Cancer Therapy: Clinical

Multicenter Phase I Trial of the Mitogen-Activated Protein Kinase 1/2 Inhibitor BAY 86-9766 in Patients with Advanced Cancer

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Abstract

Purpose: To evaluate the safety, pharmacokinetics, and pharmacodynamics of BAY 86-9766, a selective, potent, orally available, small-molecule allosteric inhibitor of mitogen-activated protein kinase 1/2 in patients with advanced solid tumors.

Experimental Design: BAY 86-9766 was administered orally daily in 28-day courses, with doses escalated to establish the maximum-tolerated dose (MTD). An expanded cohort was evaluated at the MTD. Pharmacokinetic and pharmacodynamic parameters were assessed, with extracellular signal–regulated kinase (ERK) phosphorylation evaluated in paired biopsies from a subset of the expanded MTD cohort. Tumor specimens were evaluated for mutations in select genes.

Results: Sixty-nine patients were enrolled, including 20 patients at the MTD. The MTD was 100 mg given once-daily or in two divided doses. BAY 86-9766 was well-tolerated. The most common treatment-related toxicities were acneiform rash and gastrointestinal toxicity. BAY 86-9766 was well-absorbed after oral administration (plasma half-life 12 hours), and displayed dose proportional pharmacokinetics throughout the tested dose range. Continuous daily dosing resulted in moderate accumulation at most dose levels. BAY 86-9766 suppressed ERK phosphorylation in biopsied tissue and tetradecanoylphorbol acetate–stimulated peripheral blood leukocytes. Of 53 evaluable patients, one patient with colorectal cancer achieved a partial response and 11 patients had stable disease for 4 or more courses. An ocular melanoma specimen harbored a GNAQ-activating mutation and exhibited reduced ERK phosphorylation in response to therapy.

Conclusion: This phase I study showed that BAY 86-9766 was well-tolerated, with good oral absorption, dose proportional pharmacokinetics, target inhibition at the MTD, and some evidence of clinical benefit across a range of tumor types. Clin Cancer Res; 19(5); 1232–43. ©2012 AACR.

Introduction

The RAS/RAF/MEK/ERK pathway transduces signals from cell surface receptors to the intracellular machinery involved in cell proliferation, survival, and migration (1–3). This pathway is constitutively activated in many tumors, often reflective of dysfunctional receptor tyrosine kinases or activating mutations in RAS or RAF family members (4–11). Consequently, the RAS/RAF/MEK/ERK pathway represents an attractive therapeutic target. In particular, the search for mitogen-activated protein kinase (MAPK) inhibitors has been an active area of research for two main reasons. First, the mitogen-activated protein/extracellular signal–regulated kinase (MEK) enzyme is a critical component of the pathway that transduces signals to extracellular signal–regulated kinase (ERK), which in turn phosphorylates numerous nuclear and cytoplasmic substrates involved in regulating cellular responses (1, 11–13), and second, the MEK protein structure is amenable to the design of highly selective inhibitors (14, 15). Moreover, as pathway activation occurs upstream of MEK in many human malignancies (4, 6–8), it is hypothesized that MEK inhibitors may have broad clinical use in cancer therapy.
MEK Inhibitor BAY 86-9766 in Advanced Cancer Patients

**Translational Relevance**

The article describes the phase I clinical evaluation of the mitogen-activated protein/extraacellular signal–regulated kinase (MEK)1/2 inhibitor BAY 86-9766 in patients with advanced solid tumors. The study design included extensive pharmacodynamic biomarker analyses. These biomarkers were selected for clinical evaluation based on preclinical investigation of correlates of target cell sensitivity to BAY 86-9766 and were subsequently assessed clinically within a number of analyses including ex vivo stimulated leukocytes, circulating tumor cell enumeration, and immunohistochemical and mutational analysis of serial tumor biopsies. In addition to correlating biomarker changes with clinical benefit with BAY 86-9766 treatment, these data support the hypothesis that this agent would suppress phosphorylated extracellular signal–regulated kinase (pERK) levels and may allow a basis for comparison of this agent with other MEK inhibitors. An additional clinical application in the article of data derived from preclinical studies involves the use of ADME/PK studies across multiple species to identify metabolites in humans and select the first-in-human doses and dosing regimen.

BAY 86-9766 (formerly RDEA119; VRX-621119) is a highly selective, potent, orally available, small-molecule allosteric (non-ATP–competitive) inhibitor of MEK1/2 (16). The drug binds to an allosteric site adjacent to the ATP-binding region and then interacts with ATP, the activation loop, and other surrounding residues to prevent binding of MEK to its substrate ERK, thereby blocking ERK phosphorylation (16). BAY 86-9766 inhibited cell proliferation in human cancer cell lines, including those harboring BRAF V600E mutations, and also exhibited potent antitumor activity in xenograft models (16, 17).

