RDEA119, a Potent and Highly Specific MEK Inhibitor is Efficacious in Mouse Tumor Xenograft Studies

Abstract # 4878

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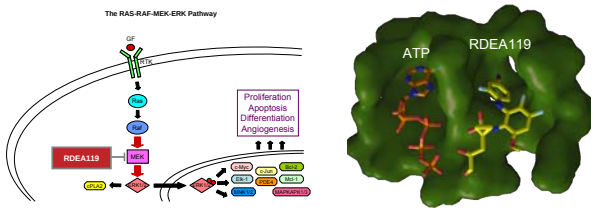
Abstract

Introduction: The RAS-RAF-MEK-ERK pathway has emerged as a significant focus for molecularly targeted oncology therapeutics. MEK inhibitors may have broad utility in the treatment of human cancers driven by activation of this pathway due to the selective phosphorylation of ERK by MEK and the highly selective inhibition of MEK displayed by this class of inhibitors. REA119 has been selected for clinical development because of its potency and favorable PK. We report here the pharmacological and pharmacokinetic properties of RDEA119 in a number of mouse tumor xenograft studies.

Methods/Results: Human tumors were implanted in nu/nu mice. RDEA119 was administered orally for 14 days once tumors were approximately 100 mm^3 in size. Tumor growth inhibition (TGI) was determined after 14 days of treatment as the reduction in the size of tumors in treated groups versus vehicle controls. The time to endpoint (TTE) was calculated as the time for the tumor to reach the specified endpoint volume or the last day of the study, whichever came first. Treatment outcome was determined from percent tumor growth delay (%TGD), defined as the percent increase in median TTE of treated versus vehicle-treated control mice. Animals were also monitored for regression responses. Levels of pERK in tumors and brain were determined by Western blots and correlated with plasma levels of RDEA119 for the pharmacodynamic/pharmacokinetic study. A number of tumor models were evaluated with different doses and dosing regimens of RDEA119. Treatment with 25 or 50 mg/kg QD of RDEA119 showed statistically significant %TGD in A375 melanoma tumors, Colo205 colon cancer tumors, and A431 epidermoid tumors. Statistically significant TGI was observed for RDEA119 dosed orally at 25 mg/kg QD for these tumor models as well as in HT29 colon cancer tumors. The effect of different dosing regimens was evaluated in A375 xenografts. Although 100 mg/kg RDEA119 given orally once every two days showed statistically significant %TGD (91%), it was not as effective as QD treatments at 25 mg/kg (143% TGD) or 50 mg/kg RDEA119 (233% TGD). BID dosing was also more effective than QD dosing as measured by %TGI. Dosing of RDEA119 at 12.5 mg/kg BID resulted in 79.5% TGI compared to 51.7% for 25 mg/kg QD of RDEA119. Dosing of RDEA119 at 25 mg/kg BID resulted in 110.1% TGI compared to 69.9% TGI for 50 mg/kg QD of RDEA119. A pharmacodynamic/pharmacokinetic study in Colo205 xenografts showed that RDEA119 inhibited pERK formation in tumors while minimal inhibition was observed in brain suggesting potent anti-tumor activity with limited CNS penetration by RDEA119.

Conclusion: We conclude that maintaining adequate MEK inhibition throughout the dosing interval is more important than peak levels due to the greater efficacy with more frequent dosing. Based on its expected longer half-life in humans than in mice, RDEA119 has the potential for use as a once- or twice-daily oral treatment for cancer.

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. RDEA119, the lead compound from Ardea's MEK Inhibitor Series 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 Schrodinger package into the co-ordinates of MEK1 (PDB 1S9J). Our preclinical data, shown here, suggest that RDEA119 has favorable in vivo properties including oral dosing and low CNS retention. We are pursuing RDEA119 for the treatment of cancer and inflammatory diseases for which MEK has been shown to play a role in the regulation of inflammatory cytokines, such as TNFα. RDEA119 is one of a platform of compounds that we are building for a variety of disease indications including cancer, inflammatory bowel disease (IBD), psoriasis and rheumatoid arthritis (RA).

