BBO-10203, a first-in-class breaker of the PI3Kα:RAS interaction, demonstrates *in vitro* and *in vivo* efficacy alone or in combination with standard-of-care therapies in solid tumor models

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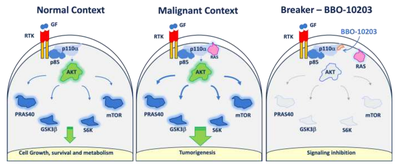
Introduction



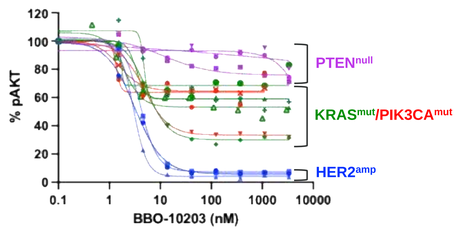
- PIK3CA is the 2nd most mutated gene in human cancers and its aberrant activation contributes to tumor development and resistance to cancer therapies[1]. While PI3Kα kinase-domain inhibitors have been developed and approved for the treatment of HR+, HER2- PIK3CA-mutated breast cancers, there remains a large unmet need to improve their safety profile due to dose-limiting hyperglycemia[2].
- Previous studies demonstrated that disrupting mutant RAS signaling through PI3Kα using genetic manipulation prevents KRAS-induced lung cancer formation[3], and in established tumors causes tumor stasis and partial regression without inducing hyperglycemia. Therefore, pharmacological disruption of the interaction of PI3Kα with RAS might provide a safer opportunity to targeting PI3Kα signaling.
- We generated BBO-10203, a first-in-class, potent, covalent inhibitor that selectively binds to PI3Kα's C242 site, blocking its interaction with the classical RAS isoforms (K/N/H-RAS).

Objectives

- Characterize the responses to BBO-10203 in various cell genotypes through pAKT inhibition.
- Evaluate its effects on glucose uptake through an oral glucose tolerance test in mice.
- Characterize BBO-10203 *in vitro* and *in vivo* efficacy alone or in combination with standard-of-care therapies in solid tumor models through growth inhibition assays and xenograft efficacy studies.

BBO-10203 inhibits cellular pAKT across various genotypes: HER2amp, KRASmut and PIK3CAmut

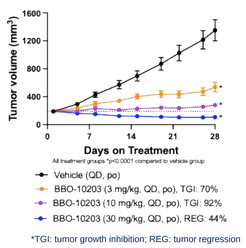
- pAKT was potently inhibited by BBO-10203 in HER2amp cell lines, while intermediate levels of pAKT inhibition were observed in KRAS/PIK3CA mutant cell lines and PTEN null cell lines were non-responsive.



BBO-10203 drives efficacy *in vivo*, without significantly increasing blood glucose levels

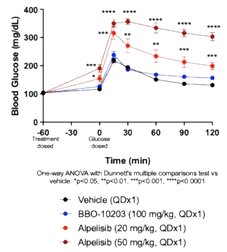
- Dose-dependent anti-tumor activity was observed in the HER2amp KRASG12C esophageal carcinoma KYSE-410 CDX model, with tumor regression observed at 30 mg/kg (left panel).
- In a glucose tolerance test in C57BL/6 mice, mice treated with alpelisib (20 or 50 mg/kg), show glucose accumulation in a dose-dependent manner, but mice treated with of BBO-10203 at 100 mg/kg show no accumulation of glucose relative to the vehicle (right panel).

KYSE-410 CDX (HER2amp and KRASG12C)



*TGI: tumor growth inhibition; REG: tumor regression

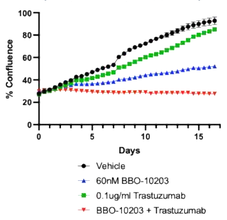
oGTT: Blood Glucose Levels



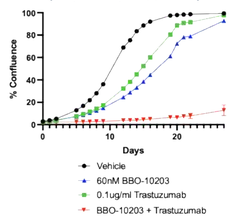
HER2+ breast cancer: BBO-10203 + trastuzumab has combination activity in the ER+ PIK3CAK111N BT-474 or ER- PIK3CAH1047R MDA-MB-453 models

- BBO-10203 is effective in combination with the anti-HER2 antibody trastuzumab in HER2+ breast cancer models BT-474 and MDA-MB-453, both *in vitro* (top panel) and *in vivo* (bottom panel) .

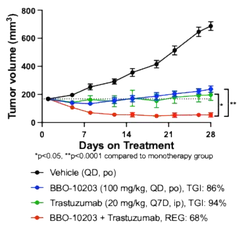
BT-474 clonogenic (ER+, HER2amp, PIK3CAK111N)



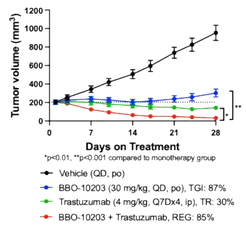
MDA-MB-453 clonogenic (ER-, HER2+, PIK3CAH1047R)



BT-474: BBO-10203 + trastuzumab



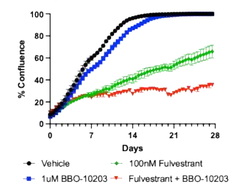
MDA-MB-453: BBO-10203 + trastuzumab



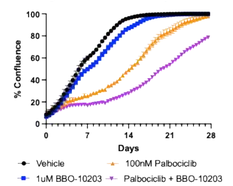
ER+/HER2- breast cancer: BBO-10203 + fulvestrant or palbociclib has combination activity in the PIK3CAE545K MCF7 model

- BBO-10203 is effective in combination with the SERD fulvestrant, or the CDK4/6 inhibitor palbociclib in MCF7.

