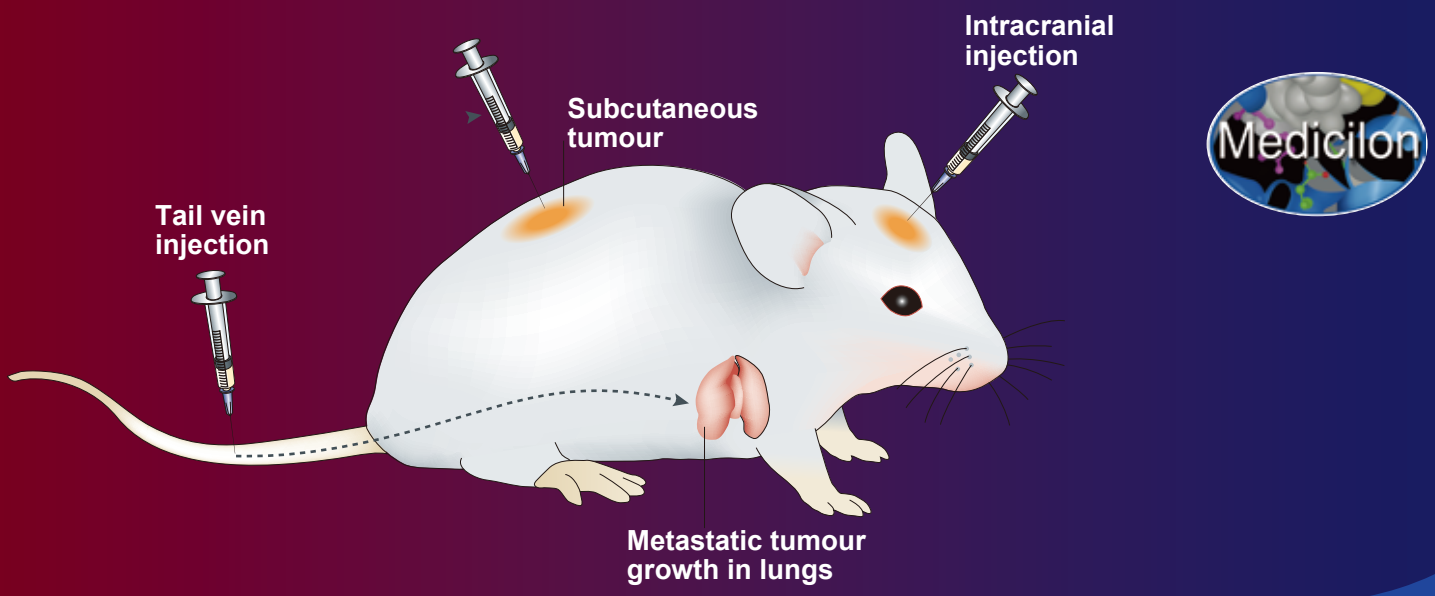


Medicilon Routes of Drug Administration Platform

Drug administration route is often classified by the location at which the drug is administered, such as oral or intravenous. The choice of routes in which the medication is given depends not only on convenience and compliance but also on the drug's pharmacokinetics and pharmacodynamic profile. Therefore it is crucial to understand the characteristics of the various routes and associated techniques as the first step before PK/PD, efficacy/pharmacology and toxicity study.

Intravenous Injection (i.v.)

Intravenous injection directly delivers the drugs to the systemic circulation. It is utilized when a rapid drug effect is desired, a precise serum drug level is needed, or when drugs are unstable or poorly absorbed in the gastrointestinal tract. It is also the route used in patients with altered mental status or severe nausea or vomiting, unable to tolerate oral medications.

