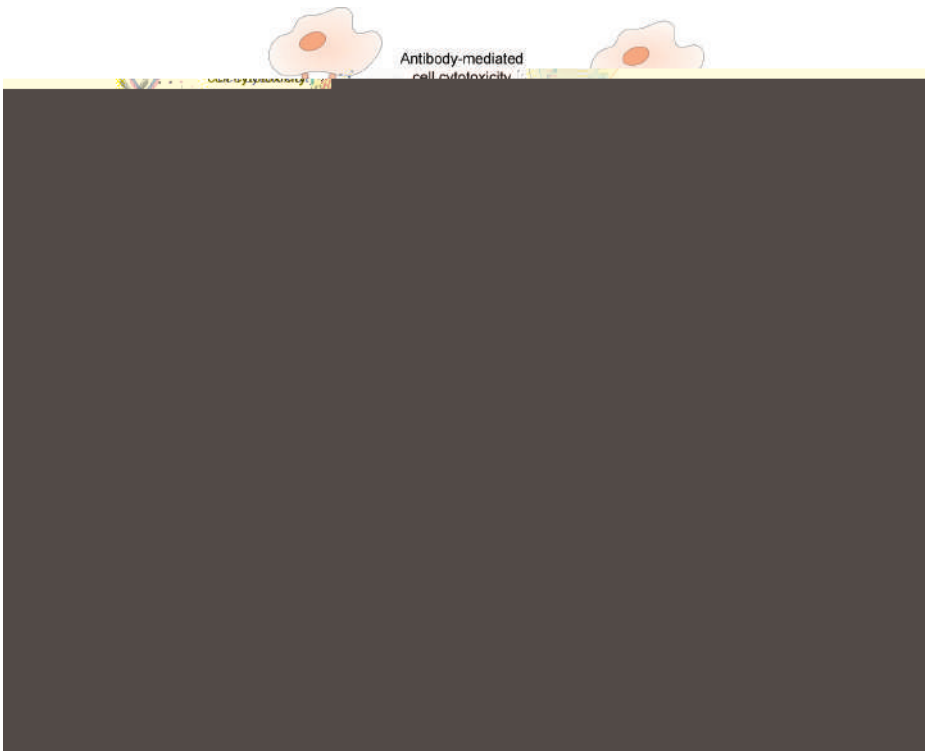
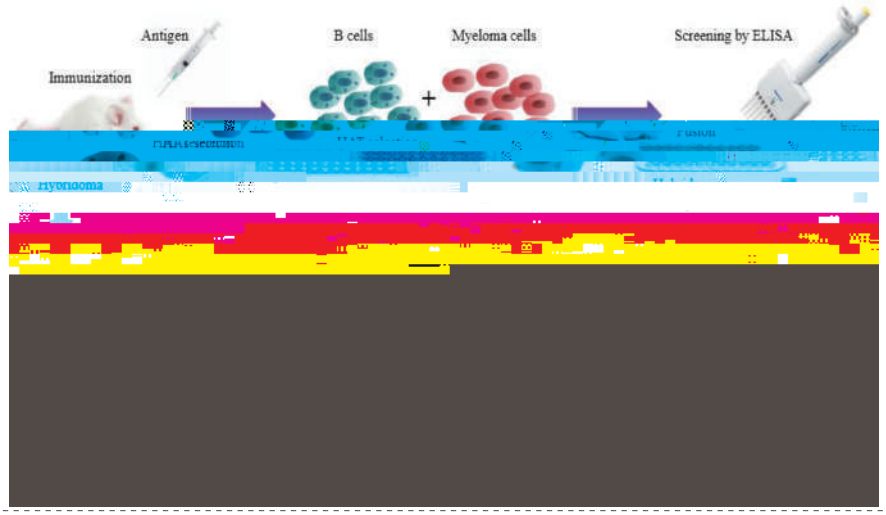
In today's pharmaceutical landscape, antibody drugs have ascended to a position of paramount importance globally. Representing pivotal therapeutic biological advancements, they stand at the forefront of life-saving disease treatments. At Medicilon, the development of antibody drugs is approached with 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.



