BBO-10203, a first-in-class, orally bioavailable, selective breaker of the RAS:PI3Kα interaction inhibits tumor growth alone and in combination with KRAS inhibitors in KRAS mutant models without inducing hyperglycemia

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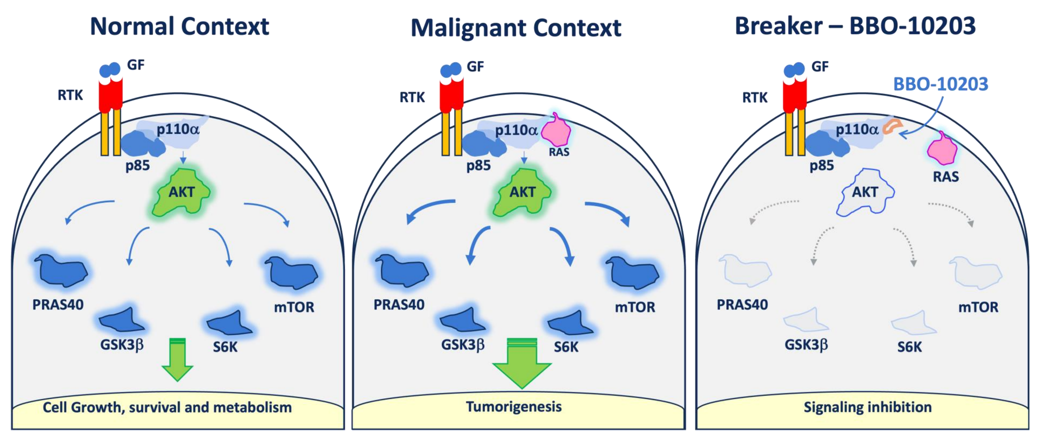
Introduction



- Aberrant activation of the PI3Kα pathway is one of the most frequent oncogenic events across human cancers and leads to promotion of tumor cell growth, survival, glucose metabolism, and acute resistance to numerous standard of care cancer therapies. While PI3Kα kinase inhibitors have been developed, a significant unmet medical need remains due to dose-limiting, on-target hyperglycemia, which can restrict target coverage, limit the number of eligible patients, and shorten the duration of treatment.
- An alternative novel strategy is to block RAS-mediated activation of PI3Kα, a signaling event prevalent mostly in malignant cells. Previous elegant preclinical studies have established that RAS activation of PI3Kα is important in tumor cells but may not be involved in normal cell types controlling glucose metabolism[1-3].
- Here, we report on BBO-10203, a first-in-class small molecule which breaks the protein-protein interaction between RAS and PI3Kα and inhibits RAS-mediated activation of the PI3Kα pathway[4].
- In addition, we evaluate the combination of BBO-10203 with the dual GTP-bound (ON) and GDP-bound (OFF) $KRAS^{G12C}$ inhibitor BBO-8520 (NCT06343402) or panKRAS inhibitor BBO-11818 (NCT06917079), since the combination of a KRAS inhibitor with a PI3Kα pathway inhibitor may maximize the response rate and reduce the development of adaptive resistance mechanisms due to full inhibition of both MAPK and PI3Kα signaling.

