# Pharmacological Targeting of Mitochondrial Quality Control Pathways Leads to Growth Inhibition in Solid Tumors *in vitro* and *in vivo*.

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## Background

Novel compounds of the chemical family 1-Thiazol-2-yl-N—3-methyl-1H-pyrozole-5-carboxylic acids have been described as antitumor agents. Two members of this family, BTM-3528 and the clinical candidate BTM-3566 have robust, single agent activity in models of DLBCL. The mechanism of action in DLBCL is dependent on activation of the mitochondrial OMA1-DELE1-HRI axis resulting in induction of the ATF4 ISR which leads to cell death [1]. Our data further indicate that, unlike DLBCL, solid tumor cell lines respond to drug by undergoing cell growth inhibition rather than apoptosis.

## Objectives

Based on the described mechanism of action, our hypothesis is that combinations with drugs that influence mitochondrial outer membrane permeabilization (MOMP) would act synergistically to increase cell death. We therefore determined the effects of combinations of BTM-3566 with BH3 mimetics targeting BCL2, BCL-XL and MCL1 on solid tumor growth and survival.

## Materials and Methods

BTM-3528 was screened across a cell line panel (Crown Bio OmniScreen) including 333 solid tumor lines and 73 hematopoietic tumor lines. Cell growth inhibition was determined using Cell Titre Glo. From this data, a panel of 6 solid tumor cell lines (renal, 786-O, 769-P, A498; uterine sarcoma (MES-SA; uterine endometrial (RL-95-2) and colorectal (HCT-116) were used to assess the effect of combinations of BTM-3566 with BH3 mimetics. Cell growth and viability was confirmed using Incucyte live cell analysis in conjunction with the cell impermeant nuclear stain YOYO-1 and Caspase 3/7 activity assays.

BTM-3566 activity was evaluated *in vivo* using cell-line derived xenograft models of colorectal tumors (HCT-116) and a selection of solid tumor PDX models. BTM-3566 was dosed orally in all cases and tumor growth inhibition was determined.

## Results

### *In Vitro* Activity in Solid Tumor Cell Lines

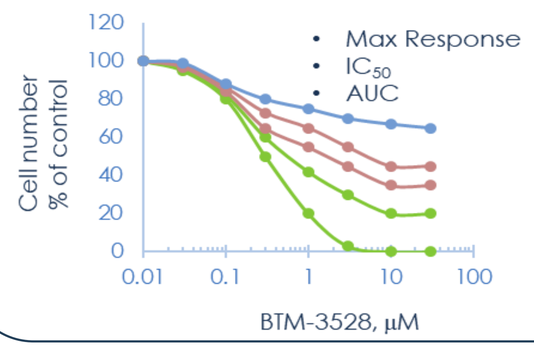


**Figure 1**: **Cell line screening identifies activity in solid tumor lines**. The activity of BTM-3528 was tested in a panel of 333 solid tumor lines. The maximum response, IC50 and Activity area (AUC) were determined for each cell line. A cutoff of AUC was used to establish responsive (AUC < 3.05) and non-responsive (AUC > 3.4) cell lines.

| Lineage | Lineage Subtype | Cell line | AUCs | IC50 (µM) | % Inhibition |
|---|---|---|---|---|---|
| Colorectal | Adenocarcinoma | HCT-116 | 2.74 | 0.92 | 80.82% |
| Kidney | Renal Clear Cell Carcinoma | 769-P | 2.8 | 1.04 | 80.28% |
| | Renal Clear Cell Carcinoma | 786-O | 3.05 | 1.15 | 71.09% |
| | Renal Cell Carcinoma | A498 | 2.77 | 0.86 | 81.18% |
| Uterus | Endometrial Adenosquamous | RL95-2 | 2.63 | 0.42 | 78.60% |
| | Uterine Sarcoma | MES-SA | 2.65 | 0.49 | 70.00% |

**Table 1: Activity of BTM-3528 in six cell lines of solid tumor origin.** All data using Cell-Titre Glo ATP to estimate cell growth inhibition. These cell lines were further used to evaluate the effects of BTM compounds in combination with BH3 mimetics.

