Pharmacology Research of ADC

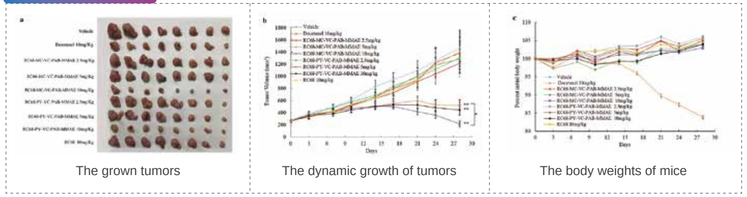
- Target antigen binding activity
- Related pharmacology of target antigen (e.g.: ADCC，CDC)
- Mechanism of payloads and metabolites (focus on the difference in pharmacology mechanism ADCs, naked antibodies, payload and metabolites).

Pharmacology Evaluation of ADC

One important pharmacological parameter of an ADC is the *in vivo* efficacy that directly reflects its potency and influences clinical trial designs. Our animal models are all established and maintained under the regulation of AAALAC. Pharmacology studies are conducted according to GLP-like standards. At present, more than 300 tumor evaluation models in six categories have been established by Medicilon.

- Tumor models for multiple tumor diseases
- Diverse selections of model types
  - Xenograft models
  - Syngeneic models
  - Orthotopic xenograft models
  - Transgenic models
  - hPBMC/CD34+ HSC humanized models
  - PDX models
- Various laboratory animal
  - Rodents: Mouse/Rat, Rabbit
  - Non-Rodents: Beagle Dog, Mini Pig, Non-human Primate

Medicilon Case:



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

In vivo antitumor activity of RC68-based ADCs.

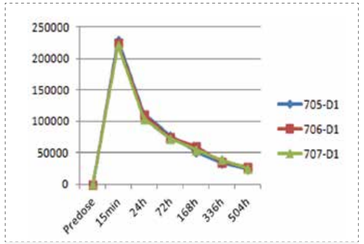
A humanized anti-EGFR monoclonal antibody (named RC68) was purifed and conjugated with MMAE using a MC-VC-PAB or PY-VC-PAB linker. The *in vivo* antitumor activity of RC68-MC-VC-PAB-MMAE and RC68- PY-VC-PAB-MMAE were performed by Medicilon.

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 by monitoring tumor volumes and their body weights were measured twice per week. At the end of the experiment, the tumors were dissected and photoimaged.

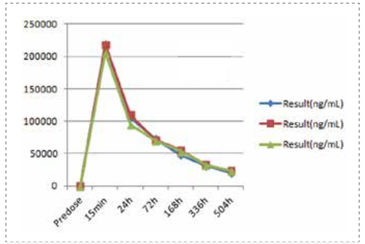
ADC Pharmacokinetics Study

ADC raises the difficulties of PK study as each component ADC molecules has unique PK characteristics. Medcilon provides high quality quantification assays for key parameters in ADC PK study, presenting accurate results.

Analyte	Description	Common analysis methods
Conjugated Anitbody	Antibody with minimum of DAR >= 1	LBA
Total Antibody	Conjugated, partially unconjugated and fully unconjugated (DAR >= 0)	LBA
Small Molecules	Released/free samllmolecule and its metabolities	LC-MS/MS
ADA	Antibodies against antibody of ADC, linker or drug	LBA



Total Ab



ADC

Benchmarking with global lab standard for results with high consistency. Developing stable and reliable methods for results with high correlation.

ADC Immunogenicity

Immunogenicity is a key parameter when evaluating biologic therapeutics. It could increase the risk for adverse effect and reduce ADC efficacy. Medicilon fully understands the compexity of ADA evaluation and offers our clients with comprehensive immunogenicity assays.

ADC Safety Assessment

Medicilon offers rigorous and specific safety assessment services strictly following S6 & S9 Regulation of ICH and in compliance with the requirement of NMPA, FDA, OECD and TGA.

