BBO-11818: an orally bioavailable, highly potent and selective non-covalent pan-KRAS(ON) and (OFF) inhibitor with robust anti-tumor activity in KRAS-mutant preclinical models

Stahlhut C.[1], Maciag A.E.[2], Sullivan K.[1#], Singh K.[1#], Gitego N.[1#], Zhang Z.[1], Chan A.[2], Sharma A. K.[2], Alexander P.[2], Shu J.[1], Yang Y.[1], Rigby M.[2], Ma R.[2], Setoodeh S.[1], Smith B.P.[2], Pei J.[3], Rabara D.[2], Larsen E.K.[2], Turner D.[2], Zhang C.[1], Feng C.[1], Feng S.[1], Stice J.P.[1], Xu R.[1], Lin K.[1], Stephen A.G.[2], Lightstone F.C.[3], Ji C.[1], Wang K.[1], Simanshu D.K.[2], Nissley D.V.[2], Wallace E.[1], Wang B.[1], Sinkevicius K.W.[1], McCormick F.[2,4], Beltran P.J.[1]

1- BridgeBio Oncology Therapeutics, South San Francisco, California.
2- NCI RAS Initiative, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, Maryland
3- Physical and Life Sciences (PLS) Directorate, Lawrence Livermore National Laboratory, Livermore, California
4- Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, California

# Equal contribution

Overview

- Oncogenic variants of KRAS drive tumor growth and metastasis through aberrant signaling, making them important therapeutic targets.[1] Inhibitors against KRAS^{G12C} have recently been approved, but a major clinical need for agents against other KRAS variants remains.[1, 2]
- We have developed BBO-11818: a potent, selective, orally bioavailable non-covalent KRAS inhibitor with activity against multiple KRAS mutants, including KRAS^{G12D} and KRAS^{G12V}.
- BBO-11818 targets KRAS in both its inactive GDP-bound and active GTP-bound states, potently suppressing MAPK signaling and inhibiting cell proliferation in KRAS-mutant cell lines.
- BBO-11818 monotherapy induces strong anti-tumor responses, including strong dose- and time-dependent inhibition of pERK and regressions at well-tolerated doses in CDX models of KRAS-mutant pancreatic, non-small cell lung, and colorectal cancer.
- In combination with BBO-10203, a selective RAS:PI3Kα breaker that blocks RAS-mediated activation of AKT; or cetuximab, an anti-EGFR monoclonal antibody, BBO-11818 shows significantly enhanced efficacy in CDX models harboring KRAS^{G12D} or KRAS^{G12V} mutations. Similarly, the combination of BBO-11818 and anti-PD-1 antibody improves survival in a KRAS^{G12D} syngeneic model.

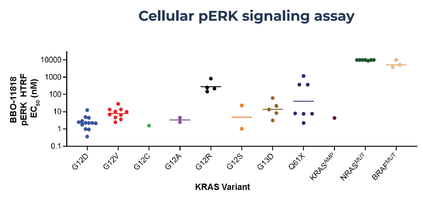
Methods

SPR: Surface plasmon resonance direct binding assays to determine affinity of BBO-11818 to GppNHp- or GDP-loaded avi-tagged KRAS proteins were performed.
Protein:protein interaction: A PPI Homogeneous Time-Resolved Fluorescence (HTRF) assay was used to determine compound effectiveness in disrupting KRAS protein and effector (RAF1) binding.
ERK phosphorylation. Cells were seeded and the next day treated with BBO-11818. Two hours post-treatment, pERK phosphorylation was assessed by HTRF.
3D viability. Cells were seeded and treated with BBO-11818 three days post-seeding after spheroid formation. Four days post-treatment, viability was assessed with the CellTiter-Glo viability assay.
Long-term 2D clonogenic assay. Cells were seeded, treated 24 hours later with BBO-11818, BBO-10203 (PI3Kα:RAS breaker) or cetuximab and incubated for 15 or 20 days. Media and compounds were changed biweekly. Confluence was measured twice daily using an Incucyte Live-Cell Analysis System.
Pharmacokinetics (PK) and pharmacodynamics (PD). Dose and time response PK/PD analyses were performed following a single oral dose of BBO-11818. Plasma and tumors were collected for PK and pERK analysis using the MesoScale Discovery platform.
***In vivo* efficacy and survival studies.** BBO-11818 efficacy was assessed following twice daily (BID) oral dosing at the indicated dose levels in cell line-derived xenograft (CDX) or syngeneic models bearing KRAS^{G12D} or KRAS^{G12V} mutations. BBO-10203 was dosed orally once daily (QD). Anti-PD-1 or cetuximab were administered twice weekly (BIW) by intraperitoneal administration. Tumor growth inhibition (TGI), mean tumor regression (REG), and number of complete regressions (CR) were calculated.
BrdU incorporation and cleaved caspase-3 assays. Capan-2 tumor-bearing mice were dosed with a single oral dose of the indicated treatments and 50 mg/kg BrdU intraperitoneally 2 hours prior to tumor collection at the indicated timepoints. Formalin-fixed tumors were prepared and sectioned. Immunohistochemistry (IHC) for BrdU and cleaved caspase-3 was performed, and positive staining for BrdU and cleaved caspase-3 was quantitated to measure levels of tumor cell proliferation and apoptosis, respectively.
Statistical analyses: Two-way repeated measures ANOVA followed by post hoc Tukey's multiple comparison test through day 15 or 16 were performed for clonogenic assays. One-way ANOVA for PD and IHC studies and two-way repeated measures ANOVA for *in vivo* efficacy studies were performed with Dunnett's test vs the vehicle group or between the indicated groups.

