Evaluation of The Efficacy of ADC Drugs *In Vitro* & *Vivo*

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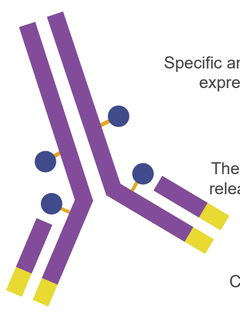
Abstract

Antibody-drug conjugates (ADCs) are an important class of therapeutics for the treatment of cancer. ADCs are potent cytotoxic agents by linking cytotoxic small molecules to monoclonal antibodies (mAbs) that directly recognize a specific antigen on tumor cell surface. Compared with the therapeutic mAbs, ADCs-derived monoclonal antibody is conjugated with cytotoxic agents which can deliver potent cellular toxins to targeted cancer cells specifically.

We started ADC non-clinical research in 2014. As of the end of 2022, We has successfully assisted in the clinical approval of **13** ADC drugs by NMPA and/or FDA and has more than 10 ADC projects under development. Up to now, Medicilon has undertaken more than 100 major IND application biopharmaceutical projects, including monoclonal antibodies, double antibodies, polyclonal antibodies, ADCs, viral vaccines and fusion proteins.

Background

Over the past couple of decades, ADCs have revolutionized the field of cancer chemotherapy. Unlike conventional treatments that damage healthy tissues upon dose escalation, ADCs utilize monoclonal antibodies (mAbs) to specifically bind tumor-associated target antigens and deliver a highly potent cytotoxic agent. The synergistic combination of mAbs conjugated to small-molecule chemotherapeutics, via a stable linker, has given rise to an extremely efficacious class of anti-cancer drugs with an already large and rapidly growing clinical pipeline.



A-Antibody
Specific antibodies against tumor antigens, expression is restricted in normal cells

D-Drug (Cytotoxic agent)
The highly potent antitumor payload released after entering the target cell

C-Conjugate (Linker)
Conjugate payloads to antibodies

Method

Target validation and binding affinity measurements

- Flow cytometry analysis
- Surface plasmon resonance (SPR)
- Enzyme-linked immunosorbent assay (ELISA)
- Homogeneous time-resolved fluorescence assay (HTRF)

***In vitro* functional study**

- ADC internalization assay
- ADC cytotoxicity analysis (cell viability, cell-cycle, apoptosis, and bystander effect)
- ADC Fc cytotoxicity (ADCC and CDC)

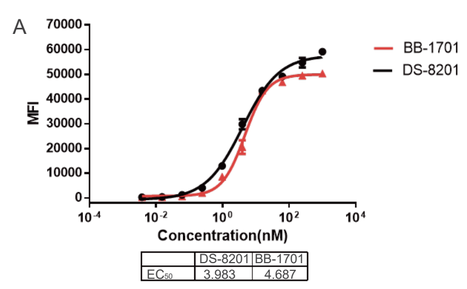
***In vivo* antitumor study**

- The grown tumors
- Dynamic growth of tumors
- The body weights of mice

Results

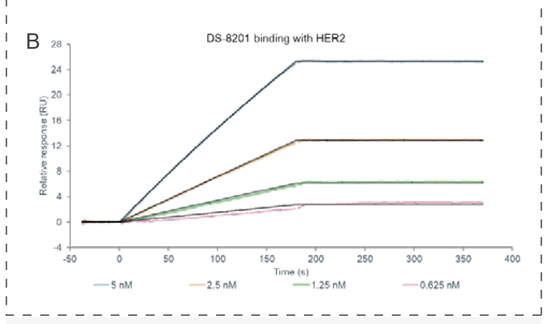
Case: ADC Binding Assay

Flow cytometry analysis of ADC binding ability to antigen-expressing cells (Figure A: HER-2 ADC DS-8202, BB-1701 binding ability test with BT-474 cells).
SPR analysis of ADC binding ability to antigenic proteins (Figure B: DS-8201 binding test to HER2 protein).



	DS-8201	BB-1701
EC_{50}	3.983	4.687

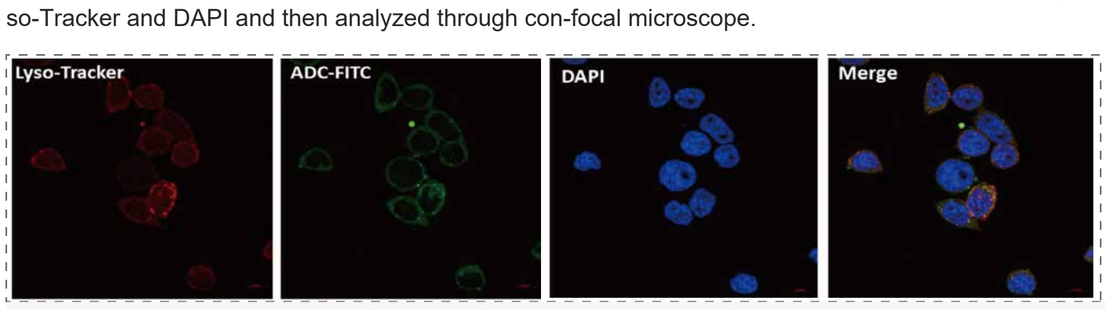
HER2 ADCs (BB-1701 & DS-8201) were incubated with N87 cells and then analyzed through FACS, MFI of PE on secondary antibodies against ADCs were calculated.