This phase I trial was designed to evaluate the safety of escalating doses to identify the maximum-tolerated dose (MTD) of BAY 86-9766 when administered daily to patients with advanced nonhematologic malignancies. Secondary objectives included characterization of single- and multiple dose pharmacokinetics, evaluation of pharmacodynamic effects in peripheral blood and tumor biopsy specimens, and evaluation of safety and tolerability of the recommended phase II dose (RP2D).

**Materials and Methods**

**Study design**

In this open-label, phase I trial, patients with advanced solid tumors received doses of BAY 86-9766 that were escalated in a modified Fibonacci design; the first 3 cohorts received doses based on body surface area rounded to the nearest milligram (2–3, 3–5, and 5–8 mg), with subsequent cohorts receiving a flat dosing schedule (10–160 mg). Three patients were initially enrolled into each cohort; they received a single dose of BAY 86-9766 either 1 hour before or 2 hours after a meal for pharmacokinetic (PK) evaluation. Seven days later, patients started a 28-day treatment course during which BAY 86-9766 was taken with or without food. Escalation to the next dose level was allowed if no dose-limiting toxicity (DLT) occurred. A DLT was defined using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 3.0, as the occurrence of any of the following drug-related toxicities: grade ≥3 nonhematologic toxicity (except grade 3 nausea, vomiting, or untreated skin rash that could be adequately controlled with medical intervention); grade ≥3 diarrhea despite the use of maximum antidiarrheal support; grade 4 thrombocytopenia; grade 3 thrombocytopenia associated with bleeding; grade 4 neutropenia lasting ≥7 days; febrile neutropenia; grade 4 anemia; grade ≥3 hemorrhage or bleeding events; grade ≥3 elevations in international normalized ratio (INR) or partial thromboplastin time (PPT) with associated bleeding; or missing ≥7 daily doses of BAY 86-9766 due to drug-related toxicity. Grade ≥3 elevations in lipase or amylase without signs of pancreatitis were not considered a DLT. If a DLT occurred in one of the initial 3 patients enrolled in a cohort, then up to 3 additional patients were enrolled and started directly on the 28-day treatment course; dose escalation was allowed if no further DLT occurred. If DLT occurred in 2 or more patients, then the next lower dose level was to be defined as the MTD, and 20 additional patients were to be enrolled in an expanded MTD cohort. Data from the initial pharmacokinetic analyses were used to determine whether dosing in subsequent cohorts would be once-a-day or twice-a-day.

Patients self-administered BAY 86-9766 during the 28-day continuous dosing period and returned to the clinic for weekly visits. In the event of DLT or grade 4 neutropenia lasting 3 or more days, BAY 86-9766 dosing was to be interrupted and resumed when toxicity resolved to grade ≤1. In cases where recovery was delayed, treatment could be reinitiated at one dose level lower. As dermatologic toxicity was expected with BAY 86-9766, patients were instructed to moisturize their skin twice-daily with a thick, alcohol-free emollient, minimize exposure to sunlight, and to report such toxicity immediately to allow rapid and aggressive treatment with both drug and nondrug therapies. Patients were allowed to receive additional 28-day courses in the absence of untoward toxicity, disease progression, or withdrawal of consent.

This study was conducted in accordance with ethical principles originating in the Declaration of Helsinki and in compliance with the International Conference on Harmonisation Good Clinical Practice and U.S. regulatory requirements. The study protocol, its amendments, and informed consent forms were approved by the Institutional Review Board for each site before patients were screened. All patients provided written, informed consent before participating in any study-related activities.
Patients

Patients were eligible for this study if they were aged 18 years or older with a histologic or cytologic confirmed advanced nonhematologic cancer for which no proven effective therapy was available, Eastern Cooperative Oncology Group (ECOG) performance status of 1 or less, and life expectancy of 12 weeks or more. Other key eligibility criteria included normal cardiac function as measured by echocardiography or multiple gated acquisition (MUGA) scan, and adequate bone marrow (platelets $\geq$ 100,000/$\mu$L, absolute neutrophil count $\geq$ 1,500/$\mu$L, and hemoglobin $\geq$ 9 g/L), liver [total bilirubin $\leq$ upper limit of normal (ULN), and aspartate aminotransferase (AST)/alanine aminotransferase (ALT) $\leq$ 2.5 $\times$ ULN], and renal (serum creatinine $ \leq $ 2 $\times$ ULN) function. Coagulation parameters were to be within normal limits for patients in the dose-escalation phase, but values that were therapeutically prolonged or not considered medically significant were allowed in the expanded MTD cohort. At least 10 patients in the expanded MTD cohort were required to have tumor accessible for biopsy. Patients were excluded if they had documented central nervous system (CNS) metastases and were receiving corticosteroids or other CNS therapies, major surgery within 30 days, evidence of uncontrolled active infection (including HIV), significant cardiac dysfunction, chronic diarrhea, other serious medical or psychiatric illness, those of childbearing potential who were unwilling to use effective contraception, or had used an investigational agent or device within the previous 28 days. As BAY 86-9766 is primarily metabolized by CYP3A4 and CYP2C19, concomitant use of inhibitors or inducers of these enzymes was to be avoided.

