Phase I Dose-Escalation Study of the Safety, Pharmacokinetics, and Pharmacodynamics of the MEK Inhibitor RO4987655 (CH4987655) in Patients with Advanced Solid Tumors

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Abstract

Purpose: This phase I study of the mitogen-activated protein/extracellular signal–regulated kinase inhibitor RO4987655 (CH4987655) assessed its maximum tolerated dose (MTD), dose-limiting toxicities (DLT), safety, pharmacokinetic/pharmacodynamic profile, and antitumor activity in patients with advanced solid tumors.

Patients and Methods: An initial dose escalation was conducted using a once-daily dosing schedule, with oral RO4987655 administered at doses of 1.0 to 2.5 mg once daily over 28 consecutive days in 4-week cycles. Doses were then escalated from 3.0 to 21.0 mg [total daily dose (TDD)] using a twice-daily dosing schedule.

Results: Forty-nine patients were enrolled. DLTs were blurred vision (n = 1) and elevated creatine phosphokinase (n = 3). The MTD was 8.5 mg twice daily (TDD, 17.0 mg). Rash-related toxicity (91.8%) and gastrointestinal disorders (69.4%) were the most frequent adverse events. The pharmacokinetic profile of RO4987655 showed dose linearity and a half-life of approximately 4 hours. At the MTD, target inhibition, assessed by suppression of extracellular signal–regulated kinase phosphorylation in peripheral blood mononuclear cells, was high (mean 75%) and sustained (90% of time >IC50). Of the patients evaluable for response, clinical benefit was seen in 21.1%, including two partial responses (one confirmed and one unconfirmed). 79.4% of patients showed a reduction in fluorodeoxyglucose uptake by positron emission tomography between baseline and day 15.

Conclusion: In this population of heavily pretreated patients, oral RO4987655 showed manageable toxicity, a favorable pharmacokinetics/pharmacodynamics profile, and promising preliminary antitumor activity, which has been further investigated in specific populations of patients with RAS and/or RAF mutation driven tumors. Clin Cancer Res; 18(17); 4794–805. ©2012 AACR.

Introduction

Constitutive activation of the Ras-regulated mitogen-activated protein kinase (MAPK) signaling cascade has been identified in various human cancers. The MAPK cascade comprises 3 enzymes (RAF/MEK/ERK) involved in regulation of cell proliferation, differentiation, survival, and migration (1, 2). Mutations of the Ras proto-oncogenes (KRAS, HRAS, and NRAS) have been found in approximately 30% of cancers (3), whereas BRAF gene mutations have been identified in up to 66% of malignant melanomas (4).

Mitogen-activated protein kinase kinase (MEK) is the only known kinase capable of phosphorylating ERK; therefore, inhibition of MEK can potentially block the activation of multiple downstream pathways. Several small-molecule inhibitors of MEK are currently being investigated (5–14). RO4987655 is a highly selective adenosine triphosphate noncompetitive oral MEK inhibitor that has shown...
Translational Relevance
The phase I and pharmacologic study has shown preliminary proof of principle that tumors with selected mutations are sensitive to mitogen-activated protein/extracellular signal–regulated kinase (MEK) inhibition. Positron emission tomography scan analysis and extracellular signal–regulated kinase phosphorylation measurement supported concentration-dependent target engagement. In addition, prolonged patient benefit could be shown in these patients. This may guide future development of the MEK inhibitor RO4987655. Evidence of pharmacologic activity, as shown by invasive and noninvasive biomarkers, combined with documented antitumor activity in this phase I study is an excellent basis for targeted development in phase II and III studies. Furthermore, despite the reported toxicities, continuous treatment at adequate levels of drug exposure was feasible in the great majority of patients.

Materials and Methods
Patient selection
Patients selected were aged 18 years or older with advanced or metastatic solid tumors for which no standard therapy was available. All patients had an Eastern Cooperative Oncology Group performance status (ECOG-PS) of ≤1, evaluable and/or measurable disease according to Response Evaluation Criteria In Solid Tumors (RECIST, v1.0; ref. 18), a life expectancy of ≥12 weeks, and adequate organ functions (see Supplementary Information for full inclusion/exclusion criteria). Patients with a history of ocular disorders or other known risk factors were excluded, as were patients who had received recent corticosteroids or hormone therapy (within 2 weeks of first planned RO4987655 dose) or recent major surgery, chemotherapy, radiotherapy, immunotherapy, or investigational agent (within 4 weeks). Unlimited prior systemic therapy for metastatic disease was permitted.

