Selective pharmacological activation of the mitochondrial protease OMA1 inhibits tumor growth and induces regression in tumors expressing low levels of FAM210B

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Abstract

We have previously described a novel compound, BTM-3566, that exhibits robust single agent activity in cell line and patient derived xenograft models of DLBCL. BTM-3566 is effective on all types of DLBCL, independent of cell of origin (COO) or genotype. In this report, we demonstrate the utility of BTM-3566 in solid tumors and provide evidence for using the expression level of FAM210B to predict response to drug.

Our data establish:

- BTM-3566 has *in vitro* activity in a broad range of solid tumor types
- *In vitro* and *in vivo* response of tumor cells to BTM-3566 is inversely correlated with the expression of the mitochondrial protein FAM210B.
- FAM210B plays a key mechanistic role in drug activity
- BTM-3566 will increase sensitivity to BH3 mimetics in specific solid tumor cell lines.

Objectives

The mechanism of action of BTM-3566 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 (see Figure 1). *In vitro* data suggested a correlation between response to drug and expression of FAM210B mRNA. We undertook *in vivo* studies in solid tumors to confirm the association of drug response with mitochondrial protein FAM210B expression. In addition, solid tumor cell lines undergo cell growth inhibition rather than apoptosis in response to drug. To further augment the utility of BTM3566 in solid tumors, we identified solid tumor cell lines wherein combinations with BH3 mimetics would lead to cell death.

Methods: Human tumor cell line were screened for response to BTM compounds (Crown Bioscience OmniScreen™). Biomarkers associated with response to compound were identified based on gene expression data found in DepMap. Patient derived xenograft models were performed with tumors sourced from Crown Bio (Crown Bio HuBase).

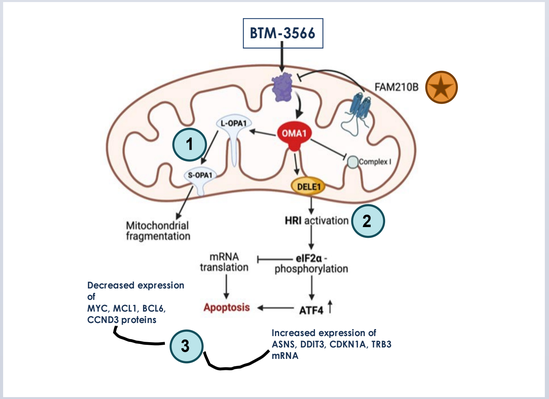

Figure 1: Model for the mechanism of action of BTM compounds. BTM compounds induce the activation of OMA1 leading to the cleavage of DELE1 and OPA1. (1) OPA1 activation leads to fragmentation of the mitochondrial network. (2) DELE1 cleavage leads to activation of HRI and (3) a reduction in pro-survival factors and downstream ATF4-ISR effector pathways leading to cell death in DLBCL cell lines.

Results

Workflow to Identify Predictive Markers of Compound Action

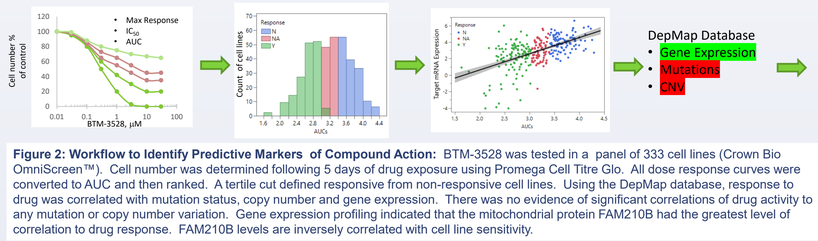

Figure 2: Workflow to Identify Predictive Markers of Compound Action: BTM-3528 was tested in a panel of 333 cell lines (Crown Bio OmniScreen™). Cell number was determined following 5 days of drug exposure using Promega Cell Titre Glo. All dose response curves were converted to AUC and then ranked. A tertile cut defined responsive from non-responsive cell lines. Using the DepMap database, response to drug was correlated with mutation status, copy number and gene expression. There was no evidence of significant correlations of drug activity to any mutation or copy number variation. Gene expression profiling indicated that the mitochondrial protein FAM210B had the greatest level of correlation to drug response. FAM210B levels are inversely correlated with cell line sensitivity.

