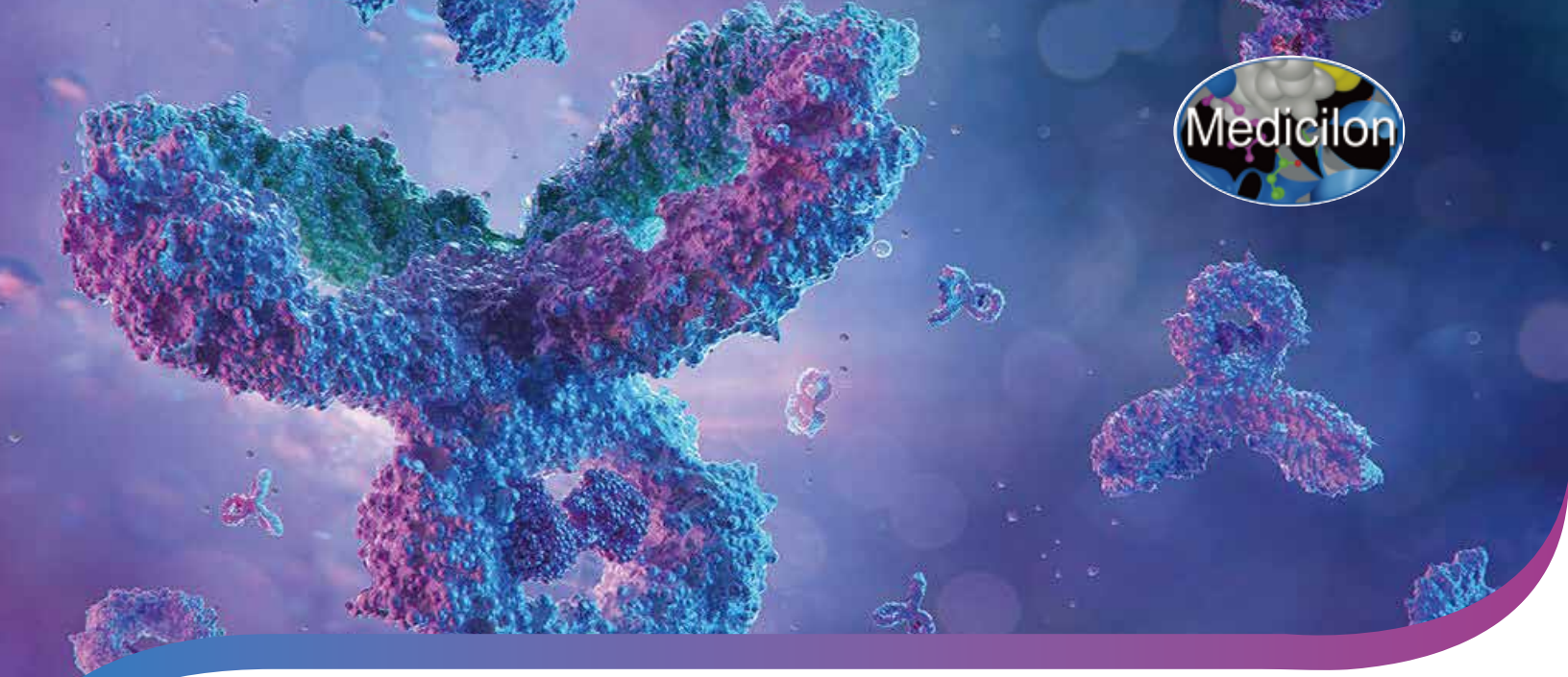


Medicilon ADC R&D Service Platform

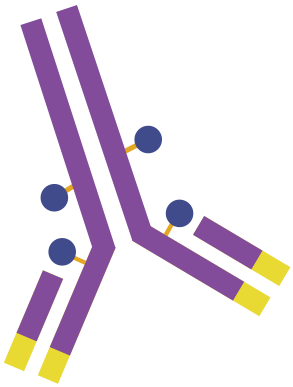
In the formulation of ADC preclinical integrated research plan, Medicilon has in-depth communication with customers. The backbone of scientific research has combined the characteristics of each case with years of practical experience and technical accumulation, and carefully submitted high-quality experimental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

A-Antibody

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

D-Drug (Cytotoxic agent)

The highly potent antitumor payload released after entering the target cell



C-Conjugate (Linker)

Conjugate payloads to antibodies

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 the end of 2023, Medicilon has successfully assisted in the clinical approval of **23** ADC drugs and has 20+ ADC projects under development.

