RDEA119/BAY 869766: A Potent, Selective, Allosteric Inhibitor of MEK1/2 for the Treatment of Cancer

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Abstract
The RAS-RAF-mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) kinase (MEK)-ERK pathway provides numerous opportunities for targeted oncology therapeutics. In particular, the MEK enzyme is attractive due to high selectivity for its target ERK and the central role that activated ERK plays in driving cell proliferation. The structural, pharmacologic, and pharmacokinetic properties of RDEA119/BAY 869766, an allosteric MEK inhibitor, are presented. RDEA119/BAY 869766 selectively binds directly to an allosteric pocket in the MEK1/2 enzymes. This compound is highly efficacious at inhibiting cell proliferation in several tumor cell lines in vitro. In vivo, RDEA119/BAY 869766 exhibits potent activity in xenograft models of melanoma, colon, and epidermal carcinoma. RDEA119/BAY 869766 exhibits complete suppression of ERK phosphorylation at fully efficacious doses in mice. RDEA119/BAY 869766 shows a tissue selectivity that reduces its potential for central nervous system–related side effects. Using pharmacokinetic and pharmacodynamic data, we show that maintaining adequate MEK inhibition throughout the dosing interval is likely more important than achieving high peak levels because greater efficacy was achieved with more frequent but lower dosing. Based on its longer half-life in humans than in mice, RDEA119/BAY 869766 has the potential for use as a once- or twice-daily oral treatment for cancer. RDEA119/BAY 869766, an exquisitely selective, orally available MEK inhibitor, has been selected for clinical development because of its potency and favorable pharmacokinetic profile. [Cancer Res 2009;69(17):6839–47]

Introduction
Cancer is the second most common cause of death in the United States (one of four deaths; ref. 1). Although progress is being made, as measured by an increase in the 5-year survival rate from ~50% in the 1970s to 66% for people diagnosed with cancer between 1996 and 2002, there is still significant unmet medical need among patients with cancer (1, 2).

The focus of much cancer drug discovery work has shifted toward targeted therapies aimed at genes and pathways that are dysfunctional in human cancer (3). Hyperactivation of cell proliferation, survival, and metastasis pathways can arise from activating gene mutations, gene amplification/overexpression, and/or loss of endogenous tumor suppressors that normally keep these pathways in check (4).

The RAS-RAF-mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase (MEK)-ERK pathway is constitutively activated in a significant proportion of human tumors often through gain-of-function mutations in RAS or RAF gene family members or through hyperactivation of upstream receptor tyrosine kinases [e.g., epidermal growth factor (EGF) receptor tyrosine kinase] that transduce signals through this pathway (5). Studies of primary tumor samples and immortalized cancer cell lines have shown constitutive activation of the RAS-RAF-MEK-ERK pathway in several human tumors, including lung, colon, melanoma, thyroid, and pancreatic cancer (6). Braf activating gene mutations are prevalent in melanoma at 66% and thyroid cancer (44% and 24% for papillary and anaplastic thyroid cancer, respectively; refs. 7–9).

Within this pathway lies MEK for which there are two highly homologous genes expressed in humans (MEK1 and MEK2; ref. 10). MEK is downstream of BRAF in the RAS-RAF-MEK-ERK pathway and is critical for transducing signals to ERK (11). BRAF activating gene mutations have been shown to predict sensitivity to MEK inhibition across a panel of cancer cell lines (12). This dependency, coupled with a unique hydrophobic pocket within the MEK enzyme that allows for the interaction of highly selective “allosteric” inhibitors, has made MEK an attractive cancer target (13). We describe the structure and activity of RDEA119/BAY 869766 (referred to in this manuscript as RDEA119), an orally available, potent, non–ATP-competitive, highly selective inhibitor of MEK1/2, which is active in human tumor xenograft models and is well tolerated within the therapeutic exposure range in animals.

Materials and Methods

The chemical name of RDEA119 is (R)-N-(3,4-difluoro-2-(2-fluoro-4-hydroxyphenylamino)-6-methoxyphenyl)-1-(2,3-dihydroxypropyl)cyclopropene-1-sulfonamide, which was synthesized at Ardea Biosciences (Fig. L1). PD0325901 (0494611 L0002): (S)-(2-fluoro-4-hydroxyphenylamino)-3-(2,3-dihydroxypropoxy)-3,4-difluoro-2-(2-fluoro-4-iodophenylamino)-benzamide can be obtained from Selleck Chemicals Co. Ltd.

