# Exploring the roles of XRCC1 and PARP (poly(ADP-ribose) polymerase) in the response to anticancer agents.

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## Background:

DNA damage is repaired by a number of different repair mechanisms such as base excision repair (BER) and non-homologous end joining (NHEJ). PARP (poly ADP-ribose polymerase) and XRCC1 play key roles in BER (Figure 1).

This project used 2 cell lines AA8 (XRCC1 wildtype) and EM9 (XRCC1 deficient) EM9 cell lines to test the sensitivity of 2 DNA damaging agents in the presence or absence of a PARP inhibitor (AG14699).

DNA damaging agents (Figure 2) include:

- Temozolomide (TMZ) – an alklyating agent which methylates DNA to produce single strand DNA breaks
- Neocarzinostatin (NCZ) – a macromoloecule chromo-protein enediyne antibiotic producing double strand DNA breaks

## Aim of the project:

- To investigate whether PARP has activity independent of XRCC1 in DNA repair i.e. has a role in multiple repair pathways
- To see if combination therapies of a DNA damaging agent and a PARP inhibitor proves a more effective anticancer therapy as inhibiting repair of severe DNA damage induces apoptosis (cell death), especially in tumours with mutations in other DNA repair pathways

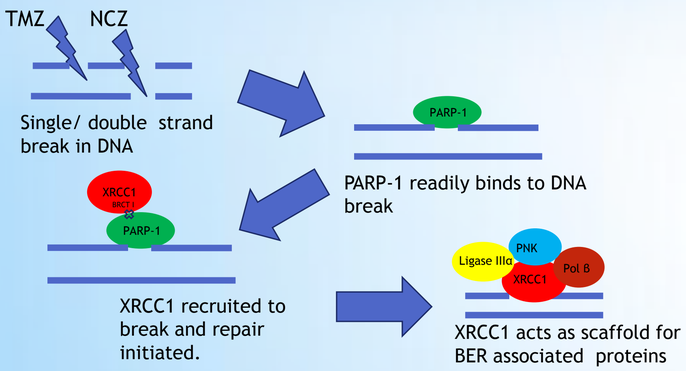



Figure 1: Schematic representation of the PARP mediated initiation of DNA repair via the BER (base excision repair) pathway.

## References:

Horton et al (2008) *Cell Res* 18(1)48-63
El-Khamisy (2003) *Nucleic acids research* 31(19)5526-5533
Curtin (2005) Review 7(4)1-20

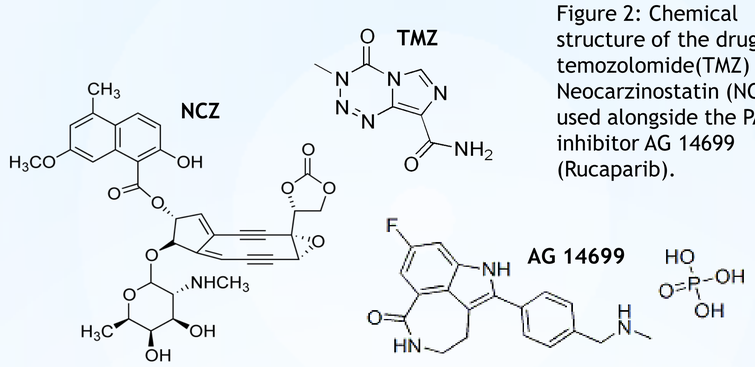
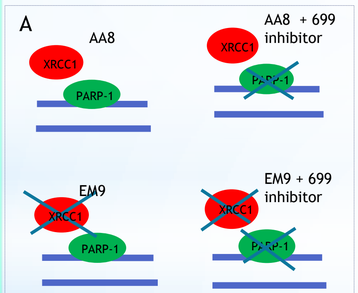



Figure 2: Chemical structure of the drugs temozolomide(TMZ) and Neocarzinostatin (NCZ) used alongside the PARP inhibitor AG 14699 (Rucaparib).

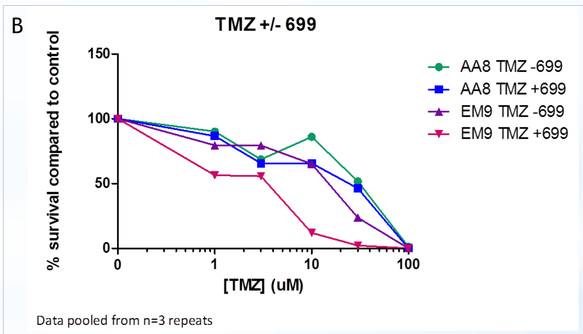
## Results:




A) Schematic representation of the DNA repair pathways affected in both the untreated and PARP inhibitor (AG 14699) treated cell lines.

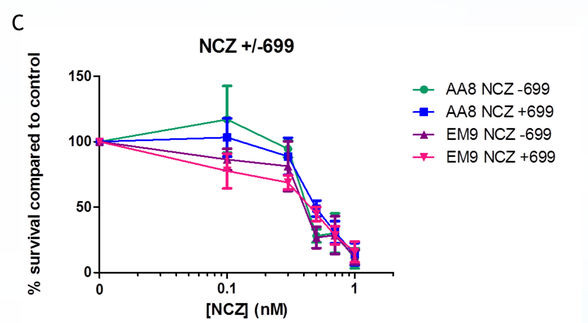
B) Graph showing cell survival of the AA8 and EM9 cell lines following treatment with and without the addition of the PARP inhibitor.

C) Graph showing cell survival following treatment with neocarzinostatin with and without the addition of the PARP inhibitor.




Data pooled from n=3 repeats

| | AA8 -699 | AA8 +699 | EM9 -699 | EM9 +699 |
|---|---|---|---|---|
| LC50 (μM) | 32.417 | 26.145 | 17.323 | 3.814 |




Data pooled from n=3 repeats

| | AA8 -699 | AA8 +699 | EM9 -699 | EM9 +699 |
|---|---|---|---|---|
| LC50 (nM) | 0.434 | 0.462 | 0.415 | 0.422 |

## Methods:

Clonogenic assays using AA8 and EM9 cells:

**Day 1**

Cells seeded into 6 well plates at different densities

**Day 2**

Cells treated with drugged media at different concentrations +/-AG 14699 PARP inhibitor, cells incubated 37oC, 5% CO2

**Day 7**

Colonies fixed with Carnoy's and stained with crystal violet prior to counting


Example of a 6 well plate set-up. Similar plate set up when testing with NCZ only and the drug + 699 PARP inhibitor combination.

## Conclusion:

- XRCC1 deficient EM9 cell line is less effective in repairing DNA damage indicated by reduced survival following treatment – due to inhibition of base excision repair (BER)
- Addition of PARP inhibitor AG 14699 further reduces survival:
  - Possible that PARP inhibition stops PARP release from the site of DNA damage and blocks other repair proteins access to site of damage
  - Possible a second independent repair mechanism is inhibited, maybe a backup NHEJ (non-homologous end joining)
  - Actual mechanism requires further study.
- Potentiating DNA damaging effect seen more in the EM9 cell line indicating that combination anticancer therapies may prove effective in cancers with mutations in DNA repair mechanisms as part of a stratified medicine approach in the future.

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