤2, and for diarrhea or any other toxicity which improved to grade ≤1 within 14 days.

Assessments

Regular assessments, including fundoscopy and of cardiac function, were carried out at baseline and throughout treatment. Disease assessment was performed at baseline and every 8 weeks according to RECIST version 1.1. (23). Adverse events (AE) were graded according to NCI Common Toxicity Criteria (CTC version 3.0).

Pharmacokinetics

Blood samples (4 mL in potassium EDTA vacutainers) for pharmacokinetics analysis were collected predose, 0.5 and 10 hours on D1/C1 following drug administration; predose on D8/C1 and predose, 1 and 3 hours postdose on D15/C1. RO4987655 levels were determined by validated LC/MS-MS (24). Pharmacokinetic parameters were calculated via standard noncompartmental methods using WinNonlin V6.1 (Pharsight Corporation).

Pharmacodynamics

Tumor biopsies. RO4987655 effects on cellular proliferation (Ki67 labeling) and target/pathway inhibition (pERK expression) were investigated by immunohistochemistry (IHC) in paired tumor biopsies (at baseline and D15/C1). A ≥20% change in biomarkers between baseline and D15/C1 was considered relevant.

IHC. Tumor biopsies were formalin fixed and paraffin embedded following standard procedures. IHC for Ki67 and pERK was conducted using the ultraView detection Kit (Ventana Medical Systems Inc.) and the iView detection Kit (Ventana) on the Ventana Benchmark XT platform according to the manufacturer’s instructions (21).

Tumor-DNA mutation analysis. Mutational analysis for patient selection was performed centrally using formalin-fixed archival tissue samples which were collected before the start with RO4987655. Biopsies were microscopically assessed to ensure ≥50% tumor content and manually microdissected. RT-PCR with fluorescence-labeled, sequence-specific probes was used to distinguish wild-type BRAF (V600) sequence (GTG) from mutant sequence (GAG). KRAS mutations were identified using an investigational assay based on PCR/melting point analysis, with fluorescence-labeled, sequence-specific probes to distinguish wild-type sequence from mutation-bearing sequences in exon 2 (codons 12/13) and exon 3 (codon 61). All assays were performed on Cobas 4800 system (Roche Molecular Systems, Inc.) according to the manufacturer’s instructions. Mutational analysis for 19 oncogenes and 225 somatic mutations (Supplementary Fig. S1) was also conducted in available DNA from fresh tumor biopsies and/or archival tumor samples at baseline using the Sequenom OncoCarta panel v1.0 (25). All 19 genes/selected 225 somatic mutations were tested in all samples and called as “non-mutated” if not otherwise reported.

Fluorodeoxyglucose positron emission tomography. Metabolic activity was investigated by 2[18F]fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) at baseline, D15/C1, and D1/C3. Baseline and follow-up PET scans were conducted on the same scanner under identical conditions. Low-dose CT scans for all PET scans served for attenuation correction. Independent analysis of PET images was conducted centrally based on European Organisation for Research and Treatment of Cancer (EORTC) guidelines (26). Lesions (≤5) with highest FDG uptake were selected at baseline for quantitative analysis using 10-mm circular regions of interest centered on maximum FDG uptake to define standardized uptake value (SUV). Changes in SUV between baseline and D15/C1 were calculated for each patient. Patients with recent history of diabetes were excluded from FDG-PET.

Statistical methods

This report includes results based on the data cutoff of September 21, 2012. The effective dose and antitumor activity were evaluated based on clinical benefit rate [CBR; defined as complete response (CR), partial response (PR), and stable disease (SD)], objective response rate (defined as CR/PR), and duration of response. Duration of response lasted from date of PR/CR to date of PD or date of death. Best overall response of SD was defined as SD lasting ≥16 weeks for melanoma/colorectal cancer and ≥8 weeks for NSCLC. Duration on treatment was defined as the time from first dose to the earlier date of last dose or data cutoff (September 21, 2012), whichever came first. Pharmacokinetics, pharmacodynamics, safety, and tumor response were analyzed by descriptive statistics. Predictive/correlational analyses were performed comparing antitumor activity and biomarkers assessed by logistic regression and ANOVA. All analyses were stratified by tumor type/mutational status. Two-sided P values were evaluated, and a P value of <0.05 was considered statistically significant.

