First-in-Human, Phase I Dose-Escalation Study of the Safety, Pharmacokinetics, and Pharmacodynamics of RO5126766, a First-in-Class Dual MEK/RAF Inhibitor in Patients with Solid Tumors

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Abstract

**Purpose:** This phase I study assessed the maximum tolerated dose (MTD), dose-limiting toxicities (DLT), safety, pharmacokinetics, pharmacodynamics, and clinical activity of the first-in-class dual MEK/RAF inhibitor, RO5126766.

**Experimental Design:** Initial dose-escalation was conducted using once daily dosing over 28 consecutive days in 4-week cycles. Further escalation was completed using 2 intermittent dosing schedules [7 days on treatment followed by 7 days off (7on/7off); 4 days on treatment followed by 3 days off (4on/3off)].

**Results:** Fifty-two patients received RO5126766 at doses of 0.1 to 2.7 mg once daily, 2.7 to 4.0 mg (4 on/3 off), or 2.7 to 5.0 mg (7 on/7 off). The most common DLTs were elevated creatine phosphokinase (CPK) and blurred vision. The MTD for each dosing schedule was 2.25 mg once daily, 4.0 mg (4 on/3 off), and 2.7 mg (7 on/7 off). The dose/schedule recommended for phase II (RP2D) investigation was 2.7 mg (4 on/3 off).

Frequent adverse events included rash-related disorders (94.2%), elevated CPK (55.8%), and diarrhea (51.9%). Cmax occurred 1 to 2 hours after dosing and mean terminal half-life was approximately 60 hours. Pharmacodynamic changes included reduced ERK phosphorylation, an increase in apoptosis in tumor tissue, and a reduction in fluorodeoxyglucose (FDG) uptake after 15 days of dosing. Three partial responses were seen: two in BRAF-mutant melanoma tumors and one in an NRAS-mutant melanoma.

**Conclusion:** This first-in-human study shows that oral RO5126766 has manageable toxicity, a favorable pharmacokinetic/pharmacodynamic profile, and encouraging preliminary antitumor activity in this population of heavily pretreated patients, achieving tumor shrinkage in around 40% of patients across all dose levels and all tumor types. Clin Cancer Res; 18(17): 4806–19. ©2012 AACR.

Introduction

The RAS-regulated RAF/MEK/ERK mitogen-activated protein kinase (MAPK) pathway plays a central role in cell proliferation, differentiation, survival, and migration (1–3). Constitutive activation of the MAPK cascade has been identified in a broad range of human cancers including pancreatic, colon, lung, ovary, and kidney cancer (4). Oncogenic RAS mutations are present in approximately 30% of all cancers, up to 50% of colorectal and thyroid cancers and 90% of pancreatic cancers (5). BRAF mutations have been identified in more than 60% of malignant melanomas and 40% to 70% of papillary thyroid cancers (6–8).

Several small-molecule inhibitors of RAF or MEK are currently under clinical investigation (9–22). However, combined inhibition of both RAF and MEK could potentially increase efficacy by exerting a superior MAPK cascade blockade. Amplification of BRAF was recently identified as a mechanism of acquired MEK inhibitor resistance and
Translational Relevance

The RAF-RAS-MEK signal transduction pathway includes oncoproteins such as BRAF and KRAS which encode proteins crucial to the signaling of other oncoproteins such as EGFR. Inhibition of this pathway can be therapeutically exploited in multiple different cancers such as melanoma, lung, colon, and low-grade ovarian cancer. RO5126766 is a first-in-class dual MEK/RAF inhibitor that allosterically inhibits BRAF, CRAF, and MEK (IC50: 0.19, 0.056, and 0.16 μmol/L, respectively). The toxicities seen with RO5126766 included rash, CPK elevation, and blurred vision, similar to other MEK inhibitors. Interestingly, there were no cases of keratoacanthomas seen, as opposed to other BRAF inhibitors, consistent with RO5126766’s additional MEK inhibitory activity. Three partial responses were seen in this phase I trial and this drug should be evaluated in molecularly targeted subsets.

inhibition of BRAF has been shown to reverse resistance to the MEK inhibitor AZD6244 in colorectal cancer (CRC) cell lines (23). In addition, a dual inhibition approach may reduce some toxicity associated with specific MEK or RAF inhibitors. For example, the selective RAF inhibitors GSK2118436 and vemurafenib (PLX4032), which inhibit mutant BRAF, are associated with high incidences (around 25%) of keratoacanthoma and squamous cell carcinomas (SCC; refs. 18, 20, 24), likely related to RAF/MEK/ERK activation in normal skin cells without RAF mutations, which may be overcome with dual RAF/MEK inhibition (25). For similar reasons, mutant RAF inhibitors should be avoided in tumors driven by RAS mutations (25). Dual mechanism BRAF and MEK inhibition using a 2-drug combination (GSK2118436 and GSK1120212) is currently under investigation (NCT01072175).

