BBO-8520, a first-in-class, direct, and covalent small molecule inhibitor of GTP-bound (ON) and GDP-bound (OFF) KRAS^{G12C}, demonstrates robust efficacy and compares favorably to GDP-bound KRAS^{G12C} (OFF) only inhibitors in preclinical models

SINGH, K[1]; STICE, JP[1]; SETOODEH, S[1]; LIN, K[1]; SINGH, D[1]; DYBA, M[2]; YANG, Y[3]; SMITH, BP[2]; RABARA, D[2]; LARSEN, EK[2]; SHARMA, AK[2]; CHAN, AH[2]; RANIERI, M[4]; MEYNARDIE, M[4]; ESPOSITO, D[2]; LIGHTSTONE, FC[3]; WONG, KK[4]; SIMANSHU, D[2]; STEPHEN, AG[2]; WANG, K[1]; NISSLEY, DV[2]; MACIAG, AE[2]; SINKEVICIUS, KW[1]; WALLACE, E[1]; WANG, B[1]; MCCORMICK, F[1,5]; BELTRAN, PJ[1]

[1]BridgeBio Oncology Therapeutics, 1 Corporate Drive, South San Francisco, California 94080 USA

[2]NCI RAS Initiative, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, Maryland 21701, USA

[3] Physical and Life Sciences (PLS) Directorate, Lawrence Livermore National Laboratory, Livermore, California 94550, USA

[4] Perlmutter Cancer Center, New York University, New York, NY, USA

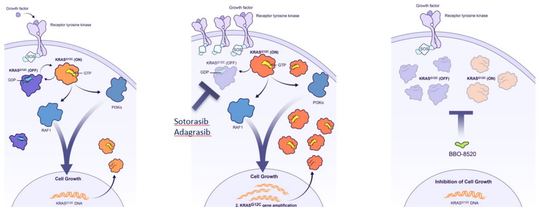
[5] Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, 1450 3rd Street, San Francisco, California 94158, USA

Introduction

Only GTP-bound KRAS^{G12C} activates effectors

Increased GTP state is a MOA of resistance to OFF inhibitors

BBO-8520 inhibits both the ON and OFF states of KRAS^{G12C}



- KRAS is a small GTPase that cycles between GTP-bound (ON) and GDP-bound (OFF) states and activates downstream MAPK and PI3K pathway signaling promoting proliferation, migration, and survival when in an active GTP-bound (ON) state.[1]
- KRAS^{G12C} mutations, found in approximately 14% of non-small cell lung cancers (NSCLCs), 3% of colorectal cancers (CRCs), and 1% of pancreatic ductal adenocarcinomas (PDACs), lead to insensitivity to GAP-mediated hydrolysis, which significantly increases the proportion of KRAS^{G12C} in the active GTP-bound (ON) state and promotes tumor cell growth.[1-2]
- Approved KRAS^{G12C} inhibitors, sotorasib and adagrasib, target the GDP bound (OFF) state and are suboptimal in terms of depth and duration of response, which is believed to stem from cancer cells' ability to increase the amount of drug-insensitive GTP-bound (ON) KRAS^{G12C}.[3]
- Preclinical data suggests combining KRAS^{G12C} inhibitors with inactive (OFF) state and active (ON) state binding are therapeutically more efficacious than either of them alone suggesting a molecule that inhibits both (OFF) and (ON) state may have better therapeutic efficacy.[4]
- To provide optimal KRAS^{G12C} target coverage, we developed BBO-8520, a next generation, potent, selective, orally bioavailable, and direct covalent dual inhibitor of both the active GTP-bound (ON) and inactive GDP-bound (OFF) forms of KRAS^{G12C}.

Methods

Maldi-TOF: Plates with GTP, GppNHp and GDP-loaded KRAS4b^{G12C}/C118S (amino acids 1-169) protein were mixed with defined dilution of tested compounds. Modified protein was measured by MALDI-TOF.

