Unveiling DDR Inhibitor Synergies: An Integrated Approach for Rapid Combination Discovery

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1 Introduction

Effective combination strategies enhance efficacy, overcome drug resistance, reduce side effects with lower doses and expand treatable indications.

We developed an integrated approach for DDR inhibitor characterisation and combination discovery that links CRISPR screens with cell panel screening.

We demonstrate this approach using PARP inhibitors as a case study.

2 CRISPR Screening Identifies drivers of Olaparib sensitivity

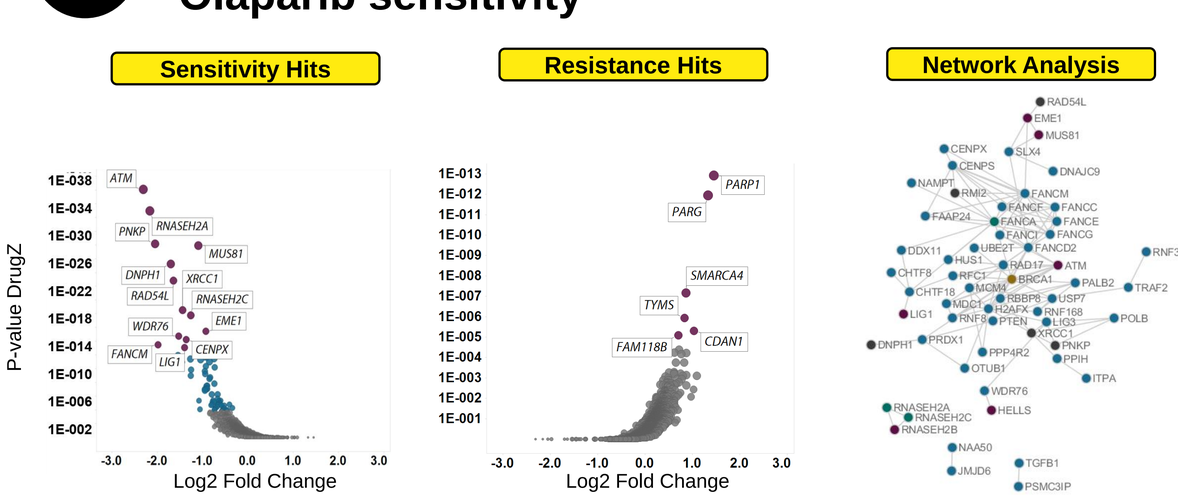

Figure 1: Whole genome CRISPRko drug-gene interaction screen using PARP1 inhibitor Olaparib in HT-29 cells. *Hit scoring for sensitivity and resistance to Olaparib. Network analysis of top scoring hits (FDR < 0.05). BRCA1 is a hit network nexus and is coloured gold for emphasis. (Blanck et al. 2020).*

