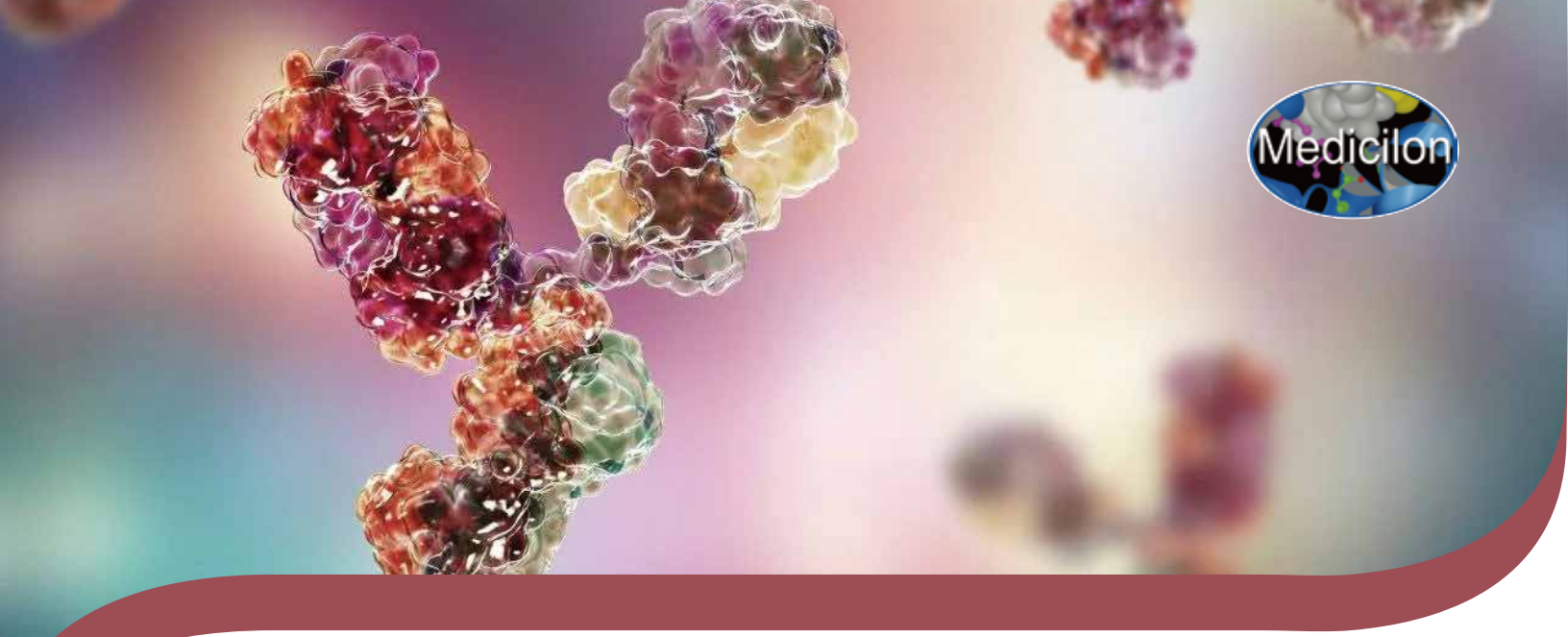
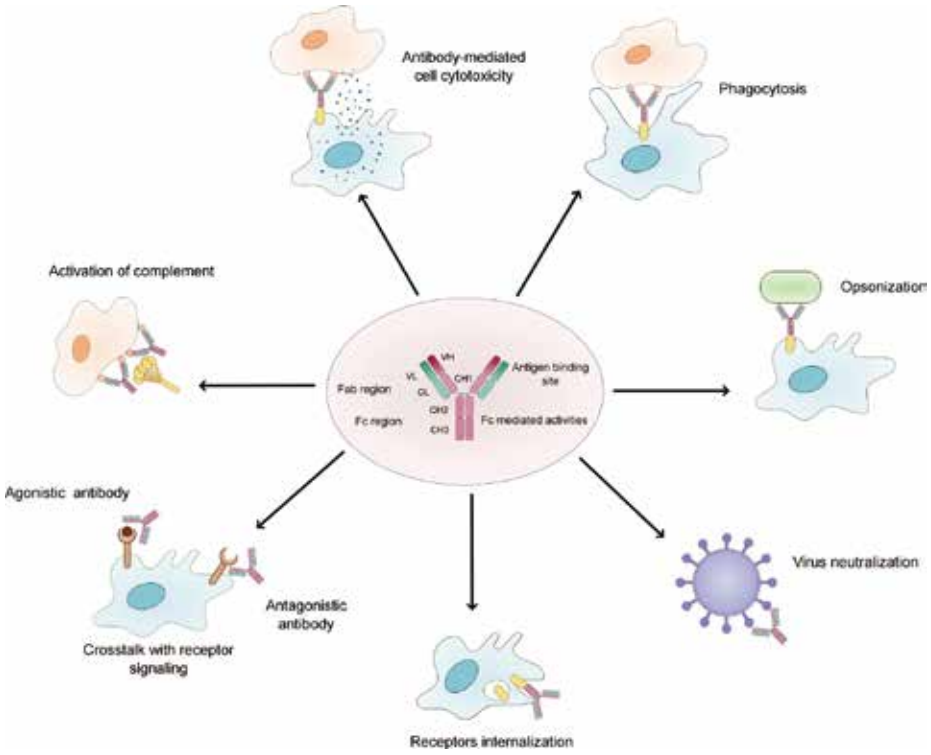


Medicilon Antibody R&D Service Platform

Nowadays, antibody drugs are increasingly important for the pharmaceutical industry across the globe. Antibody drugs are important therapeutic biological products in the evolution of life-saving disease treatment. In the formulation of antibody drugs 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. Medicilon provides antibody drugs discovery, CMC research (API + formulation), pharmacodynamics research, PK study, safety evaluation and other services. **As of the end of 2023, Medicilon has successfully assisted in the clinical approval of 32 antibody drugs and has multiple antibody projects under development.**

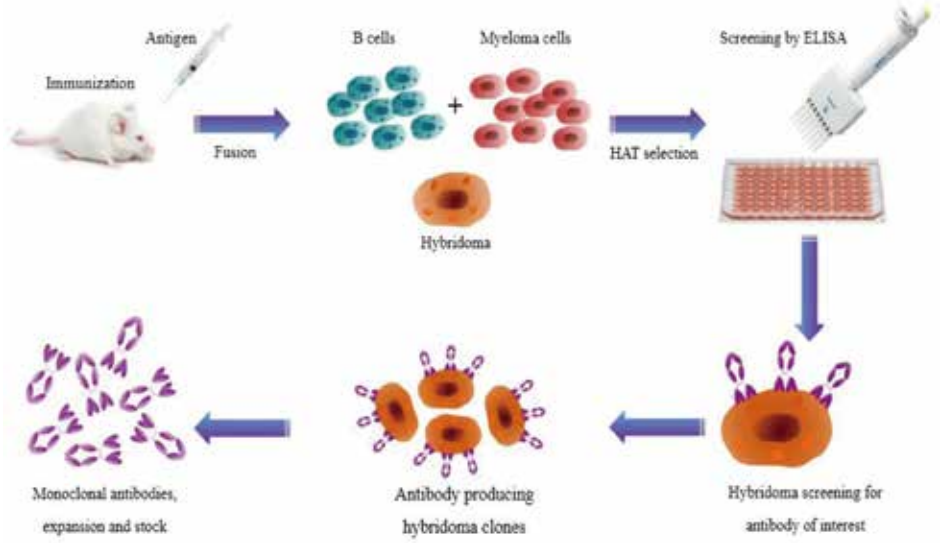


Overview of the natural function of antibodies[1]