Results

Patients

Between March 2011 to September 2012, 96 patients were enrolled, 95 of whom received RO4987655 (Table 1). All had received previous anticancer treatment, including chemotherapy, immunotherapy, targeted agents, surgery, and radiotherapy (Table 1). None had previously received MEK inhibitor but 2 patients with BRAF-mutant melanoma and 1 patient with BRAF wild-type melanoma had undergone previous BRAF inhibitor treatment.
Ninety-three patients (98%) experienced one or more treatment-related AEs. Table 2 shows the most common AEs (≥10% of patients). Most common treatment-related AEs were skin related—rash and acneiform dermatitis, gastrointestinal disorders—diarrhea, nausea, and vomiting, general symptoms—dyspnea and asthenia, and eye disorders—in particular serous retinal detachment (SRD) and blurred vision (Table 2). Forty-three patients (45%) developed SRD. Grade 1 chorioretinopathy and retinal vein occlusion were reported in 2 patients (2%) each. The majority of AEs were mild or moderate in severity, and their incidence was comparable across all four cohorts. Sixty-one patients (64%) of 95 had dose modifications and/or temporary drug interruptions due to AEs, most frequently asymptomatic blood CPK increase (n = 20; 21%), rash (n = 18; 19%), SRD (n = 14; 15%), and diarrhea (n = 8; 8%). There were no treatment-related deaths.

**Pharmacokinetics**

Pharmacokinetics results were in line with those reported earlier (21). Plasma concentrations of RO4987655 increased rapidly following oral administration. The apparent systemic clearance was approximately 3.7 L/hour. Plasma exposure seems to be dose-linear, with a coefficient of variation of approximately 50%. Mean terminal half-life was approximately 12 hours. The effective half-life of RO4987655 was about 9 hours.

**Pharmacodynamics**

In total, 81 predose and 62 postdose tumor samples were available for pharmacodynamic analysis. However, only 45 paired biopsies of 95 treated patients (47%) were assessable for pERK expression and 38 paired biopsies (40%) assessable for Ki67. Thus, tumor biomarker results could only be obtained from about 50% of the treated patients. A significant downregulation of pERK was observed across all four cohorts between baseline and D15/C1. However, a significant reduction of Ki67 expression and 38 paired biopsies was not observed across all four cohorts between baseline and D15/C1.