Study design and dose escalation
This phase I, open-label, dose-escalation study (NCT00817518) was conducted at 4 European centers, was approved by an Independent Ethics Committee, and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from all patients before carrying out any study-related procedure. RO4987655 was administered as oral capsules at least 2 hours after a light meal, followed by at least 1 hour before the next meal.

Initial escalation was conducted using a once-daily dosing schedule with oral RO4987655 administered over 28 consecutive days in 4-week cycles. On the basis of the data from a toxicity study in cynomolgus monkeys (15) and clinical data from healthy volunteers (16), a starting dose of 1.0 mg/day was chosen. A regimen of twice-daily dosing was also investigated, with a starting dose based on interim pharmacokinetics data from the once-daily regimen. A classical 3+3 dose-escalation design was used, with the dose escalated according to the grade/severity of toxicity during cycle 1. Dose escalation was conducted in 100% increments (according to nearest capsule strength) until the occurrence of grade II toxicity, after which subsequent escalation took place in increments of 50%. Following the occurrence of a grade III toxicity that was not a DLT, dose escalation was conducted in increments of 33% until the first DLT was observed. This cohort was expanded to 6 patients and if further DLTs were not observed in these 6 patients, dose escalation continued by 25% increments. Escalation was stopped if 2 or more patients in a given cohort developed a DLT and the preceding dose level expanded to 6 patients to confirm the MTD (defined as the dose level below the lowest dose at which ≥2 DLTs were seen).

No dose reductions were permitted during the first 28 days of the study (DLT evaluation period). For any given patient, a maximum of 1 dose reduction or interruption was allowed after day 28 of cycle 1. Reescalation was permitted for grade ≥II skin toxicity, which improved to grade ≤II, and for diarrhea or any other toxicity, which improved to grade ≤1 within 14 days. Patients were treated at their assigned dose until disease progression, unacceptable toxicity or patient withdrawal, whichever occurred first.

Assessments
Demographics and medical history were collected during screening. Physical examination, vital signs, and safety assessments [ECOG-PS, 12-lead electrocardiogram (ECG), hematologic/biochemistry, echocardiography/multigated acquisition (MUGA) scan, and ophthalmologic examination (fundoscopy)] were conducted at baseline/screening and throughout treatment: ECG on day 8 of cycle 1 (predose and 2 and 4 hours after drug administration); echocardiography/MUGA on day 1 of cycle 3; and all other...
assessments were done predose on days 1, 8, 15, and 22 of cycle 1, days 1 and 15 of cycles 2 and 3, and thereafter on day 1 of each cycle and at final visit. Following observation of creatine phosphokinase (CPK) elevation in 1 patient [17.0 mg, total daily dose (TDD)], CPK was measured in all subsequent patients and retrospectively in blood collected from patients receiving lower doses.

**Safety**

AEs were graded according to the National Cancer Institute Common Toxicity Criteria (v3.0; ref. 19). DLTs were defined as: grade ≥ III nonhematologic toxicity; grade ≥ III nausea/vomiting, skin rash, and/or diarrhea (despite adequate supportive care); grade ≥ III skin toxicity not reverting to grade ≤ II within 14 days of the scheduled start date; febrile neutropenia [absolute neutrophil count (ANC) <1.0 × 10^9/L and fever ≥38.5°C], and/or documented infection [ANC <1.0 × 10^9/L]; grade IV thrombocytopenia or bleeding requiring a platelet transfusion.

**Pharmacokinetics/pharmacodynamics**

Blood samples (4 mL in potassium EDTA vacutainers) were collected before dosing on days 1 and 15 of cycle 1 for pharmacokinetics analysis and at 1, 3, 7, and 12 hours following drug administration. Trough pharmacokinetics sampling was conducted predose on days 8 and 22 of cycle 1. The plasma concentration of RO4987655 was determined by a validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) method (16). Pharmacokinetics parameters were calculated via standard noncompartmental methods using WinNonlin V6.1 (Pharsight Corporation) and pharmacokinetics measurements were fitted to a pharmacokinetics/pharmacodynamics model of pERK inhibition in PBMCs using NONMEM vVI (ICON).