RO5126766 is a first-in-class dual MEK/RAF inhibitor with a novel structure based on a coumarin skeleton. RO5126766 is an allosteric inhibitor that binds directly to MEK and prevents its phosphorylation by RAF through the formation of a stable RAF–MEK complex (Ishii et al., submitted for publication). Consequently, RO5126766 inhibits both the phosphorylation of MEK by RAF and the activation of ERK by MEK. In cell-free MEK and RAF kinase assays, RO5126766 effectively inhibited activation of ERK2 by MEK1 with an IC50 of 0.16 μmol/L (SD = ±0.043) and inhibited the phosphorylation of MEK1 protein by BRAF (IC50 = 0.19 μmol/L, SD = ±0.003), BRAF (V600E; IC50 = 0.0082 μmol/L, SD = ±0.0015), and CRAF (IC50 = 0.056 μmol/L, SD = ±0.016; ref. 26). Furthermore, 10 μmol/L RO5126766 did not inhibit any of the other 256 kinases in the Ambit KINOME scan panel. RO5126766 effectively inhibited both MEK and ERK phosphorylation in a panel of human tumor cell lines including KRAS/HHAS and BRAF mutant cell lines and KRAS/HRAS and BRAF wild-type cells. This translated into potent efficacy in CRC (HCT-116, KRAS-mutant, and COLO-205, BRAF-mutant) and lung (Calu-6 and KRAS-mutant) xenograft mouse models. In KRAS-mutant xenograft models, RO5126766 inhibited growth and caused tumor regressions more effectively than another allosteric MEK inhibitor, PD0325901 (Ishii et al., submitted for publication). Preclinical data from a series of human tumor mouse xenograft models indicated an ED50 for RO5126766 of 0.03 to 0.23 mg/kg and an ED90 of 0.15 to 1.56 mg/kg. These effective doses were associated with target trough concentrations of 17 to 133 ng/L and 87 to 901 ng/mL, respectively.

The objectives of this first-in-human phase I study were to determine the maximum tolerated dose (MTD) of oral RO5126766, dose limiting toxicities (DLT), the safety and tolerability profile, the pharmacokinetic and pharmacodynamic profile, and preliminary anti-tumor activity.

Materials and Methods

Study population

Eligible patients had advanced or metastatic solid tumors of any type for which no standard therapy existed, with evaluable disease and/or measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria (version 1.0; ref. 26). Patients were aged 18 years or more with an Eastern Cooperative Oncology Group (ECOG) performance status of ≤1, a life expectancy of ≥12 weeks, and adequate bone marrow, liver and renal function, and normal calcemia and coagulation homeostasis (see Supplementary Material for full details of inclusion criteria). Patients with known allergies to components of the study drug, impaired gastrointestinal absorption, active acute, or chronic infections (including known infection with HIV, hepatitis B virus, and hepatitis C virus), a history of central nervous system metastases, bowel disease, or those with gallbladder disorders were excluded as were pregnant or lactating female patients. Any patient undergoing major surgery, chemotherapy, radiotherapy (other than palliative radiotherapy for bone pain), immunotherapy, or treatment with any investigation agent within 28 days of the first planned dose of study drug was excluded. Patients receiving hormone therapy (unless for prostate cancer), corticosteroids, or CYP3A4 inducers within 14 days, or CYP3A4 inhibitors within 7 days, of the first planned dose of study drug were also excluded. An unlimited number of prior systemic therapies for metastatic disease were allowed.

This study was approved by the 3 Independent Institutional Ethics Committees and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from all patients.

Study design

This open-label, dose-escalation study was conducted at 3 centers in France, Spain, and the United Kingdom between November 2008 and August 2010 (ClinicalTrials.gov: NCT00773526). Before the first treatment cycle, RO5126766 was administered as a single oral dose followed by a 6 to 7 day washout (run-in period, from −6 or −7 days). Initial dose-escalation was conducted in patients

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receiving oral RO5126766 once daily over 28 consecutive
days, in 4-week cycles. After thorough analysis of the safety,
pharmacokinetic and pharmacodynamic data from the
cohorts in once daily schedule, 2 intermittent dosing-regi-
ments were investigated to identify the most appropriate and
tolerable dose regimen(s) able to increase the therapeutic
window of this compound. Further escalation from the
once daily regimen MTD was conducted using 2 intermit-
tent schedules, 4 days on followed by 3 days off (4 on/3 off)
and 7 days on followed by 7 days off (7 on/7 off). These
regimens were included to identify the best regimen which
maintained therapeutic plasma levels (identified from
xenograft models) with acceptable tolerability.

The primary aim of the trial was to recommend a phase II
dose. Secondary aims included pharmacokinetic and phar-
macodynamic assessments. As efficacy was not a primary
aim, patients were not genotyped before study entry to
enrich the study with patients likely to respond to the study
agent. Samples from patients were sequenced retrospective-
ly to look for RAF and RAS mutations.

An accelerated titration design was used. The primary
endpoints were to determine the MTD and DLTs of
RO5126766. The run-in period and first 4-week cycle were
considered the interval for the determination of DLTs and
MTD. The MTD was defined as the highest dose level at
which no more than 1 out of 6 patients experienced a DLT. A
starting dose of 0.1 mg was chosen, which provided a 9.6-
fold safety factor over the highest equivalent dose tolerated
in monkeys (27).

