A novel USP1 inhibitor coupled with a novel proteomic response biomarker for precision oncology therapy

Iris Alchanati[1], Andrew Morley[2], Avital Hay-Koren[1], Fabrizio Fierro[1], Galina Otonin[1], Shay Herman[1], Alon Shtrikman[1], Yonatan Katzenelenbogen[1], Gali Arad[1], Barr Tivon[1], Dimitri Kovalerchik[1], Kirill Pevzner[1] and Eran Seger[1]

[1] Protai, Tel Aviv, Israel, [2] o2h Discovery, Cambridge, England

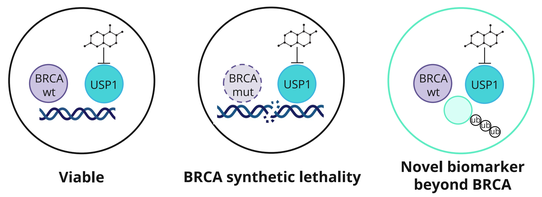
American Association for Cancer Research

Abstract number: 7122

Introduction

Ubiquitin-specific protease 1 (USP1) is a well-characterized deubiquitinating enzyme (DUB) that plays a critical role in DNA damage repair (DDR). Absence of USP1 results in reduced cell survival and disrupted genomic stability, suggesting efficacy of USP1 inhibitors (USP1i) in DDR-deficient tumors[1].

Here we present PAI-CPD1, a novel, highly potent inhibitor of USP1, showing anti-USP1 activity in both cell-free and cell-based assays, as well as in xenograft model, where it exhibits significant tumor growth inhibition compared to competing inhibitors.

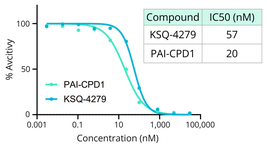


Viable | **BRCA synthetic lethality** | **Novel biomarker beyond BRCA**

While homologous recombination deficiencies (HRD) and BRCA mutations traditionally serve as biomarkers for patient selection in DDR therapies, depending solely on genomic abnormalities was shown to be insufficient, impeding clinical success. To address this limitation, we utilized Protai's AIMS™ computational platform to analyze the proteomic and post translational modifications (PTM) landscape of resistant and sensitive cell lines, both pre- and on-treatment. This analysis revealed a distinct proteomic biomarker for USP1i response, independent of HRD status, shedding light on the molecular mechanisms underlying USP1 sensitivity and promoting patient selection for USP1 monotherapy.

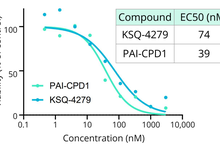
PAI-CPD1 shows high potency *in-vitro*

1A Biochemical assay (USP1-UAF1)



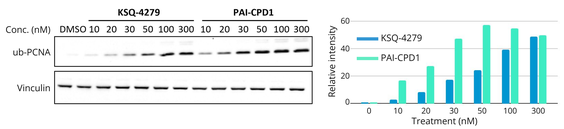
Compound	IC50 (nM)
KSQ-4279	57
PAI-CPD1	20

Cell viability assay (MDA-MB-436)

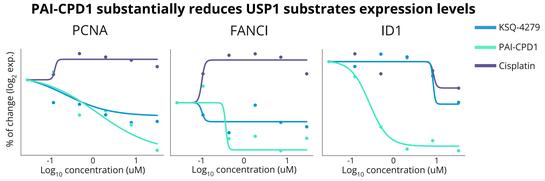


Compound	EC50 (nM)
KSQ-4279	74
PAI-CPD1	39

1B Ub-PCNA accumulation



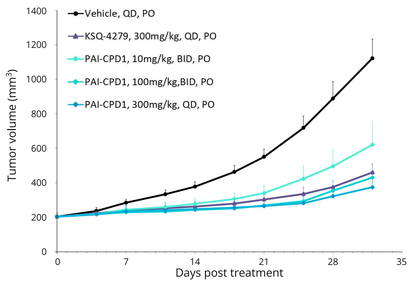
1C PAI-CPD1 substantially reduces USP1 substrates expression levels



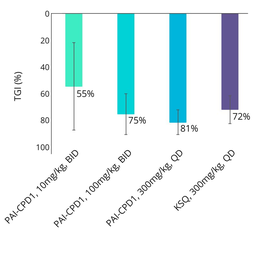
1A. PAI-CPD1 demonstrates high inhibitory potency in a cell-free assay, as well as strong cell viability inhibition of BRCA-mut TNBC cell line, outperforming KSQ-4279[2] **1B.** PAI-CPD1 exhibits dose-dependent cellular accumulation of the USP1 substrate PCNA ub-K164, indicating targeted interaction with PCNA and demonstrating slight superior efficacy compared to benchmark USP1 inhibitor. **1C.** A dose-dependent mass spectrometry (MS) proteomic analysis comparing PAI-CPD1 with KSQ-4279 at 8 hours post treatment, indicates a greater and more efficient reduction of known USP1 targets levels, implying superior efficacy in inhibiting USP1.

