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The Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Single Oral Doses of CH4987655 in Healthy Volunteers: Target Suppression Using a Biomarker

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Abstract

Purpose: CH4987655 (RO4987655) is an orally active and highly selective small-molecule MEK inhibitor. It potently inhibits mitogen-activated protein kinase signaling pathway activation and tumor cell growth, with an in vitro IC50 of 5.2 nmol/L for inhibition of MEK1/2. Single-agent oral administration of CH4987655 resulted in complete tumor regressions in xenograft models.

Experimental Design: All 40 subjects received a single oral dose followed by 72 hrs of pharmacokinetic, pharmacodynamic, and safety/tolerability assessments. The pharmacodynamics were measured by changes in phosphorylated extracellular signal-regulated kinase (pERK) levels in a surrogate tissue peripheral blood mononuclear cells ex vivo stimulated by PMA.

Results: Doses of 0.5, 1, 2, 3, and 4 mg were safe and well tolerated. No clinically significant safety event was observed. A total of 26 adverse events (n = 15) were reported: 21 mild, 5 moderate, and none severe. Moderate adverse events were experienced by one subject at 1 mg (autonomic nervous system imbalance) and three subjects at 4 mg (diarrhea, abdominal pain, autonomic nervous system and acne). CH4987655 was rapidly absorbed with a tmax of ∼1 h. Exposures were dose proportional from 0.5 to 4 mg. The disposition was biphasic with a t1/2 of ∼25 hr. Intersubject variability was low, 9% to 23% for Cmax and 14% to 25% for area-under-the-curve (AUC). pERK inhibition was exposure dependent and was greater than 80% inhibition at higher doses. The pharmacokinetic-pharmacodynamic relationship was characterized by an inhibitory Emax model (Emax ∼100%; IC50 40.6 ng/mL) using nonlinear mixed-effect modeling.

Conclusions: A significant extent of pERK inhibition was achieved for a single dose that was considered to be safe and well tolerated in healthy volunteers. (Clin Cancer Res 2009;15(23):7368-74)

The Ras/Raf/MEK/ERK [mitogen-activated protein kinase (MAPK)] pathway represents one of the best-characterized signaling pathways involved in the development and progression of human cancers. This pathway, through the Ras/Raf/MEK/ERK signal cascade, is responsible for transmitting and amplifying mitogenic signals from the cell surface to the nucleus where activated transcription factors regulate gene expression and determine cell fate. The constitutive activation of this pathway is sufficient to induce cellular transformation. Deregulation of the MAPK pathway, due to aberrant receptor tyrosine kinase activation and Ras and/or B-Raf mutations (frequently found in human cancers), represents a major factor in determining abnormal cell growth control (1). Oncogenic Ras mutations can be found in ∼30% of all human cancers. The highest incidences

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Translational Relevance

This article shows the use of a biomarker developed in the laboratory based on the mechanism of action of the drug; this biomarker was subsequently used in the clinic to evaluate suppression of the intended target or “proof of mechanism.” Preclinical efficacy studies evaluated tumor responses and, where responses were observed, explored the relationship with the extent of target suppression. In healthy volunteers, target suppression was studied in peripheral blood mononuclear cells to quickly understand if adequate target suppression can be achieved at tolerable doses. In the future, if the extent of target suppression can be correlated with tumor effects and clinical responses, suppression in PBMC can be used as a noninvasive and perhaps earlier marker of clinical response in patients, thereby facilitating personalized therapy.

of Ras mutations are found in adenocarcinomas of the pancreas (90%), colon (50%), and lung (30%; ref. 2). Mutations in B-Raf have been detected in 66% of primary melanomas and less frequently in other tumors such as colon (12%), ovarian (30%), and papillary thyroid cancers (30–70%; refs. 3–5). Aberrant activation of the MAPK pathway also correlates with tumor progression and poor prognosis in various cancer patients, such as breast, colorectal, prostate, renal cell carcinoma, non–small cell lung cancer, and melanoma patients. Therefore, there are several key components of the MAPK pathway that are attractive targets for development of therapeutic agents in cancer (6, 7).