Preparation and purification of MEK1 for structural studies. A MEK1 cDNA expression construct coding for an NH2-terminal truncation, MEK1D61 (residues 62–393 wild-type truncated form), was cloned into a bacterial expression vector as pET24b-MEK1Δ61 using NdeI/XhoI sites (10). The COOH-terminal His6-tag construct was grown at 37°C to an A600 of 1.0 and then lowered to 16°C and induced with 3.25 mmol/L isopropyl-l-thio-B-n-galactopyranoside, grown 20 to 24 h as described (10).

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

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Protein-ligand ternary complex formation and cocrystallization. RDEA119 (0.8 mM; 500 mmol/L in 100% DMSO) was added to 0.5 mL of MEK1\(D^{61}\) (3 mg/mL) and incubated overnight at 4°C, then brought to 5 mmol/L Mg-ATP and incubated 5 h at 4°C, concentrated, and mixed 1:1 with crystallization reservoir solution [250 mmol/L NH\(_4\)H\(_2\)PO\(_4\), 6% (w/v) PEG8000, 100 mmol/L malate, 10 mmol/L imidazole], and the drop was allowed to equilibrate over 1 mL of reservoir solution using the hanging drop vapor diffusion technique at 13°C. Frozen MEK1\(D^{61}/\)RDEA119 cocrystals have unit cell dimensions of \(a = 82.3\) Å, \(b = 82.3\) Å, \(c = 129.8\) Å, \(\alpha = 90^\circ\), \(\beta = 90^\circ\), and \(\gamma = 120^\circ\) and belong to the hexagonal space group P\(_6_2\).

Data collection and structure determination. A frozen MEK1\(D^{61}/\)RDEA119 crystal was mounted on an X-ray diffraction system consisting of a RUH3R X-ray generator and a RAXIS IV++ image plate area detector (Rigaku/MSC). A data set of 2.5 Å resolution was collected using 0.5° oscillations and a total \(\phi\)-rotation of 65° and processed, scaled, and reduced (14). The three-dimensional coordinates of only the MEK1 protein atoms from Protein Data Bank entry, 1S9J, were used as the phasing model using the program CNX (15). The initial model was refined against the data initially by treating the MEK1 peptide as a rigid body. Additional rounds of torsion angle annealing and manual adjustment to the model revealed strong difference density for one molecule of ATP, one magnesium ion, and RDEA119. Ligand atoms were added to the model accordingly and included in further rounds of refinement. The final \(R\) factor is 21.9% for the model, which includes MEK1 residues (62–222, 224–275, and 306–382), one molecule of ATP, one magnesium ion, 65 water molecules, and the inhibitor RDEA119. Coordinates and structural factors have been deposited into protein data bank, with PDB code 3E8N.

Biochemical kinase assays. The human MEK1 enzyme was purchased from Invitrogen. The active human MEK2 and truncated RAF1 enzyme were purchased from Millipore. Kinase inactive murine ERK2 (mERK2) K52A/T183A was affinity purified from Escherichia coli expressed using the pET21a vector. MEK1 kinase activity was determined using mERK2 K52A T183A as the substrate. Recombinant MEK1 enzyme (5 nmol/L) was first activated by 0.02 unit or 1.5 nmol/L of RAF1 in the presence of 25 mmol/L HEPES (pH 7.8), 1 mmol/L MgCl\(_2\), 50 mmol/L NaCl, 0.2 mmol/L EDTA, and 50 μmol/L ATP for 30 min at 25°C. The reactions were initiated by adding 2 μmol/L of mERK2 K52A T183A and 2.5 μCi [γ\(^{33}\)P]ATP in a total volume of 20 μL. The MEK2 kinase activity was determined similarly except that activation by RAF1 was not needed and 11 nmol/L of MEK2 enzyme (active) were used in the assays.

RDEA119 affinity

MEK1 IC\(_{50}\) = 19 nmol/L,
MEK2 IC\(_{50}\) = 47 nmol/L.
Kinase profiling was performed by Invitrogen using their SelectScreen Kinase Profiling Service. The Z'-LYTE biochemical assay was used. RDEA119 was assayed in quadruplicate at 10 μM/L against 205 kinases.