Initially, 1 patient per dose level was enrolled at the
starting dose level. If no DLT or no grade II toxicity or
non-DLT grade III-related toxicity was seen during the first
cycle at a given dose level, then the dose was escalated in
increments of 100% (dosed according to nearest capsule
strength), and one patient was enrolled in the next higher
dose level. If a grade II toxicity or non-DLT grade III toxicity
occurred at a given dose level, the following cohorts were
expanded to 3 patients and subsequent escalation was
conducted in 50% increments. If a DLT was observed at
any given dose (dose cohorts with 1 or 3 patients), this
cohort was expanded to 6 patients. If no further DLTs were
observed in the additional patients, dose escalation con-
tinued by 25%, with 3 patients recruited to the next dose
level. Escalation was stopped if more than 2 patients in a
given cohort developed a DLT. The preceding dose level
cohort was expanded to 6 patients, if this had not already
occurred, to confirm the MTD, which was defined as the
highest dose level at which no more than 1 out of 6 patients
experienced a DLT. Once a given dose level was complete,
patients who began dosing at a lower level were allowed to
increase their daily dose to the next level at the discretion
of the treating physician; these patients were not considered
for the definition of DLT if toxicities occurred in this group.
A maximum of 2 dose reductions were permitted per
patient. Re-escalation was allowed for grade 3 or more skin
toxicity that improved to grade 2 or less and, from cycle 2
onwards, for all other toxicities that improved to grade 1 or
less within 14 days. Treatment was administered until
disease progression, unacceptable toxicity, or patient with-
drawal, whichever occurred first. See Supplementary Fig. S1
for the details of the dose titration design. The dose/sched-
ule recommended for phase II (RP2D) of the study was
determined on the basis of consideration of the MTD in the
3 dose-schedules (once daily, 4 on/3 off regimen and 7 on/7
off regimen) associated with the global safety profile of the
compound across the various dose regimens, the pharma-
cokinetic and pharmacodynamic assessment (peripheral
blood mononuclear cells, PBMC), as well as the nature of
the PK/PD relationship.

Adverse events were graded according to the National
Cancer Institute Common Toxicity Criteria (NCI-CTC, v3.0;
ref. 28). DLTs were defined as: grade 3 or more non-
hematologic toxicity (nausea/vomiting, diarrhea, skin tox-
icity) if they occurred despite adequate supportive care
measures and/or temporary drug interruption, grade IV
neutropenia lasting more than 7 days, febrile neutropenia,
and/or documented infection, grade IV thrombocytopenia
or bleeding requiring a platelet transfusion. Skin toxicity
DLTs were defined as grade III requiring dose reduction or
cessation, or inability to resume dosing within 14 days.

Assessments
Medical history and demographic data were collected at
baseline. Physical examination and monitoring of vital
signs was conducted at baseline and throughout the treat-
ment period, along with safety assessments (ECOG perfor-
ance status, 12-lead ECG, hematology/biochemistry,
ECG/multigated acquisition scan, and glycemic monitoring
in patients with diabetes). Clinical safety monitoring was
conducted at baseline and throughout the study and stringent stopping
criteria were applied before dose escalations. On the basis of
toxicology studies, all toxicities were expected to be mon-
torable and manageable by dose interruption and/or dose
reduction.

Gastrointestinal disorders were monitored via adverse
events reporting; skin toxicity was monitored by clinical
examination, optional skin biopsies and pictures of rash
events, and adverse events reporting; and liver function,
renal function, and hematopoietic dysfunction were mon-
tored through laboratory parameters. Furthermore, careful
monitoring of inorganic phosphorus, serum calcium, para-
thyroid hormone, and 1,25-dihydroxyvitamin D3 levels
were included in the clinical study assessment.

Monitoring of creatine phosphokinase (CPK) levels was
introduced following the early occurrence of asymptomatic
CPK elevation (grade III) in one patient (at 2.7 mg once
daily). This CPK elevation was discovered after systematic
measurement as per site guidelines but was not prompted by
any specific safety event. Afterwards, all patients were
systematically monitored prospectively, or reported retro-
spectively in patients treated at lower doses.

At the time this study was initiated, ocular toxicity was not
established as a class effect of MEK inhibition and no
systemic ocular monitoring or toxicity management was
mandated in the protocol, with the exception of corneal
examination to detect signs of mineralization, and
fundoscopic examination, (at baseline and on day 15 of cycles 1 and 2), based on the risk of hypercalcaemia from preclinical data. Following reports of “blurred vision” as a common specific target effect of MEK inhibition (29), further ophthalmologic investigations were carried out whenever clinical symptoms occurred including sometimes, but not systematically, optical coherence tomography (OCT) and visual acuity.

Tumor response was assessed according to RECIST criteria (version 1.0) at baseline and every 2 cycles.

**Pharmacokinetics/pharmacodynamics**

Blood samples for pharmacokinetic analysis were collected during the run-in period, on day 15 of cycle 1 and on day 1 of cycle 2. Trough pharmacokinetic sampling was conducted on days 8 and 22 of cycle 1 and at occurrence of grade III or more drug-related skin toxicity. The same pharmacokinetic sampling assessment was conducted for all schedules. Noncompartmental methods were used to calculate pharmacokinetic parameters.

The biologic activity of RO5126766 was assessed by determining the inhibitory effect on the target enzyme (ERK) in surrogate tissue. Inhibition of 4-beta-phorbol-12-myristate-13-acetate (PMA)-induced phosphorylation of ERK (pERK) in PBMCs (collected during the run-in period, and on day 15 of cycle 1) was assessed using flow cytometry and results expressed as the percentage decrease in mean fluorescent intensity between baseline and post-treatment, after adjustment for non-PMA stimulated predose values (30). The specific phospho-p44/42 MAPK antibody (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).

Pharmacokinetic/pharmacodynamic measurements were simultaneously fitted to a pharmacodynamic/pharmacokinetic model of pERK inhibition in PBMCs using NONMEM (version VI, ADVAN4 and ADVAN9; Supplementary Material).

**Skin and tumor biopsies.** Skin and tumor biopsies (both optional) obtained at baseline and on day 15 of cycle 1 were analyzed by immunohistochemistry (IHC) using standard methods to investigate the effect of RO5126766 on proliferation (Ki67 labeling, using primary antibody clone 30.9, Ventana) and inhibition of pERK (using a primary antibody against phospho-p44/42 MAPK; clone D13.14.4E, Cell Signaling Technology), and by TUNEL assay (31) to investigate apoptosis (see Supplementary Material for details). An increase or decrease in biomarker level of 20% or more was considered to be significant.