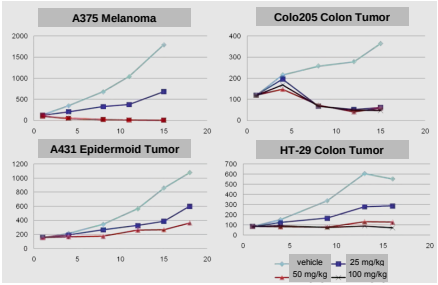
Results

Table 1. RDEA119 is a Potent Inhibitor of Cancer Cell Growth (GI_{50})

Tumor Cell Line	BRAF status	Anchorage-Dependent GI_{50} (nM ± sd)	Anchorage-Independent GI_{50} (nM ± sd)
A375 Melanoma	V600E	67 ± 12	68 ± 34
Colo205 Colon	V600E	74 ± 45	33 ± 16
HT29 Colon	V600E	70 ± 12	Not determined
A431 Epidermoid	Normal	>10,000	65 ± 19

Methods: Anchorage-dependent growth inhibition was measured using CellTiterGlo reagent after 48 hr treatment with RDEA119 of cells grown in 384-well plates. Anchorage-independent growth assays used MTS reagent after 7 days treatment with RDEA119 of cells grown in media containing 0.15% agarose or on non-binding plates (A431).

Figure 1. RDEA119 Exhibits Potent Anti-Tumor Xenograft Efficacy



Methods: Female nu/nu mice were implanted with the indicated tumor cells, which were allowed to grow to 100-200 mm^3, and then treated for 14 days with RDEA119 administered orally once a day. Average tumor volumes are graphed for the vehicle and RDEA119 treated groups.

Table 2. 25 mg/kg QD RDEA119 Exhibits Significant Tumor Growth Inhibition (TGI)

Tumor Xenograft	% TGI*	P value
A375 Melanoma	52-72**	<0.001
Colo205 Colon	70-123**	<0.001
HT29 Colon	56	<0.001
A431 Epidermoid	67	<0.001

Measured at end of once daily dosing for 14 days. %TGI=100(1-(RDEA119 treated tumor volume$_{final}$ – tumor volume$_{initial}$)/(vehicle treated tumor volume$_{final}$ – tumor volume$_{initial}$)]
**Regressions noted during course of experiment

Methods: Tumor Growth Inhibition for the groups treated with 25 mg/kg RDEA119 were calculated for the indicated tumor xenografts. The range for A375 and Colo205 represent values from 2 separate studies.

Table 3. RDEA119 has a Low ED_{50} in Colo205 Xenografts

Group	n	Treatment Regimen: Agent	Treatment Regimen: mg/kg	Initial Tumor Volume (mm^3)	Day 15 Tumor Volume (mm^3)	% TGI	C_{max} (µg/mL)	C_{min} (µg/mL)	AUC (µg-hr/mL)
1	10	Vehicle	-	185±11.1	2093±174	-	-	-	-
2	10	Paclitaxel	30	184±9.8	113±9.6	104*	-	-	-
3	10	RDEA119	2.5	184±9.8	1187±127	47*	0.99	0.003	5.5
4	10		5	183.8±9.8	1175±104	48*	1.97	0.006	11.0
5	10		10	185.1±11.7	1045±160	55*	3.94	0.012	22.0
6	10		25	185.1±11.7	762±81	70*	9.85	0.029	55.0

*P<0.001

Methods: Male nu/nu mice were implanted with Colo205 tumor cells. After 10 days, animals were randomized by tumor size (range 126-256 mm^3) and treated with paclitaxel (IV, QODx5) or vehicle or RDEA119 (PO, QDx14). Pharmacokinetic parameters were obtained from dosing Balb/c mice with 25 mg/kg RDEA119 and extrapolating values for the lower dose groups.

