# Combination therapy of DNA repair inhibitors and ionizing radiation to enhance DNA damage in 4T1 murine breast cancer and H1299 non-small cell human lung carcinoma cell lines

Vijay Patel, Broderick X. Turner Scott J. Bright, David B. Flint, David Martinus, Mandira Ben Kacem, Simona F. Shaitelman and Gabriel Sawakuchi

Department of Radiation Physics, The University of Texas MD Anderson Cancer Center, Houston, TX

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

## Background

Cancer treatments are often non-selective and non-specific towards the cancer cell line. An intentional pairing of drugs with radiation could yield improved and synergistic treatment. Both radiation types, protons and photons, yield double strand breaks, but protons deliver more clustered damage, which is more difficult to repair than photon damage. DNA repair inhibitors prevent radiation damage from being undone. We investigated the combination of DNA-repair inhibitors with radiation therapy in non-small cell human lung carcinoma H1299 and murine breast cancer 4T1 cell lines. Ceralasertib is a drug that inhibits ataxia telangiectasia and Rad3-related (ATR) kinase, a protein prominent in homologous recombination. Since protons inflict greater DNA damage, they yield the most damage to cells when paired with a DNA repair inhibitor.

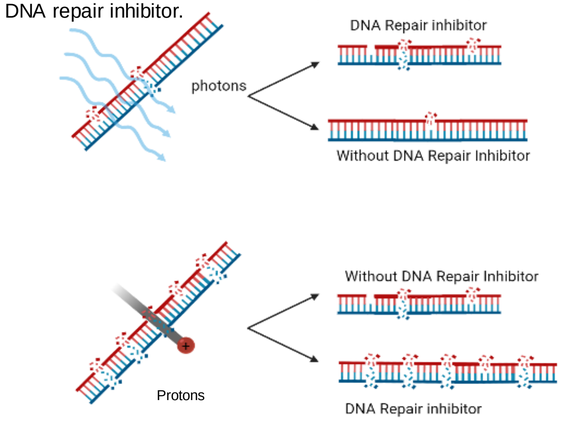


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**Figure 1**. This figure visualizes the concept of the experiment. The above image displays photon damage which has less damage overall and is sparser. The bottom image is of proton damage which is clustered and has more double strand break. Each image shows what DNA would look like without or with DNA repair inhibitors. Without DNA repair inhibitors, the DNA presents less persistent damaged.

## Hypothesis

**Proton radiation in combination with DNA repair inhibitors will lead to increased DNA damage compared to photon radiation with DNA repair inhibitors.**

## Methods

**Cell seeding**

200,000 cells were grown and seeded at a density of 200,000 and left to incubate for 24 hours.

**Drug Treatment and Radiation**

1 µM of either DMSO, Ceralasertib, or Olaparib was added to the cell condition. After one hour they were irradiated with either 5 Gy of Protons or Photons and incubated for 24 hours.

**Comet Slides**

An Alkaline Comet assay was performed. Cells were placed on comet slide in agarose gel. Once dry, gel electrophoresis was performed at a pH of 14.

**Imaging**

Cells were stained with sybr gold and imaged using Cytation 5.

**Analysis**

Images were analyzed using Comet Score. Overlapping cells and debris were removed to maintain data integrity.

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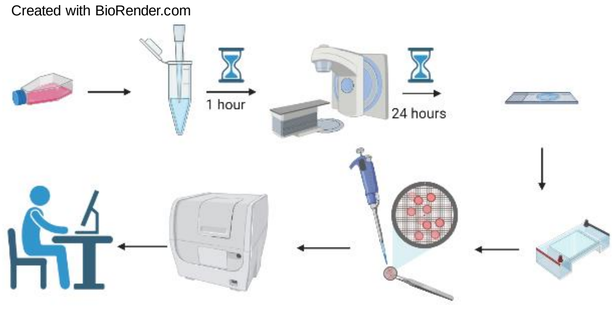


**Figure 2**. This figure demonstrates the procedure. Cells are seeded and incubated with drugs one hour prior to radiation. After 24 hours they are placed on slides and run on an Alkaline Comet Assay. Once dry, cells are stained with sybr gold and imaged using Cytation 5. The images are analyzed using comet score.