- Payload (50+): DX8951f, SN-38, DM1, DM4, Exatecan, Dxd, MMAE/MMAF, Tubulysin M, PBD dimer, Seco-Duocarmycin MA, PNU159682, Doxorubicin analog.
- Linker (200+): Various types of Cleavable and uncleavable, hydrophilic and hydrophobic, and new types of Linker, such as: Gly-Gly-Phe-Gly, VC-PAB, SMCC, N-SMP.
- Payload+Linker(30+):SN-38-PEG-PAB,DXD-GlyGlyPheGly,DXD-VCPABDM1-SMCC,DM1-SPP,DM4-SPDB, MC-VC-PAB-MMAE/MMAF, MC-Val-Ala-PBD, VC-PAB-Dolastatin 10.
- Validated ADC targets in Medicilon:Her2, Her3, Trop2, Claudin 18.2, CD33, Muc1, FR, etc.

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.

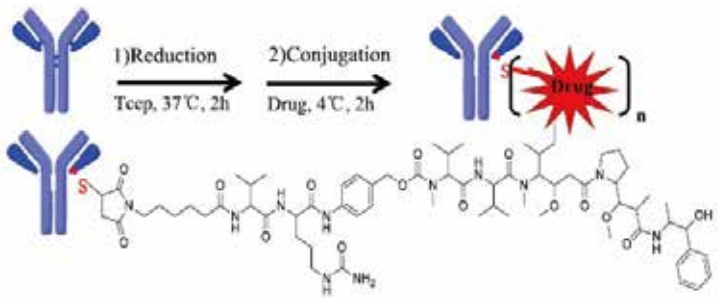
The linker connects antibody and payload, typically in cleavable or non-cleavable form. It is key to ADC stability and it determines when to release the payload.

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

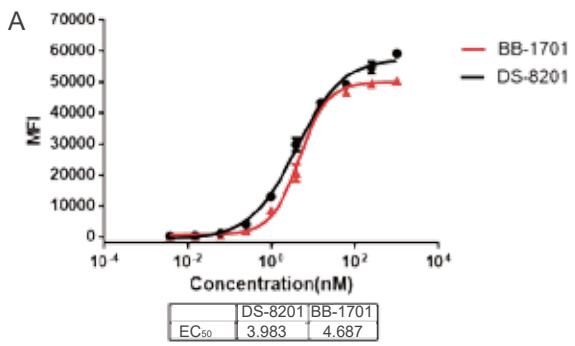
- Provide 5mg, 50mg and 500mg scale of ADC crosslinking service, timeline: 2-4weeks.
- Linker-payload: MC-MMAE, MC-MMAF, MC-GGFG-DX8951, MC-SN38 etc.
- QC methods including SEC, HIC and LC-MS/MS.
- DAR evaluation through HPLC and LC-MS/MS.



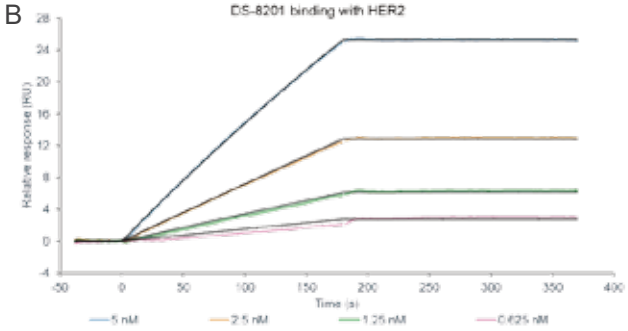
ADC *in Vitro* Analysis

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:



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



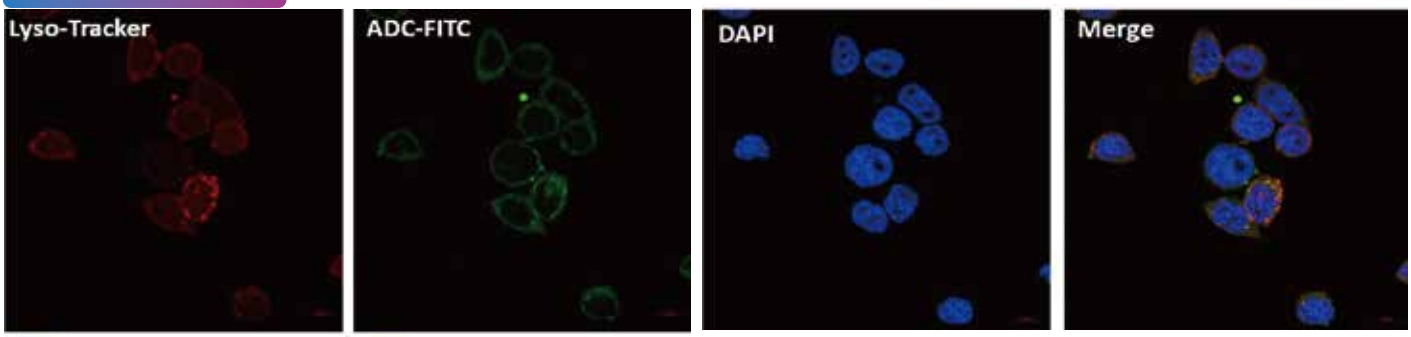
ADC Binding Assay

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-8201, BB-1701 binding with BT-474 cells which are 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:

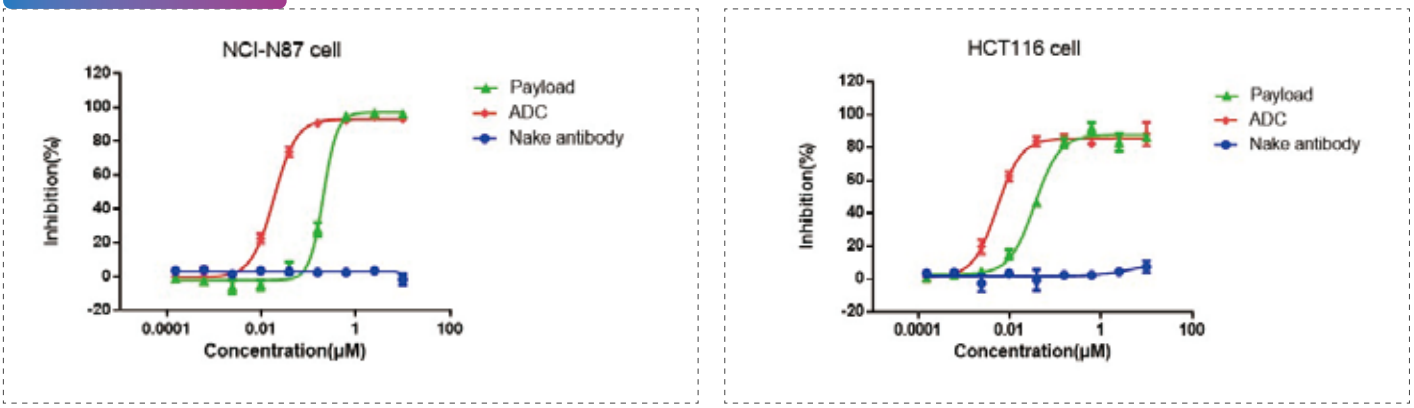


ADC Internalization: Confocal Imaging

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 was 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.

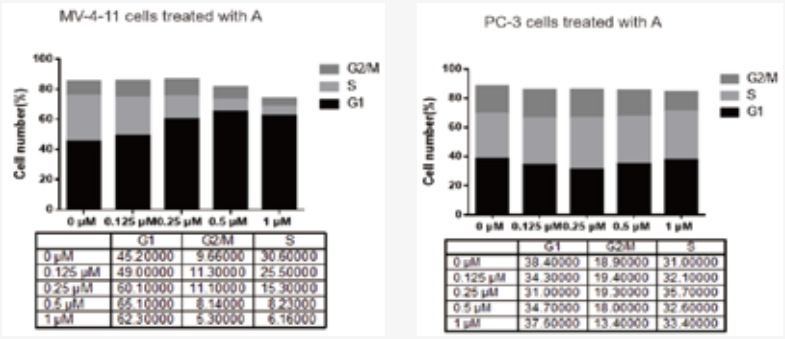
Medicilon Case:



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:



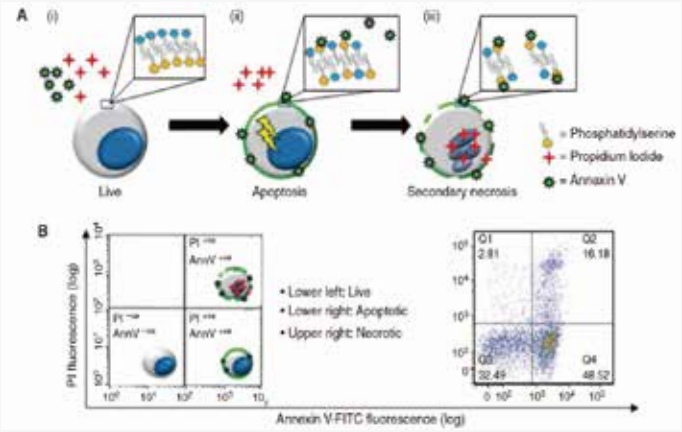
	G1	G2/M	S
0 μM	45.20000	9.66000	30.60000
0.125 μM	49.00000	11.30000	25.50000
0.25 μM	60.10000	11.10000	15.30000
0.5 μM	65.10000	8.14000	8.23000
1 μM	82.30000	5.30000	6.16000

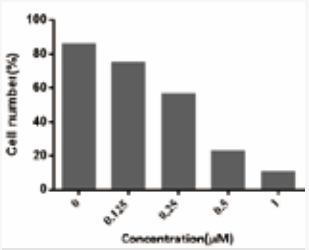
	G1	G2/M	S
0 μM	38.40000	18.90000	31.00000
0.125 μM	34.30000	19.40000	32.10000
0.25 μM	31.00000	19.30000	35.70000
0.5 μM	34.70000	18.00000	32.60000
1 μM	37.50000	13.40000	33.40000

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.

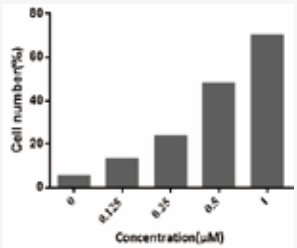
Cell Cycle Analysis

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

Pharmacology Research of ADC

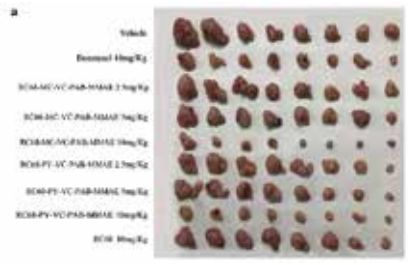
- Target antigen binding activity
- Related pharmacology of target antigen (e.g.: ADCC，CDC)
- Mechanism of payloads and metabolites (focus on the difference in pharmacology mechanism ADCs, naked antibodies, payload and metabolites).

Pharmacology Evaluation of ADC

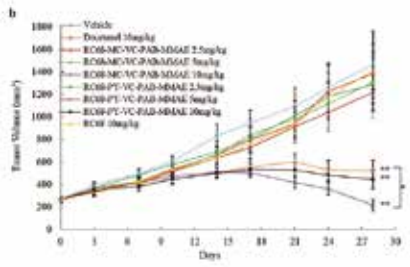
One important pharmacological parameter of an ADC is the *in vivo* efficacy that directly reflects its potency and influences clinical trial designs. Our animal models are all established and maintained under the regulation of AAALAC. Pharmacology studies are conducted according to GLP standards. At present, more than 370 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

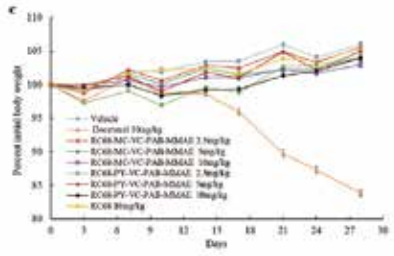
Medicilon Case:



The grown tumors



The dynamic growth of tumors



The body weights of mice

In vivo antitumor activity of RC68-based ADCs.

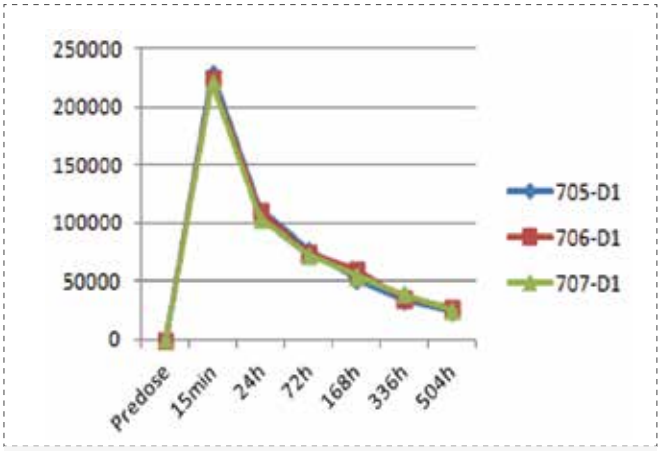
A humanized anti-EGFR monoclonal antibody (named RC68) was purifed and conjugated with MMAE using a MC-VC-PAB or PY-VC-PAB linker. The *in vivo* antitumor activity of RC68-MC-VC-PAB-MMAE and RC68-PY-VC-PAB-MMAE were performed by **Medicilon**.

