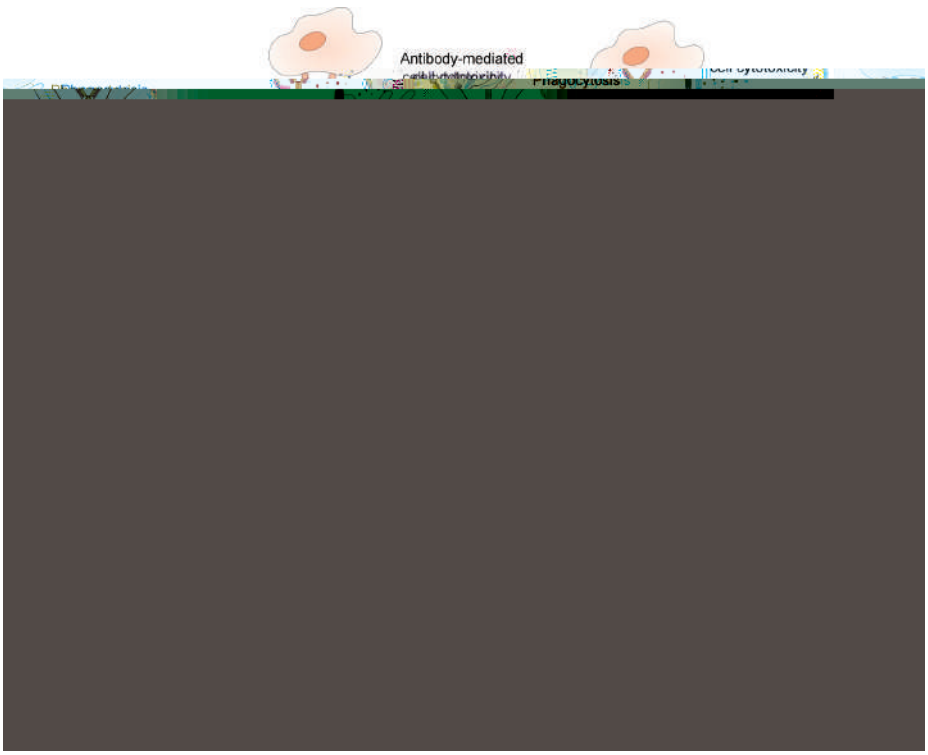
Medicilon Antibody R&D Service Platform

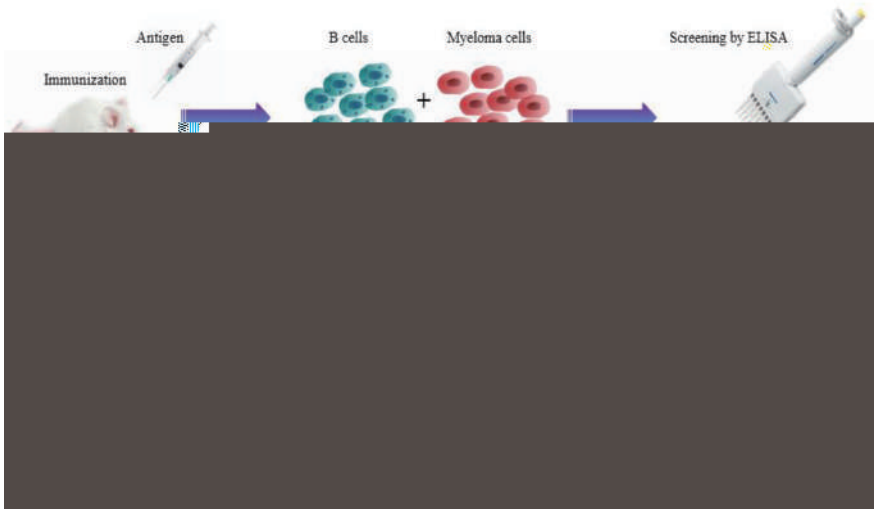
In today's pharmaceutical landscape, antibody drugs have ascended to a position of paramount importance globally. Representing pivotal therapeutic biological advancements, they stand at the forefront of life-saving disease treatments. At Medicilon, the development of antibody drugs is approached with a meticulously crafted integrated research plan, forged through comprehensive dialogue with our valued customers. Rooted in robust scientific inquiry, our methodology seamlessly harmonizes the distinctive characteristics of each case with extensive practical experience and technical acumen. **As of April 2024, Medicilon has successfully assisted clients on the IND approval of 33 antibody drugs and has multiple antibody projects on going.**



