Degrader-Antibody Conjugate Characterization and Evaluation Platform – From In Vitro Assays to In Vivo Studies

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Abstract Number:

Introduction

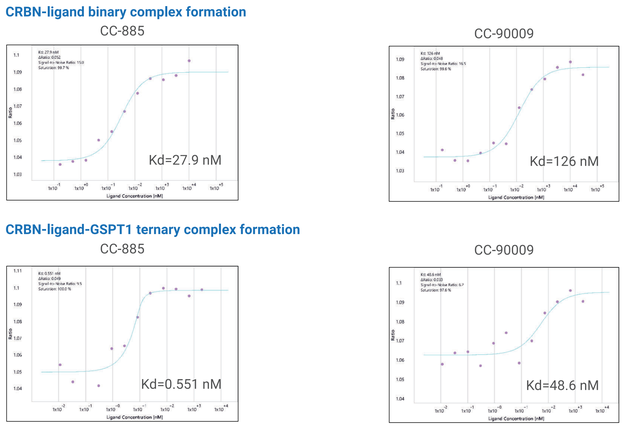
Degrader-antibody conjugates (DACs) typically consist of a tumor-targeting antibody and a conjugated degrader payload, linked via a chemical linker. By integrating the degrader payload's capacity to precisely degrade disease-causing proteins with the antibody's tumor-targeting specificity, DACs emerge as an innovative and promising therapeutic modality for cancer treatment. Herein, we present the well-established DAC integrated platform developed by ICE Bioscience, which facilitates the characterization and evaluation of DACs from in vitro assays to in vivo studies.

Methods

- **Degrader Payload Screening**
  Binary and ternary complex formation: Spectral shift, HTRF
  Target and off-target degradation: HiBiT, Flow cytometry, WB
- **Degrader-Antibody Conjugate Evaluation**
  In vitro cytotoxic effect on tumor cells: CellTiter-Glo
  Pharmacokinetics and Drug-Antibody Ratio analysis: ELISA, LC-HRMS
  In vivo anti-tumor efficacy: CDX model

Degrader: Binary and ternary complex formation

A



Compounds ID	Assay type	Kd (nM)	Cooperativity factor, α
CC-885	Binary	27.9	50.6
	Ternary	0.551	
CC-90009	Binary	126	2.59
	Ternary	48.6	

B

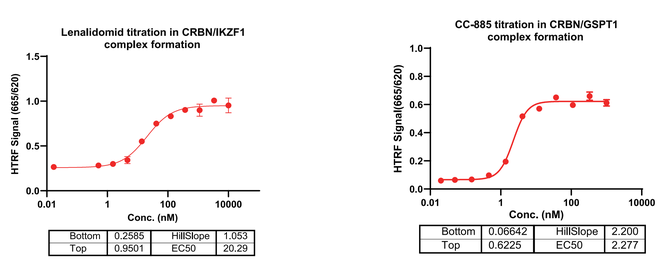


Figure 1. A, Binary and ternary complex formation was detected by the spectral shift assay. Two GSPT1 degraders exhibited cooperativity in ternary complex formation, as indicated by a cooperativity factor α > 1. B, An ICE-established HTRF assay was used to detect the formation of CRBN-degrader-IKZF1 and CRBN-degrader-GSPT1 ternary complex.

Degrader: Target and off-target degradation

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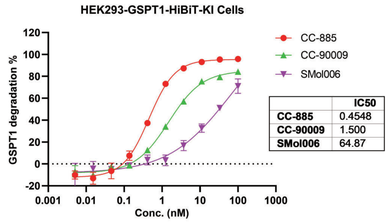

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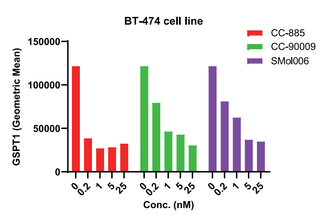

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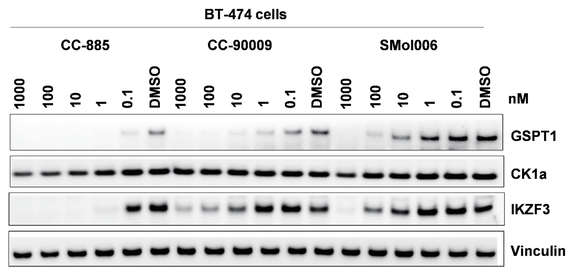
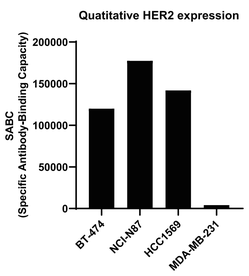


Figure 2. A-B, GSPT1 degradation was detected using the HEK293-GSPT1-HiBiT-KI cell line (A) and the flow cytometry assay in BT-474 (B). C, GSPT1 and off-targets degradation in BT474 cells was detected by WB assay. SMol006 induced weaker GSPT1 degradation and fewer off-target degradations when compared to CC-885 and CC-90009. The IKZF1 protein level is extremely low in BT474 cells for detection via WB. (SMol006 is the GSPT1 degrader payload of the Degrader-Antibody Conjugate ORM-5029.)

