Medicilon Nucleic Acid Drugs R&D Service Platform

Medicilon nucleic acid drug R&D platform provides an integrated and comprehensive solution that covers drug discovery, CMC and preclinical research services. Oriented with a rigorous scientific approach, an open-minded teamwork spirit and state-of-the-art equipment, our integrated solution will help clients and partners to fulfil their research and development mission for cutting-edge and innovative nucleic acid drugs. Our service platforms include nucleic acid drug discovery, screening and preclinical research services of pharmacology, DMPK and toxicity study for both pharmaceutical companies and academic research institutions.

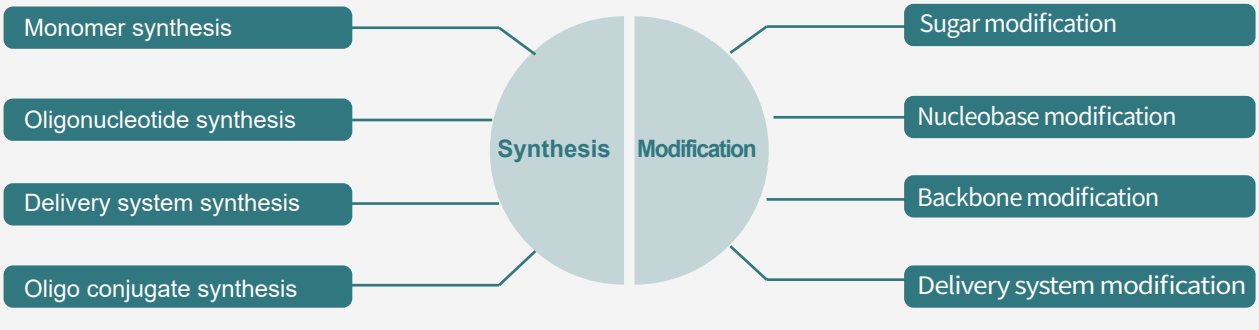
Popular Types of Nucleic Acid Drugs

ASO | **siRNA** | **mRNA** | **Aptamer**

Advantages of Nucleic Acid Drugs

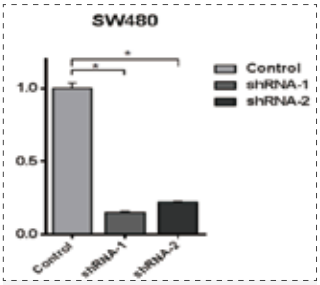
Attributed by its fast and intuitive design of base sequences, the development of nucleic acid drugs is featured with simple materials, convenient preparation processes and affordable production costs, which will greatly shorten the drug development cycle, making it possible to customize individual treatment plans. Hence, it offers a feasible solution for rare diseases and other problems currently plagued.

Synthesis & Chemical Modifications of Nucleic Acid Drugs

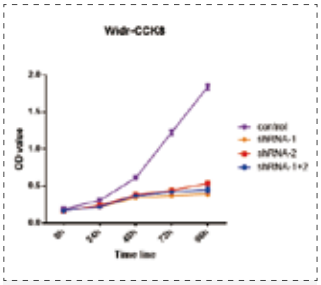


Bioactivity Screening of Nucleic Acid Drugs

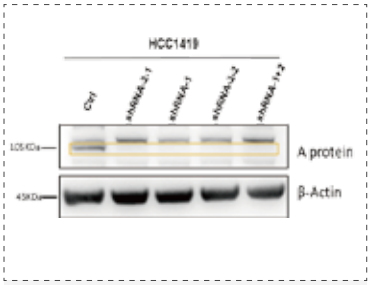
- Evaluation of binding between siRNA-GALNAc and targeted liver cells (ELISA, SPR, FP, FACS, MSD, Confocal microscope)
- Evaluation of decrease in target mRNA/protein level (RT-PCR, WB)
- Evaluation of cell phenotypes and functional regulation (Cell proliferation, Migration, Proteomics, and Transcriptome analysis).



