Does Immune Signaling Contribute to PARP inhibitor induced Synthetic Lethality?

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INTRODUCTION

- PARP inhibitors (PARPi) have been found to be most effective when targeting BRCA1 and 2 breast and ovarian cancers.

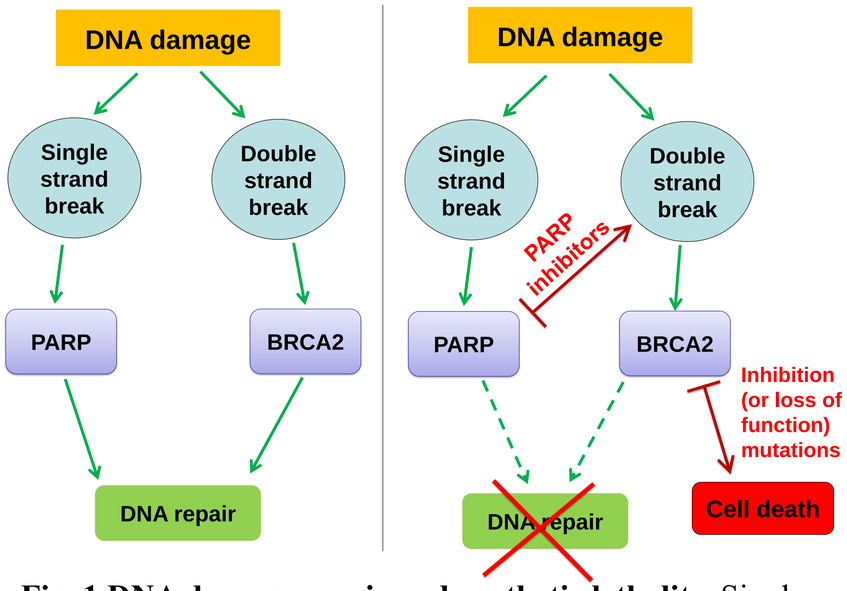


Fig. 1 DNA damage repair and synthetic lethality. Single strand breaks are repaired by PARP, so inhibiting it will lead to double strand breaks (DSBs). DSBs are repaired by BRCA2 (and BRCA1- not shown here), so if that is also defective ⟶ cell death

- Defects in both these complementary DNA repair pathways underlie the principle of **synthetic lethality**, which is thought to be the reason for PARPi mediated killing of BRCA2 defective cancer cells.
- If that were the case, PARPi should kill cells shortly after being administered (as DSBs would accumulate after every replication). However, PARPi-induced cell killing in vitro does not occur until several days after drug exposure, and the reason for this is unknown.
- PARPi recently have been found to activate pro-inflammatory cytokine production (i.e. TNFα), as does chronic inactivation of BRCA2.
- We hypothesize that PARPi induced immune signaling (i.e. TNFα) contributes to the synthetic lethality with BRCA2.

RESULTS

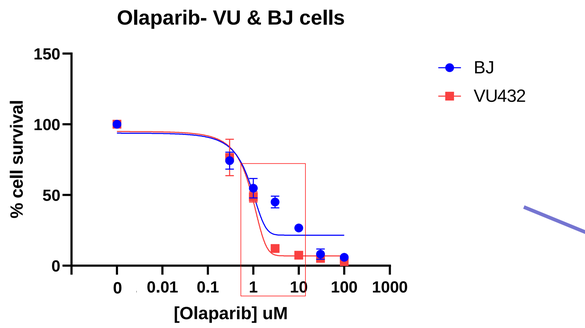
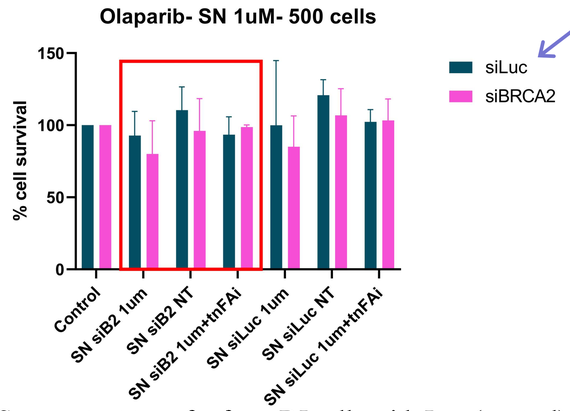


Fig. 2 Semi-log dose response curve of VU432 and BJ (wild-type) cells treated with Olaparib (0.3 to 100uM) . Used to select drug concentration for further experiments.



