Identification of SP-002, a potent USP1 inhibitor effectively inhibits HRD tumor growth and displays low hematotoxicity risk

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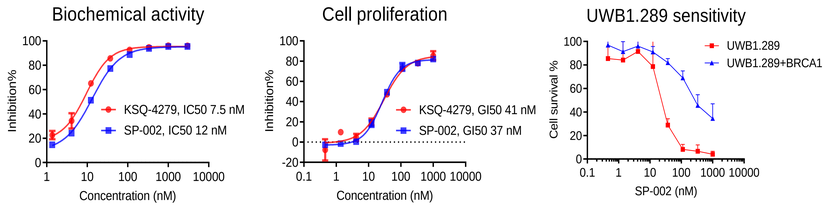
Introduction

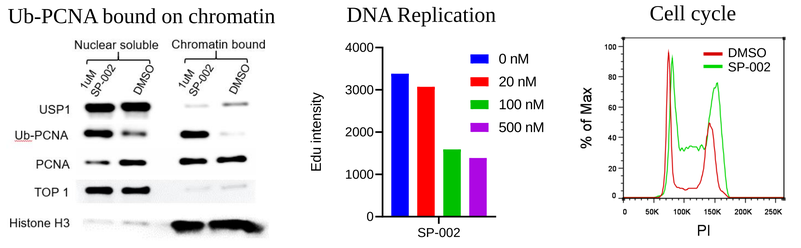
- Tumors with homologous repair deficiencies (HRD) are sensitive to agents interrupting DNA repair. The deubiquitinase USP1 involves in the DNA damage response by translesion synthesis and Fanconi anemia pathway. And the synthetic lethality of USP1 and HRD is well reported. Moreover, deficiency of USP1 or its downstream pathway leads to hypersensitivity of HRD tumors to PARP inhibition.
- In addition, target-related hematotoxicity is widely observed in serval DNA Damage Response (DDR) pathway inhibitors, including PARP and ATR inhibitors, which limits the combination therapy.
- Here, we reported SP-002, a potent USP1 inhibitor, displayed monotherapy potential and combination activity with PARP inhibitor (PARPi) in HRD cancers.

Inhibition of USP1 activity by SP-002



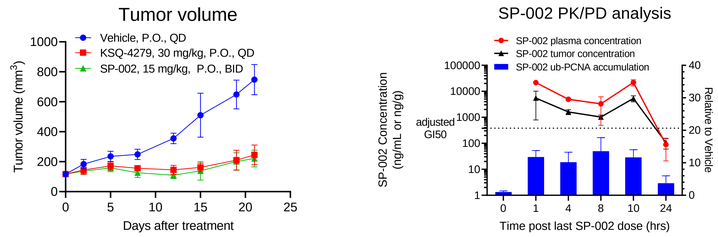
- In biochemical assay, SP-002 exhibited potent enzyme inhibition activity.
- SP-002 strongly inhibited proliferation of *BRCA1*-mutant MDA-MB-436 breast cancer cells in a 7-day proliferation assay.
- UWB1.289 and UWB1.289+BRCA1 cells were treated with SP-002 for 14 days. Cell survival was determined by CellTiter-Glo.

SP-002 induced DNA replication delay and S/G2 arrest



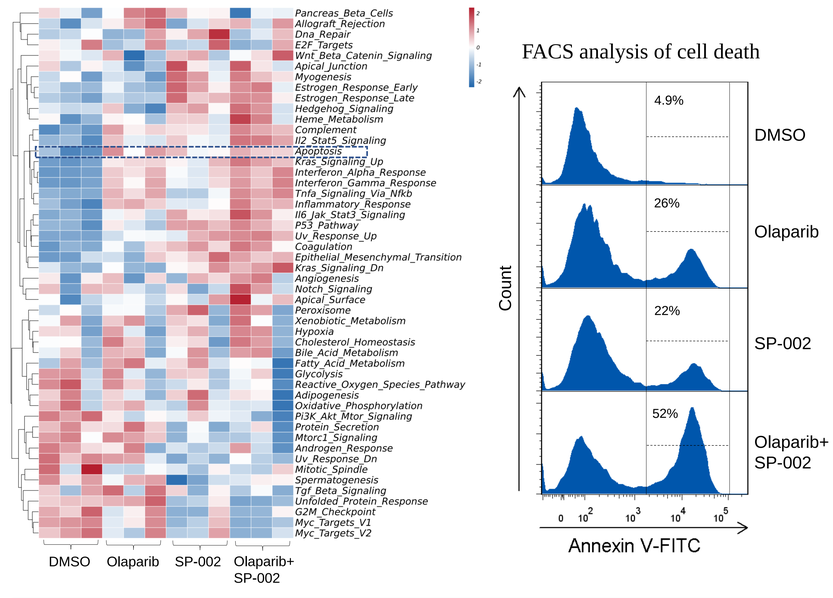
- Immunoblot analysis of ub-PCNA bound on chromatin in MDA-MB-436 cells treated with SP-002 for 0.5 hrs. SP-002 significantly induced ub-PCNA accumulation on chromatin.
- EdU intensity was determined by FACS to reflect the extent of DNA replication of MDA-MB-436 cells during S phase. MDA-MB-436 cells were treated with the indicated concentrations of SP-002 for 24 hrs.
- FACS analysis of cell cycle of MDA-MB-436 cells treated with DMSO or SP-002 for 72 hrs .

