BBO-10203, a first-in-class, orally bioavailable, selective blocker of the PI3Kα:RAS interaction inhibits tumor growth alone and in combination with standard of care therapies in breast cancer models without inducing hyperglycemia

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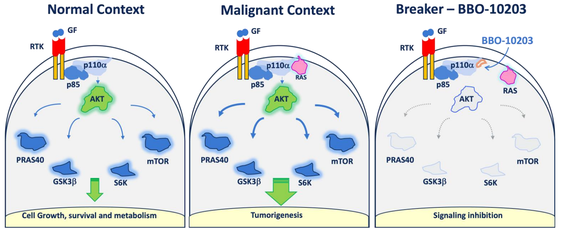
Introduction



- While small molecule inhibitors of the kinase activity of PI3Kα have been approved for the treatment of HR$^+$ HER2$^-$ breast cancer patients with PI3Kα mutant tumors, a large medical need remains to improve their safety profile due to dose-limiting on-target hyperglycemia. This toxicity may limit target coverage, the number of eligible patients, and 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 novel first-in-class covalent small molecule designed to block the PI3Kα:RAS protein-protein interaction and inhibit RAS-mediated activation of the AKT pathway via PI3Kα without the resultant hyperglycemia associated with direct inhibition of PI3Kα kinase activity.

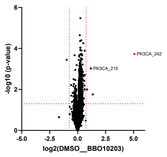
BBO-10203 covalently binds PI3Kα on cysteine 242 in the RBD, which prevents the interaction of PI3Kα with RAS

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
BT-474 full target engagement of PI3Kα RBD	10 nM
BT-474 pAKT (EC_{50})	4.4 nM
k_{inact}/K_I	7,100 $M^{-1}S^{-1}$

RBD: RAS binding domain; BT-474: Breast cancer HER2^{amp}/PIK3CA^{K111N} cell line

BBO-10203 is selective, potently inhibits cellular RAS-driven pAKT, and drives transcriptional changes which are consistent with PI3Kα-specific inhibition

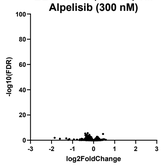
Global Cysteine Proteomics



BBO-10203 selectively binds to the cysteine 242 in PI3Kα

pAKT Inhibition in Human Breast Cancer Cell Lines

Gene Regulation



No genes are significantly differentially regulated between BBO-10203 and alpelisib

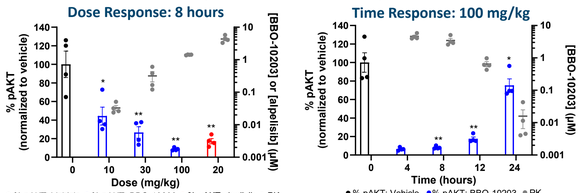
BBO-10203 is orally bioavailable and provides excellent target coverage

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

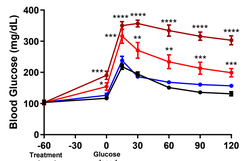
Single dose oral administration of BBO-10203 results in dose- and time-dependent inhibition of pAKT

BT-474 Breast Cancer CDX Model (ER+, HER2^{amp}, PIK3CA^{K111N})



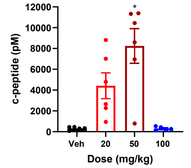
BBO-10203 does not induce hyperglycemia or hyperinsulinemia in an oral glucose tolerance test

Blood Glucose Levels



*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs vehicle

Serum C-Peptide Levels

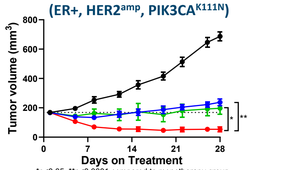


*p<0.001 vs vehicle
Timepoint: 150 minutes post glucose dose

Vehicle | Alpelisib (20 mg/kg) | Alpelisib (50 mg/kg) | BBO-10203 (100 mg/kg)

BBO-10203 shows monotherapy activity and combination activity with trastuzumab in HER2+ breast cancer models

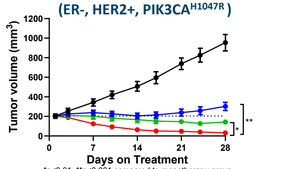
BT-474 CDX Model (ER+, HER2^{amp}, PIK3CA^{K111N})



*p<0.05, **p<0.0001 compared to monotherapy group

Vehicle | Trastuzumab (20 mg/kg) | BBO-10203 (100 mg/kg) | BBO-10203 + Trastuzumab

