A Biological and DMPK Integrated Platform for ADC Screening and Evaluation

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Abstract

Antibody-drug conjugates (ADCs) are an innovative promising class of cancer therapeutics. ADCs are comprised three key components of an antibody (monoclonal antibody or bispecific antibody), a cytotoxic payload or other novel types of payload, and a linker. ADCs integrate chemotherapy and immunotherapy by combining the potency of payloads with the specificity of antibodies[1].

ICE Bioscience has established a biological and DMPK integrated platform for ADC screening and evaluation. The platform is dedicated to support a comprehensive service portfolio with various aspects of ADC development projects.

Methods

1.Cytotoxic payload screening

- DNA Damage Repair inhibitor screening platform
- Topoisomerase I–mediated DNA relaxation assay

2.New MOA payload screening

- Targeted protein degradation assays
- STING agonist screening assays

3.Antibody screening

- Target expression quantification
- Affinity: SPR
- Cell binding affinity
- Cell internalization

4.ADC screening and evaluation

- Cytotoxicity
- Bystander effect
- Drug resistant cell line and CDX model
- DMPK

Case study: Cytotoxic payload screening DNA Damage Repair inhibitor screening platform

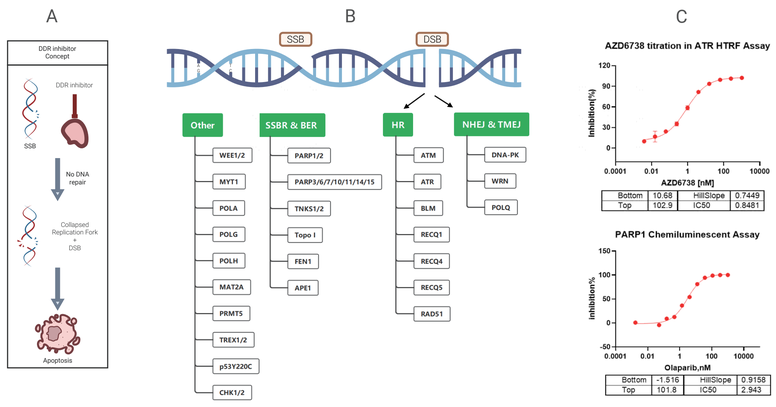
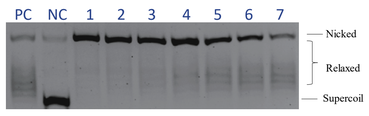


Figure 1. DNA Damage Repair (DDR) inhibitor screening platform in ICE. A. DDR inhibitor concept. B. Targets involved in ICE' s DDR inhibitor screening platform. C. ATR inhibitor and PARP1 inhibitor screening assays.

Case study: Cytotoxic payload screening Topoisomerase I– mediated DNA relaxation assay



PC: Top1+pBR322 with DMSO
NC: only pBR322 with DMSO
1-7: DXd with series dilution

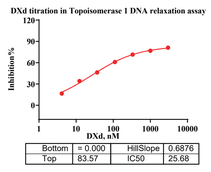


Figure 2. Inhibitory activity of DXd on Topoisomerase I (Top1). DXd prevented recombinant hTop I from converting supercoiled DNA to relaxed closed circular DNA.

Case study: New MOA payload screening Targeted protein degradation assays

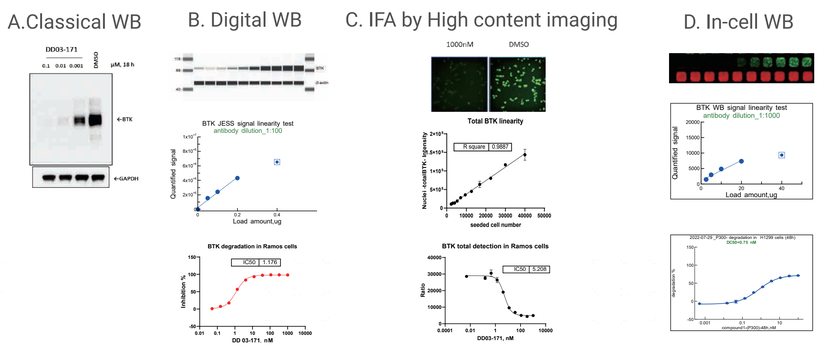
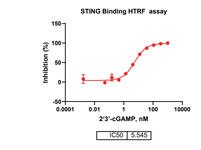


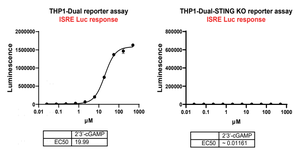
Figure 3. Targeted protein degradation assays for protein-degrader payload screening.
A. Classical WB. B. Digital WB. C. IFA by High content imaging. D. In-cell WB assays.