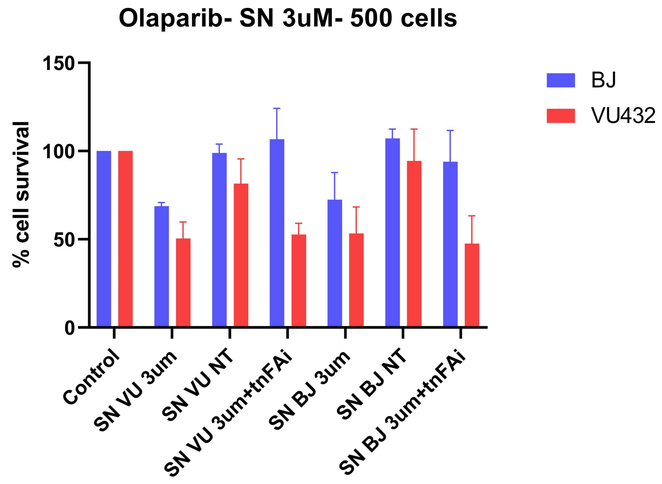


Fig. 3 Supernatant (SN) transfer from treated (Olaparib, 3uM) and untreated BJ and VU432 to naive BJ and VU432 cells. Humira (a TNFαi) was added to half of the treated supernatant before administration to naive cells. Cell survival was assessed through MTS assay after 3 days.

Fig. 4 Supernatant transfer from BJ cells with Luc (control) and BRCA2 knockdowns- treated with 1 and 3 uM Olaparib (1uM shown here)- to untreated BJ cells with Luc and BRCA2 knockdowns. Further details under 'Methods.'

Since BJ cells, unlike VU432, are rescued upon addition of TNFαi, we decided to use them in the next step (Fig 4), for BRCA2 knockout

METHODS

BRCA2 depleted BJ cells were seeded in multiple 96-well plates and treated with differing concentrations of Olaparib (a PARPi). Two and three days later, the supernatant (SN) transfer of Olaparib-treated cells was transferred to untreated cells with and without BRCA2 depletion. To test the potential involvement of TNFa, an inhibitor of TNFα was included to treated and untreated cells. Cell survival was assessed using Zen Imaging software and MTS colorimetric survival assay 5 days after initial PARPi treatment.

- **Cell culture** (BJ and VU432 cells)
  - **Supernatant transfer from treated to untreated cells**
  - **Cell splitting, seeding/plating and drug treatment**
  - **Cell harvesting for western blot**
- **MTS assay**
- **Zeiss Microscopy Zen Imaging Software**

CONCLUSION

- We wanted to see if the SN of PARPi-treated cells- theoretically containing cytokines (i.e. TNFα)- would also kill untreated cells as that would suggest inflammation signaling is the reason behind PARPi killing of BRCA2 depleted cells, as opposed to synthetic lethality by DNA repair defects.
- If cells treated with SN died while those with SN + TNFαi didn't, that would mean that TNFα is the main immune signaling factor that is involved with PARPi killing.
- As of now, we have no concrete evidence to support the hypothesis as our results aren't statistically significant. Final experiment should be repeated in case of human error.

References

1) Reisländer, T., Lombardi, E.P., Groelly, F.J. et al. BRCA2 abrogation triggers innate immune responses potentiated by treatment with PARP inhibitors

2) Heijink, A.M., Talens, F., Jae, L.T. et al. BRCA2 deficiency instigates cGAS-mediated inflammatory signaling and confers sensitivity to tumor necrosis factor-alpha-mediated cytotoxicity