Although mutations in MEK1 and MEK2 (MEK1/2) have not been observed with much frequency in cancers, overexpression of MEK is sufficient to induce cellular transformation. Thus far, the only known substrates of MEK1/2 are extracellular signal-regulated kinases 1 and 2 (ERK1/2). This unusual substrate specificity places MEK1/2 at a critical point in the signal transduction cascade, integrating many extracellular signals into the MAPK pathway. Targeting MEK1/2 with a small-molecule inhibitor could prevent all upstream aberrant oncocogenic activations (RTK, Ras, and B-Raf). In clinical studies, two highly selective MEK inhibitors, PD0325901 (Pfizer) and Arry142886/AZD6244 (Array Biopharma, licensed to AstraZeneca), have been evaluated for clinical proof of concept, and partial responses and stable disease were observed in patients with melanoma, non–small cell lung cancer, and pancreatic cancer (8).

CH4987655 (RO4987655) is a potent, highly selective non-ATP–competitive MEK inhibitor with an excellent selectivity profile in a large panel of kinase assays. It shows potent anti-tumor activity as a monotherapy and in combination with other antitumor agents (9). CH4987655 inhibited tumors in vitro in many different cancer cell lines and in vivo in various tumor models and produces significant enhancement of the antitumor activity of cisplatin, paclitaxel, and gemcitabine. We now report the results of the first study of CH4987655 in man showing that the desired level of target inhibition can be achieved with a well-tolerated oral dose.

Materials and Methods

**Study design.** This is a first-time-in-man, randomized, double-blinded, placebo-controlled, single ascending-dose study aimed to determine the safety/tolerability, pharmacokinetics, and pharmacodynamics of CH4987655 in healthy volunteers. The study used a parallel group design to reduce the extent of CH4987655 exposure in healthy subjects. This means that subjects from each dose group were different than the subjects from other dose groups. The following single doses were orally administered: 0.5, 1, 2, 3, and 4 mg using capsules of 0.5 mg CH4987655 and lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, hydroxypropylcellulose, sodium lauryl sulfate, and magnesium stearate. The placebo consisted of the same excipients. For each dose cohort, there were eight subjects (six CH4987655 treated and two placebo subjects). Subjects were dosed in a staggered manner, 2+3+3 for the first two dose cohorts followed by by-subject staggering for the remaining dose cohorts. For both staggering schemes, subjects were observed for 24 h before dosing the next subgroup of subjects.

All subjects meeting eligibility criteria were randomized and dosed on day 1 after an 8-h overnight fast. Each subject had blood collection up to 72 h post dose for pharmacokinetic assessments and up to 24 h post dose for pharmacodynamic assessments. Safety assessments were made at intervals up to 72 h post dose, and adverse event reporting were done throughout the duration of the study. After the last 72-h pharmacokinetic blood collection, all subjects left the facility and returned at a later time for follow-up within 10 to 16 d after their respective dosing date.

The study was conducted at Roche Institute of Translational and Experimental Medicine, Strasbourg, France. The study protocol and informed consent were approved by their institutional review board/independent ethics committee before the enrollment of any subject. The study was done in accordance with International Conference on Harmonization good clinical practice guidelines and the local regulatory requirements.

**Inclusion and exclusion criteria.** All subjects enrolled in the study met the following inclusion criteria: (a) healthy male or female between the age of 18 and 65 years inclusive, with a body mass index between 18 and 32 kg/m² inclusive; (b) female subjects who were surgically sterile or postmenopausal (amenorrhea for at least 12 consecutive months) confirmed by a blood hormone panel for a female without hormone replacement therapy; (c) male subjects willing to use at least one barrier method of contraception for the duration of the study and for 90 d after completion of the study; (d) agreed to abstain from alcohol consumption from 48 h before receiving study drug until 24 h after dosing on day 1 and throughout the follow-up visit; (e) was able to participate, gave written informed consent, and complied with the study restrictions; and (f) agreed not to participate in any subsequent clinical pharmacology study for 3 mo after dosing (subject to qualification and randomization into the trial).

Drug-specific exclusion criteria included the following: (a) history of gall bladder disorder or complication, including cholelithiasis; (b) history of corneal erosions, corneal degenerations, active or recurrent keratitis, and other forms of serious ocular surface inflammatory conditions; and (c) evidence of ophthalmologic abnormalities except accommodation disorders.