Overview of the natural function of antibodies[1]

Antibodies Discovery

Drawing from over a decade of expertise in tailored antibody production and bolstered by a seasoned antibody drug R&D team, Medicilon has forged a robust platform for antibody drug development. Our hybridoma technology service stands as a testament to this commitment, offering a diverse array of immune methods encompassing proteins, peptides, small molecules, and whole cells. This multifaceted approach ensures that we can effectively cater to the unique requirements



Nanobodies Discovery

Nanobodies (Nbs) stand out for their diminutive molecular weight and distinctive molecular structure, rendering them exceptionally versatile across various domains including disease diagnosis and treatment. Medicilon offers comprehensive camelid VHH antibody library construction services. This encompasses antigen preparation, immunization, and the provision of diverse antibody libraries tailored for bacterial display, nanobody library panning, ELISA verification, and other pertinent experiments.

Nanobody production scheme using a phage display library[3]

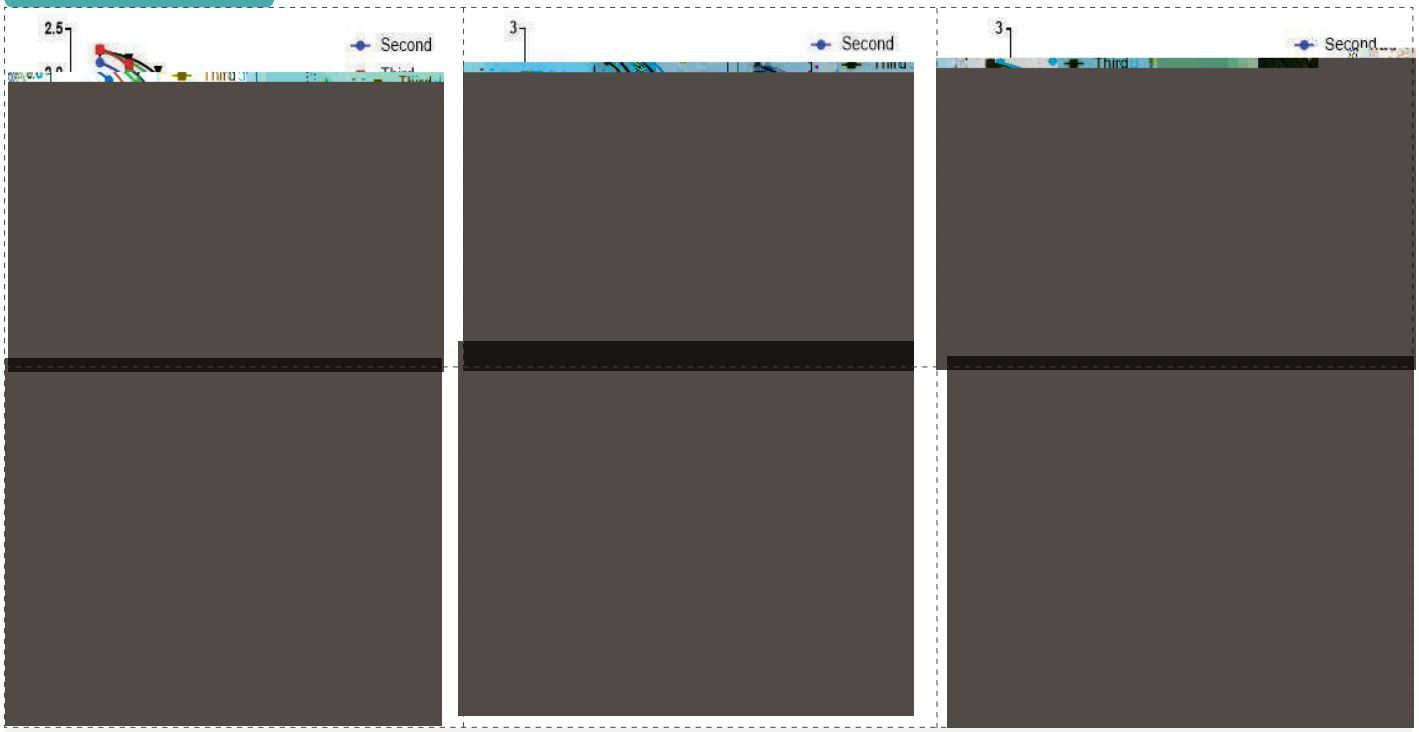
Single B Cell Antibodies Discovery Technology

