Top1i-ATRi Dual-Payload Strategy : Mechanistic Synergy and Therapeutic Potential in Drug-Resistant Colorectal Cancer

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Introduction

Dual-payload antibody-drug conjugates (ADCs) represent a next-generation therapeutic strategy designed to overcome tumor heterogeneity and drug resistance by delivering two distinct payloads with synergistic potential. This study aimed to identify an optimal dual-payload combination to address the challenge of therapeutic resistance in colorectal cancer (CRC), focusing on inhibitors of DNA damage response (DDR) pathways.

Methods

We systematically screened synergistic pairs between Topoisomerase I (Top1) inhibitors and a panel of DDR inhibitors with complementary mechanisms. Following evaluation of single-agent cytotoxicity across a CRC cell line panel, combination synergy was assessed in selected CRC models. The specific combination of the Top1 inhibitor DXd and the ATR inhibitor Ceralasertib demonstrated significant synergy in representative CRC model T-84, using both 2D and 3D cytotoxicity assays. DXd and Ceralasertib combination also showed synergistic effects in a DS-8201-resistant cell model and a Top1 inhibitor (Exatecan)-resistant CRC model. Critically, this dual-payload combination did not exhibit synergistic effects in hematotoxicity prediction assays which predict lineage-specific hematotoxicity by differentiating $CD34^+$ hematopoietic stem and progenitor cells into hematopoietic lineages. Moreover, this dual-payload combination did not show synergistic toxicity in representative cell lines of human healthy tissues. These initial results suggest a potentially wider therapeutic window for the corresponding dual-payload ADC based on these two payloads.

DDR as Potential Combination Targets for Dual-payload ADC Development

Dual-payload mechanism of action

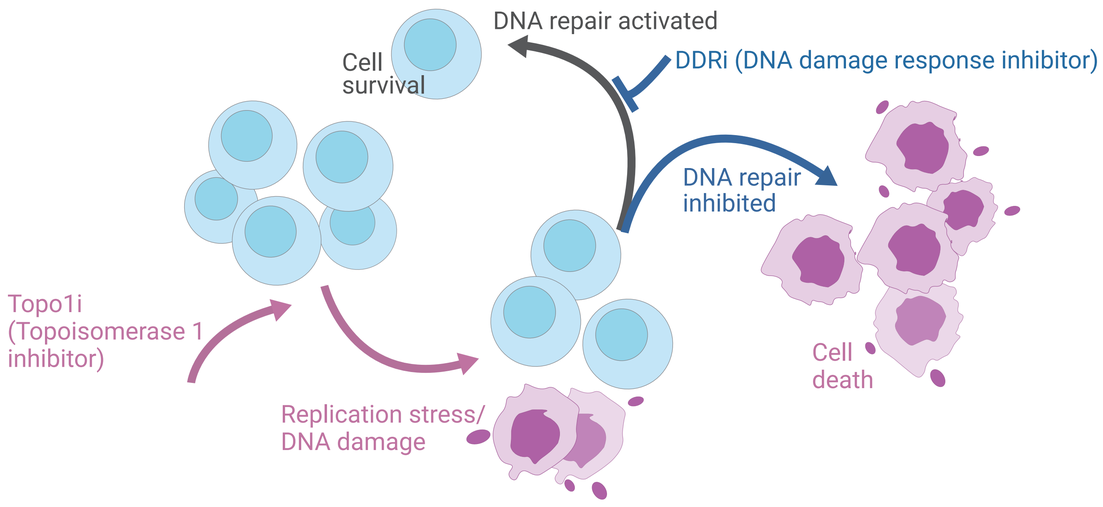

Figure 1. Schematic principle of synthetic lethality. Top1 inhibition traps DNA breaks at replication forks. Concurrent DDR blockade severs the repair escape route, leading to replication catastrophe and selective cell death, particularly in HR-deficient tumors.

Results

Cytotoxicity Screening of Different Single Payloads in the CRC Cell Panel

Payload	Target	Mechanism of action
DXd	Top1	Stabilizes Top1-cc → SSB → replication-run-off DSBs
SN38	Top1	Identical to DXd; generates replication-associated DSBs
Exatecan	Top1	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
Ceralasertib	ATR	Blocks ATR kinase → abolishes replication-stress checkpoint → fork collapse & mitotic catastrophe