Antibodies Discovery

Hybridoma Technology

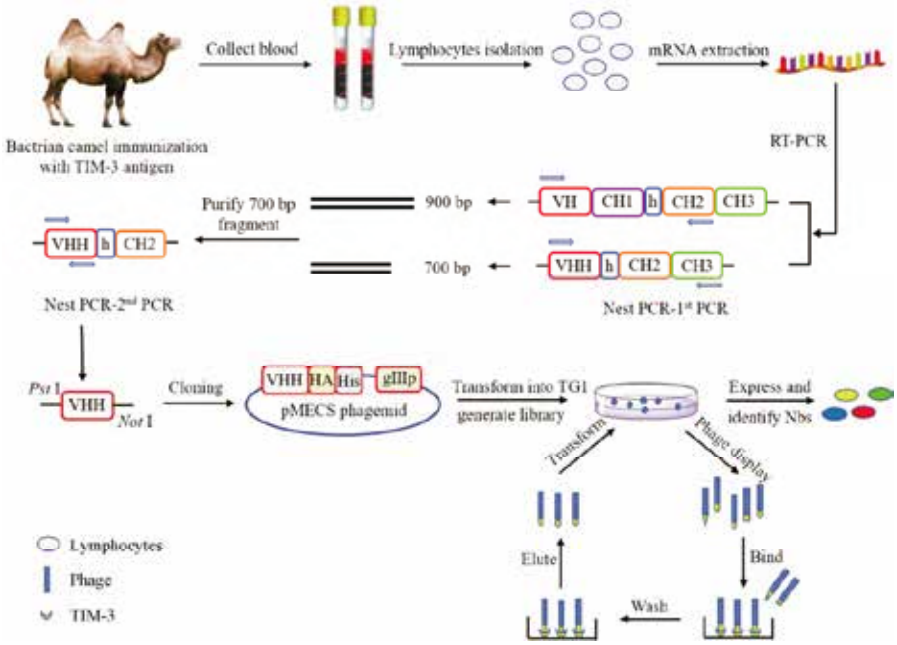
With more than 10 years of experience in customized antibody production and an experienced antibody drug R&D team, Medicilon has established an antibody drug development platform. Medicilon's hybridoma technology service can provide a variety of immune methods (proteins, peptides, small molecules, whole cells) to ensure that customer needs are met.



Production route of hybridoma technology[2]

Nanobodies Discovery

Nanobodies (Nbs) are characteristic by small molecular weight, and their unique molecular structure. These features make them suitable for many fields such as disease diagnosis and treatment. Medicilon provides camelid VHH antibody library construction services, including antigen preparation, and immunization, and provides several kinds of antibody libraries for bacterial display, nanobody library panning, ELISA verification and other related experiments.



Nanobody production scheme using a phage display library[3]

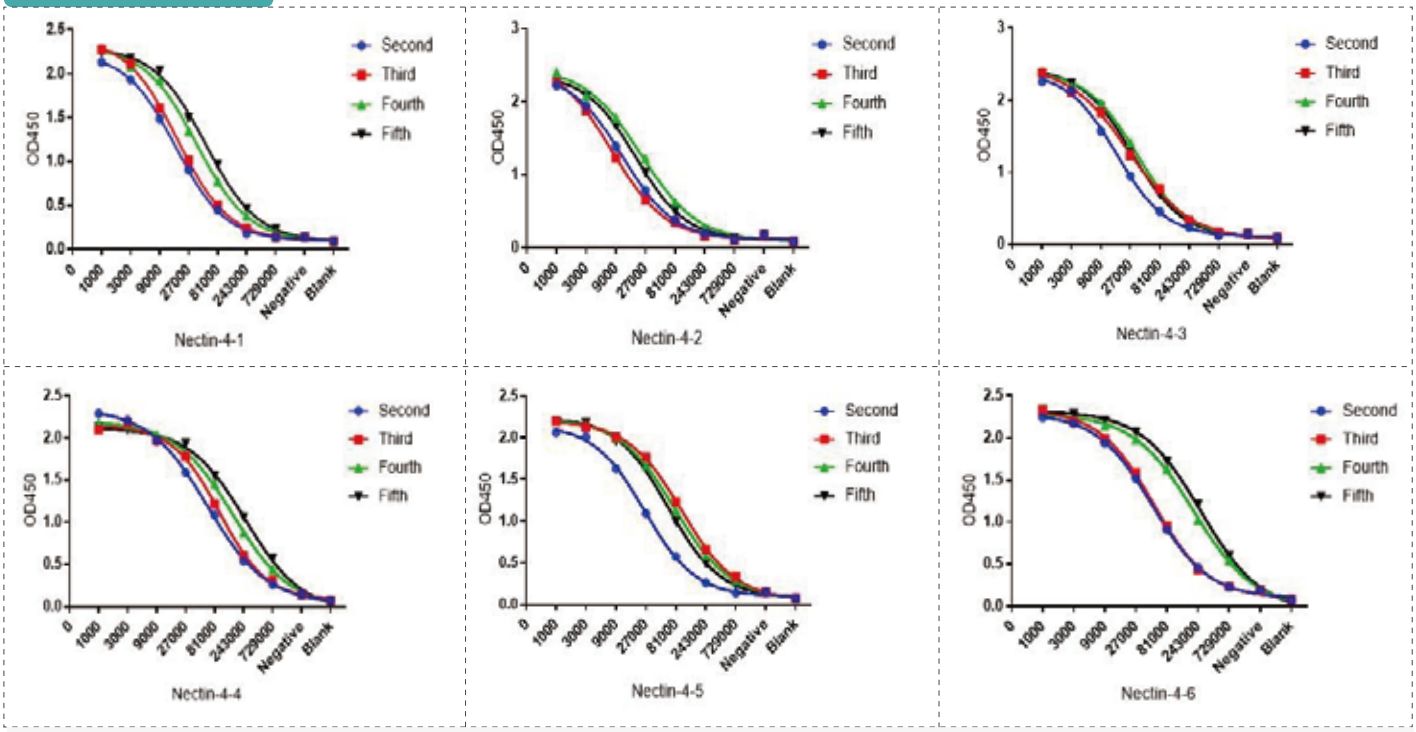
Single B Cell Antibodies Discovery Technology

Single B cell screening is a newly developed technique for rapid preparation of mab in recent years. The principle is that each B cell only contains a pair of functional heavy and light chains, and each B cell only produces a specific antibody characteristic, which can be directly amplified from a single B cell to obtain mab. This method has the advantages of fast speed, high throughput, and natural pairing of variable regions of antibody weight and light chains. It is one of the new and efficient methods for antibody discovery.

