Diverse ADC Novel Payloads, Comprehensive Screening and Evaluation Platform

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Introduction

By combining the potency of payload with the specificity of antibody, antibody-drug conjugate (ADC) is an innovative and promising drug modality for cancer therapy, possessing the advantages of both chemotherapy and immunotherapy. As ADC development continues to advance, more novel types of payloads are being actively explored.

ICE Bioscience employs different screening approaches including biochemical, biophysical, cell-based, and LC/MS-based assays for payload screening and evaluation. To be specific, the methods for cytotoxic drug evaluations include microtubule polymerization assay, DNA topoisomerase I inhibition assay, different biochemical assays targeting DNA-damage response (DDR) pathways, and NMT1/2 FI assay. For degrader payloads, spectral shifts (SPS) or HTRF assays can measure binary/ternary complex formation, while HiBiT system and Western blot (WB) analysis are commonly used to quantify target degradation. For STING agonist/antagonist payload, ICE has ready-to-use assays for detecting STING binding, STING activation and cytokine release. Additionally, cell models—such as panels of various cancer cell lines, payload- or ADC-related drug-resistant cell lines, and 3D spheroids—along with immunogenic cell death (ICD) evaluation and ADME evaluation (particularly lysosome stability and permeability assays), are valuable for the evaluation of all payload types.

Results

Representative Cytotoxic Payload Screening

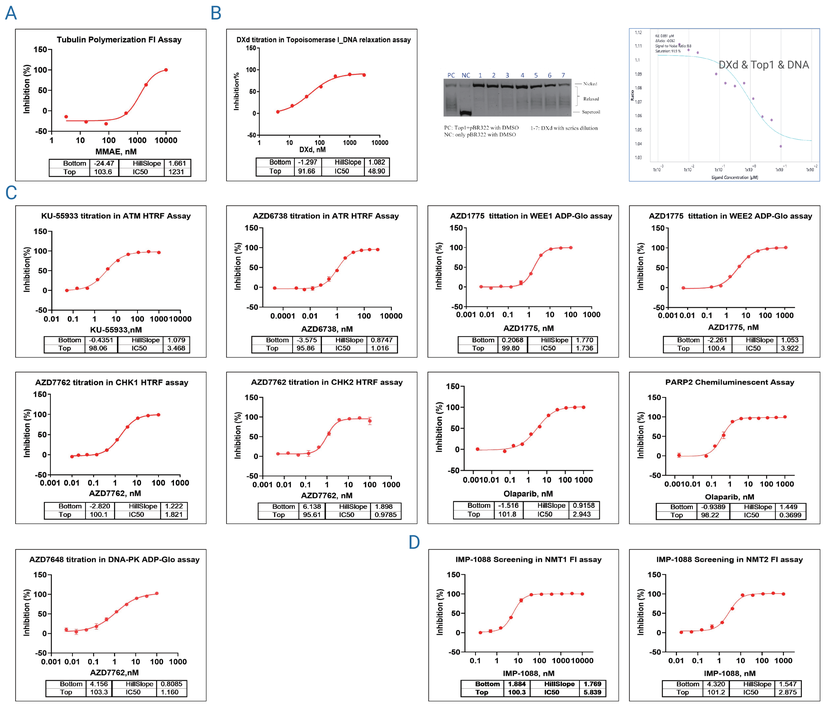
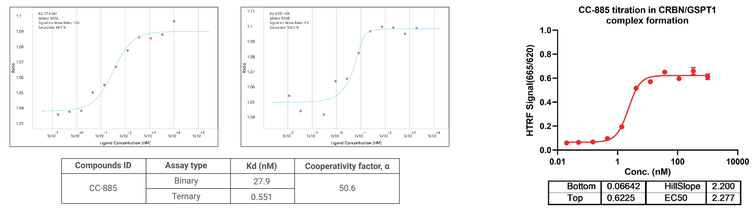


Figure 1. Cytotoxic payload screening assays. A. Inhibitory effect of MMAE on tubulin polymerization, detected by FI assay. B. Inhibitory activity of DXd on Topoisomerase I (Top 1), detected by DNA relaxation assay and spectral shift (SPS) assay. C. Different HTRF, ADP-Glo, and Chemiluminescent assays for DDR inhibitors including DNA-PK, ATM, ATR, WEE1/2, CHK1/2, and PARP1/2. D. Inhibitory effect of NMT inhibitor IMP-1088 on NMT 1/2, detected by FI assay.

Degrader Payload Screening



Compounds ID	Assay type	Kd (nM)	Cooperativity factor, α
CC-885	Binary	27.9	50.6
	Ternary	0.551	

Figure 2. Binary and ternary complex formation assays. A. CRBN-ligand binary and CRBN-ligand-GSPT1 ternary complex formation was detected by SPS assay for GSPT1 degrader CC-885. B. The formation of CRBN-degrader-GSPT1 ternary complex was detected by HTRF assay.

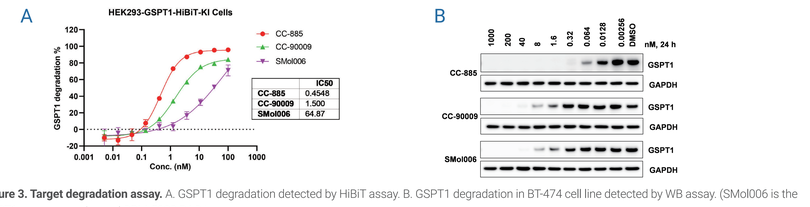


Figure 3. Target degradation assay. A. GSPT1 degradation detected by HiBiT assay. B. GSPT1 degradation in BT-474 cell line detected by WB assay. (SMol006 is the GSPT1 degrader payload of ORM-5029 DAC.)

