# Preclinical profile of RP12146, a novel, selective, and potent small molecule inhibitor of PARP1/2

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## Introduction

Poly ADP-ribose polymerase (PARP) activity involves synthesis of Poly-ADP ribose (PAR) polymers that recruit host DNA repair proteins leading to correction of DNA damage and maintenance of cell viability. PARP inhibitors have been reported to demonstrate chemo- and radio-potentiation albeit with incidences of myelosuppression upon combining with cytotoxic agents. RP12146 is a novel and potent PARP inhibitor that inhibited PARP1/2 enzyme activity with $IC_{50}$ of 0.6 & 0.5 nM, respectively, with several fold selectivity over other isoforms.

| | PARP1 | PARP2 |
|---|---|---|
| $IC_{50}$ (nM) | 0.6 | 0.5 |

**Table 1.** Enzymatic potency (*n=5*) was evaluated using a PARP Chemiluminescent Activity Assay Kit (BPS biosciences).

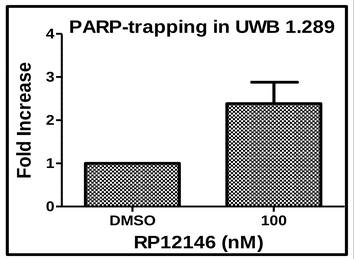



**Figure 1. PARP-trapping in UWB 1.289.** Cells were incubated with either DMSO or 100 nM of RP12146 for 24 h followed by subcellular fractioning and western-blot analysis (*n=6*). **A 2.3-fold increase in PARP-trapping observed at 100 nM in presence of RP12146**

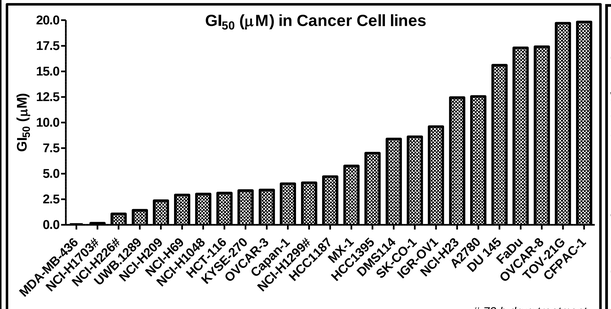



**Figure 2. Growth Inhibition in Cancer cell lines**. Cancer cell lines were incubated with either DMSO or different concentrations of RP12146 for 72 h or 144 h. **RP12146 inhibited cell growth in both BRCA mutant and non-BRCA mutant cancer cell lines with a *GI*$_{50}$ range of 0.043 to 19.83 μM in a dose dependent manner**

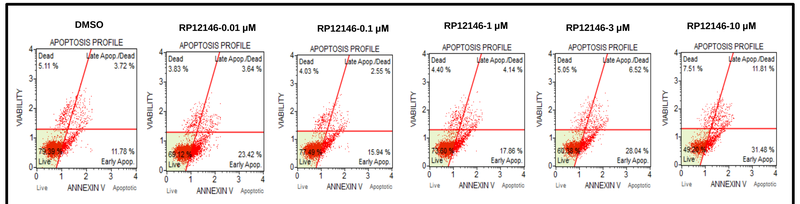



**Figure 3. Apoptosis in UWB 1.289 cells.** Cells were incubated with desired concentrations of RP12146**.** For apoptosis (120 h), cells were stained with Annexin and propidium iodide (PI) and analyzed by flowcytometry (n=3). ***RP12146 induced apoptosis in a dose-dependent manner.***

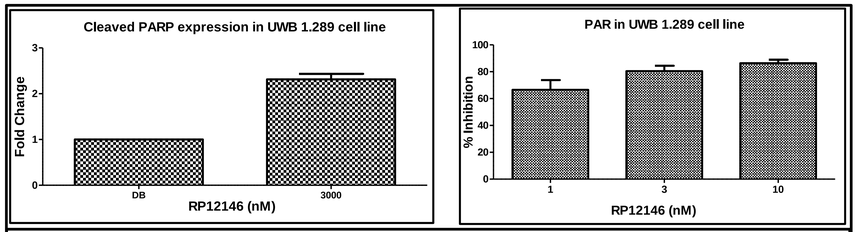



**Figure 4. Expression of downstream PAR and cleaved PARP expression was determined in UWB1.289 (BRCA1 null) cells by western blotting.** UWB1.289 cells were treated with RP12146 and incubated for 24 h for PAR and 72 h for cleaved PARP. **RP12146 inhibited PAR levels by 86% at 10 nM (N=3). At 3000 nM, RP12146 increased cleaved PARP expression by 2.31 folds compared to control in UWB.1289 cells (*N=3*).**