Ectopic overexpression of FAM210B rescues cells from the effects of BTM-3566

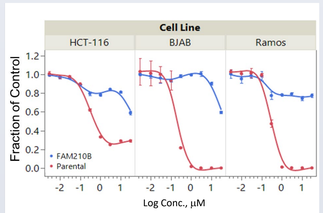

Figure 3 **Ectopic overexpression of FAM210B rescues cells from the effects of BTM-3566.** All responsive cell lines were transfected with FAM210B cDNA and selected by puromycin selection. Clones with the highest level of FAM210B expression were tested for sensitivity to BTM-3566. All cell lines proved resistant to the action of compound.

FAM210B expression levels in patient derived tumors (Crown Bio HuBase)

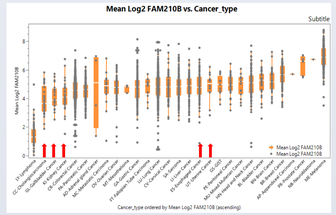

Figure 4: **FAM210B mRNA levels in patient derived tumor samples. (Crown Bio HuBase).** Tumors were biopsied and RNASeq performed on stem cells. Data are ordered by rank of FAM210B expression in each tumor type. Lymphoma has the lowest level of FAM210B expression followed by biliary tract tumors.

Select patient derived solid tumors used for testing BTM-3566 (Crown Bio HuBase)

		Log FAM210B mRNA	
Sample_Name	Cancer_type	Expression	FAM210B_Quintile
CC6204	CC-Cholangiocarcinoma	3.395	1
ES0110	ES-Esophageal Cancer	3.404	1
ES0136	ES-Esophageal Cancer	6.043	5
ES0190	ES-Esophageal Cancer	6.081	5
ES2267	ES-Esophageal Cancer	3.255	1
ES9500	ES-Esophageal Cancer	3.573	1
ES9554	ES-Esophageal Cancer	3.08	1
GL11314	GL-Gallbladder Cancer	4.05	2
KI2479	KI-Kidney Cancer	4.16	2
KI9572	KI-Kidney Cancer	3.88	2
KI9666	KI-Kidney Cancer	4.124	2
UT2449	UT-Uterine	2.895	1
UT3705	UT-Uterine	5.212	4
UT5320	UT-Uterine	6.218	5
UT9517	UT-Uterine	1.348	1
UT9535	UT-Uterine	2.936	1

Table I: **Selected Patient Derived Xenograft models chosen to test response to BTM-3566.** Identified human tumors were biopsied and RNASeq performed on stem cells. Tumors were selected to provide a range of FAM210B expression levels and binned using quintile levels determined from the total collection of tumor samples.

Testing BTM-3566 in patient derived xenografts.

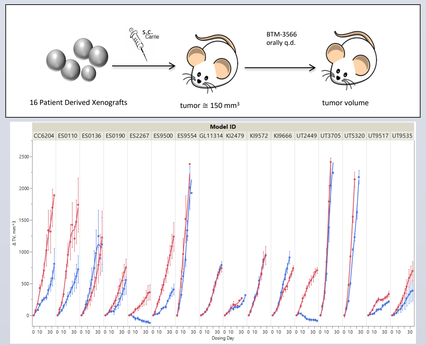

Figure 5 **Patient Derived Xenograft methods.** Subcutaneous PDX models were established in mice at Crown Bio. Randomization to establish an average tumor volume of 150 mm³ per arm. All animals were dosed with vehicle or a solution of 30 mg/kg BTM-3566 po, qd for 7 –days followed by a 7 –day holiday. The cycle was repeated for 3 dosing cycles and a total of 21 days of dosing. Tumor volume was determined every 3 days. All data are plotted as the mean change in tumor volume, n=3, +/- SEM) 🔵, Vehicle; 🔴, BTM-3566, 30 mg/kg)

BTM-3566 reduces tumor burden in patient derived xenografts.