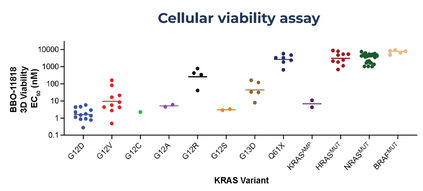
BBO-11818 is a potent and selective pan-KRAS binder and KRAS:RAF1 PPI inhibitor

		BBO-11818	
	RAS Allele	**GppNHp**	**GDP**
RAS SPR, K_D (nM)	KRAS^{G12D}	7.40	<0.003
	KRAS^{G12V}	13.2	0.370
	KRAS^{G13D}	17.5	0.300
	KRAS^{WT}	20.0	0.250
	NRAS^{WT}	>200,000	2460
	HRAS^{WT}	>200,000	831
PPI: KRAS(GTP)/RAF1 effector, IC_{50} (nM)	KRAS^{G12D}	28	
	KRAS^{G12V}	61	
	KRAS^{G12C}	47	
	KRAS^{G12R}	51	
	KRAS^{WT}	120	

BBO-11818 inhibits ERK phosphorylation and cell proliferation in KRAS-mutant cell lines



KRAS variant	Mean EC_{50} (nM)
KRAS^{G12D}	3.08
KRAS^{G12V}	9.91
KRAS^{G12C}	1.53
KRAS^{G12A}	3.46
KRAS^{G12R}	357
KRAS^{G12S}	11.9
KRAS^{G13D}	21.7
KRAS^{Q61X}	278
KRAS^{AMP}	4.35
NRAS^{MUT}	> 10 µM
BRAF^{MUT}	> 10 µM



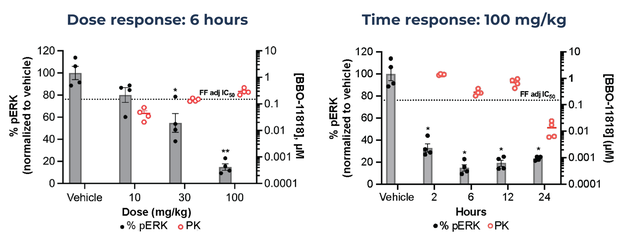
KRAS variant	Mean EC_{50} (nM)
KRAS^{G12D}	2.21
KRAS^{G12V}	31.2
KRAS^{G12C}	2.26
KRAS^{G12A}	5.32
KRAS^{G12R}	400
KRAS^{G12S}	3.09
KRAS^{G13D}	71.7
KRAS^{Q61X}	3,170
KRAS^{AMP}	7.62
HRAS^{MUT}	4,030
NRAS^{MUT}	3,720
BRAF^{MUT}	7,430

BBO-11818 has a favorable ADME and PK profile and is orally bioavailable

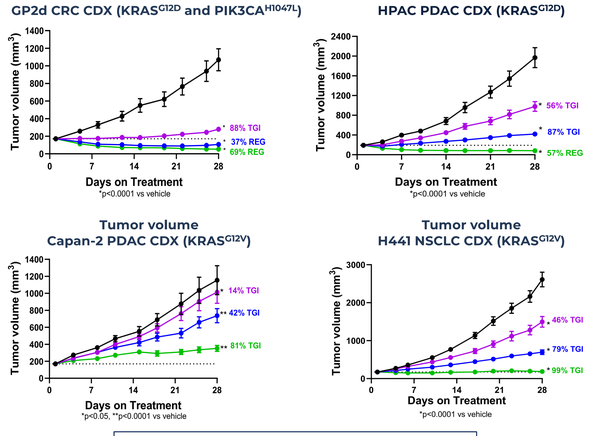
Parameter	BBO-11818
Mouse PK CL (mL/min/kg) / V (L/kg) / % F	45 / 4.9 / 18
Rat PK CL (mL/min/kg) / V (L/kg) / % F	30 / 7.8 / 16
Dog PK CL (mL/min/kg) / V (L/kg) / % F	11 / 5.8 / 28
Minipig PK CL (mL/min/kg) / V (L/kg) / % F	47 / 7.8 / 27
Selectivity: hERG & safety panel	No red flags
Minimal DDI liabilities for combinations	No predicted DDI issues

