

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

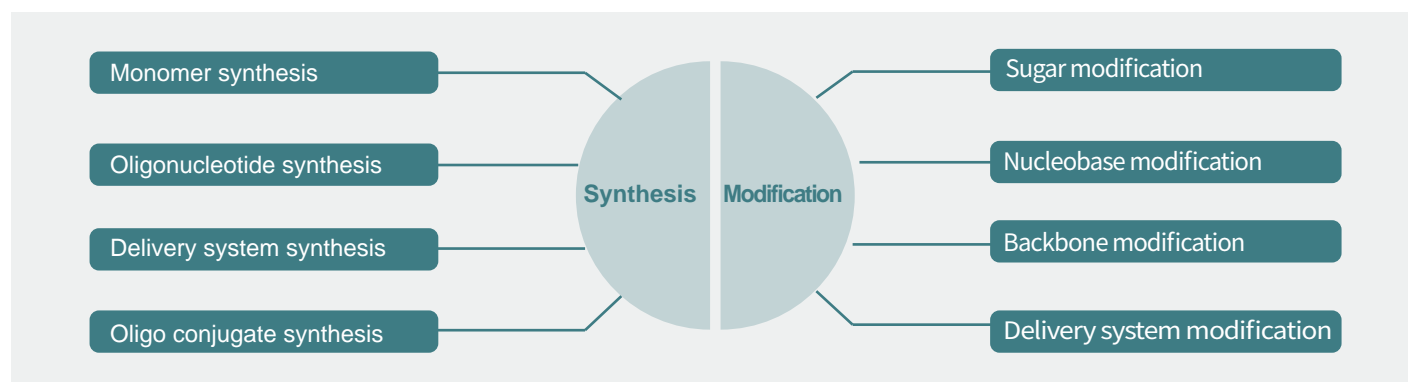
mRNA

Aptamer

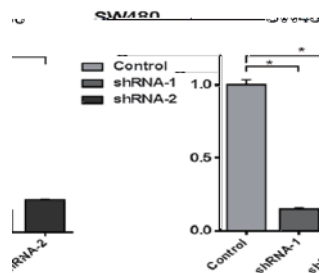
Advantages of Nucleic Acid Drugs

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.

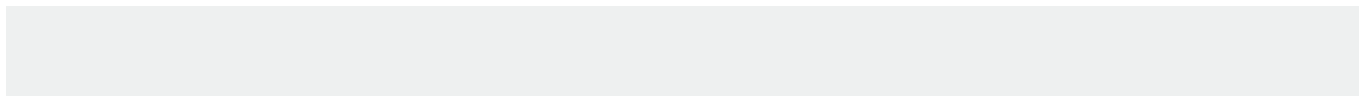
Synthesis & Chemical Modifications of Nucleic Acid Drugs



- 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).



