

Preclinical evaluation of the PARP inhibitor rucaparib in combination with PD-1 and PD-L1 inhibition in a syngeneic BRCA1 mutant ovarian cancer model

Background: Rucaparib (CO-338) is an oral small molecule inhibitor of poly(ADP-ribose) polymerase (PARP)-1, PARP-2 and PARP-3 that has shown clinical activity in patients with *BRCA1* and *BRCA2* mutated advanced ovarian cancer. Monoclonal antibodies against programmed death receptor-1 (PD-1) and programmed death-ligand (PD-L1) have also shown efficacy in advanced ovarian cancer patients. It has been reported that *BRCA1* and *BRCA2* mutated tumors have a higher mutational load and increased CD8+ T cell infiltration, suggesting that the combination of rucaparib and immune checkpoint inhibition may be complementary. However, PARP inhibition has also been reported to have an immunosuppressive effect in preclinical studies.

Methods: Subcutaneous syngeneic models using the *BRCA1* wild-type C2Km (P53^{-/-}, myc, Kras-G12D, Akt-myr) and *BRCA1* mutant BrKras (*BRCA1*^{-/-}; P53^{-/-}; myc; Kras-G12D; Akt-myr) murine ovarian cell lines were developed in the murine FVB/N background. Antibodies targeting PD-1 (RMP1-14) and PD-L1 (10F.9G2) were dosed by intraperitoneal injection at 5-10 mg/kg twice weekly, while rucaparib was administered by oral gavage at 150 mg/kg twice daily. Treatment was initiated at a tumor volume of ~150mm³ (n=15/group). Animals were dosed for 21 days, and tumors were allowed to regrow to day 76.

Results: *In vitro* cytotoxicity assays demonstrated that rucaparib was 155-fold more potent in the *BRCA1* deficient BrKras cell line (IC₅₀ = 84 nM) than the isogenic *BRCA1* wild-type C2Km cell line (IC₅₀ = 13 μM). An *in vivo* study using the syngeneic BrKras model was performed in mice treated with: vehicle, rucaparib, PD-1, PD-L1, rucaparib+PD-1, and rucaparib+PD-L1. All monotherapy and combination groups resulted in significant tumor growth inhibition and were followed for survival analysis. The median survival time (MST) and % cures (defined as undetectable growth at Day 76 post-tumor implantation) for vehicle, PD-L1, PD-1 and rucaparib monotherapy treated animals was 34 days (0%), 41 days (13%), 76 days (40%) and >76 days (56%), respectively. The rucaparib+PD-1 and rucaparib+PD-L1 combination groups demonstrated greater efficacy than the monotherapies, with a MST of >76 days (100%) and >76 days (88%), respectively. Dose response and immune profiling studies are ongoing. *In vivo* studies were also performed in the

BRCA1/2 wild-type models EMT-6, Pan02, and MC38. As expected, as a single agent, rucaparib showed limited activity in these homologous recombination competent models, whereas a range of tumor growth inhibition was observed with monotherapy PD-L1 treatment. No impact on anti-tumor activity was observed in animals treated with rucaparib+PD-L1 as compared to PD-L1 monotherapy in the *BRCA1/2* wild-type syngeneic models examined.

Conclusions: The combination of rucaparib with PD-1 and PD-L1 inhibition improved survival in a *BRCA1* mutant syngeneic model.