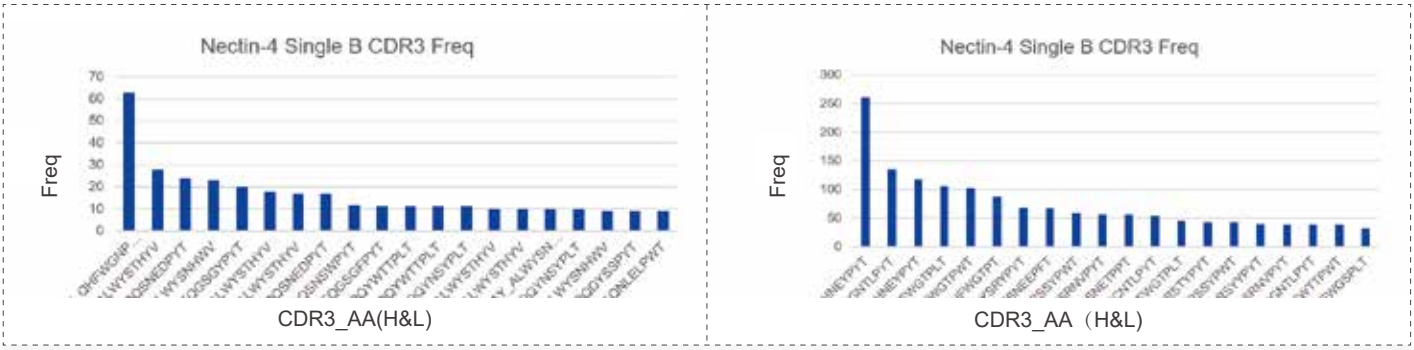
Animal Immunization	Single B Cell Sorting	Sequencing & Recombinant Antibody Expression
• Animal immunization & antibody titer test • 5-9 weeks	• B cell enrichment, antibody labeling, flow cytometry and cell sorting • 1 week	• BCR library variable region sequencing and recombinant antibody expression • 5-6 weeks