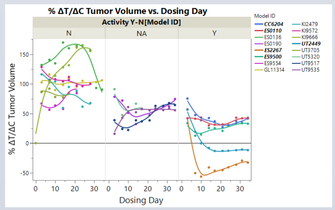

Figure 6. **Response of tumors to BTM-3566.** Response of tumor to BTM-3566 plotted as % change ratio of starting treatment tumor volume to control Tumors %ΔT/ΔC ≥ 50% were not responsive (N); %ΔT/ΔC <50% were responsive (Y). Two tumors were identified as having experienced regression %ΔT/ΔC < 0. UT2449 (regression) ES2267 (regression). ES9500, ES0110, CC6204 all responded with significant tumor growth inhibition.

BTM-3566 is effective in tumors with low FAM210B expression

Figure 7 **Responsive PDX models expressed low FAM210B** Response of tumor to BTM-3566 plotted as % of total tested tumors for each FAM210B quintile. The data indicate that in vivo tumor responsiveness is associated with low FAM210B levels as predicted by evaluation in cell lines. Additional markers have been identfied that improves the prediction of response to drug.

BTM-3566 Synergizes with the BH3 Mimetic Navitoclax

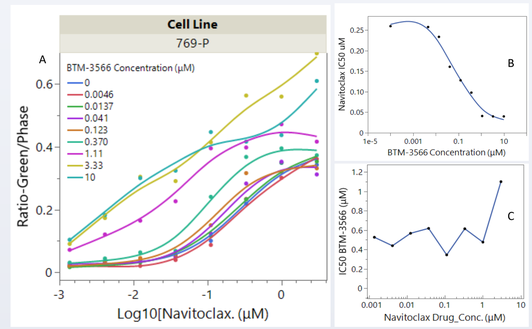

Figure 8. BTM-3566 synergizes with navitoclax to increase cell death in 769-P cells. The renal clear cell carcinoma cell line 769-P was treated with a combination of navitoclax and BTM-3566 and imaged as in Figure 2. Growth curves were integrated over 80 hours and converted to AUC. A) The ratio of the 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 IC50 of 220 nM. There is no significant cell death following BTM-3566 treatment. B) The calculated IC50 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.

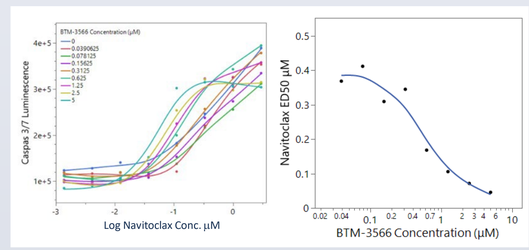

Figure 9. **BTM-3566 synergizes with navitoclax to induce Caspase 3/7 in 769-P cells**. The renal clear cell carcinoma cell line 769-P was treated with a combination of navitoclax and BTM-3566. Caspase activation was determined 24 hours following treatment using Caspase-Glo (Promega). A) BTM-3566 increases activation of Caspase 3/7 in 769-P cells in a dose dependent manner. B) The calculated ED50 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.

Summary and Conclusions

We have demonstrated that BTM-class compounds have broad, yet select, activity against both hematopoietic and solid tumors. The response of tumor cell lines is inversely correlated with expression of the gene FAM210B. Ectopic expression of FAM210B abrogates drug activity in a solid tumor line (HCT-116) and DLBCL and Burkitts lymphoma lines. These data suggest a key mechanistic role for FAM210B role in drug activity. We further demonstrate that FAM210B expression is associated with response to BTM-3566 in PDX models of solid tumors with origins in a range of tissues. We noted regression of tumor in two instances. The mechanism of action of BTM-3566, leads to a reduction in MCL1 and loosening of cristae junction, both of which may associated with sensitivity to BH3 mimetics. BTM-3566 increased the sensitivity of 769-P cells to navitoclax resulting in caspase activation and apoptotic cell death. Combinations of BTM-3566 with BH3 mimetics may therefore have utility in a number of therapeutic settings.