**Table 1. Patients characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Melanoma BRAF V600-mutated (n = 18)</th>
<th>Melanoma Not mutated for BRAF V600 (n = 23)</th>
<th>NSCLC K Ras-mutated (n = 24)</th>
<th>Colorectal cancer K Ras-mutated (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years [median (range)]</td>
<td>47 (20–47)</td>
<td>55 (29–80)</td>
<td>55 (44–69)</td>
<td>57 (36–86)</td>
</tr>
<tr>
<td>Weight, kg [median (range)]</td>
<td>71 (52–94)</td>
<td>67 (51–98)</td>
<td>67 (45–102)</td>
<td>70.5 (43–166)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>7/11 (93/61)</td>
<td>10/13 (43/57)</td>
<td>5/19 (21/79)</td>
<td>14/16 (47/53)</td>
</tr>
<tr>
<td>Race (White/Black)</td>
<td>18/0 (100/0)</td>
<td>23/0 (100/0)</td>
<td>24/0 (100/0)</td>
<td>29/1 (97/3)</td>
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<td>ECOG baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/1/2/NK</td>
<td>10/8/0/0 (56/44/0/0)</td>
<td>16/7/0/0 (70/30/0/0)</td>
<td>7/14/1/2 (30/58/4/8)</td>
<td>13/16/0/1 (43/54/0/3)</td>
</tr>
<tr>
<td>Number of metastatic sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/1/2/3</td>
<td>0/2/2/14 (0/11/1/78)</td>
<td>0/2/6/15 (0/9/26/65)</td>
<td>1/1/5/17 (4/4/21/71)</td>
<td>0/2/13/15 (0/7/43/50)</td>
</tr>
<tr>
<td>Prior brain metastases</td>
<td>3 (17)</td>
<td>0 (0)</td>
<td>6 (250)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Prior anticancer treatments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>17 (94)</td>
<td>18 (78)</td>
<td>8 (33)</td>
<td>24 (80)</td>
</tr>
<tr>
<td>Radiation</td>
<td>8 (44)</td>
<td>11 (48)</td>
<td>11 (46)</td>
<td>5 (17)</td>
</tr>
<tr>
<td>Prior systemic therapiesa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/1/2/3</td>
<td>6/4/4/4 (33/22/22/22)</td>
<td>5/7/10/1 (22/30/43/44)</td>
<td>0/2/12/10 (0/8/50/42)</td>
<td>0/1/24/5 (0/8/30/17)</td>
</tr>
<tr>
<td>Previous BRAF inhibitor</td>
<td>2 (11)</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Previous anti-CTLA4 antibodyb</td>
<td>5 (28)</td>
<td>3 (13)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Abbreviation: NK, not known.

a Including prior chemotherapy and/or immunotherapy and/or targeted agents.

b Defined as previous therapy with ipilimumab or tremelimumab.
mutant ($P < 0.02$) and BRAF wild-type melanoma ($P < 0.02$; Fig. 1; Supplementary Fig. S2).

FDG-PET analyses

FDG-PET analysis was conducted in 74 patients. A decrease in FDG uptake between baseline and D15/C1 was observed in 51 patients (69%) but in only 15 of 57 patients (26%) studied at D1/C3. The largest FDG uptake reduction was observed in melanoma (Fig. 1 and Supplementary Fig. S2). Larger FDG response was indicative of higher RECIST response rate. Patients without FDG-PET response on D15/C1 were also not responding per RECIST at a later time point (high negative predictor value). D15/C1 FDG-PET response was not predictive of later RECIST response with only 10 of 51 patients (20%) showing a decrease in FDG uptake D15/C1 achieving a RECIST response (low positive predictor value).

DNA mutational analysis

Exploratory mutational analyses were conducted for 78 tumor samples: 17 BRAF V600-mutant melanoma, 18 BRAF wild-type melanoma, 18 KRAS-mutant NSCLC, and 25 KRAS-mutant colorectal cancer. Sixty-eight samples revealed mutations (Fig. 2, Table 3): eight NRAS mutations were additionally identified among the 18 BRAF wild-type melanoma patients; 13 BRAF V600 single mutations were confirmed plus four additional concomitant mutations (located on the CDK, EGFR, MET, and RET genes, respectively) were identified in 17 BRAF V600-mutant melanomas; fourteen KRAS single mutations plus seven KRAS/PIK3CA double mutations were detected in 21 of 25 KRAS-mutant colorectal cancers (4 samples did not provide enough material for analysis; hence, PIK3CA status is unknown); 17 KRAS mutations and one double KRAS/ AKT1 mutation were found in 18 KRAS-mutant NSCLCs. Figure 2 and Table 3 show the available mutational status of tumors and their response rate.