Process of Single B Cell Antibody Production

Medicilon Case

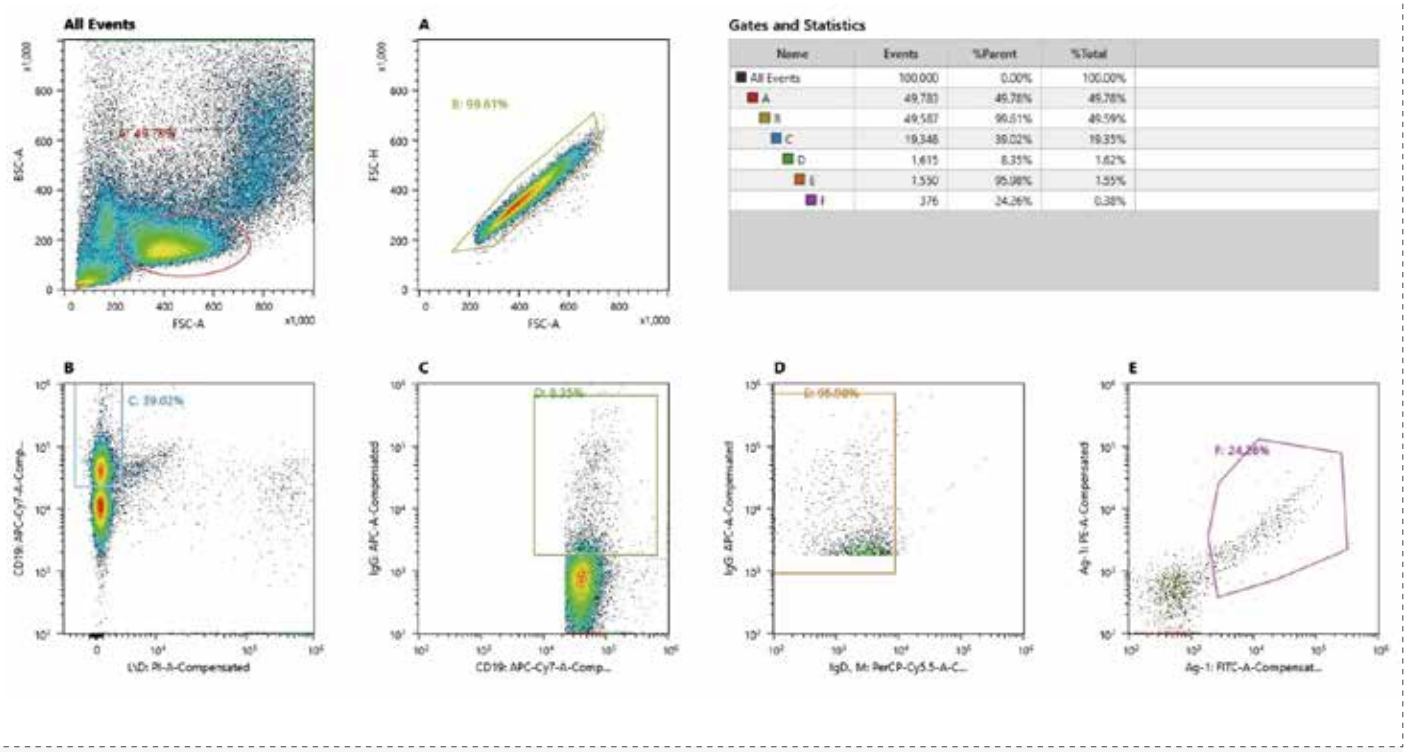


Nectin-4 Mouse Serum Titer



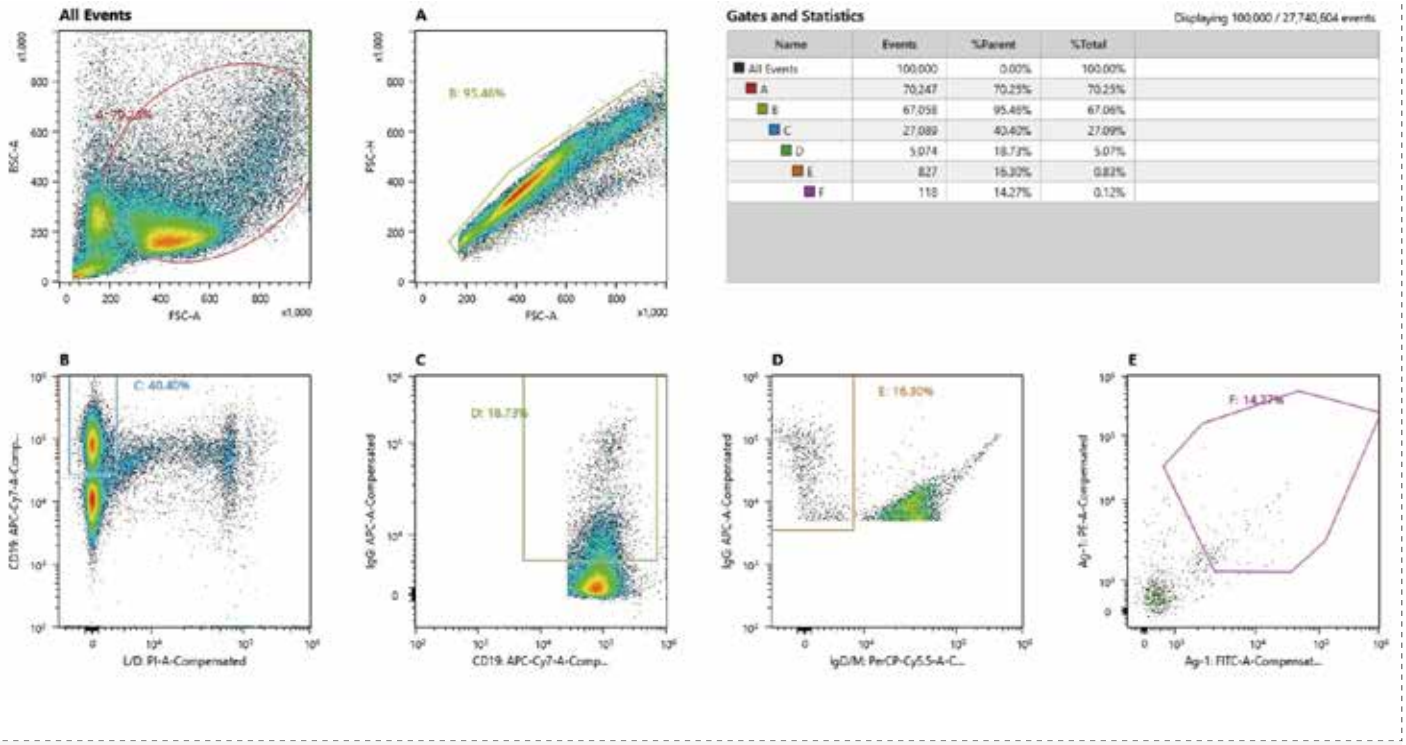
Nectin-4 (4-1) Sequencing (Top20)

Nectin-4 (4-6) Sequencing (Top20)



Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
A	49,783	49.78%	49.78%
B	49,587	99.61%	49.59%
C	19,348	39.02%	19.35%
D	1,615	8.35%	1.62%
E	1,550	95.98%	1.55%
F	376	24.26%	0.38%

Nectin-4 (4-1) Sorting Single B Cell



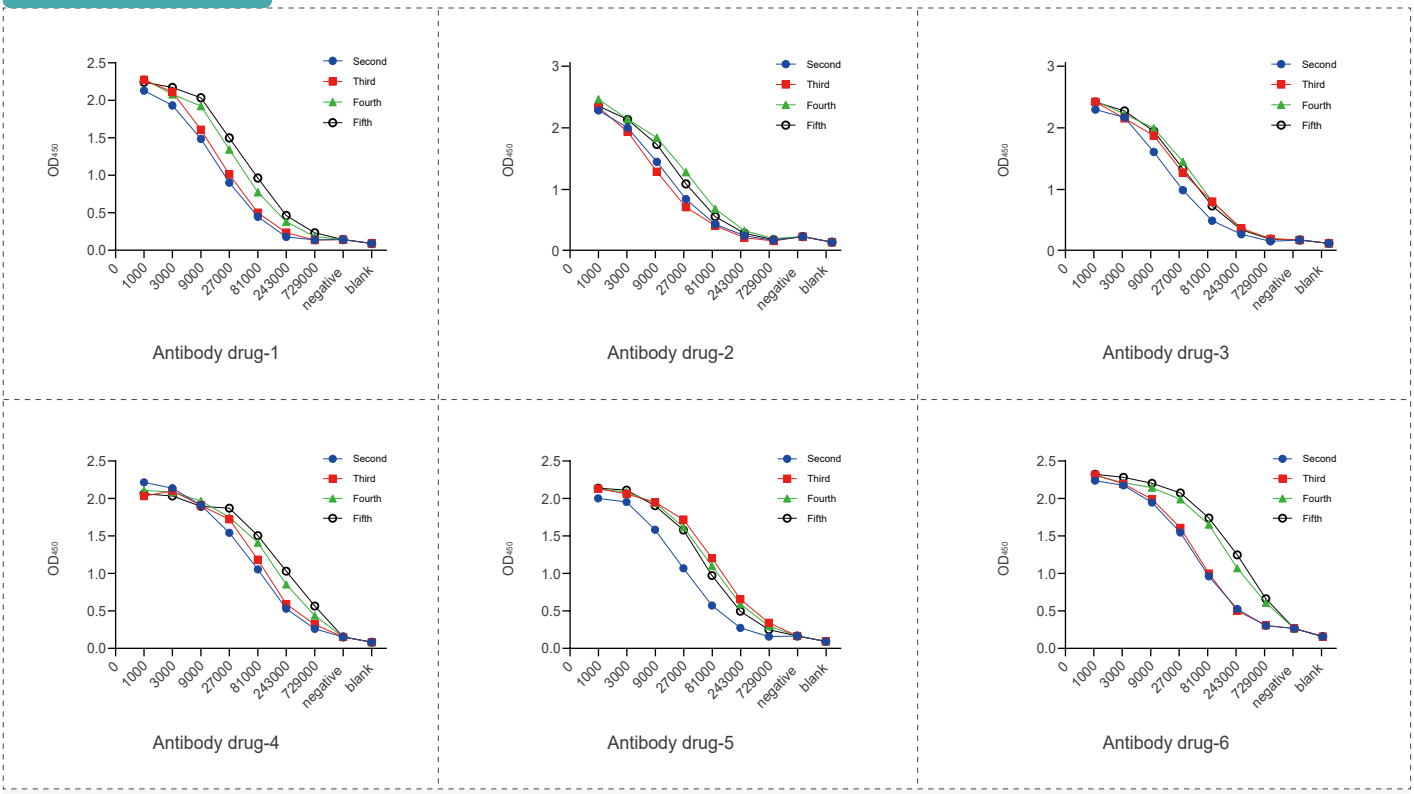
Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
A	70,247	70.25%	70.25%
B	67,058	95.46%	67.06%
C	27,089	40.40%	27.09%
D	5,074	18.73%	5.07%
E	827	16.30%	0.83%
F	118	14.27%	0.12%

Nectin-4 (4-6) Sorting Single B Cell

Anti-drug Antibodies

Highly sensitive assays for anti-drug antibodies (ADAs) are both a regulatory requirement and requisite for proper evaluation of the effects of immunogenicity on clinical efficacy and safety. Determination of ADA assay sensitivity depends on positive control antibodies to represent naturally occurring or treatment-induced ADA responses. An accurate determination of the proportion of drug-specific antibodies in these polyclonal positive control batches is critical for correct evaluation of assay sensitivity. Medicilon has rich experience in ADA development and has successfully delivered 100+ projects. Medicilon can provide high-affinity and specificity polyclonal ADA development services with less time and lower cost.

