6258: The discovery & preclinical characterization of the potent covalent KRas^{G12C} inhibitor UCT-001024

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BACKGROUND

KRAS is a frequently-mutated oncogenic driver in lung (25%), pancreatic (76%), colorectal (43%), and other tumors. The smoking-associated c.34G>T transversion mutation leading to KRas^{G12C} protein can be targeted directly by inhibitors that covalently react with the sidechain sulfhydryl group of Cys12, and thereby, prevent Ras effector binding and downstream activation of the MAPK pathway. Sotorasib and adagrasib act by this mechanism and recently received accelerated approval as second-line therapies for *KRas^{G12C}* mutated NSCLC having shown 37% (CodeBreaK 100)[1] and 43% (KRYSTAL-1)[2] objective response rates, respectively, in this patient cohort. These promising results have set the stage for combination therapy development and treatment of *KRas^{G12C}* mutated brain metastases and other tumors outside the lung. Here, we present studies characterizing the *in vitro* properties of a novel inhibitor UCT-001024, as well as preclinical modeling of its therapeutic potential, including in a brain-tropic lung cancer metastasis model. UCT-001024 has a long half-life, superior potency, CNS exposure, and excellent efficacy in preclinical models.[3,4]

DESIGN OF RAPID & SELECTIVE KRAS^{G12C} INHIBITORS

A novel inhibitor scaffold was identified by a combined structure- and ligand-based design approach. It was optimized for rapid target engagement kinetics, cellular potency and selectivity, and inertness to glutathione conjugation.

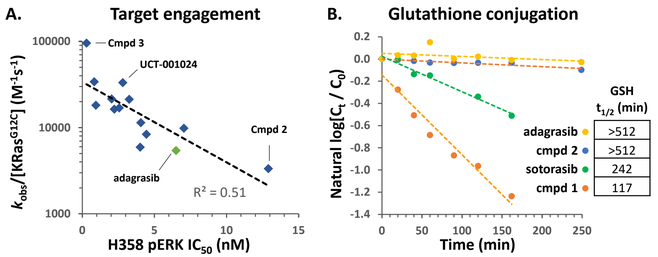


Figure 1. (A) The pseudo-first order rate constant observed *in vitro* is correlated with MAPK pathway inhibition measured in H358 cells by solution ELISA for pERK1/2. **(B)** Medchem optimization led to inhibitors, including compound **2**, which outperform adagrasib at kinetically inactivating KRas^{G12C} yet are unreactive toward glutathione conjugation at 37 °C.

INHIBITORS BIND CYS12 & STABILIZE THE SWITCH II POCKET

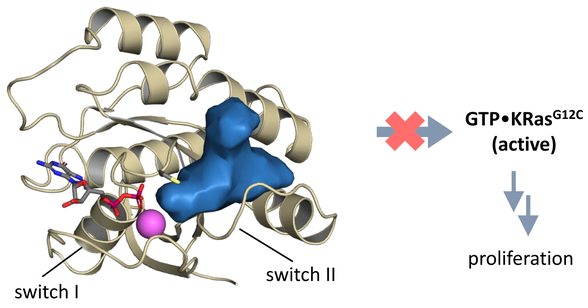


Figure 2. X-Ray co-crystal structure of inhibitor **1** in complex with KRas^{G12C}•GDP solved at 1.77Å. Inhibitor binding prevents GEF catalyzed nucleotide exchange and inhibits proliferation.