3 Longer Duration OncoSignature™ Cell Panel Screening Assays Reveal DDRi Effects

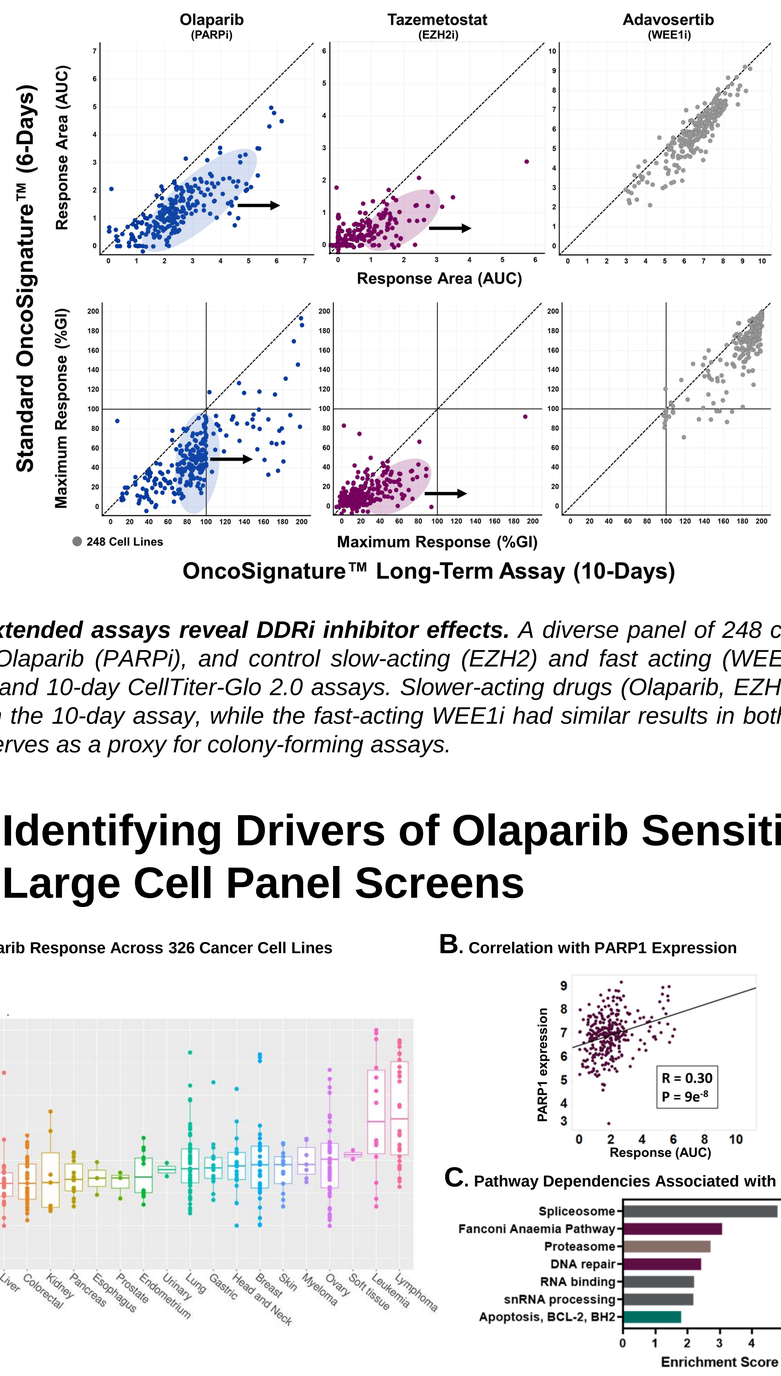

Figure 2: Extended assays reveal DDRi inhibitor effects. *A diverse panel of 248 cancer cell lines was tested with Olaparib (PARPi), and control slow-acting (EZH2) and fast acting (WEE1) inhibitor controls using 6-day and 10-day CellTiter-Glo 2.0 assays. Slower-acting drugs (Olaparib, EZH2i) showed stronger responses in the 10-day assay, while the fast-acting WEE1i had similar results in both durations. The 10-day assay serves as a proxy for colony-forming assays.*

4 Identifying Drivers of Olaparib Sensitivity in Large Cell Panel Screens

Figure 3: Pharmagenomic analysis of Olaparib using OncoSignature™. ***A.*** *Responses in 6-day CellTiter-Glo 2.0 viability assay. Correlations of drug sensitivity with PARP1 expression (**B**) or pathway dependencies (**C**) were evaluated using Depmap data (Blanck et al. 2020).*

5 Validation of Pairwise Combination Predictions

Figure 4: Validation of PARPi combinations predicted in the CRISPR and cell panel screens. ***A.*** *Heatmap of synergy scores for olaparib and talazoparib combinations with clinical-stage inhibitors against predicted targets or associated pathways, using Loewe additivity in 6-day viability assays.* ***B.*** *Example combination matrices showing: Observed Growth Inhibition (GI), Loewe Excess (positive = synergy, negative = antagonism) and Synergy scores (in gold).*

6 Evaluating Three-way DDRi combinations

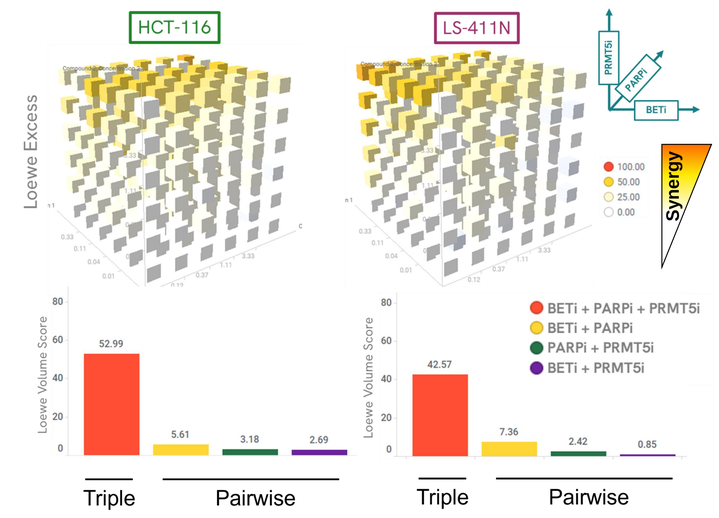

Figure 5. Enhanced synergy in triple combinations of PARP and chromatin modifier inhibitors. *Synergistic effects of pairwise versus triple combinations of Olaparib (PARPi), (+)-JQ1 (BETi), and GSK3326595 (PRMT5i) were assessed in a 4-day cell viability assay. Observed Loewe Excess values across a 6x6x6 dose matrix and Loewe Volume scores indicating overall synergy are shown.*