Materials and Methods

MALDI-TOF MS: Plates with PI3Kα protein (amino acids 157–299; RBD (RAS binding domain)) were mixed with defined dilutions of BBO-10203 and modified protein was measured using MALDI-TOF.
Isothermal Titration Calorimetry: GMPPNP-bound KRAS4b, HRAS, and NRAS (amino acids 1-169) protein was loaded into the syringe while either apo (unbound) PI3Kα-RBD or PI3Kα-RBD tethered to BBO-10203 was loaded into the cell and ITC experiments were performed.
Target engagement: BT-474 cells were treated with a titration of BBO-10203 for 4 hours and target engagement of BBO-10203 was measured through a customized MSD assay using a biotinylated breaker probe.
AKT phosphorylation: Cells were seeded, and the next day treated with a titration of BBO-10203. Four hours post-treatment, AKT phosphorylation was assessed by HTRF.
k_{inact}/K_I: BT-474 cells were treated with a titration of BBO-10203 at timepoints from 5 minutes to 4 hours and assayed for pAKT levels using HTRF. A linear regression of the natural logarithm of pAKT (%) versus incubation time was made to determine the negative slope observed rate constant (k_{obs}) for each BBO-10203 concentration, which represents the slope k_{obs}. k_{inact} and K_I were calculated using non-linear regression analysis to fit to a user-defined equation, $k_{obs} = k_{inact} * I / (K_I + I)$ where I is the BBO-10203 concentration.
Pharmacokinetic (PK) properties: BBO-10203 was administered at single dose to mice and dogs intravenously (3 mg/kg for mice and 0.5 mg/kg for dogs) and at the indicated dose levels orally. Plasma was collected and then PK parameters were assessed.
PK and pharmacodynamics (PD) studies: Dose and time response PK/PD analyses were performed in the KYSE-410 subcutaneous cell line-derived (CDX) model following a single oral dose of BBO-10203 as indicated (n=4 per group). Plasma and tumors were collected for PK analysis and pAKT analysis using MSD.
Oral glucose tolerance test study: Male C57BL/6 mice were fasted for 16 hours. Mice were randomized (n=6 per group) by fasted blood glucose levels one hour prior to oral administration of a single dose of vehicle or compounds. Fasted blood glucose levels were measured 60 mins later and then all animals were orally administered 2 g/kg glucose to begin the oGTT. Blood glucose measurements were performed at the indicated timepoints following the glucose dose and c-peptide measurements were performed using an ELISA with serum collected at 150 minutes following the glucose dose.
Efficacy studies: When subcutaneous CDX, patient-derived xenograft (PDX), or genetically engineered mouse (GEM) model tumors reached a mean size of ~180 mm^3, mice were randomized (n=10 per group) and dosed with vehicle (formulation buffer), the indicated dose levels of BBO-10203 (QD, po), BBO-8520 (QD, po), BBO-11818 (BID, po), or the indicated combinations. Tumor volumes were measured two times per week.
BrdU incorporation and cleaved caspase-3 assays. H2122 or Capan-2 tumor-bearing mice were dosed for a single day with 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.
In vivo study statistical analyses: One-way ANOVA followed by post hoc Dunnett's multiple comparisons to the vehicle group were performed for the PD, oGTT, and BrdU/cleaved caspase-3 IHC assay studies. Two-way repeated measures ANOVA were performed for the efficacy studies between the indicated groups.

BBO-10203 covalently binds PI3Kα on cysteine 242 in the RBD, breaking the interaction of PI3Kα with RAS

Assay	BBO-10203
PI3Kα-RBD MALDI-TOF MS (% modified)	>90% at 15 min
Isothermal Titration Calorimetry	No binding of KRAS/HRAS/NRAS to BBO-10203 tethered PI3Kα
Full target engagement of PI3Kα RBD	30 nM
KYSE-410 pAKT (IC_{50})	4 nM
k_{inact}/K_I	7,100 $M^{-1}S^{-1}$

RBD: RAS binding domain; KYSE-410: Esophageal carcinoma $HER2^{amp}/KRAS^{G12C}$ cell line; Note: The crystal structure of PI3Kα RBD complexed with BBO-10203 showed covalent engagement with cysteine 242 via an acrylamide warhead[4].

