Potential to improve conventional therapy using inhibitors of the DNA damage response in ovarian cancer

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Background

- Ovarian cancer is the 7th most common cause of cancer in women and less than half survive for 5 years.
- When patients relapse they may be given Gemcitabine[1], which works by stopping DNA replication in cells.

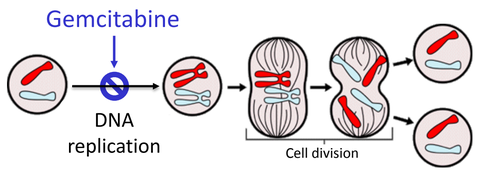


Figure 1. DNA replication occurs before each cell division. Gemcitabine stops DNA replication and leads to cell cycle arrest, thereby killing cancerous cells.[2]

- When DNA is damaged or arrested, the cell activates the DNA damage response (DDR). This may cause resistance to anticancer drugs.
- ATR and DNA-PK are key components of two different DDR pathways.
- ATR activation by gemcitabine can cause resistance.
- DNA-PK acts on different types of DNA damage but may also compete with ATR for DNA lesions[3].

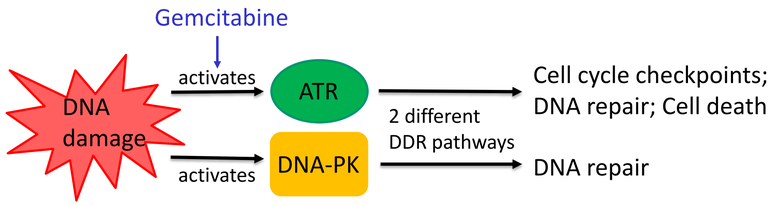


Figure 2. DNA damage activates ATR and DNA-PK pathways, leads to DNA repair and cell survival. Gemcitabine can activate ATR and cause resistance.

Aims

- To investigate the effect of ATR inhibition on the ability of gemcitabine to kill ovarian cancer cells ES-2
- To determine if DNA-PK inhibition reduces or prevents ATR activation by gemcitabine
- To see whether DNA-PK inhibitor would increase ATR inhibitor-mediated chemosensitisation of gemcitabine

Results

Gemcitabine activates ATR and ATR activation is inhibited by the ATR inhibitor, but not the DNA-PK inhibitor

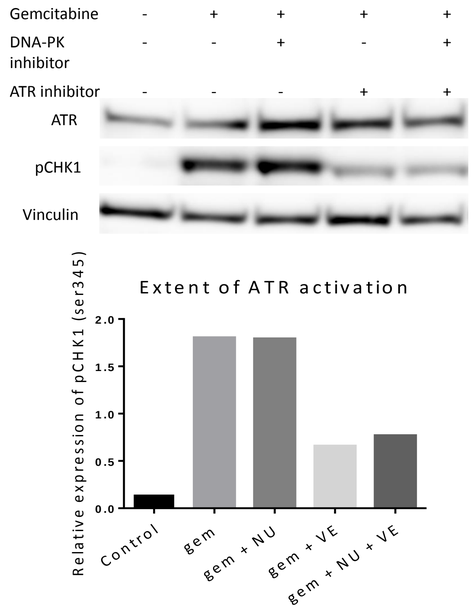


Figure 3. Activated ATR phosphorylates CHK1. This can be measured using specific antibodies by Western Blot. The thicker the band, the more phosphorylated CHK1 (pCHK1) proteins. DNA-PK inhibitor (NU) did not decrease CHK1 phosphorylation while the ATR inhibitor (VE) and combination of both DNA-PK and ATR inhibitor reduced ATR activation.

Increasing concentrations of gemcitabine reduce cell survival. The extent of cell killing by gemcitabine is increased by the ATR inhibitor but not the DNA-PK inhibitor

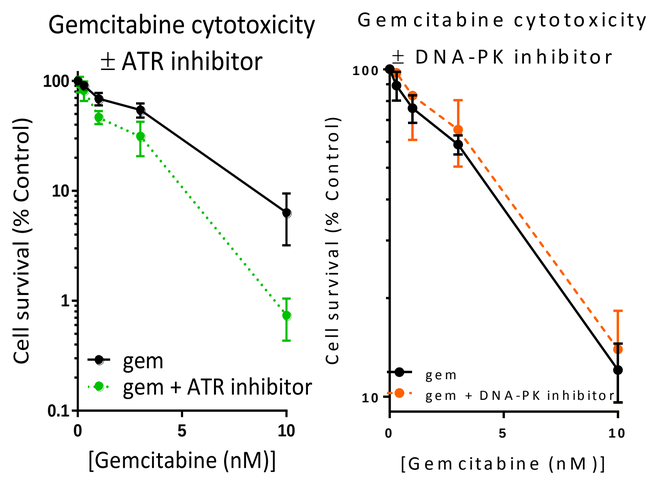
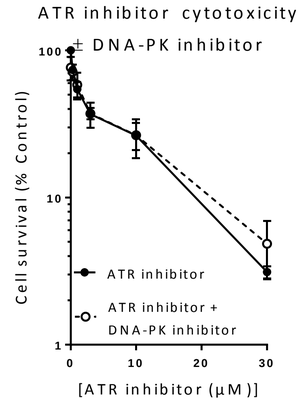


Figure 4. The survival of cells was measured by their ability to form colonies after exposure to drugs. Increasing concentrations of gemcitabine caused a progressive decrease in cell survival. The extent of cell killing by gemcitabine was increased by the ATR inhibitor but not the DNA-PK inhibitor.

Results



The DNA-PK inhibitor did not affect the ability of the ATR inhibitor to kill the cells

Figure 5. Increasing concentrations of the ATR inhibitor alone cause a progressive decrease in cell survival that was not affected by the DNA-PK inhibitor.

Conclusions

- Gemcitabine activates ATR signalling , which is inhibited by the ATR inhibitor.
- The ATR inhibitor increased the ability of gemcitabine to kill ovarian cancer cells.
- **This indicates that ATR activation by gemcitabine helps cells to survive gemcitabine treatment and so the combination of an ATR inhibitor with gemcitabine could be useful.**
- The DNA-PK inhibitor did not prevent ATR activation by gemcitabine or its inhibition by the ATR inhibitor.
- The DNA-PK inhibitor did not increase cell killing by gemcitabine or the ATR inhibitor.
- **These data suggest that DNA-PK is not involved in the resistance to gemcitabine and does not compete with ATR for the signalling of gemcitabine-induced DNA damage.**

Reference

1 Zhu X, Straubinger RM, Jusko WJ. Mechanism-based mathematical modeling of combined gemcitabine and birinapant in pancreatic cancer cells. Journal of pharmacokinetics and pharmacodynamics. 2015 Oct;42:477-96.

2 Urbano L. Meiosis: Passing on Half of Your Genes. 2013 [cited 2017 August]; Available from: http://montessorimuddle.org/2013/05/02/meiosis-passing-on-half-of-your-genes/

3 Middleton FK, Patterson MJ, Elstob CJ, Fordham S, Herriott A, Wade MA, McCormick A, Edmondson R, May FE, Allan JM, *et al.* Common cancer-associated imbalances in the DNA damage response confer sensitivity to single agent ATR inhibition. Oncotarget. 2015 Oct 20;6:32396-409.