Assessments

Patients were screened within 21 days before the first dose of BAY 86-9766, including a cardiac function assessment by echocardiogram or MUGA scan and evaluation of measurable disease by computer-assisted tomography (CT) or MRI within 28 days before dosing. The first 3 patients in each dose-escalation cohort had a physical examination, ECOG performance status assessment, vital signs measurement, laboratory testing, and adverse event and concomitant medication review on days 1, 8, 15, 22, 29, and 35 of course 1. For any additional patients in each dose-escalation cohort, patients in the expanded MTD cohort, and subsequent courses for the first 3 patients in each dose-escalation cohort, these assessments were conducted on days 1, 8, 15, 22, and 28 of all courses. Adverse events were graded by severity using the NCI CTCAE, version 3.0, and relationship to study treatment was documented by the investigator. The first 3 patients in each dose-escalation cohort had electrocardiogram (ECG) evaluations on days 22 and 35 of course 1. Additional patients in each dose-escalation cohort had ECGs conducted on days 15 and 28 of course 1. In subsequent courses for all dose-escalation patients, ECGs were conducted on day 1. Patients in the expanded MTD cohort had ECG evaluations conducted in triplicate at 3 separate time points before dosing on day 1 of course 1, at predose and 2, 3, and 4 hours postdose on day 15 of course 1, and on day 1 of subsequent courses to determine more precisely if the drug had any effect on corrected QT interval (QTc). For all patients, cardiac function was reassessed at the end of course 1 and then every 8 weeks for those continuing treatment. CT or MRI scans of measurable disease were also repeated every 8 weeks. Efficacy was evaluated in patients with measurable disease using Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0 (18).

Pharmacokinetics

For the first 3 patients in each dose-escalation cohort, serial blood samples (2.5 mL) for pharmacokinetic analysis were collected predose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 24, and 48 hours after the first dose in course 1. Pharmacokinetic sampling at the same time points, except at 48 hours, was also done on days 22 and 35 (15 and 28 days after the start of continuous dosing, respectively) of the first course. Additional samples were collected predose and at 2 and 3 hours postdose on days 8 and 15 (the first day and 8 days after the start of continuous dosing, respectively) in the first course. For patients enrolled in the expanded MTD cohort, serial blood samples were collected at predose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours on day 15 of the first course, with additional samples collected predose and at 2, 3, and 4 hours postdose on days 1 and 28 in the first course. For any additional patients enrolled in the dose-escalation cohorts, and subsequent courses for all patients, samples were collected predose and at 2, 3, and 4 hours postdose on days 1, 15, and 28. Concentrations of plasma BAY 86-9766 and its inactive metabolite M17 were determined by validated high-performance liquid chromatography tandem mass spectrometry, and pharmacokinetic parameters were calculated on the basis of concentrations measured in the serial blood samples by noncompartmental analysis using the WinNonlin program.

Pharmacodynamics

In the first 3 patients in the dose-escalation cohorts, blood was taken for the measurement of pERK on days 1 and 35 (predose), and 2, 3, and 24 (days 2 and 36) hours postdose in course 1. Leukocytes were isolated by red blood cell hypotonic lysis, recovered in cell lysis buffer, and then analyzed for pERK1/2 and total ERK1/2 content using an electrochemiluminescence (ECL) based technology from Meso Scale Discovery. Blood was also taken for enumeration of circulating tumor cells (CTC) predose on day 1, as well as on day 35 at 3 and 24 hours postdose in course 1. For additional patients in the dose-escalation cohorts and all patients in the expanded MTD cohort, blood for pERK measurement was taken on days 1 and 28 at predose and at 2 and 3 hours postdose of course 1 only. Blood for CTCs was taken in courses 1 and 2, then every other course thereafter. Samples were collected predose on day 1 (course 1 only), as well as on day 28 at 3 and 24 hours postdose. Biopsy specimens were obtained at baseline and on day 15 of the first course in a subset of the expanded MTD cohort.
and evaluated for tumor pERK and pAKT levels. Tumor biopsies (18-gauge core needle) were obtained using computed tomography or ultrasound scan guidance. Specimens were formalin-fixed and paraffin-embedded before sectioning and immunohistochemical analysis with pERK and pAKT antibodies. Nuclei and cytoplasm were scored by estimating the proportion of positive viable tumor cells multiplied by intensity of staining quantified on a 0 to 3+ scale. DNA was extracted from fixed specimens from microdissected tumor and submitted for direct sequencing of the following mutations: KRAS, BRAF, NRAS, PIK3CA, and PTEN. GNA11 and GNAQ were also sequenced on select ocular melanoma samples.

