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Activity of RP12146, a novel, selective, and potent small molecule inhibitor of PARP 1/2, in solid tumors

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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. Through 'synthetic lethality', the applicability PARP inhibitors can be expanded to cancers beyond the BRCA defects either as monotherapy or in combination with established therapy in solid tumor types. Marrow suppression with current approved PARP inhibitors limits their concurrent use with chemotherapy, and the appropriate dosage to achieve chemo-potentiation requires further assessment. Our objective therefore was to design, synthesize, and identify a potent and efficacious PARP inhibitor having a wider safety margin that allows treatment combinations with several SOC including chemotherapeutics across cancers. Herein, we describe the efficacy of RP12146 as a single agent and in combination with approved therapies in preclinical models of solid tumors.

	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).

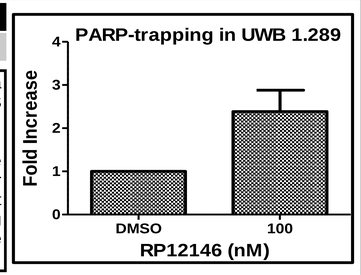
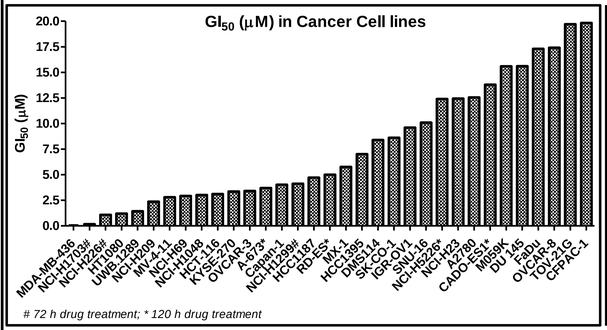


Figure 1. PARP-trapping in UWB 1.289. Cells were incubated with either DMSO or 100 nM of RP12146 for 24 h followed by subcellular fractioning and western-blot analysis (*n*=6). **A 2.3-fold increase in PARP-trapping observed at 100 nM in presence of RP12146 in line with the reported values for approved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



*# 72 h drug treatment; * 120 h drug treatment*

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**

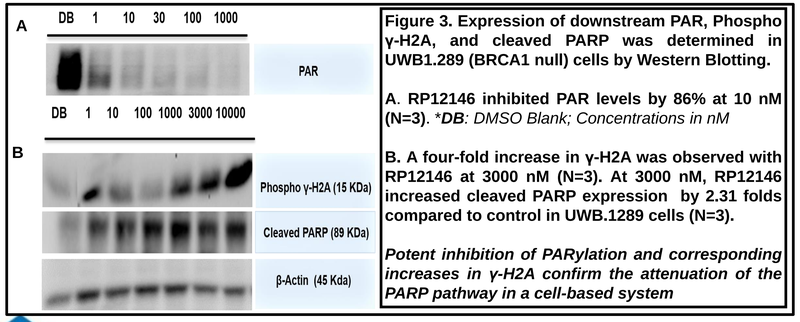
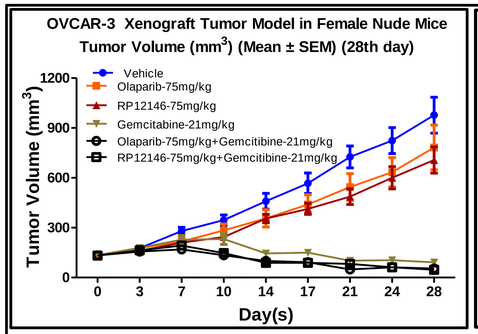


Figure 3. Expression of downstream PAR, Phospho γ-H2A, and cleaved PARP was determined in UWB1.289 (BRCA1 null) cells by Western Blotting.

A. RP12146 inhibited PAR levels by 86% at 10 nM (N=3). **DB: DMSO Blank; Concentrations in nM*

B. A four-fold increase in γ-H2A was observed with RP12146 at 3000 nM (N=3). At 3000 nM, RP12146 increased cleaved PARP expression by 2.31 folds compared to control in UWB.1289 cells (N=3).

Potent inhibition of PARylation and corresponding increases in γ-H2A confirm the attenuation of the PARP pathway in a cell-based system



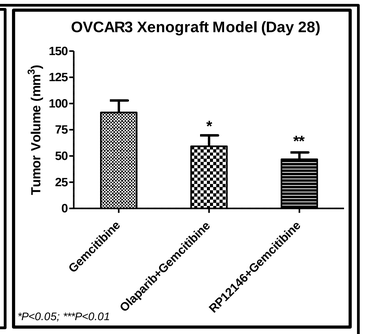
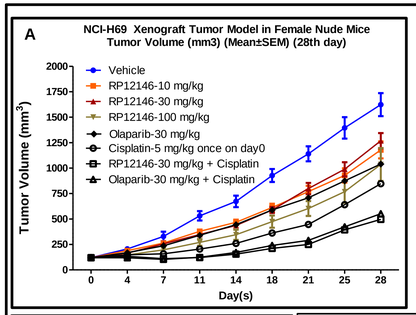
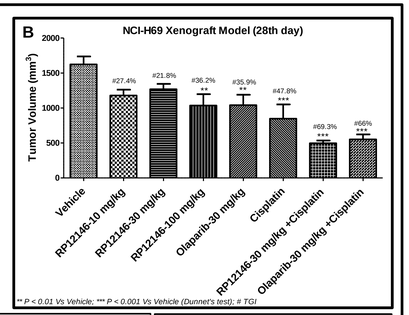
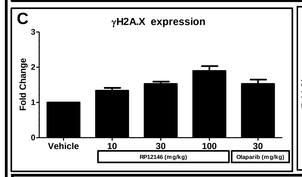
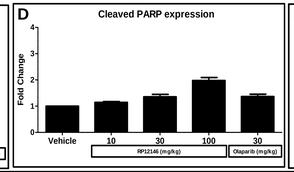