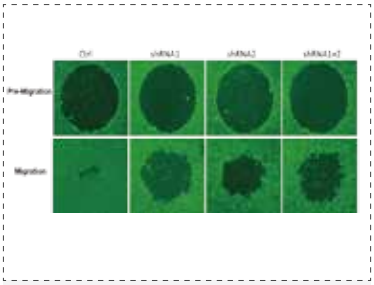
Q-PCR



Cell proliferation

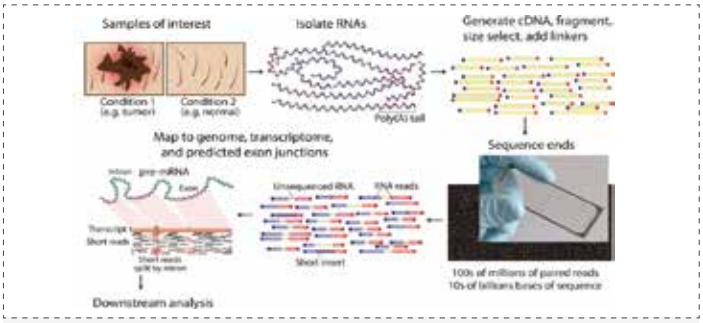


Western Blot

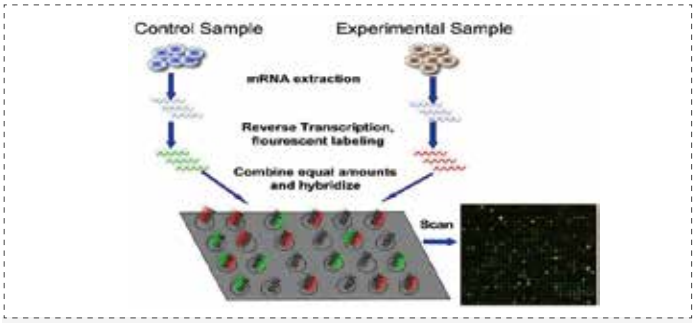


Cell migration

- Evaluation of off-target effect
  - Searching for potential off-target mRNA/protein in the database, such as NCBI, nucleotide BLAST.
  - Unbiased analysis applying RNAseq, RNA Microarray, or targeted panel analysis using Nanostring.



RNA Seq



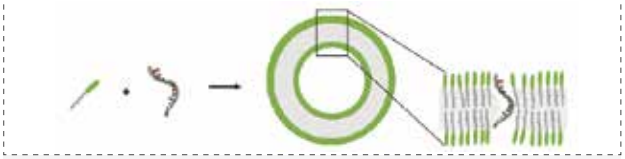
RNA Microarray

Process R&D of Nucleic Acid Drugs

Select starting materials	Choose starting materials with traits like easy to purchase, mild toxicity, good quality stability.
Process R&D of nucleic acid	Develop stable and green synthesis routes with low cost and high security.
Quality Control	Up-to-date quality control system with complete technical standard.
Select starting materials	End to end service ensuring smooth transfer.

Nucleic Acid Drugs Preparation Study

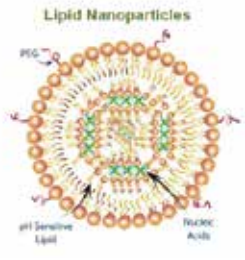
Due to their low immunogenicity, biocompatibility, and high encapsulation efficiency for oligonucleotide molecules, lipids and their derivatives have become the go-to delivery systems for nucleic acid drugs that have attracted much attention in recent years. The system is positively charged in the physiological environment.

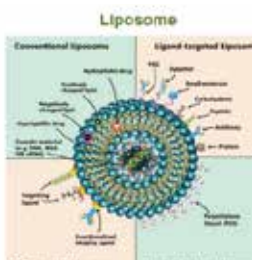


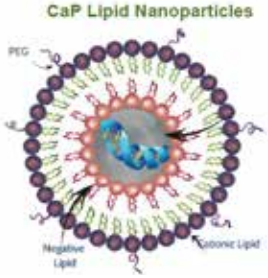
Picture Source: Science China Life Sciences volume 62,pages333-348(2019)

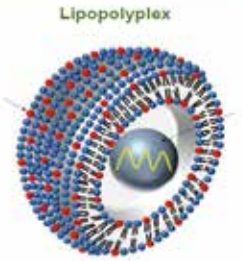
The negatively charged nucleic acid molecules are encapsulated by electrostatic action, and the positively charged surface can also help the entire carrier system to combine with the cell membrane of the target cell, thereby playing a delivery role.

Common delivery systems

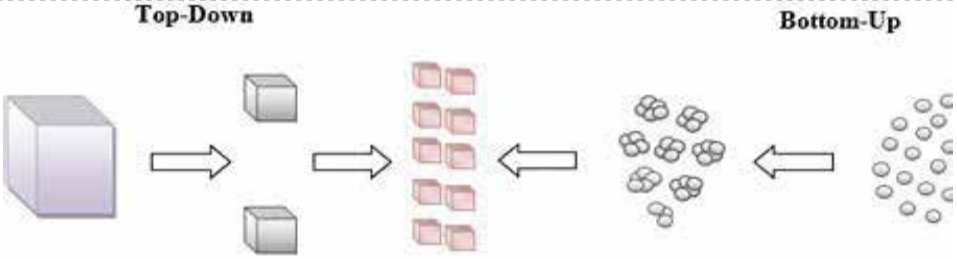








Medicilon's preparation methods of nanoparticles



Ball milling, high-pressure homogenization, ultrasound, extruding

Solvent volatilization, precipitation, T-tube, microfluidics

Traits of successful delivery systems

Easily modified, easily synthesized, easily produced.The on-target and off-target ratio of delivery should be within an acceptable range.The effective dose must be significantly lower than the toxic dose.The bioactivity of the nucleic acid should be consistent from batch to batch.In most clinical cases, repeated administration does not result in loss of efficacy or safety.

