Non-clinical identification and characterization of KRAS G12D inhibitors

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Abstract # 54

Abstract

KRAS G12D is the most prevalent KRAS oncogenic mutation and occurs frequently in pancreatic ductal adenocarcinoma (PDAC), colorectal adenocarcinoma (CRC), and non-small cell lung cancer (NSCLC; 37%, 12% and 4%, respectively). KRAS G12D cycles between inactive (GDP) and active (GTP) states to regulate the RAS/MAPK pathway. Although the rate of intrinsic and GAP (GTPase activating protein) mediated hydrolysis is significantly slower in KRAS G12D than in KRAS wild-type (KRAS WT), we were able to build upon success of targeting the GDP state of KRAS G12C and designed novel KRAS G12D lead compounds with promising in vitro and in vivo activity.

Our KRAS G12D inhibitors (ERAS G12Di's) preferentially bind to KRAS G12D GDP as indicated by differential scanning fluorimetry (DSF), block KRAS G12D SOS1-mediated nucleotide exchange, and inhibit KRAS G12D binding to the RAS binding domain of C-RAF (RAF-RBD) with single digit nanomolar potency. Our leads effectively inhibit ERK1/2 phosphorylation (pERK) in AsPC-1 PDAC cells and inhibit cell proliferation in 3-dimensional Cell-Titer Glo assays in a panel of KRAS G12D mutant PDAC, CRC, and NSCLC cell lines.

This in vitro activity translates in vivo where robust pharmacodynamic modulation in the PDAC AsPC-1 CDX model was induced. Tumor growth inhibition studies in this model revealed robust dose-dependent tumor growth inhibition and tumor regression in the absence of overt toxicity.

In summary, we have identified potent and selective KRAS G12D inhibitors with robust dose-dependent tumor growth inhibition and regression activity in a PDAC CDX model. Lead optimization is ongoing with the aim of identifying a development candidate.

1. AACR Project GENIE Consortium. AACR Project GENIE: powering precision medicine through an international consortium. *Cancer Discov.* 7, 818–831 (2017).

Results

KRAS G12D protein has an intermediate intrinsic hydrolysis rate and is insensitive to GAP mediated hydrolysis (not shown). This observation offers an opportunity to target the inactive KRAS GDP state in a similar approach that was successfully applied to development of KRAS G12C inhibitors.

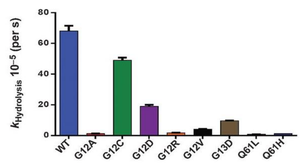

Figure 1. The intrinsic rate of hydrolysis for various KRAS mutants (adapted from Hunter et all, DOI: 10.1158/1541-7786.MCR-15-0203).

We have established a screening cascade for the identification of KRAS G12D inhibitors (G12Di's) consisting of a suite of biochemical, cell-based and in vivo assays which are outlined on Figure 2.

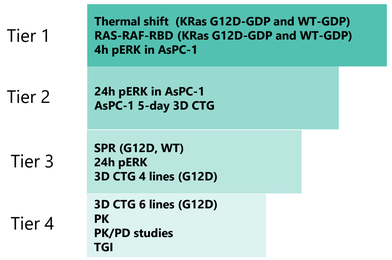

Figure 2. *In vitro* and *in vivo* screening cascade for the identification and characterization of KRAS G12D inhibitors.

Results (continued)

The increase in ERAS G12Di's melting point (surrogate for direct binding) measured against KRAS G12D GDP correlates with inhibition of ERK phosphorylation after 4h treatment in the AsPC-1 cell line. Two inhibitors with a melting point >20° C were identified which translated into low nanomolar cell potency.

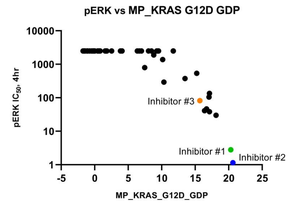

Figure 3. ERAS G12Di's cell-based activity correlates with the increase in thermal shift (melting point (MP) KRAS G12D GDP compound bound vs. DMSO control) as detected by DSF assay.

We have employed an SPR based reporter assay to extend the classical limit of multicycle kinetics studies ($T_{1/2}$ ~2h) for potent ERAS G12Di's (Figure 4). ERAS G12Di's fraction bound was calculated using this assay format and used for rank ordering of compounds.