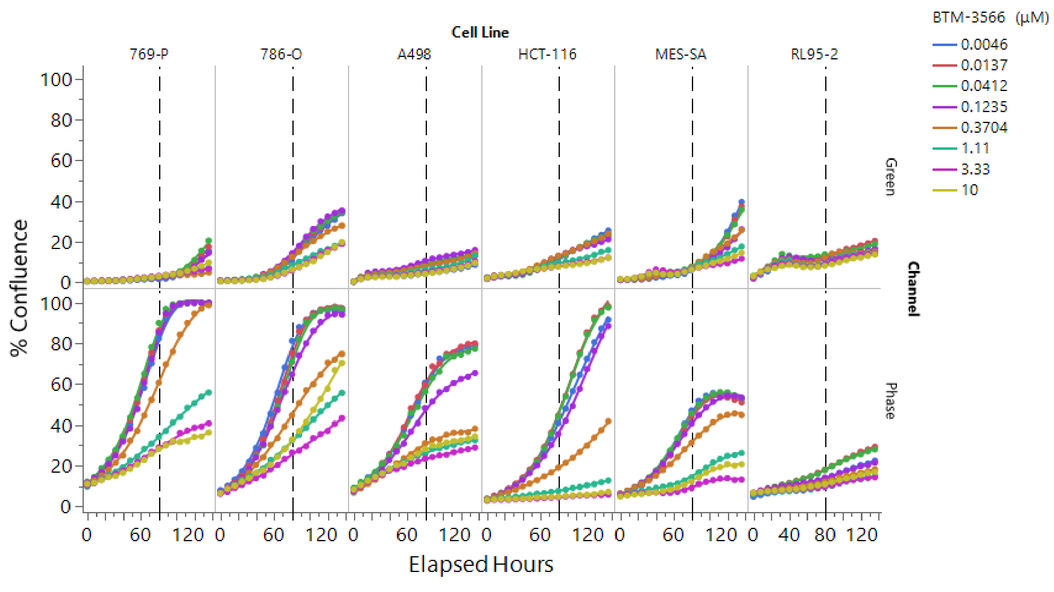


**Figure 2: BTM-3566 inhibits cell growth but does not induce cell death in solid tumor lines.** BTM-3566 was evaluated in a panel of 6 solid tumor lines using live cell brightfield imaging and scored for cell viability using green fluorescent cell impermeant dye YOYO-1. The total area of cells (Confluence) was determined for each channel. All growth curves were converted to AUC using t=80 hours as a time period capturing the linear phase of cell growth. **There is little evidence of significant cell death in any cells line following BTM-3566 treatment alone.**