**Mutation analysis.** Where sufficient archival tumor sample was available, screening for KRAS and BRAF (V600) mutations was conducted by PCR, NRAS (in melanoma), HRAS, and PI3K mutations were screened for by sequencing. PTEN loss was determined by IHC (using the anti-PTEN monoclonal antibody clone 138G6, Cell Signaling Technology).
FDG-PET. Fluorodeoxyglucose positron emission tomography (FDG-PET) imaging was conducted in all cohorts with 3 or more patients at baseline, on day 15 of cycle 1 and day 1 of cycle 3. Tumor biopsies were taken after FDG-PET scanning to avoid interference of biopsy on FDG uptake and patients with diabetes were excluded. Evaluation of FDG-PET images was conducted centrally based on published European Organisation for Research and Treatment of Cancer (EORTC) guidelines (32).

Statistical analyses
Descriptive statistics were used for the analysis of pharmacokinetics, pharmacokinetics, safety, and tumor response data. Logistic regression and analysis of variance (ANOVA) were used to assess correlations between specific adverse events and anti-tumor activity or pharmacokinetics/pharmacodynamics.

Results
The characteristics of the 52 patients who received at least one dose of RO5126766 are shown in Table 1. Twenty-five patients received RO5126766 once daily (0.1–2.7 mg), 13 patients on the 4 on/3 off schedule (2.7 mg and 4 mg), and 14 patients on the 7 on/7 off schedule (2.7–5.0 mg; Supplementary Fig. S1). Patients received a median of 2 cycles (range 1–17), with a median treatment duration of 58.0 days (range 1–410 days). None of the patients had previously been treated with a BRAF inhibitor or MEK inhibitor.

Dose-limiting toxicities
Ten DLTs were observed in 49 evaluable patients who completed the first cycle (Table 2). The MTDs for the once daily, 4 on/3 off and 7 on/7 off schedules were 2.25, 4.0, and 2.7 mg, respectively.

Two DLTs were observed at 2.7 mg once daily (grade III CPK elevation and grade III blurred vision). When the lower dose level cohort was expanded (1.8 mg once daily; n = 7), one patient experienced a DLT (grade III transaminitis). After escalation to 2.25 mg once daily, 1 out of 6 patients experienced a DLT (grade III CPK elevation), making 2.25 mg the MTD for the once daily dosing regimen. On the 4 on/3 off schedule, one patient experienced a DLT at 4.0 mg (grade III blurred vision). No further escalation was carried...
out because of the 2 early-onset (8 hours after run-in dose) DLTs observed in the 7 on/7 off regimen at 5.0 mg which was running in parallel with the 4 on/3 off schedule. With 7 on/7 off dosing, one DLT occurred at 2.7 mg (grade III capillary leak syndrome), 2 at 4.0 mg (grade III CPK elevation, and grade III febrile neutropenia), and 2 at 5.0 mg (grade III blurred vision and serous retinal detachment (SRD) associated with grade II blurred vision). All DLTs were reversible with appropriate treatment and/or drug interruption. The RP2D was 2.7 mg (4 on/3 off).

### Safety

Four-hundred and one treatment-related adverse events were reported with all patients experiencing at least one adverse event. Forty-three grade III adverse events occurred in 31 patients and 2 grade IV adverse events in 2 patients (Table 2). Rash-related toxicities (n = 49 patients), elevated CPK (n = 29 patients), diarrhea (n = 27 patients), and blurred vision (n = 22 patients) were the most frequently reported treatment-related adverse events across all dosing regimens.

### Table 2. Treatment-related toxicity (Cont’d)

<table>
<thead>
<tr>
<th>QD (mg)</th>
<th>4on/3off (mg)</th>
<th>7on/7off (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8 n = 3</td>
<td>1.2 n = 3</td>
<td>1.8 n = 7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serious Adverse events</th>
<th>All grades</th>
<th>Serious adverse events (all grades)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n(ev)</td>
<td>%</td>
<td>n(ev)</td>
</tr>
<tr>
<td>Rash</td>
<td>4</td>
<td>7.7%</td>
</tr>
<tr>
<td>Blood CPK increased</td>
<td>3</td>
<td>5.8%</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>1</td>
<td>1.9%</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>1.9%</td>
</tr>
<tr>
<td>Left ventricular dysfunction</td>
<td>1</td>
<td>1.9%</td>
</tr>
<tr>
<td>Myopathy</td>
<td>1</td>
<td>1.9%</td>
</tr>
<tr>
<td>Capillary leak syndrome</td>
<td>1</td>
<td>1.9%</td>
</tr>
<tr>
<td>Troponin increased</td>
<td>1</td>
<td>1.9%</td>
</tr>
<tr>
<td>Blurred vision</td>
<td>1</td>
<td>1.9%</td>
</tr>
<tr>
<td>Retinal detachment</td>
<td>1</td>
<td>1.9%</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>1</td>
<td>1.9%</td>
</tr>
<tr>
<td>Confusional state</td>
<td>1</td>
<td>1.9%</td>
</tr>
</tbody>
</table>

NOTE: No treatment-related toxicities were seen with daily dosing of ≤0.4 mg (n = 3 patients). Only the most severe intensity was counted for multiple occurrences of the same adverse events/serious adverse events in one individual. Grade 3 adverse events not listed in this table: increased alanine aminotransferase, increased aspartate aminotransferase, decreased blood albumin, capillary leak syndrome, febrile neutropenia, increased gamma-glutamyltransferase, genital erythema, myopathy, palmar-plantar erythrodysaesthesia syndrome, increased transaminases, increased troponin and fatigue (all n = 1). In addition, Grade 4 pulmonary embolism occurred in one patient. Serious adverse events were defined as any adverse events that 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. CPK, creatine phosphokinase; SRD, serous retinal detachment; QD, daily; 4 days on/3 days off; 7 days on/7 days off. n(pt), represents patient number; n(ev), represents event number.

