Design and discovery of BH-501242, a novel pan-KRAS on/off inhibitor targeting KRAS switch II pocket

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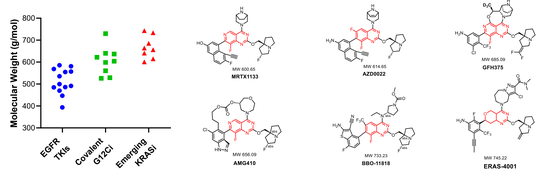
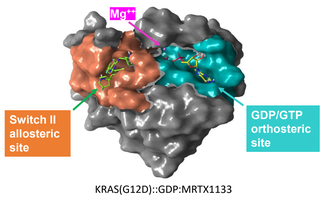
Introduction

KRAS mutations are involved in approximately 25% of all tumors and occur as oncogenic drivers in lung (32%), colorectal (40%) and pancreatic cancers (85-90%). The most common *KRAS* driver mutations are on codon 12 and 13, including G12C, G12D, G12V, G12R, G13A and G13D etc. The G12C mutation has been effectively targeted using a covalent strategy that exploits the unique reactivity of the cysteine residue to lock the KRAS G12C inhibitors in an induced Switch-II allosteric pocket. The G12D inhibitors used the salt bridge from a basic head group of the inhibitors with G12D to lock the inhibitors in the Switch-II allosteric pocket with MRTX1133 as the first to demonstrate low single digit cell potency for G12D mutation. However, MRTX1133 and many analogues have two basic centers leading to poor oral bioavailability. Since then, many medicinal chemistry efforts have been focused on replacing the basic head group with a neutral group for the improvement of oral bioavailability. Although the molecular weights were increased significantly with still less favorable ADME/PK properties, multiple KRAS inhibitors targeting G12D, G12V, or pan-KRAS mutations have advanced into clinical trials.

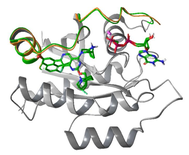


Our Design Approach for Achieving Super Cell Potency and Good ADME/PK Properties

- KRAS Switch-II inhibitors are allosteric inhibitors inside an induced pocket near switch-II range, which is available in both GTP-bound (on) and GDP-bound (off) KRAS
- Switch-II inhibitors induced a similar KRAS conformation when bound with KRAS at either "On" or "Off" state
- The allosteric Switch-II inhibitors rigidified KRAS conformation to reduce binding with GTP and disable binding with effectors



KRAS(G12D)::GDP:MRTX1133



PDB:7RPZ: KRAS(G12D)::GDP::MRTX1133
PDB:7T47: KRAS(G12D)::GCP::MRTX1133

Our Design Approach for a Novel Switch-II Allosteric Chemical Scaffold

- A pseudo-irreversible Switch-II allosteric chemical scaffold that can mimic the characteristics of the successful, covalent KRAS G12C inhibitors in clinic
- Strong induced conformational change on mutant KRAS proteins at either GTP or GDP-bound state
- High binding affinity (picomolar) and long resident time to effectively block KRAS interactions with effectors
- Novel pseudo-irreversible, pan-KRAS on/off inhibitor BH-501242 targeting KRAS Switch II pocket with prolonged slow-off rate, sub-nM cell potency and good oral bioavailability was discovered based on these design principles and medicinal chemistry lead optimization

Broad Activity Inhibiting Nucleotide Exchange

- BH-501242 showed potent activity inhibiting SOS-mediated nucleotide exchange from GDP to GTP across broad spectrum of KRAS mutations
- Sub-nM IC_{50}s were observed for key KRAS mutations, like G12D, G12V and G12C

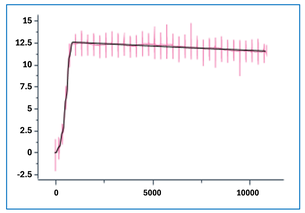
IC_{50} (nM)	G12D	G12V	G12C	G12D/T35S	G12S	G12R	WT
BH-501242	0.59	0.55	0.52	1.04	1.08	0.51	0.48

- IC_{50} values (nM) in the table above were from HTRF nucleotide exchange assay at Reaction Biology, Inc.