Medicilon's nanoparticle R&D lab

Spray dryer

Agilent InfinityLab Bio LC

Thermo U3000+CAD detector Thermo

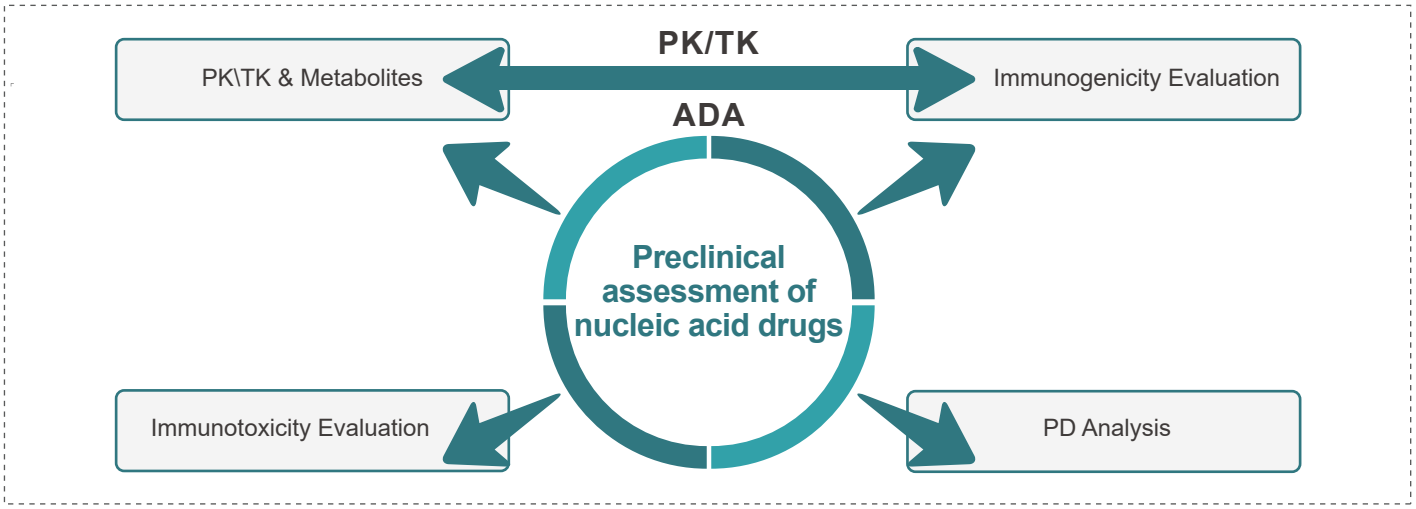
Agilent biological analyze

ZETA nanoparticle Potentiostat

Nucleic acid lipid system R&D

- Formulation: drug to lipid ratio, solvent screening, aqueous to organic solvent ratio
- Process: Preparation methods
- Stability
- Dosage form screening

Bioanalysis of Nucleic Acid Drugs



PK/TK Analysis	• Molecular hybridization-enzyme assay (H-ELISA) • Molecular hybridization-electrochemiluminescence analysis (H-ECL) • Reverse transcription fluorescence quantitative PCR (RT-qPCR) • Quantitative PCR (qPCR) • Digital Microdrop (ddPCR) • LC-MS/MS Platform
Immunogenicity Analysis	• Total Anti-Drug Antibody (ADA) Assay: MSD • Neutralizing antibody (Nab) analysis: CLBA or Cell-based Assay
PD or TOX-related Cytokine & Biomarker	• Singleplex (based on various LBA technologies) • Multiplex (Luminex, MSD, FACS CBA technologies) • FACS

Solutions for Nucleic Acid Drugs Bioanalysis

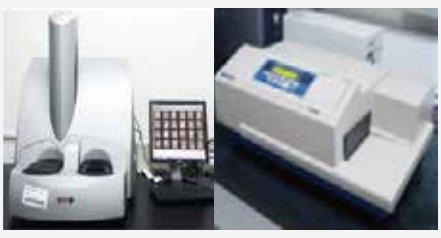
LC-MS/MS/HRMS Platform

- High specificity
- High sensitivity: ng level
- Advantages: end product detectable

qPCR/ddPCR Platform

- High specificity
- Sensitivity: Detectable within 1 log copy
- Advantage： More Sensitive

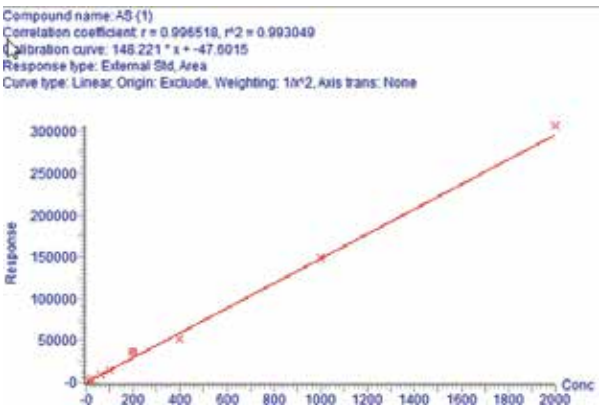
Hybridization-EIA/ECL Platform



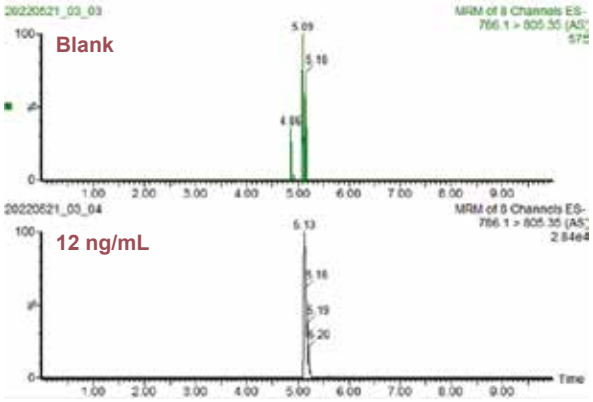
- Sensitivity: pM level
- Advantages: variable marking strategy; personalized reaction strategy.