- Maximum tolerated dose (MTD) for the regimen.
- No further escalation was conducted on the 4on/3off regimen due to two early onset (8 hours after run-in dose) DLTs observed in the 7on/7off regimen at 5.0 mg.
- Includes dermatitis aciform, rash, folliculitis, rash pustular, eczema, erythema, psoriasis, rash erythematous, rash follicular, rash papular, and rash vesicular.
- Grade 3 myopathy with muscular deficit of the scapula and pelvic belt.
- 3 serious adverse events reported in the same patient.
- 6 serious adverse events reported in the same patient.
- No associated symptoms, adverse event not due to pulmonary embolus, pericarditis, myocarditis, or rheumatoid arthritis. Coronary angiography, cardiac echography and MRI were normal.
Most rash-related adverse events were grade I or II, with no grade IV severity rash-related adverse events reported. Rash-related toxicities consisted of dermatitis acneiform \((n = 23)\), rash \((n = 12)\), folliculitis \((n = 10)\), rash pustular \((n = 4)\), eczema \((n = 2)\) and one instance each of psoriasis, rash erythematous, rash follicular, rash popular, and rash vesicular. Grade III rash-related toxicities occurred in 13 patients with once daily dosing (all at doses \(\geq 0.8\) mg, with prevalence increasing with dose), in 3 patients on the 4 on/3 off regimen (all at 4.0 mg) and in 3 patients on the 7 on/7 off regimen (2.7 mg, \(n = 1\); 4.0 mg, \(n = 2\)). No grade 3 or more rash-related toxicity occurred in the cohort who received RO5126766 at the RP2D \([2.7\text{ mg} (4\text{ on/3 off})]\). Median time to development of grade III rash-related toxicity was 34 days (range 8–143 days). Rash-related toxicity was reversible in all patients after dose reduction/interruption and/or adequate medical treatment. Three instances of treatment-related pruritus were reported; all were grade I. Additional features of skin toxicity observed included: nail changes (paronychia, onycholysis), skin fissures, hair changes (color, growth, texture), and alopecia, all occurring in less than 10% of the patients. These events occurred more often in patients receiving treatment for more than 1 month. Skin and rash-related toxicity incidence was high (\(>90\%\)) from very low doses (0.8 mg in the once daily regimen). As few patients were treated below this dose, it was not possible to identify a threshold below which the skin toxicity may be absent. From this low dose-exposure however, we did not have a clear dose relationship with respects to incidence and/or severity of skin disorders.

Treatment-related CPK elevation occurred in 55.8% of patients \((n = 29)\). Six patients experienced grade 3 or more CPK elevation; 3 on the once daily regimen \((1.8\text{ mg, 2.25 mg, and 2.7 mg})\) and 3 on the 7 on/7 off intermittent regimen \((all at 4.0\text{ mg})\). CPK elevation was generally isolated, reversible, not clinically significant, without associated clinical deterioration of cardiac and/or renal function, and no associated myolysis was observed in subsequent muscle biopsies \((n = 2)\). Ten patients with elevated CPK also experienced musculoskeletal and connective tissue disorders (myalgia, muscular weakness, myopathy, bursitis, joint stiffness, joint swelling, or muscle spasms).

Treatment-related diarrhea occurred in 51.9% of patients \((n = 27)\) and was grade 2 or less in all instances. Other frequent treatment-related gastrointestinal disorders included stomatitis in 13 patients \((25\%)\) and nausea in 10 patients \((19.2\%)\). All were grade 2 or less.

Ocular toxicity occurred in 26 patients \((50\%; n = 46\text{ events})\). The main symptoms associated with ocular toxicity were serous retinal detachment \((SRD; n = 10\text{ events in 10 patients})\) and blurred vision \((n = 33\text{ events in 22 patients})\). The majority of events were of minor severity \((grade 1)\) with only 3 \((6\%)\) patients experiencing grade III severity \((all blurred vision)\). Median time to development of grade III ocular toxicity was the day of the run-in dose \((i.e., -6; range: -6 to +15days)\). All events were reversible without specific management or after drug interruption. No retinal vein occlusion \((RVO)\) was observed.

Seventeen treatment-related serious adverse events occurred in 14 patients (Table 2), including one grade 4 pulmonary embolism \((1.8\text{ mg once daily})\). Although this mostly likely occurred because of progressing disease in the lung, relationship to study drug could not be excluded. Patients were reported with one single serious adverse events except for 2 patients in the 2.7 mg once daily regimen who reported 2 and 3 consecutive serious adverse events (see Table 2). Most serious adverse events \((9/17)\) were rash-related, CK elevation, and ocular disorders \((23\%, 17\%, and 12\%\) of the serious adverse events, respectively). Seven deaths occurred because of disease progression and were not considered treatment related.

Temporary drug interruptions occurred in 28 patients (once daily, \(n = 15\); 4 on/3 off, \(n = 7\); 7 on/7 off, \(n = 6\); median duration 7 days; range 1–21) with 15 patients experiencing 1 or more interruption. Fifteen patients \((once daily, n = 7; 4 on/3 off, n = 4; 7 on/7 off, n = 4)\) underwent dose modifications, primarily due to safety considerations by investigators and mainly rash toxicity.