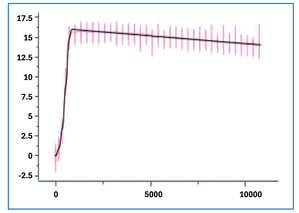
Tight Binding of BH-501242 to Both GTP and GDP-bound KRAS G12D

- BH-501242 showed tight binding to both GTP and GDP-bound KRAS G12D protein
- Long residence time (33.0 hrs with GTP and 21.4 hrs with GDP)
- High binding affinity to both "On" KRAS G12D (0.0047 nM) and "Off" KRAS G12D (0.0089 nM)

SPR of KRAS G12D with GppNHp



SPR of KRAS G12D with GDP



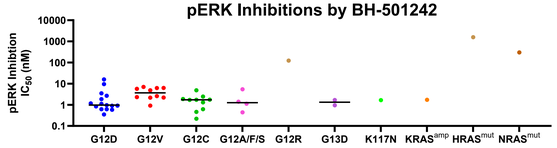
Nucleotide Bound	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)	Residence Time (hr)
GDP	$1.5x10^6$	$1.3x10^{-5}$	0.0089	21.4
GppNHp	$1.2x10^6$	$8.4x10^{-6}$	0.0047	33.0

- The SPR assays were conducted at Reaction Biology, Inc.

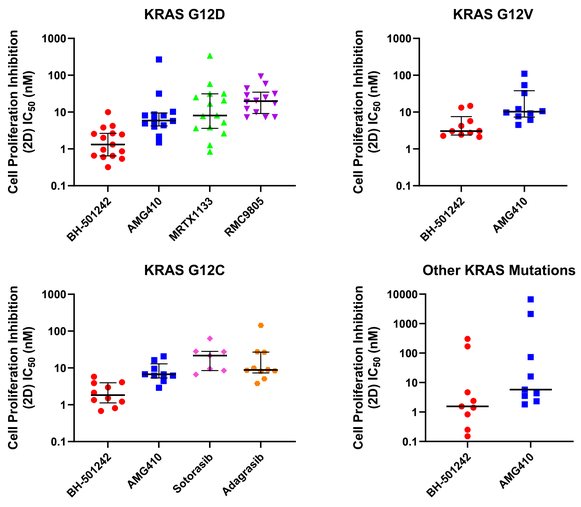
Inhibition of pERK and Cell Proliferation

- BH-501242 showed potent pERK inhibition in KRAS G12D-, G12V-, and G12C-mutant cells, with a median pERK inhibition IC_{50} of 0.96 nM, 2.58 nM and 1.71 nM, respectively
- Low single-digit-nM potency in cell proliferation inhibition (CPI) assays was achieved in G12D-, G12V- and G12C-mutant cells, with a median CPI IC_{50} of 1.31, 3.05, and 1.82 nM, respectively
- No CPI activity was observed in HRAS- and NRAS-mutant cell lines

pERK Inhibitions by BH-501242

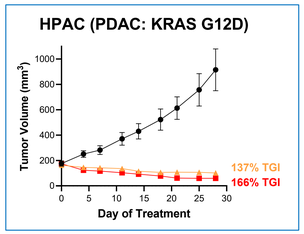


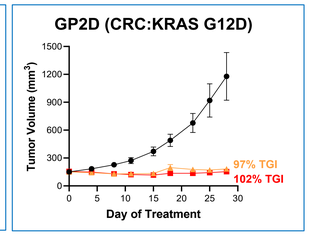
Cell Proliferation Inhibitions by BH-501242

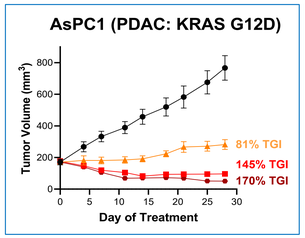


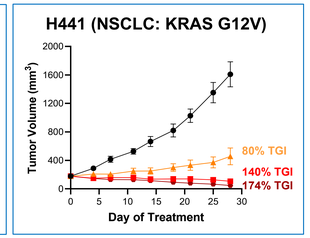
BH-501242 Anti-tumor Activity in Multiple Tumor Models

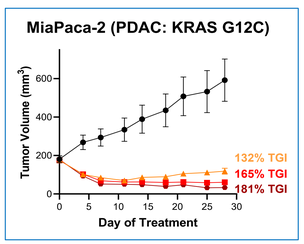
- BH-501242 achieved tumor regression in *KRAS*-G12D-mutant CDX tumor models, including HPAC and AsPC-1, and stasis in GP2D tumor model
- Tumor regression was achieved in *KRAS*-G12V-mutant H441 tumor model
- Tumor regression was achieved in *KRAS*-G12C-mutant MiaPaca-2 tumor model
- BH-501242 was well tolerated without body weight reduction or overt abnormality in tumor models