PAI-CPD1 superior efficacy in tumor growth inhibition *in-vivo*

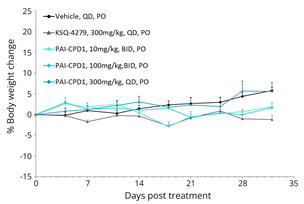
2A MDA-MB-436 xenograft (TNBC, BRCA-mut)



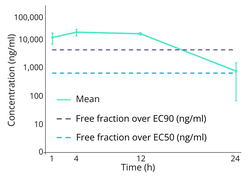
2B Tumor growth inhibition



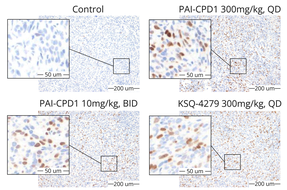
2C Body weight change



2D PK oral dosing (100mg/kg)



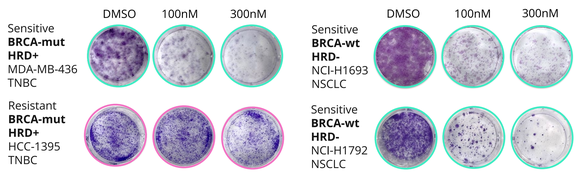
2E ubPCNA levels



2A. PAI-CPD1 shows substantial tumor growth inhibition (N=6), outperforming KSQ-4279 in a similar treatment regimen (300mg/kg QD, PO). **2B.** Tumor growth inhibition (TGI), calculated relative to control tumor volume (day 32). Max TGI = 81% **2C.** Body weight change, calculated relative to the weight on day 0. No significant weight loss observed. **2D.** PAI-CPD1 drug concentrations allow suitable coverage of free concentration over EC90 of MDA-MB-436 viability using a BID administration. **2E.** PCNA ub-K164 IHC staining was conducted 32 hours after treatment initiation, 8 hours post the final dosage for each group. Remarkable accumulation of ub-PCNA was detected in both high and low doses of PAI-CPD1.

USP1i sensitivity extends beyond BRCAness

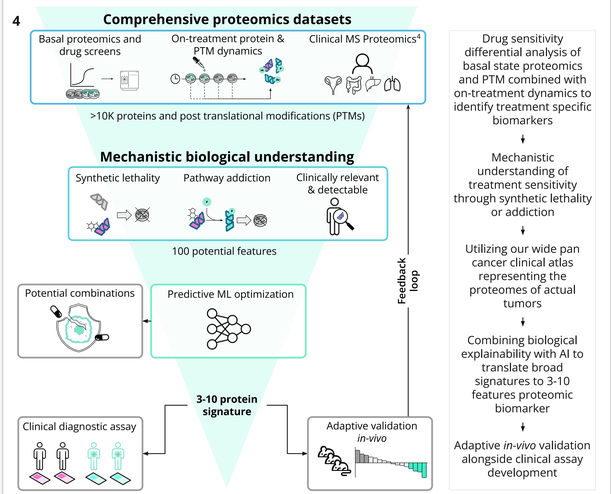
3 Colony formation assay treated with USP1 inhibitor



USP1i treatment of cell lines with diverse BRCA and HRD statuses highlights its potential efficacy both beyond and independently of BRCA-mutant and HRD+ populations.

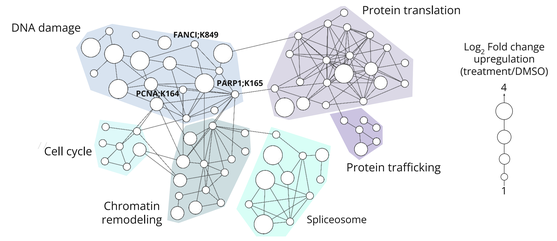
AIMS™ platform for biomarker discovery[3]

4 Comprehensive proteomics datasets



AIMS™ based ubiquitin-omics reveals USP1 roles outside DNA damage

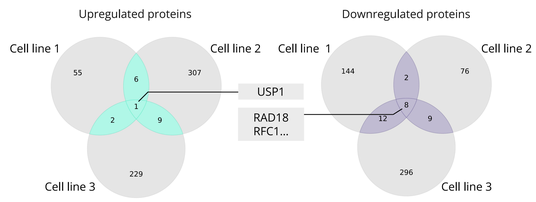
5 Ubiquitination dynamics following USP1i treatment



Protein-protein interaction (PPI) network of ubiquitination sites upregulated 2 hours post USP1 inhibition, indicating a substantial cellular impact of USP1 that extends beyond the DDR scope. Treatment/DMSO fold-change is illustrated by circle size.

