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Discovery of potent and orally available small molecule inhibitors of the SOS1-KRAS interaction

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Introduction

Abnormal activating mutations of KRAS are frequently found in many human cancers, including pancreatic ductal adenocarcinoma (PDAC), colorectal cancer (CRC), and non-small cell lung cancer (NSCLC). KRAS activating mutations lead to hyperactivation of the MAPK/ERK signaling pathway, resulting in the promotion of cell proliferation and growth. SOS1 is one of the major guanine nucleotide exchange factors (GEFs) that regulates RAS proteins including KRAS. Since SOS1 (Son of sevenless homolog 1) plays a critical role in converting the GDP-bound inactive KRAS “off” state to the GTP-bound active KRAS “on” state, disruption of SOS1 and KRAS protein-protein interaction would be effective in blocking KRAS-driven oncogenic signaling regardless of its mutation status. Importantly, SOS1 activity is crucial during the reactivation of the RAS/MAPK signaling upon the treatment of RAS/MEK/ERK inhibitors, thus SOS1 inhibitor would be an effective therapeutic option to treat RAS-driven tumors in combination with RAS/MAPK pathway inhibitors. In this study, we discovered potent, selective, and orally available small molecules that effectively disrupt the interaction between SOS1 and KRAS.

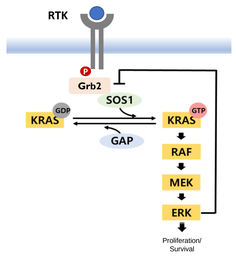


Figure 1. KRAS/MAPK pathway

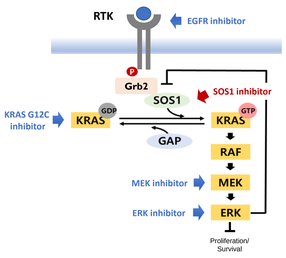


Figure 2. Combination of SOS1 inhibitor with RAS/MAPK inhibitors

Hit to Development Candidate

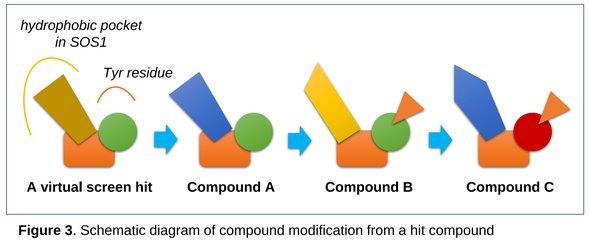
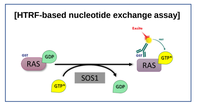


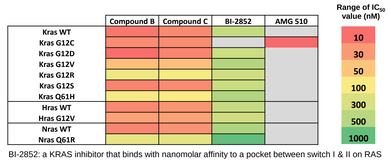
Figure 3. Schematic diagram of compound modification from a hit compound

- A hit compound was identified from a virtual screening campaign
- Through structure-based drug design, compound **A** with good *in vitro* potency and PK profile was discovered, which demonstrated good efficacy as a single agent as well as in combination with AMG 510, a KRAS G12C inhibitor
- Subsequent lead optimization led to compound **B** with excellent *in vitro* potency and efficacy. However, it exhibited time-dependent inhibition of CYP3A4 that could often result in clinically significant drug-drug interactions
- Further medicinal chemistry efforts resulted in compound **C** with a selective, orally bioavailable, and efficacious pan-KRAS SOS1 inhibitor, the preclinical studies of which are underway
- The patent for compound **C** and the analogues has been published (WO 2023022497)

Result I: *In Vitro* Assays

Evaluation of nucleotide exchange activity of compounds B and C in a pan-RAS selectivity panel



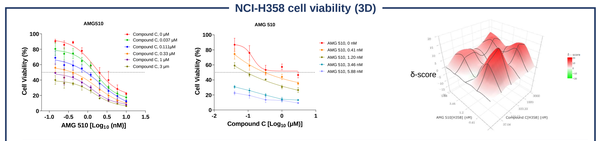


BI-2852: a KRAS inhibitor that binds with nanomolar affinity to a pocket between switch I & II on RAS

