Combined Top1 and CHK Inhibitor Dual-Payload Strategy for Synergistic Reversal of Tumor Drug Resistance

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Introduction

Dual-payload ADCs have emerged as an innovative promising next-generation ADC format, delivering two types of payloads to enhance efficacy through synergistic effects and mitigate resistance, thereby addressing the challenge of poorly responsive cancers and rapid patient relapses. The success of dual-payload ADCs hinges on the appropriate dual-payload combination.

DDR inhibitors are increasingly being evaluated as payloads for ADCs. To identify a rationally designed dual-payload combination that overcomes tumor heterogeneity and conventional drug resistance, synergistic pairs of Top 1 inhibitors and a group of DDR inhibitors with different mechanisms were screened and evaluated. A combination of a Top 1 inhibitor Exatecan and a CHK inhibitor Prexasertib showed significant synergy in cancer cell models and ADC-related drug-resistant cell models. Mechanistic study of this combination were further explored with cell cycle analysis in both LS411N and NCI-N87/DS-8201 R cells, revealing that the synergy stemmed from coordinated cell cycle arrest. Critically, the dual-payload combination did not show increased toxicity in a group of healthy human cell lines and limited or additive hematotoxicity in the CD34⁺ HSPC proliferation and differentiation assays, indicating a promising candidate payload pair for dual-payload ADC development.

DDR as potential combination targets for dual-payload ADC development

Dual-payload mechanism of action

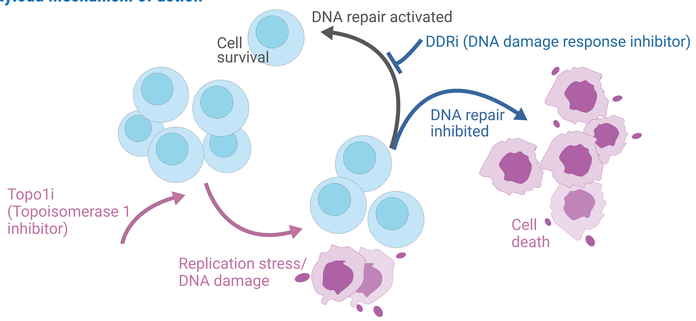
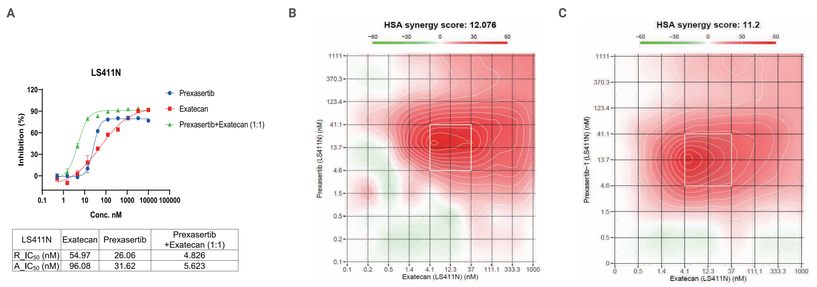


Figure 1. Schematic principle of synthetic lethality. Top1 inhibition traps DNA breaks at replication forks. Concurrent DDR blockade severs the repair escape route, leading to replication catastrophe and selective cell death, particularly in HR-deficient tumors.

