Investigation of the anti-cancer properties of glycolysis inhibitor 3-Bromopyruvate (3-BP) in combination with radiation (RT) on pancreatic cancer cell line models.

Bolutife Olagunju, Sanjit Roy, Tijana Dukic, Binny Bhandary, Zachery Keepers, Young H Ko, Hem D Shukla

Department of Radiation Oncology, University of Maryland Medical Center, Baltimore, MD

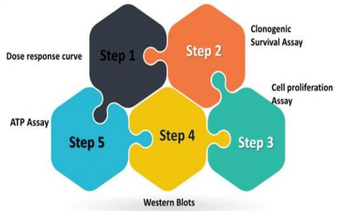
INTRODUCTION

- Pancreatic cancer (PC) is the fourth most lethal cancer in the US, with a 5-year survival rate of 12%.
- In 2023, there will be an estimated diagnosis of 64,050 new cases and 50,550 deaths.
- Current treatment options for patients with advanced pancreatic cancer include radiation therapy (RT), chemotherapy, or combination treatment (chemo-RT).
- In human cancer cells, 3-BP is transported across the membrane by the lactate/pyruvate H^+ symporter **MCT1**. Inside the cell, it becomes highly reactive, alkylating proteins with **SH groups.**
- Cancer cells rely on glycolysis as a source of energy, and they overexpress lactate transporters to import additional glucose and efflux excess lactic acid.
- 3-BP has demonstrated therapeutic potential by targeting the energy metabolism in cancer cells by inhibiting glycolysis. Specifically, 3-BP irreversibly alkylates the glycolytic enzyme, **Hexokinase II (HK II)**, disrupting glucose metabolism and leading to cancer cell death.

OBJECTIVES

This study aimed to investigate the combination of 3-BP and radiation (RT) and its effect on pancreatic tumor cell death. The study also aimed to understand the molecular mechanisms involved in the efficacy of 3-BP and radiation-induced pancreatic cancer cell death.

MATERIALS & METHODS



(A) Determination of the Dose-response curve through the treatment of PANC-1 and MIA PaCa-2 human pancreatic cancer cells with 5 FU (0-100 µM) and radiation (0-10 Gy).

(B) Clonogenic Survival Assay using Crystal violet to determine cell reproductive death after treatment with ionizing radiation and other cytotoxic agents.

(C) Cell Proliferation Assay to determine the relative rates of cell division.

(D) Western Blots using pSer552- beta catenin, Total Beta Catenin, B-actin, TGF-beta, C-Myc and MCT1 antibodies for both cell lines.

(E) An ATP assay to determine the number of viable cells in the cultures by quantifying the amount of ATP present.

RESULTS

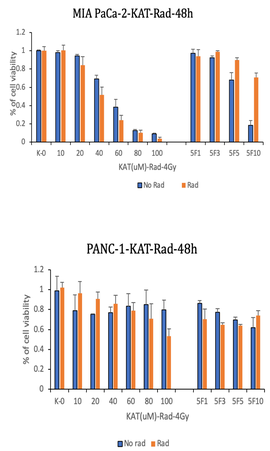


Figure 1. The dose-response curve for MIA PaCa-2 and PANC-1 cancer cells shows a decrease in % cell viability in the combination of 4 Gy radiation and various doses of KAT.

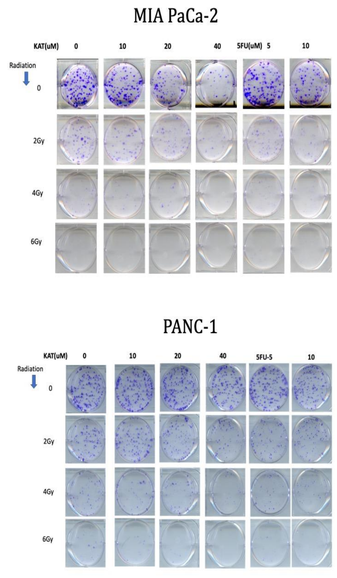


Figure 2. Comparison of Clonogenic Survival Assay for MIA PaCa-2 and PANC-1 pancreatic cancer cell lines. The figure shows a decline in the number of surviving cancer cells with increased KAT and 5-FU doses.

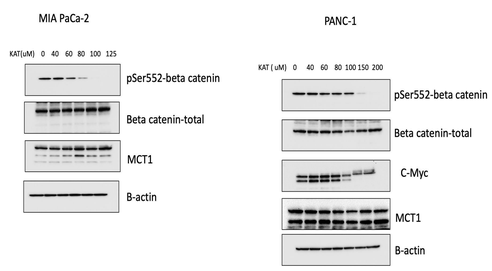


Figure 3. The Western blots show that KAT inhibits the phosphorylation (activation) of beta-catenin (pSer552) and the expression of related target proteins in a dose-dependent manner in both cell lines **without** Radiation.

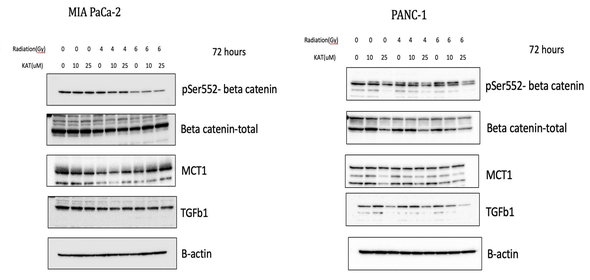


Figure 4 The Western blots show that KAT inhibits the phosphorylation (activation) of beta-catenin (pSer552) in a dose-dependent manner in both cell lines **with** Radiation. pSer552 β-catenin is an essential antibody in the PI3K AKT signal transduction pathway that regulates tumor progression.

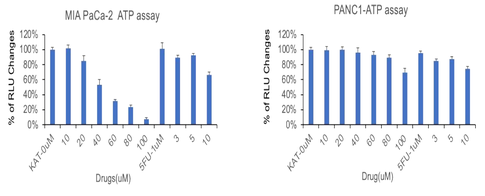


Figure 5. The ATP Assay shows that the amount of metabolically active cells decreases as the KAT dose increases in both the MIA PaCa-2 and PANC-1 cell lines.

DISCUSSION & FUTURE DIRECTIONS

- The study demonstrated that KAT dose-dependently inhibits cell growth.
- The study demonstrated that KAT inhibited clonogenic cell survival and decreased cell viability by blocking ATP generation.
- The study demonstrated that KAT dose-dependently inhibits the expression of active **Phospho-beta-Catenin at position serine 552.**
- Overall, the results of this study demonstrate that KAT, in combination with radiation, might be a very effective anti-pancreatic cancer therapy.

Future Directions:

- To conduct an **Apoptosis Assay** to determine membrane and structural changes in both pancreatic cancer cell lines (MIA PaCa-2 and PANC-1)
- To conduct a **Gamma H2AX assay** in untreated and treated cancer cells to measure **DNA damage**.
- To determine the proteins and genes targeted in the **Beta Catenin pathway**.
- Continue research on the clinical applications of 3-BP in organoid models, animal studies, and possibly clinical trials.

ACKNOWLEDGEMENTS

• This work was funded by the department of Radiation Oncology Summer Fellowship & NewGLab Pharma.

REFERENCES

The QR code to the right links to the published paper that this poster was based on. References are included in the paper.

• Roy S, Dukic T, Bhandary B, Tu KJ, Molitoris J, Ko YH, Shukla HD. 3-Bromopyruvate inhibits pancreatic tumor growth by stalling glycolysis, and dismantling mitochondria in a syngeneic mouse model. Am J Cancer Res. 2022 Nov 15;12(11):4977-4987.