Medicilon case: siRNA plasma quantification (LC-MS/MS)

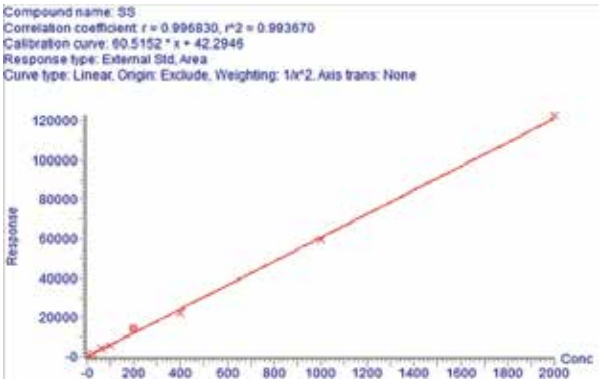
siRNA-AS linearity



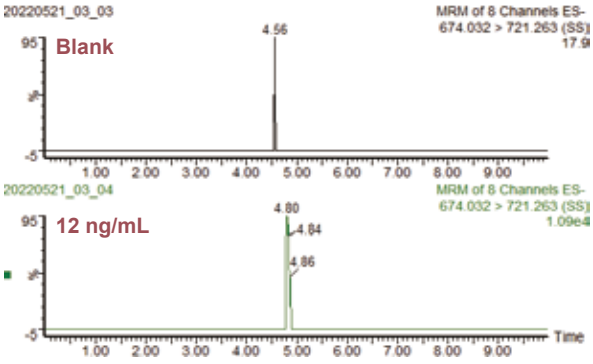
siRNA-AS 10 ng/mL



siRNA-SS linearity



siRNA-SS 10 ng/mL



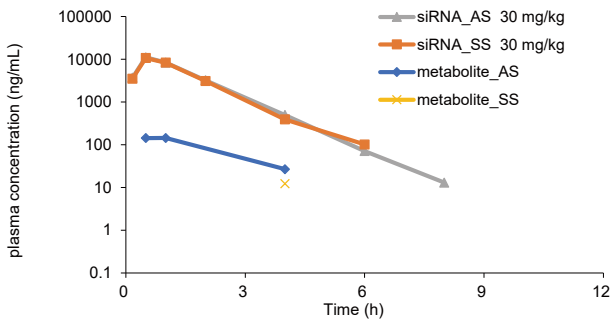
Sensitivity: 10 ng/ml

20 µL plasma!

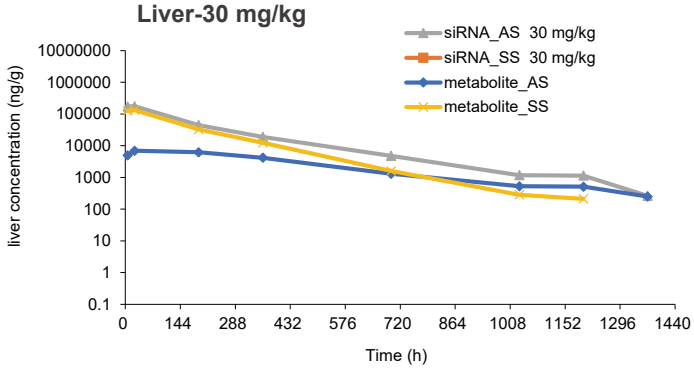
- **1344 injections (runs)**
- **Good reproducibility**
- **CV< 10%**
- **LC-MS/MS**
- **1 ng/mL feasible by MS!**

Medicilon case: siRNA and metabolite in rodent plasma and liver

Plasma-30 mg/kg



Liver-30 mg/kg



Administration Route	Dose Level mg/kg	Analyte	AUC_{last_liver} hr*ng/g	AUC_{last_Plasma} hr*ng/g	$AUC_{last\_liver}/AUC_{last\_Plasma}$
SC	30	siRNA_AS	32976645	17893	1843
SC	300	siRNA_AS	94450628	219970	429

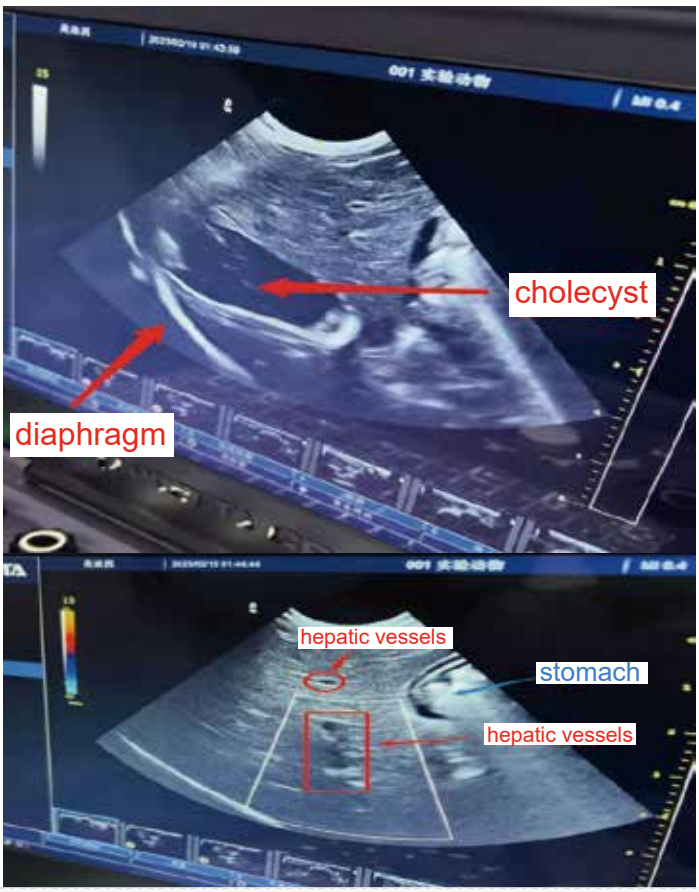
Pharmacokinetics Research of Nucleic Acid Drugs

