Discovery of potent CNS-penetrant covalent KRAS^{G12C} inhibitors

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Abstract

The RAS family of GTPases, which include KRAS, NRAS, and HRAS, are key regulators of cellular signaling, acting as molecular switches downstream of receptor tyrosine kinases (RTKs) and as key regulators of the RAS/MAPK pathway. RAS cycles between inactive (GDP) and active (GTP) states to regulate the activity of the RAS/MAPK pathway. In many cancers, activating KRAS mutations enable KRAS to persist in the active GTP-bound state, resulting in hyperactive RAS/MAPK pathway signaling that drives cell growth and survival. The KRAS G12C mutation occurs in 14% of lung adenocarcinoma[1], a cancer type that frequently metastasizes to the brain (40%)[2]. To address the high prevalence of KRAS G12C mutant CNS metastases in lung adenocarcinoma, we are developing CNS-penetrant covalent KRAS G12C inhibitors ("ERAS G12Ci's") to treat both CNS and systemic disease.

ERAS G12Ci's rapidly form covalent adducts with the KRAS G12C protein in its inactive GDP state and exhibit low nanomolar IC50's in a nucleotide exchange assay. ERAS G12Ci's demonstrate high selectivity in a cell-based proteome selectivity assay and inhibit the growth of RAS Initiative KRAS G12C mutant cells, and not RAS Initiative KRAS WT cells. ERAS G12Ci's potently inhibit cell proliferation in 3-dimensional Cell-Titer Glo (3D-CTG) assays in KRAS G12C mutant lung and pancreatic cell lines (NCI-H1373, NCI-H2122, and MIA PaCa-2). Mechanistically, ERAS G12Ci's blocked RAS-RAF complex formation and inhibited ERK1/2 phosphorylation. This *in vitro* activity translates *in vivo* where ERAS G12Ci's induce pharmacodynamic modulation in the pancreatic cancer MIA PaCa-2 model. ERAS G12Ci's significantly inhibit tumor growth in NCI-H1373 and NCI-H2122 CDX lung adenocarcinoma and MIA PaCa-2 PDAC models. No meaningful body weight loss or clinical adverse events were observed with any of these compounds.

Human efflux transporter substrate assessments indicate that ERAS G12Ci's are either not substrates or weak/modest substrates of P-gp, a crucial efflux transporter that can limit CNS penetration. In rat CNS studies, these inhibitors exhibit good CNS penetration performance as measured by brain-to-plasma partition coefficients, which are comparable to those of approved CNS-active small molecule inhibitors. Daily oral administration demonstrates dose-dependent tumor regression in intracranial and intra-carotid injection (ICA) KRAS G12C CDX models. We are optimizing multiple covalent CNS-penetrant KRAS G12C inhibitors that exhibit both CNS and systemic activity *in vivo*.

1. AACR Project GENIE Consortium. AACR Project GENIE: powering precision medicine through an international consortium. *Cancer Discov.* 7, 818–831 (2017).
2. Real world outcomes in KRAS G12C mutation positive non-small cell lung cancer. *Lung Cancer* 146, 310-317 (2020)

Results

ERAS G12Ci's showed robust rates of covalent modification of Cys12. ERAS G12Ci's blocked SOS1 mediated nucleotide exchange and selectively inhibited formation of KRAS^{G12C} with RAF-RBD (Ras binding domain of Raf1 kinase) relative to KRAS WT *in vitro* (RRB G12C and RRB WT assays, respectively)

Compound	K_{inact}/K_I ($M^{-1}s^{-1}$)	NEX IC_{50} (nM)	RRB G12C IC_{50} (nM)	RRB WT IC_{50} (nM)
ERAS-3490	9,900	41.6	10.2	>10,000
ERAS-3537	34,000	25.4	5.6	>10,000
ERAS-3788	4,900	42.3	12.6	>10,000
ERAS-4926	54,776	16.1	4.1	>10,000

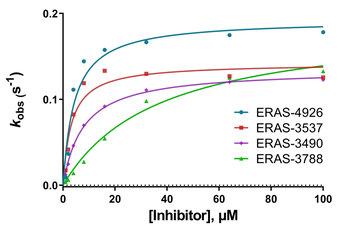


Figure 1. k_{inact}/K_I determination for ERAS G12Ci's. Data represent the mean k_{obs} measured from three experiments.