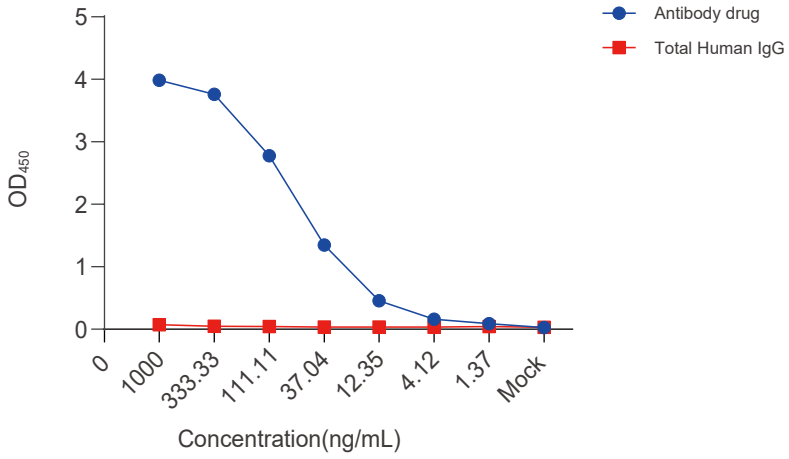
Medicilon Case



Antibody drug-1 | Antibody drug-2 | Antibody drug-3

Antibody drug-4 | Antibody drug-5 | Antibody drug-6

Serum Titer

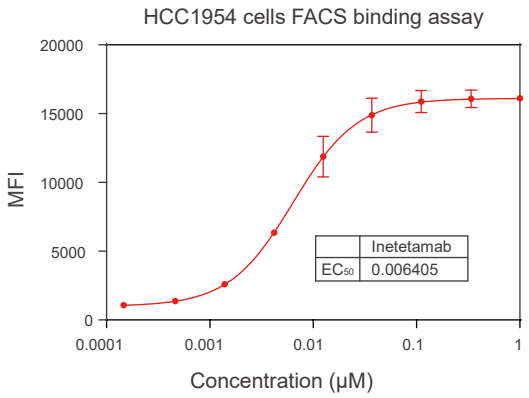


Antibody Titer after Purification

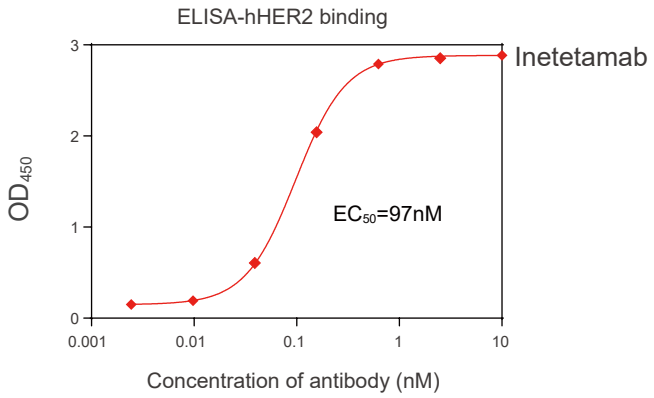
In Vitro Study of Antibodies

In vitro functional assays are crucial for the practical evaluation of a candidate antibody drug in the initial stages of research and development. These assays offer scientific evidence for validating antibody activity, understanding MoAs, and providing preliminary evidence that supports therapeutic efficacy. As such, they play a key role in the decision-making process in drug candidate selection.

Binding assay (FACS, ELISA, SPR)

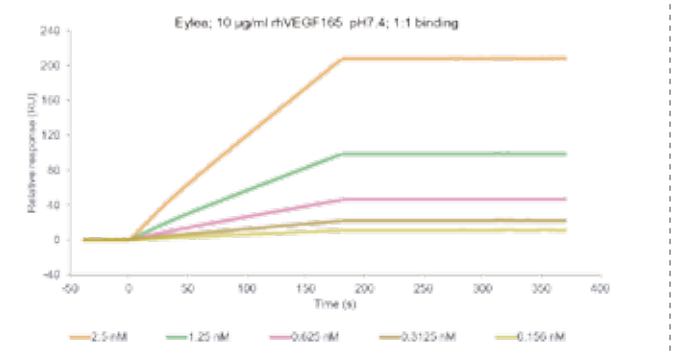


Dose-dependent binding of Inetetamab (anti-HER2) with HCC1954 cells were tested through FACS, the data showed that Inetetamab binds with HCC1954 cells with EC_{50} of 6.4 nM.

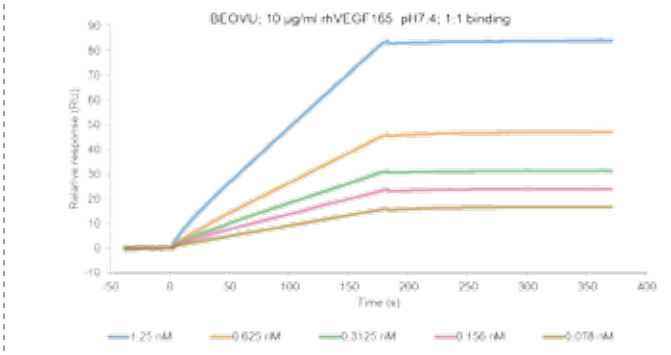


Dose-dependent binding of Inetetamab (anti-HER2) with human HER2 protein were tested through ELISA, the data showed that Inetetamab binds with HER2 protein with EC_{50} of 97 nM.

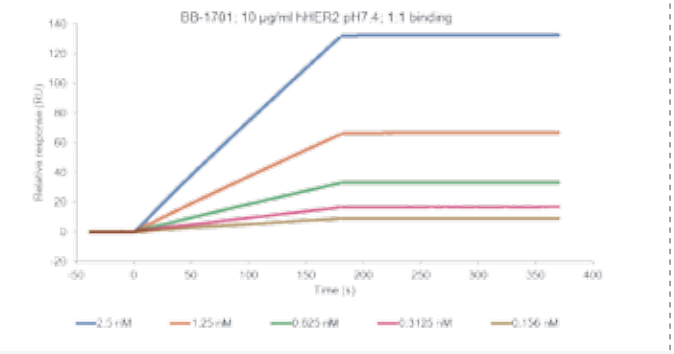
Validated targets: PD-1, PD-L1, VEGF, Nectin1, Nectin2, Nectin3, Nectin4, NECL1, NECL2, NECL3, NECL4, NECL5, EPHA1, EPHA2, EPHA3, EPHA4, EPHA5, INSR, IGF-1R, HSA, FcRN, FcRI, FcRII, FcRIII, C1q, Factor B, HER2, Transferrin, EPCR, STAT3, STAT5, STAT1, 4-1BB, SHP2, ATIII, EGFR, gp1, etc.