Medicilon Liver Biopsy Guided By B-ultrasound In Cynomolgus Monkeys Platform

The development of gene therapy and nucleic acid drugs has made the establishment of monkey models and related research a hot topic. Due to the high similarity of genetic, morphological, physiological and biochemical characteristics with humans, non-human primates, especially cynomolgus monkeys, are closest to humans in terms of evolution, and have outstanding advantages in model construction, disease mechanism research, and drug development. Many disease models have been established so far.

In the long-term dynamic experimental observation of the changes in the liver disease model of cynomolgus monkeys, due to the limitations of animal disease models and experimental objective conditions, researchers mostly obtain liver tissue pathological analysis and diagnosis of these disease models through blind puncture or surgical sampling, which not only causes great trauma to animals, complicated postoperative care, but also easily leads to various complications, which is not conducive to long-term observation of disease models.

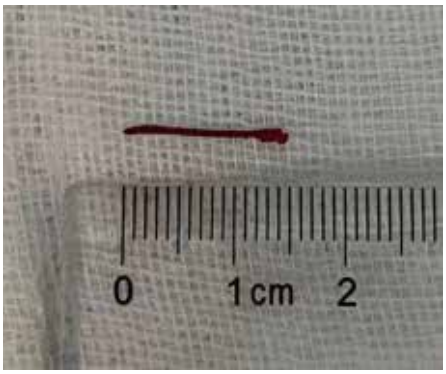
The Medicilon Liver Biopsy Guided By B-ultrasound In Cynomolgus Monkeys Platform can avoid the large blood vessels and gallbladder to the greatest extent, and has the advantages of less trauma, safe and simple puncture operation, accurate positioning, and better postoperative recovery. Medicilon Liver Biopsy Guided By B-ultrasound In Cynomolgus Monkeys Platform can dynamically display the whole process of biopsy needle insertion and material collection in real time, which greatly improves the success rate of puncture and the accuracy of experimental results. At the same time, it can be used for the preclinical PK evaluation of gene therapy drugs, which also promotes the improvement of experimental animal welfare, and provides accurate pathological basis for the dynamic monitoring and modeling progress of various liver disease models.



Liver biopsy guided by B-ultrasound in monkeys

Oligonucleotide: Monkey liver biopsy validation

Day 3	Dosing	Day 14	Day 28	Day 56
The 1st liver biopsy **Collection amount:** 10~20 mg/puncture needle		**The 2nd liver biopsy** **Collection amount:** 10~20 mg/puncture needle	**The 3rd liver biopsy** **Collection amount:** 10~20 mg/puncture needle	**The 4th liver biopsy** **Collection amount:** 10~20 mg/puncture needle



Biopsy gun & needle

Oligonucleotide: Monkey liver biopsy validation (surgery)

Day 1	Day 15	Day 29	Day 43
The 1st liver biopsy **Collection amount:** ~80-100 mg	**The 2nd liver biopsy** **Collection amount:** ~80-100 mg	**The 3rd liver biopsy** **Collection amount:** ~80-100 mg	**The 4th liver biopsy** **Collection amount:** ~80-100 mg

Surgery liver collection strategies :
recovery time between samples: 2-3 weeks (according to collection times)
monkey body weight: 3-6 kg

Oligonucleotide: Monkey muscle biopsy validation

Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 14

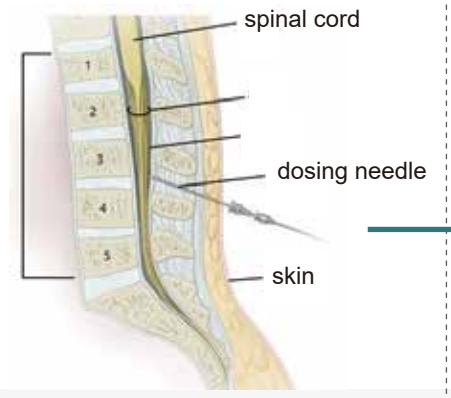
The 1st biopsy **Collection site:** Right quadriceps **surgical instruments:** surgical scissors	**The 2nd biopsy** **Collection site:** Right quadriceps **surgical instruments:** scalpel	**The 3rd biopsy** **Collection site:** Right quadriceps **surgical instruments:** biopsy punch

Animals:
select suitable animals,
body weight (monkey 3-6 kg; Dogs 8-12 kg)