**pERK inhibition.** Target inhibition of 4 beta-phorbol 12-myristate 13-acetate (PMA)-induced pERK was measured in PBMCs (collected days 1 and 15, cycle 1 from all patients) using flow cytometry as described previously (16). NONMEM was used to fit pharmacokinetics/pharmacodynamics data to a model of serum and effect compartment RO4987655 concentration versus pERK inhibition in PBMCs (Supplementary Information). pERK inhibition was calculated as the percentage decrease in mean fluorescent intensity between pre- and postdose samples, with adjustment for non–PMA-stimulated prevalues. The antibody phospho-p44/42 MAPK (ERK1/2; Thr202/Tyr204; clone D13.14.4E, Cell Signaling Technology) was used to detect endogenous levels of p44 and p42 MAPK (ERK1 and ERK2) when phosphorylated either individually or dually at Thr202 and Tyr204 of ERK1 (Thr185 and Tyr187 of ERK2).

The effect of RO4987655 on cellular proliferation (Ki67 labeling) and target inhibition (pERK expression) was investigated by immunohistochemistry (IHC) in optional skin and tumor biopsies (collected at baseline/screening and on day 15 of cycle 1). Apoptosis was analyzed in tumor biopsies by terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay. A ≥20% change in pharmacodynamics biomarkers between baseline and day 15 was considered significant. Mutational analyses for KRAS, NRAS, BRAF (V600), HRAS, PI3KCA, and PTEN loss were conducted if archival tumor samples were available.

**Immunohistochemistry.** Skin and tumor biopsies were formalin fixed and paraffin embedded according to standard procedures. IHC for Ki67 was conducted using the ultraView detection kit (Ventana Medical Systems Inc.) on a Ventana Benchmark XT platform according to the manufacturer’s instructions. Slides were dewaxed, pretreated with mild Cell Conditioning 1 buffer (CC1; Ventana) and incubated for 12 minutes with a primary antibody against Ki67 (clone 30.9; Ventana). Slides were then counterstained with hematoxylin and bluing reagent, dehydrated, and mounted. For pERK IHC, the iView detection kit (Ventana) was used on a Ventana Benchmark XT platform. Slides were dewaxed and pretreated as before, and incubated for 1 hour with a phospho-p44/42 MAPK primary antibody (ERK1/2; Thr202/Tyr204; clone D13.14.4E; Cell Signaling Technology). To reduce nonspecific staining by endogenous biotin present in cells and tissues, the Endogenous Biotin Blocking Kit (Ventana) was also used. Slides were counterstained and mounted as before.

**TUNEL assay.** Formalin-fixed tissue sections were dewaxed and washed in PBS. Sections were incubated in 3% citric acid for 1 hour to decalcify the tissue and, after 3 washes with water, epitope retrieval was conducted using proteinase K (Roche). Slides were again washed in water, and incubated with 100 μL of TUNEL reaction mixture (containing FITC-dUTP) at 37°C for 1 hour. Following another wash, slides were treated with 3% hydrogen peroxide for 15 minutes to block endogenous peroxidase activity before incubating with a secondary antibody (anti–FITC–HRP; Roche) for 45 minutes. Finally, slides were washed and 3-amino-9-ethylcarbazole substrate was added for 10 minutes. Counterstaining was conducted using hematoxylin for 30 seconds and slides were mounted using a gelatin–glycerin mounting medium.

**Mutation analysis.** Mutation analysis was conducted centrally using formalin-fixed tissue. Biopsies were first assessed to ensure at least 50% tumor cell content and manually microdissected if required. Real-time PCR with fluorescently labeled, sequence-specific probes was used to distinguish the wild-type (WT) BRAF (V600) sequence (GTG) from the mutant sequence (GAG). Mutational analyses for KRAS mutations were identified using an investigational assay based on PCR and melting temperature analysis, with fluorescently labeled, sequence-specific probes designed to distinguish the WT sequence from mutation bearing sequences in exon 2 (specifically at codons 12 and 13) and in exon 3 (specifically at codon 61). All assays were conducted on the cobas 4800 system (Roche Molecular Systems, Inc.) according to manufacturer’s instructions. NRAS (in melanoma), HRAS, and PI3K mutations were screened for by standard sequencing methods and PTEN loss was determined by IHC (antibody clone 138G6; Cell Signaling Technology).
FDG-PET. Metabolic activity of tumors was investigated by 18F-FDG positron emission tomography (FDG-PET; at baseline; day 15, cycle 1; day 1, cycle 3). Baseline and follow-up PET scans were conducted using a single scanner and under the same conditions (administered 18F-FDG activity for all scans was maintained within 10% of the calculated activity administered at baseline and the same acquisition time per bed position was used for all scans for each individual patient). Low-dose CT scans were conducted for all PET scans for attenuation correction. Independent analysis of PET images was conducted centrally based on European Organisation for Research and Treatment of Cancer guidelines (20). Lesions with the highest degree of FDG uptake were selected for quantitative analysis (up to 5) and a 10 mm circular/spherical region of interest drawn. A standardized uptake value (SUV) was measured for each selected lesion and the delta change in SUV between baseline and day 15 of cycle 1 was calculated for each patient. FDG-PET scanning took place before tumor biopsies to avoid interference on FDG uptake. Patients with a recent history of diabetes were excluded from FDG-PET analysis.