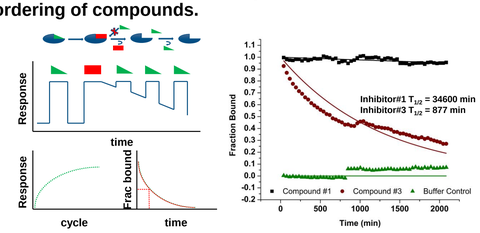

Figure 4. SPR reporter assay outline (left panel) and quantification of fraction of inhibitor ($T_{1/2}$) bound (right panel) for inhibitor #1 and inhibitor #3 using KRAS G12D GDP immobilized on the chip using direct coupling. Bulk change in baseline represents buffer change halfway through assay.

The observed long lifetime of KRAS G12D GDP protein complex with inhibitor #1 is hypothesized to favorably impact the *in vivo* pharmacodynamic (PD) response. KRAS G12D protein turnover in cells may also impact PD response. We determined protein turnover for KRAS G12D using SILAC-based proteomics and observed a half-life of 48h.

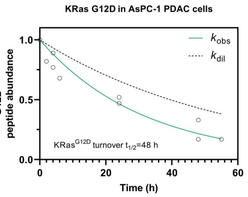

Figure 5. KRAS G12D turnover was quantified in AsPC-1 cell culture using stable isotope labeling (SILAC) and quantitative proteomics. Cells were grown to steady state in 'light' medium and then switched to 'heavy' medium at T=0. The fractional abundance of 'light' KRAS G12D (y-axis) was measured as a function of time grown in 'heavy' medium (x-axis).

ERAS G12Di's stabilized KRAS G12D GDP protein, blocked formation of KRAS G12D with RAF-RBD (Ras binding domain of Raf1 kinase; RRB assay) in biochemical assay, and potently inhibited ERK phosphorylation and proliferation in AsPC-1 cells.

Compound	MP (ΔºC)	RRB G12D-GDP IC_{50} (nM)	RRB G12D-GMPPNP IC_{50} (nM)	NEX IC_{50} (nM)	AsPC-1 pERK-HTRF IC_{50} (nM), 4 h	AsPC-1 3D-CTG IC_{50} (nM), 5 day
Inhibitor #1	20.3	7.35	11.2	42	2.81	15.08
Inhibitor #2	20.6	9.22	15.8	52	1.15	3.56

ERAS G12Di's potently inhibited cell proliferation across the panel of KRAS G12D mutant cell lines representing different tumor types and exhibit ~100x or greater selectivity over WT in engineered cell lines.

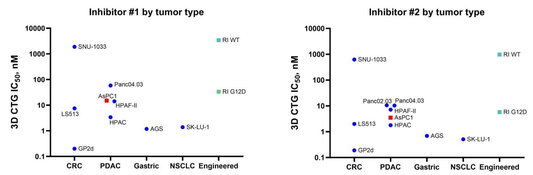

Figure 6. KRAS G12Di's #1 (left panel) and #2 (right panel) inhibit proliferation of KRAS G12D cell lines representing different tumor types.

ERAS G12D inhibitor #2 showed low risk of deleterious off-target activity in cellular thermal shift studies (CETSA™), with one protein (c-FOS) significantly destabilized at 0.5 μM of the compound.

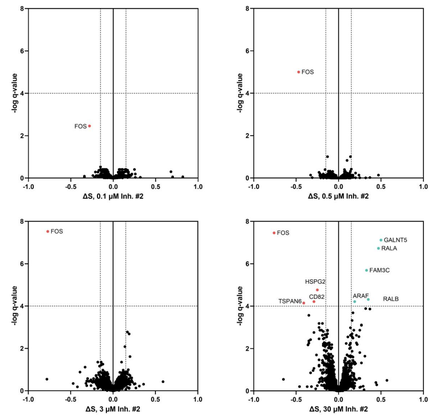

Figure 7. Volcano plots show proteins with significant destabilization (red) or stabilization (green) in response to treatment with ERAS G12D inhibitor #2 (0.1-30 μM). The x-axis, ΔS, is the difference in thermal stability due to treatment, with ±0.15 as the significance threshold (dashed lines). A total of 6108 proteins from AsPC-1 cell lysates were analyzed using a compressed cellular thermal shift assay (CETSA™, Pelago).

