Advancing ADC Development: Assessment of Dual-Payload Synergistic Effect using Normal Cancer and Resistant Cell Panels

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Introduction

Antibody-drug conjugates (ADCs), combining the precision of antibodies with the potency of cytotoxic drugs, represent a promising anticancer therapy. However, current ADCs face significant challenges, including non-responsive cancers and rapid patient relapse, primarily due to tumor heterogeneity and drug resistance. To address these issues, dual-payload ADCs have emerged as a novel strategy, delivering two cytotoxic agents to enhance efficacy through synergistic effects, mitigate resistance, and offer flexible dosing. Despite their potential, the development of dual-payload ADCs remains complex. Cell panel-based studies and drug-resistant cell lines provide powerful tools to explore effective dual-payload ADCs by evaluating drug combination synergies and elucidating resistance mechanisms.

DDR Targets as Potential Combination Targets for Dual-Payload ADC Development

Dual-payload mechanism of action

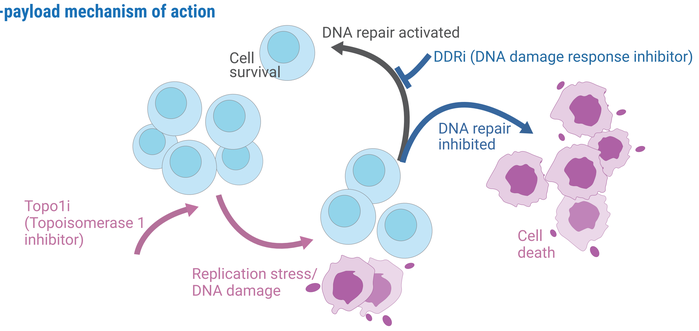

Figure 1. Schematic principle of synthetic lethality. TOPO1 inhibition traps DNA breaks at replication forks. Concurrent DDR blockade severs the repair escape route, leading to replication catastrophe and selective cell death, particularly in HR-deficient tumors.

Screening of Different Single Payloads in the CRC Cell Panels

Molecule	Target	Mechanism
Dxd	TOPO1	Stabilises TOPO1-cc → SSB → replication-run-off DSBs
SN38	TOPO1	Identical to Dxd; generates replication-associated DSBs
Exatecan	TOPO1	Generates replication-associated DSBs
Azenosertib	WEE1	Blocks WEE1 → abolishes G2/M checkpoint → forces cells with DNA damage into premature mitosis → mitotic catastrophe & apoptosis;
Prexasertib	CHK1/2	Prevents CHK1/2 activation → bypasses S/G2 arrest → cells enter mitosis with broken DNA
Lartesertib	ATM	Blocks ATM kinase→Replication-fork collapse & mitotic catastrophe
Ceralasertib	ATR	Blocks ATR kinase → abolishes replication-stress checkpoint → fork collapse & mitotic catastrophe
MMAE	Tubulin	Binds β-tubulin → blocks microtubule polymerisation → mitotic arrest → apoptosis

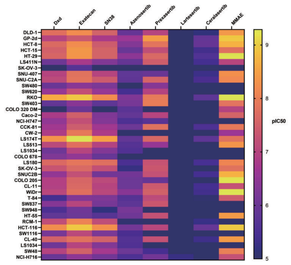

Figure 2. Single payload activity across a CRC cell panel.TOPO1 inhibitor, DDR related Targets(ATR,ATM, WEE1, CHK1/2) and Tubulin inhibitors treated Cells for 3 days and viability was quantified by CTG at the endpoint; pIC50 values are shown in the heat-map (Orange = high potency, blue = low potency).