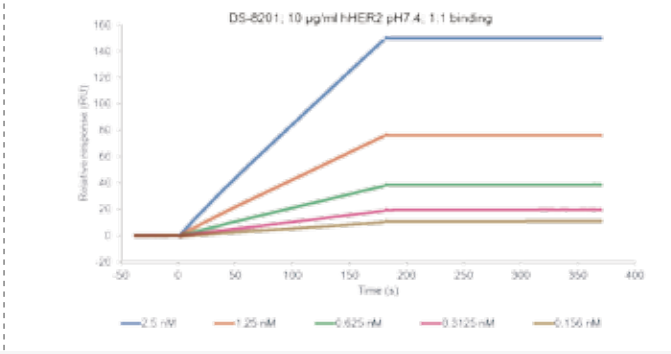
Eylea binding with hVEGF



BEOVU binding with hVEGF

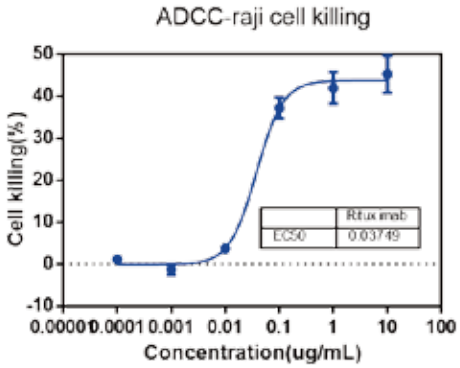


BB-1701 binding with hHER2

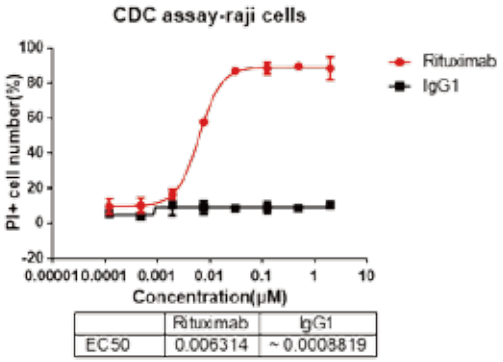


DS8201 binding with hHER2

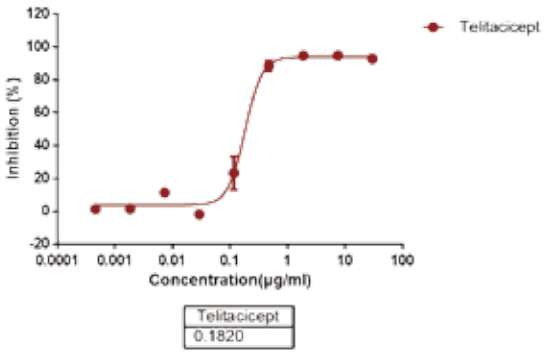
Functional assay for antibodies



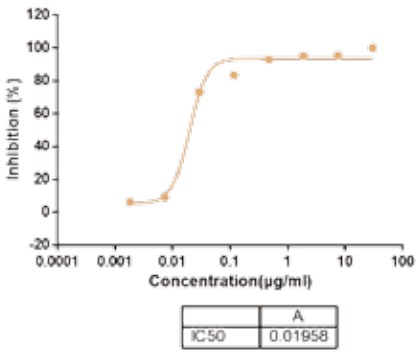
Raji cells were mixed with human PBMCs, and different doses of Rituximab was added to induce ADCC, the killing of raji cells were detected through FACS(CFSE labeling of raji).



Raji cells were mixed with human AB serum, and different doses of Rituximab was added to induce CDC, the killing of raji cells were detected through FACS(PI staining of raji).



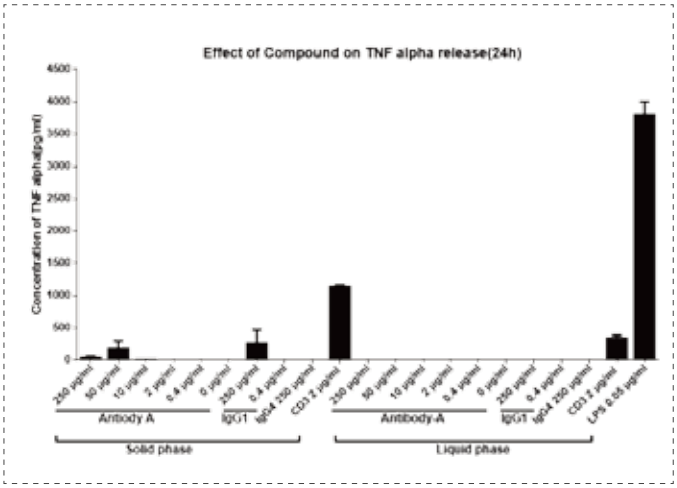
B cells were treated with Teliacicept and induced with Baff for 72 hours, H3-thymidine incorporation were analyzed through scintillation counting.



T cells were treated with compound A and induced with ICOSL for 72 hours, H3-thymidine incorporation were analyzed through scintillation counting.

Cytokine release assay

- Human PBMCs were treated with antibody A/IgG1 and anti-CD3/LPS for 25 hours(Liquid phase or solid phase), TNFa level were analyzed through Luminex kit.
- Cytokine release assay were performed following ICH guidance, IL-2, IL-6, IL-10, IFN-γ and TNF-α must be tested, usually antibodies were treated to the PBMCs under solid phase and liquid phase, at least 3 donors will be tested. ELISA, Luminex, CBA and MSD methods will be used for detection of cytokines. OKT3 was used as positive control.
- The purpose of this assay is to evaluate the potential antibody induced cytokine release effects to prevent from the occurrence of strong cytoking release storm in clinical trials.



CMC Research of Antibodies

Medicilon can provide API process development and preparation R&D services for antibodies. Medicilon has established a platform for the development of cGMP APIs and has developed GMP APIs for clinical trials for innovative drug companies. Medicilon team continues to assist the development of antibodies through rigorous design of experiments (DOE), professional R&D technology, standardized project management, efficient communication, etc.

Medicilon Case:LQ036

In February 2022, Shanghai Novamab Biopharmaceuticals Co., Ltd. (Novamab), LQ036 - recombinant anti-IL-4Rα single-domain antibody nebulizer (Pichia pastoris), a core drug for the treatment of moderate to severe asthma, was successfully approved for IND by NMPA.

In the development process of the world's first inhaled nanobody drug for the treatment of moderate and severe asthma, Medicilon relies on the Medicilon Inhalation Drug Research and Development Platform to assist LQ036 to complete the quality research services of inhaled preparations with high quality and efficiency, and provided strong support for the project to be approved for clinical use.

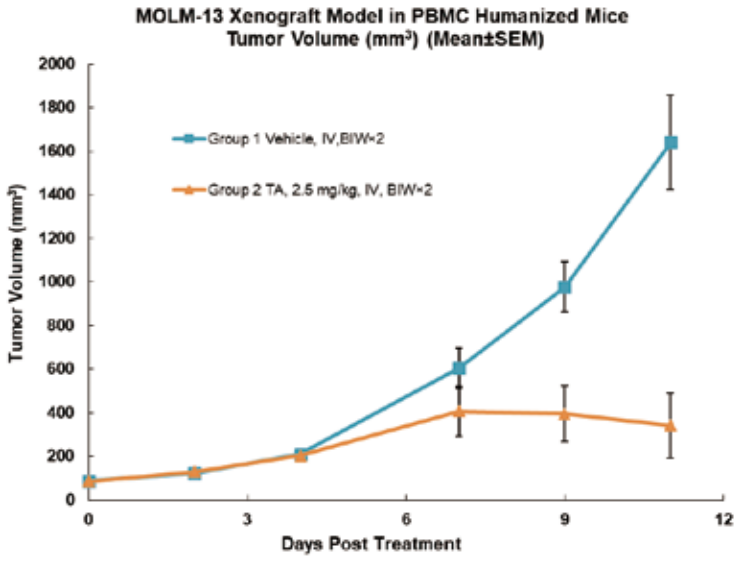
Pharmacology Evaluation of Antibodies

Medcilon provides mature models for evaluating the efficacy of antibodies *in vivo*. 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.