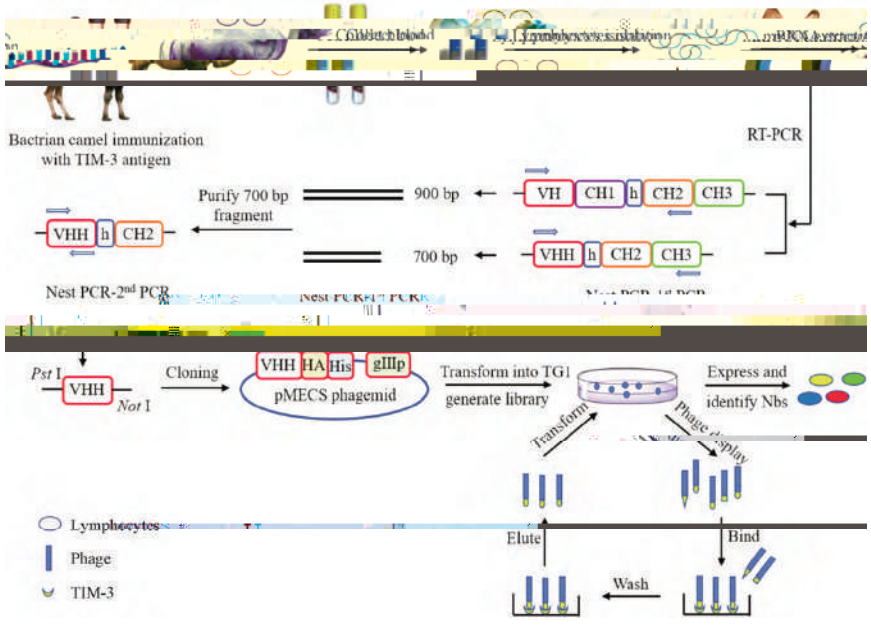
Overview of the natural function of antibodies[1]

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 of our valued clientele, delivering solutions tailored to their specific needs.



Production route of hybridoma technology[2]

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.

