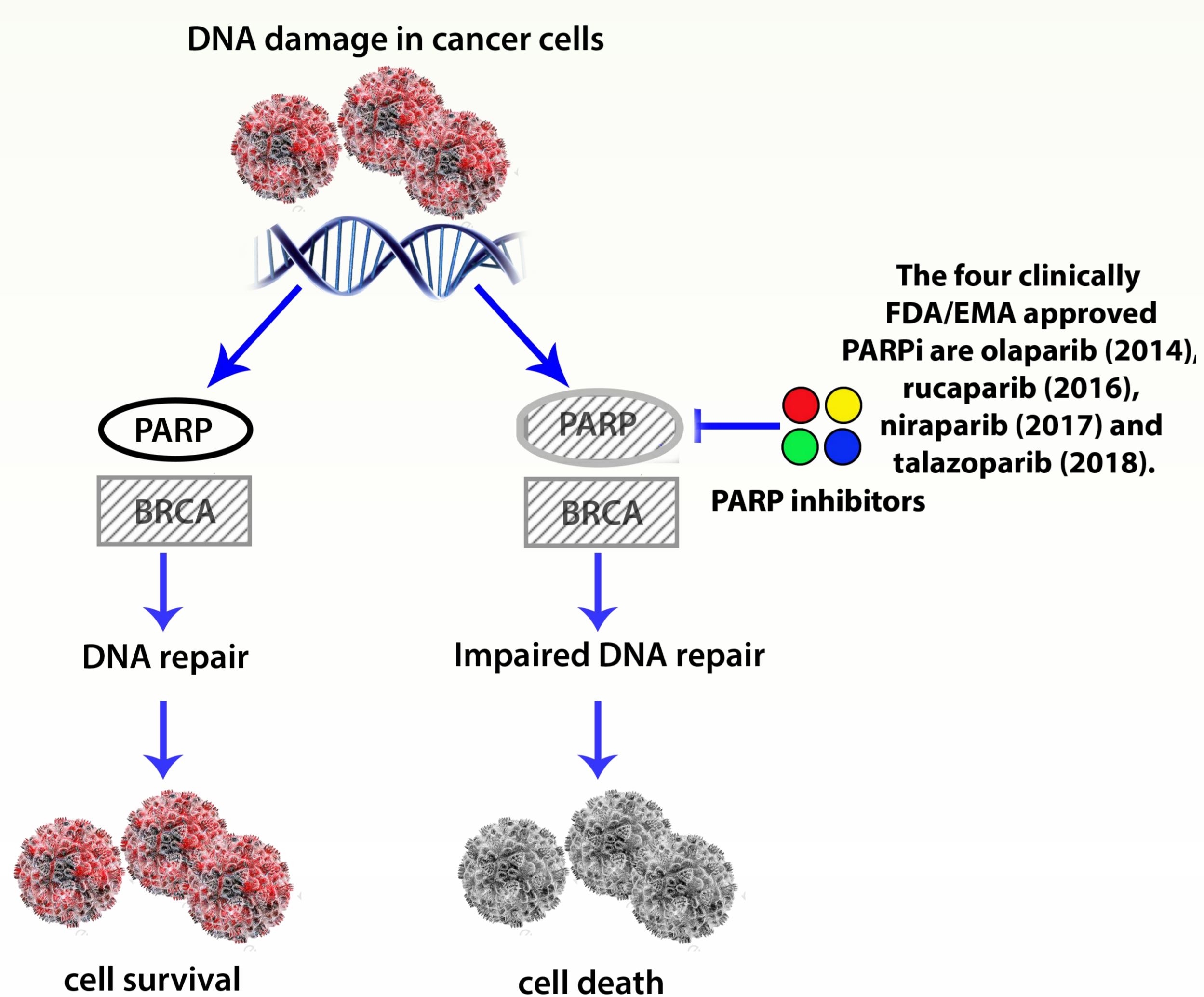# A cell-based neuroprotection assay targeting poly (ADP-ribose) polymerase (PARP) reveals oncology-based potency ranking of clinically used PARP inhibitors (PARPi)

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1. Introduction

- Poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) selectively kill cancer cells that harbour mutation in tumour suppressor genes, such as *BRCA1* and *BRCA2*.

- Four PARPi are currently used in the clinic as monotherapy for *BRCA*-mutated cancers.

- PARPi have also been suggested for repurposing for neurological and neurodegenerative indications, wherein excessive PARP activation leads to neuronal death and PARP inhibition is neuroprotective [1].

DNA damage in cancer cells

The four clinically FDA/EMA approved PARPi are olaparib (2014), rucaparib (2016), niraparib (2017) and talazoparib (2018).

