

RDEA436, a Novel MEK Inhibitor with Favorable Pharmacokinetic Properties

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Abstract # 4895

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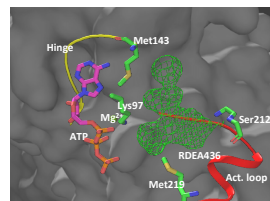
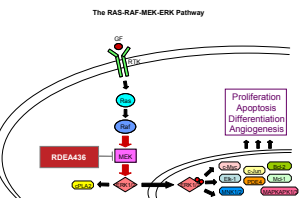
Abstract

Introduction: The RAS-RAF-MEK-ERK pathway has emerged as a significant focus for molecularly targeted cancer therapy. Several MEK inhibitors are being developed for the treatment of human cancers driven by activation of this pathway, including RDEA119. We have identified another novel series of potent MEK inhibitors that differ in pharmacological properties from MEK inhibitors currently in the clinic. RDEA436 has been selected from this series for clinical development.

Methods/Results: A radiometric enzyme assay that measured RDEA436 inhibition of MEK phosphorylation of ERK resulted in IC_{50} values of 11 nM for MEK1 and 23 nM for MEK2. X-ray crystallography of the complex of MEK1-ATP-Mg-RDEA436 showed binding of RDEA436 to an allosteric pocket in MEK. Of 210 kinases tested, only MEK1 & 2 were significantly inhibited at 10 μ M. ELISA analysis of cellular phospho-ERK levels was used to determine MEK inhibition by RDEA436 in A375 human melanoma cells in the presence of human, monkey, bovine, mouse and rat serum. RDEA436 inhibited MEK activity with EC_{50} values ranging from 2.7-4.8 nM in the presence of 1% serum. Increasing the serum concentration to 50% shifted the EC_{50} values to 28-121 nM. These shifts in EC_{50} values were also noted with PD-325901 and ARRY-142886 although the magnitude of the shifts varied with the species of serum. In 50% mouse serum PD-325901 was the most potent MEK inhibitor with EC_{50} of 30 nM while RDEA436 and ARRY-142886 had EC_{50} values of 57 and 654 nM, respectively. However, in 50% human serum RDEA436 was the most potent MEK inhibitor with an EC_{50} of 57 nM while PD-325901 and ARRY-142886 had EC_{50} values of 189 and 422 nM, respectively. These shifts in EC_{50} values in the presence of serum or serum proteins and the relative ranking of potencies depending on serum source were also observed in other tumor cell lines. In human cell lines A375 (melanoma), SK-Mel-28 (melanoma), Colo205 (colon cancer), and HT29 (colon cancer), RDEA436 inhibited anchorage-dependent proliferation with mean GI_{50} values of 8, 13, 12, and 9 nM, respectively. RDEA436 also inhibited anchorage-independent growth with GI_{50} values of 2 nM in A375 and Colo205 cells. Flow cytometry revealed a G1/S phase arrest in RDEA436-treated A375 cells, and at concentrations up to 100 μ M, RDEA436 showed negligible cytotoxicity in primary hepatocytes. PK analysis in mice and rats showed oral bioavailability for RDEA436 of 85% and 29%, respectively. A 5 mg/kg oral dose of RDEA436 in rats resulted in a plasma AUC of 8.27 hr \cdot μ g/mL while the AUC levels in the brain were 1.05 hr \cdot μ g/mL.

Conclusion: RDEA436 is a novel MEK inhibitor with favorable pharmacological and pharmacokinetic and a low potential for CNS penetration. RDEA436 is being further evaluated in a pharmacodynamic/pharmacokinetic study in tumor bearing mice and in a micro-dose study in humans.

Introduction



Defects in the RAS/RAF/MEK/ERK signaling pathway (above left) are closely associated with the development of human tumors, such as melanoma, colon, lung and thyroid cancers. RDEA436 is a potent, non-ATP competitive, highly-selective inhibitor of mitogen-activated ERK kinase (MEK) that is currently being developed for oncology and inflammatory diseases. Shown above on the right is a model of RDEA436 in the allosteric pocket of MEK1. The electron density of RDEA436 was calculated using PyMol after docking into MEK1 (PDB 1S9F) using Glide (Schrodinger). Our preclinical data, presented here, show that RDEA436 is a potent in vitro and in vivo inhibitor of MEK, has favorable PK properties with low CNS penetration, and had a long half-life in a human micro-dose study indicating the potential for once daily dosing in humans.