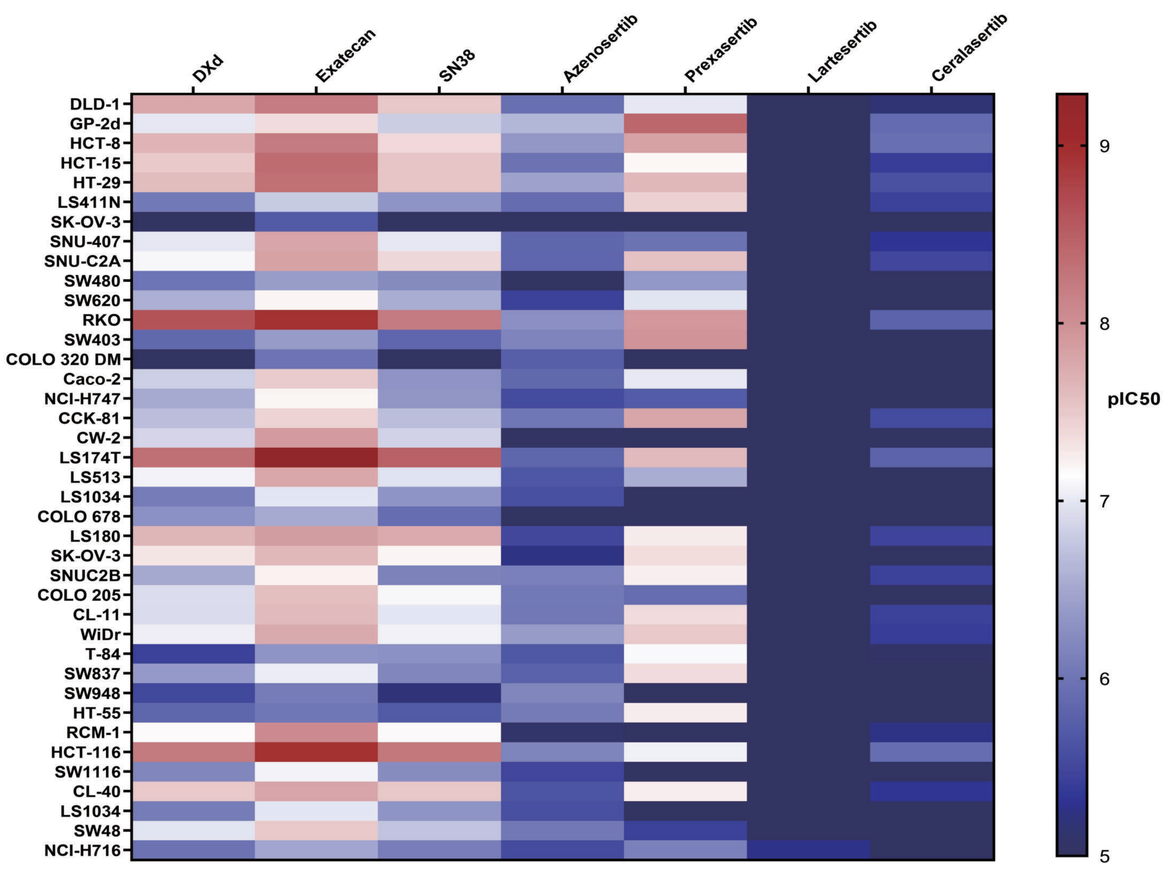

Figure 2. Cytotoxic activities of different single payloads across a CRC cell panel. 39 CRC lines were treated with individual Top1 inhibitor or DDR related targets (ATR, WEE1, CHEK1/2) inhibitors and cell viability was quantified by CTG; pIC_{50} ($-\log_{10}(IC_{50})$) values were shown in the heat-map (dark red = high potency, dark blue = low potency).

Combination of Ceralasertib and DXd Showed Synergistic Efficacy *in vitro*

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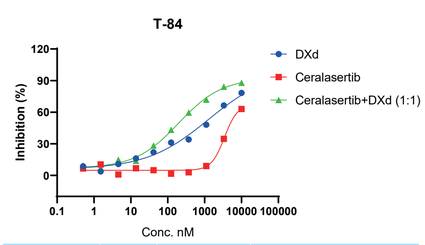
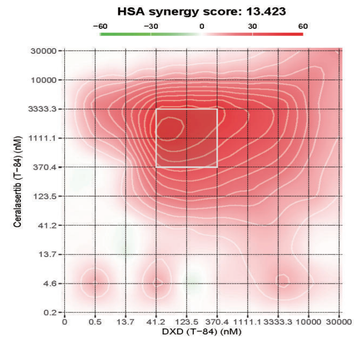


T-84	DXd	Ceralasertib	Ceralasertib+Dxd(1:1)
R_IC_{50} (nM)	1255	3462	198.9
A_IC_{50} (nM)	990.4	5118	203.2