Statistical analysis

Safety parameters were summarized descriptively for all patients who received at least one dose of BAY 86-9766. The median time to progression (TTP) was assessed by Kaplan–Meier methodology. Statistical analysis of pERK values from ex vivo stimulated leukocytes was conducted using one-way ANOVA and Dunnett’s posttest for comparison to baseline predose values. Summarized leukocyte data are presented as geometric means due to the multiplicative nature of this endpoint, which is consistent with this type of analysis (19). PK/PD modeling was conducted using a nonlinear fit Emax model with Emax set to 100%. Statistical analysis of CTC data was done using both paired t tests and one-way ANOVA where appropriate. Statistical analysis of marker data from matched biopsies was done using a paired t test to assess statistical significance of the observed changes. Data from the once-a-day and twice-a-day groups of the expanded MTD cohort were combined for this analysis.

Results

Patient disposition and baseline characteristics

This open-label, phase I trial in patients with advanced solid tumors was conducted at 3 U.S. sites from November 2007 to November 2010, with 3 patients still ongoing at the time of the data cutoff. Sixty-nine patients were enrolled and received at least one dose of BAY 86-9766, including 49 patients in the dose-escalation phase and 20 patients in the expanded MTD cohort (Supplementary Fig. S1). Overall, 57 patients (83%) completed at least 1 course of treatment. Sixty-eight of the 69 patients who received BAY 86-9766 were evaluable for treatment exposure (1 patient enrolled in the 100-mg dose-escalation cohort received an initial dose for pharmacokinetic analysis but discontinued before beginning the first course) and received a median of 56 doses (range, 4–669) and a median total dose of 2,630 mg (range, 16–23,950 mg). The median duration of treatment was 1.9 courses (range, 0.1–13.8 courses). In the expanded MTD cohort, the median number of doses was 49 (range, 4–110) and 109 (range, 18–325), respectively, for the 100-mg once-a-day and 50-mg twice-a-day dose levels; the median total dose was 4,175 mg (range, 400–11,000 mg) and 5,450 mg (range, 900–16,250 mg), respectively, and the median duration of treatment was 1.8 (range, 0.1–3.9) and 1.9 courses (range, 0.3–5.8 courses), respectively. Patient characteristics are presented in Table 1.

Safety

All 69 (100%) patients reported at least 1 adverse event, with a total of 998 adverse events reported. Treatment-related adverse events were reported in 61 patients (88%), most commonly acneiform dermatitis (33%), diarrhea (32%), nausea (29%), lymphedema (28%), vomiting (26%), fatigue (26%), and maculopapular rash (20%; Table 2). There was no evidence of a dose–response relationship, and most treatment-related adverse events were grade 1 or 2. Treatment-related grade 3 adverse events were lymphedema (n = 2; 1 each at 100 and 160 mg), fatigue (n = 1; 60 mg), acneiform dermatitis (n = 1; 100 mg), diarrhea (n = 1; 160 mg), and abdominal pain (n = 1; 50 mg twice-a-day). The overall incidence of treatment-related adverse events declined with each treatment course from 87% in course 1 to 49%, 23%, 10%, and 9% in courses 2 to 5, respectively. Thirty-six (52%) patients reported at least 1 serious adverse event (SAE). All treatment-related SAEs were reported at dose levels ≥100 mg, including 2 (18%) and 2 (40%) patients in the 100- and 160-mg dose-escalation cohorts, respectively, and 2 (20%) and 3 (30%) patients in the 100-mg once-a-day and 50-mg twice-a-day groups of the expanded MTD cohort, respectively. There were 10 deaths that were attributed to adverse events during the study or within 28 days after the last dose, including disease progression in 4 (6%) patients. Importantly, no adverse events that led to death were considered treatment-related.