Single B cell screening represents a cutting-edge technique for the swift generation of monoclonal antibodies (mAbs) in recent years. Its underlying principle lies in the fact that each B cell harbors a singular pair of functional heavy and light chains, with each B cell generating a specific antibody trait. Leveraging this principle, mAbs can be directly amplified from individual B cells, facilitating rapid acquisition. This method offers notable advantages including rapidity, high throughput capabilities, and the natural pairing of variable regions of antibody heavy and light chains. It stands as one of the novel and efficient approaches to antibody discovery in contemporary biotechnology.

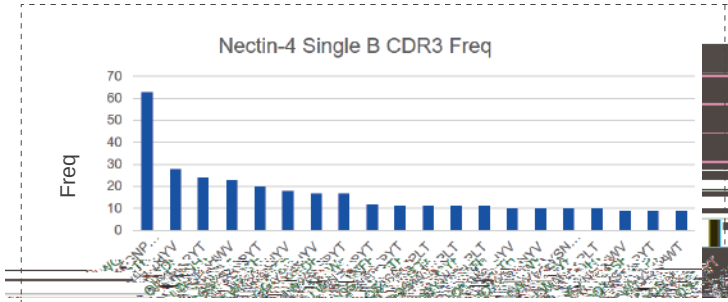
Animal Immunization	Single B Cell Sorting	Sequencing & Recombinant Antibody Expression
Animal immunization & antibody titer test	• B cell enrichment, antibody labeling, flow cytometry and cell sorting	• BCR library variable region sequencing and recombinant antibody expression
5-9 weeks	• 1 week	• 5-6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

