



# Combination of ATR Inhibition and X-Ray Irradiation Radiosensitizes Cancer Cells

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## Background

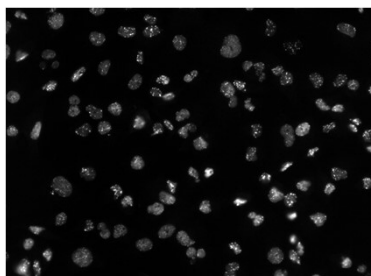
Efforts to suppress cancer cells after radiotherapy fail due to their ability to repair radiation-induced damage. One pathway to repair DNA double-strand breaks (DSBs) is homologous recombination (HR). HR is assisted by Ataxia telangiectasia and Rad3-related protein (ATR), which phosphorylates checkpoint kinase 1 (CHK1) upon exposure to single-stranded DNA, which promote HR factors like Rad51. Combining ATR Inhibitors (ATRi) with DSB-induced radiation sensitizes cancer cells by forcing them to find alternative pathways such as non-homologous end joining (NHEJ), which is not as precise in DSB repair as HR. Thus, we experiment with the combined effect of x-rays and ATRi on cancer cell DSB repair.

## Hypothesis

We hypothesize that increasing concentrations of ATRi will result in reduced number of foci per nuclei at early time points while later time points will show increased number of foci per nuclei.

## Methods

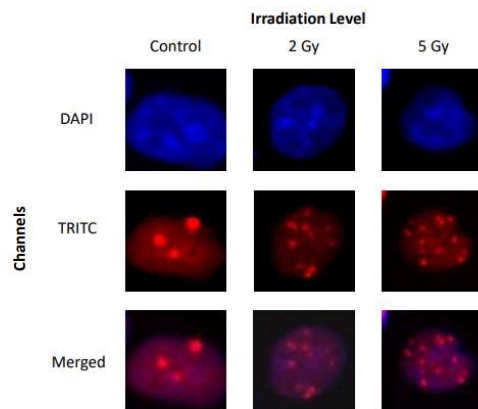
H1299 (lung cell carcinoma) and Panc1 (pancreatic cell carcinoma) cell lines were irradiated by 6 MV x-rays with and without ATRi (0.1-1  $\mu$ M, AZD6738). Immunofluorescent staining with the DSB-repair protein 53BP1 was then performed. Cells were then fixed at 4-, 8-, and 24-hour timepoints.



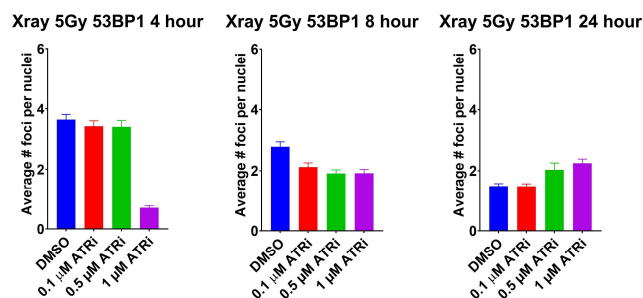
**Fig. 1** 53BP1 foci overlayed on nuclei 4 hours post x-ray irradiation of Panc1 cell line

## Results

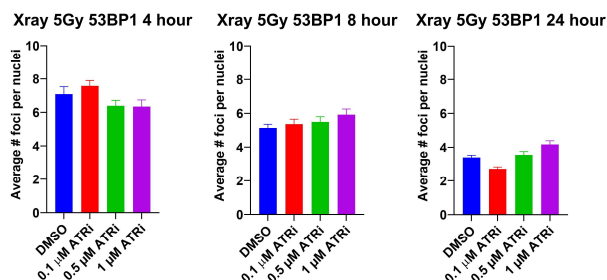
4 hours post-irradiation, increasing levels of ATRi were shown to decrease 53BP1 foci in both Panc1 and H1299 cell lines, as shown in figures 3 and 4, respectively. 8 hours post-irradiation, increasing levels of ATRi were shown to decrease 53BP1 foci in Panc1 but increase 53BP1 foci in H1299. However, at the 24-hour fixation, increasing levels of ATRi were shown to increase 53BP1 foci for both cell lines.



**Fig. 2** Panc1 cells were given 1  $\mu$ M ATRi at DMSO control, 2Gy, and 5Gy irradiation levels. DAPI, TRITC, and a combined DAPI-TRITC channel were shown, displaying 53BP1 foci



**Fig. 3** 53BP1 foci count 4-, 8-, and 24-hours post x-ray irradiation of Panc1 cell line



**Fig. 4** 53BP1 foci count 4-, 8-, and 24-hours post x-ray irradiation of H1299 cell line

## Conclusions

Addition of ATRi radiosensitizes Panc1 and H1299 cells by inhibiting DSB repair. The TRITC channel displays 53BP1 foci that appear during repair pathways, which activate at higher levels of irradiation. Our results show that, at higher levels of x-ray irradiation, more 53BP1 foci appear per nucleus, an example shown in figure 2. This results from the fact that photons cause DSBs, which cells repair through several pathways. Thus, x-rays irradiation is a viable source of cancer cell damage. Our results also show that, 4 hours post-irradiation, cells of either cell line with 1  $\mu$ M concentration ATRi were hindered in their ability to repair the DNA damage in comparison to cells with lower concentrations of ATRi and DMSO control. 24 hours post-irradiation, early lesions of DSBs in these cancer cells lingered with higher concentration ATRi, forcing such cells to find alternate DNA repair pathways to repair this damage. These results show that inhibiting ATR sensitizes pancreatic and lung cancer cell lines to x-ray irradiation. This sensitization increases at higher levels of irradiation.

## Resources

### ImageJ

<https://imagej.net/software/fiji/downloads>

### CellProfiler

<https://cellprofiler.org/releases>

### MATLAB

[https://www.mathworks.com/products.html?s\\_tid=gn\\_ps](https://www.mathworks.com/products.html?s_tid=gn_ps)