BBO-11818, an orally bioavailable, highly potent and non-covalent pan-KRAS inhibitor demonstrates robust anti-tumor activity in KRAS-mutant preclinical models

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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.

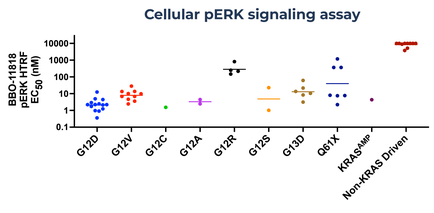
Methods

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 14 or 15 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 14 or 15 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.

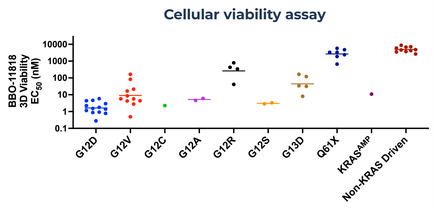
BBO-11818 is a potent and selective pan-KRAS binder and KRAS:RAF1 PPI inhibitor

		BBO-11818	
	RAS Allele	**GppNHp**	**GDP**
RAS SPR, K_D (nM)	$KRAS^{G12D}$	17	<0.01
	$KRAS^{G12V}$	18	0.031
	$KRAS^{G13D}$	26	0.28
	$KRAS^{WT}$	40	0.23
	$NRAS^{WT}$	>20000	2700
	$HRAS^{WT}$	>20000	880
PPI: KRAS(GTP)/RAF1 effector, IC_{50} (nM)	$KRAS^{G12D}$	28	
	$KRAS^{G12V}$	59	
	$KRAS^{G12C}$	46	
	$KRAS^{G12R}$	49	
	$KRAS^{WT}$	117	

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



KRAS variant	Mean EC_{50} (nM)
$KRAS^{G12D}$	2.95
$KRAS^{G12V}$	9.95
$KRAS^{G12C}$	1.53
$KRAS^{G12A}$	3.46
$KRAS^{G12R}$	357
$KRAS^{G12S}$	11.9
$KRAS^{G13D}$	19.8
$KRAS^{Q61X}$	278
$KRAS^{AMP}$	4.35
Non-KRAS Driven	> 10 μM

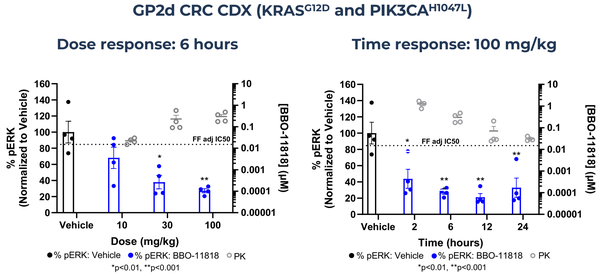


KRAS variant	Mean EC_{50} (nM)
$KRAS^{G12D}$	2.21
$KRAS^{G12V}$	28.9
$KRAS^{G12C}$	2.26
$KRAS^{G12A}$	5.32
$KRAS^{G12R}$	400
$KRAS^{G12S}$	3.09
$KRAS^{G13D}$	71.7
$KRAS^{Q61X}$	3170
$KRAS^{AMP}$	10.9
Non-KRAS Driven	5,220

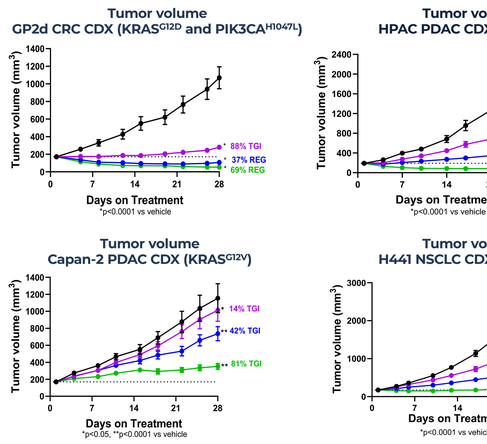
BBO-11818 has a favorable ADME and PK profile and is orally bioavailable

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

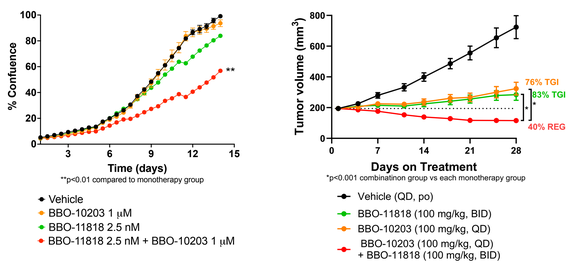
BBO-11818 demonstrates dose- and time-dependent inhibition of pERK in a $KRAS^{G12D}$ model



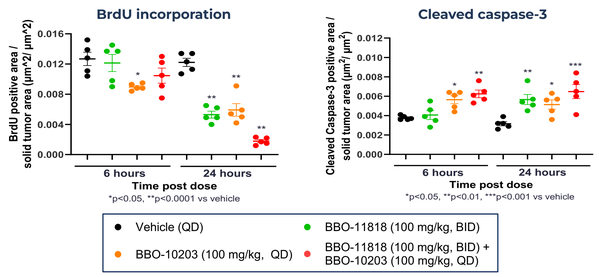
BBO-11818 demonstrates efficacy in $KRAS^{G12D}$ and $KRAS^{G12V}$ CDX models



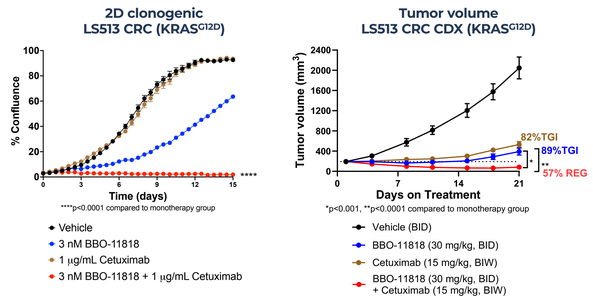
BBO-11818 and BBO-10203 (RAS:PI3Kα breaker) show combination effect *in vitro* and *in vivo*



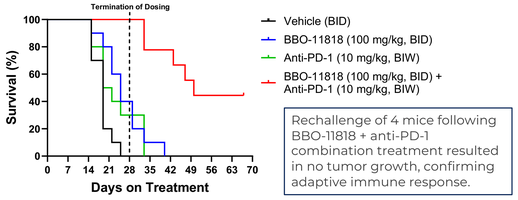
Efficacy of BBO-11818 and BBO-10203 combination is driven by decrease in tumor cell proliferation and increase in apoptosis



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 will enroll patients globally with $KRAS^{G12D}$ or $KRAS^{G12V}$ tumors.

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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