Medicilon Case: siRNA monkey PK/PD study

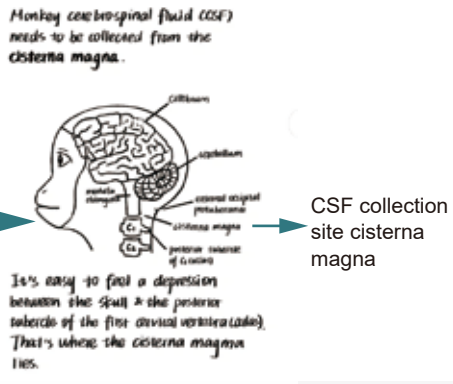
Compound	Monkey Matrix	Animal Number	BA Assay
siRNA IV infusion	Plasma Muscle biopsy Liver biopsy	N=2	▪ Cytokine study ▪ Complement study ▪ Lipid study ▪ Cir-luc mRNA ▪ IHC slide ▪ hELISA study ▪ MSTN Protein
	Plasma Muscle biopsy	N=2	▪ hELISA study ▪ NHP mRNA ▪ NHP MSTN Protein ▪ IHC slide ▪ Cytokine study ▪ Complement study

Medicilon Case: Monkey IT validation work flow by concentration



monkey IT dosing schematic lumbar

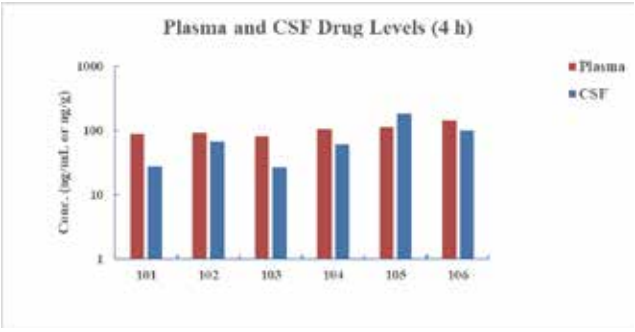
monkey dose location: lumbar
X-ray imaging monitoring

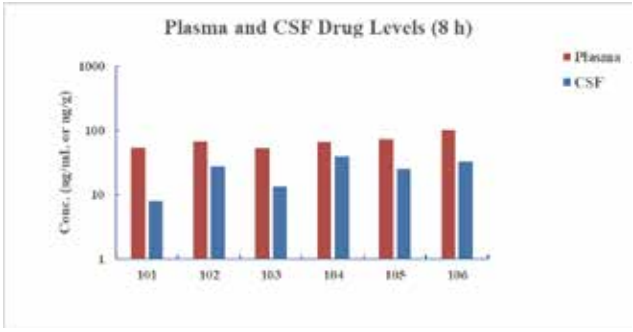


monkey CSF collection schematic

Group No.	Test Material	Dose Level	Route & Regimen	Dose Rate	Plasma & CSF Collection
1	MED-002	6 mg/Monkey	IT on Day 1 6 monkeys	2 mL (Infusion, 3 min) Location: Lumbar	Post-dose at 4 h and 8 h

Medicilon Case: Monkey IT validation results by concentration





The coefficient of variation (CV%)

Plasma: 21.1%~26.4% **CSF: 48.2%~74.3%** **Success rate: 6/6, 100%**

Group No.	Test Material	Dose Level	Route & Regimen	Dose Rate	Plasma & CSF Collection
1	MED-002	6 mg/Monkey	IT on Day 1 6 monkeys	2 mL (Infusion, 3 min) Location: Lumbar	Post-dose at 4 h and 8 h

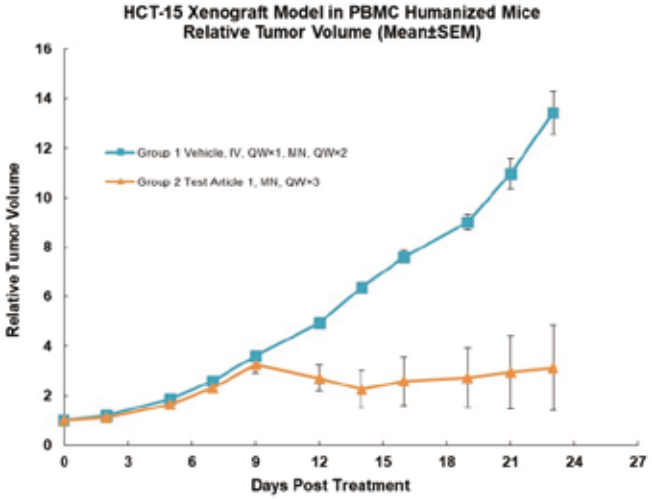
Pharmacology Evaluation of Nucleic Acid Drugs

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

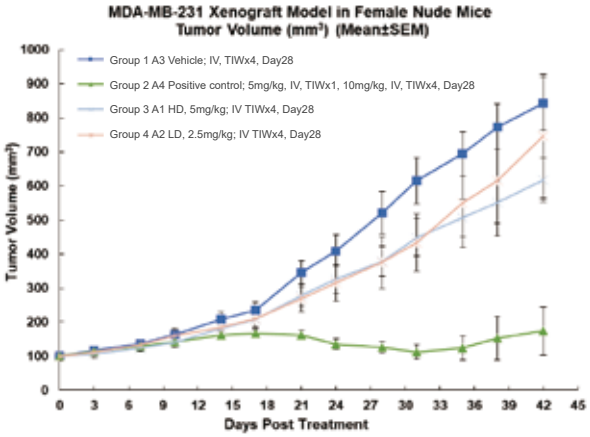
- Rodents: Mouse/Rat, Rabbit
- Non-Rodents: Beagle Dog, Mini Pig, Non-human Primate