**Safety and tolerability.** Safety assessments included hematology, biochemistry, and urinalysis done at screening, predose, at intervals up to 72 h after each dose, and at follow-up. A 24-h urine sample was tested for calcium, creatinine, and phosphates. Additional safety assessments included electrocardiograms, ophthalmology tests (visual acuity, fundoscopy, Schirmer's test), abdominal ultrasound, and fecal occult blood test (FOBT). Adverse event reporting was done throughout the entire duration of the treatment period and were categorized as mild (grade 1), moderate (grade 2), or severe (grade 3).

**Pharmacokinetics.** Blood samples (4 mL, potassium EDTA vacutainers) were taken up to 72 h and all urine samples were collected in 6- or
12-h periods for 48 h after dosing. Concentrations of CH4987655 in plasma and urine were determined by a validated liquid chromatography tandem mass spectrometry (LC/MS/MS) method. Plasma samples from the 4 mg dose group were initially screened for human metabolites using LC/MS (ESI Q-TOF). Samples from the later time points, 4 to 8 and 12 to 72 h, were pooled by subject. To get a quantitative estimate of the parent and metabolite, plasma samples were pooled across subjects by time point and analyzed by LC/MS. Peaks were quantified using calibration curves. Data analyses were done using noncompartmental methods through PhasitWINNONLIN software v.5.2.1.

Pharmacodynamics. The effect on target suppression was measured using a surrogate tissue, peripheral blood mononuclear cells (PBMC). Assessments of drug effects or "proof of mechanism" were measured by the level of phosphorylation of ERK (pERK) before and after drug administration at doses that were considered to be tolerated and safe.

In the study, ~2 mL blood samples were taken at intervals up to 24 h postdose. Because the basal pERK level was expected to be low in the normal surrogate tissue, blood samples were stimulated with phorbol 12-myristate 13-acetate (PMA) to activate the MAPK pathway in the blood cells. Following PMA stimulation, blood cells were fixed with formaldehyde and red blood cells were lysed by the addition of a Triton X-100/PBS solution to a final concentration of 0.1%, which allowed samples to maintain their in vivo MEK/ERK status. The resulting cells were stained with two antibodies (anti-human CD3 and anti-pERK1/2). The CD3 antibody was used to identify T-lymphocytes, thus allowing pERK levels to be analyzed only in gated CD3-positive lymphocyte population. The pERK level was measured by flow cytometry (10). The mean fluorescence intensity was measured. The mean fluorescence intensity in predosed blood samples was set as control (100%), and the percentage of pERK inhibition was determined based on the decreased mean fluorescence intensity levels in various dosed subjects against the control samples. Correlation between that of CH4987655 concentration and %pERK change for each subject was done initially using simple time course plots. This allowed for selection of the most appropriate model.

Result

Subject demographics. A total of 40 healthy subjects were enrolled in and completed the study: 10 subjects in the placebo group and 6 subjects each dose groups. Single doses of 0.5, 1, 2, 3, and 4 mg were tested in the study. Although 4 mg was the highest dose tested, it was not considered the maximum tolerated dose because it did not meet the clinical safety stopping criteria.

### Table 1. Summary of subject demographics

<table>
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<th>1 MG (n = 6)</th>
<th>2 MG (n = 6)</th>
<th>3 MG (n = 6)</th>
<th>4 MG (n = 6)</th>
<th>All cohorts (N = 40)</th>
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Most subjects were white (98%) and male (98%) and all were between 18 and 55 years of age. All 40 subjects were included in the pharmacokinetic, pharmacodynamic, and safety assessments. A summary of subject demographics, by dose groups, is presented in Table 1.

Safety. All enrolled subjects (n = 40) were evaluable for safety. Only 15 of the 40 subjects experienced adverse events: 2 of 10 subjects in the placebo group, 5 of 6 in the 0.5 mg group, 1 of 6 in the 1 mg group, 1 of 6 in the 2 mg group, 0 of 6 in the 3 mg group, and 6 of 6 in the 4 mg group. Most adverse events were of mild intensity (21 of 26) and none were severe. Autonomic nervous system imbalance was reported with moderate intensity in the 1 mg group, and autonomic nervous system imbalance (i.e., vasovagal reaction without loss of consciousness), diarrhea, abdominal pain, and acne were reported with moderate intensities in the 4 mg group.