HER2 protein was immobilized on M5 chip, DS-8201 was serial diluted and injected into the chip, binding affinities of HER2 and DS-8201 was analyzed through Biacore 8K.

Case: ADC Internalization: Confocal Imaging

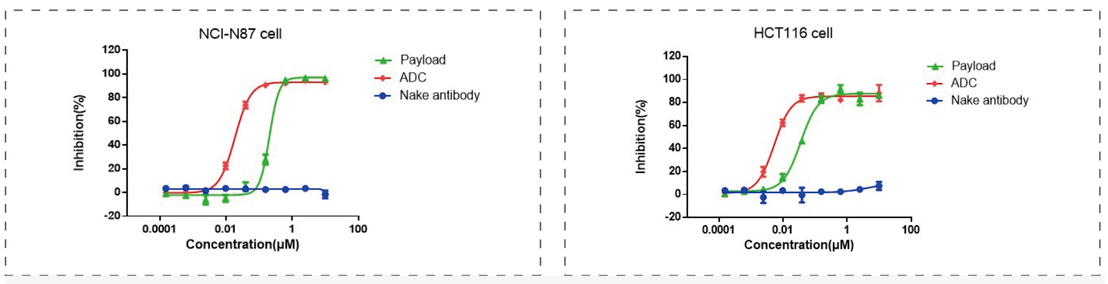
OVCAR-3 cells were incubated with FITC-labeled ADC for 24 hours, the cells were incubated with Lyso-Tracker and DAPI and then analyzed through con-focal microscope.



In vitro FITC-labeled ADC internalization assay in OVCAR-3 cells

Case: Cytotoxicity of Payloads or ADC

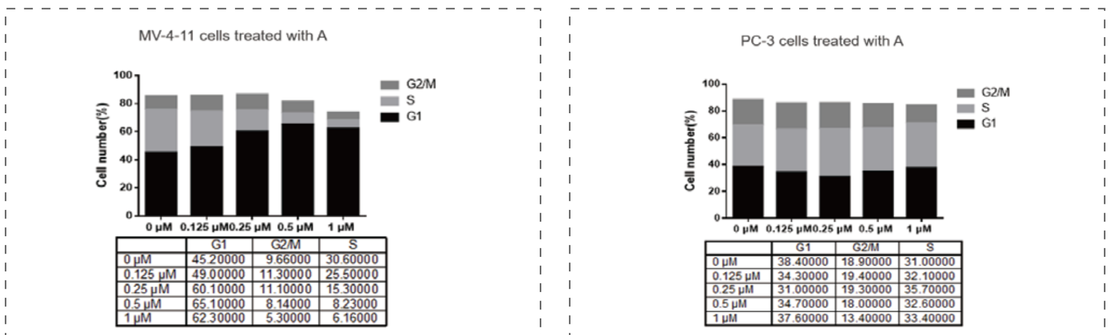
ADC and payload were incubated with target cells, cell viability were analyzed through CCK-8, CTG and MTT. (Figure A. B: Toxicity of Dxd to NCI-N87 and HCT116 cells were analyzed through CCK-8 assay kit). Dxd displays the anti-proliferation effect on NCI-N87 and HCT116 *in vitro*.



In vitro cell viability in NCI-N87 and HCT116 cells

Case: Cell Cycle Analysis

MV-4-11 cells (human myelomonocytic leukemia cells) and PC-3 cells (human prostate cancer cells) were treated with Compound A and stained with PI for FACS-based cell cycle analysis. The data showed that Compound A significantly blocked the cell cycle of MV-4-11 cells, but had no significant effect on PC-3 cells. This indicates that Compound A is indication-specific.