RAS-RAF PPI: A protein:protein interaction (PPI) Homogeneous Time-Resolved Fluorescence (HTRF) assay was used to determine the effectiveness of compounds in disrupting KRAS protein and effector (RAF1) binding. Avi-KRAS^{G12C} (amino acids 2-169) GTP or GppNHp and RAF1 RBD-3xFLAG (amino acids 51-131) were used.

ERK phosphorylation: Cells were seeded, and the next day treated with a titration of BBO-8520. Two hours post-treatment, pERK phosphorylation was assessed by HTRF.

3D viability: Cells were seeded and after 2 days treated with a titration of BBO-8520 for 4-7 days. Cell viability was assessed by 3D CTG.

RAS-RAF ELISA: MIA PaCa-2 cells were treated at 2, 5, 10, 15, 30 or 60 minutes with BBO-8520, sotorasib, adagrasib, or divarasib and luminescence were measured following RAS-RAF ELISA Kit from Abcam.

PK properties: BBO-8520 was administered at single dose of 1 or 3 mg/kg intravenously and 5, 10, 30 or 100 mg/kg orally. Plasma was collected and then PK parameters were assessed.

Pharmacokinetics (PK) and pharmacodynamics (PD) studies: Dose and time response PK/PD analyses was performed in the MIA PaCa-2 subcutaneous Matrigel plug model following a single oral dose of BBO-8520 as indicated. Plasma and tumors were collected for PK and pERK analysis using MSD.

Efficacy studies: BBO-8520 efficacy was assessed following once daily (QD) oral dosing of the indicated dose levels of BBO-8520 in cell line-derived xenograft (CDX) models, patient-derived xenograft (PDX) models, genetically engineered mouse models (GEMM), or liver syngeneic tumor models bearing KRAS^{G12C} mutations. For the MIA PaCa-2 sotorasib-resistant CDX model, tumor-bearing mice were treated daily orally with 10 mg/kg sotorasib until resistance developed (tumors reached ~200 mm^3) and then mice were dosed with the indicated treatments. Anti-PD-1 was administered twice weekly (BIW) as indicated by intraperitoneal administration. Tumor growth inhibition (TGI), mean tumor regression (REG), and number of complete regressions (CR) were calculated.

KRAS amplification assay: Standard methods were used to extract genomic DNA from tumors and measure levels of KRAS amplification using pre-designed ddPCR copy number assay probes for human KRAS and the reference gene RPP30.

Statistical analyses: One-way ANOVA for the PD studies and two-way repeated measures ANOVA for the efficacy studies were performed with Dunnett's test vs the vehicle group or between the indicated groups. Log-rank (Mantel Cox) tests were performed for the survival analyses.

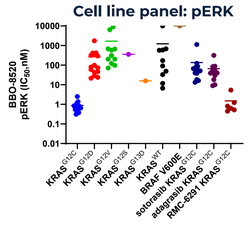
Results

BBO-8520 modifies both GDP- and GTP-bound KRAS^{G12C} and inhibits effector binding

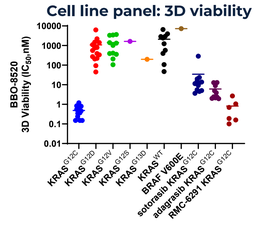
Compounds	MALDI % modification 15 min			PPI: KRAS^{G12C}/RAF1 (μM)		k_{inact}/K_i ($M^{-1}s^{-1}$)	
	GDP	GppNHp	GTP	GppNHp	GTP	GDP	GTP
BBO-8520	100	100	89	0.026	0.10	2,743,000	20,000
Sotorasib	88	0	3	>30	>30	11,000	0
Adagrasib	82	0	1	>30	>30	180,000	0

BBO-8520 shows sub-nanomolar potency in multiple KRAS^{G12C} models and is selective for KRAS^{G12C}