- Evaluation of off-target effect



RNA Seq

Control Sample
Experimental Sample

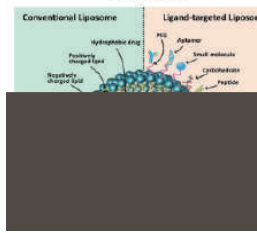
RNA Microarray

Common delivery systems

Lipid Nanoparticles



Liposome



Medicilon's preparation methods of nanoparticles

Top-Down

Bottom-Up



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

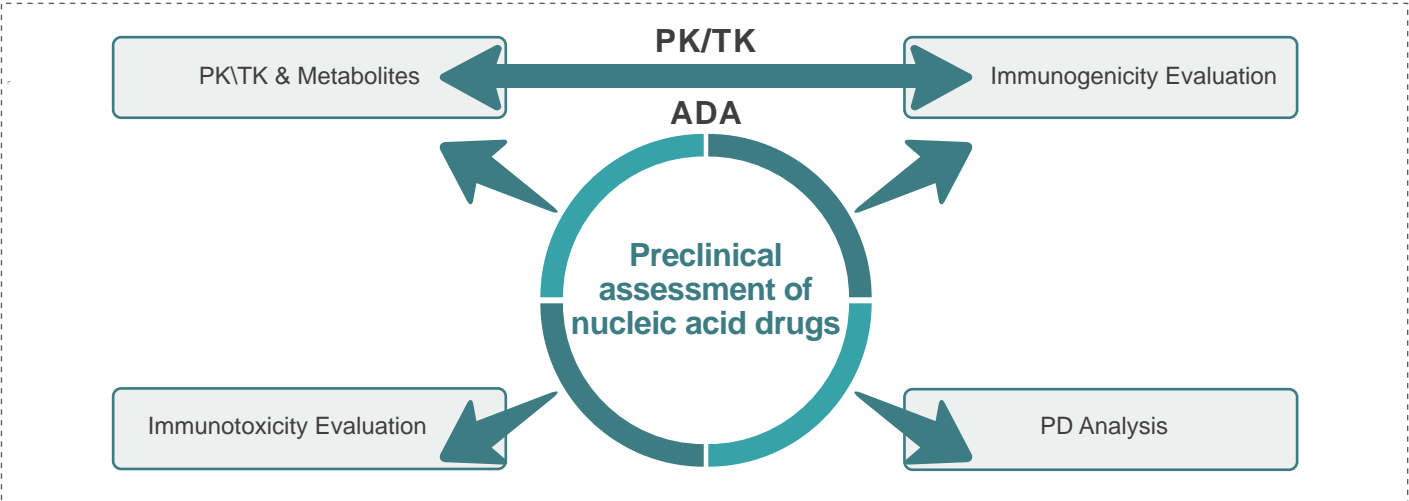


ZETA nanoparticle
Potentiostat

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



<p>PK/TK Analysis</p>	<ul style="list-style-type: none"> • 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
<p>Immunogenicity Analysis</p>	<ul style="list-style-type: none"> • Total Anti-Drug Antibody (ADA) Assay: MSD • Neutralizing antibody (Nab) analysis: CLBA or Cell-based Assay
<p>PD or TOX-related Cytokine & Biomarker</p>	<ul style="list-style-type: none"> • 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



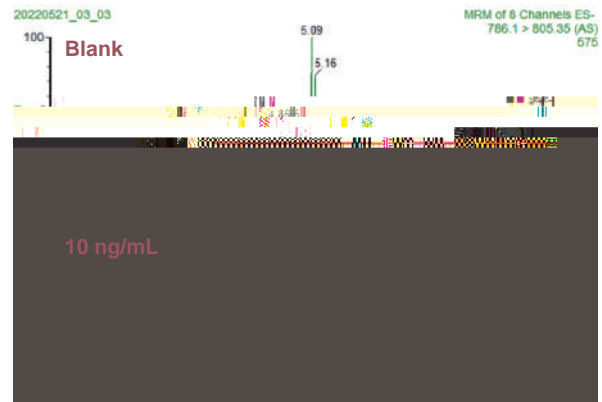
- Sensitivity: pM level
- Advantages: variable marking strategy; personalized reaction strategy.