Treatment-related dermatologic toxicity was observed in 50 patients (73%), primarily acneiform dermatitis (33%), maculopapular rash (20%), and rash (12%); dry skin, macular rash, papular rash, and pustular rash (9% each) and erythematous rash (6%) were also observed. It should be noted that pustular rash and erythematous rash were reported at dose levels ≥40 mg only, and the incidence of acneiform dermatitis was higher in the 50-mg twice-a-day group (90%) in the expanded MTD cohort compared with the 100-mg dose-escalation cohort (27%) and the 100-mg once-a-day group in the expanded MTD cohort (30%). Dermatologic toxicity responded to aggressive treatment with high-emollient lotion combined with steroid and/or antibiotic therapy for higher grades of toxicity. Of the 126 treatment-related dermatologic adverse events, 81 were reported during course 1. The majority were grade 1 or 2, and there were no grade 4 reported. The few grade 3 dermatologic adverse events reported during the study occurred at dose levels ≥100 mg.

Gastrointestinal toxicities of nausea, vomiting, and diarrhea represented the second most common toxicity observed in this study. The severity of these events was primarily grade 1 and 2. One patient in the 160-mg once-a-day group experienced a grade 3 DLT of diarrhea and discontinued from the study due to this adverse event as well as an adverse event of grade 2 vomiting.
Although CNS adverse events were expected in patients treated with a MEK inhibitor, they were reported infrequently in this study, with the most common treatment-related CNS toxicity being abnormal dreams. Importantly, all cases of abnormal dreams were classified as grade 1. Grade 3 CNS toxicities were reported at dose levels of 100 mg. Treatment-related ocular toxicity occurred in 7 patients (10%). It should be noted that in 6 of these 7 patients, the maximal severity was grade 1. Neurologic toxicity led to treatment discontinuation in 4 patients due to hemorrhagic stroke (2–3 mg; not related to study drug), syncope (100 mg; not related to study drug), somnolence (160 mg; possibly related to study drug), and neurotoxicity (50 mg twice-a-day; possibly related to study drug). Reversible ocular toxicities of chorioretinopathy (grade 1) and retinal vein occlusion (grade 2) were observed in 1 patient each in the expanded MTD cohort (100-mg once-a-day and 50-mg twice-a-day groups, respectively). The patient with retinal vein occlusion was treated with intravitreous bevacizumab, resulting in resolution to baseline vision.

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Dose-escalation cohorts (n = 49)</th>
<th>Expanded MTD cohort</th>
<th>All patients (N = 69)</th>
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<td>64.7 (45–79)</td>
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<td>Male</td>
<td>29 (59)</td>
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<td>20 (41)</td>
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<td>10 (100)</td>
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<td>ECOG performance status, n (%)</td>
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<td>19 (39)</td>
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<td>3 (30)</td>
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<tr>
<td>1</td>
<td>30 (61)</td>
<td>8 (80)</td>
<td>7 (70)</td>
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<td>Years since diagnosis, mean</td>
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<td>Prior cancer therapy, n (%)</td>
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<td>Baseline signs/symptomsb, n (%)</td>
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<td>3 (30)</td>
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<tr>
<td>Nausea</td>
<td>15 (31)</td>
<td>2 (20)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>11 (22)</td>
<td>4 (40)</td>
<td>3 (30)</td>
</tr>
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Abbreviation: BID, twice-a-day; QD, once-a-day.
aIncludes breast cancer and gynecologic malignancy (2 patients each in the dose-escalation cohorts), gastric cancer, thyroid cancer, gastrointestinal stromal tumor, malignant mesothelioma, and malignant peripheral epithelial cell sarcoma originating from the abdomen (1 patient each in the dose-escalation cohorts), and non-melanoma skin cancer (1 patient in the expanded MTD cohort).
bAll patients had at least 1 sign/symptom at baseline.
Cardiovascular toxicity was observed infrequently. In a formal triplicate ECG study for QTc evaluation in the expanded MTD cohort, there were no shown effects of BAY 86-9766 on cardiac repolarization. There was no evidence of different effects on ECG parameters of the 100-mg once daily dose compared with the 50-mg twice daily dose. Routine ECGs in the dose-escalation cohorts showed sporadic prolongations in QTc. Nine patients with routine single ECGs showed a prolongation of >30 milliseconds from baseline to a post-baseline value >450 milliseconds (including 3 patients with a QTc >500 milliseconds). All but one of these routine ECG changes was considered not clinically significant. Mean left ventricular ejection fraction values remained greater than 50% from baseline throughout the study for the majority of patients in all treatment groups. Two patients had postbaseline abnormal clinically significant left ventricular ejection fraction values associated with adverse events. One patient in the 10-mg cohort had a grade 1 adverse event of an abnormal MUGA scan that was unlikely related to treatment and 1 patient in the 100-mg once daily group of the expanded MTD cohort had a grade 3 SAE of ventricular dysfunction that was treatment-related and led to study discontinuation.