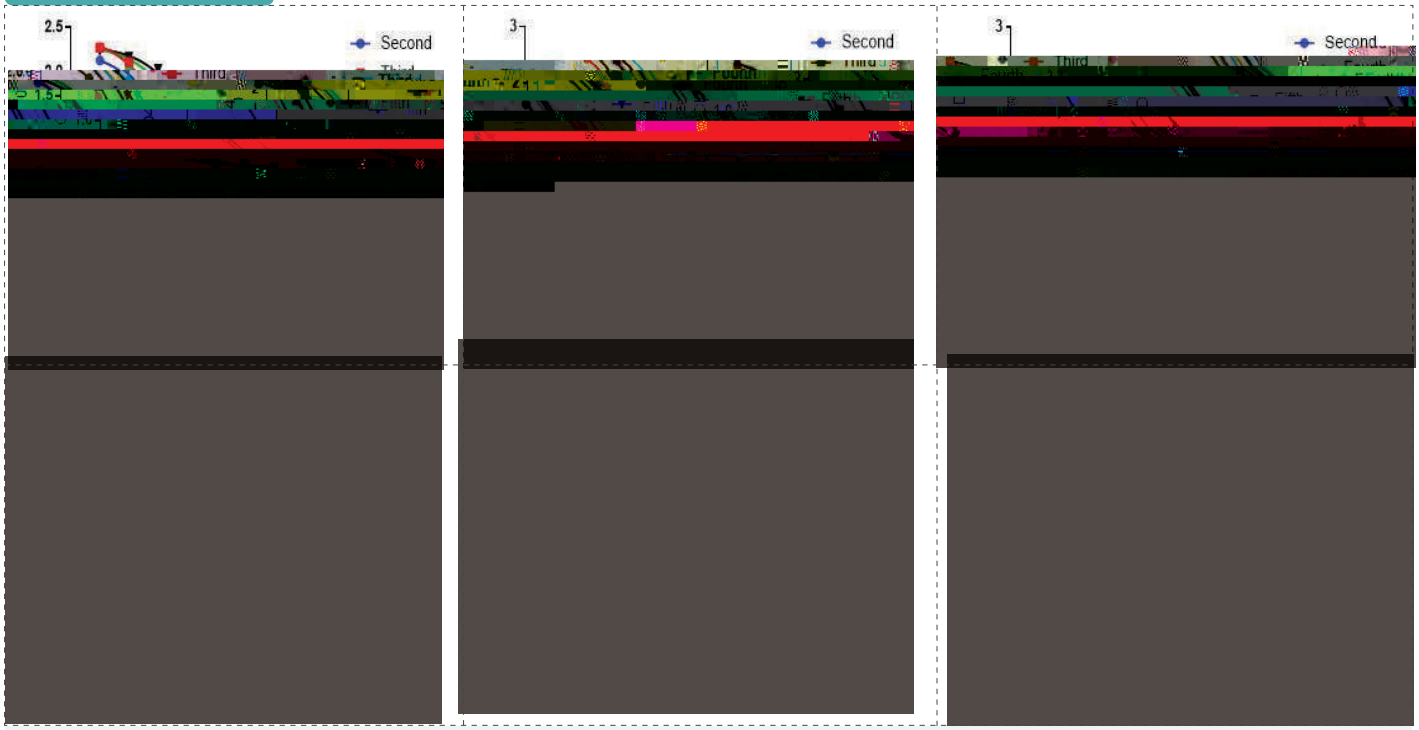


Nanobody production scheme using a phage display library[3]

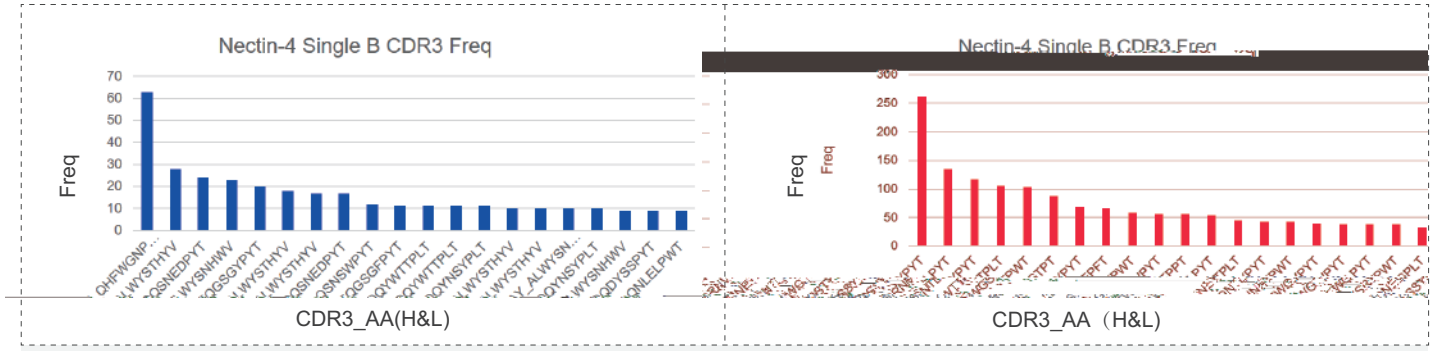
Single B cell screening represents a cutting-edge technique for the swift generation of monoclonal antibodies (mAbs) in recent years. Its underlying principle lies in the fact that each B cell harbors a singular pair of functional heavy and light chains, with each B cell generating a specific antibody 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 & 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