BBO-11818 demonstrates dose- and time-dependent inhibition of pERK in a KRAS^{G12D} model

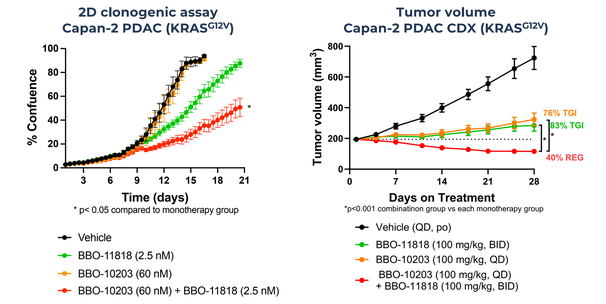
HPAC PDAC CDX (KRAS^{G12D})



BBO-11818 demonstrates efficacy in KRAS^{G12D} and KRAS^{G12V} CDX models

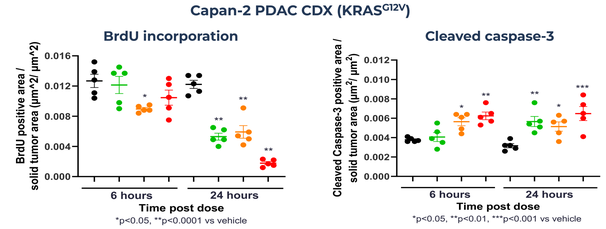


BBO-11818 and BBO-10203 (RAS:PI3Kα breaker) show a combination effect *in vitro* and *in vivo*

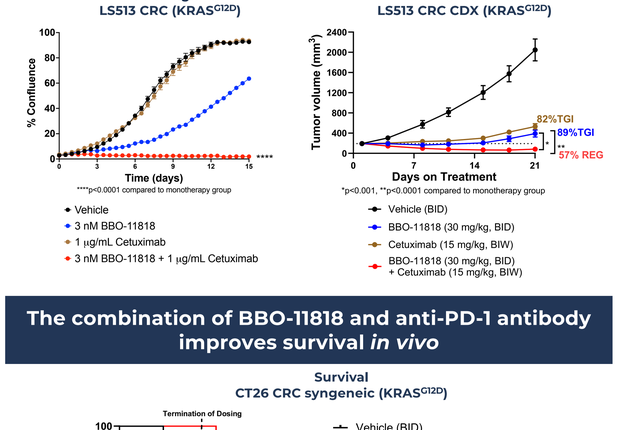


Efficacy of BBO-11818 and BBO-10203 combination is driven by decrease in tumor cell proliferation and increase in apoptosis

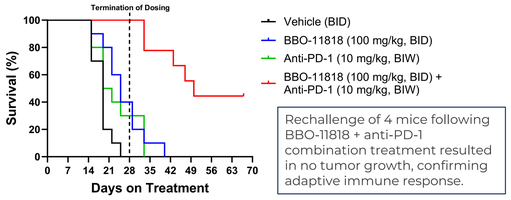
Capan-2 PDAC CDX (KRAS^{G12V})



BBO-11818 and EGFR inhibitors demonstrate combination benefit *in vitro* and *in vivo*



The combination of BBO-11818 and anti-PD-1 antibody improves survival *in vivo*



Rechallenge of 4 mice following BBO-11818 + anti-PD-1 combination treatment resulted in no tumor growth, confirming adaptive immune response.

Conclusion

- BBO-11818 is a potent pan-KRAS inhibitor targeting both GTP-bound and GDP-bound forms of KRAS, with good selectivity over HRAS and NRAS.
- BBO-11818 potently inhibits ERK phosphorylation and proliferation in KRAS-dependent cell lines *in vitro*.
- BBO-11818 has favorable PK and oral bioavailability and shows dose- and time-dependent inhibition of pERK in *in vivo* PD studies.
- BBO-11818 demonstrates robust *in vivo* efficacy in KRAS^{G12D} and KRAS^{G12V} CDX models.
- BBO-11818 exhibits combination effect with the RAS:PI3Kα breaker BBO-10203 and cetuximab *in vitro* and in CDX models.
- The efficacy of the BBO-11818 and BBO-10203 combination is driven by a robust decrease in tumor cell proliferation and increase in apoptosis.
- BBO-11818 also shows a combination benefit with anti-PD-1 treatment, resulting in complete tumor regressions in the CT26 syngeneic model.
- The Phase 1a/1b KONQUER-101 study (NCT06917079) has been initiated and is enrolling patients globally with KRAS G12A, G12C, G12D, G12S, or G12V mutation, or KRAS-amplification.

References and acknowledgements

1- Prior, Ian A., Fiona E. Hood, and James L. Hartley. The frequency of Ras mutations in cancer. *Cancer research 2020*;80(14): 2969-2974.
2- Liu J, Kang R, Tang D. The KRAS-G12C inhibitor: activity and resistance. *Cancer gene therapy. 2022*;29(7):875-8.

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