**Tumor cell lines and media.** A375, SK-Mel-28, A431, Colo205, HT-29, MDA-MB-231, and BxPC3 cell lines were obtained from the American Type Culture Collection. A375, A431, MDA-MB-231, and SK-Mel-28 cells were grown in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS), 1 mM/L sodium pyruvate, 0.1 mM/L nonessential amino acids, 2 μM/L L-glutamine, 100 units/mL penicillin, and 0.1 mg/mL streptomycin. HT-29 cells were grown in McCoy's 5A medium supplemented with 10% FBS, 2 mM/L L-glutamine, 100 units/mL penicillin, and 0.1 mg/mL streptomycin. BxPC3 cells were grown in RPMI 1640 supplemented with 10% FBS, 1 mM/L sodium pyruvate, 10 mM/L HEPES, 2 mM/L L-glutamine, 100 units/mL penicillin, and 0.1 mg/mL streptomycin. Cells were maintained at 37°C, 5% CO₂, and 100% humidity.

**Cellular MEK inhibition.** A375, A431, SK-Mel-28, Colo205, HT-29, and BxPC3 cells were plated onto 96-well tissue culture plates (Corning) at densities of 2 × 10⁴, 1.5 × 10⁴, 0.75 × 10⁴, 2 × 10⁴, and 2 × 10⁵ per well, respectively, with appropriate growth medium. Plates were incubated overnight to allow cells to adhere. The diluted compounds were added to PBS-washed cells, incubated at 37°C for 20 min in 1% FBS, washed with PBS, and stored at −70°C until further processing.

MEK activity was assayed by measuring the phosphorylation of ERK1 and ERK2 using a phospho-ERK1/2 ELISA kit (BioSource). The mean absorbance measured at 450 nm was converted to percent activity, and average percent activity values were determined from duplicate wells.

**Cell growth inhibition assays.** For anchorage-dependent growth inhibition experiments, cells were plated in white 384-well plates at 1,000/20 μL/well or white 96-well microplates at 4,000/100 μL/well. After 24-h incubation at 37°C, 5% CO₂ and 100% humidity, compound was incubated for 48 h at 37°C and assayed using CellTiter-Glo. For the 96-well anchorage-independent growth assay, wells of an “ultralow binding” plate (Corning) were filled with 60 μL of a 0.15% agarose solution in complete RPMI 1640. Then, 60 μL of complete RPMI 1640 containing 9,000 cells in 0.15% agarose were added per well. After 24 h, 60 μL of a 3× drug solution in agarose-free complete RPMI 1640 were added. After 7, 36, 36 μL of 6× 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt reagent (CellTiter 96 Aqueous, Promega) were added per well. After 2 h at 37°C, absorbance at 490 nm was determined on the M5 plate reader (Molecular Devices). Nonlinear curve fitting was performed using GraphPad Prism 4.

**Human tumor xenograft studies.** Female athymic nude mice (nu/nu, Harlan) were used for all the efficacy studies except for the Colo205 study 2, which used male mice. Studies were performed at Piedmont Research Center in compliance with Association for Assessment and Accreditation of Laboratory Animal Care standards and approved by the review board at Piedmont Research Center. Mice were injected s.c. in the flank with 1 × 10⁶ tumor cells (Colo205 and A431) or 1 × 10⁵ tumor fragments (A375 and HT-29). Tumor volumes were monitored by caliper measurements using the formula tumor volume (mm³) = (w² × l)/2, where w = width and l = length in mm of the tumor. For efficacy analysis, treatment was initiated when tumors were 80 to 185 mm³. RDEA119 was administered by oral gavage once daily or twice daily for 14 d or once every 2 d for 14 doses.