UCT-001024 IS EFFICACIOUS IN A H2030-BrM3 (NSCLC) BRAIN-TROPIC METASTASIS MODEL

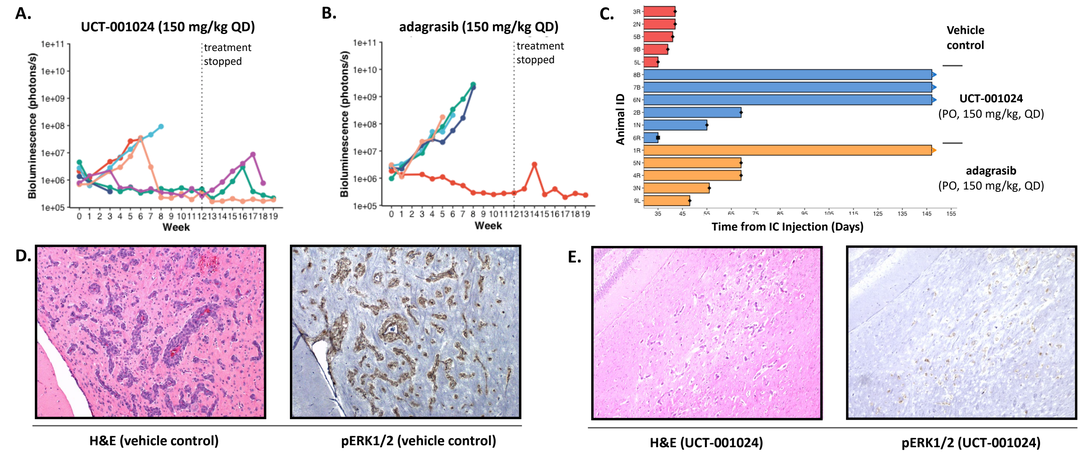


Figure 3. The brain-seeking subclone H2030-BrM3 (KRas^{G12C} + GFP/FLuc) was used as a model for lung cancer-derived brain metastases.[5] CD-1 mice were inoculated by intracardiac injection (500k cells) via the left ventricle and allowed to rest for 12 days before beginning daily treatment with UCT-001024 (150 mg/kg, QD), adagrasib (150 mg/kg, QD), or control vehicle. **(A, B)** Tumor growth was monitored by *in situ* bioluminescent imaging. **(C)** Treatment with UCT-001024 conferred significant survival benefit over the vehicle control group. Brain tissue sections (Day 7) from **(D)** control, and **(E)** UCT-001024 treated animals show reduced tumor burden and decreased MAPK pathway activity as measured by inhibition of ERK1/2 phosphorylation.

UCT-001024 EXERTS STRONG ANTI-TUMOR EFFECTS IN LUNG AND COLORECTAL CDX MODELS

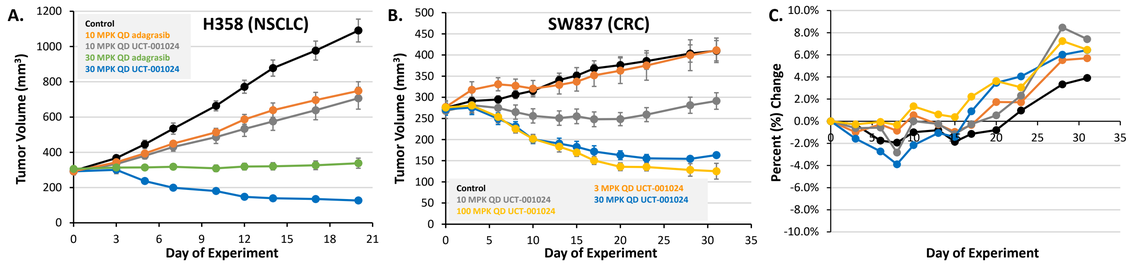


Figure 4. NCI-H358 **(A)** and SW837 **(B)** xenograft studies in female CD-1 nude mice. **(C)** Animal weight changes from SW837 study. 8 animals were used per arm. Tumor measurements were made using calipers. Error bars are ± SD.

KRAS^{G12C} INHIBITORS ARE ANTI-PROLIFERATIVE & MUTANT-SPECIFIC IN A PANEL OF HUMAN CANCER CELL LINES

A.

Cell line	KRas status	UCT-001024	Cmpd 1	Cmpd 2	Cmpd 3	adagrasib	sotorasib
		IC$_{50}$ (nM)					
MIA PaCa-2	G12C	0.5	< 3.2	13	< 0.04	11	5
NCI-H1385	G12C	0.5	< 3.2	3.3	< 0.04	13	14
NCI-H358	G12C	2.5	< 3.2	15	*nd*	22	12
CALU-1	G12C	4.4	*nd*	87	0.2	106	28
NCI-H2030	G12C	12	< 3.2	< 3.2	< 0.04	79	*nd*
H1373	G12C	29	*nd*	*nd*	0.1	141	*nd*
NCI-H2122	G12C	1000	372	652	< 0.04	958	1618
NCI-H647	G12D	1000	8270	10000	1000	950	10000