BBO-10203 is orally bioavailable and oral administration results in robust dose- and time-dependent inhibition of pAKT and efficacy

In Vivo PK Results

Species	Parameters	BBO-10203
Mouse	IV Cl (mL/min/kg) / $t_{1/2}$ (hr) / V_{ss} (L/kg)	26 / 0.86 / 1.2
	%F @ 30 / 100 / 300 / 600 / 1000 mg/kg PO	24 / 31 / 30 / 25 / 38
Dog	IV Cl (mL/min/kg) / $t_{1/2}$ (hr) / V_{ss} (L/kg)	16 / 6.9 / 3.7
	%F @ 10 / 30 / 100 mg/kg PO	63 / 63 / 82

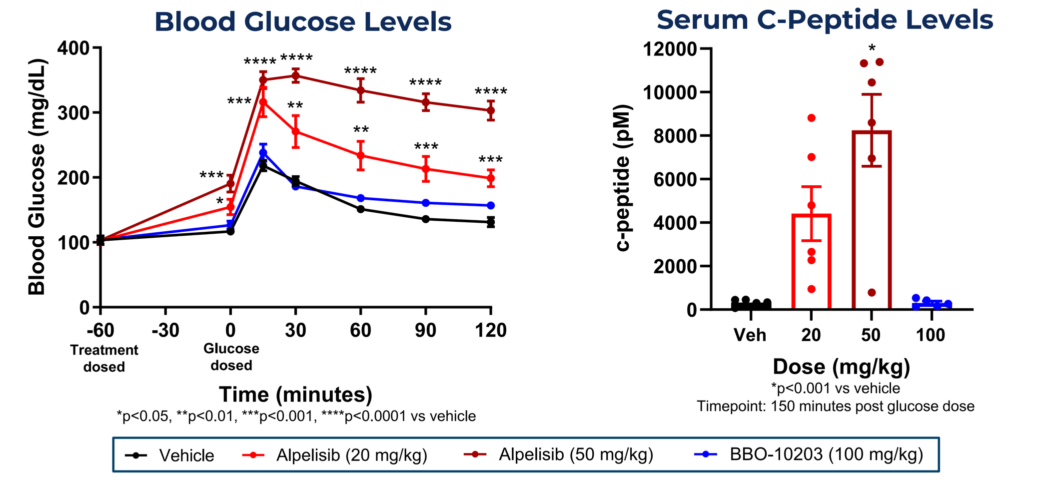
IV: intraveneous; Cl: clearance; $t_{1/2}$: half life; Vss: volume of distribution; %F: oral bioavailability; PO: oral

In Vivo PK/PD and Efficacy Results

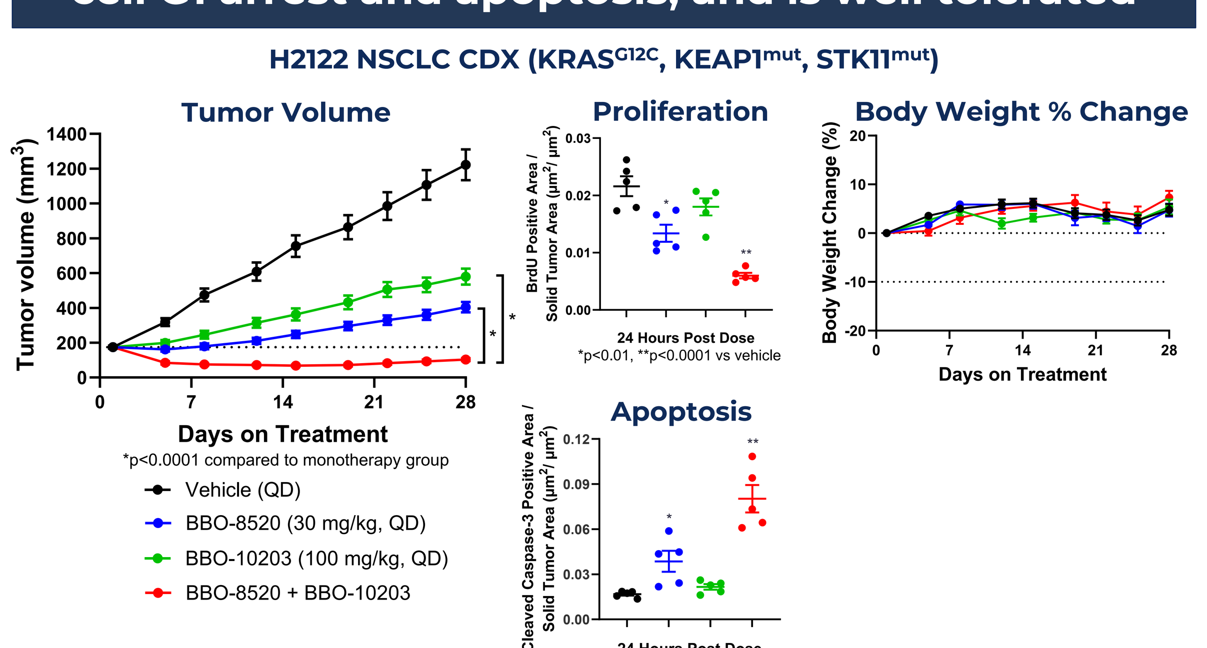
Model	Assay	BBO-10203
KYSE-410	Dose response PK/PD	EC_{50}: 45 nM, EC_{90}: 720 nM
	Time response PK/PD	70% pAKT suppression at 24 hours post 30 mg/kg dose
	Efficacy	ED_{50}: 2.5 mg/kg, ED_{90}: 4.0 mg/kg