Tumor response
Tumor assessments according to RECIST criteria (version 1) were conducted at screening, every 2 cycles and on suspicion of disease progression.

Statistical analyses
Pharmacokinetics/pharmacodynamics, safety, and tumor response data were analyzed by descriptive statistics. Correlations between specific AEs and antitumor activity or pharmacokinetics were assessed by logistic regression and ANOVA.

Results
Forty-nine patients were enrolled between January 2009 and June 2010 (Table 1), all received at least 1 dose of RO4987655. The most common tumor types were melanoma (n = 27, 55.1%; including choroidal melanoma, n = 9) and colorectal cancer (CRC, n = 11, 22.4%). None of the patients had previously received treatment with a MEK inhibitor. Thirteen patients received RO4987655 once daily (1.0–2.5 mg) and 36 received RO4987655 twice daily (3.0–21.0 mg TDD; Supplementary Fig. S1). Patients received a median of 2 treatment cycles (range, 0–12; 93.8% of patients completed at least 1 cycle), with a median duration of treatment of 57 days (range, 2–337).

Four DLTs were observed during the first 28 days of treatment, all with twice-daily dosing (Table 2). At 8.5 mg twice daily (TDD, 17 mg), 1 patient experienced grade III elevated CPK. No further DLTs were observed when this cohort was expanded to 9 patients in total. After escalation to 10.5 mg twice daily (TDD, 21 mg), 3 patients experienced DLTs (grade III blurred vision, grade III elevated CPK, and grade IV elevated CPK). Accordingly, 8.5 mg twice daily (TDD 17.0 mg) was defined as the MTD. All DLTs were reversible.

Safety
Patients experienced 189 treatment-related AEs, including 20 grade III AEs (in 17 patients) and 2 grade IV AEs (in 2 patients). The most common AEs (≥10% of patients; Table 2) were skin toxicity [rash related, n = 45 (92% of patients); dry skin, n = 7 (14%); skin fissures, n = 6 (12%)] and gastrointestinal [diarrhea, n = 16 (33%); nausea, n = 7 (14%); vomiting, n = 6 (12%); stomatitis, n = 5 (10%)]. Grade III/IV AEs were primarily limited to twice-daily dosing of ≥5.0 mg. Among the rare (<10% patients) grade ≥III toxicities, isolated and reversible grade III neutropenia occurred in 2 patients in the 1.5 and 10 mg dose cohorts and 1 case of reversible grade III anemia occurred in the 13 mg dose cohort treated by transfusion on day 63. One patient was reported with a grade III left ventricle ejection fraction.
Table 2. Treatment-related toxicity

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Total daily dose (mg)</th>
<th>Once-daily dosing&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Twice-daily dosing&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>1.5 (n = 4)</td>
<td>3 (n = 3)</td>
</tr>
<tr>
<td>DLTs</td>
<td></td>
<td></td>
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<tr>
<td>Grade III blurred vision (n = 1)</td>
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<td></td>
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<tr>
<td>Grade III elevated CPK (n = 2)</td>
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<tr>
<td>Grade IV elevated CPK (n = 1)</td>
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<tr>
<td>Treatment-related AEs</td>
<td>AEs (all grades)</td>
<td></td>
<td></td>
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<tr>
<td>Rash related&lt;sup&gt;d&lt;/sup&gt;</td>
<td>45</td>
<td>91.8</td>
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<tr>
<td>Diarrhea</td>
<td>16</td>
<td>32.7</td>
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<tr>
<td>Eye related&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Elevated CPK</td>
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<td>Fatigue</td>
<td>8</td>
<td>16.3</td>
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<tr>
<td>Dry skin</td>
<td>7</td>
<td>14.3</td>
<td></td>
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<tr>
<td>Nausea</td>
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<td>14.3</td>
<td></td>
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<tr>
<td>Skin rashes</td>
<td>6</td>
<td>12.2</td>
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<tr>
<td>Vomiting</td>
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<td>12.2</td>
<td></td>
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<tr>
<td>Stomatitis</td>
<td>5</td>
<td>10.2</td>
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<tr>
<td>Asthenia</td>
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<td>6.1</td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
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<td>6.1</td>
<td></td>
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<tr>
<td>Anemia</td>
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<td>4.1</td>
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<tr>
<td>Decreased appetite</td>
<td>2</td>
<td>4.1</td>
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<td>Depression</td>
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<td>2.0</td>
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<tr>
<td>Decreased Ejection Fraction</td>
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<td>2.0</td>
<td></td>
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<tr>
<td>Treatment-related SAEs (all grades)</td>
<td>n</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>1</td>
<td>2.0</td>
<td></td>
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<tr>
<td>Anemia&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>2.0</td>
<td></td>
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<tr>
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<td>Asthenia</td>
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<td>2.0</td>
<td></td>
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<tr>
<td>Decreased appetite</td>
<td>1</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Chorioretinopathy</td>
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<tr>
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<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Blurred vision</td>
<td>1</td>
<td>2.0</td>
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</tbody>
</table>

