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 chemotherapeutic potential in various cancers. RP12146 is a novel and potent PARP inhibitor that inhibited PARP1/2 enzyme activity with IC₅₀ of 0.6 & 0.5 nM, respectively, with several fold selectivity over other isomeric PARP inhibitors.

Table 1. Enzymatic potency was evaluated using a PARP-Chemiluminescent Activity Assay Kit (Biosciences).

Table 2. Biological potential of RP12146 was assessed in vitro.

Figure 1. PARP-trapping in UWB1.289. Cells were incubated with either DMSO or 100 nM of RP12146 for 24 h followed by subcellular fractioning and western blot analysis (n=3). A 2.3-fold increase in PARP-trapping was 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. RP12146 inhibited cell growth in both BRCA mutant and non-BRCA mutant cancer cell lines with a GI₅₀ range of 0.243 to 19.83 μM in a dose dependent manner.

Figure 3. Apoptosis in UWB1.289. Cells were incubated with desired concentrations of RP12146. For apoptosis (120 h), cells were stained with Annexin V and propidium iodide (PI) and analyzed by flow cytometry (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 (Kd). At 3000 nM, RP12146 increased cleaved PARP expression by 2.31 folds compared to control in UWB1.289 cells (Kd).

Figure 5. A. Expression of downstream Phospho γH2A 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 γH2A. A four-fold increase was observed with RP12146 at 3000 nM (Kd). B. Expression of Phospho γH2A, Cleaved PARP, 8-Amin in UWB1.289 (BRCA1 null) cell lines.

Figure 6. Cell cycle in UWB1.289. 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 15, 30, 100 mg/kg/BID was tested in subcutaneous OVCAR-3 human ovarian cancer 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