- Single dose/Repeat dose toxicity (With TK)
- Tissue cross-reactivity
- ADA test.

SHANGHAI MEDICILON INC.

Address: No.585 Chuanda Road, Pudong New Area, Shanghai (Headquarters)
Email: marketing@medicilon.com Website: www.medicilon.com
Tel: +86 (21) 5859-1500 (main line)

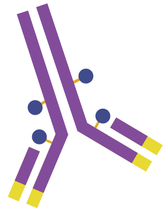
Medicilon ADC R&D Service Platform

In the formulation of ADC preclinical integrated research plan, Medicilon has in-depth communication with customers. The backbone of scientific research has combined the characteristics of each case with years of practical experience and technical accumulation, and carefully submitted high-quality experimental plans and results to customers.

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

C-Conjugate (Linker)
Conjugate payloads to antibodies

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



Solutions

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. As of May 2022, Medicilon has successfully assisted in the clinical approval of 10 ADC drugs and has multiple ADC projects under development.

- Provides toxin small molecules: DM1, MMAE, Exatecan, Dxd, SN38, etc
- Provides the targets: Her2, Her3, Trop2, Claudin 18.2, CD33, Muc1, FR, etc
- Has rich experience in developing and validating analysis methods for different targets, and can effectively analyze the expression level and accessibility of targets according to needs, and provide constructive suggestions for target selection.

MEDICILON

Synthesis of ADC Payloads

Medicilon's compound library has a variety of chemical ADC payloads with different mechanisms of action for customers to choose from. At the same time, ADCs can be customized and synthesized according to the specific needs of customers.

- Tubulin inhibitors
- DNA damaging agents
- Immunomodulators

Provides 6 payloads of all marketed ADCs
Provides 20+ payload derivatives of marketed ADCs
Provides independent R&D payloads

Medicilon High Potency Laboratory

Conjugate Strategies of ADCs

The three main components of ADCs are the antibody, the linker, and the payload

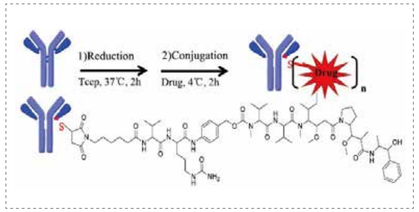
The antibody is responsible for target engagement, it can be in form of Mab, Fab, Bispecific Ab or nanobody.

The linker connects antibody and payload, typically in a form of cleavable or non-cleavable. It is key to ADC stability and responsible when to release the payload.

The payload is a highly potent toxin with defined mode of action. It is responsible for killing cancer cells.

Medicilon Case:

ADC crosslinking strategy based on cysteine

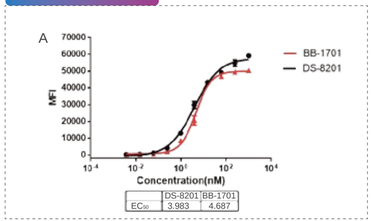


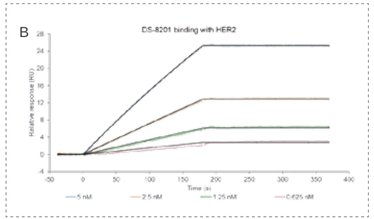
- Provide 5mg, 50mg and 500mg scale of ADC crosslinking service, timeline: 2-4weeks.
- Linker-payload: MC-MMAE, MC-MMAF, MC-GGFG-DX8951, MC-SN38 etc.
- QC methods including SEC, HIC and LC-MS/MS.
- DAR evaluation through HPLC and LC-MS/MS.

ADC *in Vitro* Analysis

With more than 200 cancer cell lines, the Medicilon Biological team has a wide selection of ADC target protein positive and negative tumor cells. In addition, the Medicilon Biological team has extensive experience in cell labeling and FACS-based cell viability analysis to help screen optimal antibodies.