Cell line panel: pERK

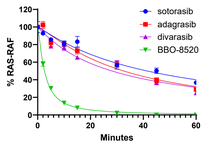


Cell line panel: 3D viability



BBO-8520 maintains potency in active state of KRAS^{G12C}

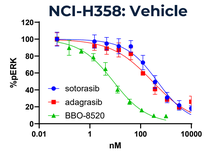
MIA PaCa-2 RAS-RAF ELISA: Measurement of activated (GTP) RAS



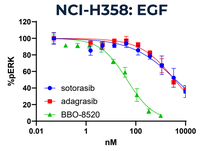
Compound	Time to 50% Inhibition (Minutes)	% sotorasib, Time to 50% Inhibition
sotorasib	24.6	100
adagrasib	26.3	106.8
divarasib	17.8	72.4
BBO-8520	4.1	16.7

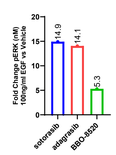
Growth Factor Treatment: Measurement of potency when there is more GTP-bound KRAS^{G12C}

NCI-H358: Vehicle



NCI-H358: EGF



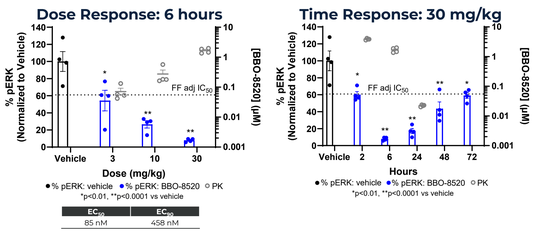


BBO-8520 has a favorable ADME and PK profile and is orally bioavailable

Parameter	BBO-8520
Whole blood stability $T_{1/2}$ minutes H / C / D / R / M	> 371 for all species
Mouse: Cl, $T_{1/2}$, Vss, F	5 mL/min/kg, 2.7 hr, 1.0 L/kg, 37%
Rat: Cl, $T_{1/2}$, Vss, F	28 mL/min/kg, 3.4 hr, 7.0 L/kg, 14%
Dog: Cl, $T_{1/2}$, Vss, F	16 mL/min/kg, 4.1 hr, 4.1 L/kg, 23%
Minipig: Cl, T½ , Vss, F	64 mL/min/kg, 2.6 hr, 7.8 L/kg, 48%
Cyno: Cl, $T_{1/2}$, Vss, F	30 mL/min/kg, 2.6 hr, 3.7 L/kg, 6%

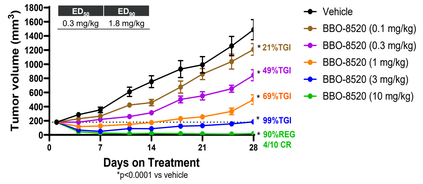
BBO-8520 demonstrates dose- and time-dependent Inhibition of pERK in PD studies

MIA PaCa-2 (KRAS^{G12C}) PDAC Matrigel Plug Model

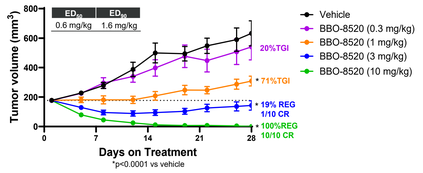


BBO-8520 shows robust dose dependent efficacy in PDAC and NSCLC KRAS^{G12C} CDX models and GEM models

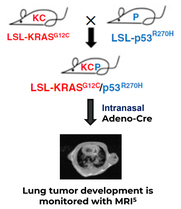
MIA PaCa-2 (KRAS^{G12C}) PDAC CDX Model



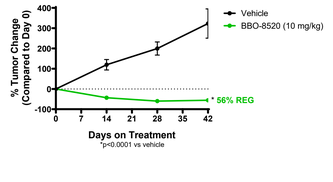
NCI-H358 (KRAS^{G12C}) NSCLC CDX Model



KRAS^{G12C}; Tp53^{R270H} (KCP) NSCLC GEMM

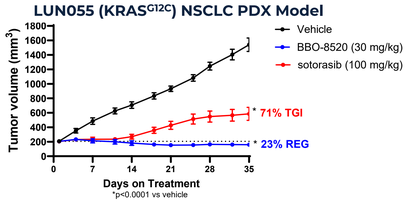


Lung tumor development is monitored with MRI[5]