**nd* = not determined

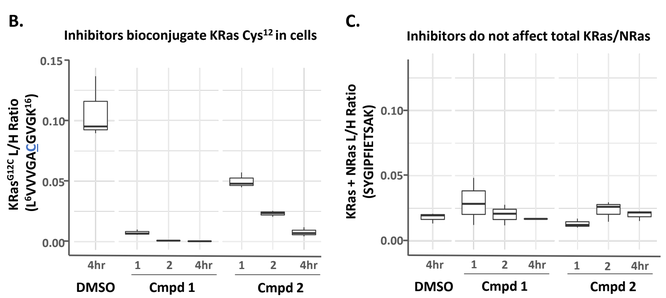
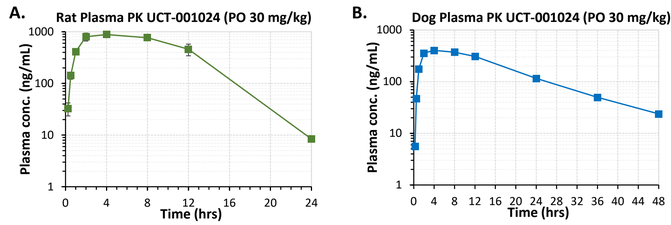


Figure 5. (A) *In vitro* proliferation IC$_{50}$ was determined by cell counting following treatment with varying concentrations of inhibitor for 6 days. **(B)** Proteomics MS was used to monitor target engagement in NCI-H358 cells treated with 100 nM compound **1** or **2** by the SILAC method. Both compounds achieve >90% depletion of the KRas^{G12C} peptide within 4hrs. **(C)** No effect was observed on the absolute concentration of total KRas and NRas.

UCT-001024 ACHIEVES SUSTAINED PLASMA EXPOSURE IN RODENTS AND DOGS



C.

	dose	C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0-\infty}$ (hr·ng/mL)	V_z/F (L/kg)	$t_{1/2}$ (hr)
Rat	PO, 30 mg/kg	896	3.3	11057	8.6	2.2
Dog	PO, 30 mg/kg	405	3.0	8306	64	10

Figure 6. Mean plasma concentrations following a single oral dose of UCT-001024 in **(A)** Sprague-Dawley rats and **(B)** Beagle dogs. **(C)** Pharmacokinetic parameters for each. Error bars are ± SD.

UCT-001024 SHOWS FAVORABLE CARDIAC SAFETY *IN VITRO*

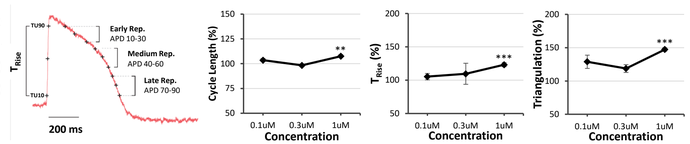


Figure 7. Over a therapeutically relevant concentration range, UCT-001024 minimally affects the cycle length, depolarization, and repolarization of spontaneously beating hiPSC derived cardiomyocytes (iCELL^2) *in vitro* suggestive of low TdP risk.[6] Error bars are ± SEM. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001

SUMMARY & CONCLUSIONS

- UCT-001024 is a next-generation, long half-life KRas^{G12C} inhibitor that shows rapid, selective, and irreversible target engagement *in vitro*.
- In a panel of seven *KRAS^{G12C}* mutated cancer cell lines, UCT-001024 inhibits proliferation 5- to 28-fold more potently than adagrasib and sotorasib.
- UCT-001024 is orally bioavailable and shows sustained exposure and large volume of distribution in mice, rats, and dogs and is projected to have a human half life >20 hrs.
- In a brain-tropic mouse model of metastatic lung adenocarcinoma, once daily UCT-001024 (150 mg/kg, PO) attains therapeutic unbound drug concentrations in whole brain and CSF, potently inhibits downstream ERK activation, and exerts anti-tumor efficacy and pro-survival benefit superior to adagrasib at the same dose.
- Using an *in vitro* hiPSC-derived cardiomyocyte assay, we find that UCT-001024 minimally affects the optical action potential over a therapeutically relevant concentration range suggestive of low human TdP risk.
- UCT-001024 has entered IND-enabling studies and is being developed for combination with other 1200 Pharma oncology therapeutics.

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