Medicilon Case: mRNA in PBMC humanized mice



- **Animals:**
  Female NOG mice
- **Tumor Cells:**
  HCT15, $2x10^6$/mouse
- **PBMC:**
  $5x10^6$/mouse
- **Treatment:**
  Intracutaneous injection

Medicilon Case: Comparing different drug delivery methods of mRNA

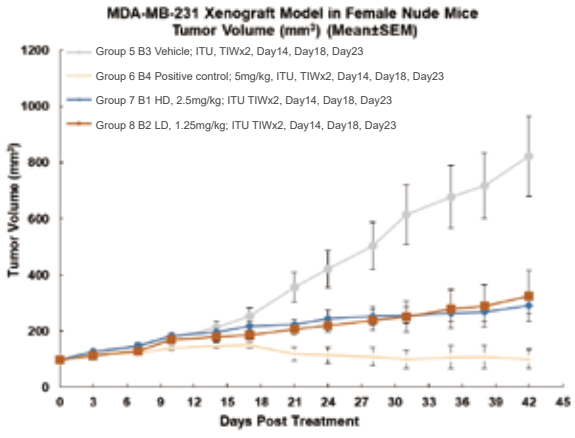


Animals: Female BALB/c Nude mice

Cells: MDA-MB-231, $5x10^6$/mouse

Model Establishment: Right flank SC injection

Treatment: **IV** injection; TIW (three times a week); Group3, 4: mRNA (LNP) group.