Results (continued)

ERAS G12Ci's exhibited favorable proteome-wide selectivity

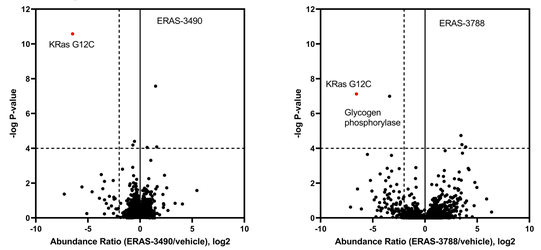


Figure 2. Cysteines targeted for covalent modification across the proteome of NCI-H358 cells following a 4 hr treatment with ERAS-3490 and ERAS-3788 were identified by LC-MS/MS.

ERAS G12Ci's blocked cellular RAS/RAF-RBD complex formation in KRAS^{G12C} mutant lung and pancreatic cell lines

Compound	NCI-H1373-GFP IC_{50} (nM)	NCI-H2122-GFP IC_{50} (nM)	MIA PaCa-2 IC_{50} (nM)	NCI-H23-GFP IC_{50} (nM)
ERAS-3490	16.86	127.5	272.7	78.09
ERAS-3537	17.07	50.23	79.94	19.26
ERAS-3788	18.77	42.22	68.88	34.37
ERAS-4926	6.11	56.32	139.1	24.71

ERAS G12Ci's modulated phosphorylation of ERK1/2 in MIA PaCa-2 KRAS^{G12C} cell line

Compound	MIA PaCa-2 pERK-HTRF IC_{50} (nM)	
	2 hr	16 hr
ERAS-3490	105.6	11.70
ERAS-3537	139.5	12.25
ERAS-3788	90.67	16.34
ERAS-4926	54.18	8.49

ERAS-3490 selectively inhibited cell proliferation in KRAS^{G12C} vs non-KRAS^{G12C} cell lines

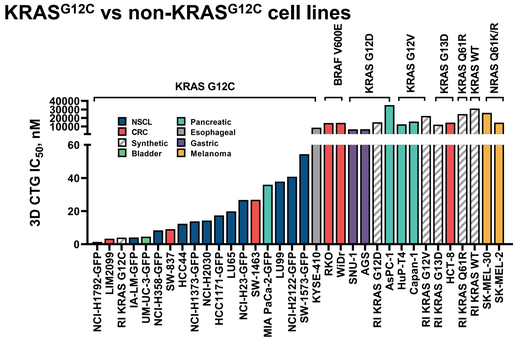


Figure 3. ERAS-3490 effects on 3D cell viability (5 days post treatment) and proliferation across a panel of KRAS^{G12C} and non-KRAS^{G12C} cell lines. ERAS-3490 exhibited >7,000-fold selectivity for KRAS^{G12C} versus KRAS^{WT} by comparison of 3D CTG IC50 values from synthetic cell lines harboring these respective mutations (RI KRAS^{WT} : RI KRAS^{G12C}) and >950-fold selectivity when comparing all non-KRAS^{G12C} cell lines to all KRAS^{G12C}-harboring cell lines.

Properties of CNS-penetrant ERAS G12Ci's

Parameter	3490	3537	3788	4926	Reference compounds
P-gp substrate ratio[1]	↑ 1.5	↑ 5.0	↑ 4.0	↑ 3.5	30.9[2]
Rat brain / plasma (%)	↑ 52%	↑ 68%	↑ 11%	↑ 21%	1 - 6%
Rat brain concentration (ng / g)	↑ 156	↑ 290	↑ 91	↑ 170	6 - 36
Mouse AUC_{po}/D (hr*kg*ng/mL/mg)	↑ 693	↔ 535	↔ 326	↔ 614	102 - 637

Arrows indicate change in values relative to sotorasib and adagrasib, whose values are listed in the reference compounds column.

[1] P-gp substrate ratios were characterized in a P-gp expressing MDCK cell line. Per compound, a P-gp substrate ratio was calculated by dividing its efflux ratio in absence of a P-gp inhibitor by its efflux ratio in presence of a P-gp inhibitor. Compounds with lower P-gp substrate ratios are less likely to be P-gp substrates.

[2] The P-gp substrate ratio was characterized for a single reference compound.

ERAS-3490 demonstrated dose proportional target engagement and inhibited RAS-RAF binding and ERK phosphorylation in MIA PaCa-2 CDX model

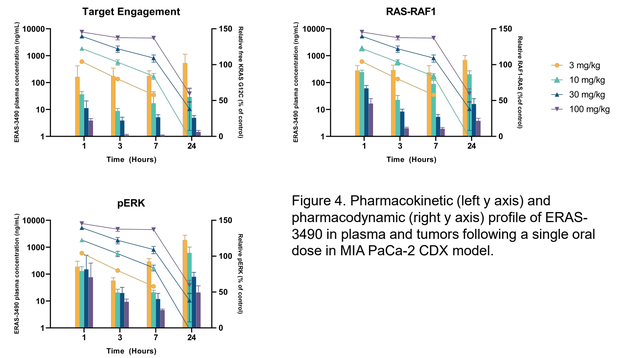


Figure 4. Pharmacokinetic (left y axis) and pharmacodynamic (right y axis) profile of ERAS-3490 in plasma and tumors following a single oral dose in MIA PaCa-2 CDX model.