SP-002 suppressed *in vivo* tumor growth



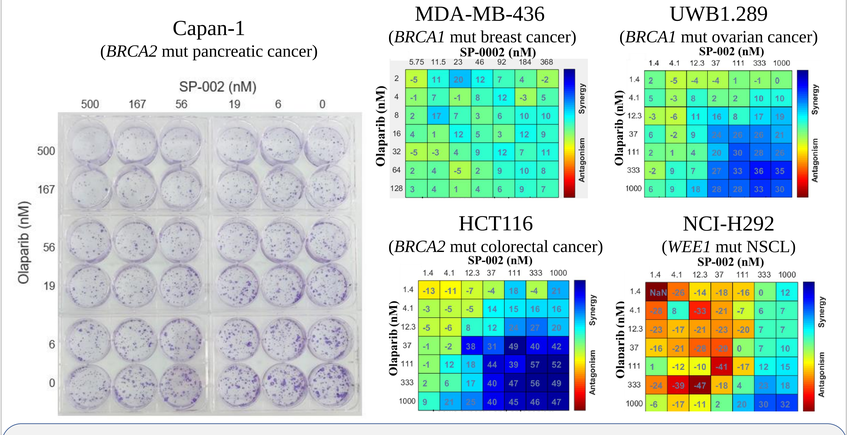
- SP-002 led to tumor growth inhibition in MDA-MB-436 xenograft model without changing the mice's body weight.
- SP-002 concentrations in plasm and tumor tissue were determined at the indicated time points. Un-PCNA accumulation in tumor tissues was analyzed by western blot.

Combination of SP-002 and PARPi significantly enhanced cell death



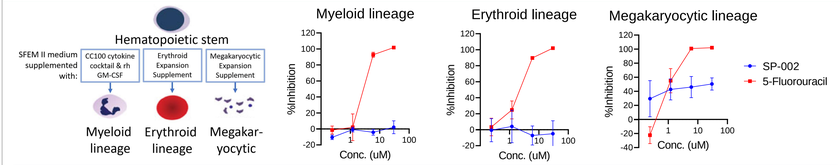
- MDA-MB-436 cells were treated with 500 nM Olaparib or 500 nM SP-002 as monotherapy or combination for 48 h, followed by total RNA harvest and RNA-seq analysis. Gene set variational analysis reveals relative enrichment of apoptosis hallmark.
- MDA-MB-436 cells were treated with the indicated 500 nM compounds for 5 days. Dead cells were stained with Annexin V and analyzed by FACS.

Synergistic activities of SP-002 and PARPi in a panel of HRD cell lines



Synergistic activities of SP-002 and Olaparib were observed in Capan-1 cells in a 14-day colony formation assay. The synergistic activities were also evaluated in a 7-day CellTiter-Glo assay in variable other HRD cells, including MDA-MB-436 breast cancer cells, UWB1.289 ovarian cancer cells, HCT116 colorectal cancer cells and NCI-H292 NSCL cells.

In vitro hematological toxicity



CD34$^+$ bone marrow cells were differentiated as above hierarchy chart for 14 days into myeloid, erythroid and megakaryocytic lineages in the presence of SP-002 and a positive control 5-Fluorouracil.

Summary

- SP-002 exhibited potent *in vitro* USP1 inhibitory activity, which induced ub-PCNA accumulation on chromatin, delayed DNA synthesis, and eventually arrested cell cycle in S/G2 phase. *In vivo*, SP-002 suppressed tumor growth in a xenograft mice model without affecting mice body weight.
- SP-002 promoted significant cell death either as monotherapy or in combination with PARPi. Different SP-002 activity was shown on UWB1.289 cells with/without BRCA1 mutation, suggesting the dependency of SP-002 activity on HRD. SP-002 and PARPi show synergistic effects in variable HRD tumors, indicating wide potential patient opportunities.
- Minimal *in vitro* hematological toxicity was observed in an *in vitro* hematotoxicity assay.