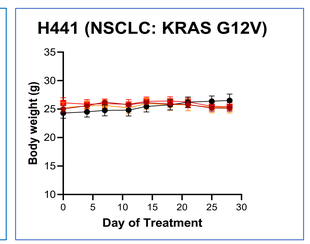






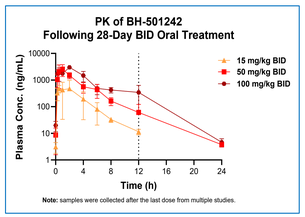


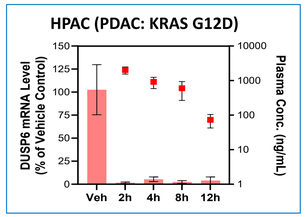




PK Exposure and PK/PD Consistent with Efficacy in Tumor Model

- BH-501242 PK exposures were dose-dependent as studied in tumor models
- PK/PD was studied using DUSP6 as biomarker
- >90% suppression of DUSP6 within dose interval was associated with tumor regression observed at 100 mg/kg BID dose





ADME/PK Properties of BH-501242 and Analog BH-501352

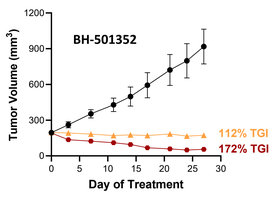
- The ADME/PK properties of BH-501242 and its close analog BH-501352 have been profiled, with good bioavailability achieved in multiple species

Profiling Assays	BH-501242	BH-501352
LogD / pK_a	3.44 / 8.51	3.74 / 8.49
Human LM CL_{int} (mL/min/mg)	108	53
Human HEP CL_{int} CL_{int} (mL/min/10^6Cells)	34	18
MDCK P_{app} AB/BA [10^(-6)cm/s]	0.13 / 19.3	0.18 / 31.5
Human PPB (%)	96.7%	98.5%
Mouse PK CL (mL/min/kg)/V (L/kg)/F%	16.6 / 2.17 / 11.3%	9.19 / 2.22 / 29.5%
Rat PK CL (mL/min/kg)/V (L/kg)/F%	54.7 / 11.1 / 21.4%	40.7 / 9.6 / 21.3%
Dog PK CL (mL/min/kg)/V (L/kg)/F%	24.8 / 6.9 / 6.4%	11.9 / 6.6 / 15.3%
Monkey PK CL (mL/min/kg)/V (L/kg)/F%	N/A	27.8 / 9.7 / 6.2%

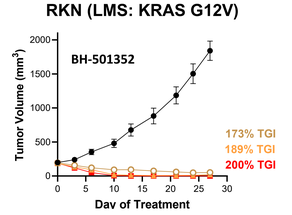
BH-501352 Demonstrated Comparable Activities as BH-501242

Cell Line (KRAS Mutations)	Cell Proliferation Inhibition IC_{50} (nM)	
	BH-501242	BH-501352
GP2D (G12D)	0.54	0.69
SW620 (G12V)	2.26	1.61
H358 (G12C)	2.97	4.68

HPAC (PDAC: KRAS G12D)



RKN (LMS: KRAS G12V)



Vehicle BID | 5 mg/kg BID | 15 mg/kg BID | 50 mg/kg BID | 100 mg/kg BID

Conclusion

- BH-501242 is a novel pan-KRAS inhibitor offering pseudo-irreversible allosteric binding to mutant KRAS with a prolonged residence time
- Sub-nM to single-digit nM cell potency as measured by cell proliferation and pERK inhibition were achieved across cell lines with KRAS G12D, G12V, G12C, and G12A, G12F, G12S, G13D, and K117N, etc.
- Oral bioavailability was demonstrated across species
- Anti-tumor efficacy was demonstrated in CDX tumor models with KRAS G12D, G12V, or G12C mutation with regression observed at ≥50 mg/kg BID dose
- BH-501242 was well tolerated as observed in the CDX tumor model studies
- Further profiling of BH-501242 and close analog BH-501352 is ongoing

Note: All reference compounds in this poster are proxy ones from commercial vendors.

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