Dual-Payload Screening Strategy

Fixed Ratio Combination Study and CI (Combination index) Calculation

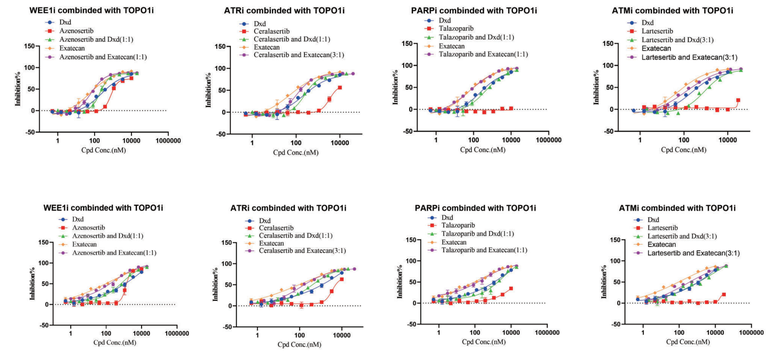

Figure 3. Fixed-ratio combination matrix of DDR inhibitors (WEE1i, ATRi, PARPi, ATMi) with TOPO1 inhibitors (Dxd or Exatecan) in LS411N (upper panel) and T-84 (lower panel) cells. Cells were exposed to 3-day constant-ratio combinations and viability was quantified by CTG.

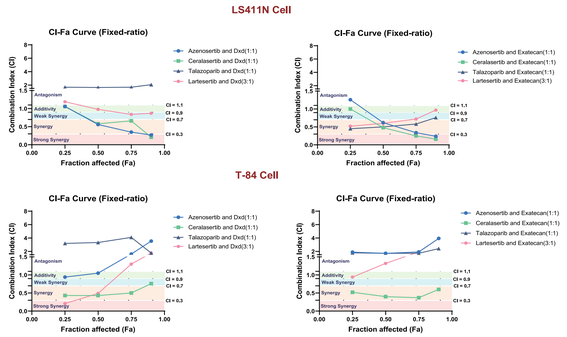

Figure 4. Chou–Talalay CI-Fa curves for fixed-ratio DDRi + TOPO1i combinations in LS411N (upper two panels) and T-84 (lower two panels) cells. dashed grey lines indicate synergy (0.9), additivity (1.1) and strong-synergy (0.7) boundaries. Color code: red ≤ 0.3 (strong synergy), orange 0.3-0.7(synergy), blue 0.7–0.9 (weak synergy), green 0.9-1.1 (additive), no color ≥ 1.1 (antagonism).

Matrix Combination Study to Confirm the Synergistic Effect of Dual Payloads

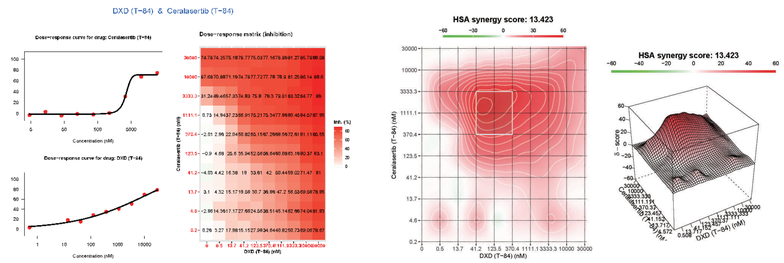

Figure 5. Matrix-combination synergy maps for dual-payload ATRi + TOPO1i pairs. Cells were exposed to 10 × 10 dose matrices (0.1–10 000 nM, 3-fold dilution) for 72 h; viability was assessed by CTG. The left column shows single-agent dose–response curves, while the remaining grids reveal pronounced synergy for Ceralasertib with Dxd (HSA score > 13 across ≥ 2 log concentration windows), confirming strong synergistic activity in T-84 cells.