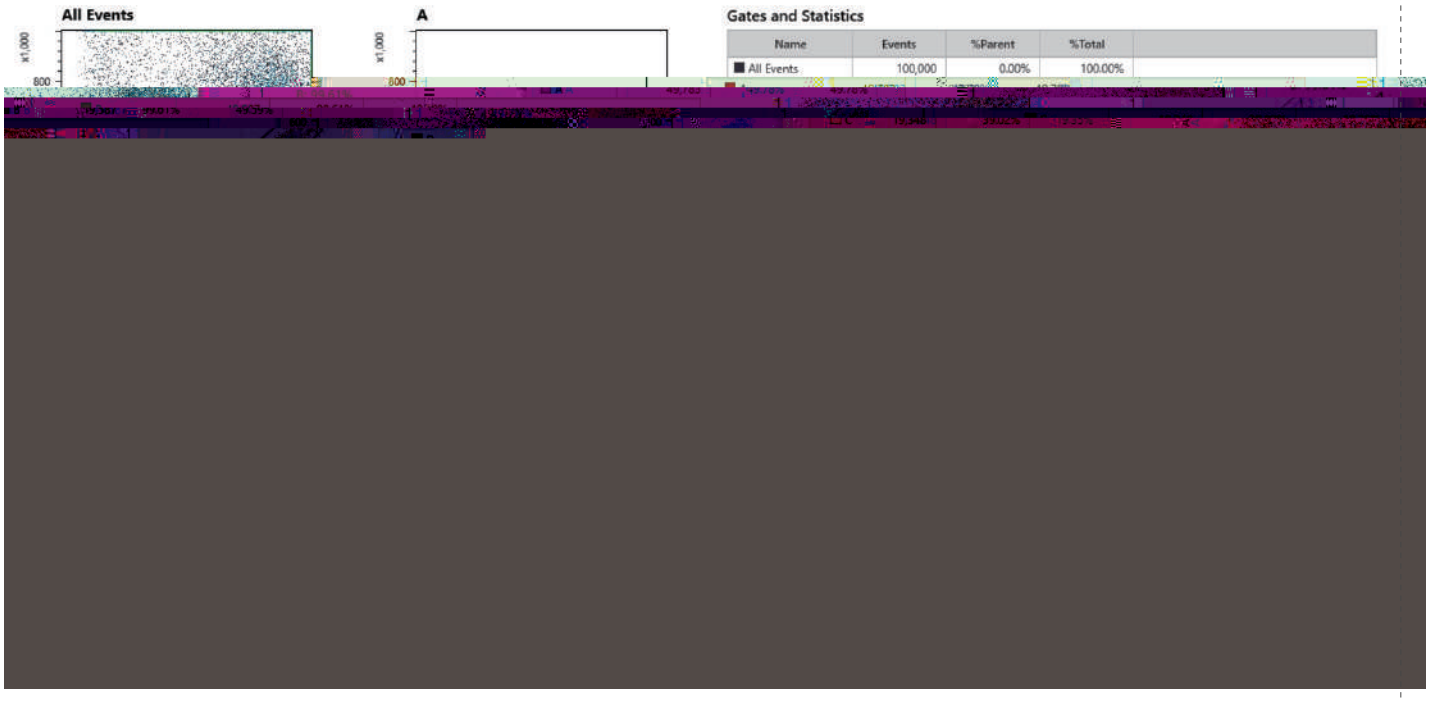


Nectin-4 Mouse Serum Titer



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

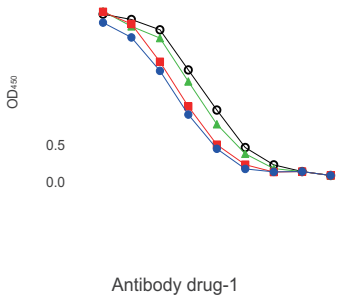
Nectin-4 (4-6) Sequencing (Top20)



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

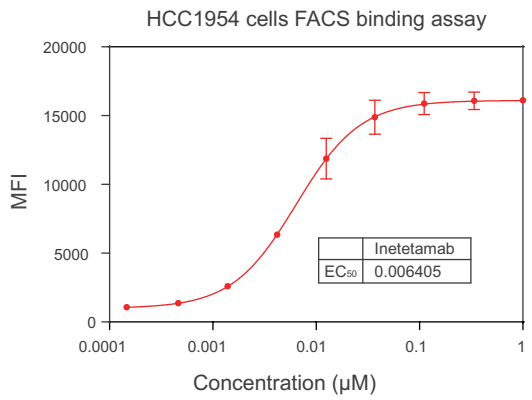
Nectin-4 (4-6) Sorting Single B Cell

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 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.

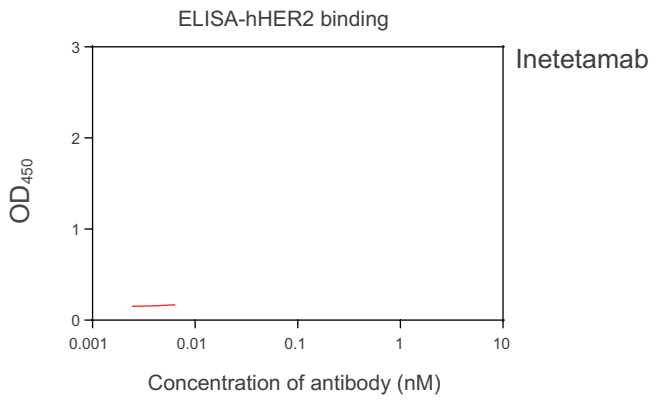


Antibody Titer after Purification

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.

