Use of ATR-PARP inhibitor combination therapy to treat and generate an immune response in PARP inhibitor resistant breast cancer is questioned

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Introduction

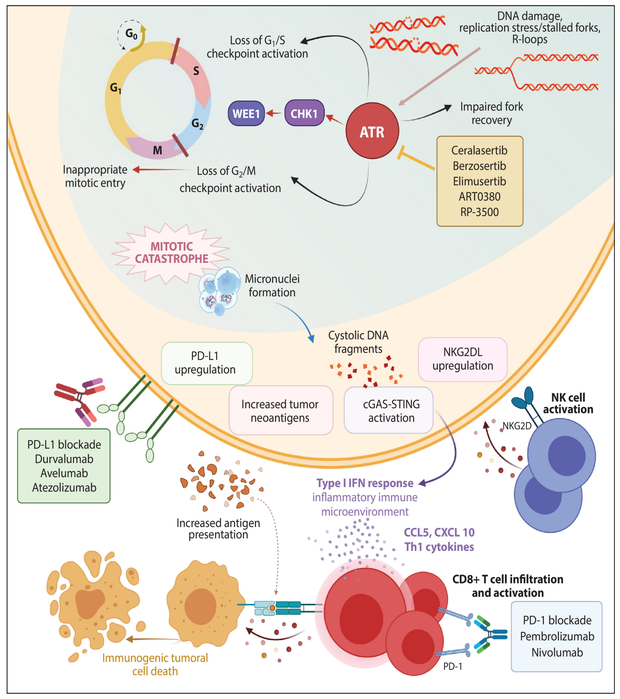

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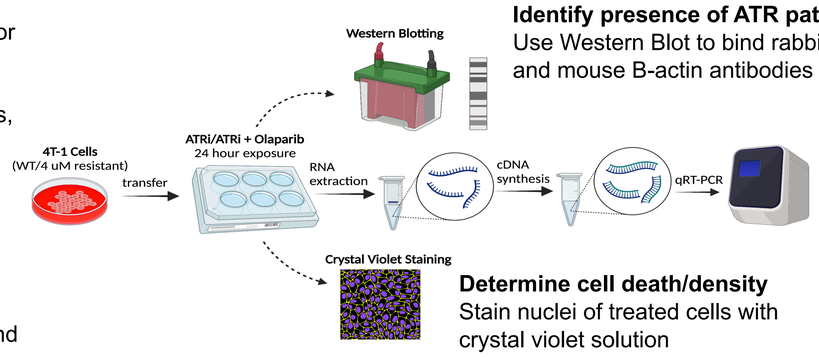
Figure 1

- Clinically, PARP inhibitor (PARPi) Olaparib is used to treat ovarian and breast cancer patients with BRCA1/2 mutations and those without. However, tumors will inevitably develop resistance to PARPi[1].
- Treatments in development to overcome this resistance are urgently needed in clinical settings. Currently, combination therapies of PARPi, ATR inhibitors (ATRi), and PD-1/PD-L1 inhibitors are being tested in clinical trials[2]. A key question remains: **Can ATRi/PARPi-ATRi be used to re-sensitize PARPi resistant tumors?**
- ATRi forces improper mitotic entry despite unrepaired DNA lesions. This creates cytosolic DNA fragments that triggers the cGAS-STING pathway for a Type I IFN response, such as the production of CCL5 and CXCL10. Consequently, these chemokines/cytokines promote T-cell chemotaxis and antitumor effects[3] (Fig. 1). The second key question is: **Can ATRi exert antitumor effects, in addition to causing unresolved genomic lesions and cell death, by generating an immune response?**

Methodology

Cell culturing and treatment

- 4T-1 mouse cells [Wild Type (**WT**) or 4 uM Olaparib resistant cells (**Resistant**)], which closely mimic human Stage IV breast cancer cells, are cultured and transferred onto six-well plates
- 24-hour exposure to either:
  - **ATRi treatment:** ATRi (AZD6738) of 0, 1, 2, or 5 uM
  - **ATRi-PARPi treatment:** ATRi of 0, 1, 2, or 5 uM and 4 uM Olaparib