Animals: Female BALB/c Nude mice

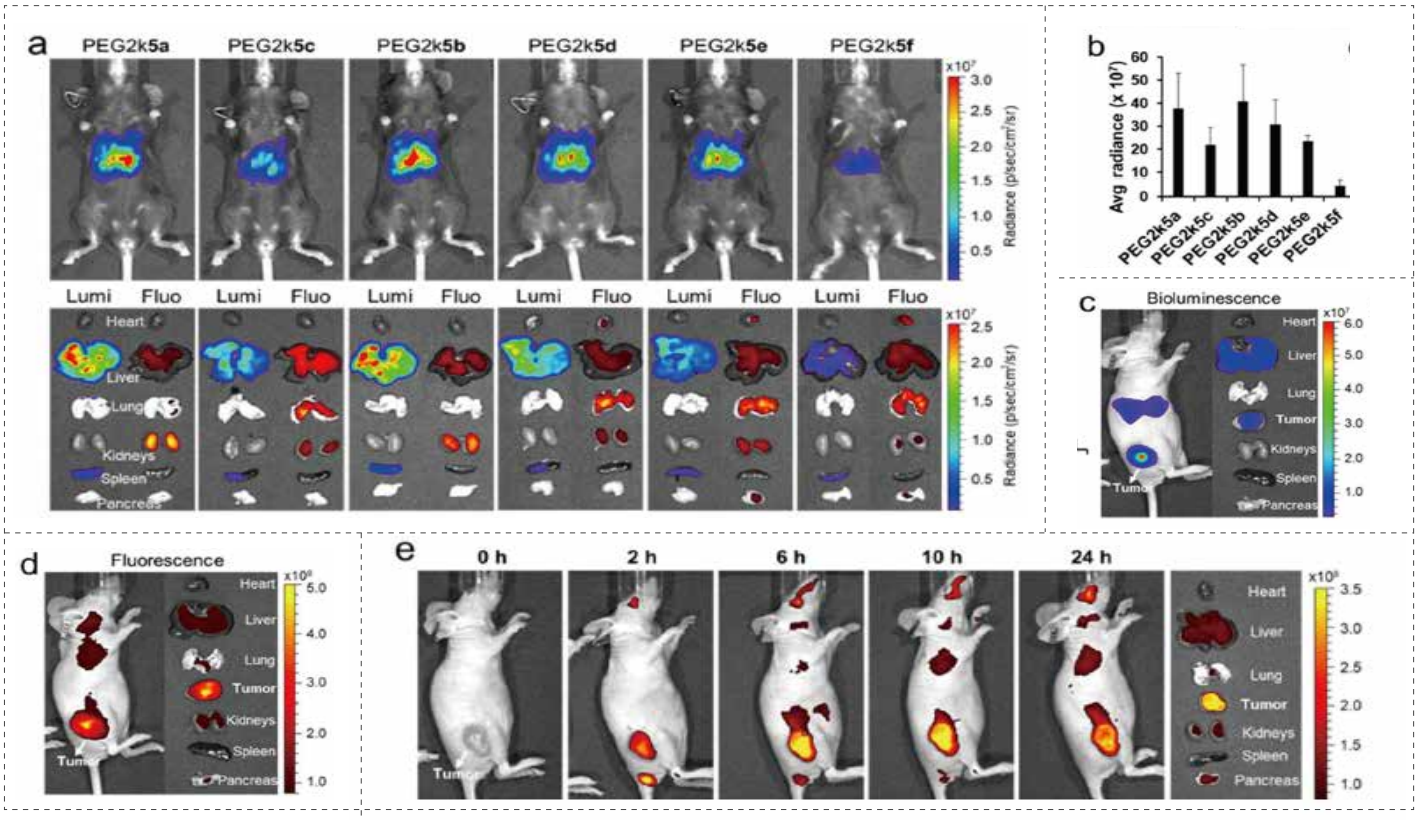
Cells: MDA-MB-231, $5x10^6$/mouse

Model Establishment: Right flank SC injection

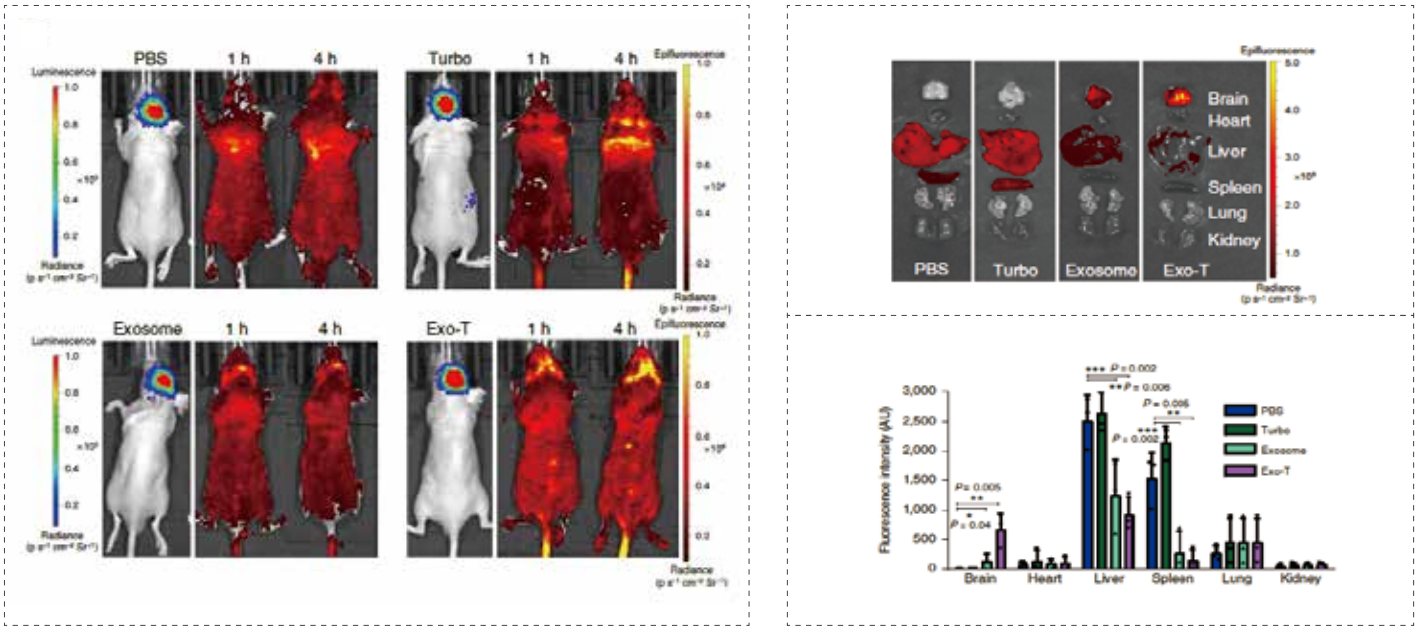
Treatment: **Intratumor** injection; TIW (three times a week); Group 7, 8: mRNA (LNP) group.

Medicilon Case: *In vivo* imaging to study the *in vivo* distribution of nucleic acid drugs

- Dendrimer LNP
- Luc mRNA; IV and intra-tumoral



- CNP-generated exosome
- Delivery of PTEN mRNA by Glioma-directed Exosome EXO-T,IV



Syngeneic mouse models

Cancer Type	Cell Line
Bladder Cancer	MB49
Brain Cancer	G261
Breast Cancer	4T1, EMT6, JC, EO771
Colon Cancer	CT26.WT, MC-38, Colon26
Leukemia	C1498, L1210, WEHI-3
Liver Cancer	H22, Hepa 1-6
Lung Cancer	LLC1, KLN205
Lymphoma	A20, EL4, L5178-R, E.G7-OVA
Mastocytoma	P815
Melanoma	B16-F10, Clone-M3
Pancreas Cancer	Panc 02
Renal Cance	RENCA
Myeloma	J558

Humanized mouse models

Cancer Type	Cell Lines in PBMC or HSC CD34$^+$ Humanized Mice
Brain Cancer	U-87 MG
Breast Cancer	HCC1954, MDA-MB-231, JIMT-1
Colon Cancer	HT29, LoVo, Ls174T, HT-15
Gastric Cancer	NCI-N87, NUGC-4
Leukemia	THP-1
Lung Cancer	HCC827, NCI-H1975, NCI-H292, A549
Lymphoma	Raji, TMD8, MOLM-13
Melanoma	A375
Myeloma	RPMI-8226, NCI-H929, MM.1S
Ovarian Cancer	OVCAR-3
Pancreatic Cancer	Capan-2
Renal Cancer	786-O
Skin Cancer	A431

Luciferase Cell Line	G261-luc, 4T1-luc, MC38-luc, H22-luc, B16-F10-luc, LLC1-luc

MEDICILON

Email: marketing@medicilon.com

Address: 50 Soldiers Field Place, Boston, MA 02135

Website: www.medicilon.com **Tel:** +1(626)986-9880