BBO-10203, a first-in-class breaker of the RAS:PI3Kα interaction, inhibits tumor growth alone and in combination with fulvestrant or ribociclib in breast cancer models without inducing hyperglycemia

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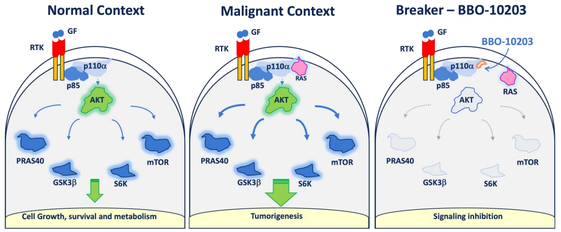
Introduction



- While PI3Ka kinase inhibitors have been approved for the treatment of HR$^+$ HER2$^-$ breast cancer patients with PI3Kα mutant tumors, 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 covalent small molecule which breaks the protein-protein interaction between RAS and PI3Kα, inhibits RAS-mediated activation of the PI3Kα pathway, and does not induce hyperglycemia[4].

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
IC_{50} and full target engagement of PI3Kα RBD	2.6 nM and 30 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 ER+, HER2^{amp}, PIK3CA^{K111N} cell line; Note: The crystal structure of PI3Kα RBD complexed with BBO-10203 showed covalent engagement with cysteine 242 via an acrylamide warhead and when the cysteine 242 was mutated to serine, BBO-10203 no longer potently inhibited the KRAS:PI3Kα interaction[4].

Oral administration of BBO-10203 results in robust PK, 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

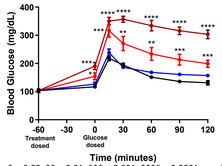
In Vivo PK/PD and Efficacy Results

Model	Assay	BBO-10203
BT-474	Dose response PK/PD*	pAKT EC_{50}: 35 nM, EC_{90}: 856 nM
	Time response PK/PD**	83% pAKT suppression at 12 hours
	Efficacy**	88% TGI

BT-474: Breast cancer ER+, HER2^{amp}, PIK3CA^{K111N} cell line; *at 8 hours, **100 mg/kg QD BBO-10203

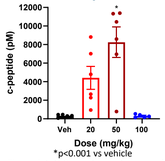
BBO-10203 does not induce hyperglycemia or hyperinsulinemia in an oral glucose tolerance test

Blood Glucose Levels



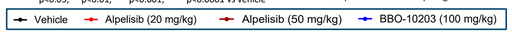
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs vehicle

Serum C-Peptide Levels



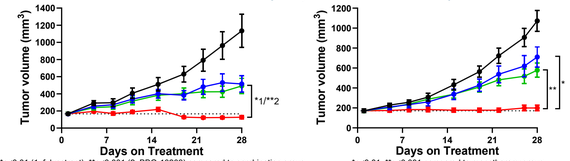
*p<0.001 vs vehicle

Timepoint: 150 minutes post glucose dose



BBO-10203 shows activity alone and in combination with fulvestrant or ribociclib in an ER+ HER2- breast cancer model with a PIK3CA helical domain mutation

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

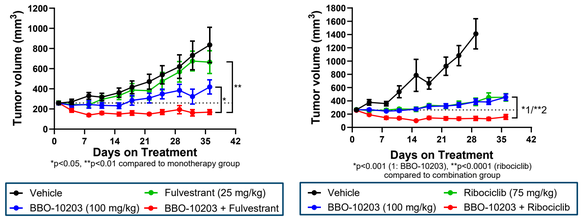


*p<0.01 (1: fulvestrant), **p<0.001 (2: BBO-10203) compared to combination group

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

BBO-10203 shows activity alone and in combination with fulvestrant or ribociclib in an ER+ HER2- breast cancer model with a PIK3CA kinase domain mutation

T47D CDX Model (ER+, HER2-, PIK3CA^{H1047R})

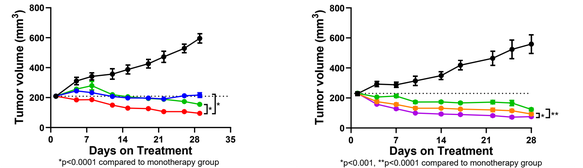


*p<0.05, **p<0.01 compared to monotherapy group

*p<0.001 (1: BBO-10203), **p<0.0001 (ribociclib) compared to combination group

Similar efficacy is observed with BBO-10203 or STX-478 (LY4064809), a PIK3CA mutant-selective inhibitor, in an ER+ HER2- breast cancer model with a PIK3CA kinase domain mutation

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

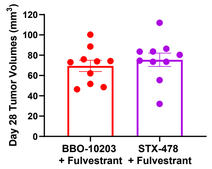


*p<0.0001 compared to monotherapy group

*p<0.001, **p<0.0001 compared to monotherapy group

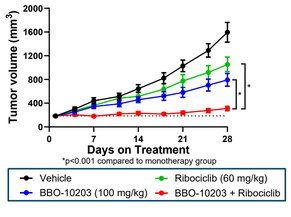
Comparison of Day 28 Tumor Volumes

BBO-10203 + Fulvestrant vs STX-478 + Fulvestrant



BBO-10203 shows activity alone and in combination with ribociclib in an ER+ HER2- breast cancer model with wild-type PIK3CA

HBCx-34 PDX Model (ER+, HER2-, PIK3CA^{WT})



*p<0.001 compared to monotherapy group

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 fulvestrant or ribociclib in ER+ HER2- breast cancer models with mutant or wild-type PIK3CA.
- 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 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, pAKT 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 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.
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 or patient-derived xenograft (PDX) tumors reached a mean size of 165 to 265 mm^3, mice were randomized (n=10 per group) and dosed with vehicle (BBO-10203 formulation buffer), the indicated dose levels of BBO-10203 (QD, po), fulvestrant (Q7D, sc), ribociclib (QD, po), STX-478/LY4064809 (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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References

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