Process of Single B Cell Antibody Production

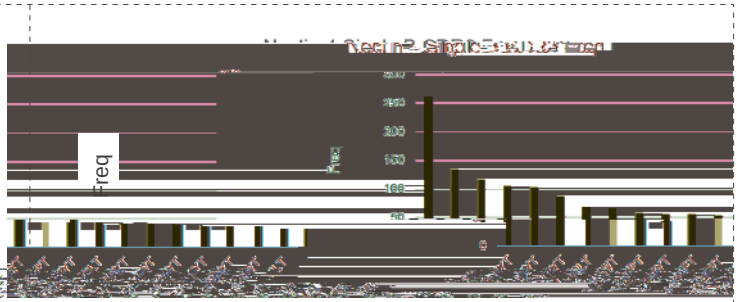
Medicilon Case



Nectin-4 Mouse Serum Titer



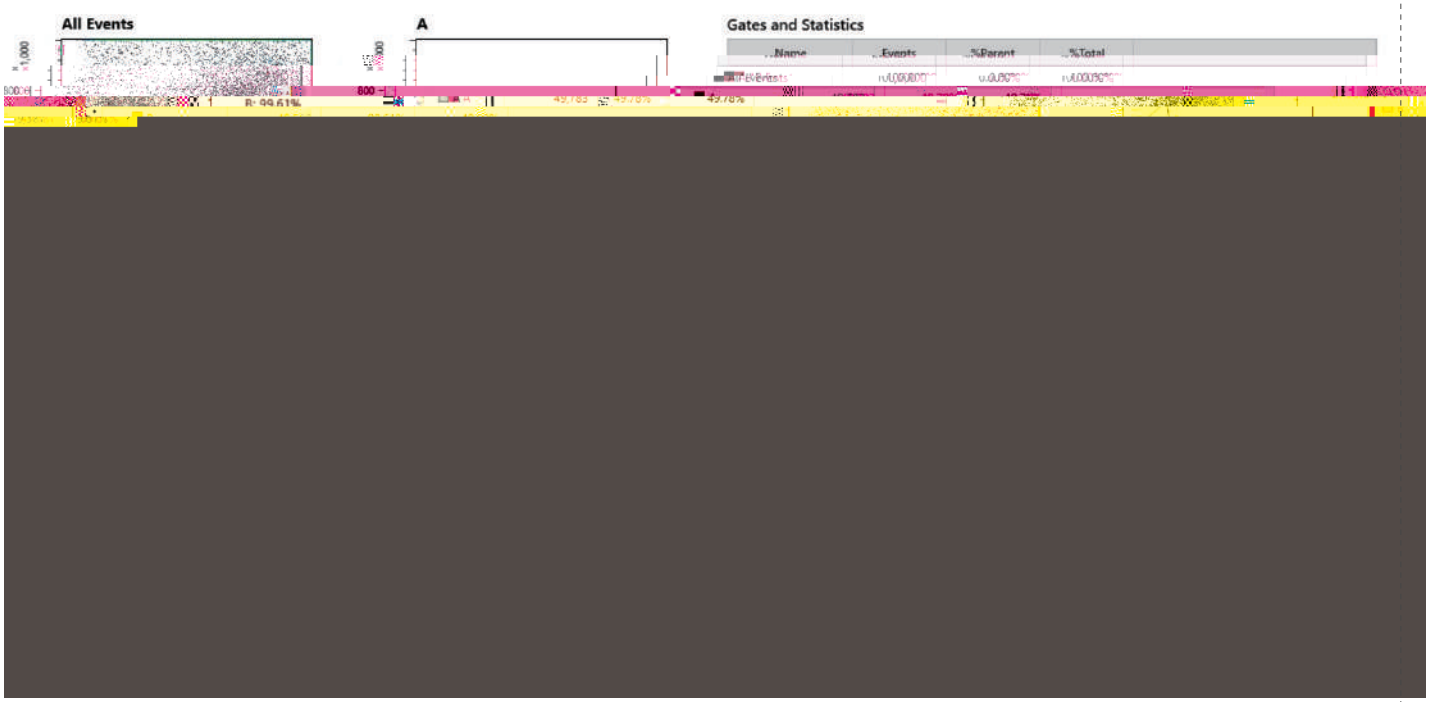
CDR3_AA(H&L)



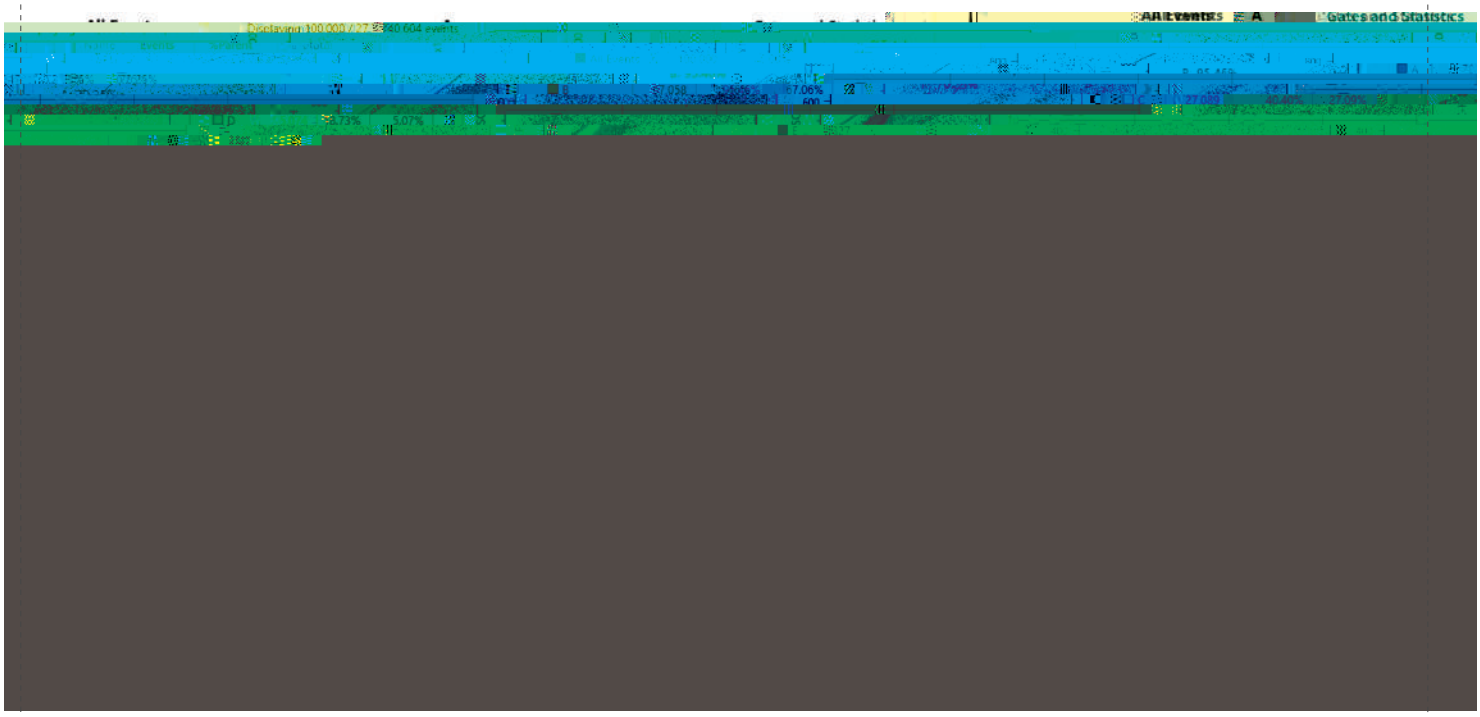
CDR3_AA（H&L)