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

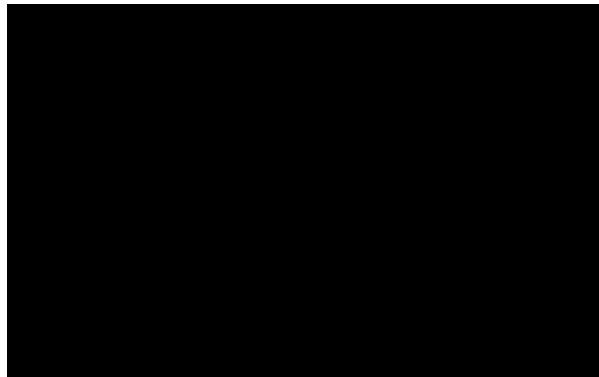
Compound A siRNA-AS linearity



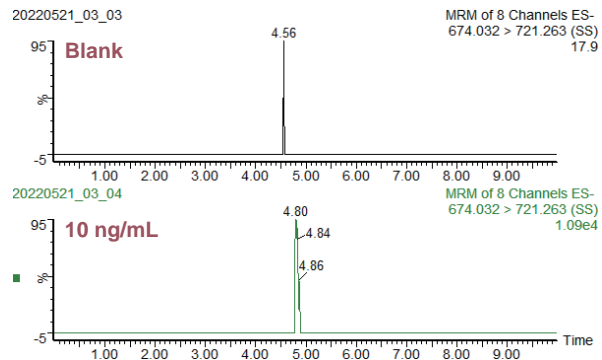
Compound A siRNA-AS



Compound A siRNA-SS linearity



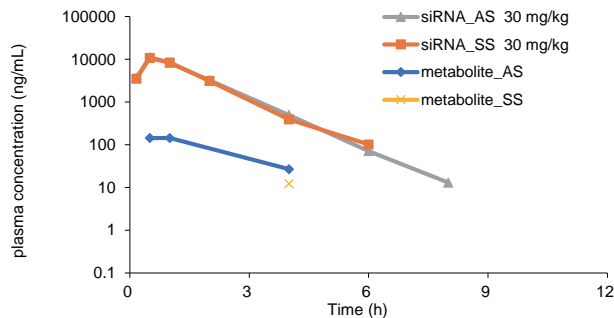
Compound A siRNA-SS



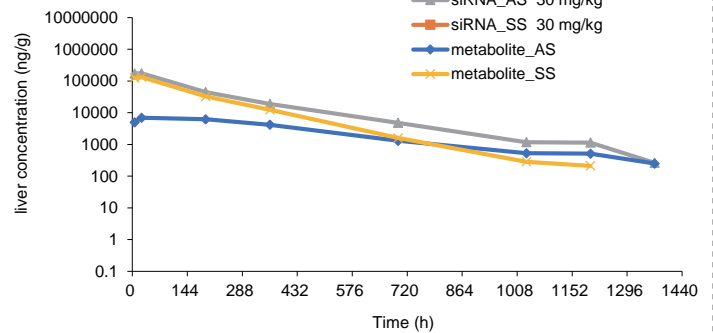
- Sensitivity: 1-10 ng/mL
- 20 μ L plasma
- 1344 injections (runs)
- Good reproducibility
- CV < 10%
- LC-MS/MS
- 1 ng/mL feasible by MS