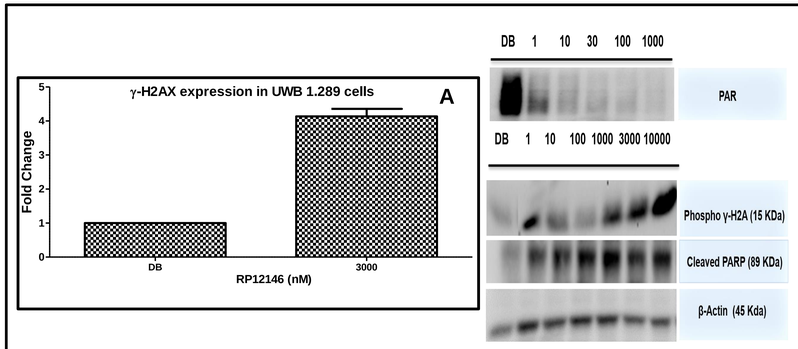



**Figure 5. A. Expression of downstream Phospho γ-H2A was determined in UWB1.289 (BRCA1 null) cells by western blotting**. UWB1.289 cells were treated with RP12146 and incubated for 96 h and lysate was analyzed for Phospho γ-H2A**. A four-fold increase was observed with RP12146 at 3000 nM (*N=3*). B. Expression of PAR, Phospho γ-H2A , Cleaved PARP, β-Actin in UWB1.289 (BRCA1 null) cell lines.**

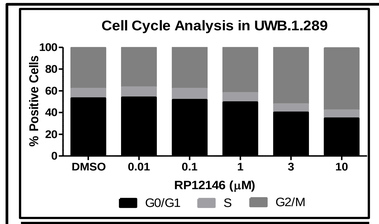
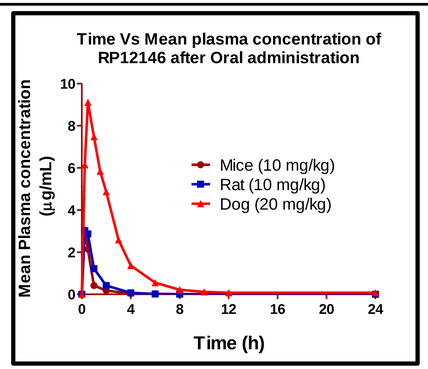



**Figure 6. Cell cycle in UWB 1.289 cell.** Cells were incubated with desired concentrations of RP12146**.** For cell cycle (72 h), cells were stained with propidium iodide and analyzed by flow cytometry (n=3). ***RP12146 caused cell cycle arrest in G2/M arrest phase in a dose-dependent manner.***



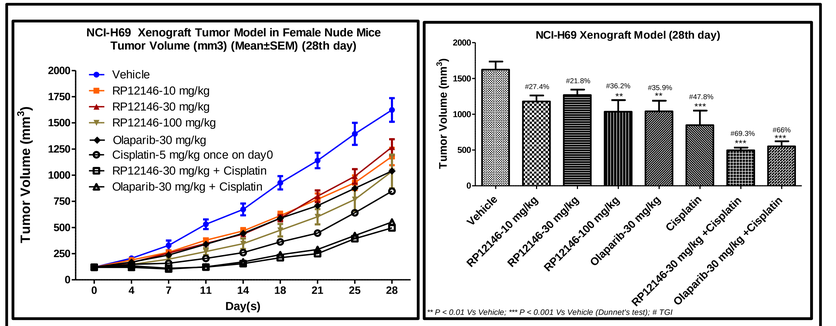




**Figure 7. Anti-tumor activity in NCI-H69 Xenograft**. RP12146 at 10, 30, 100 mg/kg/BID was tested in subcutaneous Small Cell Lung Cancer (NCI-H69) Xenograft model. **RP12146 exhibited anti-tumor potential with TGI of 36.2% as a single agent.**

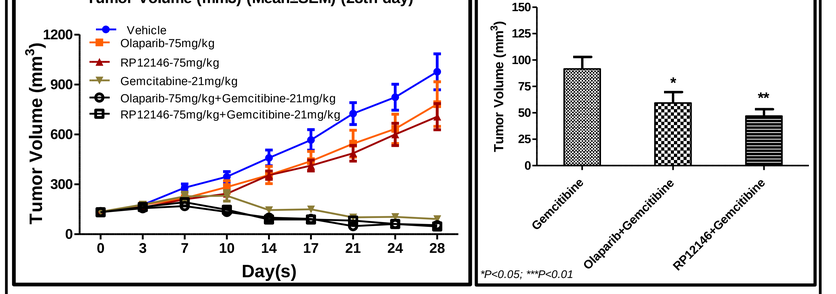



**Figure 8. Anti-tumor activity in OVCAR-3 Xenograft**. RP12146 at 75mg/kg/BID was tested in subcutaneous OVCAR-3 human Ovarian cancer xenograft model. **RP12146 exhibited anti-tumor potential with TGI of 28% as a single agent in OVCAR-3 Xenograft model.**

## SUMMARY & CONCLUSIONS

- RP12146 is a potent, small molecule selective PAPR 1/2 inhibitor
- Demonstrated growth inhibitory activity in BRCA mutant and non-BRCA mutant cancer cell lines.
- Demonstrated anti-tumor activities in both tumor size and tumor weight in OVCAR-3 xenograft model.
- Phase-1 trials in solid tumors is planned for H1 2021