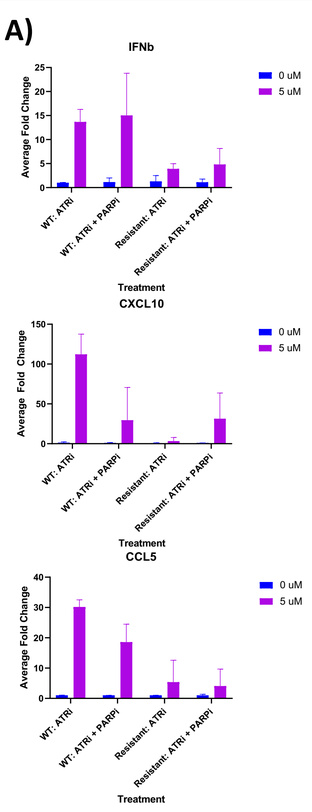


Identify presence of ATR pathway proteins
Use Western Blot to bind rabbit p-CHK1, rabbit p-RPA32/RPA32, and mouse B-actin antibodies to the membrane

Detect level of gene expression (CXCL10, CCL5, IFNb, Actin)
After RNA extraction and cDNA synthesis, qRT-PCR is run to measure the average fold change of gene expression

Determine cell death/density
Stain nuclei of treated cells with crystal violet solution

Results



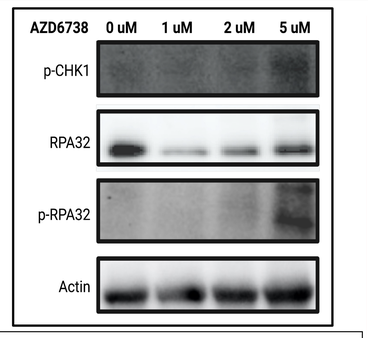


Figure 2:
A) ATRi and ATRi-PARPi treatments exhibit differential effects in WT and PARPi resistant cells regulating Type 1 INF pathway CXCL10, CCL5, IFNb expression. WT 4T-1 cells were exposed to indicated concentrations of ATRi/ATRi-PARPi treatment for 24 hours. qRT-PCR measured level of gene expression. (Mean±SD)
B) ATRi induces DNA damage at high concentrations. WT 4T-1 cells were treated with ATRi at indicated concentrations for 24 hours. Proteins were extracted for Western Blot analysis. DNA damage markers used were p-CHK1, RPA32 and p-RPA32.

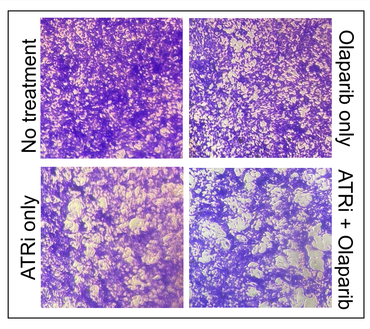
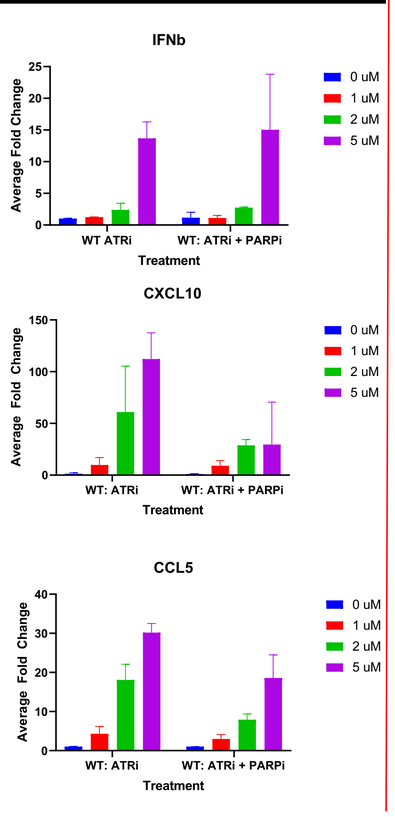


Figure 3:
A) ATRi-PARPi combination exhibits synergy in inhibiting cell survival. 4T-1 WT cells were moved to six-well plates and exposed to treatments indicated in the figure for 24 hours: *ATRi 5 uM and/or PARPi Olaparib 10 uM*. Cell survival was measured using crystal violet staining.
B) ***ATRi-PARPi combination does not show synergy in promoting a Type 1 IFN response of CCL5, CXCL10, and IFNb expression***. WT cells were treated with ATRi or ATRi-PARPi treatment for 24 hours at indicated concentrations. RNA was extracted for RT-PCR analysis. The bar graphs represent three experiments. (Mean±SD)



Results cont.