Case study: New MOA payload screening STING agonist screening assays

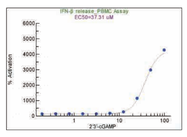
A.SITNG binding assay



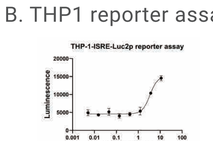
C. THP1-Dual reporter assay

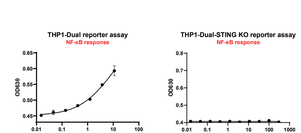


D. Human PBMC cytokine release assay



B. THP1 reporter assay





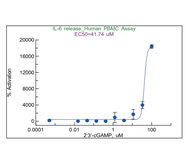
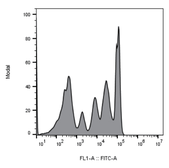


Figure 4. STING agonist payload screening assays. A. SITNG HTRF binding assay. B. THP1 reporter assay. C. THP1-Dual reporter assay. D. Human PBMC cytokine release assay.

Case study: Antibody screening

Quantitative analysis of antigen membrane expression

A. Histogram of Calibration Bead populations



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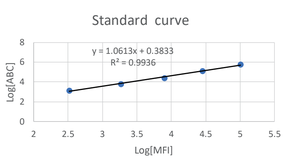

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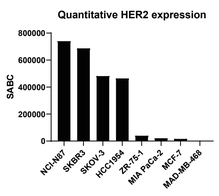
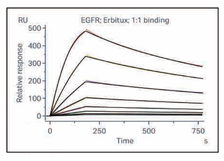


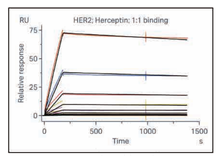
Notes:Specific Antibody-Binding Capacity (SABC)
SABC = ABC – BAE
Antibody-Binding Capacity (ABC) Background Antibody Equivalent (BAE)

Figure 5. Quantitative analysis of HER2 antigen membrane expression in different cancer cell lines with high/medium/low/negative HER2 expression. A. Histogram of Calibration Bead populations. B. Standard curve. C. Quantitative analysis of HER2 membrane expression.

Affinity - SPR



Erbitux - EGFR



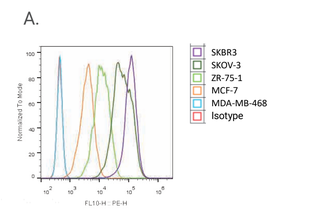
Herceptin – HER2

Sample	ka (M-1 s-1)	kd (s-1)	KD (M)
Erbitux	9.87E+05	1.47E-03	1.49E-09
Herceptin	2.04E+04	7.28E-05	3.56E-09

Figure 6. SPR analysis of Trastuzumab (Herceptin) binding affinity to HER2-ECD antigen and Cetuximab (Erbitux) binding affinity to EGFR-ECD antigen using Biacore 8K.

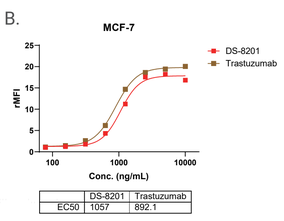
Cell binding

A.



Trastuzumab

B.

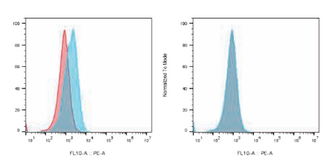


	DS-8201	Trastuzumab
EC50	1057	892.1

Figure 7. Cell binding assays. A. Cell binding activity of Trastuzumab in different cancer cell lines with different HER2 expression levels. B. Cellular affinity comparison of DS-8201 and the corresponding antibody Trastuzumab on MCF-7 cell line.

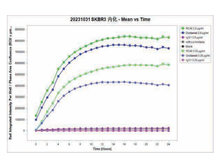
Cell internalization

A.



Antibody Isotype

B.



C.

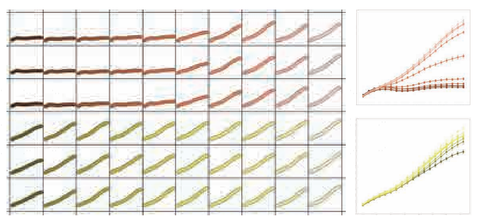
Lysosome Antibody Hoechst Merge

Figure 8. Antibody internalization evaluation. A. Antibody internalization assessed by flow cytometry B. Kinetic monitoring of antibody internalization with Incucyte. C. Internalized antibody co-localization with lysosome, detected by High content imaging system.