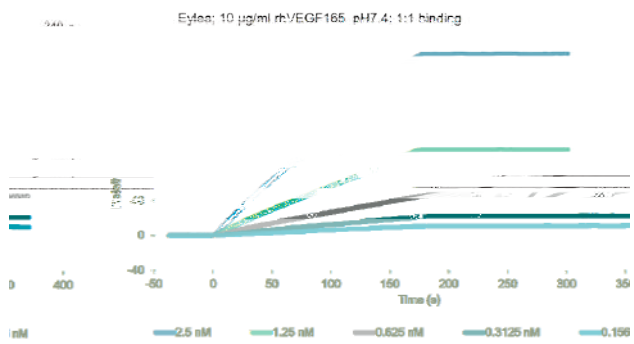


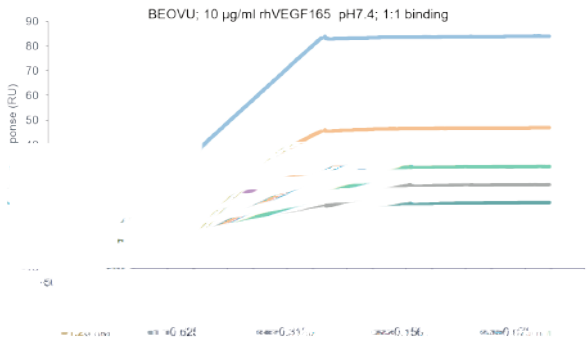
Dose-dependent binding of Inetetamab (anti-HER2) with HCC1954 cells were tested through FACS, the data showed that Inetetamab binds with HCC1954 cells with EC_{50} of 6.4 nM.



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.

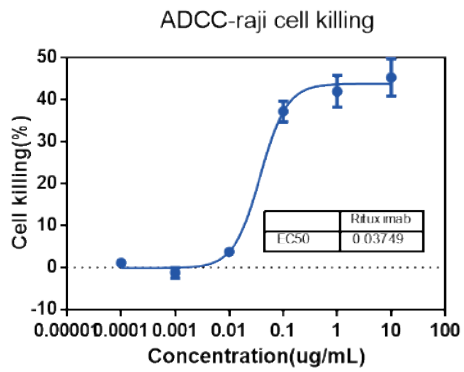
: PD-1, PD-L1, VEGF, Nectin1, Nectin2, Nectin3, Nectin4, NECL1, NECL2, NECL3, NECL4, NECL5, EPHA1, EPHA2, EPHA3, EPHA4, EPHA5, INSR, IGF-1R, HSA, FcRN, FcRI, FcRII, FcRIII, C1q, Factor B, HER2, Transferrin, EPCR, STAT3, STAT5, STAT1, 4-1BB, SHP2, ATIII, EGFR, gp1, etc.



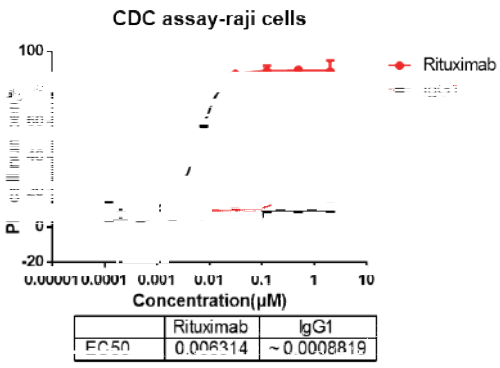


Eylea binding with hVEGF

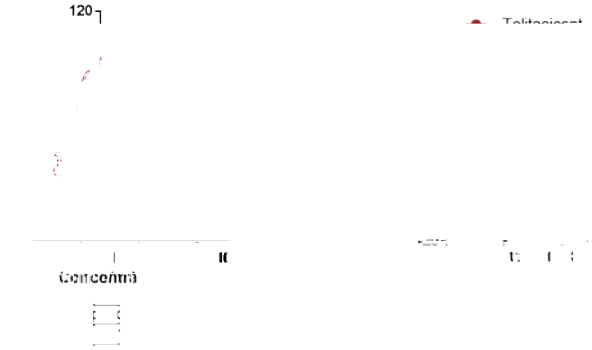
BB-1701 binding with hHER2



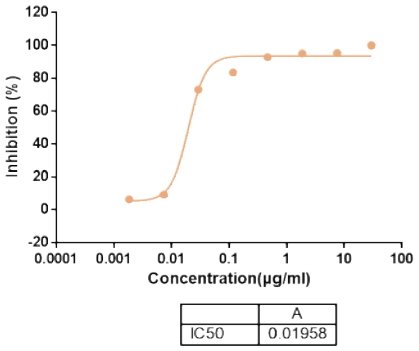
Raji cells were mixed with human PBMCs, and different doses of Rituximab was added to induce ADCC, the killing of raji cells were detected through FACS(CFSE labeling of raji).