Medicilon case: siRNA and metabolite in rodent plasma and liver

Plasma-30 mg/kg



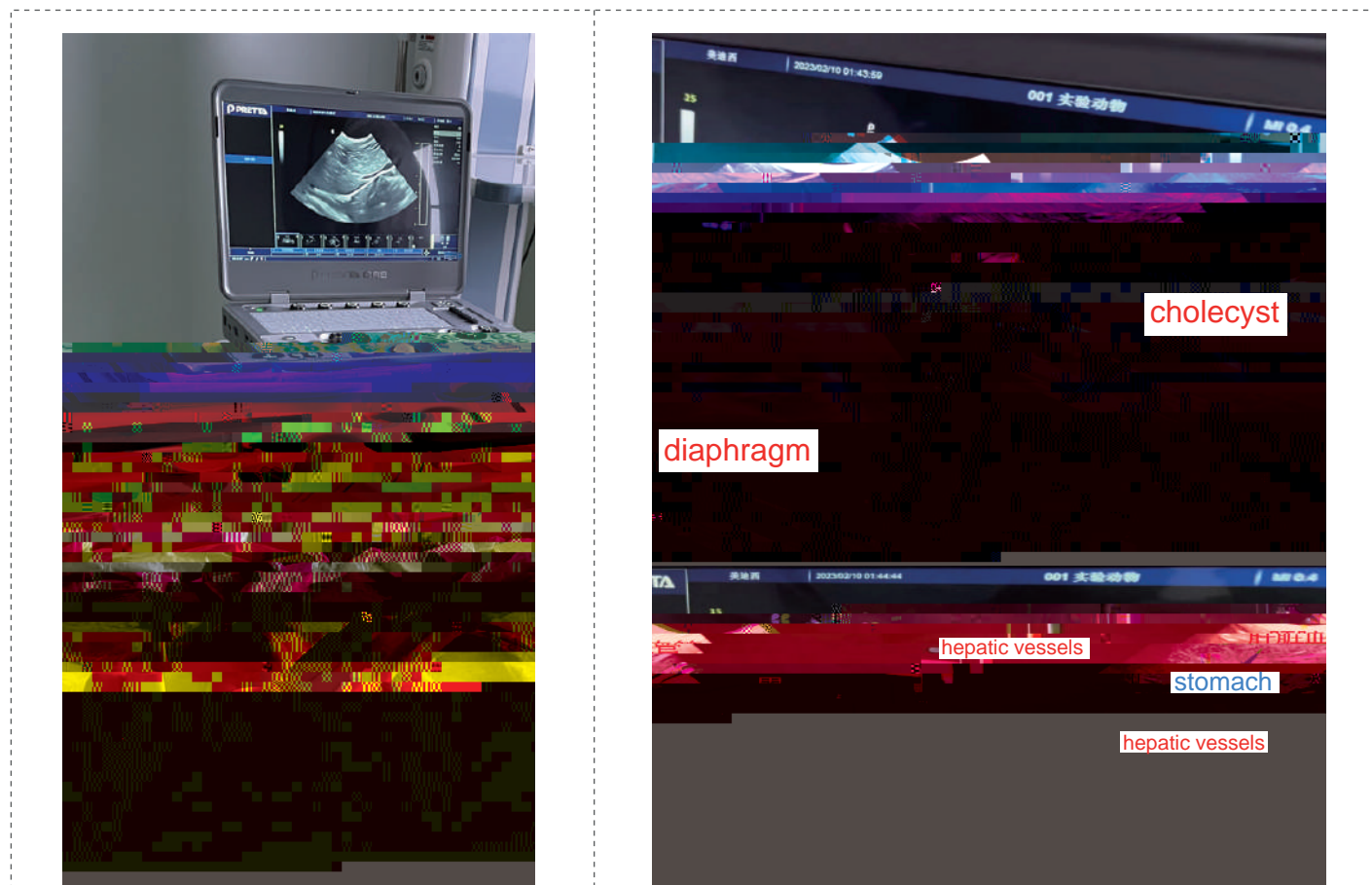
Liver-30 mg/kg



Administration Route	Dose Level mg/kg	Analyte	AUC _{last_liver}	AUC _{last_Plasma}	AUC _{last_liver} /AUC _{last_Plasma}
			hr*ng/g	hr*ng/g	
SC	30	siRNA_AS	32976645	17893	1843
SC	300	siRNA_AS	94450628	219970	429

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



Medicilon Case: siRNA monkey PK/PD study

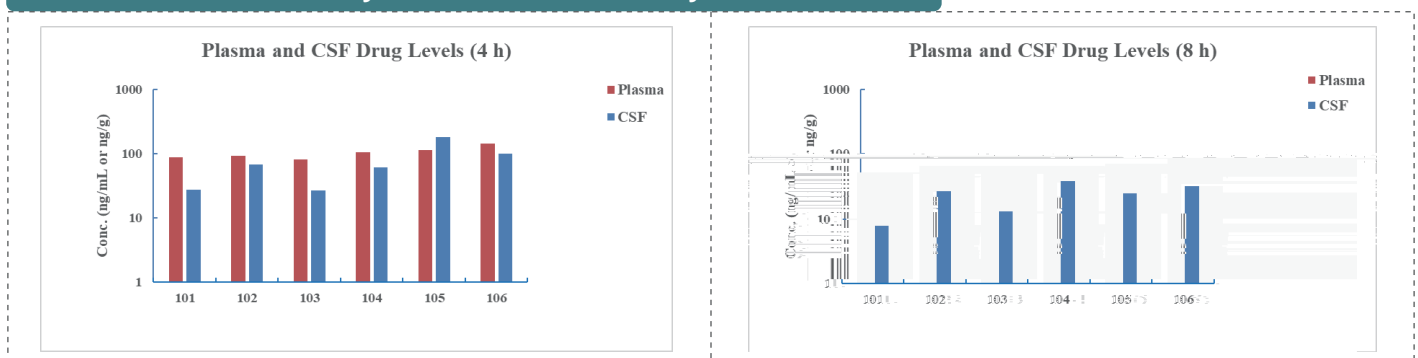
Compound	Monkey Matrix	Animal Number	BA Assay
siRNA IV infusion	Plasma Muscle biopsy Liver biopsy	N=2	<ul style="list-style-type: none"> Cytokine study Complement study Lipid study Cir-luc mRNA IHC slide hELISA study MSTN Protein
	Plasma Muscle biopsy	N=2	<ul style="list-style-type: none"> hELISA study NHP mRNA NHP MSTN Protein IHC slide Cytokine study Complement study