## BTM-3566 inhibits tumor growth *In vivo*

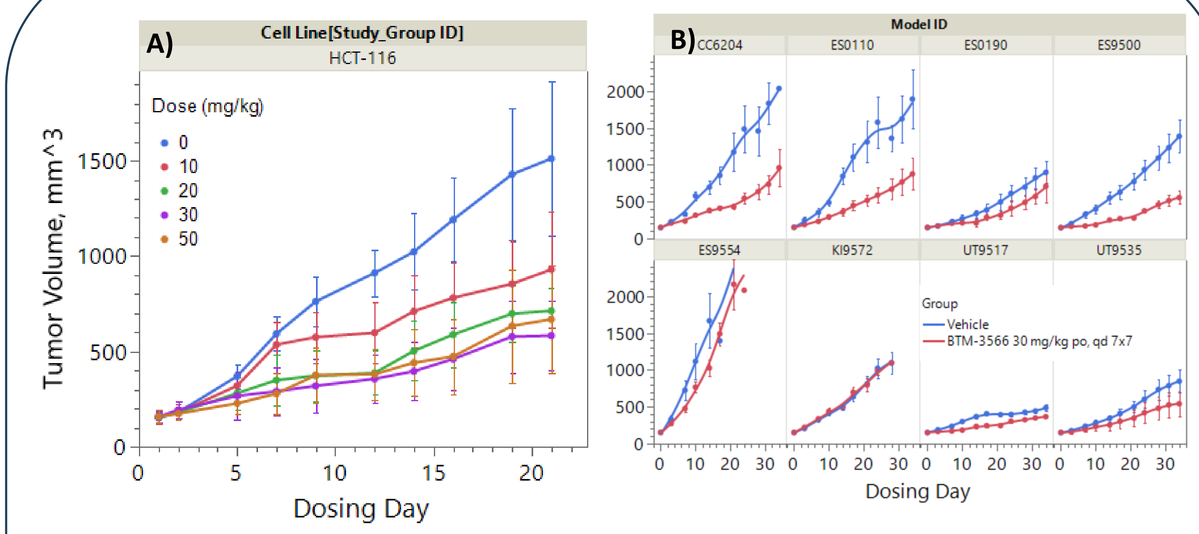


**Figure 3**: **BTM-3566 inhibits tumor growth *in vivo* . *A)*** BTM-3566 was tested in a cell line xenograft model (HCT-116) of colorectal cancer. BTM-3566 treatment (po, qd for 21 days) results in a dose dependent inhibition of tumor growth. **B)** 8 PDX models were also tested using a single dose of BTM-3566 (20 mpk dosed po, qd for 21 total days). CC = Choriocarcinoma; ES = Esophogeal carcinoma; K = Kidney; UT = Uterine. Three models demonstrated ~ 60 TGI , similar to that observed using HCT-116 cell model. The data suggest that BTM-3566 has tumor growth inhibiting activity in a subset of solid tumors. To achieve regression will require identification of agents that synergize with BTM-3566.