ADC and Payload Resistant Cell Line Panel for Dual-Payload Screening

ADC-Related Resistant Cell Lines Generation

Resistant Cell Lines	Resistance	Resistance Index (RI#)	Status	Method	Cancer Type
NCI-N87/DS-8201 R	ADC& Payload	>100	MoA Study*	Natural Resistance (in vitro)	Gastric tubular adenocarcinoma
DLD-1/Exatecan R	ADC& Payload	>100	MoA Study*	Natural Resistance (in vitro)	Colon adenocarcinoma
GP2d/Dxd R	ADC & Payload	>10	MoA Study*	Natural Resistance (in vitro)	Colon adenocarcinoma
GP2d/SN-38 R	ADC & Payload	>5	MoA Study*	Natural Resistance (in vitro)	Colon adenocarcinoma
SK-OV-3/MMAE R	ADC & Payload	>20	MoA Study*	Natural Resistance (in vitro)	Ovarian serous cystadenocarcinoma
NUGC-4/MMAE R	ADC & Payload	>5	Stability Test	Natural Resistance (in vitro)	Gastric signet ring cell adenocarcinoma
SK-BR-3/hABCB1 OE	ADC & Payload	Validated	Validated	Overexpression	Breast adenocarcinoma
SK-BR-3/hABCG2 OE	ADC & Payload	Validated	Validated	Overexpression	Breast adenocarcinoma
JIMT-1/hABCB1 OE	ADC & Payload	Validated	Validated	Overexpression	Breast ductal carcinoma
JIMT-1/hABCG2 OE	ADC & Payload	Validated	Validated	Overexpression	Breast ductal carcinoma

RI#: fold increase in IC_{50} (resistant vs parental)
MoA Study*: The MoA study combines flow-cytometry–based target detection with transcriptomic (RNA-seq) and genomic (WES) profiling.

ADC and Payload Resistant Cell Panel for Dual Payload Screening

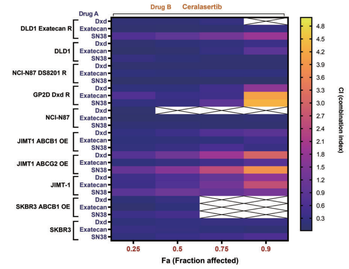

Figure 6. Synergy landscape of TOPO1–DDR combinations across ADC-resistant and wild-type cancer cell lines. Heat map shows the Chou–Talalay combination index (CI) calculated at Fa = 0.25, 0.5, 0.75 and 0.9 (columns) for 10 ADC-related resistant or WT cell lines (rows) and six fixed-ratio drug pairs (matrix columns). Crosses mark conditions where accurate CI could not be determined because of incomplete curves or maximum inhibition < 50 %.

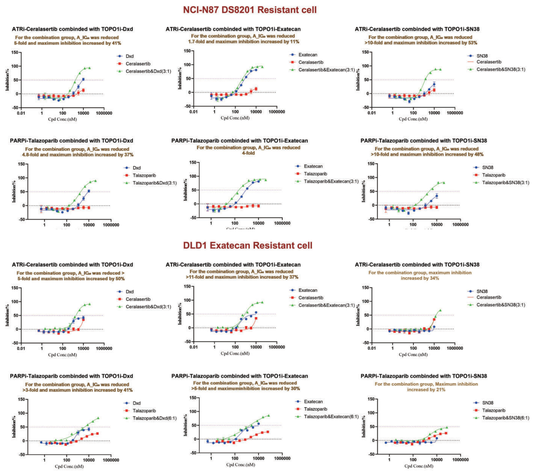

Figure 7.Dose–response relationships and the corresponding synergy assessment for DDR-inhibitor (Ceralasertib, Talazoparib) plus TOPO1-inhibitor (Dxd, Exatecan, SN-38) combinations in the NCI-N87 DS8201 resistant cell and DLD1 Exatecan resistant cell models. Although incomplete single-agent curves prevented accurate CI determination for some conditions (hence their absence from the Figure 5 heat map), marked left-shifts of IC_{50} (≥ 3-fold) are evident throughout, signifying potent synergy and partial reversal of DS8201 or Exatecan resistance. These data provide mechanistic support for dual-payload ADC strategies that co-deliver DDRi and TOPO1i payloads to overcome HER2-ADC refractoriness.

Summary

In this study, we first profiled a colorectal-cancer cell panel with single-payload agents, identified inherently resistant models, and then used these hits to anchor a dual-payload combination screen. LS411N and T-84 cells were subsequently selected for two orthogonal synergy assays—fixed-ratio combination/CI analysis and a 10 × 10 matrix synergy map—to obtain rigorous, quantitative interaction metrics. Finally, we generated an ADC and payload-resistant cell panel to test whether dual-payload combinations can overcome either free-payload or ADC-evoked resistance. ATR inhibitors—and, in some contexts, PARP inhibitors—consistently produced the strongest synergies with TOPO1 inhibitors, providing a clear mechanistic rationale for co-loading these agents in novel dual payload ADCs.