**Pharmacokinetics**

Following oral administration of a single dose of RO5126766, plasma concentrations increased rapidly with \(C_{\text{max}}\) reached 1 to 2 hours after dosing with a mean terminal half-life of approximately 60 hours (Fig. 1A). Apparent systemic clearance was 6 mL/min. Plasma exposure of RO5126766 increased approximately dose-proportionally during run-in (Fig. 1B) and at steady state (Fig. 1C). In the once daily cohort, steady state was reached by day 15 and drug accumulations of between 2- and 7-fold were observed. Plasma exposure on the 4 on/3 off and 7 on/7 off intermittent regimens was notably different due to the 3- or 7-day washout period, respectively (Supplementary Table S1). At the MTD for once daily dosing \((2.25\text{ mg})\), \(C_{\text{max}}\) and AUC\(_{0–24\text{ h}}\) were 106.3 ng/mL and 1639.2 ng·hr/mL, respectively, during run-in and 343.8 ng·mL and 6017.4 ng·mL·hr, respectively, at steady state. PK parameters (Supplementary Table S1) were in-line with what was predicted by preclinical models (Ishii et al., submitted for publication). Increased steady-state plasma exposure tended to associate with increased CPK elevation (ANOVA; \(P = 0.05\)); however, CPK was not always measured at lower doses.

**Pharmacodynamics**

The relationship between RO5126766 plasma concentration and pERK level in PBMCs was characterized by a direct link pharmacokinetic/pharmacodynamic model (Fig. 2A).

**pERK inhibition in PBMCs.** The relationship between RO5126766 plasma concentration and pERK level in PBMCs was characterized by a direct link pharmacokinetic/pharmacodynamic model (Fig. 2A).

**Tumor/skin biopsies.** Six of 11 patients with paired tumor biopsies showed a reduction in pERK expression in tumor cells by 20% or more, of which 2 showed a reduction of more than 90% between baseline and day 15 (Fig. 2B). pERK expression in epidermal keratinocytes in normal skin decreased by 20% or more from baseline to day 15 in 14 of 40 patients (Fig. 2B), increased by 20% or more in 4 patients, and was unchanged in 16 patients. IHC for pERK...
failed for 6 patients. Cellular proliferation decreased (20% or more decrease in Ki67) in 6 of 11 tumors between baseline and day 15, with the remaining 5 showing no change. Five of 10 tumor pairs showed an increase of 20% or more in apoptotic cells between baseline and day 15, 3 showed a decrease of 20% or more, and 2 showed no change. No correlations were found between changes in biomarker levels and tumor response, mutational status, or exposure.

The 4 on/3 off regimen at a dose of 2.7 mg was assessed as being the best tolerated of all dose regimens. No DLTs or grade III toxicity (including skin toxicity) occurred in the 5 patients treated at this dose, and this dose regimen maintained a sustained duration of target inhibition (>90% pERK inhibition in PBMC for >50% of time) in more than 70% of the treated patients. The 2.7 mg dose was just below the MTD (4.0 mg) of the 4 on/3 off regimen and hence, defined as the RP2D for the next part of the study. In comparison, sustained target inhibition and grade III skin toxicity were reported at the MTD in 92% and 50% of patients in the once daily regimen and 45% and 40% of patients in the 7 on/7 off regimen, respectively.

**Efficacy**

Forty-five patients were evaluable for tumor response (RECIST). All patients enrolled in the study were heavily pretreated with a median of 3 lines of previous chemotherapy (range 1–12). There were 3 partial responses (PR; Fig. 3), all occurring in patients with melanoma. Two of these patients had tumors with BRAF [V600E] mutation (a 35-year-old man treated by 2 previous lines of treatment [(dacarbazine plus oblimersen then sorafenib) and a 64-year-old woman also treated by 2 previous lines of treatment (interferon and dacarbazine, cisplatin, and vinblastine)]. The other patient was a 48-year-old woman with an NRAS-mutant [Q61K] melanoma previously treated with 3 lines of therapy (interferon, dacarbazine, and fotemustine). The median duration of these responses was 60.0 days (range 57–137) and these patients were treated for a median of 117 days (range 109–211). Disease stabilization through to week 16 was achieved in 9 patients and in 2 patients for more than 1 year. Tumor shrinkage was observed in 18 of 45 evaluable patients including minor response (≥10% shrinkage) in 9 of 45 evaluable patients. Decrease in tumor size at week 8 was significantly associated with duration of grade III rash up to day 57 (P = 0.01).

Seventeen of the 39 analyzed tumors contained mutations in either \( \text{BRAF} \), \( \text{NRAS} \)/\( \text{HRAS} \), or \( \text{PI3KCA} \) and one patient showed loss of PTEN staining (Table 1). PTEN was intact in all 3 patients achieving PR.