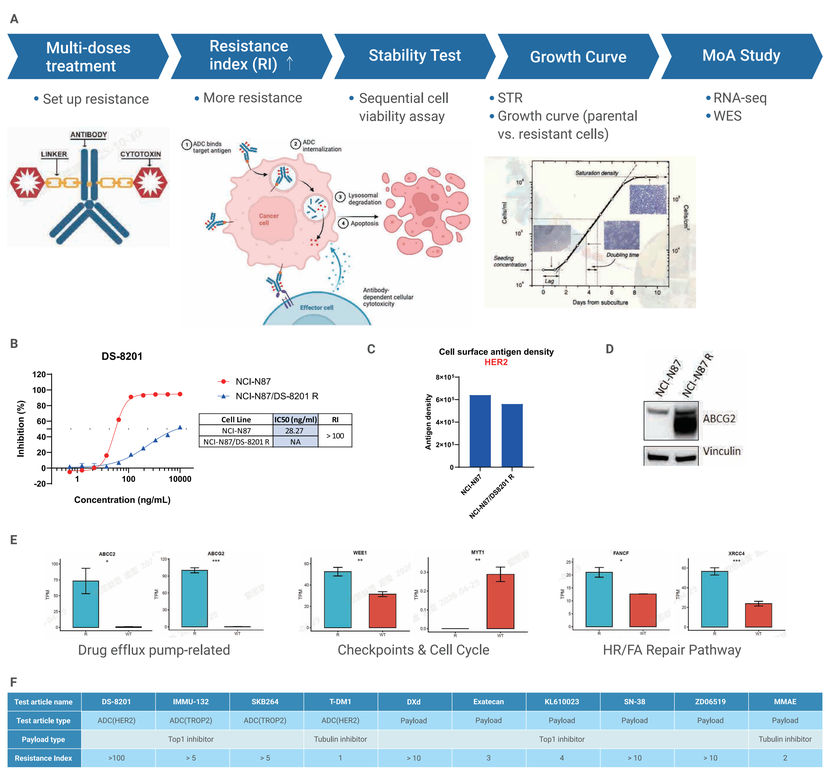
Results

Combination of Prexasertib (a CHK inhibitor) and Exatecan (a Top1 inhibitor) showed in vitro synergistic effects.



LS411N	Exatecan	Prexasertib	Prexasertib +Exatecan (1:1)
R_IC_{50} (nM)	54.97	26.06	4.826
A_IC_{50} (nM)	96.08	31.62	5.623

Figure 2. Combination of Prexasertib and Exatecan showed synergistic effects in LS411N 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 Prexasertib group. B-C. Matrix combinations of dual-payload were tested in 2D (B) and 3D (C) LS411N cell models with 10×10 dose matrices. Grid data revealed pronounced synergy for Prexasertib and Exatecan (HSA synergy scores >10 in both 2D and 3D models), demonstrating synergistic activities.



Test article name	DS-8201	IMMU-132	SKB264	T-DM1	DXd	Exatecan	KL610023	SN-38	ZD06519	MMAE
Test article type	ADC(HER2)	ADC(TROP2)	ADC(TROP2)	ADC(HER2)	Payload	Payload	Payload	Payload	Payload	Payload
Payload type	Top1 inhibitor			Tubulin inhibitor	Top1 inhibitor					Tubulin inhibitor
Resistance Index	>100	> 5	> 5	1	> 10	3	4	> 10	> 10	2

Figure 3. Construction, verification and resistant mechanism exploration of NCI-N87/DS-8201 resistant (R) cell model. A. Schematic workflow for the in vitro construction of drug-induced resistance cell lines. B. Resistance verification with dose-response inhibition curves of DS-8201 in parental and resistant cells. C. Quantitative detection of cell surface HER2 antigen density in parental and resistant cells. D. Western blot analysis of ABCG2 efflux transporter expression in parental and resistant cells. E. Transcriptional expression changes of key resistance-related genes detected by transcriptome sequencing, grouped into drug efflux pumps, cell cycle/checkpoint regulators, and HR/FA DNA damage repair pathway genes. F. Cross-resistance profiling of NCI-N87/DS-821R cells against multiple ADCs and free payloads.

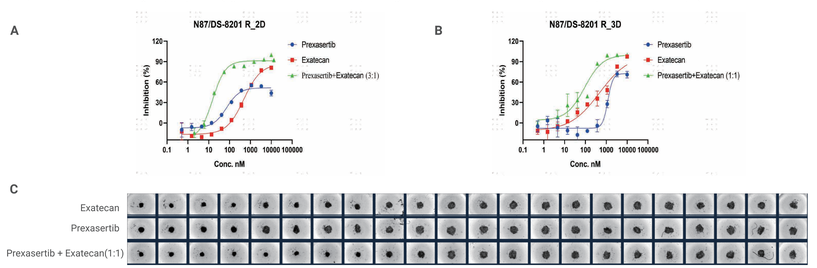


Figure 4. Fixed-ratio combination of Prexasertib and Exatecan displayed synergistic effects in NCI-N87/DS-8201 R cell models. A. The combination group showed 5-fold A_IC_{50} left-shift and ~40% maximum inhibition improvement compared to Prexasertib group in 2D model. B. The combination group displayed slight A_IC_{50} difference and ~30% maximum inhibition improvement compared to Prexasertib group in 3D model. C. Representative images of 3D tumor spheroids treated with different drugs. These data indicated the potential of dual-payload combination to overcome resistance of DS-8201.

