#1678 Integrated ADC Platform: from Discovery to Functional Evaluation

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Introduction

Antibody-Drug Conjugates (ADCs) represent a rapidly advancing modality with elegantly simple and compelling mechanisms. They have proven successful not only in combating cancer but have also recently expanded into other therapeutic areas such as autoimmune diseases. Despite these advancements, challenges such as new payload exploration, antibody penetration, increasing ADC uptake and processing, and overcoming ADC resistance continue to persist.

Our comprehensive antibody discovery platform excels in generating high-affinity antibodies against challenging antigens, particularly multi-pass transmembrane proteins. By combining this with advanced high-throughput screening capabilities, we efficiently identify potent antibody candidates, thereby accelerating the antibody discovery process.

To further expedite ADC discovery with high efficiency, we have developed a state-of-the-art high-throughput conjugation platform integrated with various downstream biological assays. Utilizing parallel synthesis and analytics, we can generate and evaluate ADCs with diverse linkers and payloads at microgram to milligram scales within 2-3 weeks. This facilitates the simultaneous construction of hundreds of conjugate variants per run, allowing for rapid cytotoxicity and stability assays to triage and prioritize candidates for further development.

In addition to high-throughput antibody screening and conjugation, our well-established full-spectrum ADC platform provides comprehensive services from payload assessment and ADC *in vitro* efficacy evaluation to supporting in-depth mechanistic exploration.

Key words: Antibody-Drug Conjugates (ADCs), Cancer, Autoimmune diseases, High-throughput screening, Payload exploration, ADC resistance

Comprehensive Antibody Discovery Platform

	Phage Display	Hybridoma	Single B-cell sorting
Primary Screening Throughput (Candidate Antibodies)	10^9–10^11	10^3-10^4	10^4–10^6
Types	VHH, scFv, Fab	IgG, Fab, scFv natural sequences	
Antigen type	Peptide, protein, VLP, payload, cells	Peptide, protein, payload, oligonucleotides	
Species	Human/camel/…	Mouse	Mouse/Rat/Rabbit
Timeline	3-4 weeks *	13-16 weeks *	5-6 weeks immunization + 1-2 days *
Features	• Large library size, increasing the probability of identifying hits • Off-the-shelf ready to go • Well suited for high-throughput • Cost-effective • Well-established platform and workflow • Versatile panning strategy	• Higher success rate > 95% • Natural antibody sequence • Antibody with high affinity • Cost-effective • Well-established platform and workflow	• Natural antibody sequence, better for druggability • More effective for identifying natural conformations or epitopes of membrane proteins • Naturally high affinity, no additional maturation required • High screening efficiency

* Additional 1 month for antibody expression and affinity validation

State-of-the-art High-throughput Conjugation Platform

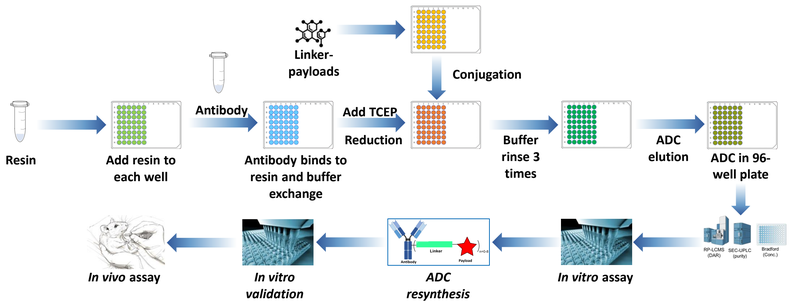


Figure 1: ADC D2B work flow in WuXi Biology, WuXi AppTec

Time Saving [2-3 weeks/cycle]: High-throughput ADC conjugation in 96-well plate
Cost Saving: Nanomole-scale synthesis, reagent saving, rapid purification
Various linker/payload stock: 40+ linker and 70+ payloads next to synthetic building blocks