KYSE-410: Esophageal carcinoma $HER2^{amp}/KRAS^{G12C}$ cell line

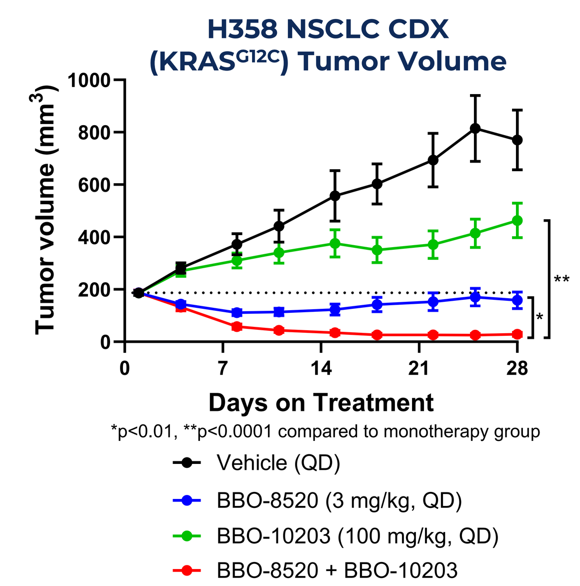
BBO-10203 does not induce hyperglycemia or hyperinsulinemia in an oral glucose tolerance test

