BBO-11818, an orally bioavailable, highly potent and non-covalent pan-KRAS inhibitor demonstrates robust anti-tumor activity in KRAS^{G12D} and KRAS^{G12V} preclinical models

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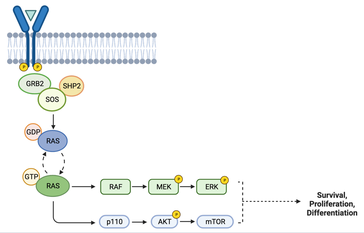
Background

- RAS is the most frequently mutated oncogene in cancer, and KRAS is the most mutated protein in the RAS family. Oncogenic variants of KRAS drive tumor growth and metastasis through aberrant signaling.[1]
- Inhibitors against KRAS^{G12C} have only recently been approved, and there is a major unmet clinical need for other RAS variants including KRAS^{G12D} and KRAS^{G12V}.[1, 2]
- BBO-11818 is a small molecule inhibitor against multiple oncogenic KRAS variants.
- BBO-11818 targets both GDP- and GTP-bound KRAS, shows high potency against KRAS^{G12D} and KRAS^{G12V} and has favorable PK/PD profile and efficacy *in vitro* and *in vivo*.



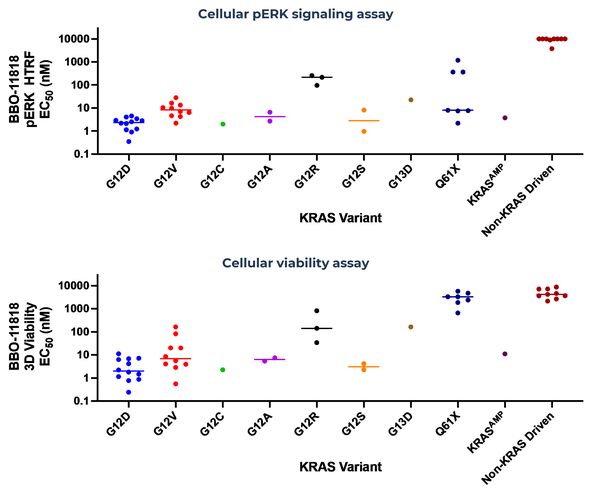
BBO-11818 is a potent and selective pan-KRAS:RAF1 PPI inhibitor

		BBO-11818	
	RAS Allele	GppNHp	GDP
RAS SPR, KD (nM)	KRAS G12D	17	<0.1
	KRAS G12V	18	<0.1
	KRAS G13D	23	0.28
	KRAS WT	40	0.23
	NRAS WT	>100000	270
	HRAS WT	>100000	88
PPI: KRAS(GTP)/RAF1 effector, IC_{50} (nM)	KRAS G12D	65	
	KRAS G12V	86	
	KRAS G12C	84	
	KRAS G12R	170	
	KRAS WT	160	

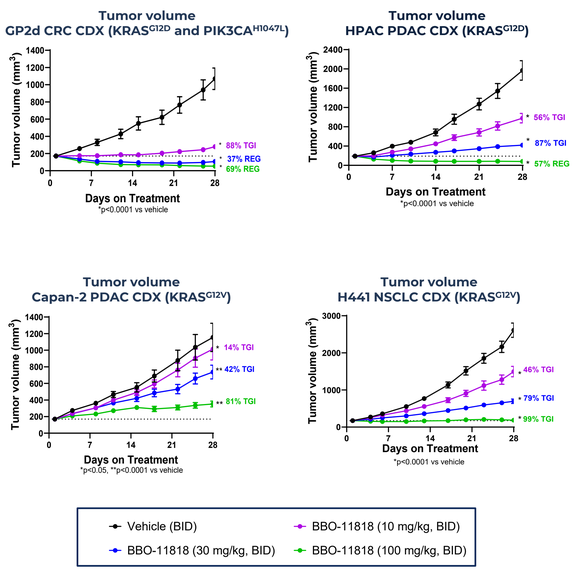
Methods

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 3 days post-seeding after the formation of spheroids. 4 days post-treatment, viability was assessed by CTG.
Long-term 2D clonogenic assay. Cells were seeded and the next day treated with BBO-11818, BBO-10203 (PI3Kα:RAS breaker) and cetuximab and incubated for 14 or 15 days. Media and compounds were changed biweekly. Confluence was measured twice daily by Incucyte Live-Cell Analysis System.
Pharmacokinetics (PK) and pharmacodynamics (PD). Dose and time response PK/PD analyses were performed in the GP2d subcutaneous tumor model following a single oral dose of BBO-11818. Plasma and tumors were collected for PK and pERK analysis using MSD.
***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 efficacy studies were performed with Dunnett's test vs the vehicle group or between the indicated groups.

