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Evaluation of PARP inhibitors: performed on BMG LABTECH's FLUOstar® Omega

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- **Cellular PARP assay and cell viability assay utilized to evaluate PARP inhibitors**
- **BMG LABTECH's FLUOstar® Omega allows versatility in choice of assay read out, allowing rapid detection of luminescence, fluorescence and absorbance**

Introduction

Poly ADP-ribosylation is a post-translational modification of proteins that plays a crucial role in regulating DNA repair. Poly (ADP-ribose) polymerase (PARP) transfers ADP-ribose to itself and other nuclear proteins such as histones. The substrate for that reaction is NAD+. PARP inhibition has been demonstrated to potentiate the cytotoxicity of anti-cancer drugs and ionising radiation. Therefore much effort has been put into the development of specific PARP inhibitors.

To evaluate such inhibitors, we have first used a specific PARP activity assay, to monitor their ability to inhibit endogenous PARP activity contained within a colon cancer cell line. This assay requires protein concentration determination (BCA assay - absorbance) followed by luminescent detection.

Secondly, we have used the AlamarBlue viability assay (fluorescent read-out) to evaluate the ability of a PARP inhibitor to potentiate the effects of the chemotherapeutic agent temozolomide to stimulate cell death in a colon cancer cell line. All measurements were performed using a FLUOstar Omega multi-detection microplate reader from BMG LABTECH.

Materials & Methods

- Bicinchoninic Acid Protein Detection Kit from Sigma-Aldrich
- Universal Chemiluminescent PARP assay kit including white plates from Trevigen
- clear and black 96 well plates from Fisher
- Temozolomide from Sigma-Aldrich
- AlamarBlue from Invitrogen

Sample	Absorbance 560 nm
LoVo Control	0.767
LoVo 1 nM PARP Inhibitor	0.774
LoVo 3 nM PARP Inhibitor	0.791
LoVo 10 nM PARP Inhibitor	0.778
LoVo 30 nM PARP Inhibitor	0.762
LoVo 100 nM PARP Inhibitor	0.75
LoVo 300 nM PARP Inhibitor	0.736
Blank	0.075

Universal Chemiluminescent PARP assay

LoVo cells were treated with PARP inhibitor at a range of concentrations (1-300 nM) for 1h before cells were harvested and lysed in PARP Buffer.
A BCA protein assay was carried out in a 96 well microplate, following manufacturer's instructions adapted for a 96 well plate. The plate was read at 560 nm using the FLUOstar Omega.

A standard curve was generated for the BCA protein assay (Figure 1) and from this the protein concentration of the lysates were determined, and adjusted to 40 µg per sample.

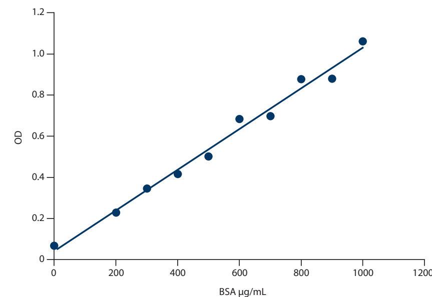


Fig. 1: BSA standard curve (linear regression fit performed using GraphPad Prism).

Lysates were then screened for PARP activity following manufacturer's instructions for determining PARP activity in cell and tissue extracts.
The assay measures the incorporation of biotinylated poly (ADPribose) onto histone proteins in a 96 well strip format. Detection is carried out using a Streptavidin-Horseradish Peroxidase (HRP) conjugate. Following addition of the HRP substrate, the resulting luminescent signal was read using the FLUOstar Omega equipped with luminescence optic.

AlamarBlue Viability Assay

LoVo cells were seeded in 96 well black plates at 5000 cells per well and allowed to adhere overnight, prior to addition of compound or vehicle control.
0- 300 µM temozolomide was added to cells, with and without 300 nM PARP inhibitor for 72 h. AlamarBlue 10 % (v/v) was then added to cells, and incubated for a further 6 h at 37°C. Live, metabolically active cells convert the AlamarBlue substrate to a fluorescent product, which was then detected using the FLUOstar Omega (Excitation 544 nm and Emisson 590 nm).

Rev. 04/2008
Keywords: AlamarBlue, Cell proliferation, Cell viability, HRP, PARP

Results & Discussion

Universal Chemiluminescent PARP assay

Following luminescence measurement, data was analysed using GraphPad Prism (Figure 2). The PARP inhibitor inhibits 50 % PARP activity at a concentration of 14 nM.

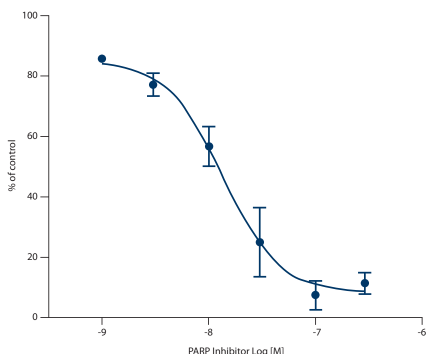


Fig. 2: Cellular PARP activity in LoVo cells treated with PARP inhibitor for 1h.

AlamarBlue Proliferation Assay

Following measurement of fluorescent product, data was analysed using GraphPad Prism (Figure 3). Temozolomide as a single agent leads to very little cell death, with an IC_{50} >300 µM.

However, addition of PARP inhibitor in combination with temozolomide, leads to a significant increase in cell death, with an IC_{50} of 60 µM. This represents >5-fold enhancement of cell death.

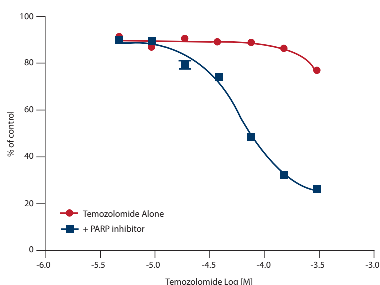
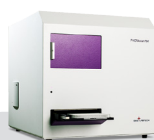


Fig. 3: Proliferation Assay performed in LoVo cells treated with Temozolomide alone, or in combination with 300 nM PARP inhibitor.

Note: PARP inhibitor alone does not stimulate cell death.

Conclusion

The ability of the FLUOstar Omega to measure absorbance, luminescence and fluorescence, facilitates simple, rapid measurement of all aspects of our evaluation, using a single machine.



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