Figure 4. Anti-tumor activity in OVCAR-3 Xenograft. RP12146 at 75mg/kg/BID was tested in subcutaneous OVCAR-3 human Ovarian cancer xenograft model. **RP12146 exhibited anti-tumor potential with TGI of 28% as a single agent in OVCAR-3 Xenograft model.**









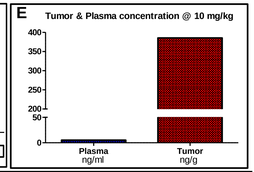
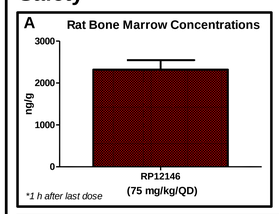
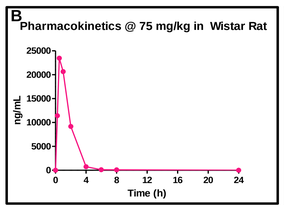


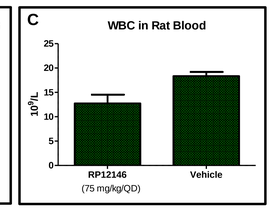
Figure 5. Anti-tumor activity in NCI-H69 Xenograft.
A & B: RP12146 at 10, 30, 100 mg/kg/BID was tested in subcutaneous Small Cell Lung Cancer (NCI-H69) Xenograft model. **RP12146 exhibited anti-tumor potential with TGI of 27.4%, 21.8% and 36.2% at 10, 30, and 100 mg/kg as single agent. RP12146 @ 30 mg/kg in combination with cisplatin exhibited a TGI of 69.3 %.**
C: γH2A was measured by Western Blotting. RP12146 increased γH2A.x expression by 1.3, 1.5, 1.8-fold in the 10, 30, and 100 mg/kg groups respectively, compared to vehicle group.
D. Cleaved PARP was measured by Western Blotting. RP12146 increased cleaved PARP expression by 1.1, 1.3, 2.0-fold in the 10, 30, and 100 mg/kg groups respectively, compared to vehicle group.
E. Concentrations of RP12146 were higher in tumor (>70-fold) compared to plasma at terminal collection indicating preferential distribution of the drug into tumor cells.

Taken together, preferential distribution of RP12146 into tumor inhibited PARP-related markers that translated into attenuation of tumor growth.

Safety







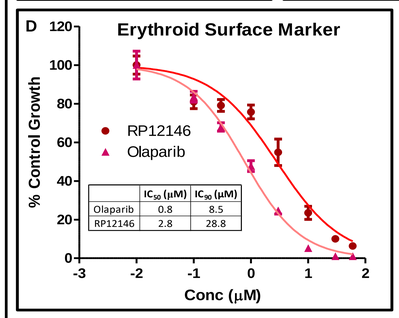


Figure 6. A 7-day Repeat dose study in Wistar Rats and HemaTox™ assay (Erythroid). Wistar rats were dosed with RP12146 at 75 mg/kg/PO for 7 days. Bone marrow (**A**) and blood (**B & C**) were processed and RP12146 concentrations/hematology were analyzed**. *Unlike the known myelosuppressive effects of the earlier PARPi, RP12146 did not cause any myelosuppression/ leucopenia as reflected by normal circulating WBC presumably due to the relatively lower distribution into the bone marrow.***

D. Human CD34+ bone marrow cells (HemaTox™ assay STEMCELL Technologies, Canada) were exposed to RP12146 for 7 days and erythroid surface marker was measured by flowcytometry. **RP12146 inhibited growth of erythroid progenitors in the micromolar range indicating a potentially safer profile compared to the approved PARP inhibitors.**

Clinically known adverse events such as anemia, leukopenia, neutropenia, lymphocytopenia, and gastric disturbances reported for the first-generation PARP inhibitors were not encountered upon repeat dose administration of RP12146 over a 28-day period in rat and dog even at the highest dose tested. Findings include:

- No effect on food intake or any abnormal clinical signs
- Slight reductions in body weight only at the highest dose tested
- No significant changes in the hematology parameters
- No toxicologically relevant clinical chemistry parameters at any of the doses tested
- No changes in organ weights, gross pathology, or histopathology

Summary & Conclusions

- RP12146 is a next generation potent, small molecule selective PARP 1/2 inhibitor with a wide therapeutic window with no evidence of dose limiting toxicities reported for the first-generation inhibitors.
- RP12146 demonstrated growth inhibitory activity in solid tumor cell lines with downstream modulation of relevant pharmacodynamic markers in line with its modulatory effect on PARP enzymes.
- In addition, RP12146 was able to control tumor growth as monotherapy and in combination with standard of care that may help in selecting optimal treatment regimens for clinical testing. Impressive translational activity manifested by an increase in γH2A and cleaved PARP was observed in the animal studies.
- Investigations in preclinical models aimed at the ability of RP12146 to translate the tumor control without dose-limiting toxicities observed by currently marketed PARP inhibitors revealed: a) relatively lower distribution into bone marrow noticed without any deleterious effects on WBC; and b) lack of effect on bone marrow cell proliferation at the clinically relevant concentration range.
- Observations from the IND enabling animal tox studies further confirmed the differentiation of RP12146 with a very low or no potential to cause clinically reported adverse effects of PARP inhibitors and thus support the combinations with SOC in various cancers to exploit the target-related activity to a maximum
- RP12146 is currently being evaluated in Phase 1/1b trials in Europe across a panel of solid tumor indications.

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