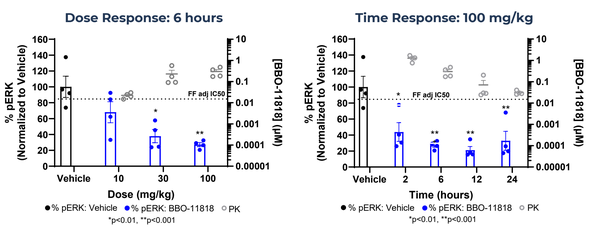
BBO-11818 inhibits ERK phosphorylation and cell proliferation in KRAS-mutant cell lines



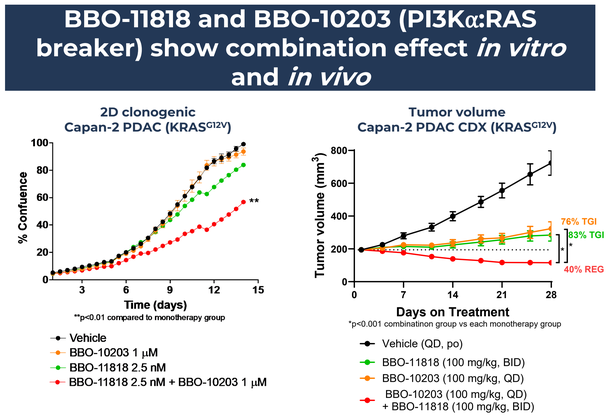
BBO-11818 is efficacious in KRAS^{G12D} and KRAS^{G12V} CDX models



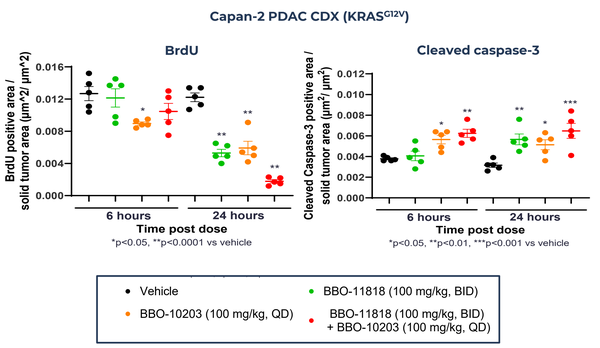
BBO-11818 *in vivo* pharmacokinetics and pharmacodynamics



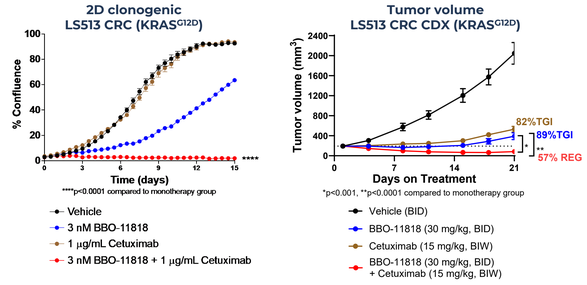
BBO-11818 and BBO-10203 (PI3Kα:RAS breaker) show combination effect *in vitro* and *in vivo*



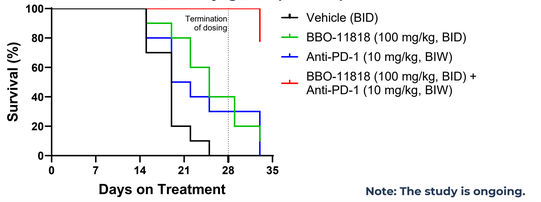
Efficacy of BBO-11818 and BBO-10203 combination is driven by a decrease in tumor cell proliferation and increase in apoptosis



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



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



Note: The study is ongoing.

Conclusion

- BBO-11818 is a potent pan-KRAS inhibitor targeting both "ON" (GTP-bound) and "OFF" (GDP-bound) forms of KRAS, with good selectivity over HRAS and NRAS.
- BBO-11818 potently inhibits ERK phosphorylation and cell proliferation of KRAS-dependent tumors *in vitro*.
- BBO-11818 has favorable PK and oral bioavailability and demonstrates robust *in vivo* efficacy in KRAS^{G12D} and KRAS^{G12V} CDX models.
- BBO-11818 demonstrates dose- and time-dependent inhibition of pERK in PD studies.
- BBO-11818 exhibits combination effect with the PI3Kα:RAS breaker BBO-10203 and cetuximab in KRAS^{G12D} and KRAS^{G12V} *in vitro* and CDX models.
- Efficacy of BBO-11818 and BBO-10203 combination is driven by a robust decrease in tumor cell proliferation and increase in apoptosis.
- The combination of BBO-11818 and anti-PD-1 antibody improves survival in a KRAS^{G12D} syngeneic model.
- IND submission is planned in 2024.

References and acknowledgements

- Prior, Ian A., Fiona E. Hood, and James L. Hartley. The frequency of Ras mutations in cancer. *Cancer research 2020*;80(14): 2969-2974.
- 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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- This work was performed in collaboration with FNL and LLNL.

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