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).

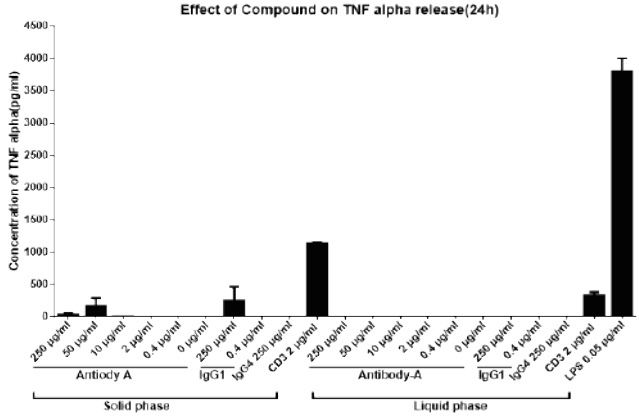


B cells were treated with Teliacicept and induced with Baff for 72 hours, ^{3}H-thymidine incorporation were analyzed through scintillation counting.



T cells were treated with compound A and induced with ICOSL for 72 hours, ^{3}H-thymidine incorporation were analyzed through scintillation counting.

- 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.

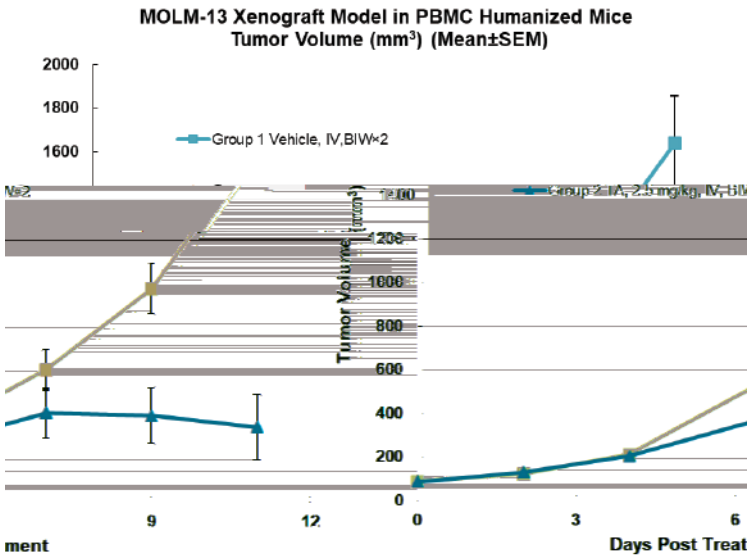


Medicilon offers comprehensive API process development and preparation R&D services tailored specifically for antibodies. Leveraging our established platform for cGMP API development, we have successfully produced cGMP APIs for clinical trials, supporting innovative drug companies in their endeavors. 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.

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.

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 tumor evaluation models across six categories, providing a comprehensive platform for evaluating the efficacy of ADCs and other therapeutic agents.

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



- **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

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

Mean Tumor Volum (mm^3)		

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

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 .

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

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 . On the basis of these results, a phase I clinical study is ongoing in patients with advanced solid tumors (NCT02499224).

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

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 .

Dose (mg kg^{-1} day^{-1})	Day	Statistic	C_{max} (ng ml^{-1})	C_{max}/Dose (kg × ng ml^{-1} mg^{-1})	T_{max}[a](h)	T_{last}[a](h)	AUC_{Tlast} (ng × h ml^{-1})	$AUC_{0\text{-}168hr}$ (ng × h ml^{-1})	$AUC_{0\text{-}168hr}$/Dose (kg × ng × 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]

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.

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.

In May 2022, Jimincare's lgE antibody drug JYB1904 has been approved for clinical trials. JYB1904 is a new anti-lgE 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.

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.

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.

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 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.

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.

:

[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.

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