7 Evaluating DDRi-Irradiation Synergies

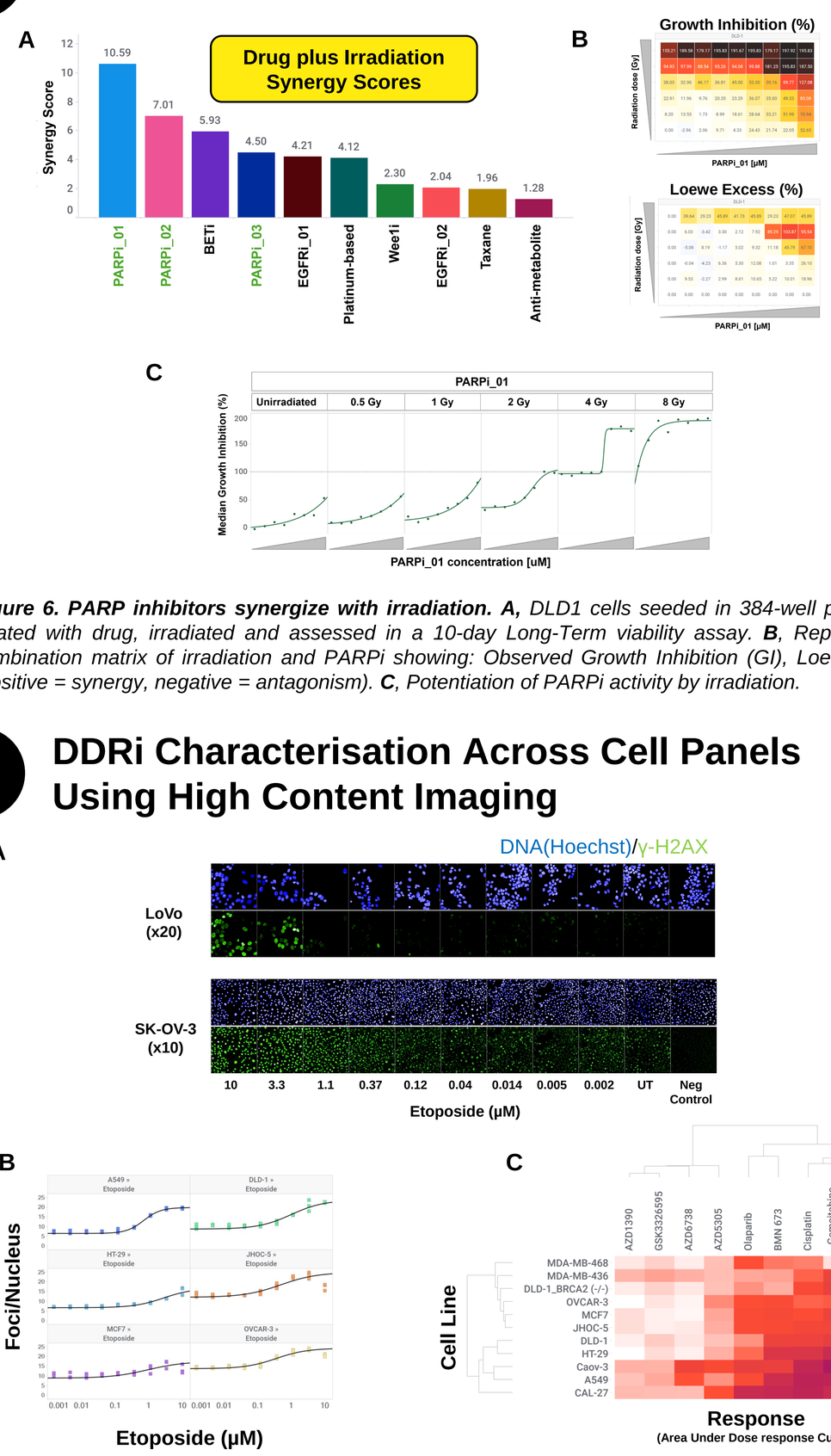

Figure 6. PARP inhibitors synergize with irradiation. A, *DLD1 cells seeded in 384-well plates were treated with drug, irradiated and assessed in a 10-day Long-Term viability assay.* ***B****, Representative combination matrix of irradiation and PARPi showing: Observed Growth Inhibition (GI), Loewe Excess (positive = synergy, negative = antagonism).* ***C****, Potentiation of PARPi activity by irradiation.*

8 DDRi Characterisation Across Cell Panels Using High Content Imaging

Figure 7. Cell Panel Screening Reveals Diverse DNA Damage Responses. *Representative images of γ-H2AX immunostaining after 24h treatment with a library of DNA damage compounds. B) Dose-response curves demonstrating data robustness. C) Hierarchical clustering of single-agent responses across the panel (darker red indicates stronger response).*

7 Summary

We describe an integrated approach for rapid DDR inhibitor combination discovery that links pooled CRISPR screens with large cell panel screens.

This identified sensitivity/resistance mechanisms and potential therapeutic combinations.

Using PARP inhibitors, we found significant overlap in sensitivity genes and identified synergistic interactions with inhibitors of DNA Damage response, cell survival and division pathways, chromatin modifiers and with irradiation.

Blanck, M. *et al.* (2020) A flexible, pooled CRISPR library for drug development screens, *The CRISPR Journal*, 3(3), 211-222

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