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 result in DNA repair proteins leading to correction of DNA damage and maintenance of cell stability. 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. Marine sponges with current approved PARP inhibitors limit 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.

Table 1. Enzymatic potency (% inhibition) was evaluated in PARP Cholinesterase Activity Assay Kit (BPS Biosciences).

<table>
<thead>
<tr>
<th>PARP1</th>
<th>PARP2</th>
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<td>IC50(nM)</td>
<td>0.6</td>
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<tr>
<td>IC100(nM)</td>
<td>0.5</td>
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Figure 1. PARP-trapping in UW1 1.289. Cells were incubated with either DMSO or 10 ng/ml of RP12146 for 24 h followed by subcellular fractioning and western blot analysis (n=3). A 2.3-fold increase in PARP-trapping observed at 100 ng/ml in presence of RP12146 in line with the reported values for approved PARP inhibitors.

Figure 2. Growth inhibition in Cancer cell lines. Cancer cell lines were incubated with either DMSO or different concentrations of RP12146 for 72 or 144 h. RP12146 showed growth inhibition in both BRCA mutant and non-BRCA1/2 mutant cancer cell lines with a GI50 range of 0.043 to 19.85 μM in a dose dependent manner.

Figure 3. Expression of downstream PARP, Phospho- γH2AX, and cleaved PARP was determined in UW1.289 (BRCA1 null) cells by Western Blotting. A. RP12146 inhibited PAR levels by 80% at 10 nM. B. DMSO Blank, Concentrations in nM.

A. RP12146 inhibited PAR levels by 80% at 10 nM (NC3). B. DMSO Blank, Concentrations in nM.

B. A four-fold increase in γH2AX was observed with RP12146 at 3000 nM (NC3). At 100 ng/ml, RP12146 increased cleaved PARP expression by 2.31 folds compared to control in UW1.289 cells (NC3).

Cleaved PARP expression in JHY and DKO Hela cells by Western Blotting. RP12146 increased cleaved PARP expression by 1.3, 1.5, 1.8-fold in the 10, 30, and 100 mg/kg groups respectively, compared to vehicle group.

Cleaved PARP expression measured by Western Blotting. RP12146 increased cleaved PARP expression by 1.3, 1.5, 1.8-fold in the 10, 30, and 100 mg/kg groups respectively, compared to vehicle group.

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D. Concentration of RP12146 were higher in tumor (20-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.

Figure 4. Anti-tumor activity in OVCAR-3 Xenograft Tumor model in Female Nude Mice. RP12146 at 70 mg/kg 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 was tested in subcutaneous Small Cell Lung Cancer (NCI-H69) Xenograft model. RP12146 showed anti-tumor potential with TGI of 21.8% and 38.2% at 10, 30, and 100 mg/kg respectively. RP12146 (10 mg/kg in combination with cisplatin exhibited a TGI of 69.9%.

Figure 6. A 7-day repeat dose study in Wistar Rat and Female Nude Mice. A. Plasma and Tumor concentration at 10 mg/kg of RP12146 for 7 days and erythroid surface marker was measured by HemaTox. RP12146 inhibited growth of erythroid progenitors in the monocytes range indicating a potentially safer profile compared to the approved PARP inhibitors.

Safety
- No effect on food intake or any abnormal clinical signs
- Significant elevations in body weight only at the highest dose tested
- No significant changes in the hematology parameters
- No toxicologically relevant chemical 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 inhibition activity in solid tumor cell lines with downstream modulation of relevant pharmacodynamic markers in vivo with 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 yH2AX 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 to lack of effect on bone marrow cell proliferation at the clinically relevant concentration range.
- Observations from the 7D receiving animal toxic studies further confirmed the differentiation of RP12146 with a very low or no cause to clinically reported adverse effects of PARP inhibitors and thus support the combinations with SOC to 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.

American Association for Cancer Research (2022)