Changes in laboratory parameters were consistent with the health status of the study population; no dose-related trends were observed. Nine patients (13%) had grade 3 clinically significant laboratory values, including 2 patients with AST elevations and 1 patient with an elevated lipase that was a DLT. Importantly, all grade 3 clinically significant laboratory values resolved to either within-range values or out-of-range but not clinically significant values at the last available testing date.

**DLTs, dose reductions, and study discontinuation**

Six patients in the dose-escalation cohorts experienced a total of 8 DLTs, all of which were classified as grade 3 and considered possibly related to study treatment (Supplementary Table S1). No single DLT was reported by more than 1 patient, although 2 patients had DLTs affecting the skin and subcutaneous tissue body system [1 patient (100 mg) with skin pain and papular rash, and 1 (160 mg) with macular rash], and 2 patients had DLTs affecting the nervous system [presyncope and somnolence (both at 160 mg)]. Three patients in the 160-mg cohort had DLTs, and consequently the MTD was defined as 100 mg. All DLTs resolved during the study.

Six patients (9%; 5 in the dose-escalation cohorts and 1 in the expanded MTD cohort) had adverse events that required dose reductions, including 2 patients who required dose reduction from 160 to 100 mg, and 33 other patients (48%) had adverse events that led to dose interruptions or stopping. One patient receiving 100 mg once daily in the expanded MTD cohort had a dose reduction to 50 mg for chorioretinopathy. Dose reduction led to adverse event resolution in all 6 cases.

**Pharmacokinetics**

Following oral administration at doses ranging from 2 to 160 mg, BAY 86-9766 was readily absorbed with median
T<sub>max</sub> of approximately 2 hours. Average BAY 86-9766 half-life of approximately 12 hours was estimated after single dosing on course 1, day 1. Geometric means of BAY 86-9766 daily dosing plasma concentration–time profiles on day 22 are shown in Fig. 1, and pharmacokinetic parameters are presented in Supplementary Tables S2 and S3 for the once-daily and twice-daily dosing cohorts respectively. C<sub>max</sub> and steady-state area under the curve (AUC)<sub>0-24</sub> values increased nearly dose proportionally in the 2- to 100-mg dose range with daily dosing. Continuous daily dosing resulted in moderate BAY 86-9766 accumulation at most dose levels, including the 50-mg twice-a-day group in the expanded MTD cohort, where approximately a 2-fold accumulation was observed. BAY 86-9766 exposure on days 22 and 35 was generally comparable, indicating that a steady-state condition was achieved within 2 weeks of continuous dosing. The half-life of (inactive) metabolite M17 averaged approximately 14 hours, and geometric mean metabolite M17-to-BAY 86-9766 molar ratios for C<sub>max</sub> and AUC were typically less than 30% in the dose range studied. It should be noted that the pharmacokinetic parameters evaluated were generally comparable between the 50-mg twice-a-day or 100-mg once-a-day MTD dosing regimens.

Pharmacodynamics

12-O-Tetradecanoylphorbol-13-acetate (TPA) is a phorbol ester that directly stimulates PKC activating MAPK signaling. BAY 86-9766 suppressed pERK levels in TPA-stimulated leukocytes, with up to 100% inhibition seen 3 hours after a single 50-mg dose (geometric mean: 83%). When expressed according to plasma drug levels, BAY 86-9766 suppressed pERK levels in a concentration-dependent manner with a 95% confidence interval (CI) ED<sub>50</sub> of 156 to 254 ng/mL (Fig. 2A). BAY 86-9766 inhibited pERK by up to 93% (geometric mean: 67%) during the pharmacokinetic trough at the MTD (Table 3). Moreover, BAY 86-9766 reduced pERK levels up to 89% in tumor biopsy specimens collected after 15 days of continuous dosing in a subset of 11 patients from the expanded MTD cohort for which biopsy specimens were available (Fig. 2B). The immunohistochemical staining of pERK in matched pre and post BAY 86-9766 biopsies from a patient with BRAF V600K melanoma is shown in Fig. 2C and D. Tumors without detectable activating mutations tended to have lower baseline levels of pERK, and BAY 86-9766 exposure did not produce the same magnitude of pERK suppression as was observed in tumors possessing mutations in either KRAS, PIK3CA, BRAF, or PTEN. In contrast, BAY 86-9766 did not affect pAKT levels (data not shown). In an exploratory analysis, BAY 86-9766 did not significantly affect CTC load or pERK in CTCs after 2 courses of continuous dosing (data not shown).