NOTE: Data cutoff: January 28th 2011. Only the most severe intensity was counted for multiple occurrences of the same AE in one individual. Adverse events occurring in >10% of patients are shown above the dotted line.

<sup>a</sup>Only one grade III toxicity (neutropenia) was reported in only one dosing-cohort in the once-daily regimen (1.5 mg once daily).

<sup>b</sup>The most common AEs were reported at grade III or IV severity in the twice-daily dosing cohorts. Two grade IV AEs occurred, both elevated CPK (8.5 and 10.5 mg, twice daily). No grade V AEs occurred. Nausea/vomiting, skin rash, and/or diarrhea were only considered a DLT if they reached grade III severity despite adequate supportive care measures. SAEs were defined as any AE, which was fatal; life-threatening; required in-patient hospitalization or prolongation of existing hospitalization; resulted in persistent or significant disability/incapacity; was a congenital anomaly/birth defect; was medically significant or required intervention to prevent one or other of the outcomes listed above. No SAEs occurred in once-daily dosing cohorts.

<sup>c</sup>No further DLTs were observed when the 17.0 mg (twice daily) cohort was expanded to 9 patients.

<sup>d</sup>Includes dermatitis acneiform, rash, dermatitis, papular rash, folliculitis, and genital rash.

<sup>e</sup>No further DLTs were observed when the 17.0 mg (twice daily) cohort was expanded to 9 patients.

<sup>f</sup>Includes blurred vision, photopsia, corneal erosion, dry eyes, periorbital edema, chorioretinopathy, punctate keratitis, and retinal vein occlusion.

<sup>g</sup>No signs of chronic or acute gastrointestinal bleeding were observed and no hemoglobinuria was recorded.
fraction decrease in the 13 mg dose cohort which occurred on day 56 when drug was stopped because of the progression of the disease. The other grade III toxicities included general disorders (asthenia, depression, decreased appetite) and skin disorders (Table 2). Most rash-related toxicities were grade I/II, with 6 patients experiencing grade III events and no grade IV events reported. Median time to development of grade III rash was 49.5 days (range, 9–146). Skin toxicity developed primarily in the face, upper trunk, and back; comprising red papulopustules and crusts, occasionally accompanied by swelling (mainly the nose). Severe psychologic impacts were reported in patients experiencing substantial alterations in appearance.

Sixteen eye-related AEs occurred in 13 [27%] patients [including blurred vision, photopsia, corneal erosion, dry eyes, periorbital edema, chorioretinopathy, punctate keratitis, and retinal vein occlusion (RVO)]. Blurred vision was associated with fluid accumulation in the subretinal space, identified by optical coherence tomography (OCT), resulting in serous retinal detachment (SRD) in one patient. Two patients experienced grade III ocular toxicity [RVO (8.5 mg twice daily) and blurred vision (10.5 mg twice daily)]. Median time-to-onset of ocular toxicity was 12 days (range, 1–175 days), and median duration of toxicity was 14 days (range, 2–104 days). Ocular toxicity resolved either spontaneously or with drug interruption (2 grade II and 2 grade III) except for 1 patient with grade III visual disturbances associated with RVO who improved to grade I at study completion, and 1 with grade I blurred vision whose condition remained unresolved at study completion.