MDA-MB-453 CDX Model (ER-, HER2+, PIK3CA^{H1047R})

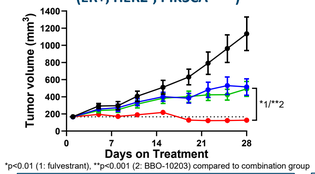


*p<0.01, **p<0.001 compared to monotherapy group

Vehicle | Trastuzumab (4 mg/kg) | BBO-10203 (30 mg/kg) | BBO-10203 + Trastuzumab

BBO-10203 shows monotherapy activity and combination activity with fulvestrant in ER+ HER2- breast cancer models

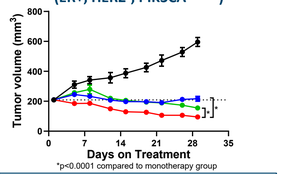
MCF7 CDX Model (ER+, HER2-, PIK3CA^{E545K})



*p<0.01 (1: fulvestrant), **p<0.001 (2: BBO-10203) compared to combination group

Vehicle | Fulvestrant (25 mg/kg) | BBO-10203 (100 mg/kg) | BBO-10203 + Fulvestrant

EFM-19 CDX Model (ER+, HER2-, PIK3CA^{H1047L})

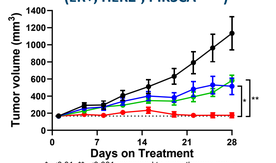


*p<0.0001 compared to monotherapy group

Vehicle | Fulvestrant (25 mg/kg) | BBO-10203 (100 mg/kg) | BBO-10203 + Fulvestrant

BBO-10203 shows monotherapy activity and combination activity with CDK4/6 inhibitors in ER+ HER2- breast cancer models

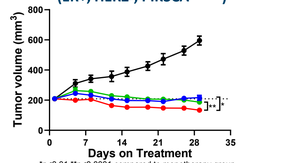
MCF7 CDX Model (ER+, HER2-, PIK3CA^{E545K})



*p<0.01, **p<0.001 compared to monotherapy group

Vehicle | Palbociclib (10 mg/kg) | BBO-10203 (100 mg/kg) | BBO-10203 + Palbociclib

EFM-19 CDX Model (ER+, HER2-, PIK3CA^{H1047L})



*p<0.01, **p<0.0001 compared to monotherapy group

Vehicle | Ribociclib (75 mg/kg) | BBO-10203 (100 mg/kg) | BBO-10203 + Ribociclib

Conclusions

- BBO-10203 blocks RAS-mediated activation of PI3Kα, strongly inhibits pAKT signaling in tumor cells without affecting glucose metabolism, and shows robust monotherapy activity and combination activity with SOC agents in HER2+ or HER2- breast cancer models with PI3Kα mutations.
- The phase 1 BREAKER-101 (NCT06625775) trial is underway.

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 bound into the syringe while either apo (unbound) PI3Kα-RBD or PI3Kα-RBD tethered to BBO-10203 was bound into the cell and ITC measurements 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, pAKT phosphorylation was assessed by HTRF.
k_{obs}/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.
Global cysteine proteomics: Cysteine proteome analysis on BT-474 whole cell lysates after a 4-hour treatment with vehicle (DMSO) or 200 nM BBO-10203 was performed. For the statistical analyses, a two-tailed Student's t-test was performed.
Gene regulation: BT-474 cells were treated with DMSO or 300 nM BBO-10203 or alpelisib for 24 hours and RNA-Seq was performed. The gene hit counts table was used for downstream differential expression analysis. Genes with an adjusted p-value <0.05 and absolute log2 fold change > 1 were considered differentially expressed genes for each comparison.
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.
Pharmacokinetics (PK) and pharmacodynamics (PD) studies: Dose and time response PK/PD analyses were performed in the BT-474 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.
oGTT 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 tumors reached a mean size of 165 to 210 mm^3, mice were randomized (n=9-10 per group) and dosed with vehicle (BBO-10203 formulation buffer), the indicated dose levels of BBO-10203 (QD, po), trastuzumab (Q7D, ip), fulvestrant (Q7D, sc), palbociclib (BID, po), or ribociclib (QD, po) or the indicated combinations. Tumor volumes were measured two times per week.
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 and oGTT studies. Two-way repeated measures ANOVA were performed for the efficacy studies between the indicated groups.

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