Results

Table 1. RDEA436 is a Potent Non-ATP Competitive Inhibitor of MEK1 and MEK2

RDEA436	MEK1 $IC_{50} \pm$ std. dev. (nM)	MEK2 $IC_{50} \pm$ std. dev. (nM)
RDEA436	11 \pm 4	23 \pm 1
RDEA119	21 \pm 1	50 \pm 7
PD-325901	17 \pm 4	69 \pm 9
ARRY-142886	282 \pm 56	Not determined

Methods: MEK1 (Invitrogen) and MEK2 (Upstate Biotechnology) activity was measured by incorporation of radioactivity from [γ -³²P]ATP into kinase inactive ERK2. Nonlinear regression analysis was performed using GraphPad to determine IC_{50} values for MEK inhibitors. Values shown are an average of 2 independent experiments.

- RDEA436 at 10 μ M inhibited only MEK1 and MEK2 out of 210 kinases
- RDEA436 is a highly potent and selective inhibitor of MEK1/2

Table 2. RDEA436 Inhibits Cellular MEK Activity in Tumor Cell Lines

Cell Line	Tumor Type	BRAF status	$EC_{50} \pm$ std. dev. (nM)
A375	Melanoma	V600E	3.5 \pm 0.3
SK-Mel-28	Melanoma	V600E	4.0 \pm 0.4
Colo205	Colon	V600E	1.2 \pm 0.3
HT29	Colon	V600E	3.5 \pm 0.01
A431	Epidemioid	wt	4.3 \pm 0.3
Bx-PC3	Pancreatic	wt	9.8 \pm 1.8

Methods: Cells in 96-well plates were treated with serial dilutions of RDEA436 in media containing 1% FBS. After 20 min incubation, pERK levels were determined with a pERK1/2 ELISA kit (Bioss USA).

Table 3. RDEA436 Exhibits Higher Potency in Human Serum than other MEK Inhibitors

Serum (%)	MEK Inhibitor EC_{50} Values (nM)				
	RDEA436	RDEA119	PD-325901	ARRY-142886	
Human	1	2.7	4.0	3.2	12.1
	10	20.0	32.0	29.0	79.0
50	57.0	111.0	194.0	439.0	
Bovine	1	3.8	12.8	2.5	13.2
	10	10.0	156.0	16.0	76.0
50	17.0	60.0	13.0	168.0	
Mouse	1	4.3	9.7	1.4	31.4
	10	17.0	60.0	13.0	168.0
50	50.0	224.0	29.0	678.0	

Methods: EC_{50} values were determined in A375 cells as above except that 1% FBS was replaced with the indicated amounts of serum.

- MEK inhibition is highly dependent upon protein binding across species
- RDEA436 and RDEA119 are more potent than PD-325901 and ARRY-142886 in the presence of 50% human serum; PD-325901 is the most potent in the presence of 50% mouse serum
- Similar activity in mouse and human serum with RDEA436 should allow for better extrapolation between xenograft and human doses

Table 4. RDEA436 is a Potent Inhibitor of Cancer Cell Growth (GI_{50})

Tumor Cell Line	GI_{50} (nM)	
	Anchorage-dependent	Anchorage-independent
A375	7.9 \pm 1.2	2.2 \pm 0.7
SK-Mel-28	13.1 \pm 0.9	Not determined
Colo205	11.8 \pm 2.1	1.8 \pm 0.5
HT29	8.7 \pm 0.1	Not determined

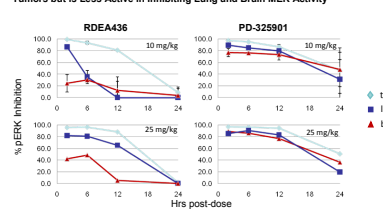
Methods: Anchorage-dependent growth inhibition was measured using CellTiterGlo reagent after 72 hr treatment with RDEA436 of cells grown in 96-well plates. Anchorage-independent growth assays used MTS reagent after 7 days treatment with RDEA436 of cells grown in media containing 0.15% agarose. Media contained 10% FBS.