Nine (18%) patients experienced elevated CPK including 4 grade III (1 at 2.0 mg twice daily, 2 at 8.5 mg twice daily, and 1 at 10.5 mg twice daily) and 2 grade IV events (at 8.5 mg twice daily and 10.5 mg twice daily). CPK elevation was reversible with drug interruption and was asymptomatic in most patients and not associated with either clear rhabdomyolysis symptoms or cardiac dysfunction. Three cases of grade I myalgia (in 3 patients), 1 grade I joint swelling, 1 grade II joint stiffness, 1 grade II neck pain, 1 grade I pain in an extremity, and 1 grade II muscular weakness were reported in association with CPK elevation.

Eight patients experienced dose reductions due to treatment-related AEs (1 patient at 1.5 mg once daily and 4.0/3.5 mg twice daily, and 3 patients at 8.5 mg twice daily and 10.5 mg twice daily), including 5 patients who experienced more than one AE-related dose interruption (1 patient at 1.5 mg once daily and 2 patients each at 8.5 mg twice daily and 10.5 mg twice daily). Of the 9 patients receiving RO4987655 at the RP2D (8.5 mg twice daily), the median duration of dosing was 87.5 days (range, 50–194 days) in the 6 patients who did not undergo dose modification. Median time to dose modification in the remaining 3 patients was 37 days (range, 14–51).

Eleven patients experienced temporary drug interruptions due to AEs (1 each at 1.5 mg once daily, 2.0 mg once daily, 4.0/3.5 twice daily, and 5.0 mg twice daily; 2 at 6.5 mg twice daily and 10.5 mg twice daily; and 3 patients at 8.5 mg twice daily). The median duration of interruption was 7 days (range, 1–21; Supplementary Table S1).

Seven patients experienced 8 treatment-related serious AEs (SAEs; all grade II/III; Table 2). Five of the SAEs were resolved, 2 with dose modifications, 2 without dose modifications, and 1 with treatment discontinuation. The remaining 3 SAEs were unresolved. Two deaths following disease progression were not considered to be treatment related.

**Antitumor activity**
Clinical benefit [defined as partial response (PR) or stable disease (SD) lasting ≥16 weeks] was seen in 8 of the 38 evaluable patients (21.1%; Fig. 1), including one confirmed and one unconfirmed PR in patients with skin melanomas (mutational status unknown). These patients received treatment with RO4987655 for 113 and 224 days (mean 168.5 days) and responses lasted for 48 and 168 days (mean 108 days). Six other patients achieved SD lasting >16 weeks: 3 patients with melanoma, 2 patients with choroidal melanoma, and 1 patient with a rectal adenocarcinoma. The median percentage change in tumor size at maximum reduction from baseline in evaluable patients was 9.8% (range −66.9% to 101.4%).

**Pharmacokinetics**
PK was assessed in 43 patients (87.8%). Plasma concentrations of RO4987655 increased rapidly following oral administration. For the majority of patients, maximum plasma drug concentration (C_{max}) was reached approximately 30 to 60 minutes after dosing (Fig. 2A, Table 3). Mean terminal half-life was approximately 4 hours. Plasma exposure increased approximately dose proportionally on day 1 (Fig. 2B) and increased linearly with dose at steady state (Fig. 2C). Intrapatient variability in plasma exposure was limited (Table 3). At the MTD, C_{max} and area under the plasma concentration–time curve (AUC_{0–12h}) were 425 ng/mL and 1,660 ng·h/mL, respectively, at day 1 and 530 ng/mL and 2,577 ng·h/mL, respectively, at steady state. The mean accumulation index (AUC_{day 15}/AUC_{day 1}) was 1.53 (range, 1.15–1.96). Increased steady-state plasma exposure was significantly associated with occurrence of grade II/III rash (logistic regression, P = 0.01) and showed a trend toward association with CPK elevation (ANOVA; P = 0.07).

**Pharmacodynamics**
**pERK inhibition in peripheral blood lymphocytes.** Assessment of target suppression was evaluated by measuring the extent of pERK inhibition in a surrogate tissue, PBMC. The relationship between exposure (RO4987655 plasma concentration) and pharmacodynamics effect (pERK inhibition in PBMCs) was characterized by a direct link pharmacokinetics/pharmacodynamics (effect compartment) model, which revealed 70% to 80% pERK inhibition at plasma concentrations of >200 ng/mL (Fig. 3A).