Degrader: Binary and ternary complex formation

A



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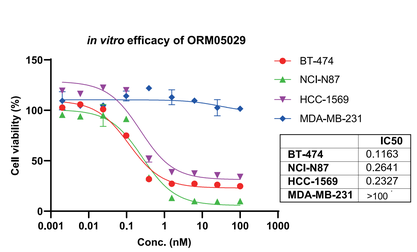

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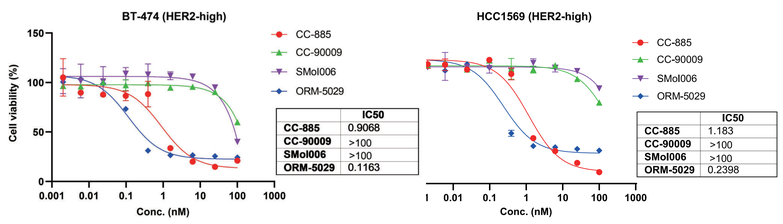

Figure 3. A, Quantitative analysis HER2 expression levels in different cell lines via flow cytometry. B, In vitro efficacy evaluation of ORM-5029 across cell lines with different HER2 expression levels using CellTiter-Glo. C-D, Comparison of the cytotoxic effects between GSPT1 degraders and ORM-5029 in HER2-high BT-474 and HCC1569 cell lines. The cytotoxicity of ORM-5029 is highly correlated with the HER2 expression level on cells, and ORM-5029 exhibited a superior cytotoxic effect compared to GSPT1 degraders.

DAC: Pharmacokinetics and Drug-Antibody Ratio analysis

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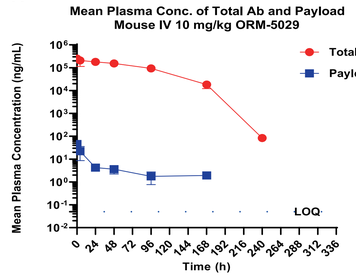

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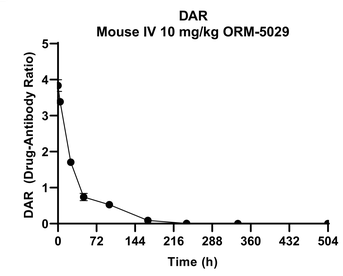

Figure 4. A, After a single administration of ORM-5029 at 10 mg/kg, pharmacokinetic analysis of the total antibody and payload was performed via ELISA and LC-MS/MS, respectively. B, DAR detection of ORM-5029 was conducted via IC-LC-HRMS (immunocapture-liquid chromatography-high-resolution mass spectrometry). Each assay used N=3 mice. ORM-5029 had a half-life of approximately 34 hours, with payload release showing a clearance of 332,926 mL/day/kg. The DAR value changes were well correlated with the ORM-5029 pharmacokinetic profile.

DAC: In vivo anti-tumor efficacy

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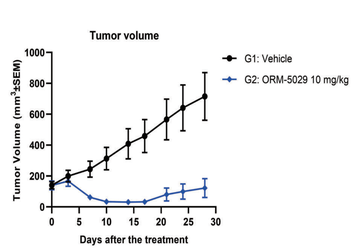

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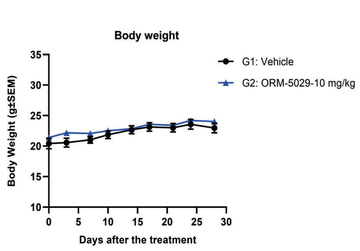

Figure 5. A-B, The in vivo anti-tumor efficacy of ORM-5029 was evaluated in HER2-high breast cancer HCC1569 cell-derived xenograft after a single 10 mg/kg intravenous administration. Each group used N=3 mice. ORM-5029 showed in vivo efficacy without affecting body weight.

Summary

With 15 years of experience in early drug discovery—from target validation to preclinical candidate identification—ICE offers screening and evaluation services to support projects in verifying the efficacy and mechanism of action of degrader payloads, antibodies and DACs. This integrated platform significantly accelerates the timeline of DAC research and development.

References

- Tsuchikama, K. et al. Exploring the next generation of antibody-drug conjugates. Nat Rev Clin Oncol. 21(3):203-223 (2024).
- Saini, S. et al. Assessment in Breast Cancer Patients from Phase I Clinical Trial Of ORM-5029, a Potent GSPT1 Degrader. AACR 2023. Abstract #2118.