Protein dynamics analysis provides insights into USP1 cellular functions

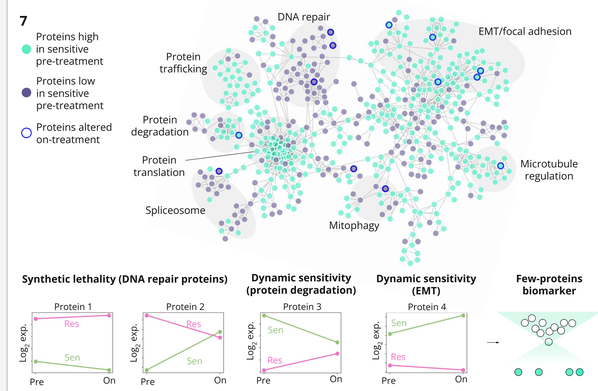
6 Altered proteins following USP1i treatment in sensitive cells



Protein dynamics analysis of three cell lines sensitive to USP1 inhibition reveals both unique and mutual upregulated and downregulated proteins 24 hours post treatment (p-value < 0.05). These findings indicate a specific cellular response to USP1 inhibition, shedding light on both known and novel key players of its mechanism of action.

AIMS™ platform identifies predictive biomarker for USP1

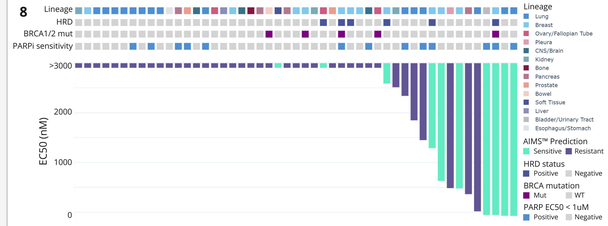
7



Pre-treatment proteomics analysis identifies differentially expressed proteins in sensitive and resistant cells (PPI network, p-value < 0.05). Proteins enriched in either group are colored as indicated. Integration of on-treatment protein and PTM dynamics (outlined in blue), highlights treatment-specific patterns, enabling a deep biological understanding of treatment-related vulnerabilities. AIMS™ biomarker, generated via AI, translates broad pathway signatures and protein patterns into minimal-feature biomarker.

AIMS™ biomarker predicts USP1i sensitivity in cell lines regardless of BRCA/HRD status

8



Cell line screen (N=49) demonstrates AIMS™ biomarker precision in predicting USP1i sensitivity, independently of BRCA mutation and HRD status. Cell lines from various lineages (indicated by color), underwent 10 days viability assay and categorized as either predicted-sensitive (turquoise) or predicted-resistant (purple) to USP1 inhibition, based on their AIMS™ biomarker score. EC50 exceeding max dosage assigned as >3000nM. No correlation between PARP1i and USP1i (predicted or actual) sensitivity was observed.

Summary

- PAI-CPD1, a novel USP1 inhibitor, exhibits remarkable potency and has superior *in-vivo* efficacy compared to other USP1 inhibitors.
- Using the AIMS™ platform for biomarker discovery, a unique and specific biomarker for USP1 monotherapy was identified.
- AIMS™ biomarker identifies populations predicted to respond to USP1i treatment, extending beyond BRCA mutations or HRD status.
- The program is advancing towards IND and clinical trial assay by the end of 2025.
- Utilizing AIMS™ platform, Protai is actively working on validating potential new combinations involving USP1.

References

1. García-Santisteban et al. Mol Cancer (2013)
2. Cadzow et al. European Journal of Cancer (2020)
3. Arad et al. Phospho-proteomic dynamics reveal DNA damage and repair signatures of drug sensitivity [abstract]. In: Proceedings of the 115th Annual Meeting of the American Association for Cancer Research; 2024 April 5-10; San Diego, CA. Philadelphia (PA): AACR; 2024. Abstract #5130
4. Simchi et al. A large scale proteogenomic atlas for precision oncology [abstract]. In: Proceedings of the 115th Annual Meeting of the American Association for Cancer Research; 2024 April 5-10; San Diego, CA. Philadelphia (PA): AACR; 2024. Abstract #6241