**Tumor/skin biopsies.** Between baseline and day 15, pERK expression in tumor biopsies decreased by ≥20% in 6 of 11 evaluable patients and increased by ≥20% in the
other (Fig. 3B, Supplementary Table S2). One tumor showed >90% reduction in pERK expression. Paired pre-treatment and posttreatment normal skin biopsies were available from 20 patients; 5 showed a decrease in pERK expression of ≥20% by day 15, 14 showed no change, and IHC failed in 1 patient (Fig. 3B, Supplementary Table S2). One skin biopsy showed >90% pERK reduction. Most tumor and skin biopsies showed no change in cell proliferation (Ki67 labeling) between baseline and day 15 (Supplementary Table S2). Three of 5 paired tumor biopsies showed no change in apoptotic signal between baseline and day 15 (TUNEL assay; Supplementary Table S2). No correlations were observed between changes in biomarker levels and tumor response, mutational status, or exposure.

**Mutational analysis.** Mutational analyses were conducted for tumor samples from a total of 30 patients: 22 samples were suitable for assessment of *BRAF V600* and *KRAS* mutations, 21 were suitable for *NRAS*, 18 for *HRAS* and *PI3K*, and 10 samples were suitable for assessment of *PTEN* loss. Of the 30 tumor samples assessed, 8 revealed mutations (Table 1) including 2 melanomas with *BRAF* (V600) mutation, 5 CRC with *KRAS* mutation and 1 CRC with both *KRAS* and *PI3K* mutations. Figure 1 shows the mutational status of tumors that were evaluable for tumor response and are not shown. b.i.d., twice daily; q.d., once daily.

In patients with advanced solid tumors, oral RO4987655 was moderately tolerated with manageable toxicity and showed a favorable pharmacokinetics/pharmacodynamic profile and encouraging antitumor activity. The safety profile of RO4987655 in this study was consistent with data from healthy volunteers with no new safety signals being identified. The management and treatment of safety events was facilitated by the short half-life of RO4987655. The MTD of RO4987655 was 8.5 mg twice daily (17.0 mg TDD). DLTs were grade III blurred vision (n = 1) and grade III/IV elevated CPK (n = 3), all of which were reversible without treatment.

The most frequent RO4987655-related AEs were skin toxicity and gastrointestinal disorders. MEK inhibitor class effects included rash (91.8%) and eye-related toxicity (26.5%). Previous studies with MEK inhibitors have reported rash, diarrhea, nausea, fatigue, and visual disturbances as the most common treatment-related AEs.
An indirect comparison between RO4987655 and the phase I published data from other MEK inhibitors suggests that RO4987655 has a comparable safety profile with a higher frequency of rash-related toxicity (92% vs. 38%–79%), but a generally lower incidence of diarrhea (32% vs. 32%–55%), nausea (14% vs. 29%–54%), and eye-related toxicity (27% vs. 33%–50%; refs. 5, 8, 13, 21, 22), while CPK elevation which was observed regularly in this study, has so far not been observed with other MEK inhibitors (5, 13, 21, 23).

Ocular toxicity is a known class effect of MEK inhibitors and was also observed in this study. Two episodes of blurred vision even occurred very early in treatment, after 1 or 2 days of dosing. The majority of visual symptoms reported in this study were due to SRD, but OCT was not systematically conducted, preventing accurate evaluation of the incidence of associated SRD. Although ocular toxicities can be alarming for both patients and physicians, all cases of SRD reported in this study were reversible without any specific treatment and without long-term damage. This is in line with blurred vision reported in other studies (5, 8, 13). Other class-related ocular complications in this study occurred less frequently than SRD and include ocular hypertension, which can be detected by regular measure of intraocular pressure, and RVO, that can be detected early by regular fundus photographs. Because the pathogenesis of MEK-related eye disorders remains unknown and experience with chronic administration of MEK inhibitors is limited, careful monitoring of ocular disorders should be implemented in further clinical trials with MEK inhibitors to ensure adequate management of patients.

CPK elevation was reported in this study and considered related to RO4987655. Systematic measurement of CPK in this study may have generated a higher incidence of reports. Most elevated CPK episodes were asymptomatic and no RO4987655-related cardiovascular disorders or rhabdomyolysis were observed. The mechanism behind the observed CPK elevation remains unknown at present. A direct role of MEK inhibition cannot be excluded as the MAPK pathway plays a key role in regulation of muscle cell signaling (24–27).

RO4987655 monotherapy showed encouraging antitumor activity as measured by RECIST. Like other MEK inhibitors, RO4987655 showed clinical activity against melanoma (13, 21–23). Seven of the 8 patients who achieved clinical benefit with RO4987655 had melanoma tumors, including 2 PRs (1 confirmed, 1 unconfirmed; both at 8.5 mg twice daily). No clear correlation existed between response and mutational status; however, the number of patients with mutation data was limited. On the basis of the safety and pharmacokinetics/pharmacodynamics profile presented here, a dose regimen of 8.5 mg twice daily (17.0 mg TDD) RO4987655 is recommended for phase II studies.