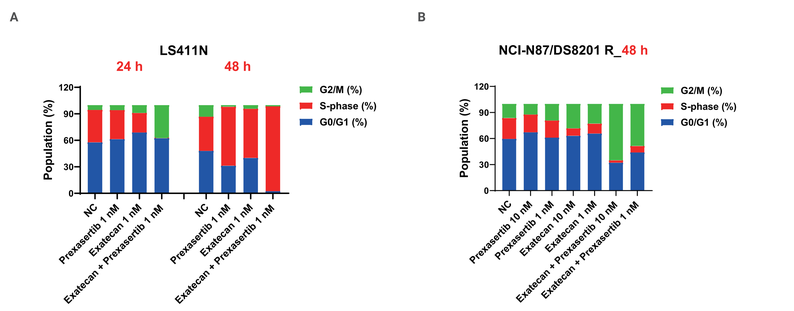


Figure 5. Cell cycle analysis of LS411N and NCI-N87/DS-8201 R cells treated with dual-payload combination showed coordinated cell cycle arrest. A. LS411N cells were treated with 1 nM of individual payload or the combination for 24 h and 48 h. The combination group induced coordinated G2/M phase arrest or significant S-phase accumulation after 24 h or 48 h incubation. B. NCI-N87/DS-8201 R cells were treated with different concentrations of test articles for 48 h. The combination group triggered robust G2/M phase arrests in a dose-dependent manner.

Dual-payload combination exhibited minimal additive toxicity in vitro

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

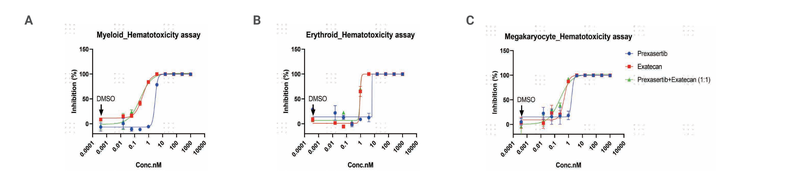


Figure 6. Fixed-ratio combination of dual-payload exhibited minimal additive effects in hematotoxicity prediction assays. CD34⁺ hematopoietic stem and progenitor cells were differentiated into 3 different hematopoietic lineages to predict lineage-specific hematotoxicity in vitro. Drugs were added while 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 Exatecan group in all these 3 assays.

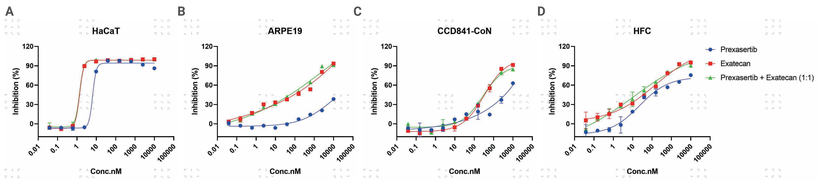


Figure 7. Fixed-ratio combination of dual-payload did not show increased toxicity in representative cell lines of human healthy tissues. Cytotoxicity of dual-payload tested in A. HaCaT (skin; keratinocyte), B. ARPE19 (eye; retinal pigmented epithelium), and C. FHC and CCD841-CoN (colon) showed similar or comparable toxicity with the Exatecan groups.

Summary

This study established a rational strategy for dual-payload screening and demonstrated that simultaneously targeting Top1 and CHK can effectively overcome conventional and acquired resistance with synergistic effects, and minimal additive toxicity in vitro. The strategies and findings from this study can accelerate innovative research on dual-payload ADCs, especially in the proof-of-concept stage for dual-payload combination as a promising strategy to overcome cancer heterogeneity and resistance.