KRAS G12D protein is detected and showed concentration-dependent increase in thermal stability of KRAS G12D-specific peptide. c-FOS is intrinsically unstable and stabilized by ERK phosphorylation. Destabilization of c-FOS is a result of MAPK pathway inhibition. No stabilization of [HN]RAS specific peptide is detected.

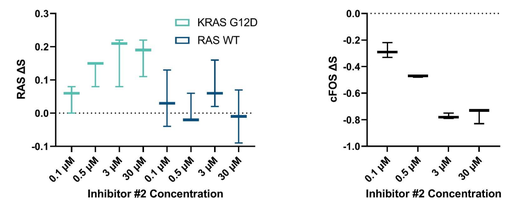

Figure 8. Left plot shows the change in thermal stability observed for the KRAS G12D-specific peptide (LVVVGADGVGK) and [HN]RAS-specific peptide (LVVVGAGGVGK). Right plot shows the observed destabilization of c-FOS at each concentration.

ERAS G12Di's demonstrated dose-dependent inhibition of ERK and RSK phosphorylation in AsPC-1 CDX model.

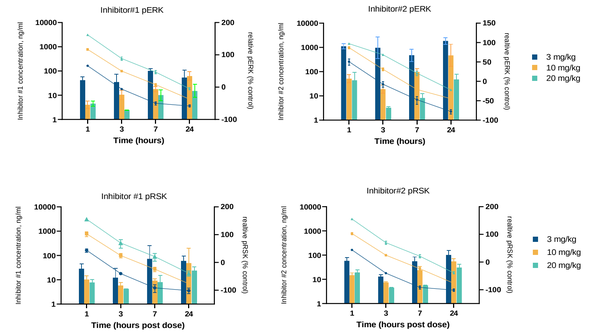

Figure 9. Pharmacokinetic (left y axis) and pharmacodynamic (right y axis) profile of inhibitor #1 and inhibitor #2 plasma and tumors following a single intraperitoneal (IP) dose in AsPC-1 CDX model. *Error bars*, SEM.

ERAS G12Di's demonstrated robust anti-tumor activity in KRAS G12D pancreatic cancer CDX models.

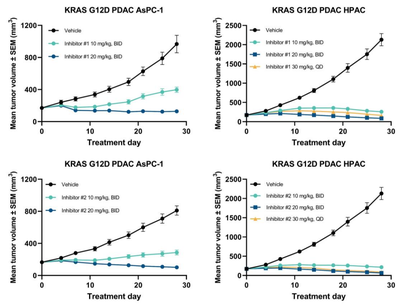

Figure 10. Immunodeficient mice bearing the indicated tumor xenografts were IP dosed with Inhibitor #1 and Inhibitor #2 as indicated. Tumors were measured on the indicated days and mean tumor volumes were plotted. *Error bars*, SEM.

Conclusions

- Erasca has identified KRAS G12D inhibitors that exhibit robust systemic activity in CDX models of pancreatic cancer
- ERAS G12Di's induced significant thermostabilization of KRAS G12D GDP state, and inhibit Raf-RBD binding in vitro to both GDP and GMPPNP bound KRAS G12D
- Inhibitor #1 formed a stable long-lived complex with KRAS G12D GDP in vitro. Binding kinetics of inhibitor #1 combined with slow KRAS G12D protein turnover in cells may provide a foundation of favorable PD response in vivo
- ERAS G12Di's potently inhibited cell proliferation in a panel of KRAS G12D cell lines representing different tumor types and RAS/MAPK signaling (pERK) in KRAS G12D cells
- ERAS G12D inhibitor #2 showed dose-dependent KRAS G12D target engagement in AsPC-1 cell line and minimal off-target binding events
- c-FOS destabilization was identified and could be used as a potential biomarker of MAPK pathway inhibition upon treatment with ERAS G12Di's
- ERAS G12Di's mediated significant and sustained modulation of ERK1/2 and RSK phosphorylation in an AsPC-1 mouse model
- ERAS G12D inhibitors #1 and #2 showed robust antitumor activity in KRAS G12D mutant AsPC-1 and HPAC pancreatic cancer CDX models