Various laboratory animal

- Rodents: Mouse/Rat, Rabbit
- Non-Rodents: Beagle Dog, Mini Pig, Non-human Primate

Medicilon Case: CD3+ Bispecific Antibody

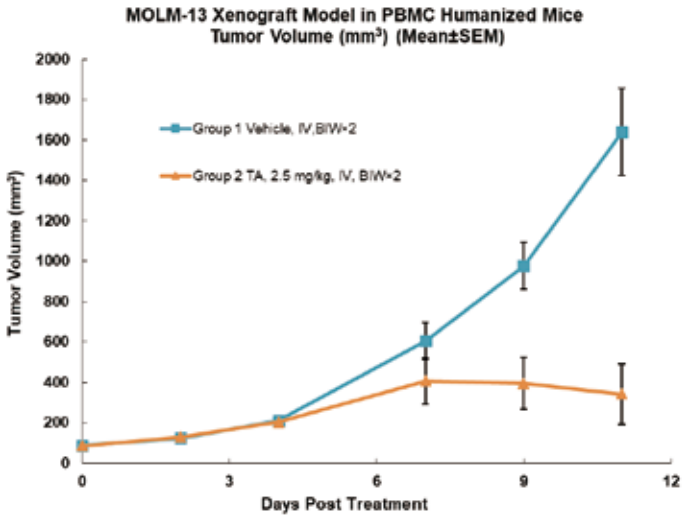


- **Animals:**
  Female NOG mice
- **Tumor Cells:**
  Tumor mixed hPBMC (1:1) with 50% Matrigel, SC
- **PBMC:**
  injected before tumor cells inoculation
- **Treatment:**
  Bispecific antibody

Mean Tumor Volum (mm^3) Day 14

Group	TV	$\%TGI_{TV}$	*P* Value
Vehicle	1639.53	/	/
Antibody	340.90	90.38	<0.001

Medicilon Case: CD3+ Bispecific Antibody



- **Animals:**
  Female NOG mice
- **Tumor Cells:**
  Tumor, 2x10^6/mouse with 50% Matrigel, SC
- **PBMC:**
  Injected after tumor cells inoculation
- **Treatment:**
  Bispecific antibody

Mean Tumor Volum (mm^3) Day 17

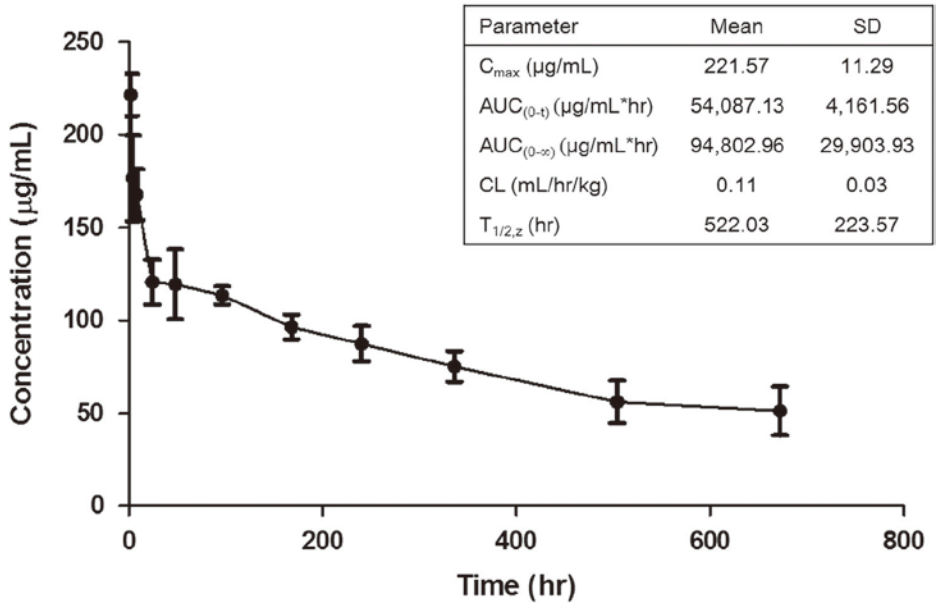
Group	TV	%TGI$_{TV}$	*P* Value
Vehicle	1963.22	/	/
Antibody	522.83	73.37	<0.01

Pharmacokinetic (PK) Studies of Antibodies

Medcilon provides high quality quantification assays for key parameters in antibodies PK study, presenting accurate results.

Medicilon Case: Pharmacokinetics of YYB-101 in cynomolgus monkeys

The pharmacokinetics of YYB-101 was investigated in four male cynomolgus monkeys after a single intravenous injection (10 mg/kg). The YYB-101 serum T_{max} was 2 h, C_{max} was 221.57 μg/mL, and $AUC_{(0-\infty)}$ was 94 802.96 μg/mL*h. The $t_{1/2Z}$ was ~21.7 days and clearance was 0.11 mL/kg/h. This study was conducted by **Medicilon**.



Parameter	Mean	SD
C_{max} (μg/mL)	221.57	11.29
$AUC_{(0-t)}$ (μg/mL*hr)	54,087.13	4,161.56
$AUC_{(0-\infty)}$ (μg/mL*hr)	94,802.96	29,903.93
CL (mL/hr/kg)	0.11	0.03
$T_{1/2,z}$ (hr)	522.03	223.57

Pharmacokinetic profile of YYB-101 in cynomolgus monkeys[4]

Medicilon Case: Anti-drug antibodies of YYB-101 in cynomolgus monkeys

Anti-drug antibodies were detected on day 1 in one female monkey at 50 mg/kg per day YYB-101 but were not detected in samples collected from this animal on day 29 or 85. Anti-drug antibodies were detected in only one animal at a single time point, and little cross-reactivity to normal tissue was observed. This study was conducted by **Medicilon**. On the basis of these results, a phase I clinical study is ongoing in patients with advanced solid tumors (NCT02499224).

Safety Evaluation of Antibodies

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 Case: Toxicokinetics of YYB-101 in cynomolgus monkeys

Following intravenous administration of YYB-101, the mean systemic exposure ($AUC_{0\text{-}168h}$) and Cmax values of YYB-101 increased proportionally with dose. The mean peak and trough serum concentrations of YYB-101 appeared to approach steady state following the four weekly infusions of YYB-101. Serum concentrations were quantifiable in recovered animals 63 days after the last dose (~2.8% of C_{max} from day 22). Systemic exposure ($AUC_{0\text{-}168h}$) increased with repeated intravenous administration of YYB-101, with accumulation ratios ranging from 2.38 to 2.95. This study was conducted by **Medicilon**.