Antitumor activity

In total, 80 of 95 patients were evaluable for efficacy assessment (Table 3). PR was achieved in four (23.6%) of 17 patients with BRAF V600-mutated melanoma (Fig. 2A, Table 3), including one receiving study treatment/C21 52 weeks (Fig. 3). SD for/C21 16 weeks was noted in 5 patients (29%). The 2 patients who were treated previously with vemurafenib experienced PD (Table 3). In 20 patients with melanomas without BRAF V600 mutation, four (20%) PRs and four (20%) SD were reported (Fig. 2B, Table 3). The patient with NRAS-mutant melanoma who experienced PR received study treatment for 323 days. In 18 cases of KRAS-mutant NSCLC, two PRs were confirmed, including one on treatment for 56 weeks, as well as 8 (44%) with SD $\geq$8 weeks (Fig. 2C, Table 3). All 25 patients with KRAS-mutant colorectal cancer had PD (Fig. 2D, Table 3). The median duration of treatment across all subgroups was 63 days (range, 5–394) with the longest median duration of treatment being in BRAF-mutant melanomas at 113 days (range, 18–366; Fig. 3, Table 3).

Discussion

Our findings show that RO4987655 has clinical activity in patients with BRAF V600-mutated melanoma, BRAF wild-type melanoma, and KRAS-mutated NSCLC, but not...
in KRAS-mutated colorectal cancer. The safety profile of RO4987655 was at the level predicted from the phase I/part 1 selection of the MTD with no new safety signals being identified. Evidence of target modulation and early biologic activity was shown among all indications independent of tumor mutation status.

Similar to other MEK inhibitors (12, 18, 20), rash and diarrhea were the most frequent treatment-related AEs. The most common ocular toxicity was SRD occurring in 45% of all patients. The incidence of SRD was higher compared with other MEK inhibitors (17, 18), which may reflect the high rate and systematic monitoring of eyes disorders in the

Figure 1. Biologic activity across the four cohorts. Evidence of early biologic effect, as measured by FDG-PET, was shown for all four cohorts, with markedly lower effect in colorectal cancer than in other tumor types. Evidence of pathway inhibition effects (%pERK) was observed across all cohorts, with strongest effect in patients with BRAF V600-mutant melanoma. Early evidence of pharmacodynamic effects and blockade of cellular proliferation (%Ki67 decrease) was observed only in patients with BRAF V600-mutant melanoma.

Figure 2. Tumor percent change from baseline and exploratory genotyping in the four cohorts. A, BRAF V600-mutant melanoma. B, non-BRAF V600-mutant melanoma. C, KRAS-mutant NSCLC. D, KRAS-mutant colorectal cancer.
The mechanism of MEK-related eye disorders remains unknown and should be monitored closely in trials with similar compounds.

Evidence of pharmacodynamic drug effect was observed with a metabolic response in all four cohorts, with markedly larger effects in those in which objective tumor shrinkage was observed. Our study confirms the high negative predictive value of FDG for MEK inhibition and potential for FDG-PET to predict early nonresponders (27). We also noted a significant decrease of pERK phosphorylation in all cohorts, but an effect on proliferation (as measured by Ki67) was only observed in the two melanoma cohorts. These results may indicate an attenuated effect on the tumor by RO4987655 in KRAS-mutant NSCLC and particularly in colorectal cancer. However, it should be taken into consideration that the tumor biomarker results (assessed by IHC) were only obtained from about 50% of the treated patients due to limitation in the amount of tumor materials. The high FDG response on D15/C1 coupled with limited effects on cellular proliferation suggests that change in FDG uptake might not be related reduction in the number of viable tumor cells but instead reflects an effect on glucose uptake and utilization. This effect is rapid, reversible, and does not seem sufficiently sustained to induce apoptosis or necrosis and generate a response at later time points.