Epithelial and gastrointestinal types of adverse events were considered drug related. Of the 21 adverse events, there were diarrhea (5), abdominal pain (2), acne (1), and single incidences of upper abdominal pain and flatulence. The remaining adverse events were either remotely or unlikely related to drug treatment. They included fatigue (1), asthenia, headache, influenza-like illness, pharyngolaryngeal discomfort, phlebitis, and micturition urgency.

There were no deaths or serious adverse events during the study. There were no clinically significant changes in laboratory results. There were no grade 4 laboratory abnormalities reported, and there were no grade 2 or grade 3 laboratory abnormalities reported in more than one subject per dosing cohort for any of the parameters presented. There were no clinically significant changes in vital signs at any dose level after a single dose administration of CH4987655. There were no abnormal findings in overall electrocardiogram (ECG) interpretations, although a few subjects had abnormalities reported on individual parameters. In particular, there were no individual QTcB or QTcF values ≥450 ms and no individual changes from time-matched baseline in QTcB or QTcF of ≥60 ms at any dose level after a single dose administration of CH4987655. There was no medically relevant positive Schirmer’s test. There were no abnormal visual acuity tests, fundoscopy examinations, or slit lamp tests.

One subject (subject 1030, 4 mg) had an abdominal ultrasound that was positive for steatosis at follow-up (day 12). This was most likely a hepatic manifestation of food excess (sugar and fat). Total cholesterol, triglycerides, and alanine aminotransferase were above the limit of normal correlating with steatosis. According to the clinical site, a day 24 abdominal ultrasound scan showed regression of steatosis. Aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transferase were in the normal range; however, cholesterol and triglycerides were slightly elevated. At a later follow-up, an abdominal ultrasound scan still showed some steatosis; however, it was determined to be unrelated to drug.

There were five CH4987655 subjects with positive FOBT, which occurred on day 3. The three subjects with positive FOBT from the 4 mg dose group experienced gastrointestinal toxicities. All repeat FOBTs at follow-up were negative for all subjects.

Pharmacokinetics. Table 2 summarizes the mean plasma pharmacokinetic parameters by dose groups. Figure 1 (top) provides the mean plasma concentration time profiles.

CH4987655 was rapidly absorbed with a median $t_{\text{max}}$ of ~1 hour (range of 0.5-2 hours). The disposition seemed to be biphasic with a terminal elimination $t_{1/2}$ of ~25 hours. The variability was low, ranging from 9% to 23% for $C_{\text{max}}$ and 14% to 26% for area-under-the-curve (AUC). Apparent clearance, CL/F, apparent volume of distribution, Vd/F, and the terminal elimination rate constant, $\lambda_z$, are listed in Table 2. As shown in Fig. 2, $C_{\text{max}}$ and AUC seemed to be dose proportional within the dose range tested.

The result of metabolite profiling identified the presence of CH4987655, CH5046399, and oxidative metabolites. CH5046399, the ring-open form metabolite, was a major metabolite as evident by major peaks in human plasma, whereas the oxidative metabolites were considered minor metabolites. CH5034027, the glucuronide, was not detected.

The total percent recovery of CH4987655 in urine was very low in all dose groups. The highest individual value was 2% in the 4 mg dose group. The mean total percent drug recovery was 0.6% to 1% across all dose groups.

Pharmacodynamics. As shown in Fig. 1 (bottom), pERK inhibition seemed to be dose dependent. Visual inspection suggested a direct relationship between CH4987655 concentration and pERK. This was evident by maximum pERK inhibitions, which corresponded with the maximum CH4987655 concentration, both of which occurred at ~1 to 2 hours postdose followed by parallel decline.

Characterization of the relationship between %pERK inhibition and its corresponding CH4987655 concentrations were done using an $F_{\text{max}}$ model and data were pooled for all subjects from all dose cohorts.

$$\% \text{pERK Inhibition} = \frac{F_{\text{max}} \cdot \text{Conc}^\gamma}{IC_{\gamma0}^\gamma + \text{Conc}^\gamma}$$
where Conc is the CH4987655 concentration, $E_{\text{max}}$ is the maximum pERK inhibition, $IC_{50}$ is the CH4987655 concentration at 50% of maximum pERK inhibition, and $\gamma$ is the Hill coefficient, fixed to 1 in the final model.