Case study: ADC screening and evaluation

Cytotoxicity

A. Cell growing curves



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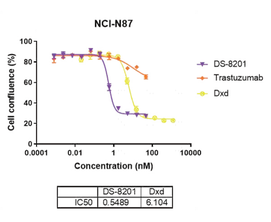
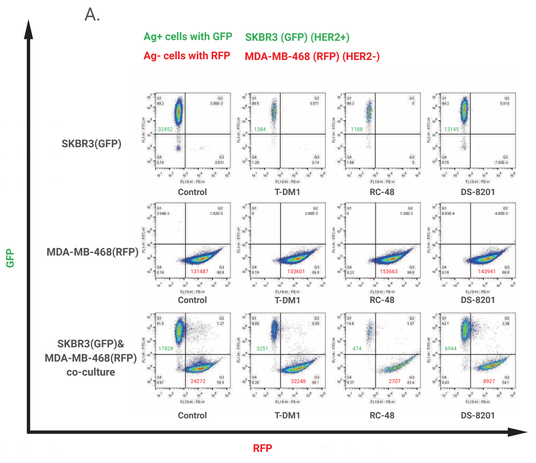


	DS-8201	Dxd
IC50	0.5489	6.104

Figure 9. Cell killing activity of DS-8201 and DXd in NCI-N87 cell line. A. Cell growing curves monitored by Incucyte. B. IC50 shifted ~10 times lower after conjugation to antibody.

In vitro bystander effect detection assays

A.



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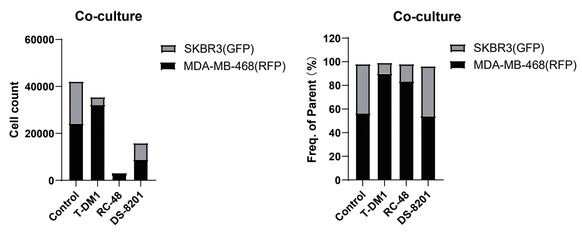
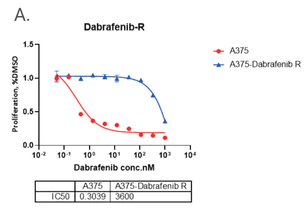


Figure 10. *In vitro* bystander effect assay of DS-8201 and RC-48 in engineered breast caner cell lines detected by flow cytometry. T-DM1 is negative control ADC for bystander effect assay. A. Flow cytometry results of SKBR3(GFP)&MDA-MB-468(RFP) co-culture after ADC treatment. B. GFP and RFP cell count and cell count ratio in the co-culture system.

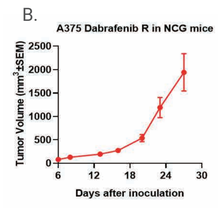
Case study: Drug resistant cell line and CDX model

A.



	A375	A375-Dabrafenib R
IC50	0.3039	3600

B.



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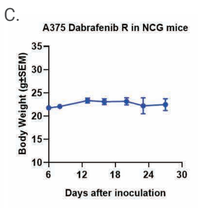
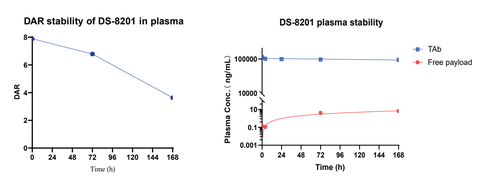


Figure 11. Development of drug resistance melanoma CDX model with A375 Dabrafenib-R cell line. A. Generation of A375-Dabrafenib R cell line. B and C. CDX tumor growth (B) and body weight (C) curves after inoculation.

Case study: DS-8201 Plasma Stability



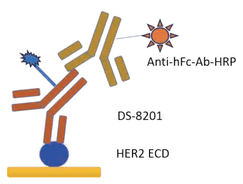


Figure 12. *In vitro* stability of DS-8201 in plasma. A. DAR stability of DS-8201 in plasma. B. Concentration of free payload and total Antibody (TAb) in DS-8201 treated plasma. DS-8201 was incubated in plasma at 37 ℃ with different time points.

Conclusions

With 14 years of experience in providing early drug discovery services from target validation to pre-clinical candidate identification, ICE Bioscience has established a biological and DMPK integrated platform for ADC screening and evaluation. The platform is dedicated to support a comprehensive service portfolio with various aspects of ADC development projects, including antibody screening (target validation, affinity, binding, internalization), new payload (Topoisomerase I inhibitors, tubulin binders, etc.) or linker-payload screening (ADC characterization, bystander effect, etc.), efficacy and safety evaluation of the ADCs (gene editing and drug resistant cell line generation, cytotoxic effect, MOA, DMPK, etc.), in the process of ADC screening or PCC evaluation.

Acknowledgements

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References

[1] Tsuchikama K, Anami Y, Ha SYY, Yamazaki CM. Exploring the next generation of antibody-drug conjugates. Nat Rev Clin Oncol. 2024 Mar;21(3):203-223.