Current treatment progress in advanced melanoma has been focusing on relatively common BRAF V600 mutations (>50% melanomas; ref. 4). Recently, the BRAF inhibitors vemurafenib and dabrafenib, the MEK inhibitor trametinib, and the combination of the MEK inhibitor, trametinib, and the BRAF inhibitor, dabrafenib have been approved for the treatment of BRAF V600-mutated metastatic melanoma based on phase II/III clinical trials (8, 28–30). Besides BRAF inhibition, downstream targeting of the MAPK pathway has evolved as a further interesting target. Recently, the MEK inhibitor MEK162 has shown activity in melanoma harboring NRAS or V600 BRAF mutation (17). A further MEK inhibitor currently under investigation in advanced melanoma and in GNAQ/GNA11-mutant uveal melanoma is selumetinib (19, 31, 32). Our study demonstrated clinical efficacy with the MEK inhibitor RO4987655 both in BRAF V600-mutated melanoma and in non–BRAF V600-mutated melanoma, including NRAS-mutated melanoma. However, based on the well-established role of the cytotoxic T-lymphocyte antigen 4 (CTLA-4) inhibitor ipilimumab (33, 34) and the emerging data with the new immune checkpoint inhibitors, i.e., programmed death 1 (PD-1; refs. 35–37) and programmed death ligand 1 (PD-L1) inhibitors (38, 39), as well as the combination of both (40), the role of RO4987655 as single agent in patients with BRAF wild-type melanoma is limited. Further, the anti–PD-1 antibody, nivolumab, is being tested in a pivotal registration phase III trial as monotherapy versus dacarbazine in patients with BRAF wild-type metastatic melanoma (NCT01721772). If successful, this trial will establish an attractive future option for patients with BRAF wild-type melanoma. However, a small window of opportunity for combinations with MEK inhibitors exists for BRAF wild-type patients progressing following initial immunotherapy. Because a recent double-blind

<table>
<thead>
<tr>
<th>Table 3. Clinical activity</th>
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<tr>
<td><strong>Overall response</strong></td>
</tr>
<tr>
<td>Patients, n</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Melanoma, BRAF V600 mutated&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Previous BRAF inhibitor</td>
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<tr>
<td>Melanoma, non-BRAF V600 mutated</td>
</tr>
<tr>
<td>NRAS</td>
</tr>
<tr>
<td>Non-NRAS</td>
</tr>
<tr>
<td>NRAS unknown</td>
</tr>
<tr>
<td>NSCLC, KRAS mutated&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Colorectal cancer, KRAS mutated</td>
</tr>
<tr>
<td>KRAS&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>KRAS/PK3CA</td>
</tr>
<tr>
<td>KRAS mutated/PK3CA status unknown</td>
</tr>
</tbody>
</table>

<sup>a</sup>Best overall response of SD was defined as SD lasting ≥16 weeks for melanoma/colorectal cancer and ≥8 weeks for NSCLC.

<sup>b</sup>Defined as CR, PR, and SD.

<sup>c</sup>Inclusive BRAF V600/CDK, BRAF V600/RET, BRAF V600/EGFR, BRAF V600/MET.

<sup>d</sup>Inclusive KRAS/AKT1.

<sup>e</sup>Inclusive KRAS/CDK and KRAS/MET.
randomized phase II trial of docetaxel with or without the MEK inhibitor selumetinib in BRAF wild-type melanoma showed no significant improvement in progression-free survival compared with docetaxel alone (41). chemotherapy combinations may not be the best choice for this population. A possible strategy in BRAF wild-type melanoma after immunotherapy failure could be a combination of a MEK inhibitor with other new emerging signal transduction inhibitors. Moreover, in BRAF V600-mutant melanoma, the combination of the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib is superior to single-agent treatment (30).

