USP1 inhibitor synthetic lethality in BRCA1/2-mutant cancer is driven by PCNA ubiquitination

Abstract # 2603

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Abstract

CRISPR-based functional genomic screening is a powerful approach for identifying novel classes of synthetic lethal drug targets. Here, we define the deubiquitinating enzyme USP1 as a synthetic lethal target in cancers with underlying DNA repair vulnerabilities. A highly potent and selective small molecule USP1 inhibitor conferred a viability defect in BRCA1-mutant, but not WT cell lines by activating replication stress. Genome-wide CRISPR screening uncovered RAD18 and UBE2K, which promote PCNA mono- and poly-ubiquitination respectively, as key mediators of USP1-BRCA1/2 dependency. Increased cellular mono- and poly-ubiquitination reduced PCNA protein levels, and restoration of PCNA protein expression rescued USP1 inhibitor sensitivity. USP1 dependency is associated with upregulated RAD18 and UBE2K expression, suggesting that elevated PCNA ubiquitination in the context of BRCA1/2 deficiency mediates USP1 synthetic lethality. Interestingly, USP1, but not PARP1/2 inhibition, elicited a viability defect in a subset of BRCA1/2 WT lung cancer cell lines, indicative of novel synthetic lethal interactions unique to USP1. Moreover, dual inhibition of PARP1/2 and USP1 are strongly synergistic in PARP1/2 inhibitor-responsive cell line models. Strong *in vivo* anti-tumor activity across multiple tumor models was demonstrated with USP1 inhibition alone and in combination with the PARP1/2 inhibitor olaparib. Our studies suggest that USP1 and PARP1/2 inhibitors target BRCA1/2-mutant cancer though distinct yet synergistic mechanisms. As such, USP1 inhibitors may provide novel treatment strategies for PARP1/2 inhibitor-resistant and -naïve BRCA1/2-mutant cancer.

USP1 is a synthetic lethal target in BRCA1/2 mutant cancer

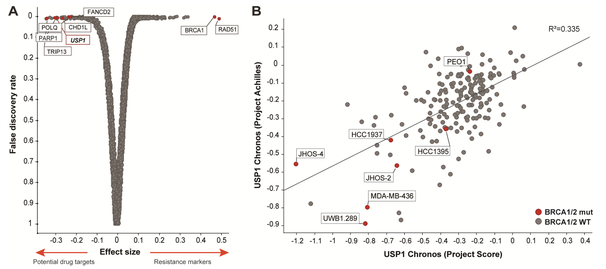

Figure 1: USP1 was discovered as a synthetic lethal target in BRCA1 mutant cell lines. (A) Analysis of CRISPR screens performed in 30+ BRCA1 WT vs. mut cell lines. USP1 was identified as a synthetic lethal target consistent with published findings (Lim et al., 2018). (B) USP1 dependency in BRCA1/2 WT and mut cell lines based on projects Achilles and Score (DepMap Public, Q1 2022).

Tango lead series USP1 inhibitors are highly potent and selective

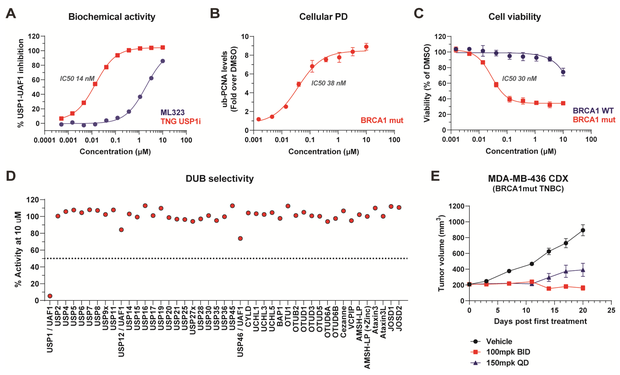

Figure 2: Characterization of USP1 inhibitors representative of Tango lead series. (A) Activity of TNG USP1i and ML323 in an USP1-UAF1 Ubiquitin-Rhodamine 110 cleavage assay. (B) Pharmacodynamic modulation of ub-PCNA by TNG USP1i in MDA-MB-436 cells. (C) Anti-proliferative activity of TNG USP1i in non-isogenic BRCA1 WT and mutant breast cancer cell lines. Data are represented as mean ± SD. (D) DUB (deubiquitinating enzyme) panel profiling of TNG USP1i at 10 μM using Ubiquigent DUB*profiler*™. (E) *In vivo* efficacy of TNG USP1i against MDA-MB-436 xenograft model. Data are represented as mean ± SEM.