**FDG-PET**

A decrease in \(^{18}\text{F-FDG}\) uptake between baseline and day 15 was observed in 71% of patients (29/41; Fig. 4A). No relationship between dose/exposure and change in FDG uptake from baseline to day 15 was seen in the overall population; however, in patients with melanoma, there was a dose-dependent change in FDG uptake as dose of
Figure 2. A, RO5126766 plasma concentration versus PBMC pERK activity (all doses). B, case study: immunohistochemistry for pERK and Ki67 expression showing a reduction between baseline and day 15 in (i–iv) tumor and (v–viii) skin biopsies from a patient with a metastatic cutaneous melanoma with NRAS mutation (dosing cohort 11, 4.0 mg (4on/3off)). Measurements were fitted to a PK/PD model of pERK inhibition in PBMCs. $I_{\text{max}}$ was investigated by objective function profiling. $E_{\text{max}}$, 90%; $I_{\text{max}}$, 90%; IC50, 56 ng/mL (BSV, 67%). Mean pERK activity (fluorescence intensity) is represented by the blue line, and confidence intervals are shown as red lines. IC50 corresponded to an RO5126766 dose of approximately 0.6 mg QD. IC50 was achieved by 6 patients [1.8 mg QD, n = 1; 2.7 mg QD, n = 1; 2.7 mg (4on/3off), n = 1; 4.0 mg (4on/3off), n = 1; 4.0 mg (7on/7off), n = 2]. No pERK inhibition of >95% was seen. At the RP2D of 2.7 mg (4on/3off), the mean pERK inhibition was 65%, with 72% of patients showing pERK inhibition of <50% IC50, half maximal inhibitory concentration; $I_{\text{max}}$, maximum inhibitory effect; BSV, between-subject variability). Tumor [(i), Ki67 baseline; (ii), Ki67 day 15; (iii), pERK baseline; (iv), pERK day 15] and skin biopsies [(v), Ki67 baseline; (vi), Ki67 day 15; (vii), pERK baseline; (viii), pERK day 15] showing a reduction in pERK expression and Ki67 labeling between baseline and day 15. Patient 1005 had metastatic disease present in the lymph nodes, soft tissue, and pancreas. RO5126766 was administered at 4.0 mg (4on/3off), then 2.7 mg (4on/3off) from week 5 onward and 1.8 mg (4on/3off) from week 14 onward, for a total duration of 16 weeks. A PR was achieved on cycle 3 (confirmed on cycle 4) and the patient eventually progressed on cycle 5.
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Figure 3. Percentage change in tumor size at maximum reduction from baseline. Forty-five patients were evaluable for tumor response. Seven patients were not evaluable for tumor response assessment at day 1 of cycle 3. Red lines indicate the RECIST cutoff for progressive disease (−20%) and PR (−30%). Fifty patients were withdrawn from treatment: 45 due to disease progression and 5 due to reasons other than progression (adverse events, treatment refusal, insufficient therapeutic response, protocol violation, n = 1). Tumors with ongoing response.

RO5126766 increased [−15% at 1.2 mg once daily (n = 1), −43% at 2.25 mg once daily (n = 4), −70% at 2.7 mg once daily (n = 1)]. An example of the FDG-PET imaging data for one patient is shown in Fig. 4B.

A weak relationship was observed in the overall tumor population between the change in $^{18}$F-FDG uptake between baseline and cycle 1, day 15, and the change in the longest diameter of target lesions as measured by RECIST on cycle 3, day 1 ($R^2 = 0.04$; data not shown).

Discussion

We report the first-in-human study of the dual MEK/RAF inhibitor RO5126766. In this population of heavily pretreated patients, oral RO5126766 showed a challenging but manageable toxicity profile. The MTD for each dosing schedule were defined as 2.25 mg (once daily), 4.0 mg (4 on/3 off), and 2.7 mg (7 on/7 off). RO5126766 had a favorable pharmacokinetic/pharmacodynamic profile and encouraging preliminary anti-tumor activity in this population of heavily treated patients, achieving tumor shrinkage in more than 40% of patients across all dose levels. On the basis of observed DLTs, MTDs, safety, pharmacokinetic/pharmacodynamic assessments and efficacy, a dose regimen of 2.7 mg (4 on/3 off) has been selected for further investigation in phase II trials. Potential drawbacks of an intermittent schedule are primarily the periods of time that target inhibition is not present leading to lack of efficacy and increased ability of the cancer cells to regrow and cause resistance. This, however, had to be pragmatically balanced with increased tolerance of intermittent schedules.

RO5126766 PK were linear and time-independent with a substantially longer half-life than has been reported for other specific MEK inhibitors (19, 21, 33). Plasma concentrations and exposure increased dose-proportionally and all doses of RO5126766 achieved plasma concentrations known to be effective in preclinical models (Ishii et al., submitted for publication). The relatively long half-life reported in this study (~60 hours) should facilitate daily and intermittent dosing schedules for RO5126766, ensuring that serum levels remain above the target threshold. However, this extended half-life may also limit the recovery of normal tissues. Several additional intermittent regimens (including weekly and biweekly) were investigated by simulating their pharmacokinetic/pharmacodynamic profile (data not shown). These scenarios yielded consistently inferior predictions for RO5126766 exposure, and the extent of pERK inhibition in the PBMCs, and were therefore not further considered.