PARP inhibitors

DNA repair — cell survival
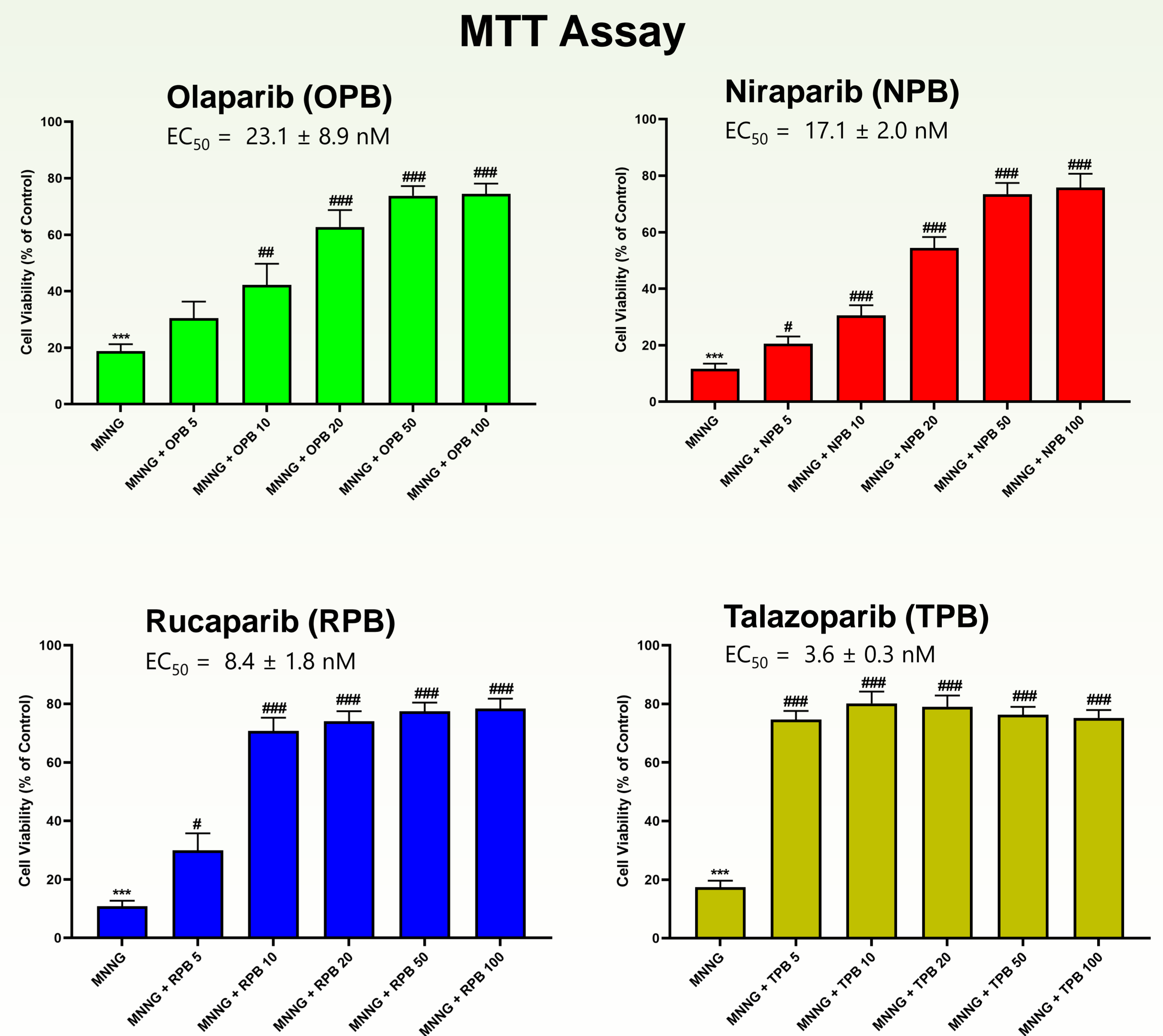
Impaired DNA repair — cell death

2. Aim

To examine whether our cell-based assay, originally designed for interrogating PARP-mediated neuronal death, is adaptable to other application contexts by revealing the established, oncology-related rank order of potencies for the PARPi.

3. Methods

- PARP-dependent cell death was induced in HeLa cells by treatment with the alkylating agent, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (50µM, 25 minutes) [2].

- Cells were treated with each of four PARP inhibitors: Olaparib, niraparib, rucaparib and talazoparib (each up to 10µM).

- Protection against cell death (evidence of PARP inhibition) was assessed 24 h after MNNG treatment using the MTT assay.

- EC_{50} values reported as means ± SEM; n=3-6.

- Statistical analysis (in GraphPad 9.2.0): ANOVA with Tukey's post-hoc test; P<0.05 considered statistically significant.

4. Results

MTT Assay

Olaparib (OPB) — EC_{50} = 23.1 ± 8.9 nM

Niraparib (NPB) — EC_{50} = 17.1 ± 2.0 nM

Rucaparib (RPB) — EC_{50} = 8.4 ± 1.8 nM

Talazoparib (TPB) — EC_{50} = 3.6 ± 0.3 nM

Figure 1: Each of the four PARPi (5-100nM) shows concentration-dependent protection against MNNG-induced reduction in cell viability, as quantified by the MTT assay.
***P<0.001 with respect to the control; ###P<0.001, ##P<0.05 and #P<0.01 with respect to MNNG alone.

Table 1: EC_{50} values (Mean ± SEM) for the four PARPi and their relative potencies. The EC_{50} values revealed their potencies in the following order: talazoparib > rucaparib > niraparib > olaparib.

	EC_{50} /IC_{50} (nM)		Potency ratio vs. olaparib	
	EC_{50} (our **cell-based** assay)	IC_{50} (literature-reported, **cell-free** assay)	Our Cell-based Assay	Literature-reported Cell-free Assay
Olaparib	23.1 ± 8.9	5	1	1
Niraparib	17.1 ± 2.0	3.8	1.3	1.3
Rucaparib	8.4 ± 1.8	2	2.8	2.5
Talazoparib	3.6 ± 0.3	0.57	6.4	8.8

5. Conclusions

- Although the cell-based EC_{50} values are higher than the literature-reported IC_{50} values in cell-free assays, the order of potency was the same and the relative potencies compared to olaparib were similar [2].

- Our cell-based assay is therefore robust for the characterisation of PARPi for oncological and repurposing applications.

References:
1. Fatokun AA et al. (2013). Identification through high-throughput screening of 4'-methoxyflavone and 3',4'-dimethoxyflavone as novel neuroprotective inhibitors of parthanatos. *Br. J. Pharmacol.* **169**: 1263-78.
2. Slade D (2020). PARP and PARG inhibitors in cancer treatment. *Genes Dev.* **34**:360-394.