Table 5. Comparison of MEK Inhibitor Activities on Growth Inhibition of A375 Cells

MEK Inhibitor	GI_{50} (nM)	
	Anchorage-dependent	Anchorage-independent
RDEA436	7.9 \pm 1.2	2.2 \pm 0.7
PD-325901	7.5 \pm 1.8	2.2 \pm 0.4
ARRY-142886	99 \pm 5.3	23.4 \pm 3.9

Methods: Anchorage-dependent and independent growth inhibition was measured as described above.

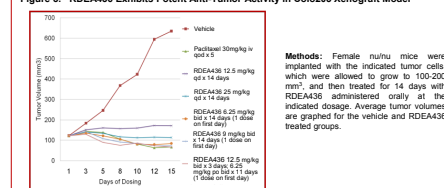
Figure 2. RDEA436 is a Potent Inhibitor of MEK in A375 Mouse Xenograft Tumors but is Less Active in Inhibiting Lung and Brain MEK Activity



Methods: Pharmacodynamic Study of RDEA436 in A375 Xenografts. Female nu/nu mice implanted with A375 tumors were given a single dose of vehicle, RDEA436 (left panels) or PD-325901 (right panels) at 5, 10, or 25 mg/kg. pERK levels were determined in tumor, lung, and brain samples collected at 2, 6, 12, and 24 hr post-dose. Tumor, lung, and brain samples were homogenized and analyzed by western blot using antibodies to either pERK1/2 or total ERK1/2. Secondary antibodies were tagged with IR dyes, and imaged using the Li-COR Odyssey Infrared Scanner. Average pERK inhibition values were determined by comparison to vehicle treated samples and are shown for the 10 and 25 mg/kg dose groups.

- RDEA436 has favorable brain/plasma profile compared to PD-325901

Figure 3. RDEA436 Exhibits Potent Anti-Tumor Activity in Colo205 Xenograft Model



Methods: Female nu/nu mice were implanted with the indicated tumor cells, which were allowed to grow to 100-200 mm³, and then treated for 14 days with RDEA436 administered orally at the indicated dosage. Average tumor volumes are graphed for the vehicle and RDEA436 treated groups.

Table 6. RDEA436 Plasma Concentrations Producing Anti-Tumor Activity in Colo205 Model

Dose (mg/kg)	C_{max} (μ g/mL)	C_{12h} (ng/mL)	AUC_{0-12h} (hr \cdot ng/mL)
6.25	2.45	6.7	5.69
12.5	4.99	13.3	11.4

Figure 4. Plasma Concentrations following a Single Oral 10 μ g Dose of RDEA436 in Healthy Subjects

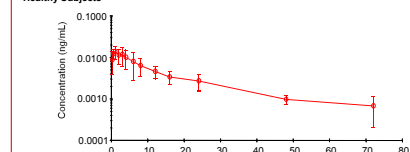


Table 7. RDEA436 has a Long Half-Life in Humans and has the Potential for Once a Day Dosing

	T_{max} (hr)	C_{max} (ng/mL)	$T_{1/2}$ (hr)	C_{12h} (ng/mL)	AUC_{0-12h} (hr \cdot ng/mL)	AUC_{0-24h} (hr \cdot ng/mL)	$t_{1/2}$ (hr)
N	4	4	4	4	4	4	4
Mean	0.813	0.0159	72.0	0.006978	0.204	0.227	19.7
CV%	46.2	18.0	0.001	69.0	33.1	27.5	45.7

- Based on the results from the Colo205 xenograft study and the human micro-dose study, the target dose in patients is approximately 20-30 mg/day

Conclusions

- RDEA436 is a novel, potent, broadly active MEK inhibitor, with a low EC_{50} in human serum.
- RDEA436 has lower potential for CNS toxicity than PD-325901.
- Results from a human micro-dosing experiment indicates that RDEA436 has a ~20 hour half-life and favorable pharmacokinetic properties.
- Modeling of the human data provides an estimate of 20-30 mg/day to produce anti-tumor activity.
- Based on its pharmacological and pharmacokinetic profile, RDEA436 has been designated a clinical development candidate and should enter Phase 1 in 2H2008.