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

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



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



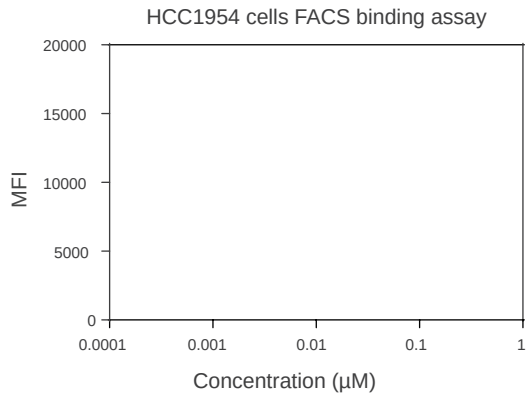
Nectin-4 (4-6) Sorting Single B Cell

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 determina-

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.

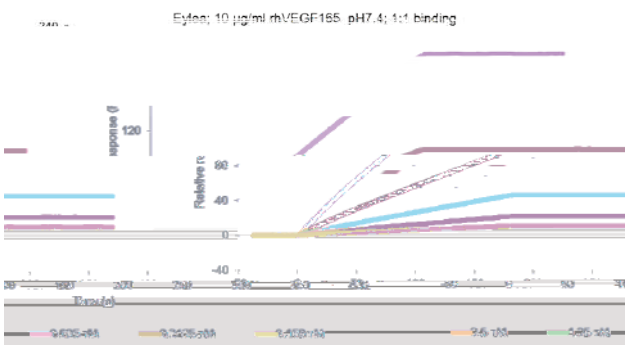
Binding assay (FACS, ELISA, SPR)

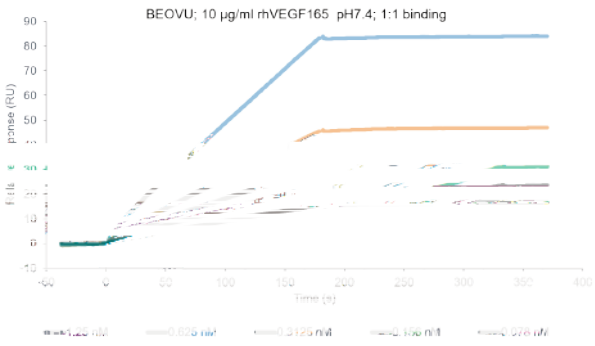


Dose-dependent binding of Inetetamab (anti-HER2) with HCC1954 cells were tested through FACS, the data showed that Inetetamab binds with HCC1954 cells with EC_{50} of 6.4 nM.

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.

