Differences in durability of PARP inhibition by PARP inhibitors in ovarian cancer cells

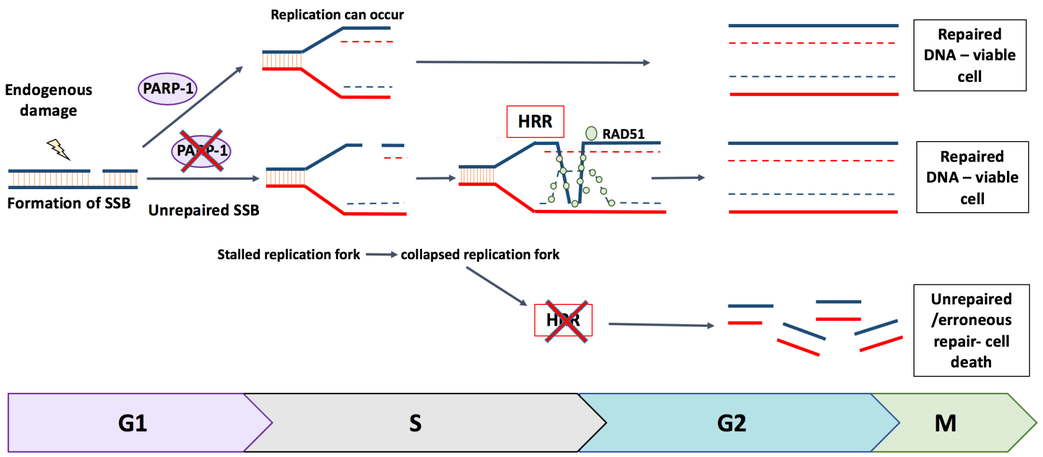
Hannah L Smith [1], Elaine Willmore [2] and Nicola J Curtin [1].

[1] Newcastle Centre for Cancer, Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne NE2 4HH, UK
[2] Cancer Research UK Drug Discovery Unit, Newcastle Centre for Cancer, Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne NE2 4HH, UK

Background

- Poly (ADP-ribose) polymerase (PARP) is the first line of defence against the commonest endogenously-induced DNA damage: single strand breaks (SSB), by promoting their repair.
- PARP inhibitors (PARPi) exploit defects in homologous recombination repair (HRR) to selectively kill tumour cells [1].
- To effectively target these HRR defective tumours PARP must be continuously inhibited, allowing cells to go through S-phase whilst inhibited [2, 3].
- 4 PARPis (rucaparib, olaparib, niraparib and talazoparib) are approved for use in cancer patients using daily dosing schedules [4].
- Pre-clinical data from 2014 showed rucaparib caused PARP inhibition in tumour xenografts for at least 7 days after a single dose [5].

Our aim was to we determine if persistent PARP inhibition is unique to rucaparib or a class effect common to all approved PARPi.



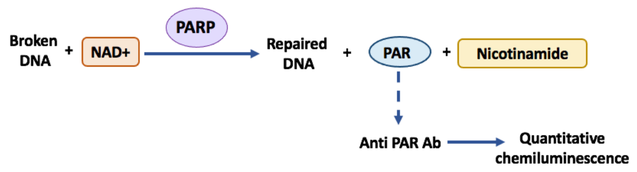
Synthetically lethal relationship between PARP and HRR defective cells. Endogenously generated SSB are repaired continuously by PARP dependent repair mechanisms. When PARP is inhibited unrepaired SSB collide with replication forks causing them to stall and collapse resulting in DSBs which can only be repaired by HRR during S and early G2 phase. If HRR is defective, e.g. due to *BRCA* mutation the DNA cannot be repaired accurately, resulting in cell death.

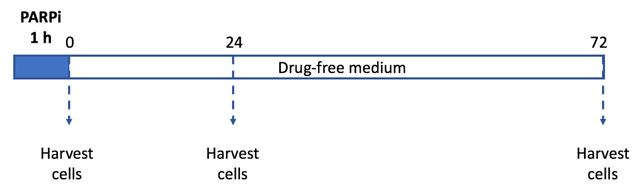
Conclusions

- Rucaparib is unique in its ability to cause persistent PARP inhibition compared to other PARPis and it is not a class effect.
- These data have important clinical implications for the different uses of PARPi: for single agent activity exploiting HRR defects durable PARP inhibition is required. In contrast, for combinations with cytotoxic agents causing DNA SSBs (e.g temozolomide, topotecan, radiotherapy) less durable PARPi may be less toxic.
- These data suggest that the current twice daily dosing approved for rucaparib treatment may not be necessary. Further studies are needed to determine whether less frequent dosing would have equivalent anticancer activity.