## Results

- H1299 had increased DNA% damage
  - Proton+Ceralasertib was 1.27x more effective than photons+Ceralasertib
- 4T1 had a similar trend
  - Proton+Ceralasertib was 1.14x more effective than photons+Ceralasertib
  - More trials are needed to determine significance

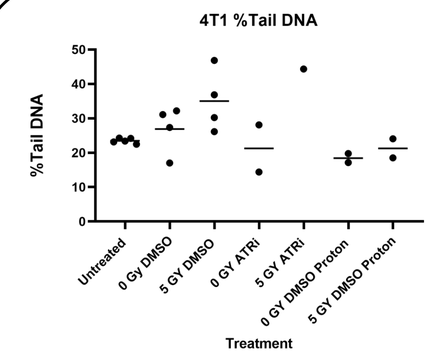


**Figure 3.** This figure shows the average amount of DNA in comet tails of each condition in 4T1. Radiation with DNA repair inhibitors were the most effective option, however a lack of additional proton trials yielded less accurate results. An important trend is that the combination therapy was more effective than isolated therapies

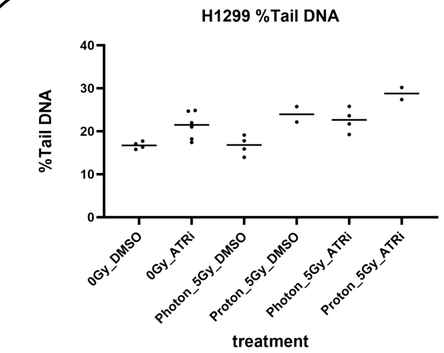


**Figure 4.** This figure shows the average amount of DNA in comet tails of each condition in H1299. Proton+ATRi yielded the greatest DNA damage out of all combinations. In each instance of drug with protons or photons, protons yielded greater damage

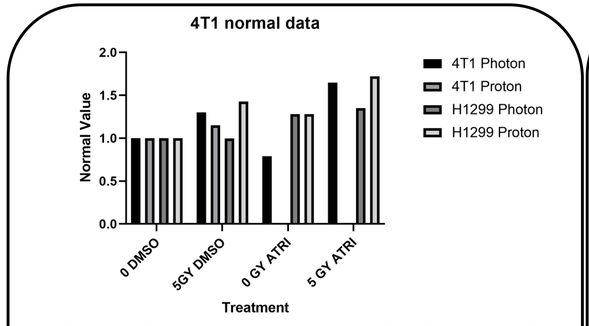


**Figure 5**. This figure shows the comparative effectiveness of each condition compared to the control: 0Gy DMSO. Combination therapies were marginally more effective than isolated treatments, and protons were the most effective compared to the controls of that specific trial.

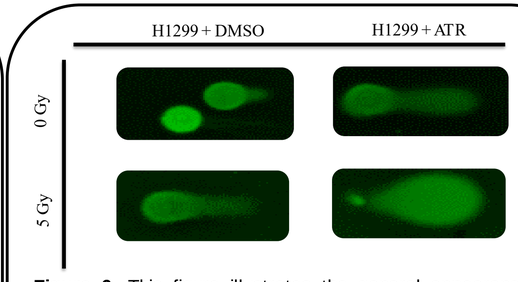


**Figure 6**. This figure illustrates the general appearance of comet tails 24-hours after being treated with photon irradiation and DMSO or 1 µM ATR. The green color is Sybr Gold stain, which highlights nuclear DNA. It shows the general appearance of comet tails of after treatment in the H1299 lung cancer cell line in vitro.

## Conclusions

- Combined DNA repair inhibitors with radiation was the most effective
  - Protons with inhibitors was the most effective
- Similar trends were seen between both cell lines
  - Could imply applications to other cell lines
- DNA repair inhibitors could be tailored to cell lines

## Future Directions

- Test PARP inhibitors in 4T1
- Additional proton trials for 4T1
- Determine efficacy in additional cell lines
- Test treatment viability in animal models

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