USP1 inhibitor activity is BRCA1/2^{mut}-selective and correlated with olaparib activity

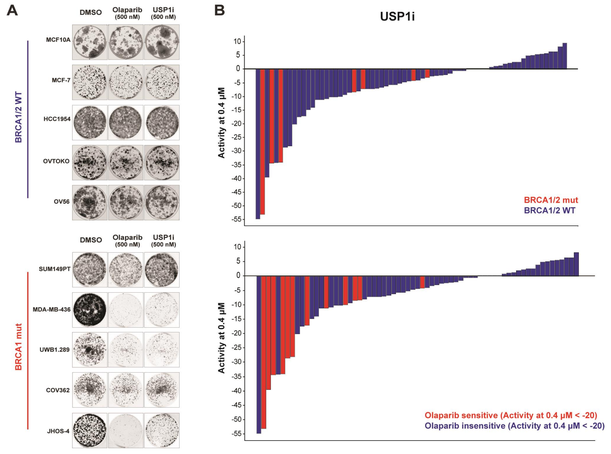

Figure 3: Activity of USP1i in breast and ovarian cancer cell lines. (A) Clonogenic assays were performed in BRCA1 mutant and WT cell lines. In brief, cells were incubated for 10-17 days in the presence of DMSO, 500 nM olaparib, or 500 nM USPi. Media was replaced every 3-4 days, and colonies were fixed and visualized with crystal violet. (B) TNG USP1i activity was profiled in 68 breast and ovarian cancer cell lines using a 7-day CellTiter-Glo assay. Activity at 0.4 μM is reported as % DMSO – 100. BRCA1/2 mutant cell lines (top panel) and olaparib sensitive cell lines irrespective of BRCA1/2 mutation status (bottom panel) are colored in red.