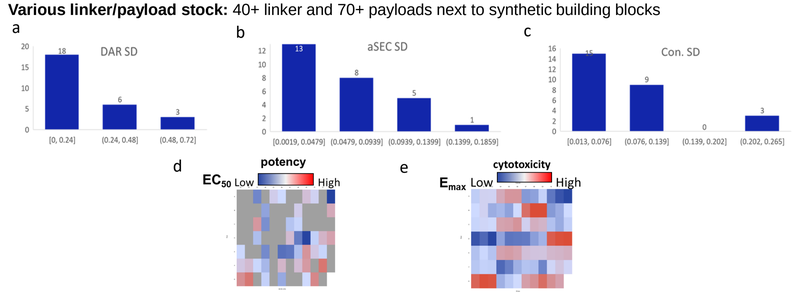


Figure 2: ADC D2B testing results in reaction plate heatmap view

- Most antibody recover rates between 30%-70%, SD < 0.1 mg/mL;
- 80% of ADCs shows aSEC purity >50%, SD < 10%; DAR SD = 0.29 for DAR8.
- The most potent ADC exhibits an EC50 of 6.79 nM with an Emax of 67.0%.

Conventional Conjugation Platform

Antibody engineering & expression

Linker/payload synthesis

Peptide Linker
Disulfide Linker
Thioether Linker
Glucuronide Linker
Phosphoramidate Linker
PEG Linker

Conjugation strategy

Lysine-based conjugation
Cysteine-based conjugation
Site-specific conjugation by mutagenesis
Site-specific conjugation by enzyme

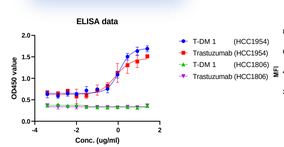
ADC characterization

ADC-specific quality attributes
Physiochemical characterization
In vitro and *in vivo* validation

Full-spectrum *In Vitro* ADC Evaluation Platform

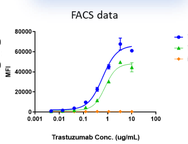
ADC binding affinity

Cell-based ELISA



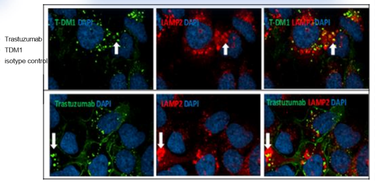
	T-DM 1 (HCC1954)	Trastuzumab (HCC1954)
EC50	1.137	0.7404

Cell-based FACS



	Trastuzumab	TDM1	isotype control
EC50	0.6135	0.7851	~ 0.01470

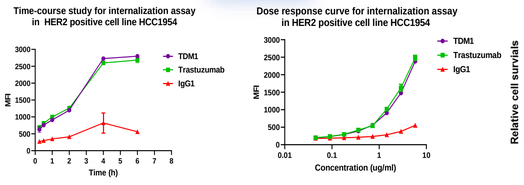
ADC internalization trafficking



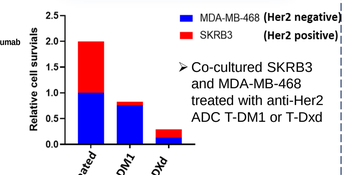
Validation of Lysosomal trafficking
- **LAMP2**: Lysosomal markers

ADC Internalization Evaluation

FACS



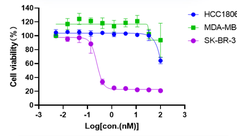
Bystander Effect via FACS



➢ Co-cultured SKRB3 and MDA-MB-468 treated with anti-Her2 ADC T-DM1 or T-Dxd

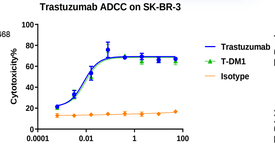
In vitro Efficacy Evaluation

ADC Cell Viability

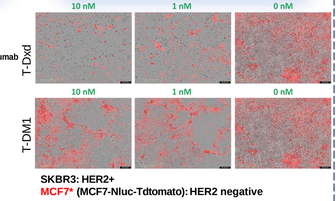


	HCC1806	MDA-MB-468	SK-BR-3
IC50 (nM)	>100	>100	0.244

ADC evaluation by ADCC



Bystander Effect via IncuCyte



SKBR3: HER2+
MCF7* (MCF7-Nluc-Tdtomato): HER2 negative

Figure 3: ADC drug evaluation toolbox enabling systematic analysis of key biological processes of ADCs covering target binding, efficacy to MOA test.

Summary

Our comprehensive ADC platform is designed to support a broad spectrum of activities, spanning from early-stage drug discovery to overcoming emerging challenges in ADC therapy. By harnessing cutting-edge approaches in antibody discovery, linker-payload conjugation, and *in vitro* ADC evaluation studies, we are dedicated to advancing the development of innovative ADC therapeutics that offer enhanced efficacy and safety.

References

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