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 difficulties in PK study as each component ADC molecules has unique PK characteristics. Combining our antibody department and pharmacology department expertise, Medicilon provides high quality quantification assays for each key parameter in ADC PK study, presenting accurate results.

Description		
Conjugated Anitbody	Antibody with minimum of DAR ≥ 1	LBA
Total Antibody	Conjugated, partially unconjugated and fully unconjugated (DAR >= 0)	LBA
Small Molecules	Released/free samll molecule 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 potential risk of adverse effects and reduced ADC efficacy. Medicilon fully understands the complexity 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

Medicilon Assisted Projects

DAC-002

In July 2020, DAC Biotech's new ADC drug development, TROP2-ADC—DAC-002 was approved of clinical study by NMPA for the indication of solid malignant tumor. DAC-002 is an ADC anti-Trop2 monoclonal antibody conjugated by an intelligent ligand against Tubulysin B analogue. It is used to treat Trop2 triple negative breast cancer, small cell lung cancer, non-small cell lung cancer and pancreatic cancer.

Medicilon completed preclinical pharmacokinetic and toxicological studies in this project, accelerating the development process.

Muc1-ADC

In July 2021, a recombinant humanized anti-MUC1 monoclonal antibody-Tub201 coupling agent (hereinafter referred to as "Muc1") for the treatment of advanced solid tumor class 1 ADC drug injection from Dac Biotechnology was approved for clinical use. This is the first clinically approved Muc1-ADC drug in China.

Medicilon has provided a full set of preclinical research services including pharmacology, pharmacokinetics and safety evaluation in the research and development of new Muc1 drugs, helping the project to be successfully approved for clinical trials.

BAT8006

In May 2022, Bio-Thera Solutions, Ltd. (Bio-Thera) has been approved for clinical application of BAT8006 for injection, a product under development for the treatment of advanced solid tumors. BAT8006 is composed of a recombinant humanized anti-FRα antibody and a toxic small molecule topoisomerase I inhibitor connected by a self-developed cleavable linker. BAT8006 has efficient anti-tumor activity, and the toxin small molecule has strong cell membrane penetration ability.

During the R&D of BAT8006, Medicilon's ADC preclinical research and development service platform has extensive practical technology and experience in the field of ADC drug preclinical R&D, followed the ICH guidelines S6 and S9 and combined with the specific situation of the BAT8006 project, to customize a personalized safety evaluation plan and overcome the complexity and diversity of drug-to-antibody ratio (DAR), stability, for BAT8006.

KM501

In March 2023, Xuanzhu Biopharmaceutical (Xuanzhu) and its wholly-owned subsidiary Beijing Xuanzhu Bio, obtained clinical trial approval for the double-antibody ADC drug KM501. This product is suitable for the treatment of advanced/metastatic solid tumors with positive/expression, amplification or mutation of HER2, including related advanced tumors with low expression of HER2. The drug is the world's first double-antibody ADC drug that completely knocks out fucose, and is expected to become the "Best in Class" drug.

Medicilon, as a partner of Xuanzhu, provided KM501 with GLP-compliant preclinical research services based on the Medicilon Antibody Development Service Platform, including pharmacokinetic studies and safety evaluation.

HB0052

In November 2023,Huaota Biotech received a notification from the US FDA, agreeing that the third-generation ADC project HB0052 developed by the company targeting the CD73 antigen will enter clinical trials. This is the first antibody conjugate drug project approved by Huaota Biotech to enter clinical trials by the FDA.

As a partner of Huaota, Medicilon relied on its antibody drug conjugate R&D service platform and its professional technical capabilities and rich project experience to provide HB0052 with R&D of the drug metabolism and safety evaluation tests that complied with GLP standards to ensure high-quality and efficient advancement.

Reference:

Zhuanglin Li, et al. Monomethyl auristatin E-conjugated anti-EGFR antibody inhibits the growth of human EGFR-positive non-small cell lung cancer. Cancer Chemother Pharmacol. 2019 Jul;84(1):61-72. doi: 10.1007/s00280-019-03848-9.

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