Medicilon Case: Monkey IT validation work flow by concentration



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

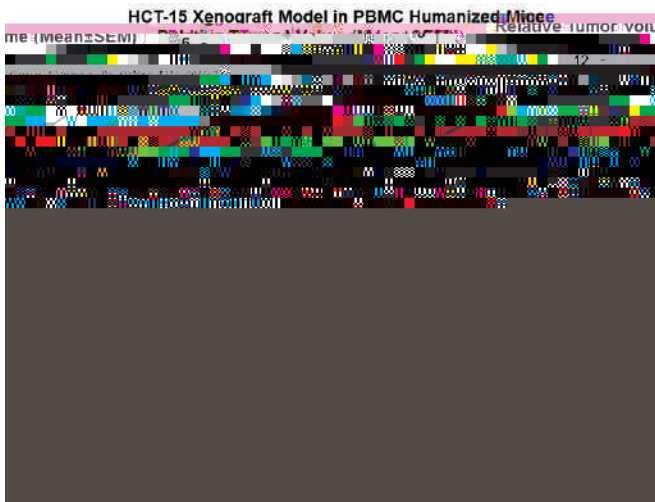
Pharmacology Evaluation of Nucleic Acid Drugs

Medicilon 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

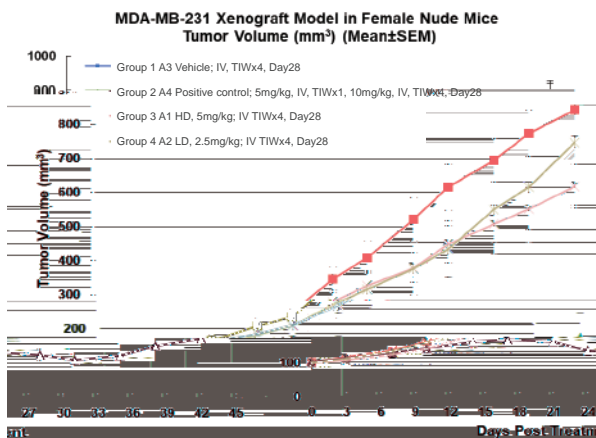
- Rodents: Mouse/Rat, Rabbit
- Non-Rodents: Beagle Dog, Mini Pig, Non-human Primate

Medicilon Case: mRNA in PBMC humanized mice



- **Animals:**
Female NOG mice
- **Tumor Cells:**
HCT15, 2×10^6 /mouse
- **PBMC:**
 5×10^6 /mouse
- **Treatment:**
Intracutaneous injection

Medicilon Case: Comparing different drug delivery methods of mRNA



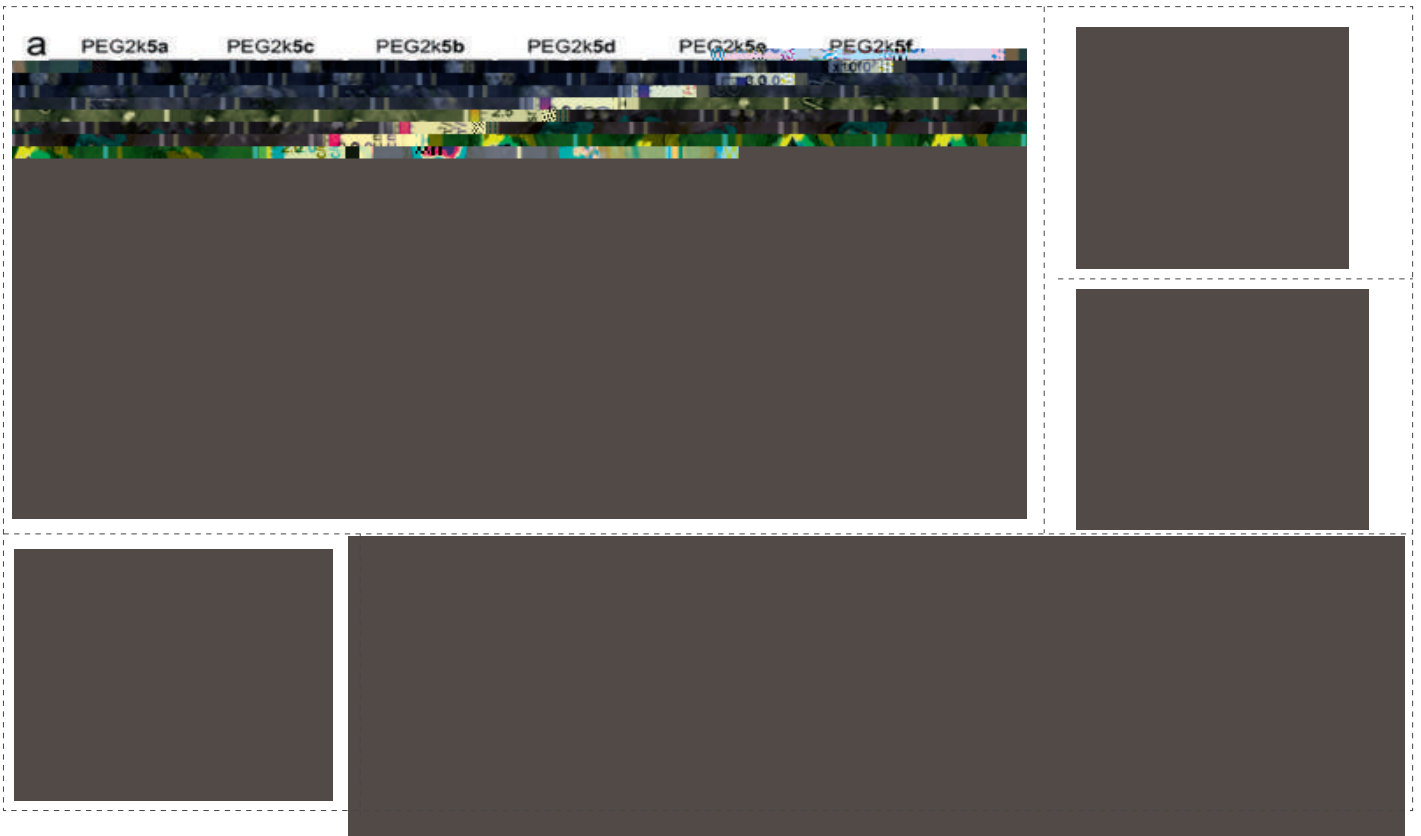
- Group 5 B3 Vehicle; ITU, TIWx2, Day14, Day18, Day23
- Group 6 B4 Positive control; 5mg/kg, ITU, TIWx2, Day14, Day18, Day23
- Group 7 B1 HD, 2.5mg/kg; ITU TIWx2, Day14, Day18, Day23
- Group 8 B2 LD, 1.25mg/kg; ITU TIWx2, Day14, Day18, Day23