B



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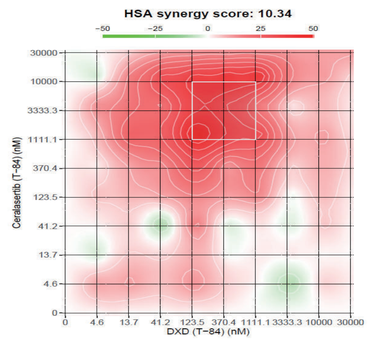
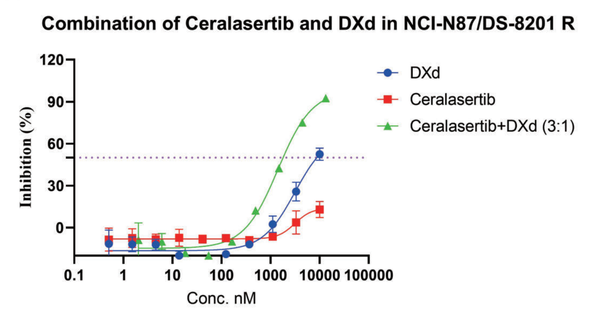


Figure 3. Combinations of Ceralasertib (ATR inhibitor) and DXd (Top1 inhibitor) showed synergistic effects in T-84 with either fixed-ratio combination or matrix combination cytotoxicity assays. A. 1:1 ratio combination of dual-payload demonstrated ~5-fold A_IC_{50} left-shift compared to DXd group. B-C. Matrix combinations of dual-payload were tested in 2D (B) and 3D (C) T-84 cell models with 10×10 dose matrices. Grid data revealed pronounced synergy for Ceralasertib and DXd (HSA synergy scores >10 in both 2D and 3D models), demonstrating synergistic activities.

A



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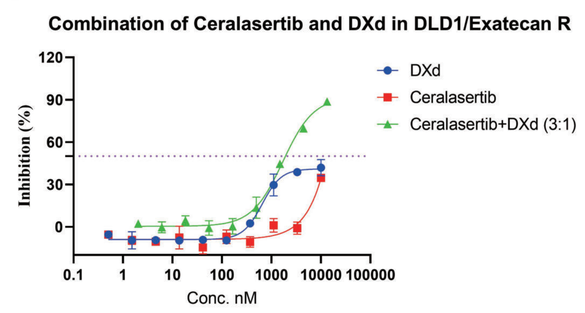
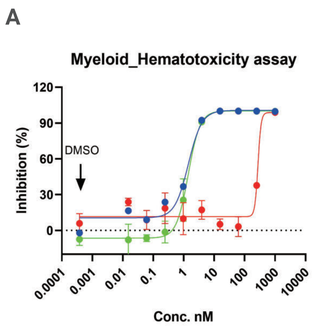


Figure 4. Fixed-ratio combinations of Ceralasertib and DXd displayed synergistic effects in both NCI-N87/DS8201 resistant (R) and DLD1/Exatecan resistant (R) cells. A. The combination group showed 5-fold A_IC_{50} left-shift and ~40% maximum inhibition improvement compared to DXd group in NCI-N87/DS8201 R cell line. B. The combination group displayed significant A_IC_{50} difference and ~50% maximum inhibition improvement compared to DXd group in DLD1/Exatecan R cell line. These data indicated the potential of dual-payload combination to overcome resistance of DS-8201 and Exatecan in the tested models.

Dual-payload Combination did not Exhibit Synergistic *in vitro* Toxicity

Progenitor Cell Type	Detected Pathway	Predictive Toxicity
Primitive erythropoietic progenitor cell: $CD34^+$ HSPCs induced into the erythropoietic lineage	Erythropoietic lineage	Anemia
Primitive granulocyte-macrophage (GM) progenitor cell: $CD34^+$ HSPCs induced into the myeloid-GM lineage	Myeloid-GM lineage	Neutropenia, Leukopenia
Primitive megakaryocyte progenitor cell: $CD34^+$ HSPCs induced into the megakaryocyte lineage	Megakaryocyte lineage	Thrombocytopenia

A



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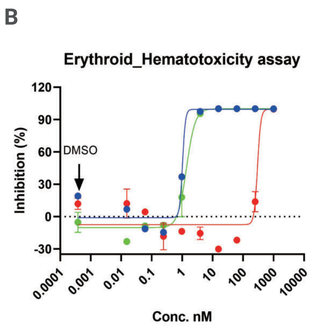