STING Agonist/Antagonist Payload Screening

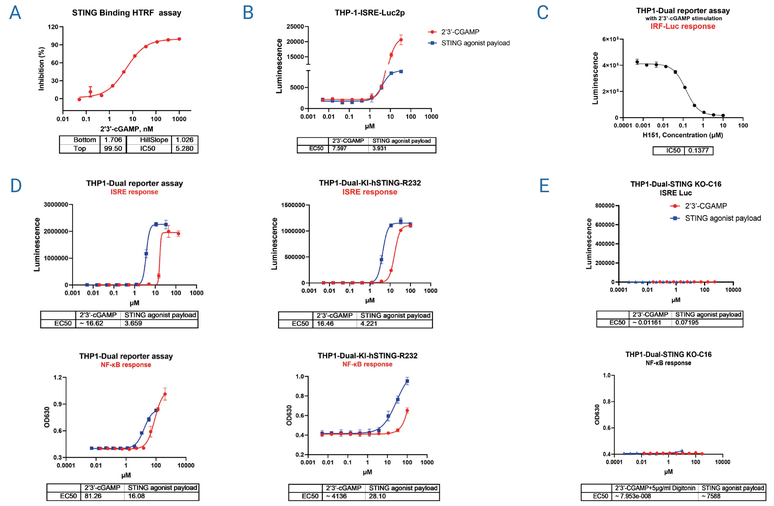


Figure 4. Binding activity and potency evaluation of STING agonist or antagonist. A. Biochemical binding assay. B. THP1-ISRE reporter assay. C. THP1-Dual reporter assay for STING antagonist evaluation with 2'3'-cGAMP stimulation. D. THP1-Dual reporter assay of different STING variants for STING agonist evaluation. E. THP1-Dual STING-KO reporter assay to evaluate non-specific activities. Assays for STING agonist can also be used for antagonist screening.

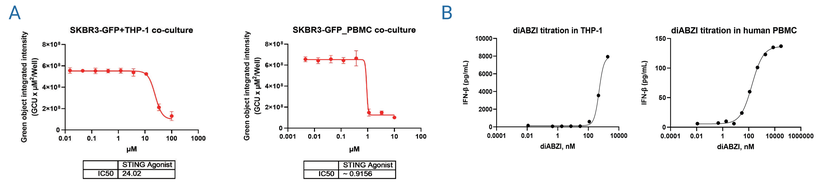
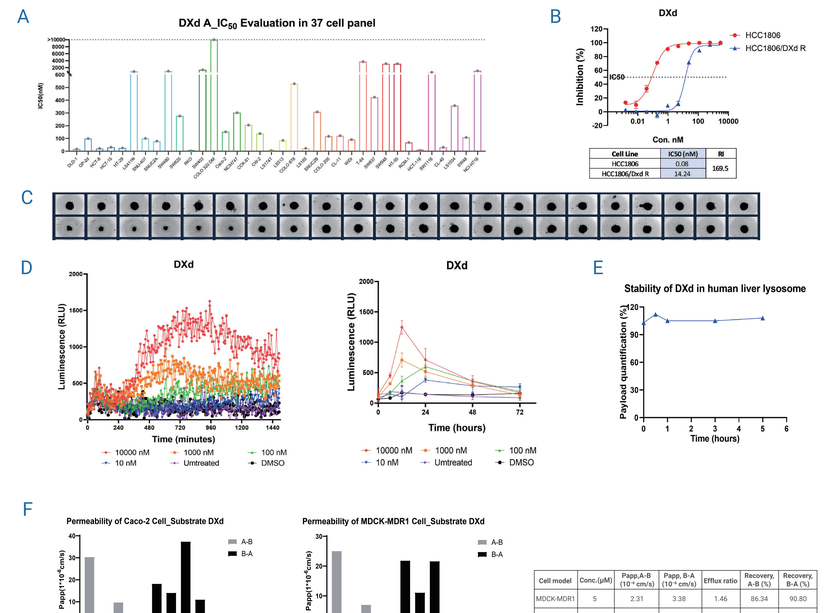


Figure 5. Co-culture and cytokine release analysis. A. Cell killing activity of STING-agonist payload in SKBR3-GFP+THP1 and SKBR3-GFP+human PBMC co-culture systems. B. Cytokine release analysis. IFN-β in different culture supernatants (THP-1 and human PBMC) induced by STING agonist diABZI were detected by ELISA.

Common Assays for Screening of Different Payload Types



Cell model	Conc.(μM)	Papp,A-B (10⁻⁶ cm/s)	Papp, B-A (10⁻⁶ cm/s)	Efflux ratio	Recovery, A-B (%)	Recovery, B-A (%)
MDCK-MDR1	5	2.31	3.38	1.46	86.34	90.80
Caco-2	5	2.27	10.98	4.84	87.27	97.71

Figure 6. Common assays for assessing all different types of payloads. A. Cytotoxicity evaluation of payload (DXd) in CRC cell panel. B. Resistance index (RI) verification of DXd-resistant HCC1806 cell line. C. 3D cytotoxicity evaluation. D. Real-time extracellular ATP detection assay and the parallel detection for different time-points to evaluate payload's immunogenic cell death (ICD) effect. E. Lysosome stability assay for payload. F. Payload permeability evaluated with different cell models.

Summary

With 16 years of experience spanning from target validation to preclinical candidate identification, our well-established integrated ADC platform provides comprehensive support for early-stage drug discovery for payload, antibody and ADC. It enables efficient payload screening and evaluation for both traditional ADCs and emerging novel ADC/XDC formats with novel payloads—including cytotoxic agents, novel degraders, and immune-related payloads, etc.—thereby significantly accelerating early-stage ADC research.