**Pharmacodynamic/pharmacokinetic studies.** Mice bearing tumors (250–350 mm³) were dosed by oral gavage with RDEA119 at 2.5, 5, 10, or 25 mg/kg, PD325901 at 10 mg/kg, or vehicle. Three mice from each group were sacrificed at 2, 6, 12, and 24 h after dosing, and plasma, brain, lung, and tumor samples were collected and frozen. Tissue samples were thawed on ice and homogenized in Cell Extraction Buffer (BioSource) at 10% (v/w) ratio for tumor and 20% (v/v) ratio for brain and lung. Homogenates were incubated on ice for 1 h and centrifuged at 500 × g for 5 min. Lysates were transferred to microcentrifuge tubes and further clarified by centrifugation at 14,000 × g for 5 min. Lysates were subjected to Western blot analysis probed with a mixture of phospho-p44/42 MAPK antibody and p44/42 MAPK antibody (Cell Signaling). Rat pharmacokinet/pharmacodynamic studies were conducted as follows: animals from each treatment group were sacrificed at 48 h after dose. Blood samples (~1 mL) were collected for plasma bioanalysis of RDEA119 and PD325901 concentrations by centrifugation at 2,000 rpm for 10 min. Plasma supernatants were aspirated and placed in labeled tubes and stored at −20°C until analysis. Brain and lung samples were also collected (whole organ), weighed, and flash frozen in liquid nitrogen for analysis of total ERK and pERK levels.

**Plasma samples** were analyzed for RDEA119 using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The method involved the addition of the internal standard ([¹³C₆]RDEA119), protein precipitation with acetonitrile, and final analysis by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS). An API 5000 triple quadrupole mass spectrometer was used to monitor the precursor—product ion transitions of m/z 573—394 and m/z 579—400 for RDEA119 and [¹³C₆]RDEA119 in positive electrospray ion mode. The calibration curves covered the concentration range from 10 to 10,000 ng/mL.

**Results**

RDEA119 is an orally bioavailable small molecule with a molecular weight of 572 g/mol. We conducted a variety of preclinical in vitro and in vivo studies to determine (a) potency against its molecular target MEK, (b) selectivity relative to other kinases, (c) antiproliferative activity in cell culture systems using both cancer and normal cells, (d) in vivo antitumor efficacy, (e) pharmacokinetic relationships between antitumor efficacy and brain retention, and (f) downstream consequences of MEK inhibition on proliferation and/or apoptosis.

**Interaction between RDEA119 and the MEK enzyme: X-ray cocrystal structure.** RDEA119 potently inhibited MEK activity in enzyme inhibition assays in a non–ATP-competitive manner (MEK1 IC₅₀ = 19 nmol/L, MEK2 IC₅₀ = 47 nmol/L) determined through incorporation of radioactive phosphate from ATP into ERK as substrate.

To determine the mechanism of inhibition of RDEA119, we cocrystallized RDEA119 with a truncated version of MEK1 that was amenable to crystallization (10). The RDEA119-MEK complex structure reveals that RDEA119 binds to an allosteric site adjacent to the Mg-ATP binding region and interacts extensively with ATP, the activation loop, and other surrounding protein residues through hydrogen bonding and hydrophobic interactions (Fig. 1B and C). This interaction site is similar to the one identified for the MEK inhibitor PD-318088 (PDB ID: 1ISJ; ref. 10). Notably, the sulfonamide moiety hydrogen bonds with the basic side chain of the Lys⁹⁷, a conserved residue believed to be important for the catalytic activity of protein kinases. The iodine is in electrostatic interaction with backbone C = O of Val¹²⁷, whereas one of the two fluorines is involved in hydrogen bonding with −NH of Ser¹¹². This inhibitor also forms several contacts to Asp²⁰⁸, Phe²⁰⁹, and Gly²¹⁰, also known as the DFG motif that is shared across several families of protein kinases. The diol extension of RDEA119 interacts heavily with oxygen atoms of the α and γ phosphate groups of the ATP cofactor, whereas the cyclopropyl of RDEA119 forms contacts with the side chain of Met²³⁹. RDEA119 is also engaged in hydrophobic contacts with side chains of residues Ile⁹⁷, Leu¹¹², Leu¹¹⁸, Phe¹²³⁹, Ile¹³⁵, Met¹⁴⁶, Asp¹⁵⁰, Cys²⁰⁷, Asp²⁰⁸, Phe²⁰⁹, Gly²¹⁰, Val²¹¹, Ser²¹², Leu²¹⁵, and Ile²¹⁸. In addition, unlike PD325901, RDEA119 has a unique cyclopropyl group that is in direct hydrophobic contacts with the side chain of Met²³⁹ in the A-loop, further stabilizing it in the closed “active” conformation of the enzyme. Such a binding mode thus suggests a noncompetitive mechanism of inhibition of RDEA119 against MEK1, which allows ATP binding but
precludes binding to the substrate ERK, thus preventing ERK phosphorylation (10).