All model fittings were done through the nonlinear mixed effect model (NONMEM) using NONMEM software ver 6.0. Both placebo- and nonplacebo-corrected pERK data were used in the fitting. The $IC_{50}$ estimate based on placebo-corrected data was deemed more reliable as it accounted for any time-dependent variations in pERK, which may, in part, be explained by a reflection of the intrinsic variability of the assay. Individual pERK data were corrected at each time point for placebo effect by subtracting the mean pERK data taken from combined placebo subjects. The results of placebo-corrected fitting are shown in Fig. 3. Because $E_{\text{max}}$ was estimated to be close to 100%, it was fixed to 100% in the final model. The $IC_{50}$ was estimated to be 40.6 ng/mL with a coefficient of variation of 8%, suggesting a range of $IC_{50}$ in the population from 30 to 50 ng/mL. An additive residual variance was estimated to be 15.5%, which corresponded with a pERK suppression of 3.8%.

Discussion

A first-in-man study conducted in healthy volunteers presented the opportunity to characterize the pharmacokinetics, pharmacodynamics, and safety/tolerability of CH4987655 without the confounding factors that exist in patient populations, such as decreased organ function, the effect of concomitant medications, and disease-related adverse events. In addition, the data obtained from this study allowed for a starting dose in the subsequent patient study to start at a dose level that better approximated the potential active dose and also allowed for a reduced pharmacokinetic sampling strategy that alleviated patients from undergoing extensive blood collection.

In healthy volunteers, single doses up to 4 mg were considered safe and tolerated. As expected for MEK inhibitors, epithelial and gastrointestinal types of adverse events were observed, i.e., diarrhea, abdominal pain, acne, and upper abdominal pain and flatulence. This was consistent with preclinical toxicology data, reported adverse events from other MEK inhibitors already tested in clinic, and the mechanism of action of the drug. In the gastric epithelium, the MAPK pathway is involved in the maintenance of mucosal integrity and thus any interference with this pathway is expected to lead to gastrointestinal toxicities (11). In the skin, MAPK pathway activation is required for keratinocyte proliferation, survival, and motility as well as the process of differentiation and keratinization, and, similarly, interference with this pathway is expected to lead to skin toxicities. Both epithelial and gastrointestinal adverse events were dose dependent, with increasing frequency and severity at higher doses.

CH4987655 showed rapid absorption with a median $t_{\text{max}}$ of 1 hour and a disposition phase with a terminal elimination $t_{1/2}$ of ~25 hours. Doses were proportional and the variability was low for both $C_{\text{max}}$ and AUC. The proposed metabolism pathway from in vitro studies is shown in Fig. 4. Pharmacokinetic data from the current study showed that <2% of CH4987655 was eliminated unchanged in the urine, suggesting that CH4987655 was 13.8%, which corresponded with a pERK suppression of 3.8%.
was extensively metabolized before elimination. The result of metabolite profiling confirmed that CH5046399, the ring-open form, was possibly a major metabolite. Because the oxidative metabolites were shown to be minor metabolites, this suggested that metabolism through CYP450 was possibly a minor metabolism pathway.

For most cancer types, it is inconvenient to measure the target of interest directly in tumor tissues. However, mouse xenograft data show good correlation between inhibition of pERK in PBMCs and tumor, both of which also correlated with tumor growth inhibition (12); thus, it was considered reasonable to study the target effect in PBMCs as a surrogate tissue. One of the limitations of PBMCs is that they do not account for mutations of the MAPK pathway, which may alter response to drug treatment. For example, patients with KRas or BRaf mutations may preferentially respond to MEK inhibitors due to constitutive activation of the MAPK pathway. However, although the magnitude of MAPK pathway activation may differ between that of PMA-stimulated PBMC and KRas or BRaf mutated tumor types, the drug effect can still be determined because it is calculated as a percentage change from baseline. ERK is the sole identified substrate of MEK and CH4987655 is a specific MEK inhibitor, as evidenced by the AMBIT kinase panel, which showed no activity against any other kinases. Therefore, not only can the drug effect through pERK inhibition be measured in PBMC but any downstream responses are likely linked to suppression of the intended target, MEK.