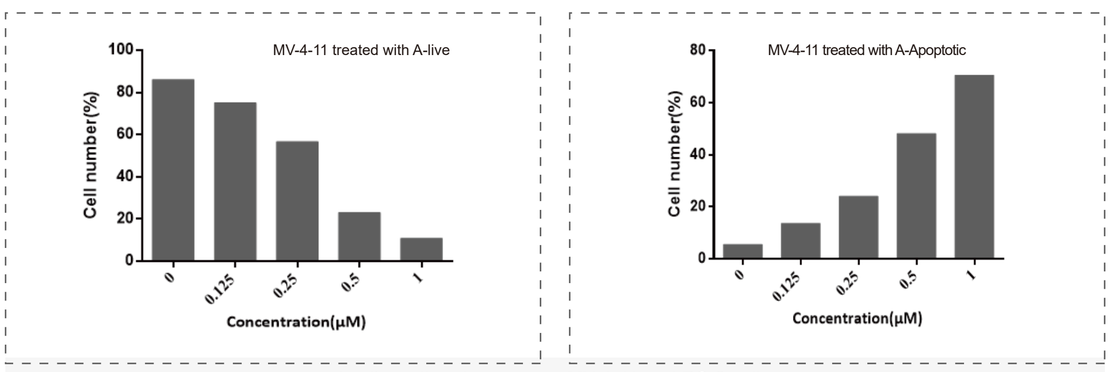
	G1	G2/M	S
0 μM	45.20000	9.66000	30.60000
0.125 μM	49.00000	11.30000	25.50000
0.25 μM	60.10000	11.10000	15.30000
0.5 μM	65.10000	8.14000	8.23000
1 μM	62.30000	5.30000	6.16000

	G1	G2/M	S
0 μM	38.40000	18.90000	31.00000
0.125 μM	34.30000	19.40000	32.10000
0.25 μM	31.00000	19.30000	35.70000
0.5 μM	34.70000	18.00000	32.60000
1 μM	37.60000	13.40000	33.40000

In vitro cell cycle assay on MV-4-11 and PC-3 cells

Case: Apoptosis Analysis

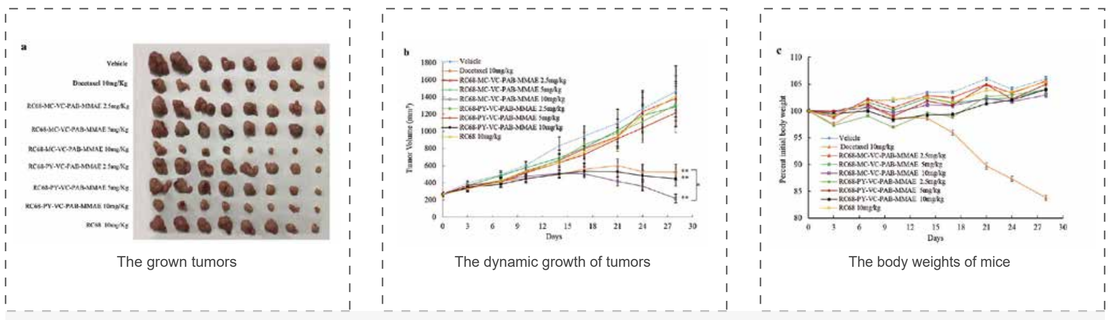
MV-4-11 cells were treated with Compound A and stained with PI/Annexin V for FACS-analysis. The data shown that Compound A promotes the apoptosis of MV-4-11 cells.



In vitro cell cycle assay on MV-4-11 and PC-3 cells

Case: *In vivo* antitumor efficacy of ADC

A humanized anti-EGFR monoclonal antibody (named RC68) was purifed and conjugated with MMAE using a MC-VC-PAB or PY-VC-PAB linker. BALB/c nude mice were implanted subcutaneously with H125 cells and when the solid tumor reached 100-300 mm^3, the mice were randomized and treated intravenously with indicated drug weekly. The effect of each treatment on the growth of tumors was measured for tumor volumes and their body weights were measured twice per week. Treatment with 10 mg RC68-MC-VC-PAB-MMAE or RC68-PY-VC-PAB-MMAE significantly inhibited the growth of H125 tumors in mice, but did not affect their body.



The grown tumors | The dynamic growth of tumors | The body weights of mice

In vivo efficacy study of H125 NSCLC models

Summary

- We have successfully established a series of *in vitro* assays and *in vivo* models to study the function and mechanism of action of ADC.
- We have established the state-of-the-art platform to support the evaluation of the efficacy of ADC or the combination strategy of ADC and other anti-cancer therapy in the process of pre-clinical drug discovery, such as target validation, efficient internalization, cytotoxic effects and *in vivo* efficacy testing.
- One important pharmacological parameter of an ADC is the *in vivo* efficacy that directly reflects its potency and influences clinical trial designs. In terms of *in vivo* ADCs assessment, our abundant model resource facilitates the process of *in vivo* ADCs evaluation, Our animal models are all established and maintained under the regulation of AAALAC. Pharmacology studies are conducted according to GLP-like standards. At present, we have established more than 300 tumor evaluation models in six categories, and we have already assess the efficacy of ADC or the combination treatment of ADC and other anti-tumor drugs in several animal models.

References

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