Animals: Female BALB/c Nude mice
 Cells: MDA-MB-231, 5×10^6 /mouse
 Model Establishment: Right flank SC injection
 Treatment: **IV** injection; TIW (three times a week);
 Group3, 4: mRNA (LNP) group.

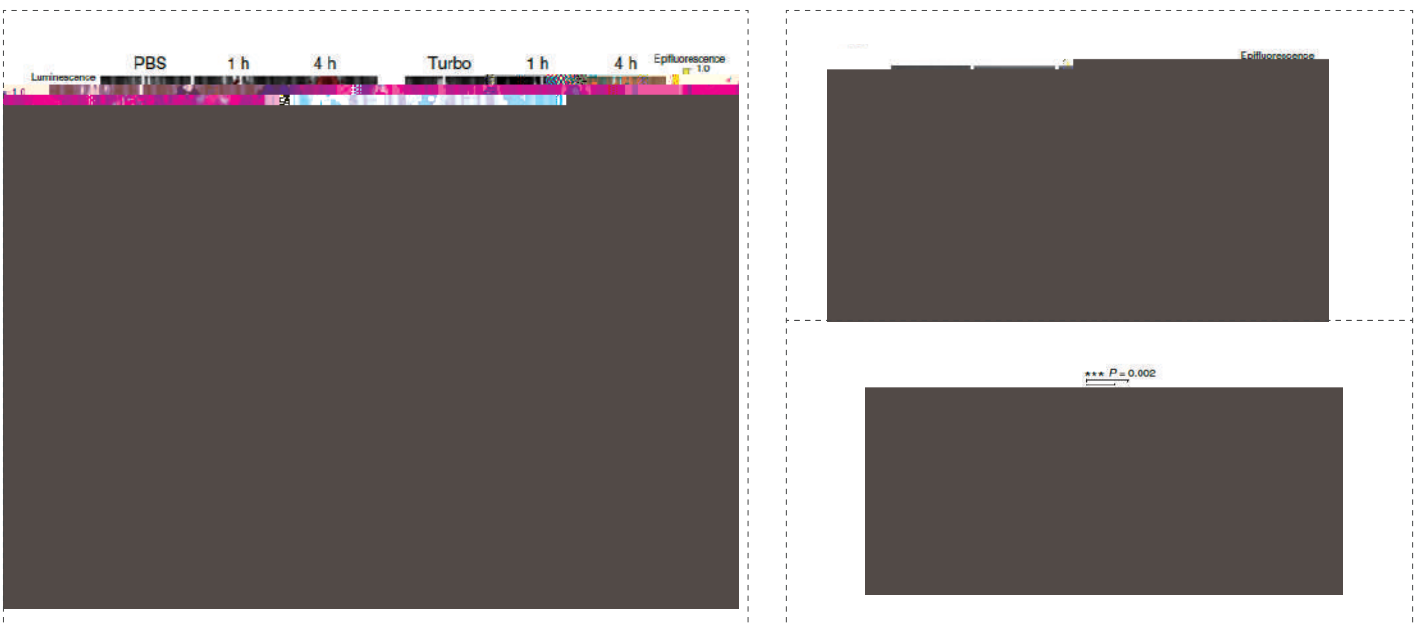
Animals: Female BALB/c Nude mice
 Cells: MDA-MB-231, 5×10^6 /mouse
 Model Establishment: Right flank SC injection
 Treatment: **Intratumor** injection; TIW (three times a week);
 Group 7, 8: mRNA (LNP) group.

