

# RDEA119: A Potent and Highly Selective MEK Inhibitor for the Treatment of Cancer

Poster # 577

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## Abstract

### Introduction:

RDEA119, a novel, highly selective MEK1/2 inhibitor currently in clinical trials for the treatment of cancer, is capable of inhibition of MEK1/2 at nanomolar concentrations.

This molecule exhibits superb pharmacokinetic properties in man consistent with once per day dosing while maintaining constant drug levels in the pharmacologic range as determined by measurement of pharmacodynamic markers in treated patients.

### Results and Methods:

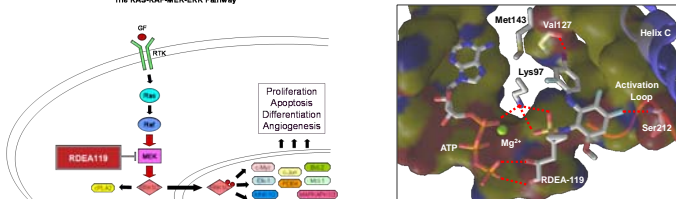
The compound exhibits activity in both subcutaneous (melanoma, colon) as well as orthotopic xenograft models including orthotopic hepatoma and orthotopic colon cancer. For these models, the caecum wall or liver of female BALB/c nu/nu mice was inoculated with human HT 29 colorectal adenocarcinoma cells or Hep3B2.1-7 tumor cells, respectively. Beginning 20 days post-inoculation, 21 days of oral dosing with RDEA119 was initiated and tumor number and weights were assessed. Because RDEA119 interacts solely with MEK1/2, as determined by SelectScreen kinase Profiling (Invitrogen) against 205 other kinase targets (>100 fold selectivity), this indicates that these tumors exhibit growth dependence on the MEK pathway. We noted that after withdrawal of compound, certain tumors resumed growth in some of these xenograft models. We therefore tested whether RDEA119 induces a cytostatic response or a cell death response. A375 melanoma cells were treated for 24 hr with RDEA119, washed, permeabilized and stained with propidium iodide and analyzed for cell cycle status. RDEA119 inhibited A375 cell proliferation by inducing cell cycle arrest rather than apoptosis as demonstrated by measuring both cellular membrane integrity (adenylate kinase release) and cell cycle analysis showing a G1 phase cell cycle arrest. We examined the ability of RDEA119 to synergize with multiple anti-tumor agents in vitro and measured cell death response in both BRAF wildtype and mutant cell lines. Significant synergy was observed with several combinations, the magnitude of synergy ranged from 5-80 fold.

### Conclusions:

Thus, RDEA119 represents a new potential weapon for use as both single agent in selected cancers and in combination with other active agents in a broader array of cancers.

## Introduction

### The RAS-RAF-MEK-ERK Pathway

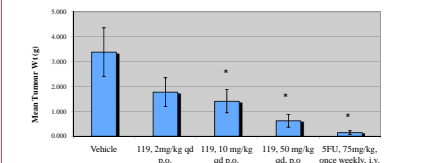
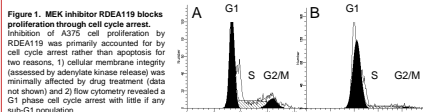


Defects in the RAS/RAF/MEK/ERK signaling pathway (above left) are closely associated with the development of human tumors, such as melanoma, colon, lung and thyroid cancers. RDEA119, the lead compound from Ardea's MEK inhibitor program, is a potent, non-ATP competitive, highly-selective inhibitor of mitogen-activated ERK kinase (MEK) that is currently in Phase 1 clinical development. The model shown above right was obtained by docking RDEA119 using Glide in the Schrödinger package into the co-ordinates of MEK1 (PDB:1SSJ). We are pursuing RDEA119 for the treatment of cancer and inflammatory diseases for which MEK has been shown to play a role.

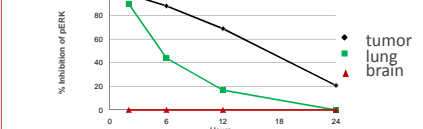
## Results

Cell Line	Tumor Type	BRAF Status	EC <sub>50</sub> ± sd (nM)		GI <sub>50</sub> ± sd (nM)	
			1% FBS	Dependent	Dependent	Independent
A375	Melanoma	V600E	6.7 ± 0.7	67 ± 12	65 ± 17	