PK analyses showed that RO4987655 was absorbed rapidly, reaching $C_{\text{max}}$ 0.5 to 1 hour after dosing, and that plasma concentration and exposure increased approximately dose proportionally. The pharmacokinetics of
RO4987655 was linear, time independent, and consistent with an earlier study in healthy volunteers, with the exception of terminal half-life, which was much shorter than previously reported (~4 hours vs. 25 hours, respectively; ref. 16). Although the reason for this remains unclear, it may indicate that the longer sampling period used previously allowed for a more accurate assessment of the terminal phase half-life: RO4987655 was monitored for 72 hours postdosing in healthy volunteers but only 12 hours in this study because of the inclusion of twice-daily dosing. The influence of food on the absorption of RO4987655 remains to be determined. Data from recent studies with other MEK inhibitors are conflicting; although administration with a high-fat meal was shown to increase exposure to the oral inhibitor CI-1040, exposure to selumetinib was reduced when administered with food (28, 29).

Evidence of biologic activity was shown by FDG-PET, particularly in melanoma where decrease in FDG uptake appeared to be associated with dose and drug exposure. In addition, a weak relationship was observed between change in FDG uptake (between baseline and day 15) and RECIST tumor assessment. All patients achieving a PR or SD lasting >16 weeks showed a reduction in FDG uptake by day 15, suggesting that an FDG decrease was necessary, but not sufficient, for later tumor response. Furthermore, FDG-PET data correlated with PBMC pERK inhibition; larger changes from baseline were associated with increased inhibition of pERK. Clinical studies investigating other signal transduction inhibitors, like imatinib (in gastrointestinal stromal tumors and soft-tissue sarcomas; refs. 32, 33) and erlotinib (in NSCLC; refs. 34, 35) and the chemotherapy agent irinotecan (in CRC; ref. 36), have supported the role of FDG-PET as a predictive marker of clinical activity, although this needs large-study confirmation.

There is currently an unmet need for effective treatment of patients with tumors containing KRAS mutations and patients with WT BRAF melanoma (37–41). Although the mutational analysis in this study was limited, MEK inhibition may offer a therapeutic option independent of KRAS and BRAF mutation state, most likely in combination with chemotherapy and/or another targeted agent. Recent preclinical data show that combined RAF/MEK inhibitors can block ERK activation in resistant cells and may delay emergence of resistance (42, 43). Studies with other MEK inhibitors are investigating combinations with AKT inhibitors, PI3K inhibitors, and chemotherapy agents such as paclitaxel and docetaxel (44–48). The optimum partners for RO4987655 remain to be determined; however, in vitro and in vivo data show that combination with PI3-kinase pathway inhibitors (mTOR, PI3K inhibitors; ref. 15), other targeted agents, or chemotherapy agents (cisplatin,
paclitaxel, and gemcitabine) may potentiate RO4987655’s antitumor activity.

Single-agent RO4987655 is currently under investigation in an expansion of this study in 4 parallel patient cohorts including patients with: (i) melanoma tumors carrying the \textit{BRAF} (V600) mutation, (ii) melanoma tumors not carrying the \textit{BRAF} (V600) mutation, (iii) NSCLC carrying \textit{KRAS} mutations, and (iv) CRC carrying \textit{KRAS} and/or \textit{BRAF} (V600) mutations. The primary endpoint of this expansion cohort study will be to investigate the efficacy of single-agent RO4987655 in these specific tumor genotypes, using approximately 20 patients per cohort. Further development of RO4987655 will involve combination with chemotherapy or other signal transduction inhibitors.

In summary, oral RO4987655 was reasonably well tolerated in patients with advanced or metastatic solid tumors,
but often resulted in skin toxicity (91.8%: primarily facial, and with psychosocial impact reported). RO4987655 revealed a safety profile comparable with other MEK inhibitors. The main DLTs were reversible blurred vision and elevated CPK. At the RP2D, high (>100%) and sustained (>1C_{90} for >90% of time) pERK inhibition was observed in PBMCs, and plasma drug concentrations were in the range predicted to be efficacious in preclinical models. Metabolic and anatomic responses were observed in all tumor types, but particularly in patients with melanoma tumors.

Disclosure of Potential Conflicts of Interest

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