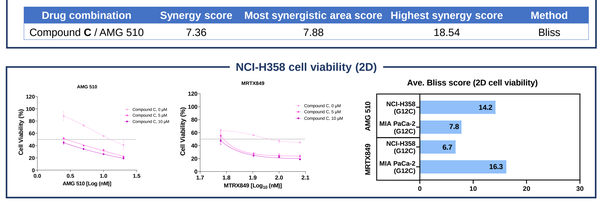
- The HTRF-based nucleotide exchange assay was performed with mutant and wild type RAS proteins
- Compounds **B** and **C** effectively inhibited the activation of various mutant and wild type RAS proteins (K-, H- and N-RAS)

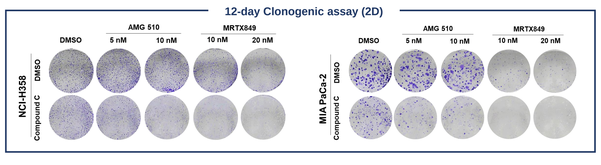
In vitro evaluation of combination of compound C and RAS/MAPK pathway inhibitors

❑ KRAS G12C inhibitors (AMG 510 and MRTX849)

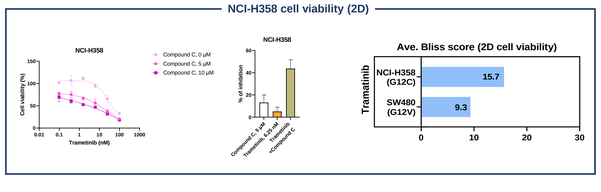


Drug combination	Synergy score	Most synergistic area score	Highest synergy score	Method
Compound **C** / AMG 510	7.36	7.88	18.54	Bliss

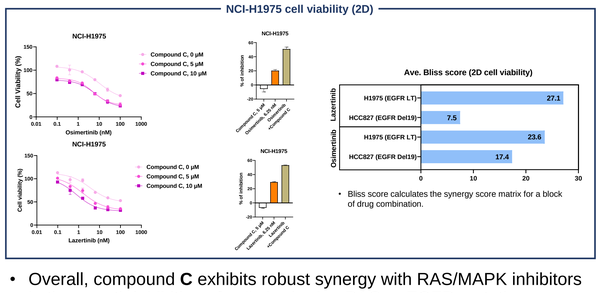




❑ MEK inhibitor (Trametinib)



❑ EGFR inhibitors (Osimertinib and Lazertinib)



- Bliss score calculates the synergy score matrix for a block of drug combination.

- Overall, compound **C** exhibits robust synergy with RAS/MAPK inhibitors in the growth inhibition of cells harboring RAS or EGFR mutations

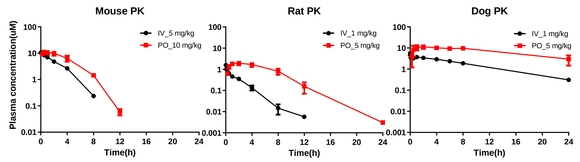
Result II: Pharmacokinetic Profiles

❑ ADME profile of compound **C**

Assays	Values
MDCK-MDR1 (P_{app}, 10^{-6} cm/s)	P_{app}(A to B): 4.66, P_{app} (B to A): 23.77; ER 5.10
Microsomal stability CL_{int} (mL/min/kg) [mouse/rat/dog/monkey/human]	100 / 22 / <13.8 / <13.0 / <8.6
CYP450 inhibition (IC_{50}, μM) [1A2/2B6/2C8/2C9/2C19/2D6/3A4]	>50 / >50 / >50 / 23.8 / >50 / >50 / >50
TDI of CYP3A4 (shifted IC_{50}, μM) [3A4-T/3A4-M]	>50 / >50
PPB (%) [mouse/rat/dog/human]	92.2 / 74.7 / 89.2 / 83.8

- Compound **C** has excellent ADME profile and low drug-drug interaction (DDI) risk