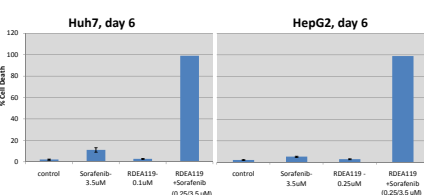
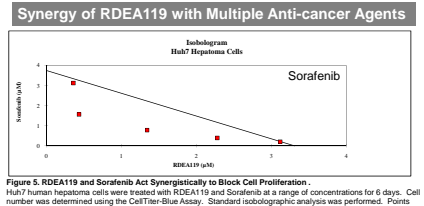
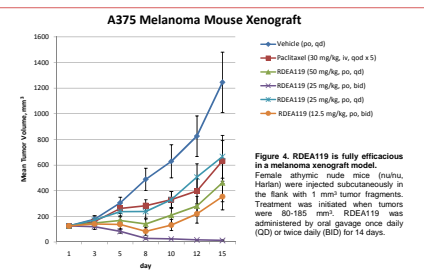
**Table 1. RDEA119 suppresses both anchorage dependent and independent growth of Melanoma.**  
 A375 cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS). Compounds were added to PBS-washed cells. Incubated at 37°C for 20 min in 1% FBS, washed with PBS. MEK activity was assayed by measuring the phosphorylation of ERK1 and ERK2 using a phospho-ERK1/2 ELISA kit (BioSource, Camarillo, CA). For anchorage-dependent growth inhibition experiments, cells were incubated for 48 hr at 37°C and ATP levels were determined using CellTiter Glo (Promega, Madison, WI). For the anchorage-independent growth assay, cells were inoculated in a 0.15% agarose solution in complete RPMI. After 7 days, MTS reagent (CellTiter 96 Aqueous, Promega) was used to measure growth.



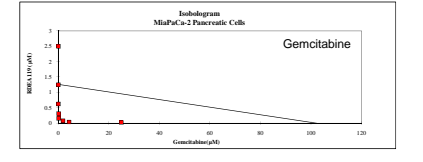
**Figure 2. RDEA119 is efficacious in an orthotopic model of hepatoma.**  
 BALB/c nu/nu mice were inoculated with Hep3B cells in the liver. Treatment started 14 days after inoculation, and animals were treated for 18 days, following which tumors were excised, cleaned, and weighed. \*p < 0.05. Vehicle vs treatment, using the Tukey-Sidak Method.



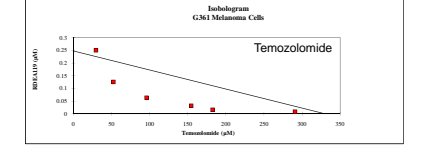
**Figure 3. RDEA119 concentrations are higher in tumors than in non-target tissues.**  
 Mice with Colo205 tumors were dosed by oral gavage with RDEA119 at 10 mg/kg. Tumor, lung and brain sample homogenates were lysed on ice for 1 hr and centrifuged, subjected to western blot analysis and probed with a mixture of Phospho-p44/42 MAP Kinase antibody and p44/42 MAP Kinase Antibody (Cell Signaling, Danvers, MA).



**Figure 6. RDEA119 and Sorafenib act synergistically to induce cell death of hepatoma cell lines.**  
 After 6 days of treatment, cell death was determined by trypan blue staining followed by counting using light microscopy. Five hundred cells from randomly chosen fields were counted, and the number of blue-staining dead cells were expressed as a percentage of the total number of cells counted.



**Figure 7. RDEA119 and Gemcitabine Strongly Synergize to Block Pancreatic Cancer Cell Proliferation.**  
 MiaPaCa-2 pancreatic cancer cells were treated with RDEA119 and Gemcitabine for 3 days. Cell number was determined using the CellTiter-Blue Assay. Standard isobolographic analysis was performed. Points below the isobolic line (Fw=50) indicate a synergistic interaction.



**Figure 8. RDEA119 and Temozolomide Act Synergistically to Block Cell Proliferation.**  
 G361 melanoma cells were treated with RDEA119 and Temozolomide for 3 days. Cell number was determined using the CellTiter-Blue Assay. Standard isobolographic analysis was performed. Points below the isobolic line (Fw=50) indicate a synergistic interaction.

## Conclusions

- RDEA119 is a selective, allosteric inhibitor of MEK 1/2 that acts to inhibit cell proliferation by blocking the progression after the G1 phase of the cell cycle.
- RDEA119 produces robust anti-tumor activity in a variety of tumor cell lines and xenograft models including orthotopic models of hepatoma.
- RDEA119 exhibits substantial synergistic activity with several anti-cancer agents, which may enable either lower doses or better efficacy. Combination clinical trials are being initiated to explore this option.