Dose ($mg\,kg^{-1}\,day^{-1}$)	Day	Statistic	C_{max} ($ng\,ml^{-1}$)	C_{max}/Dose ($kg \times ng\,ml^{-1}mg^{-1}$)	T_{max}[a](h)	T_{last}[a](h)	AUC_{Tlast} ($ng \times h\,ml^{-1}$)	$AUC_{0\text{-}168hr}$ ($ng \times h\,ml^{-1}$)	$AUC_{0\text{-}168hr}$/Dose ($kg \times ng \times h\,ml^{-1}\,mg^{-1}$)	R[b]
10	1	N	6	6	2 (2–2)	168 (168 –168)	6	6	6	NA
		Mean	363 000	3 6300			31 000 000	31 000 000	3 100 000	NA
		s.d.	24 200	2420			2 980 000	2 980 000	298 000	NA
		CV%	7	7			10	10	10	NA
	22	N	6	6	2 (2–2)	168 (168 –168)	6	6	6	6
		Mean	738 000	73 800			77 200 000	77 200 000	7 720 000	2.49
		s.d.	44 300	4430			10 700 000	10 700 000	1 070 000	0.286
		CV%	6	6			14	14	14	11
50	1	N	6	6	2 (2–2)	168 (168 –168)	6	6	6	NA
		Mean	1 950 000	39 000			163 000 000	163 000 000	3 250 000	NA
		s.d.	289 000	5770			13 100 000	13 100 000	262 000	NA
		CV%	15	15			8	8	8	NA
	22	N	6	6	2 (2–2)	168 (168 –168)	6	6	6	6
		Mean	3 650 000	73 000			387 000 000	387 000 000	7 730 000	2.38
		s.d.	75 200	1500			34 800 000	34 800 000	696 000	0.119
		CV%	2	2			9	9	9	5
200	1	N	10	10	2 (2–2)	168 (168 –168)	10	10	10	NA
		Mean	7 330 000	36 600			666 000 000	6 660 000 000	3 330 000	NA
		s.d.	670 000	3350			61 500 000	61 500 000	308 000	NA
		CV%	9	9			9	9	9	NA
	22	N	10	10	2 (2–96)	168 (168 –1512)	10	10	10	10
		Mean	17 800 000	89 200			3 180 000 000	1 980 000 000	9 910 000	2.95
		s.d.	4 350 000	21 700			1 160 000 000	652 000 000	3 260 000	0.841
		CV%	24	24			36	33	33	29

Toxicokinetic parameters on days 1 and 22 following intravenous infusion of YYB-101 at 10, 50, or 200 mg/kg perday in cynomolgus monkeys[4]

Medicilon Assisted Projects

BAT6021 Injection and BAT6005 Injection

In October 2021, Bio-Thera Solutions, Ltd. (Bio-Thera)'s BAT6021 injection and BAT6005 injection of innovative drugs have been approved for clinical use, which means the new progress has been made in the field of tumor treatment. BAT6021 and BAT6005 are anti-TIGIT monoclonal antibodies, which are intended to be developed for tumor treatment. **As a partner of Bio-Thera, Medicilon provided preclinical research services such as safety evaluation and pharmacokinetics for BAT6021 injection and BAT6005 injection.**

BAT7104 Injection

In October 2021, Bio-Thera's PD-L1/CD47 bispecific antibody BAT7104 injection has been granted implicit permission for clinical trials, and the approved indication is advanced malignant tumors. In preclinical studies, BAT7104 can effectively block the combination of the two pathways, mediate T cell activation and trigger phagocytosis of macrophages. **As a long-term partner of Bio-Thera, Medicilon was honored to cooperate with Bio-Thera in the research and development of BAT7104 injection. Under the GLP laboratory environment and operating specifications, comprehensive preclinical research services for BAT7104 injection (including pharmacokinetics and safety evaluation) were completed, providing a professional guarantee for the efficient and high-quality clinical approval of BAT7104 injection.**

JYB1904

In May 2022, Jimincare's IgE antibody drug JYB1904 has been approved for clinical trials. JYB1904 is a new anti-IgE recombinant humanized monoclonal antibody targeted therapy drug. JYB1904 injection has excellent clinical therapeutic potential and can provide a potential new solution for the clinical treatment of allergic diseases such as moderate to severe asthma. **Medicilon provided JYB1904 with a comprehensive preclinical study (including pharmacokinetics and safety evaluation) that complied with GLP specifications with compliant, efficient and high-quality services.**

CC312

In June 2022, CytoCares Inc. (CytoCares) obtained the FDA's implied approval for the IND application of its first CD19/CD3/CD28-targeting trispecific antibody CC312. This is the first trispecific antibody in China and the third in the world to enter the clinical development stage based on the CD28 costimulatory signal. CC312 showed significant pharmacodynamic effects and good safety in preclinical studies on hematological tumors. **Medicilon provided CC312 with a comprehensive preclinical study (including pharmacokinetics and safety evaluation) that complied with GLP specifications with compliant, efficient and high-quality services.**

BAT2022

In June 2022, Bio-Thera Solutions, Ltd. (Bio-Thera) announced that BAT2022 for injection has obtained a clinical trial approval. BAT2022 for injection is a bispecific neutralizing antibody independently developed by Bio-Thera, which is intended to be used for the treatment of new coronary pneumonia caused by the infection of the COVID19 and its mutants. **Medicilon was honored to participate in the R&D project of Bio-Thera BAT202 for injection, providing a safety evaluation test that complied with the GLP specification and assisting the project successfully obtain clinical approval with compliant, efficient and high-quality services.**

GT90008

In October 2022, the PD-L1/TGF-β dual-target antibody (GT90008) of Kintor was approved for clinical trials. GT90008 is a PD-L1/TGF-β dual-target antibody, which can simultaneously inhibit the high activity of PD-L1 and TGF-βR2, and has the potential to become the best drug in its class. **In the research and development of GT90008, Medicilon provided GLP-compliant (including pharmacokinetics and safety evaluation) and comprehensive preclinical research services of pharmacokinetics, the research and development of the entire project is progressing smoothly and efficiently.**

HCW9218

In October 28, HCW Biologics Inc. (hereinafter referred to as "HCW") fusion protein complex HCW9218 was approved by the FDA for cancer treatment trials. As a heterodimeric, bifunctional fusion protein complex, HCW9218 contains the extracellular domain of TGF-β receptor II and IL-15/IL-15 receptor α complex, which can effectively activate and proliferate NK cells and $CD8^+$ T cells, enhance the cytotoxicity of cells against tumor targets, optimize the efficacy of chemotherapy and reduce the side effects of chemotherapy. **As a partner of HCW, Medicilon has set up a team of research experts in accordance with the principle of "case by case" in view of the HCW9218 project's key technical points. Analysis and exploration, and finally Medicilon established an analysis method suitable for HCW9218 under the GLP laboratory environment and operating specifications, provided preclinical pharmacokinetics and safety evaluation studies, and fully contributed to the high-quality and efficient completion of the project. In addition, Medicilon's preclinical pharmacology and toxicology research team relied on the comprehensive and mature SEND data conversion platform in software, technology, specifications, quality and other aspects to help HCW9218 successfully apply for FDA and promote it to enter the clinical trial stage.**

NB002

In July 2023, Neologics Bioscience announced that its research and development pipeline NB002 for the treatment of solid tumors has successfully passed the review of the US FDA and agreed to conduct phase I clinical trials. NB002 is a monoclonal therapeutic antibody targeting a unique epitope of TIM-3, which has a significant single-drug anti-tumor therapeutic effect. **As a partner of Neologics Bioscience, Medicilon provided preclinical research services such as safety evaluation and pharmacokinetics for NB002, and assisted its IND application successfully obtain FDA clinical approval.**

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