RDEA119 significantly inhibits only MEK1/2 relative to 205 other kinases in a multikinase screen (data not shown). MEK1 and MEK2 were inhibited 97% and 99%, respectively, when tested at 10 μmol/L. Four other kinases (BRAF, VAF599E, COT, and RAF1) showed inhibition of >90%; however, these used MEK1 in a cascade assay format and the inhibition in these assays is due to MEK1 inhibition rather than direct inhibition of the other kinases by RDEA119. The kinase-specific assay conditions use a cascade format that has RAF1/MEK/ERK all together in the assay, which phosphorylate an ERK substrate. Therefore, a MEK inhibitor will be flagged as a RAF1 inhibitor in this assay due to reduction of the ERK-specific peptide.

Cellular MEK potency. RDEA119 potently inhibited MEK activity as measured by phosphorylation of ERK1/2 across several human cancer cell lines of different tissue origins and BRAF mutational status with EC_{50} values ranging from 2.5 to 15.8 nmol/L (Table 1A). RDEA119 was significantly bound by human serum albumin (hSA) in these assays as shown by 10- to 30-fold loss of potency in the presence of 1% FBS + 45 mg/mL hSA compared with 1% FBS alone (Table 1A). hSA (45 mg/mL) is the midpoint of the normal serum albumin range in human serum (35-55 mg/mL). Mouse and human plasma protein binding of RDEA119 is 99.6% and 99.8%, respectively (data not shown). These results speak to the availability of the compound in plasma and may affect the activity of a compound in vivo; thus, potency in a high protein environment is reduced, but this effect is likely small in humans. The species binding data points towards a smaller free fraction of compound in mouse models, which in turn may underestimate the potency of RDEA119 in man.

Growth inhibition potency and effect of BRAF status. RDEA119 inhibited anchorage-dependent growth of human cancer cell lines harboring the gain-of-function V600E BRAF mutant with EC_{50} values ranging from 67 to 89 nmol/L (Table 1A). In contrast, RDEA119 had significantly less growth-inhibitory potency against cell lines with wild-type BRAF (A431 cells) or MDA-MB-231 cells harboring a BRAF mutation G46V that shows minimal (<2-fold increase) enhancement of inherent kinase activity (Table 1A; ref. 16). Interestingly, under anchorage-independent conditions, EC_{50} values for all cell lines tested were similar (40-84 nmol/L; Table 1A). MDA-MB-231 and A431 cells were significantly more sensitive to RDEA119 under anchorage-independent conditions, suggesting increased dependence on the MEK pathway. These concentrations of RDEA119 had minimal or no effect on the growth of primary human hepatocytes or human renal proximal tubule epithelial cells (data not shown). Inhibition of A375 cell proliferation by RDEA119 was primarily accounted for by cell cycle arrest rather than apoptosis for two reasons: (a) cellular membrane integrity (assessed by adenylate kinase release) was minimally affected by drug treatment (data not shown) and (b) flow cytometry revealed a G_1-phase cell cycle arrest with little if any sub-G_1 population (Supplementary Data).

Antitumor efficacy of RDEA119 in multiple human tumor xenograft models. The human melanoma A375 tumor xenograft was found to be sensitive to RDEA119 treatment (Table 1B; Fig. 2A) with 54% and 68% tumor growth inhibition (TGI) seen with 25 and 50 mg/kg/d administered orally on a once daily × 14 schedule. Significant tumor growth delay (TGD) and regressions were also observed in A375 tumors on this once-daily schedule. For example, five to eight complete or partial responses (CR/PR) and up to six tumor-free survivors (TFS) were observed (Table 1B). We also observed a schedule-dependent antitumor efficacy profile for RDEA119 in A375 tumor xenografts. For example, administering RDEA119 every other day at 100 mg/kg was less effective than daily dosing with either 25 or 50 mg/kg (Table 1B). Interestingly, when RDEA119 was dosed on a twice-daily schedule, it was more effective than once-daily schedules (Table 1B; Fig. 2B). This effect correlates with C_{min} levels as depicted in Fig. 2B. Taken together with the observation that A375 tumors rapidly regrew after the 14-day course of treatment (data not shown), our results suggest that continuous dosing with RDEA119 will be necessary to maximize in vivo antitumor activity.