Efficacy

Fifty-three patients were evaluable for efficacy. One patient (2%) achieved a partial response (PR) and 11 patients (16%) had stable disease for at least 4 courses. An additional 15 patients had stable disease lasting for shorter times as shown in Fig. 3A. A waterfall plot of best treatment response is shown in Fig. 3B. The PR occurred in a 63-year-old man with heavily pretreated colorectal cancer who received 50 mg twice-a-day. It should be noted that this tumor was wild-type for all mutations tested. As of the data cutoff, his target lesions had decreased by 18% and 35% after 2 and 4 courses of treatment, respectively. This patient later went on to complete 16 courses before leaving the study due to disease progression. Prolonged stable disease was observed in patients with colorectal cancer, ocular melanoma, ovarian cancer, breast cancer, adrenal cancer, and liposarcoma. Notably, an ocular melanoma patient with prolonged stable disease, who later went on to complete 28 courses, was positive for GNAQ<sup>Q209L</sup> and PTEN<sup>R173S</sup> mutations. The PTEN mutation was identified only in the postdose biopsy and not in the predose biopsy. For the entire efficacy population as of the data cutoff, median
Figure 2. A, percentage inhibition of pERK in TPA-stimulated leukocytes according to plasma BAY 86-9766 concentration. Data are based on 84 pharmacodynamic and pharmacokinetic samples collected before and at 2, 3, and 24 hours after steady-state dosing. B, individual MTD patients with matched pre- and postdose tumor pERK immunohistochemical data. Tumor mutational background denoted as K (KRAS), B (BRAF), P (PIK3CA), PT (PTEN), G (GNAQ), and WT (wild-type for all tested). Dosing schedule was either 100 mg once-a-day or 50 mg twice-a-day. Representative (C) pre- and (D) postdose patient biopsy sections stained with anti-pERK antibodies. BID, twice-a-day; QD, once-a-day.

Table 3. Stimulated leukocyte pERK response comparing 50 mg twice-a-day versus 100 mg once-a-day BAY 86-9766 at days 1 and 28

<table>
<thead>
<tr>
<th>Timing</th>
<th>BAY 86-9766 dose</th>
<th>n</th>
<th>Maximum</th>
<th>Mean</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 single</td>
<td>50 mg BID</td>
<td>10</td>
<td>100</td>
<td>83</td>
<td>38</td>
</tr>
<tr>
<td>Dose $T_{\text{max}}$</td>
<td>100 mg QD</td>
<td>13</td>
<td>100</td>
<td>72</td>
<td>29</td>
</tr>
<tr>
<td>Day 28 steady</td>
<td>50 mg BID</td>
<td>5</td>
<td>89</td>
<td>70</td>
<td>2</td>
</tr>
<tr>
<td>State predose $^c$</td>
<td>100 mg QD</td>
<td>6</td>
<td>93</td>
<td>64</td>
<td>23</td>
</tr>
</tbody>
</table>

Abbreviation: BID, twice-a-day; QD, once-a-day.
$^a$Dose-escalation and expanded MTD cohort patients grouped for analysis.
$^b$Inhibition values at maximum day 1 plasma concentration.
$^c$Inhibition values taken before day 28 daily dosing.
TTP was 63 days (range, 1–428+ days). Across the dose-escalation cohorts, the median TTP ranged from 49 to 331 days, with no dose-related trends. In the 100-mg once-a-day group in the expanded MTD cohort, median TTP was 67 days (range, 8–112 days). In the 50-mg twice-a-day group, the median TTP could not be estimated because the survival distribution function did not reach 0.5 (range, 12–169 days).

Discussion

BAY 86-9766 is a highly selective, potent, orally available, small-molecule allosteric inhibitor of MEK1/2.
Given the activation of the RAS/RAF/MEK/ERK pathway in many tumors, this agent has therapeutic potential. In this phase I study of patients with advanced solid tumors, the MTD and RP2D were identified as 100 mg, either once-daily or in 2 divided doses. BAY 86-9766 plasma Cmax values were generally comparable after 50-mg twice-a-day and 100-mg once-a-day dosing. Daily oral dosing of BAY 86-9766 was well tolerated by patients in this study, with a safety profile consistent with that of other MEK inhibitors, including AZD6244 (selumetinib), PD0325901, trametinib, and RO4987655 (19–22). The most common treatment-related adverse events with BAY 86-9766 were dermatologic toxicities (acneiform dermatitis and maculopapular rash), gastrointestinal toxicity (diarrhea, nausea, and vomiting), lymphedema, and fatigue. Dermatologic toxicity was expected on the basis of clinical experience with other MEK inhibitors (21–24), and consequently the study protocol included a plan to minimize the incidence and severity of dermatologic toxicity by implementing an aggressive pharmacologic and nonpharmacologic approach upon the early identification of any skin toxicities. Interestingly, rash (papular and macular) was identified as a DLT in only 2 of 49 patients (4%) in the dose-escalation cohorts, and grade 3 acneiform dermatitis was identified in only 1 of 69 patients (1%) overall. In contrast, rash was a frequent DLT in clinical studies with other MEK inhibitors (19–22).