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



CDX Models

Cancer Type	Cell Line
Brain cancer	CHP-212, LN-229, U-87 MG, U-251 MG
Breast cancer	BT474, HCC70, HCC1569, HCC1806, HCC1954, JIMT-1, MCF7, MDA-MB-231, MDA-MB-436, MDA-MB-468, MX-1, SUM 149PT, ZR-75-1
Cervix	Hela, SiHa
Colon cancer	COLO 205, DLD-1, HCT-8, HCT-15, HCT-116, HT-29, LIM-1215, LoVo, Ls174T, NCI-H508, RKO, SW48, SW480, SW620, SW837
Endometrium	AN3 CA, HEC-1-A, HEC-1-B, RL95-2
Esophageal	KYSE-520
Fibrosarcoma/Soft tissue	HT-1080
Gastric cancer	Hs 746T, MKN-45, NCI-N87, NUGC-4, SNU-16
Head and neck cancer	CAL-27, Detroit562, FaDu, TT
Leukemia	CCRF-CEM, HEL, HL-60, Jurkat E6.1, JVM-3,K-562, MOLM-13, MOLM-16, MOLT-3, MOLT-4, MV-4-11, OCI-AML-3, TF-1a, THP-1, U-937
Liver cancer	HCCLM3, Hep G2, HuH-7
Lung cancer	A427, A549, Calu-1, Calu-3, Calu-6, DMS114, EBC-1, HCC827, MOTO-211H, NCI-H69, NCI-H292, NCI-H358, NCI-H460, NCI-H520, NCI-H522, NCI-H526, NCI-H727, NCI-H820, NCI-H1299, NCI-H1373, NCI-H1568, NCI-H1581, NCI-H1650, NCI-H1703, NCI-H1975, NCI-H2122, NCI-H2228, NCI-H3122, NCI-H3255, PC9
Lymphoma	Daudi, DB, DOHH2, HH, JeKo-1, Karpas299, MAVER-1, Mino, OCI-LY10, OCI-LY19, Raji, RL, SR, SU-DHL-1, SU-DHL-2, SU-DHL-4, SU-DHL-6, TMD-8, WSU DLCL2
Melanoma	A375, C32
Myeloma	MM.1R, MM.1S, NCI-H929, OPM-2, RPMI-8226
Osteosarcoma	SJSA-1
Ovary	A2780, OVCAR-3, OVCAR-8, PA-1, SK-OV-3
Pancreatic cancer	AsPC-1, Bx PC-3, Capan-1, Capan-2, CFPAC-1, HPAF-II, Mia PaCa-2, PANC-1, SU.86.86, SW1990
Prostate	22RV1, DU145, LnCap, LNCaP-F877L mut, PC-3, Vcap
Renal cancer	786-O, A498, ACHN, OS-RC-2
Skin cancer	A-431
Urinary bladder cancer	5637, HT1376, RT4, UM-UC-3