RDEA119 also showed potent TGI in the human colon carcinoma Colo205 tumor xenograft. Doses of 25 and 50 mg/kg produced marked TGI during drug treatment (e.g., 25 mg/kg TGI = 123%; Table 1B; Colo205 study 1). Note that TGI >100% occurs when the tumor shrinks below its starting volume. Tumor regressions were also noted with seven PRs, two CRs, and two TFS observed within the nine animals treated with 25 mg/kg, and nine PRs seen in the 50 mg/kg treated group. An addition study was performed in Colo205-bearing animals to estimate an ED_{50} value using lower doses of RDEA119 on a once daily × 14 schedule (2.5-25 mg/kg, oral). In this experiment, we again found that RDEA119 produced marked TGI during drug treatment (e.g., 25 mg/kg TGI = 68%; Table 1B, Colo205 study 2). The ED_{50} value for TGI within this study ranged between 2.5 and 10 mg/kg for which 43% to 53% TGI was seen across these treatment arms (Table 1B).

In addition, we found that human A431 cells in which the ERK pathway is constitutively activated due to the overexpression of the EGFR receptor (17) and human colon cancer HT-29 cells that harbor a BRAF V600E mutation also exhibited inhibition of tumor growth in the presence of RDEA119 (Table 1B). A dose of 25 mg/kg once daily × 14 produced 56% and 67% TGI for HT-29 and A431 tumors, respectively. During the 14 days of oral treatment with RDEA119, there was no significant loss in body weights across the 0 to 50 mg/kg treated animals (data not shown).

Pharmacokinetics of RDEA119 in mice. The pharmacokinetics of RDEA119 in mice was assessed over a 24-hour time course following oral exposure to a single dose of 25 mg/kg. RDEA119 was rapidly absorbed (T_{max} = 2 hours) with a total serum C_{max} of 9.85 μg/mL (see Fig. 3A). The C_{min} values at 12 and 24 hours were 1.37 and 0.03 μg/mL, respectively. The t_{1/2} of RDEA119 following oral exposure in the mouse was 2.6 hours and the AUC_{0-24h} was 55 (μg-h/mL). We simulated the pharmacokinetics of the more efficacious twice-daily 12.5 mg/kg oral dose of RDEA119 and project a total serum C_{max} of ~5 μg/mL (see Fig. 3A) with C_{min} values at 12 and 24 hours of 1.2 μg/mL. In comparison, the concentrations of RDEA119 necessary to inhibit (a) cellular pERK in vitro was 1.4 to 9.0 ng/mL (conversion from nmol/L to ng/mL = 0.572) or (b) anchorage-independent cell proliferation in vitro was 23 to 48 ng/mL across the cancer cell lines tested in 10% FBS (see Table 1A and B).

Pharmacokinetics/pharmacodynamics of RDEA119. The levels of pERK, a biomarker of MEK activity, were monitored over a 24-hour period in tumors, lung, and brain after a single dose of RDEA119 or another MEK inhibitor PD325901 at multiple dose levels. RDEA119 strongly suppressed pERK levels in Colo205 tumors for up to 12 hours compared with the vehicle-treated group (Fig. 3B). The relationship between MEK inhibition and plasma levels of RDEA119 is depicted in Fig. 4B. An EC_{50} value of 42 ng/mL (73 nmol/L) for MEK inhibition in tumors was obtained.
Table 1. RDEA119 efficacy in cancer cell lines and tumor xenograft models

A. In vitro activity of RDEA119 in human tumor cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tumor type</th>
<th>BRAF status</th>
<th>EC50 ± SD (nmol/L)</th>
<th>GI50 ± SD (nmol/L)*</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1% FBS +45 mg/mL hSA</td>
<td>Anchorage-dependent</td>
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<td>V600E</td>
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B. Response summary of RDEA119 in xenograft models

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<th>T-C(^\d)</th>
<th>% TGD(^\d)</th>
<th>P(^\d) Regression</th>
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<td>6.6</td>
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NOTE: Multiple xenograft models were tested with RDEA119, including A375 melanoma, Colo205 colon cancer, A431 epithelial human squamous carcinoma, and HT-29 colon adenocarcinoma.

Abbreviations: ND, not determined; qd, once daily; bd, twice daily; qod, every 2 days.

