Sensitization of PANC1 Cells by ATR inhibition

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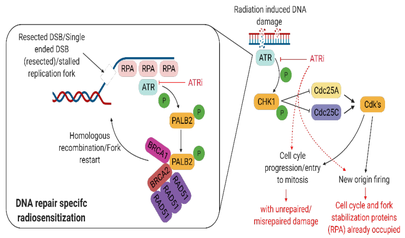
Background

- Combining drugs with radiation has been shown to have a synergistic effect. This effect can be quantified by pH3 population and cell cycle distribution.

ATR is a DNA repair protein involved in repairing double strand breaks, which is caused by radiation. Phospho-histone-3 is a marker used to quantify mitotic population.

ATR inhibition (ATRi) in other works has been sown to enhance cell death (add references). Previous studies have also shown that ATRi disrupts cell cycle arrest at G2, resulting in more damaged cells going into mitosis, increasing genotoxicity and cytotoxicity[3]

Figure 1. Radiation causes double strand breaks (DSBs) and single strand breaks (SSBs), which activates DNA repair proteins like ATR. ATR activates Checkpoint Kinase 1 (CHK1) by phosphorylation, preventing cancer cells' progression into the mitotic phase. Using an ATR inhibitor, one can cause these damaged cells to enter mitosis as ATR will dephosphorylate CHK1

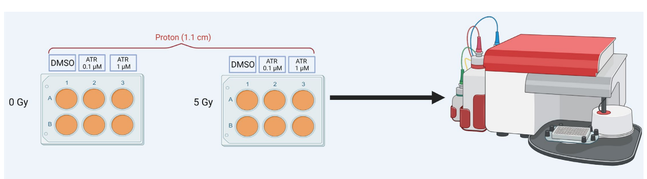


Hypothesis

Cells treated with ATR inhibitor and radiation are expected to have a higher PH3 population and have a smaller G2 peak.

Methods

PANC-1 (epithelial pancreatic carcinoma) were treated with DMSO, and 0.1 µM or 1 µM ATRi (AZD6738). Cells were then irradiated with 6 MV x-rays or 9.9 keV/ µm protons. The cells were incubated for 48 hours before being fixed with ethanol and run in the Flow Cytometer (BD Accuri C6). The data was then analyzed in FlowJo 10.7.1



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Results

ATRi is shown to stop G2 blockage (**Figure 2**) regardless of whether proton or photon radiation was used.
Cell cycle analysis showed that both X-rays alone and protons alone arrest cells in G2 so that cells can repair DNA. However, ATRi significantly disrupts G2 arrest for both X-rays and protons. PH3 population is generally higher in both the 24hr and 48hr groups when combined with protons+ATRi. We initially tried a time point of 24 hour, but saw an insignificant difference between 1 µM ATRi and DMSO treatment groups. A more noticeable difference was observed in the one 48 hr trial. The reason why it was necessary to see the PH3 population at 48 hr is because PANC1 has a long doubling time (approximately 56 hrs [1]), thus it was expected that there would be more PH3 at 48 hr compared to 24 hr. Time constraints prevented additional trials at 48 hr.

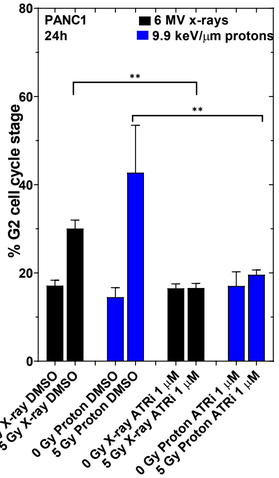
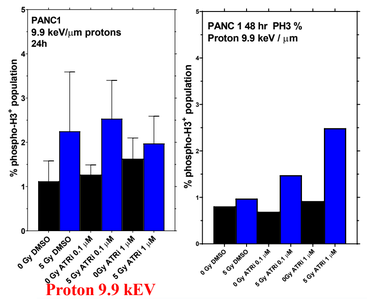


Figure 2. Cell cycle analysis for 24 post-radiation in PANC1 cells showing inhibition of G2 blockage by ATRi combined with protons or X-rays.



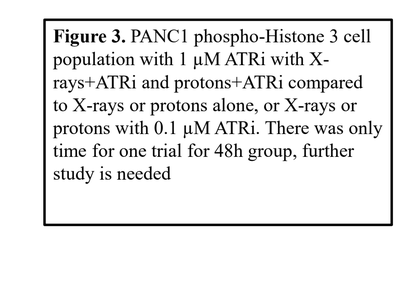
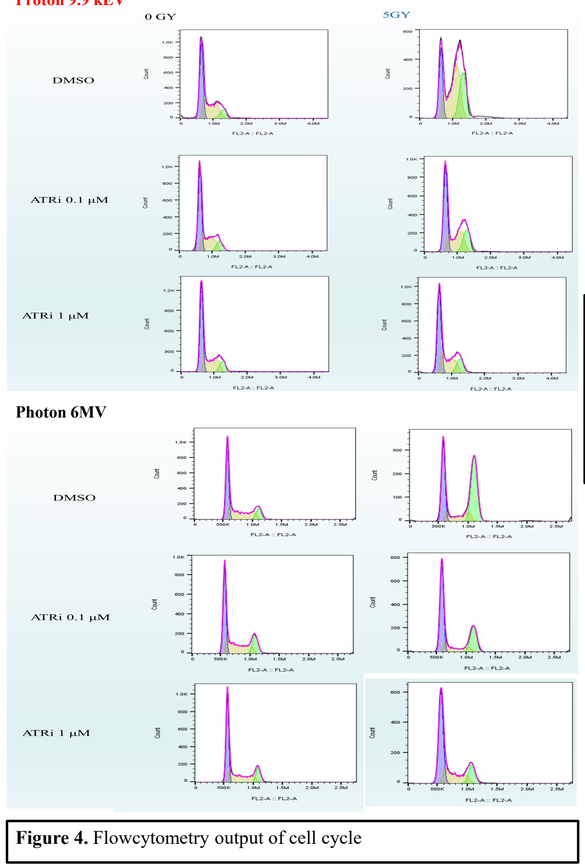


Figure 3. PANC1 phospho-Histone 3 cell population with 1 µM ATRi with X-rays+ATRi and protons+ATRi compared to X-rays or protons alone, or X-rays or protons with 0.1 µM ATRi. There was only time for one trial for 48h group, further study is needed



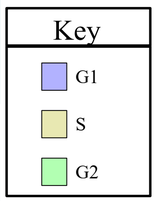


Figure 4. Flowcytometry output of cell cycle

Conclusions

Our initial findings indicate robust G2 checkpoint deactivation after both photon and proton treatment with ATRi. Protons + ATRi disrupted the cell cycle checkpoint and increased the number of cells in mitosis compared to photons + ATRi. Our results suggest that ATRi is effective at inducing sensitivity of cancer cells to radiation, possibly because of the the role of ATRi in phosphorylating CHK1. Further research is necessary to understand the how long to incubate between treatments.

References

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2. Vitti, E. T. and J. L. Parsons (2019). "The Radiobiological Effects of Proton Beam Therapy: Impact on DNA Damage and Repair." Cancers (Basel) 11(7).
3. Tu, X., et al. (2018). "ATR Inhibition Is a Promising Radiosensitizing Strategy for Triple-Negative Breast Cancer." Molecular Cancer Therapeutics 17(11): 2462.

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