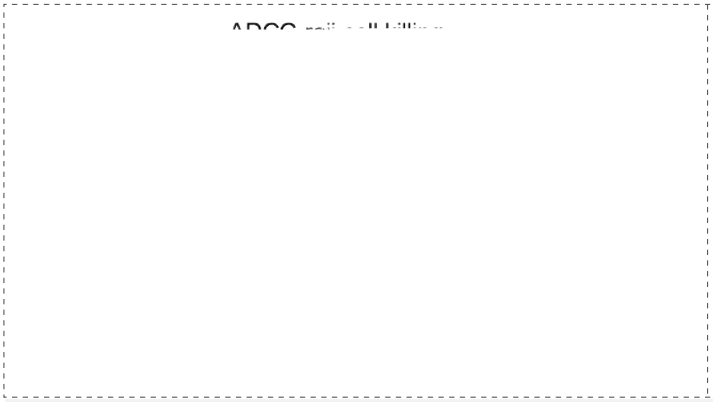




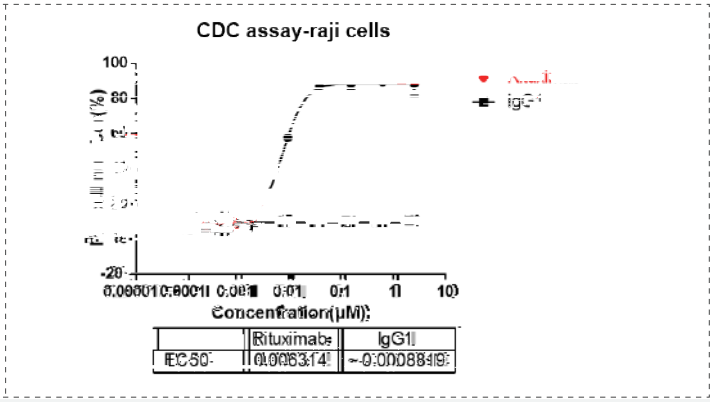
Eylea binding with hVEGF

BB-1701 binding with hHER2

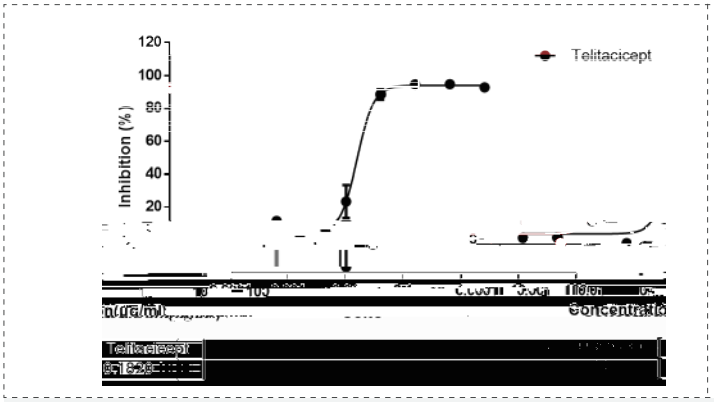
Functional assay for antibodies



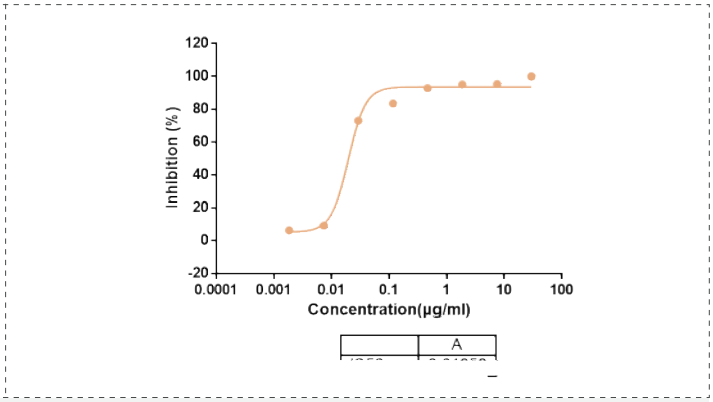
Raji cells were mixed with human PBMCs, and different doses of Rituximab was added to induce ADCC, the killing of raji cells were detected through FACS(CFSE labeling of raji).



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).