Neurotoxicity has also been reported with MEK inhibitors. Transient and reversible blurred vision occurred with AZD6244 (19), and acute neurotoxicity with visual disturbances, balance, and gait disorders was seen with PD0325901 (20). Retinal vein occlusion was reported during prolonged treatment in patients with underlying risk factors for retinopathy. In the present study, neurological toxicity was seen with BAY 86-9766 at doses ≥ 100 mg, presenting most frequently as abnormal dreams in 7 patients. BAY 86-9766 showed evidence of clinical benefit in 12 of 69 patients (17%). One patient achieved a PR and 11 patients had prolonged stable disease of at least 4 courses. Importantly, the clinical benefit associated with BAY 86-9766 was evident in multiple patients across a range of tumor types including colorectal cancer, melanoma, and sarcoma, suggesting that BAY 86-9766 may possess broad use in solid tumors. The clinical benefit rate with BAY 86-9766 was consistent with rates observed with other MEK inhibitors in phase I, including patients with colorectal cancer, melanoma, non–small cell lung cancer, pancreatic cancer, and hepatobiliary cancer (19–21, 24–30). Of note, the clinical benefit of MEK inhibitors in non-melanoma is typically characterized by prolonged stable disease, whereas objective responses were only occasionally observed. The presence of the GNAQ Q209L and PTEN R173S mutations in the patient with ocular melanoma achieving prolonged stable disease may suggest that patients with this genotype should receive combination therapy with MEK and PI3 kinase inhibitors (31). If GNAQ Q209L mutation commonly segregates with secondary mutations, it may explain the failure of GNAQ/GNA11 mutations to predict a beneficial response to MEK inhibitors, as observed with trametinib monotherapy (21). These findings suggest that MEK inhibitors should be used in combination with other active agents or as a targeted therapy against tumors with sensitizing mutational backgrounds to optimize the anti-cancer benefit.

The extent of inhibition of the MAPK pathway needed for efficacy with MEK inhibitors remains unclear. Recent data with the BRAF inhibitor PLX4032 suggests that more than 90% kinase inactivation is necessary to obtain clinical response in BRAF-positive melanoma (32). Consistent with this observation, resistance to MEK inhibitors occurs, in part, due to amplification of RAS and BRAF, resulting in augmented ERK activity requiring higher concentrations of MEK inhibitors to inhibit ERK activity (33). It should be noted that BAY 86-9766 inhibited TPA-induced ERK phosphorylation in leukocytes by an average of 70% at trough pharmacokinetic. BAY 86-9766 reduced pERK levels, but not pAKT levels, in tumor biopsies in a subset of the expanded MTD cohort. BAY 86-9766 was able to reduce ERK phosphorylation (27%–89% pERK inhibition) to a similar degree in tumors harboring KRAS and BRAF mutations. In contrast, tumors not harboring activating MAPK pathway mutations tended to show no significant inhibition of ERK phosphorylation in response to BAY 86-9766. However, it should be noted that in this study, it is possible that biopsies taken at day 15 of course 1 may not represent an accurate pharmacodynamics picture for long-term response. Indeed, the tumor from the patient with colorectal cancer who achieved a PR while receiving 50 mg twice-a-day of BAY 86-9766 did not show any pERK reduction at day 15 of course 1.

In summary, the observations obtained from this phase I study of BAY 86-9766 in patients with advanced solid tumors warrants continued clinic investigation administered at the MTD of 100 mg, given as 50 mg twice-a-day or 100 mg daily. BAY 86-9766 exhibited a favorable safety profile, good oral absorption, and dose proportional pharmacokinetics and pharmacodynamic profile. Ultimately, efficient clinical development of MEK inhibitors will be augmented by the simultaneous development of patient selection strategies to determine rational combinations with cytotoxic and molecular-targeted agents in the appropriate disease setting.

Disclosure of Potential Conflicts of Interest
G.J. Weiss has honoraria from speakers bureau of Genentech, Pfizer, Quintiles, and Medscap. J.N. Miner is employed as Senior Director by AstraZeneca. R.L. Dubowy has ownership interest (including patents) in Stock ownership. No potential conflicts of interest were disclosed by the other authors.

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