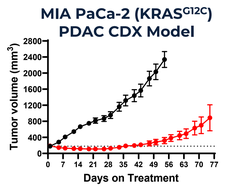
BBO-8520 maintains potency in the LUN055 PDX model, a RET overexpressing, KRAS^{G12C} GTP bound (ON) model

LUN055 (KRAS^{G12C}) NSCLC PDX Model

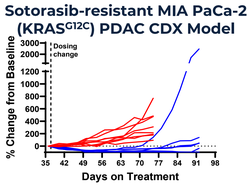


BBO-8520 retains activity in sotorasib-resistant CDX models with KRAS amplification

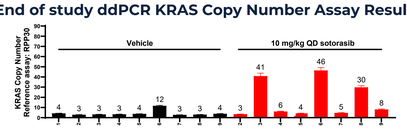
MIA PaCa-2 (KRAS^{G12C}) PDAC CDX Model



Sotorasib-resistant MIA PaCa-2 (KRAS^{G12C}) PDAC CDX Model

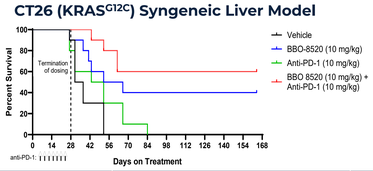


End of study ddPCR KRAS Copy Number Assay Results



BBO-8520 + anti-PD-1 cures 60% of mice bearing the KRAS^{G12C} CT26 syngeneic liver tumors

CT26 (KRAS^{G12C}) Syngeneic Liver Model



Group (n=10)	Day 164			Median Overall Survival (day)
	p value vs vehicle	p value vs combo	Survival	
Vehicle	-	-	0%	34
BBO-8520 (10 mg/kg)	0.0240	0.1932	40%	59
Anti-PD-1 (10 mg/kg)	0.3952	0.0033	0%	48
BBO-8520 (10 mg/kg) + Anti-PD-1 (10 mg/kg)	0.0001	-	60%	Undefined

Conclusions

- BBO-8520 is a dual inhibitor of both the active GTP-bound (ON) and inactive GDP-bound (OFF) forms of KRAS^{G12C}.
- BBO-8520 is highly potent and selective for KRAS^{G12C} with superior K_{inact}/K_I
- BBO-8520 shows sub-nM IC_{50} in KRAS^{G12C} driven cancer cell lines in pERK and 3D viability in vitro assays and maintains potency in active state of KRAS^{G12C}.
- BBO-8520 demonstrates strong PD and efficacy in KRAS^{G12C} models and enhanced target coverage prevents adaptive resistance mechanisms to KRAS^{G12C} (OFF) inhibitors.
- The Phase 1a/1b ONKORAS-101 trial in KRAS^{G12C} positive non-small cell lung cancer is currently ongoing (NCT06343402).

References and Acknowledgements

1. Moore et al, RAS-targeted therapies: is the undruggable drugged?, Nat Rev Drug Discov, 2020, 19(8):533-552
2. Ostrem et al, K-Ras (G12C) inhibitors allosterically control GTP affinity and effector interactions, Nature, 2013, 503(7477): 548-551
3. Ryan et al, KRAS^{G12C}-independent feedback activation of wild-type RAS constrains KRAS^{G12C} inhibitor efficacy. Cell Reports, 2022, 39(12): 110993
4. Nokin et al. 2024, RAS-ON inhibition overcomes clinical resistance to KRAS G12C-OFF covalent blockade, 2024, Nat Commun, 15:7554
5. Li et al, Assessing therapeutic efficacy of MEK Inhibition in a KRAS^{G12C}-Driven Mouse Model of Lung Cancer, Clin Cancer Res, 2018, 24:4854-4864

This work was performed in collaboration with:

RAS INITIATIVE — Lawrence Livermore National Laboratory — NYU Langone Health