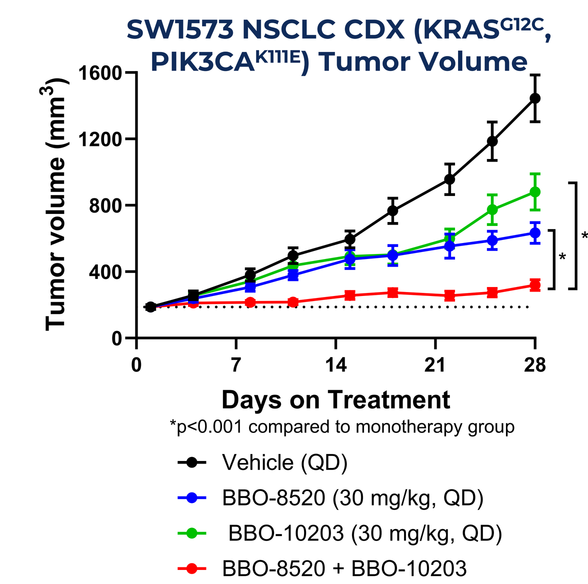


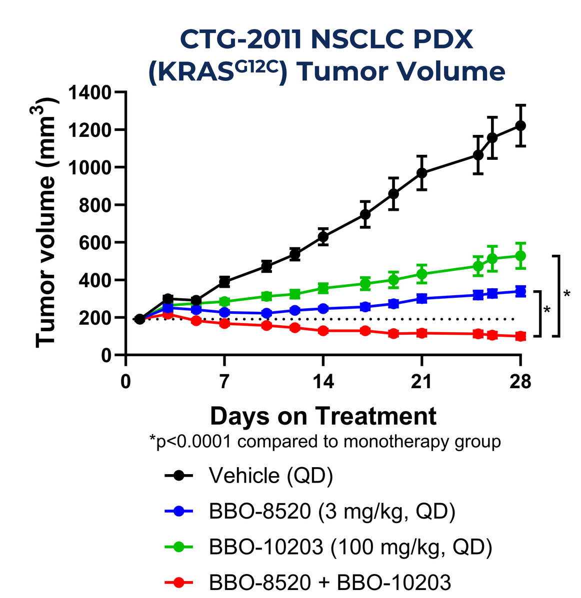
The combination of BBO-8520 and BBO-10203 shows robust activity, drives regressions by inducing tumor cell G1 arrest and apoptosis, and is well tolerated

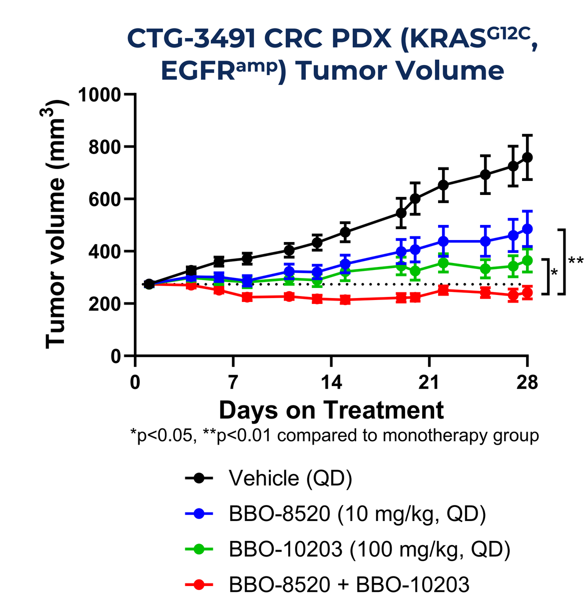


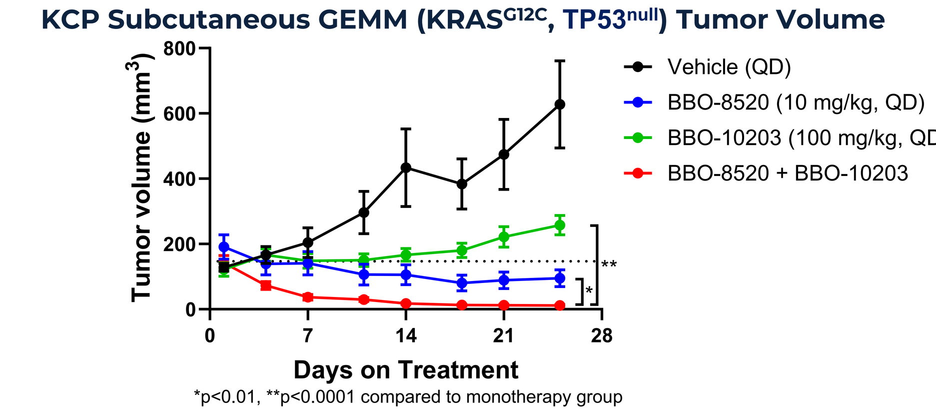
BBO-10203 shows monotherapy activity and strong combination activity with BBO-8520 in $KRAS^{G12C}$ CDX, PDX, and GEM models