Methods

A biochemical GCLP-validated assay was conducted on permeabilised cells to measure PARP activity using oligonucleotide to mimic DNA breaks and an excess of the substrate NAD+ [6]. The assay based on the following reaction:

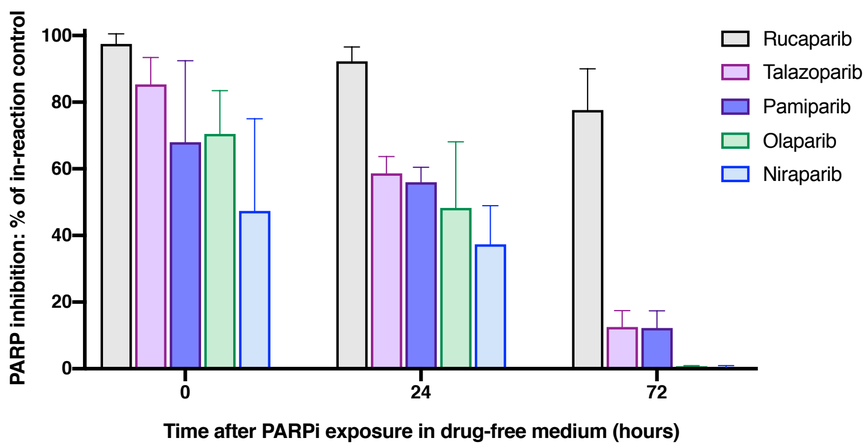




IGROV-1 ovarian cancer cells were treated with 1 µM rucaparib, olaparib, niraparib, talazoparib or pamiparib for 1 hr before drug was washed off and replaced with fresh media. Cells were harvested and cellular PARP activity was measured and compared to untreated control and where 1 µM was added directly to permeabilised cells in the reaction.

Results

Rucaparib, olaparib, niraparib, pamiparib and talazoparib each inhibited PARP activity >99% in permeabilised cells with 1 µM added to the reaction.



- Rucaparib, olaparib, niraparib, talazoparib and pamiparib each inhibited PARP activity in permeabilised cells >99% when 1µM was present during the reaction.
- Only rucaparib maintained this level of inhibition in cells harvested immediately after exposure to PARPi, with the other inhibitors only inhibiting PARP 47-85%, suggesting failure to achieve adequate intracellular concentrations or wash-out during harvesting.

- After 24 h in drug-free medium rucaparib-induced PARP inhibition was maintained at 92.3 ± 4.3% but was much less with talazoparib (58.6 ±5.0%), pamiparib (56.0 ± 4.5%) olaparib (48.3 ± 19.8%) and niraparib (37.3 ± 11.6%)
- PARP inhibition declined with time but in rucaparib-treated cells was maintained for 72h in drug-free medium (77.7 ± 12.3%). This sustained PARP inhibition was not observed with the other PARPis. PARP inhibition was only 12.3 ± 5.2% and 12.5 ± 4.9% 72h after talazoparib and pamiparib, respectively, and undetectable with olaparib and niraparib.

[1] Li, H.; Liu, Z.; Wu, N.; Chen, Y.; Cheng, Q. and Wang, J. PARP inhibitor resistance: the underlying mechanisms and clinical implications. *Molecular Cancer* **2020**, *19*. [2] Javle, M. and Curtin, N. J. The role of PARP in DNA repair and its therapeutic exploitation. *British Journal of Cancer* **2011**, *105*. [3] Michelena, J.; Lezaja, A.; Teloni, F.; Schmid, T.; Imhof, R. and Altmeyer, M. Analysis of PARP inhibitor toxicity by multidimensional fluorescence microscopy reveals mechanisms of sensitivity and resistance. *Nature Commun* **2018**, *9*. [4] Jiang, X.; Li, W.; Li, X.; Bai, H. and Zhang, Z. Current status and future prospects of PARP inhibitor clinical trials in ovarian cancer. *Cancer Manag Res* **2019**, *11*. [5] Murray, J.; Thomas, H.; Berry, P.; Kyle, S.; Patterson, M.; Jones, C.; Los, G.; Hostomsky, Z.; Plummer, E. R.; Boddy, A. V. and Curtin, N. J. Tumour cell retention of rucaparib, sustained PARP inhibition and efficacy of weekly as well as daily schedules. *British Journal of Cancer* **2014**, *110*. [6] Plummer, R.; Jones, C.; Middleton, M.; Wilson, R.; Evans, J.; Olsen, A.; Curtin, N.; Boddy, A.; McHugh, P.; Newell, D.; Harris, A.; Johnson, P.; Steinfeldt, H.; Dewji, R.; Wang, D.; Robson, L. and Calvert, H. Phase I study of Poly (ADP-ribose) Polymerase Inhibitor, AG014699, in Combination with Temozolomide in Patients with Advanced Solid Tumors. *Clinical Cancer Research* **2008** *14*.