CH4987655 showed dose- and concentration-dependent pERK inhibition in PBMCs. Exposures of CH4987655 up to 4 mg dose spanned a wide concentration range covering pERK inhibition from the no effect level to near the maximum effect of 100%, thereby allowing complete characterization of the exposure-effect relationship. Consistent with in vivo preclinical data, the relationship was characterized by an E_{\text{max}} model. Assuming that the effect, pERK inhibition, is driven mainly by CH4987655 concentration, and that CH4987655 pharmacokinetics remain linear after repeated dosing, simulations of multiple dosing at various doses and dosing regimen provided projections of CH4987655 concentration and pERK inhibition at steady state. Nonparametric simulations of CH4987655 concentration suggested that, at steady state, either continuous once-daily dosing or twice-daily dosing will maintain pERK inhibition above IC_{50} over a 24-hour interval.

The pharmacokinetic variability was rather low, but the pharmacodynamic variability was high, thereby producing some noise around the pharmacokinetic-pharmacodynamic relationship. Intrinsic pERK assay variability may contribute to the variability; however, a more controllable means of reducing variability may be through the use of crossover designs provided that repeated drug exposure to healthy subjects poses minimal safety risks. A crossover design should reduce both intrasubject pharmacokinetic and pharmacodynamic variabilities, which in turn will reduce overall data variability.

In the era of translational medicine, it is of interest to retrospectively evaluate if preclinical data translate to clinical data. The current study provides an example of how a biomarker was developed in preclinical research and brought forward in the clinic for testing the effect of a drug on a target that is related to disease biology. As shown by Yoshimura et al. (12), a mouse xenograft model showed good correlation of target suppression as measured by pERK inhibition between that of the surrogate tissue (PBMC) and tumor tissue, which ultimately showed a correlation to the extent of tumor growth inhibition. In cynomolgous monkeys, pERK inhibition measured in PBMC was similar to that observed in the mouse xenograft model. Upon availability of clinical data in patients, a correlative analysis will be done to understand this translational aspect. For the moment, the preclinical data provide some approximation of the extent of target suppression in either PBMC or tumor tissue, which may be required for activity.

One of the other MEK inhibitors, AZD6244, which is already being studied in the clinic, used similar pERK assays in both PBMC and tumor tissues for assessment of target suppression (13). Along with preclinical data, they also provide some approximation of the extent of target suppression in either PBMC or tumor tissue required for activity. An AZD6244 phase I study in patients showed up to 90% pERK inhibition (geometric mean = 51%) in PBMC at steady state when 50 to 300 mg doses were administered on a continuous twice-daily schedule (t_{1/2} ∼ 8 hours). At 100 mg administered on a twice-daily schedule, pERK inhibition was maintained for ∼ 12 hours above EC_{50} (14). This was based on a C_{\text{min}} range of 0.13 to 0.29 μg/mL and an estimated EC_{50} of 0.15 μg/mL. Tumor biopsies collected 2 to 4 hours after a dose of either 100 or
200 mg also showed a geometric mean pERK reduction of 79% from the basal level. Although AZD6244 clinical data presented preliminary evidence of target suppression as measured by pERK inhibition in both PBMC and tumor biopsies, further evidence will be necessary to show that pERK can be inhibited consistently in selected tumor types at tolerable doses. The link between that of pERK inhibition will also need to be validated to that of clinical outcome in future studies. If the link can be established, the extent and duration of pERK inhibition correlating with clinical response can be determined. The current ongoing CH4987655 patient study will continue to explore these correlations and activity thresholds. If all of these relationships can be confirmed, then a model can be developed linking pERK inhibition in PBMC to tumor tissue and to clinical response. In addition, if preclinical to clinical relationships can be confirmed, a model bridging preclinical to clinical outcome can also be incorporated. A validated model may be useful in early development for other MAPK inhibitors or it may help project clinical outcome for alternative dose and dosing regimens that have not yet been tested in the clinic. Although it is clear that uncertainties remain and further exploratory evaluations are pending, pERK inhibition in PBMC presently serves as an acceptable biological marker for preliminary assessment of target suppression for MEK inhibitors in early clinical development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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