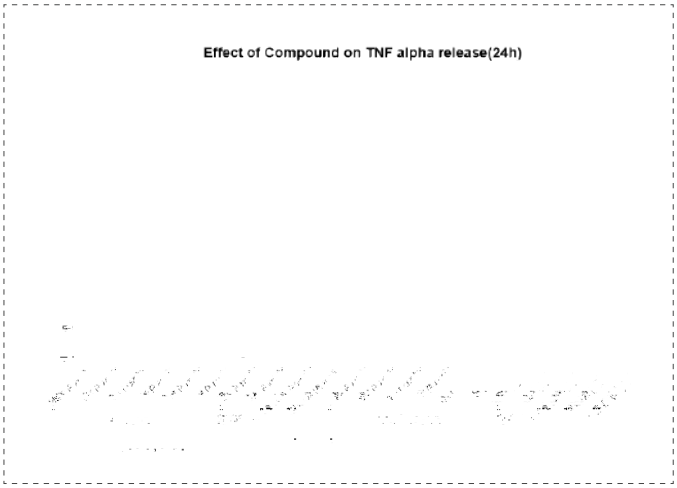
B cells were treated with Teliacicept and induced with Baff for 72 hours, ^{3}H-thymidine incorporation were analyzed through scintillation counting.



T cells were treated with compound A and induced with ICOSL for 72 hours, ^{3}H-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 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 offers comprehensive API process development and preparation R&D services tailored specifically for antibodies. Leveraging our established platform for cGMP API development, we have successfully produced cGMP APIs for clinical trials, supporting innovative drug companies in their endeavors. Our dedicated team remains committed to aiding antibody development through meticulous design of experiments (DOE), leveraging professional R&D technologies, adhering to standardized project management practices, and facilitating efficient communication channels.

Medicilon Case:LQ036

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.

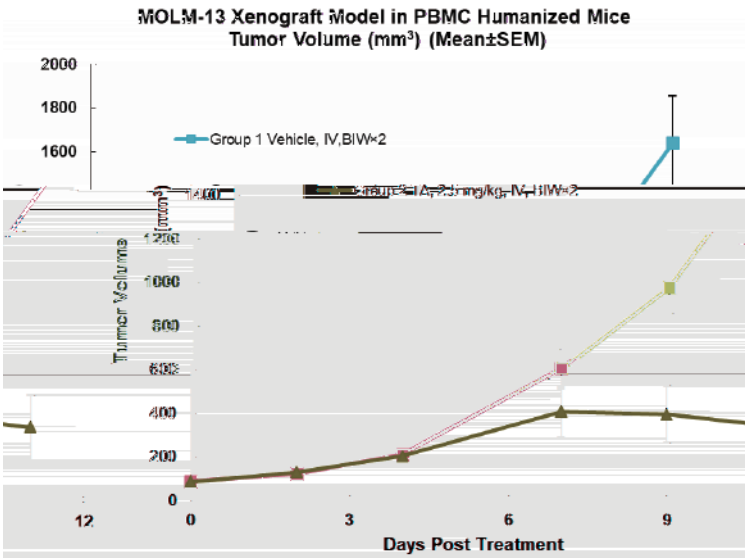
Pharmacology Evaluation of Antibodies

Medicilon offers a range of mature *in vivo* models for evaluating antibody efficacy, all meticulously established and maintained in compliance with AAALAC regulations. Our pharmacology studies adhere to GLP-like standards, ensuring robust and reliable results. Currently, Medicilon has established **400+** tumor evaluation models across six categories, providing a comprehensive platform for evaluating the efficacy of ADCs and other therapeutic agents.

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

			P
Vehicle	1639.53	/	/
An body	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

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} $_{1/2Z}$ was ~21.7 days and clearance was 0.11 mL/kg/h. This study was conducted by **Medicilon**.

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.**

In May 2022, Jimincare's lgE 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.

GT90008

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

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ate NK cells and $CD8^+$ T cells, enhance the cytotoxicity of cells against tumor targets, optimize the efficacy of chemother apy 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

References：

[1] Ivana Spasevska. An outlook on bispecific antibodies: Methods of production and therapeutic benefits.

[2] Abdullah F U H Saeed, et al. Antibody Engineering for Pursuing a Healthier Future. Front Microbiol. 2017 Mar 28;8:495. doi: 10.3389/fmicb.2017.00495.

[3] MA Lin-lin, et al. Construction and screening of phage display library for TIM-3 nanobody. Acta Pharmaceutica Sinica 2018, 53 (3): 388-395.

[4] Hyori Kim, et al. Preclinical development of a humanized neutralizing antibody targeting HGF. Exp Mol Med. 2017 Mar 24;49(3):e309. doi: 10.1038/emm.2017.21.