MEK and BRAF inhibitors are under investigation in pretreated patients with advanced NSCLC. Single-agent selumetinib showed limited activity in a randomized phase II versus single-agent pemetrexed in unselected second-line patients with NSCLC (42). Data from NSCLC cell lines strongly suggest that those with RAS mutations are sensitive to selumetinib (43). Therefore, a signal finding randomized phase II was performed comparing the combination of docetaxel and selumetinib in KRAS-mutated NSCLC with docetaxel alone. The combination gave a better response rate (40% vs. 0%) and significantly improved progression-free survival (44). Toxicity of the combination has led to an ongoing randomized phase II trial (NCT01750281) investigating different docetaxel doses in combination with MEK inhibitor versus docetaxel monotherapy to better understand the toxicity/efficacy ratio of combinations of MEK inhibition and chemotherapy. The promising CBR ≥ 8 weeks of 56% (10/18; 2 PR/8 SD) with the MEK inhibitor RO4987655 as single agent in 18 patients with KRAS-mutated NSCLCs in third-line and significant metabolic responses demonstrated with FDG-PET/CT in this cohort underline that further investigation of MEK inhibitors preferably in combination with conventional cytotoxics and/or targeted agents in KRAS-mutated NSCLC may be justified. However, given the fact that patients with wild-type NSCLC were excluded from this expansion study, it is not possible to determine whether activity of RO4987655 in NSCLC is related to KRAS mutation status. This is particularly important, because in melanoma, activity was also seen in patients with BRAF wild-type melanoma. Furthermore, a phase I/ib study combining the oral MEK inhibitor trametinib with docetaxel or pemetrexed in KRAS-mutant and wild-type NSCLC demonstrated tolerability and clinical activity in both settings (NCT01192165; refs. 45, 46). Of note, 1% to 5% of NSCLCs harbor BRAF V600 mutations (4, 5). In these patient groups, BRAF inhibitors are currently being investigated (NCT01336634; ref. 47). Combinations of MEK inhibitors with BRAF inhibitors may also be of interest.

**Figure 3.** Duration of treatment in the four cohorts.
Despite metabolic responses on FGDPET/CT, we saw no clinical activity in KRAS-mutant colorectal cancer. The effect on Ki67 expression in paired biopsies was also not significant, including a few cases with upregulation of Ki67 in matched on-treatment samples. Coexistence of KRAS and PIK3CA mutations has been demonstrated in colorectal cancer tumors (48). Thus, single-agent administration of MEK inhibitors in colorectal cancer may not be advisable based on primary resistance mechanisms, but combinations with other signal transduction inhibitors may yet prove effective. In our study, we observed that the majority of tumors bearing double activating PIK3CA/KRAS mutations cluster among the less responsive patients. Despite insufficient sample size to derive robust conclusions, this may identify a particular aggressive tumor phenotype displaying double mutations, and this would indicate combined treatment with PI3K inhibitors. In addition, preclinical results also predict primary resistance of colorectal cancer cell lines to MEK inhibitors and confirm that there is a strong biologic rationale to combine these drugs with PI3K inhibitors or mTOR inhibitors (49, 50).

In conclusion, further development of MEK inhibitor RO4987655 based on its toxicity/efficacy profile and early clinical results in this expansion phase I may potentially be pursued in BRAF wild-type melanoma in combination with other emerging signal transduction inhibitors after immuno-therapy failure. Initial observations of clinical activity in NRAS-mutated melanoma need further confirmation. In KRAS-mutated NSCLC, combinations with taxanes, other cytokstatics or PI3K inhibitors and/or mTOR inhibitors may be rational approaches. In colorectal cancer, MEK inhibitor monotherapy is not effective and combinations with PI3K and/or mTOR inhibitors may potentially be of interest.

Disclosure of Potential Conflicts of Interest

I. Zimmer and W.E. Eberhardt report receiving speakers bureau honoraria from and are consultants/advisory board members for Bristol-Myers Squibb and Roche. F. Barlesi and J.-C. Soria are consultants/advisory board members for Roche. J.-P. Spano reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Roche. M.R. Middleton is a consultant/advisory board member for and reports receiving speakers bureau honoraria from Roche and commercial research grants from Astazeneca, GlaxoSmithKline, and Roche. E. Calvo reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Novartis. L. Paz-Ares reports receiving speakers bureau honoraria from Roche. J. Larkin is a consultant/advisory board member for Bristol-Myers Squibb, GlaxoSmithKline, Novartis, and Pfizer. M. Paques is a consultant/advisory board member for Merck Serono. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions


Analysis and interpretation of data (e.g., statistical analysis, bios- 


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L. Zimmer, F. Barlesi, M. Martinez-Garcia, V. Dieras, J.H.M. Schellens, W.E.E. Eberhardt, E. Guarin, V. Meresse, J.-C. Soria


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