Medicilon Case:





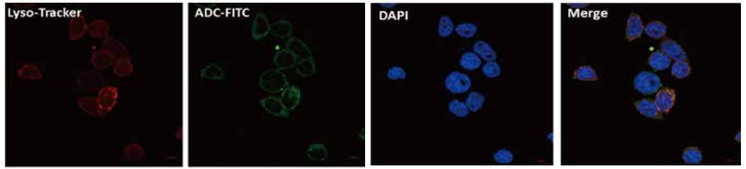
ADC Binding Assay

A: 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.

B：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.

- FACS-based ADC binding assay (Figure A: HER-2 ADC DS-8202, BB-1701 binding with BT-474 cells which is HER2 highly expressing cells).
- SPR analysis of ADC with antigen in protein level (Figure B: DS-8201 binding with HER2 protein). Provide Kd, Kon, Koff values for binding.
- Provide other methods for ADC binding, e.g. ELISA and HTRF.

Medicilon Case:

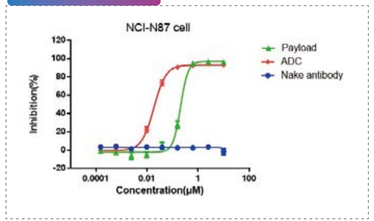


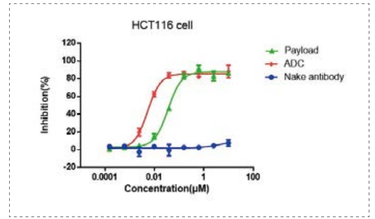
ADC Internalization: Confocal Imaging

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.

- ADC were labeled with fluorophore（e.g. ADC-FITC, ADC-Cy5).
- Internalization of ADC-FITC were analyzed through con-focal (co-localization of lyso-tracker with ADC-FITC indicating internalization of ADC).
- Internalization could also be analyzed through LICOR (In cell Western) and FACS.

Medicilon Case:

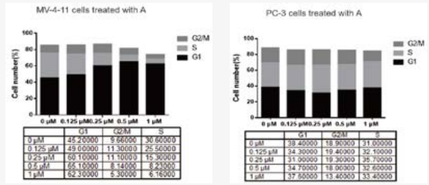




Cytotoxicity of Payloads or ADC

Payload, nake antibody and ADC were incubated with target cells, cell viability were analyzed through CCK-8, CTG and MTT.

Medicilon Case:

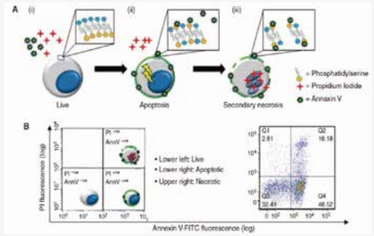


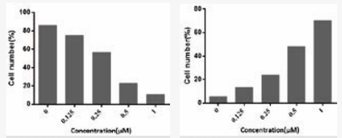
	MV-4-11 G1	MV-4-11 G2/M	MV-4-11 S	PC-3 G1	PC-3 G2/M	PC-3 S
0 μM	45.25000	9.56000	30.60000	38.40000	18.90000	31.00000
0.125 μM	49.00000	11.30000	25.50000	34.30000	19.40000	32.10000
0.25 μM	60.10000	11.10000	15.30000	31.90000	19.30000	35.70000
0.5 μM	66.10000	8.14000	8.23000	34.70000	18.90000	32.60000
1 μM	62.30000	5.30000	6.16000	37.80000	13.40000	33.40000

Cell Cycle Analysis

MV-4-11 cells and PC-3 cells were treated with compound A and stained with PI for FACS-based cell cycle analysis. The data shown that compound A dramatically block the cell cycle of MV-4-11 cells and did not affect PC-3 cells too much.

Medicilon Case:





MV-4-11 treated with A-live

MV-4-11 treated with A-Apoptotic

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.

Apoptosis Analysis

MEDICILON