USP1 dependency is mediated by PCNA ubiquitination

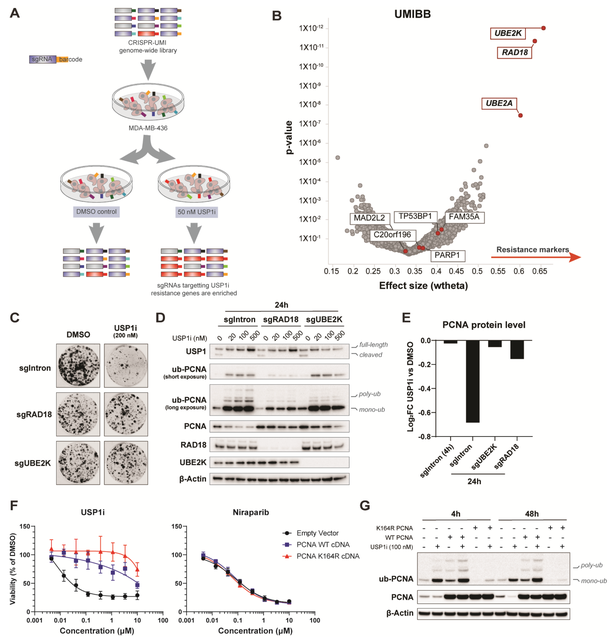

Figure 4: PCNA ubiquitination and protein loss mediate USP1i activity. (A) Schematic showing CRISPR-UMI (unique molecular identifiers) screen workflow. A custom genome-wide CRISPR-UMI library was constructed by linking randomized barcodes with sgRNAs (Michlits et al., 2017). MDA-MB-436 cells stably expressing Cas9 were transduced with the CRISPR-UMI library and cultured for 21 days +/- USP1 inhibitor. (B) NGS results were analyzed UMIBB, a novel non-parametric Bayesian method where the probability of UMI count depletion or enrichment among the total number of UMIs represented in each sgRNA is modeled by a beta-binomial distribution (AACR 2022 Abstract #1224). UBE2K, RAD18, and UBE2A sgRNAs were the most significantly enriched with USP1 inhibition. (C) Isogenic RAD18 or UBE2K null cell lines were constructed by transducing targeting sgRNAs into MDA-MB-436 cells stably expressing Cas9. Clonogenic assays and (D) immunoblotting analysis were performed in these cell lines +/- USP1i. RAD18 or UBE2K depletion notably reduced PCNA mono- and poly-ubiquitination, respectively, in the presence of USP1i. (E) Quantitation of total PCNA protein levels using LC-MS/MS in WT control, RAD18, or UBE2K null MDA-MB-436 cells following USP1i or mock treatment. Values are represented as $\log_2$ fold change from DMSO. (F) 7-day CellTiter-Glo assay with USP1i (left) or niraparib (right), and (G) immunoblotting analysis of isogenic MDA-MB-436 cells stably expressing empty vector, WT or K164R ub-dead PCNA. Data are presented as mean ± SD.

USP1 inhibition induces replication stress and DNA damage

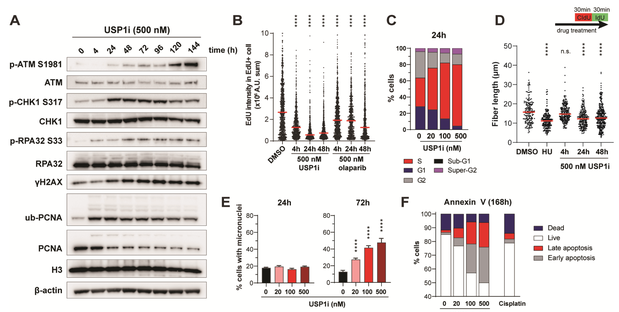

Figure 5: USP1 inhibition reduces the rate of DNA synthesis and causes DNA damage. (A) Immunoblotting analysis of the DNA damage sensing pathway in MDA-MB-436 cells in a time course of USP1i treatment. (B) Quantitation of EdU intensity in MDA-MB-436 cells pulsed-labelled with EdU +/- USP1 inhibitor at indicated time points. (C) Flow cytometry-based cell cycle analysis of MDA-MB-436 cells treated with DMSO or USP1i and stained with propidium iodide. (D) Replication fork speed in MDA-MB-436 cells +/- USP1i, measured with DNA fiber assay following the labeling schematic as shown. (E) Quantitation of micronuclei formation in MDA-MB-436 cells +/- USP1i based on fluorescence imaging. (F) Cell death in MDA-MB-436 after prolonged treatment with DMSO or USP1i was measured using a flow cytometry-based Annexin V assay. Statistical significance was evaluated by ANOVA followed by Dunett's post hoc test, comparing treatment conditions against DMSO control, **** P<0.0001, n.s. not significant.