- 50% TGI is between 2.5-10 mg/kg RDEA119
- With a ~2 hour half-life in mice, trough levels with once-daily dosing are low across this dose range and may explain the flat dose-response curve

More Frequent Dosing of RDEA119 in Mice for Optimal Efficacy

Figure 2. Tumor Growth Inhibition with A375 Xenografts

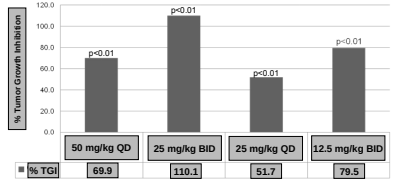


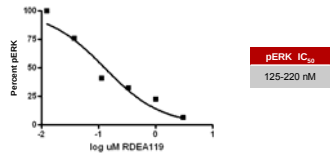
Table 4. Plasma Concentrations in Mice for Doses above

AUC (µg·hr/ml)	132.5	117	66.5	78
Cmax (µg/ml)	23.8	10.2	11.9	7.8
Cmin (µg/ml)	0.06	1.24	0.03	0.49
Cmin Free Fraction (ng/ml)	0.117	2.48	0.059	0.986

Methods: Female nu/nu mice were implanted with A375 tumor cells, which were allowed to grow to 100-200 mm^3, and then treated for 14 days with RDEA119 administered orally once a day (QD) or twice a day (BID). Measured at end of once-daily dosing for 14 days. %TGI=100*(1-(RDEA119 treated tumor volume$_{final}$ – tumor volume$_{initial}$)/(vehicle treated tumor volume$_{final}$ – tumor volume$_{initial}$)] Statistical Significance = Logrank test

- Greatest %TGI was associated with the highest trough concentrations; neither C_{max} nor AUC correlated with efficacy
- Based on the free fraction of RDEA119 observed with optimal doses evaluated in this study, target trough plasma concentrations in humans are 250 – 625 ng/ml

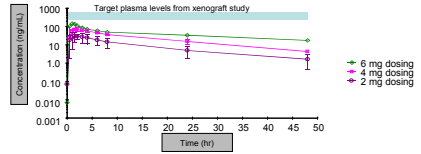
Figure 3. RDEA119 is a Potent Inhibitor of pERK in Human Whole Blood



Methods: Whole heparinized blood was diluted in RPMI media 3x and treated with and without the phorbol ester TPA (Phorbol-12-myristrate-13 acetate) in the presence or absence of the indicated concentrations of RDEA119 for 10 min. at 37ºC. Peripheral blood mononuclear cells were isolated by 2 successive ammonium chloride washes, a 1x wash in PBS and lysed in cell extraction buffer (Invitrogen). pERK and total ERK were measured using a Mesoscale discovery platform, the percent pERK detected is shown in the graph.

- Target plasma levels from xenograft studies are consistent with significant pERK inhibition in humans

Figure 4. RDEA119 Plasma Levels Following Single Oral Doses in Cancer Patients



- The long plasma half-life of RDEA119 in humans (16-26 hours) and low peak-to-trough ratio allows for QD dosing
- Sustained trough levels above 250 ng/ml should be achieved with 20 mg QD
- Plasma levels with target human dose should be well below the highest no-effect levels observed in rat 28-day safety study

Conclusions

- RDEA119 is a potent inhibitor of MEK1/2 that suppresses tumor cell growth in vitro and in vivo.
- BRAF status determines sensitivity to growth inhibition by RDEA119 in anchorage-dependent growth but not anchorage-independent growth or in xenografts.
- Maintaining adequate MEK inhibition throughout the dosing interval appears to be more important than peak levels due to the greater efficacy with more frequent dosing.
- RDEA119 has a favorable pk profile in humans, with the projected therapeutic dose, based on xenograft results, of 20 mg/day in humans.
- These studies support the continued development of RDEA119, currently in Phase 1 clinical trials in cancer patients.