Abstract #306679: BXQ-350 targets to the lysosome and kills glioblastoma (GBM) cells via activation of apoptotic caspases in vitro.

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Background:

Apoptosis is a programmed cell death mechanism where cells respond to internal or external stimuli by initiating a cascade of events and enzymes leading to cell death. One of the hallmarks of cancer is the ability of tumor cells to resist these apoptotic stimuli. This allows tumor cells to have aberrant metabolisms, such as sphingolipid metabolism in tumor cell lysosomes, or mutations which would normally commit cells to death. Cancer cells contain an overabundance of aberrant lysosomes in order to deal with their metabolic deficiencies. The lysosomes become the metabolic hub of the cell which can be shut off to induce cell death. Saposin C (SapC), the protein component of BXQ-350, is a lysosomal protein involved in sphingolipid metabolism. Removing resistance, shortcutting steps leading to apoptosis, or correcting sphingolipid metabolism can result in the death of these tumor cells. Targeting the aberrant lysosomes of cancer cells can speed up this process.

Methods:

The GBM cell line, Gli36ΔEGFR, was used in Caspase 3/7 and 9 activity assays along with parallel MTT cytotoxicity assays. Cells were treated with 9uM to 30uM BXQ-350 in triplicate and incubated for 24 hours at 37°C then read via their respective assay.

The GBM cell line U87 MG was used to determine lysosomal targeting. U87 MG cells were treated with 10uM BXQ-350 and incubated at 37°C overnight. Immunofluorescence methods were used to stain cells with anti-SapC (RFP) and anti-LAMP1 (GFP) antibodies and a nuclear counterstain (DAPI). Images were collected using the Biotek Cytation 5.

Conclusion

Targeting the lysosomes of cancer cells is a point of interest in cancer research. While they give cancer an advantage, the lysosomes are also primed to be turned against these cells to cause death. BXQ-350 targets to these lysosomes where it is able to initiate a cascade of caspases causing apoptosis in GBM cells.

Caspases 3/7 are effectors caspases that are needed to commit a cell to apoptosis. Their rise in activity, in conjunction with the rise of Caspase 9, the initiator caspase, forces these cell into an apoptotic cell death. This removes one of the major barriers to fighting cancer.

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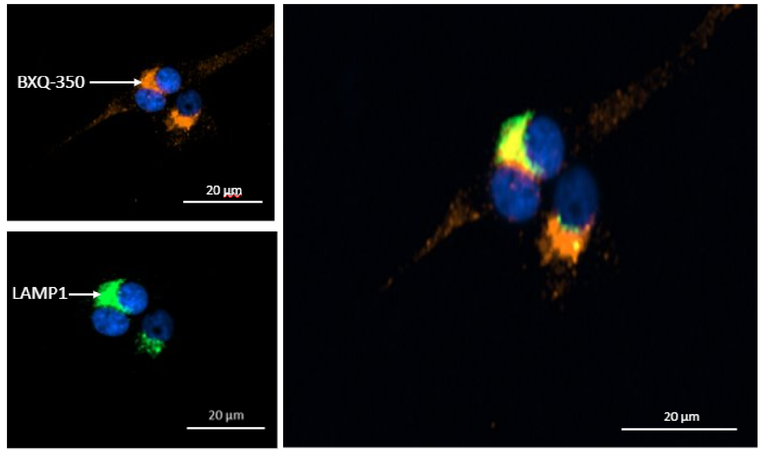
Results:

BXQ-350 was observed to co-localize to LAMP1, a lysosomal membrane protein in GBM cells. This signifies BXQ-350 targets to the numerous lysosomes of GBM cells.



BXQ-350 mediated cell death is correlated with a rise in Caspase 3/7 and Caspase 9 activity. The caspase activity levels did not rise until after BXQ-350 passed its IC50 and stayed elevated. Caspases 3/7 levels showed higher activity compared to untreated than Caspase 9.