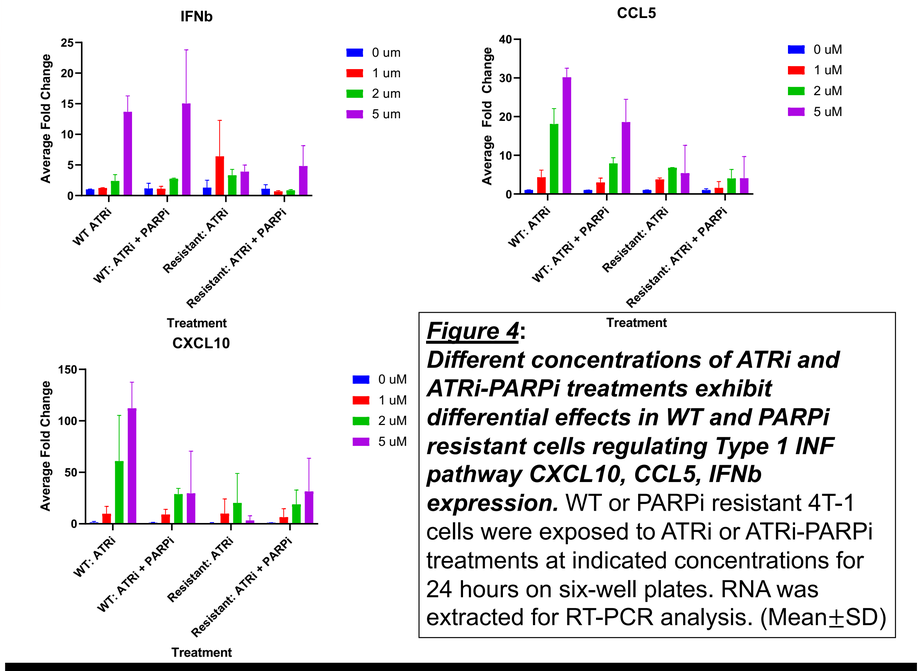


Figure 4:
Different concentrations of ATRi and ATRi-PARPi treatments exhibit differential effects in WT and PARPi resistant cells regulating Type 1 INF pathway CXCL10, CCL5, IFNb expression. WT or PARPi resistant 4T-1 cells were exposed to ATRi or ATRi-PARPi treatments at indicated concentrations for 24 hours on six-well plates. RNA was extracted for RT-PCR analysis. (Mean±SD)

Conclusion

- In this experiment, we observed ATRi at high concentrations displayed a DNA damage response, likely through alternative phosphorylation signaling pathways (Fig 2 B).
- Our data showed that ATRi and ATRi-PARPi can induce Type 1 INF response in WT cells but not in PARPi resistant cells (Fig 2 A, Fig 4).
- ATRi-PARPi synergistically induces cell death in WT cells but does not synergistically promote the activation of Type 1 INF response in WT cells (Fig 3, Fig 4).

Discussion and Future Directions

- These experiments were conducted in a 4T-1 breast cancer mouse cell line. To confirm these observations, additional cell lines, cancer types, and replicates need to be further tested.
- We speculate the generation of DNA fragments from the damaged genome in the ATRi-PARPi combination or in the resistant line is not as efficient as the ATRi alone or in WT cells, leading to differential effects in Type 1 interferon induction. To gain mechanistic understanding, the effect of ATRi and ATRi-PARPi on DNA damage signaling and cytosolic DNA accumulation should be examined using Western blot and Picogreen. This may explain the differential effects in WT and resistant cell lines, as well as the differential effects in ATRi and ATRi-PARPi treatment.
- Alternative signalling pathways such as ATM, DNA-PK or AKT could be analyzed to explain p-CHK1 and p-RPA32 in the presence of ATRi or ATRi-PARPi.
- These observations need to be further tested in pre-clinical animal models to potentially guide the choice of ATRi in PARPi resistant tumors.

References

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