❑ PK profiles of compound **C**



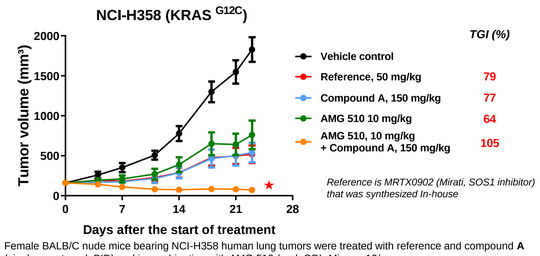
Species	Mouse	Rat	Dog
CL (mL/min/kg) | Vd_{ss} (L/kg) | $T_{1/2}$ (h) | AUC_{inf} (μmol.h/L) | %F	6.68 | 0.91 | 1.51 | 50.7 | 96.8	19.1 | 2.18 | 1.27 | 13.4 | 147	0.88 | 0.49 | 6.09 | 221 | 91

Male CD-1(CR) Mouse, n=3/group. Male SD Rat, n=3/group, Male Beagle Dog, n=3/group

- Compound **C** has low plasma clearance and high bioavailability across species
- Especially, high exposures in rats and dogs enable a once-daily dose of compound **C**

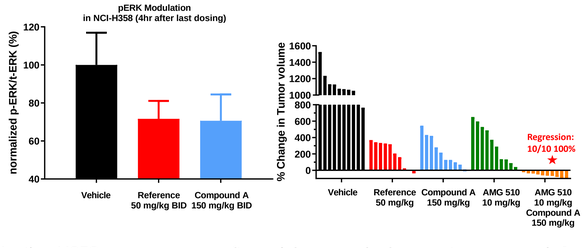
Result III: *In Vivo* Xenograft Model

❑ Efficacy study of compound **A**



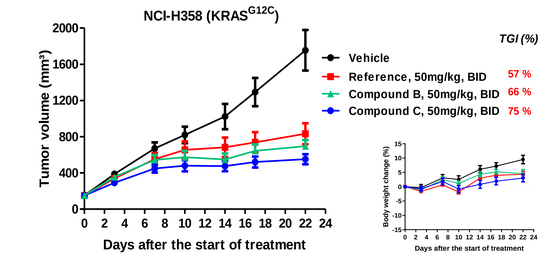
Reference is MRTX0902 (Mirati, SOS1 inhibitor) that was synthesized In-house

Female BALB/C nude mice bearing NCI-H358 human lung tumors were treated with reference and compound **A** (single agent, oral, BID) and in combination with AMG 510 (oral, QD); Mice n=10/group

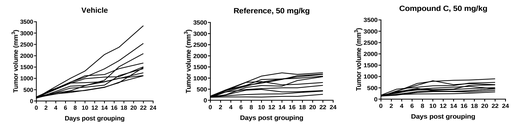


- In the H358 mouse xenograft model, as a single agent, compound **A** showed comparable tumor growth inhibition when compared to the reference compound, which was also correlated with pERK modulation
- Significant regression was observed when combined with AMG 510, a KRAS G12C inhibitor

❑ Efficacy study of compounds **B** and **C**



Female BALB/C nude mice bearing NCI-H358 human lung tumors were treated with Reference, compound **A** and **B** (single agent, oral, BID); Mice n=10/group



- In the same tumor model, compounds **B** and **C** showed equivalent or superior tumor growth inhibition with no tolerability issue compared to the reference compound at the same dose oral (50 mg/kg BID)
- The individual tumor growth curves in the compound **C**-treated group showed relatively low variability in drug response

- In the end-of-study PK analysis, compounds **B** and **C** displayed higher plasma concentrations than that of the reference compound at 4 h after the last dosing
- Dose-dependent efficacy study is underway to understand PK/PD relationship and efficacy, along with combination studies

Summary

- Compound **C**, a selective, orally bioavailable, and efficacious SOS1 inhibitor, was achieved from a virtual screening hit through structure-based drug design
- Compound **C** has good ADME profile, high unbound fractions across species, and no TDI risk, leading to excellent PK profiles in mice, rats, and dogs
- Compound **A**, an in vivo tool compound, demonstrated strong synergy effect with AMG 510, a KRAS G12C inhibitor
- Compound **C** exhibited robust synergy with RAS/MAPK inhibitors in the growth inhibition of cells harboring RAS or EGFR mutations
- In the NCI-H358 mouse xenograft model, compound **C** showed a robust tumor growth inhibition. Currently, combination studies of compound **C** are being conducted in several xenograft mouse models based on the synergistic results in the cell viability assays
- Compound **C** was selected to be assessed in preclinical studies, aiming to perform GLP tox study by the end of 2023

Acknowledgement

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