## BTM-3566 Synergizes with the BH3 Mimetic Navitoclax

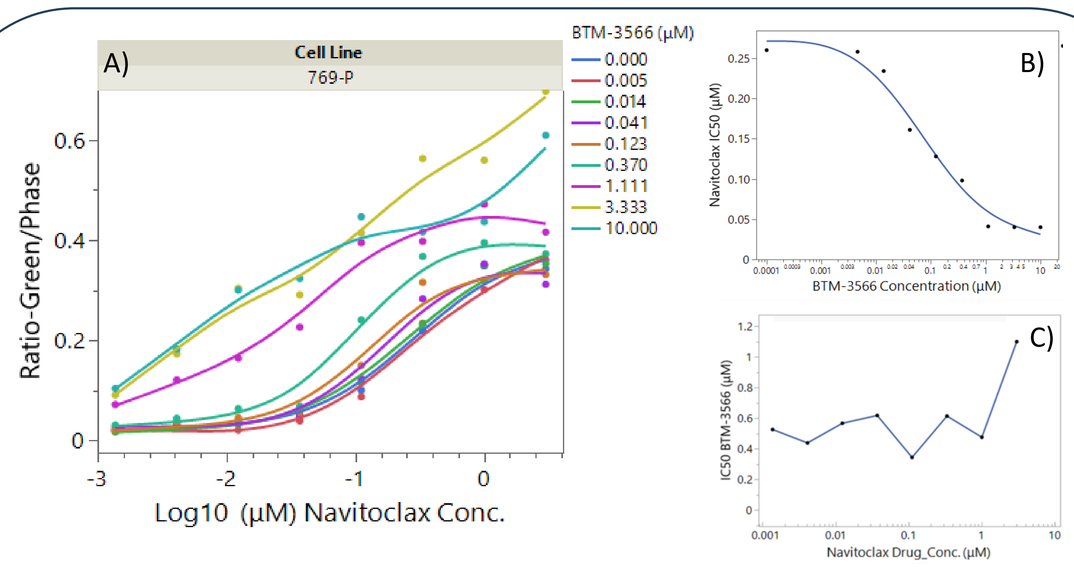


**Figure 4 BTM-3566 synergizes with navitoclax to increase cell death in 769-P cells.** The renal clear cell carcinoma cell line 769-P was treated with a combination of navitoclax and BTM-3566 and imaged as in Figure 2. Growth curves were integrated over 80 hours and converted to AUC. A) The ratio of the AUC of the % confluence of imaged cells in the green fluorescent channel and brightfield is used as an estimate of cell viability. 769-P cells are sensitive to navitoclax alone with an $IC_{50}$ of 220 nM. There is no significant cell death following BTM-3566 treatment. B) The calculated $IC_{50}$ of navitoclax decreases from 220nM to 46 nM with increasing BTM-3566. C) There is no change in the potency of BTM-3566 with increasing navitoclax concentration.

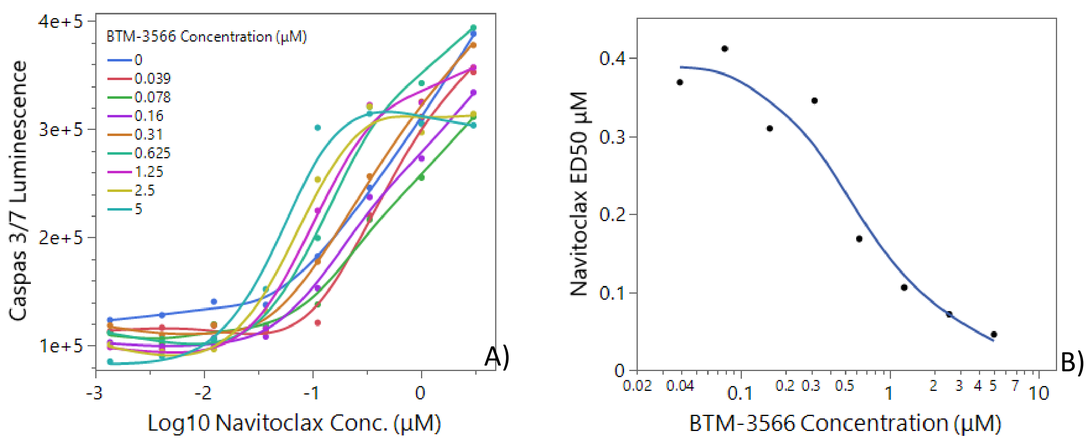


Figure 5. **BTM-3566 synergizes with navitoclax to induce Caspase 3/7 in 769-P cells**. The renal clear cell carcinoma cell line 769-P was treated with a combination of navitoclax and BTM-3566. Caspase activation was determined 24 hours following treatment using Caspase-Glo (Promega). A) BTM-3566 increases activation of Caspase 3/7 in 769-P cells in a dose dependent manner. B) The calculated $ED_{50}$ for navitoclax mediated induction of Caspase 3/7 decreases with increasing concentrations of BTM-3566 in 769-P cells. **BTM-3566 increases the sensitivity of 769-P cells to navitoclax dependent induction of the intrinsic pathway of apoptosis.**

## Conclusions

BTM-3566 targets mitochondrial quality control pathways leading to cell growth arrest in sensitive solid tumor line of diverse origins. BTM-3566 is orally bioavailable and successfully inhibits tumor growth *in vivo*. To extend the use of drug in solid tumors we demonstrated that BTM-3566 augments the activity of the BH3 mimetic navitoclax *in vitro* against certain solid tumor lines. We are advancing efforts to demonstrate synergy *in vivo* and to identify markers that predict activity in drug combinations with BH3 mimetics suitable for clinical use.

## Contact

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## References

1. Schwarzer A, Oliveira M, Kleppa M-J, Slattery SD, Anantha A, Cooper A, et al. Targeting Aggressive B-cell Lymphomas through Pharmacological Activation of the Mitochondrial Protease OMA1. Mol Cancer Ther. 2023;22:1290–303.