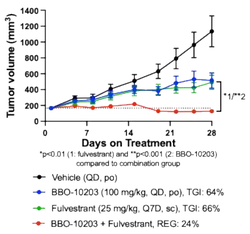
Clonogenic: BBO-10203 + fulvestrant



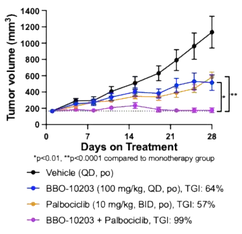
Clonogenic: BBO-10203 + palbociclib



Tumor Volume: BBO-10203 + fulvestrant



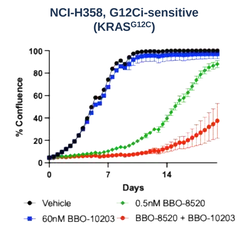
Tumor Volume: BBO-10203 + palbociclib



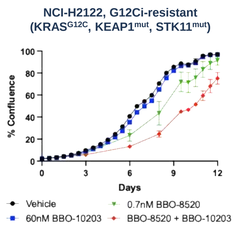
KRASG12C NSCLC: BBO-10203 + BBO-8520 (KRASG12C DUAL inhibitor) has combination activity in the NCI-H358 and NCI-H2122 models

- BBO-10203 displays combination benefit with the KRASG12C inhibitor BBO-8520 in G12Ci-sensitive and resistant models.

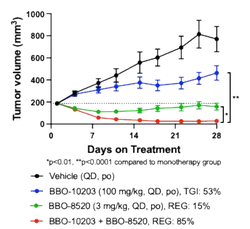
NCI-H358, G12Ci-sensitive (KRASG12C)



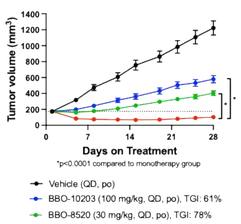
NCI-H2122, G12Ci-resistant (KRASG12C, KEAP1mut, STK11mut)



NCI-H358 : BBO-10203 + BBO-8520

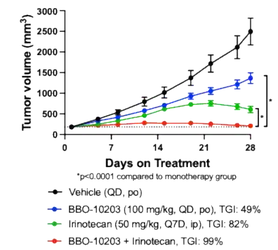


NCI-H2122: BBO-10203 + BBO-8520



KRAS mutant CRC: BBO-10203 + irinotecan has combination activity in the KRASG12V SW620 CDX model

- BBO-10203 is effective in combination with the chemotherapy irinotecan in the colorectal cancer KRASG12V CDX model SW620.



Conclusion

- BBO-10203 blocks RAS-mediated activation of PI3Kα in tumor cells without affecting glucose metabolism.
- BBO-10203 demonstrates combination benefit *in vitro* and *in vivo* with targeted and conventional anti-cancer therapies across multiple indications, including HER2+ breast cancer, ER+/HER2- breast cancer, KRAS G12C NSCLC and KRAS mutant CRC.
- BBO-10203 will enter phase 1 clinical trials (Breaker-101) in 2H 2024.

Materials and Methods

- HTRF Human Phospho-AKT1/2/3 (Ser473) Detection Kit (Cat # 64AKSPEH) was purchased from Revvity for the pAKT inhibition measurement. Cells were seeded in 100µl of complete growth media in a 96-well plate, placed in a 37◦C incubator, and allowed to adhere overnight. The next day cells were treated with a 9-point dose titration of BBO-10203 resuspended in 0.1% dimethyl sulfoxide (DMSO) starting at 3 µM in 1:3 dilution increments using a Tecan D300e. 1 µM AMG511 was dosed as a positive control to assess the maximal inhibition of the assay. Cells were treated for 4 hours with compounds.
- In clonogenic assays, cells were seeded at 24-well plates using 500ul of complete media and left to adhere overnight. Compounds were added in duplicate to cells next day and media/compounds were replenished twice every week. Cell confluence was monitored and analyzed in Incucyte S3.
- For the cell line-derived (CDX) xenograft efficacy studies, immune compromised mice were inoculated with human tumor cells. When CDX tumors reached a mean size of 165 to 205 mm^3, mice were randomized into treatment groups (n=9-10 per group) and dosed with vehicle (BBO-10203 formulation buffer), the indicated dose levels of BBO-10203, trastuzumab, fulvestrant, palbociclib, or BBO-8520, or the combinations. Subcutaneous tumor volumes were measured two times per week. Tumor growth inhibition (TGI), an indicator of antitumor effectiveness, was calculated on the final day of the study. Tumor regression (REG) was also assessed and defined as a tumor with a smaller tumor volume compared to the 1st day of dosing. Two-way repeated measures or mixed-effects ANOVA followed by post hoc Dunnett's multiple comparisons test of the means was applied over the indicated number of days using GraphPad Prism software.
- For the oGTT study, male C57BL/6 mice started the fast 16 hours prior to the study. 1-hour prior to the oGTT, blood was collected, and blood glucose levels were measured. Mice were then randomized into four groups (n=6 per group) by fasted blood glucose and orally administered 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 15, 30, 60, 90, and 120 minutes following the glucose dose. Individual mouse glucose concentrations were recorded. One-way ANOVA of all group means followed by post hoc Dunnett's multiple comparisons were performed at each time point.

References and Acknowledgements

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