ERAS-3490 demonstrated promising anti-tumor activity in KRAS^{G12C} pancreatic and lung cancer CDX models

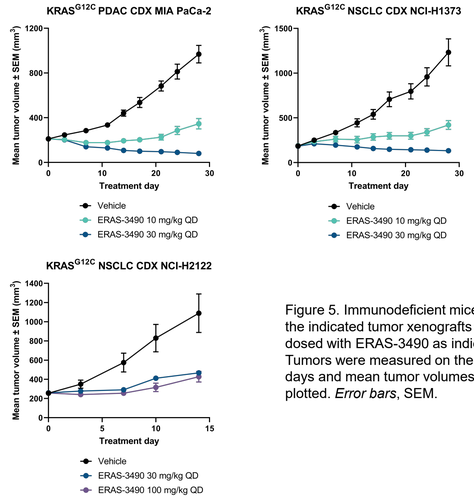
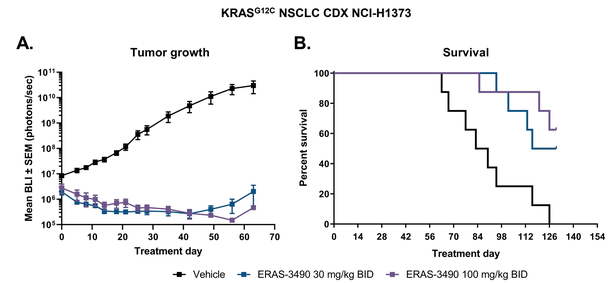


Figure 5. Immunodeficient mice bearing the indicated tumor xenografts were orally dosed with ERAS-3490 as indicated. Tumors were measured on the indicated days and mean tumor volumes were plotted. *Error bars*, SEM.

ERAS-3490 showed dose dependent survival benefit in NCI-H1373-luc brain tumor-bearing mice



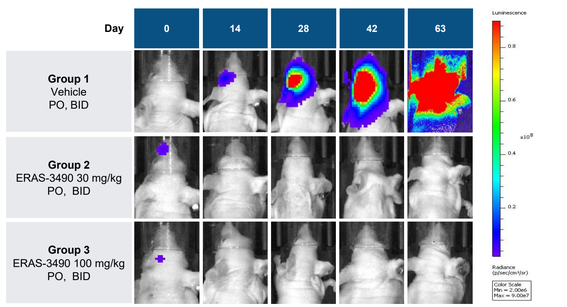


Figure 6. Mice were intra-cranially implanted with luciferase-expressing NCI-H1373 cells via intra-carotid injection and treated as indicated. **A.** tumor growth was monitored as a function of bioluminescence (BLI) and mean BLI is plotted as a function of treatment day. *Error bars*, SEM. **B.** Kaplan-Meier survival analysis of treated groups. **C.** Representative bioluminescence images of mice from treatment groups are shown.

Conclusions

- Erasca has developed CNS-penetrant KRAS^{G12C} covalent inhibitors that show robust systemic and CNS activity in CDX models
- ERAS G12Ci's show promising CNS penetration potential, as measured by lower in vitro P-gp substrate ratios of 1.5–5.0 and higher in vivo brain/plasma concentration ratios of 11-68% and brain concentrations of 91-290 ng/g in SD rats
- ERAS G12Ci's potently inhibit cell proliferation and RAS/MAPK signaling (RAS-RAF1 and pERK) in KRAS^{G12C} cell lines, including NCI-H358, MIA PaCa-2, and RI KRAS G12C
- ERAS G12Ci's selectively bind to the KRAS^{G12C} peptide in the H358 cell line and show dose-dependent target engagement in MIA PaCa-2 xenograft
- ERAS-3490 mediates significant and sustained modulation of KRAS^{G12C}/RAF-RBD binding and ERK1/2 phosphorylation in a MIA PaCa-2 mouse model (in vivo)
- ERAS-3490 shows robust antitumor activity in KRAS^{G12C} mutant MIA PaCa-2, NCI-H1373, and NCI-H2122 CDX subcutaneous models and NCI-H1373-luc intracranial model
- ERAS-3490 shows dose-dependent survival benefit in a KRAS^{G12C} NSCLC brain metastases model