PDX Models

Cancer Type	Cell Line
Breast cancer	201B, 203B
Cervix	371Ce
Colon cancer	002C, 008C, 011C, 013C, 015C, 016C, 020C, 021C, 057C, 058C, 059C, 060C, 061C, 062C, 064C, 065C, 069C, 070C, 072C, 075C, 076C, 084C, 087C, 088C, 095C, 102C, 104C, 110C, 116cC, 117C, 128C, 143C
Endometrium	361En
Esophagus cancer	341Es
Gastric cancer	091Ga, 092Ga, 122Ga, 126Ga, 142Ga, 145Ga, 254Ga, 256Ga, 258Ga, 259Ga, 381Ga
Head and neck cancer	281T, 285HN, 284HN, 286HN
Leukemia	291Le, 292Le, 293Le, 294Le, 295Le
Liver cancer	212Li, 213Li, 214Li, 216Li
Lung cancer	028Lu, 047Lu, 050Lu, 053Lu, 054Lu, 263Lu, 264Lu, 265Lu, 267Lu
Lymphoma	244Ly, 245Ly
Myeloma	321Bm
Ovary	271O, 272O, 273O, 274O
Pancreatic cancer	221Pa, 222Pa, 223Pa, 224Pa, 225Pa, 226Pa, 228Pa
Prostate	351Pr, 353Pr, 354Pr, 355Pr
Renal cancer	301R, 303R, 304R
Sarcoma	332Sa, 333Sa, 334Sa
Urinary bladder cancer	232U, 234U, 235U, 236U

♥ Syngeneic Models

Cancer Type	Cell Line
Brain cancer	Neuro-2a
Breast cancer	4T1, EMT6, EO771, JC
Colon cancer	Colon26, CT26.WT, MC-38
Leukemia	C1498, L1210, WEHI-3
Liver cancer	H22
Lung cancer	KLN205, LLC1, M109
Lymphoma	A20, E.G7-OVA, EL4, L5178-R
Mastocytoma	P815
Melanoma	B16, B16-F0, B16-F10, Clone-M3, YUMM1.7
Myeloma	J558
Pancreas cancer	KPC, Panc 02
Prostate	RM-1
Renal cancer	RENCA
Sarcoma	S180, WEHI-164
Urinary bladder cancer	MB49

♥ Humanized Models (PBMC, HSC CD34+)

Cancer Type	Cell Lines
Brain cancer	U-87 MG
Breast cancer	HCC70, HCC1954, JIMT-1, MDA-MB-231, MDA-MB-468
Colon cancer	HT-15, HT29, LoVo, Ls174T
Gastric cancer	NCI-N87, NUGC-4
Leukemia	MOLM-13, THP-1
Liver cancer	Hep G2, HuH-7
Lung cancer	A549, HCC827, NCI-H292, NCI-H838, NCI-H1975
Lymphoma	Raji, TMD8
Melanoma	A375
Myeloma	MM.1S, NCI-H929, RPMI-8226
Ovarian cancer	OVCAR-3
Pancreatic cancer	Capan-2
Renal cancer	786-O, A498
Skin cancer	A431
Urinary bladder cancer	UM-UC-3

♥ Orthotopic Models

Cancer Type	Orthotopic Model (Human)	Orthotopic Model (Mouse)
Brain cancer	LN299-luc, U87-MG-luc, U251-luc	G261-luc
Breast cancer	BT-474-luc, MCF-7-luc,MDA-MB-231-luc	4T1-luc
Colon cancer	HCT-116-luc, HT29-luc	MC38-luc
Gastric cancer	NCI-N87-luc	/
Leukemia	K562-luc, MOLM-13-luc, MV-4-11-luc, Nalm-6-luc, THP-1-luc	/
Liver cancer	HuH-7-luc, Hep G2-luc	H22-luc
Lung cancer	A549-luc, NCI-H460, NCI-H1650-luc, NCI-H1975-luc	LLC1-luc
Lymphoma	Raji-luc, JeKo-1-luc	/
Melanoma	A375-luc	B16-F10-luc
Myeloma	NCI-H929-luc, MM.1S-luc, OPM-2-luc	/
Ovary	OVCAR-3-luc, SK-OV-3-luc	/
Pancreatic cancer	Mia-Paca 2-luc, PANC-1-luc	Panc 02-luc
Prostate	PC-3-luc	/
Renal cancer	A498-luc	/
Sarcoma	HT1080-luc, SJSA-1-luc	/
Urinary bladder cancer	UM-UC-3-luc	MB49-luc

Luciferase Cell Line

G261-luc, 4T1-luc, MC38-luc, H22-luc, B16-F10-luc, LLC1-luc



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