Tolerability of RO5126766 was similar to that reported for specific MEK and RAF inhibitors (18–22, 29, 33–38). The most frequent adverse events were rash-related toxicities, CPK elevation, diarrhea, and ocular toxicities. All rash-related toxicity was reversible after dose reduction/interruption and/or adequate treatment. Ocular toxicity (50.0%) was more commonly reported than with other MEK inhibitors (CI-1040, 22.4%; AZD6244, 12.3%; PD-0325901, 33.3%; refs. 9, 33, 39); however, only 3 instances of grade III ocular toxicity (all blurred vision) and no grade IV events were reported. Blurred vision was usually acute, transient, associated with SRD when it could be documented by an OCT and always reversible spontaneously and/or with temporary drug interruption. The high incidence of ocular toxicity observed in this study may reflect the detailed ophthalmic investigations that were introduced in response to reports of ocular toxicity as a class effect of MEK inhibitors. However, as OCT was not conducted systematically in this dose-escalation study, particularly at lower doses, the true incidence of SRD in patients treated with RO5126766 may be underestimated. While no long-term damage has been observed to date in patients reporting instances of SRD, the long-term risk of repeated retinal detachment remains unknown. Comprehensive ophthalmic assessments including visual acuity tests, slit lamp examination,
A, relative change (%) in FDG-PET from baseline to day 15 of cycle 1. B, a comparison of FDG-PET uptake at baseline (i and iii) and day 15 of cycle 1 (ii and iv) and CT scan images at baseline (v) and day 1 of cycle 3 (vi). FDG-PET was conducted at baseline and at day 15 of cycle 1 in 41 patients (78.8%; QD, n = 21; intermittent dosing, n = 20) including 18 patients with melanoma tumors. Lesions with the highest degree of FDG uptake were selected for quantitative analysis (up to 5) and a 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. Patient 1106 with melanoma expressing the BRAF (V600) mutation, treated with 2.7 mg (QD) RO5126766. CT images show a 36% reduction in target lesion size, indicating partial response.
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OCT, fundoscopy, and intraocular pressure measurements have been implemented for future trials. Importantly, and in contrast to the MEK inhibitor PD-0325901 (33), no cases of RVO were observed with RO5126766, even in patients exposed to long treatment duration (≥52 weeks).

CPK elevation was common and was defined as a DLT in 3 patients. Episodes of elevated CPK remained asymptomatic in the majority of cases. The exact mechanism underlying CPK elevation is still under investigation. No RO5126766-related cardiovascular disorders or rhabdomyolysis were observed. As the RAS/RAF/MEK (MAPK/ERK) pathway is at the centre of muscle signalling networks (40–43), a direct role of MEK inhibition in CPK elevation cannot be excluded; however, to date, CPK elevations have not been commonly reported with other MEK and BRAF inhibitors. High exposure at steady state was significantly associated with an increased blood CPK. In this heavily pretreated population, the median duration of treatment was short (58 days), limiting the evaluation of accumulating toxicity and/or long term toxicity. However accumulating toxicity was not reported by any patient in the study, including the 9 and 2 patients who remained on study for more than 16 weeks and more than 1 year, respectively.

Of note, no keratocanathoma or SCC were reported in this study of RO5126766 compared with the high incidence seen with the RAF inhibitors GSK2118436 and PLX4032, which has been linked to activation of ERK signaling in normal tissue without RAF mutations (18, 20, 24, 25). Furthermore, 65% (26/40) of normal skin biopsies analyzed in this study showed a significant (i.e., >20%) reduction in pERK.

Target inhibition, studied by quantifying pERK in PBMCs, was concentration dependent and consistent with that seen with other MEK inhibitors (19, 21, 33). A reduction in pERK between baseline and day 15 was seen in approximately half the tumors and a third of the skin biopsies. Increases in Ki67 in some skin samples may be related to skin toxicity. This was accompanied by a reduction in tumor cell proliferation and an increase in apoptosis, each seen in half the post-treatment tumor biopsies analyzed. There was no clear relationship between dose and any of the biomarkers analyzed; however, the small number of biopsy pairs available limits any conclusions that can be drawn.

Objective responses included 2 confirmed and 1 unconfirmed PR in patients with melanoma. One patient in each of the 3 dosing schedules achieved PR. Efficacy findings were supported by FDG-PET imaging data with 71% of patients experiencing a reduction of FDG uptake after 15 days of treatment. However, not all the patients responding on day 15 with 18F-FDG PET achieved a response according to RECIST. This suggests that an early metabolic response was a necessary (but not in itself sufficient) indicator for later RECIST response. RO5126766 seemed to be particularly active against melanoma, similar to other MEK and RAF inhibitors (18, 20, 29, 44). RAF inhibitors such as GSK2118436 and vemurafenib (RG7204) have shown high response rates and improvement in progression-free survival (PFS) in patients with melanoma expressing the BRAF (V600) mutation (18, 20, 24, 34), but are ineffective in tumors with wild-type BRAF. In this study, RO5126766 achieved 2 PRs in patients with BRAF (V600)-mutant melanomas and one confirmed PR in a BRAF-wild-type melanoma tumor (although this tumor did contain an NRAS mutation). This novel activity, compared with specific RAF inhibitors, in a patient wild-type for BRAF may reflect the dual MEK/RAF inhibitory properties of RO5126766.

In summary, dual RAF/MEK inhibition by oral RO5126766 seems to be a feasible anti-cancer strategy. Toxicity was high but acceptable and adverse events were mechanistically related to MEK and RAF inhibition (CPK elevations, rash, and ocular toxicity) and were reversible. Target inhibition was shown in tumor and normal tissue and evidence of clinical activity was seen. Unlike specific RAF inhibitors, response was not limited to BRAF-mutated tumors, and anti-tumor activity was seen in tumors with BRAF and NRAS mutations. Further evaluation of RO5126766 is warranted and a study including patients with KRAS/NRAS and BRAF-mutant solid tumors is planned.

Disclosure of Potential Conflicts of Interest
F. Kaeber-Bodere and J.-C. Soria are consultants/advisory board members of F. Hoffmann-La Roche Ltd.

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References


First-in-Human, Phase I Dose-Escalation Study of the Safety, Pharmacokinetics, and Pharmacodynamics of RO5126766, a First-in-Class Dual MEK/RAF Inhibitor in Patients with Solid Tumors

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