\(^\d\) GI50 = concentration resulting in 50% of the maximal growth inhibition induced by 1 μmol/L RDEA119.

\(^\d\) Anchorage-dependent growth was assessed after 48-h exposure to RDEA119.

\(^\d\) Anchorage-independent growth was assessed after 7-d exposure to RDEA119 in 0.15% agarose.

\(n\) = number of animals at the end of 14-d dosing period.

\(\% TGI = 100 \times [1 - (\text{RDEA119-treated tumor volume}_{\text{final}} - \text{tumor volume}_{\text{initial}}) / \text{vehicle-treated tumor volume}_{\text{initial}} - \text{tumor volume}_{\text{initial}}] \)

\(\% TGD** = \% TGI / \% TGI_{\text{vehicle}}\)

\(\text{P}^{\dagger} \) = regression analysis

\(\text{PR}^{\dagger\dagger} \) = percent reduction

\(\text{CR}^{\dagger\dagger} \) = complete response

\(\text{TFS}^{\dagger\ddagger} \) = time to first success

in this study. In brain samples, MEK activity was not sufficiently inhibited by RDEA119 to calculate an EC50 value but would likely require >3,000 ng/mL in plasma to achieve 50% inhibition of MEK activity in brain (Fig. 3C). Interestingly, other MEK inhibitors block pERK equally well in brain and in lung. Figure 4A to D shows a comparison of pERK inhibition by RDEA119 and PD325901 at 10 mg/kg in the three different tissues in nude mice. In all three tissues, the PD325901 compound blocks phosphorylation of ERK.
similarly. In contrast, RDEA119 exhibits tissue selectivity, showing little or no inhibition in brain, significantly lower effect in lung, and sustained efficacy in tumor tissue. The levels of RDEA119 in the lung are similar to the concentration in plasma, whereas the levels in the brain are much lower than in the plasma, suggesting that RDEA119 has low central nervous system (CNS) penetration. In contrast, concentrations of PD325901 are higher in the brain than in plasma (Fig. 4).

Rat tissue samples were evaluated for pERK inhibition 48 hours after oral dosing with RDEA119 and PD325901. MEK activity in the brain was inhibited by PD325901 in a dose-dependent manner. No significant inhibition of MEK was shown for RDEA119 at any dose tested. Plasma concentrations were correlated with MEK inhibition in brain homogenates. Figure 4 shows dose-dependent inhibition of brain pERK levels only for PD325901, which had an EC_{50} value of 641 ng/mL. There was very little pERK inhibition in the brain for RDEA119 (Fig. 4A–B).

We next compared the efficacy of the PD and RDEA compounds in two different xenograft models (Table 2). These were a colon cancer line, Colo205, and a melanoma line, A375. These were tested in a standard xenograft model with oral dosing for both compounds for 14 days. Tumor volumes were measured along with tumor-free survival. The data are shown in tabular form for both experiments. In both colon and melanoma models, the efficacy and potency for each compound were similar and not significantly different. Both compounds significantly suppressed tumor volume in treated mice. The MEK inhibitors showed a higher number of tumor-free survivors in both models compared with the reference treatment.

**Discussion**

The attractiveness of MEK as a pharmaceutical target has been enhanced by the discovery of highly selective non–ATP-competitive inhibitors of MEK1/2 (18–21). Such selectivity is achieved by the interaction of the inhibitor with a hydrophobic pocket in MEK that interferes with subsequent binding and hydrolysis of ATP (10). Through cocrystallization of RDEA119 with human MEK1, we have found that RDEA119 binds in this MEK hydrophobic pocket and blocks MEK enzymatic activity (Fig. 1). First-generation MEK inhibitors (e.g., CI-1040) had poor exposure in patients, preventing sustained MEK inhibition in tumor tissue...
Second-generation inhibitors (e.g., PD325901), although significantly more potent and more water soluble, have been hampered by side effect issues, including ocular and neurologic toxicities, presumably due to accumulation of such agents in the brain and other neural tissue (25). Thus, significant interest has arisen in the field for the discovery and development of potent and selective MEK inhibitors that lack CNS retention. As such, we focused our efforts on designing a MEK inhibitor that retains high potency and selectivity to MEK over other kinases and good pharmaceutical properties (solubility, oral bioavailability, etc.) while avoiding CNS retention compared with first-generation agents such as PD325901.