USP1 inhibition is synergistic with PARP1/2 inhibitors

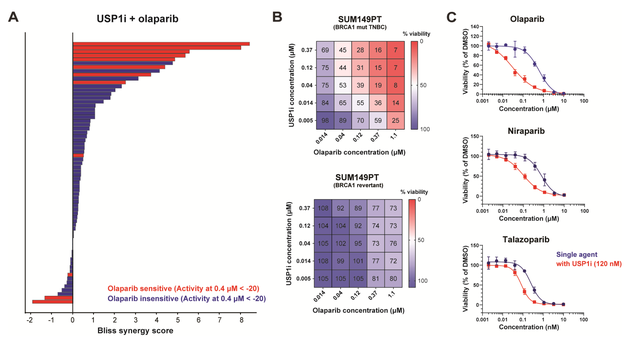

Figure 6: USP1i and PARPi are synergistic. (A) 68 ovarian and breast cancer cell lines were profiled in a 9-point USP1i + olaparib dose response matrix with a 7-day CellTiter-Glo assay. Synergy score was calculated based on the Bliss model. Olaparib sensitive cell lines (activity < -20% at 0.4 μM) are highlighted in red. (B) USP1i + olaparib combination dose response matrix in SUM149PT BRCA1 isogenic cell lines measured using a 7-day CellTiter-Glo assay. SUM149PT BRCA1 revertant cell line was generated by CRISPR-Cas9 targeting of the BRCA1 mutation site, followed by talazoparib selection and single clone isolation (Dréan et al., 2017). BRCA1 reversion was confirmed by immunoblotting and sequencing. (C) Dose response of indicated PARPi in SUM149PT, with and without USP1i co-treatment. Dose response curves were measured using 7-day CellTiter-Glo assay, and data are represented as mean ± SD.

Tango USP1 inhibitors have single agent and combination activities *in vivo*

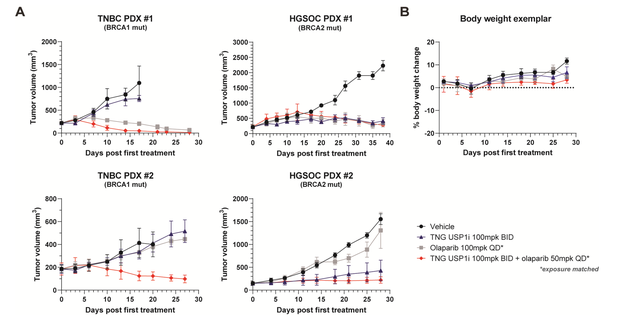

Figure 7: Anti-tumor activity of TNG USP1i single agent and in combination with olaparib. (A) BRCA1/2 mutant PDX models were treated with TNG USP1i, olaparib, or in combination for the indicated time periods. Olaparib doses were chosen to be clinically relevant and were adjusted in combination with USP1i to deliver equivalent exposures to single agent. (B) % body weight change in representative study. Data are presented as mean ± SEM.

USP1 inhibitors may have clinical opportunity outside of BRCA1/2 mutant tumors

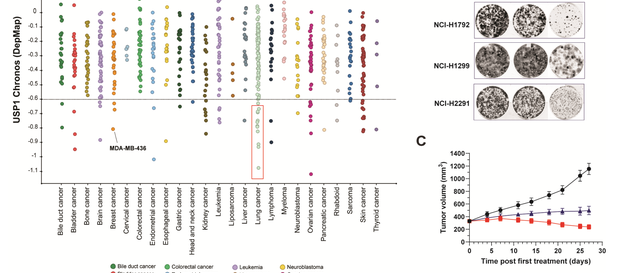

Figure 8: A subset of BRCA1/2 WT lung cancer cell lines are sensitive to USP1i. (A) USP1 dependency across multiple cancer lineages in DepMap Public (Q1 2022) CRISPR dataset. (B) Clonogenic assays showing response to USP1i but not olaparib in USP1 dependent lung cancer cell lines. (C) *In vivo* efficacy of TNG USP1i in a BRCA1/2 WT lung cell line xenograft model. Data are presented as mean ± SEM.

Summary

- **USP1 is synthetic lethal in BRCA1/2 mutant tumors**
- **USP1 dependency is mediated by PCNA ubiquitination and consequent PCNA protein loss**
- **CRISPR knockout of RAD18/UBE2A and UBE2K, but not PARPi resistance genes, rescue USP1i sensitivity**
- **USP1i and PARPi are synergistic in BRCA1/2 mutant tumors**
- **Tango lead series USP1 inhibitors are highly potent and selective**
  - ***In vitro* selectivity against BRCA1 mutant and PARPi sensitive cell lines**
  - ***In vivo* single agent and combination activity against multiple BRCA1/2 mutant xenografts**
- **A subset of BRCA1/2 WT lung cancer cell lines are sensitive to USP1i, representing additional patient expansion opportunity**

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