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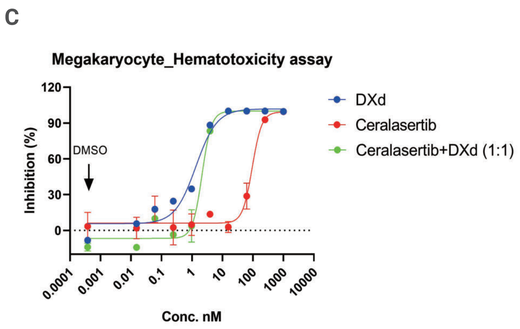

Figure 5. Fixed-ratio combinations of dual-payload did not exhibit synergistic effects in hematotoxicity prediction assays. $CD34^+$ hematopoietic stem and progenitor cells were differentiated into 3 different hematopoietic lineages to predict lineage-specific hematotoxicity *in vitro*. Drugs were added while inducing lineages (A. Erythropoietic, B. Myeloid, and C. Megakaryocyte lineages). The combination group did not show significant IC_{50} shift or maximum inhibition improvement compared to DXd group in all these 3 assays.

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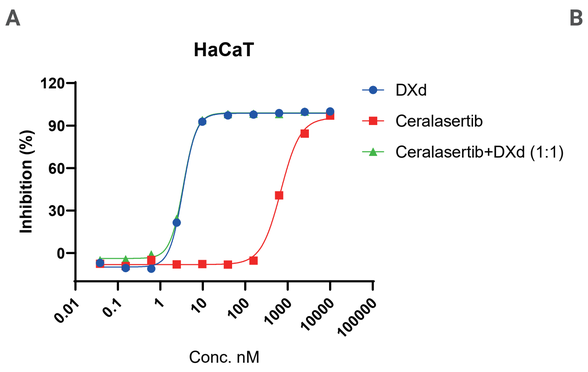

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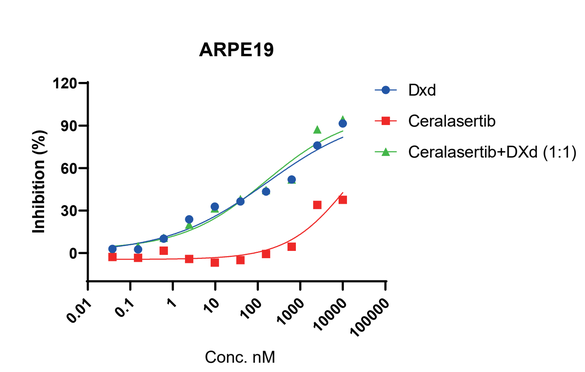

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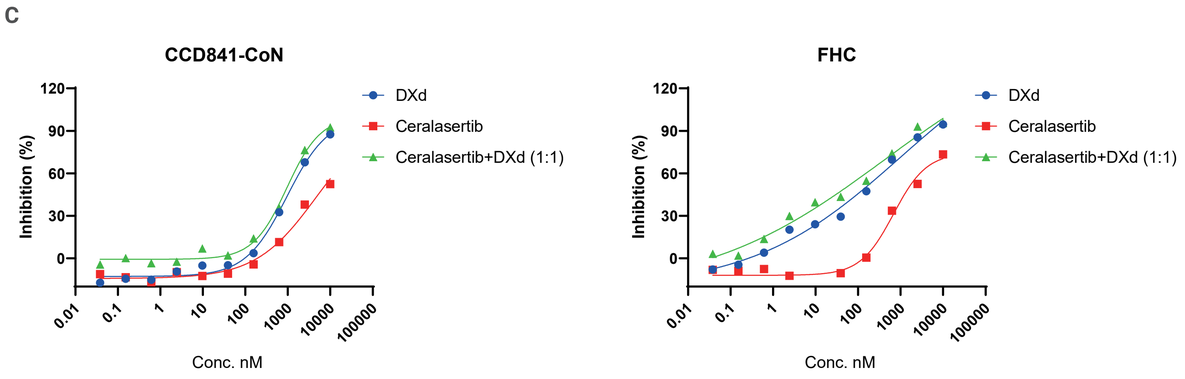

Figure 6. Fixed-ratio combinations of dual-payload did not show synergistic toxicity in representative cell lines of human healthy tissues. Cytotoxicity of dual-payload tested in A. HaCaT (skin; keratinocyte), B. ARPE19 (eye; retinal pigmented epithelium), and C. FHC and CCD841-CoN (colon) showed similar or comparable toxicity with the DXd groups.

Summary

Our findings establish a rational strategy for dual-payload screening and provide insights that targeting complementary DDR pathways can effectively overcome conventional and acquired resistance in CRC without inducing more observed hematotoxicity or healthy tissue toxicity. This strategy could accelerate the development of more durable dual-payload ADC therapies, particularly in the proof-of-concept stage for dual-payload combinations.