The data described in this article show that RDEA119 is a highly potent and selective inhibitor of MEK1/2 activity through its interaction with MEK. X-ray crystallographic analysis reveals that RDEA119 binds to an allosteric pocket adjacent to the ATP binding site, locking the enzyme in a catalytically inactive conformation. When bound to MEK, RDEA119 interacts directly with ATP, the activation loop, and other surrounding protein residues through both hydrogen bonding and hydrophobic interactions, resulting in significant affinity for the binding pocket. RDEA119 had preferential growth-inhibitory activity in cancer cells harboring the \( \text{BRAF} \) gain-of-function mutation, V600E, consistent with the activities of other MEK inhibitors.
However, this differential sensitivity was only seen in our hands under anchorage-dependent (cell growth on plastic) conditions. Anchorage-independent growth in a semisolid medium showed no clear sensitivity differences in the cancer cell lines tested. Further studies will be necessary to explain the mechanistic basis for this differential sensitivity. However, it seems that growth on plates reduces or bypasses the need for MEK activity for cell proliferation in some cell lines.

The antitumor activity of RDEA119 was shown across a variety of human tumor xenografts, including the human melanoma A375 tumor and the human colon cancer xenograft Colo205. Both tumors express activated \textit{BRAF} mutations (see Table 1A). We also

### Table 2. RDEA119 and PD325901 efficacy in Colo205 and A375 tumor xenograft models

<table>
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<th>Group</th>
<th>n</th>
<th>Colo205 xenograft</th>
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<th>T-C</th>
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<th>No. TFS</th>
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<td>27%</td>
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<td>18</td>
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Abbreviation: TTE, time to end point.
observed dosing schedule dependency in the degree of antitumor activity observed in vivo (see Table 1B and Fig. 2), with twice-daily dosing being more effective than less frequent dosing. Pharmacokinetic analysis of RDEA119 concentrations in the plasma of mice was assessed from a single dose of RDEA119 (see Fig. 3A). The twice-daily and every other day pharmacokinetics of RDEA119 were then modeled from this actual data set. Superimposition of these pharmacokinetic profiles illustrated that the degree of antitumor activity seen with RDEA119 in these human tumor xenografts is more likely to correlate with maintaining plasma levels of RDEA119 above 1,240 ng/mL (from xenografts is more likely to correlate with maintaining plasma antitumor activity seen with RDEA119 in these human tumor xenografts) for 24 hours rather than high peak levels (C_{max}). This is consistent with findings that once RDEA119 dosing stopped, tumors rapidly regrew in mice harboring residual tumor mass. These data suggest that continuously inhibiting MEK activity in a patient’s tumor throughout the period between doses will be important to achieve and sustain antitumor efficacy.

To assess the potential for brain penetration and activity, we compared the ability of RDEA119 to inhibit pERK levels in the brain, lung, and tumor tissues of tumor-bearing mice and found that at least 76-fold lower plasma levels of RDEA119 were required to inhibit 50% of the pERK level in tumor versus brain. Testing of other MEK inhibitors, including PD325901, revealed little or no difference in brain, tumor, and lung penetration versus plasma for these other compounds (Figs. 3B and C and 4A–D). We have tested mouse MEK in vitro and in vivo and see no difference in affinity or inhibitory activity between the species (data not shown). These experiments suggest that RDEA119 exhibits reduced potential for brain-related side effects and may preferentially accumulate in tumor tissue. To ensure that comparable pERK suppression in tumors correlated with efficacy in vivo, we tested both compounds side by side in two 14-day xenograft models. As shown in Table 2, each compound was equally efficacious at blockade of tumor growth at both 25 and 50 mg/kg. These data are consistent with the pERK suppression detected in tumors from the 1-day dose experiment for both compounds (Fig. 4) and is also consistent with the comparable affinities for the MEK enzyme for these two compounds. The differences noted for these molecules give further weight to the notion that alterations in chemical structure with similar effects on affinity can differentially affect activity in vivo.

In summary, RDEA119 is a highly potent and selective inhibitor of MEK1/2 whose encouraging preclinical pharmacology profile supported entry into development for the treatment of cancer. RDEA119 is currently undergoing phase I clinical trials in late-stage cancer patients refractory or intolerant to other anticancer therapies.

Disclosure of Potential Conflicts of Interest
All authors were employees of sponsoring company.

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References
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