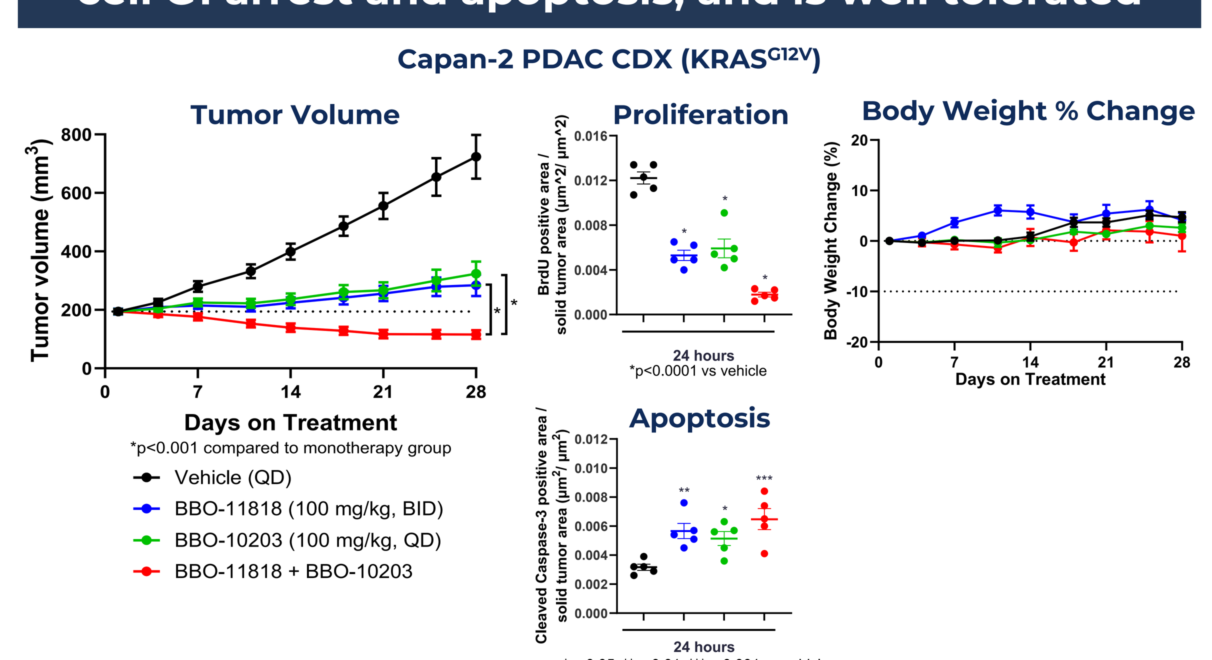




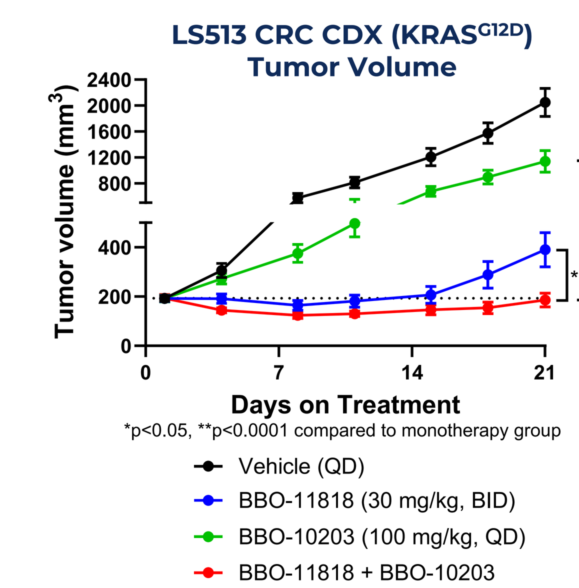


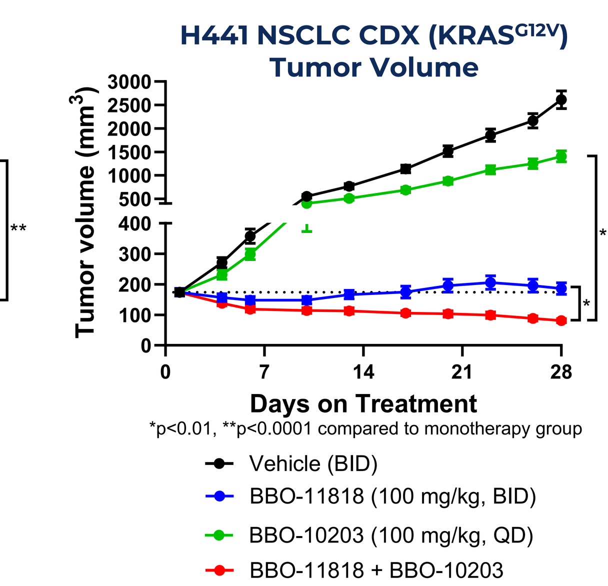


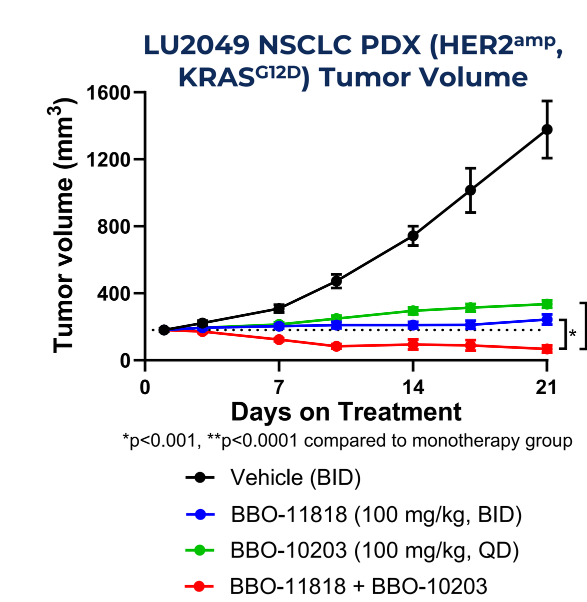
The combination of BBO-11818 and BBO-10203 shows robust activity, drives regressions by inducing tumor cell G1 arrest and apoptosis, and is well tolerated

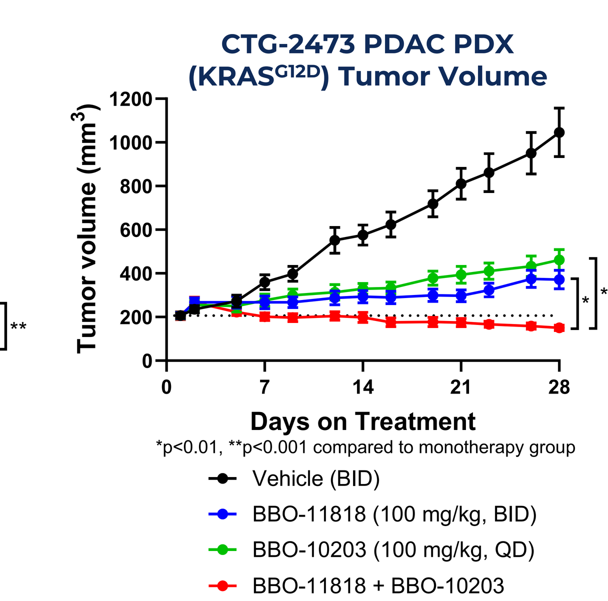


BBO-10203 shows monotherapy activity and strong combination activity with BBO-11818 in $KRAS^{G12D}$ and $KRAS^{G12V}$ CDX and PDX models









Conclusions

- BBO-10203 blocks RAS-mediated activation of PI3Kα and strongly inhibits pAKT signaling in tumor cells without affecting glucose metabolism.
- BBO-10203 shows robust monotherapy activity and combination activity with KRAS inhibitors in a panel of NSCLC, CRC, and PDAC CDX, PDX, and GEM models bearing KRAS mutations.
- These combinations induce deep tumor regressions through direct effects on tumor cell proliferation and apoptosis and are well tolerated.
- BBO-10203 is being evaluated in KRAS mutant solid tumors as a monotherapy in a phase 1 clinical trial (NCT06625775) and will be evaluated in combination with KRAS inhibitors in future trials.

References and Acknowledgements

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