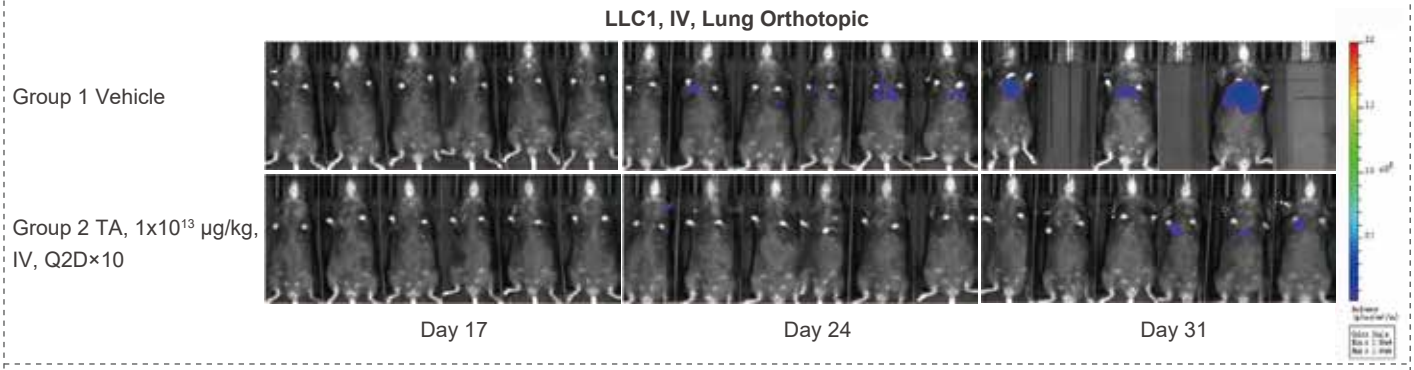
Advantages:

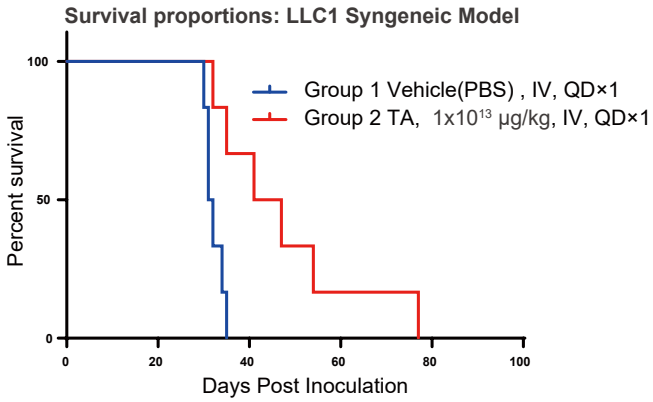
- Rapid onset of action
- Predictable way of action and almost complete bioavailability
- The problems of oral drug administration can be eliminated by avoiding the gastrointestinal tract
- The best way of administration in very ill and comatose patients who cannot ingest anything orally

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

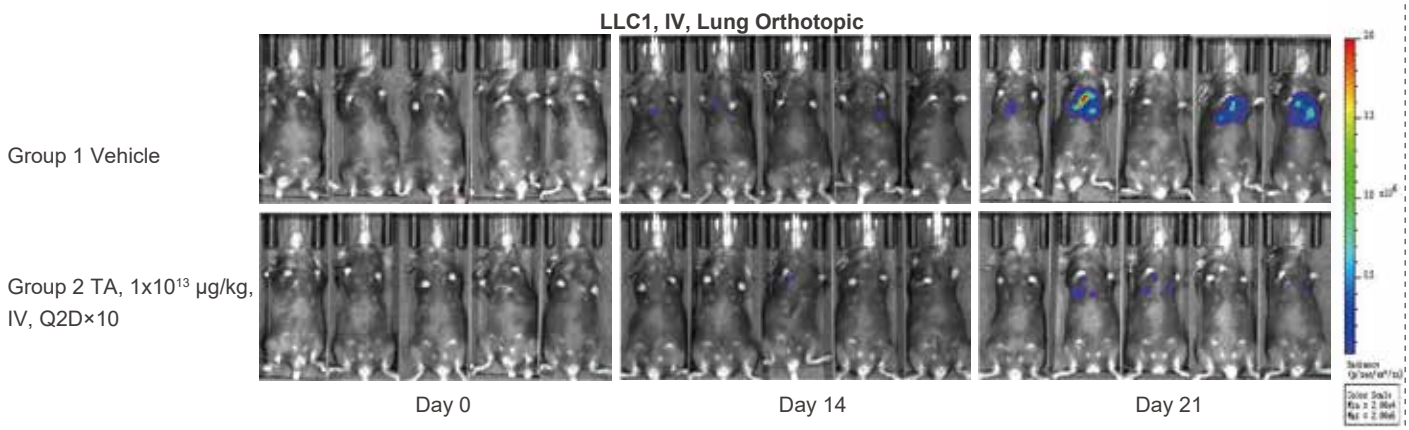
Medicilon Case: Prophylactic cancer vaccines

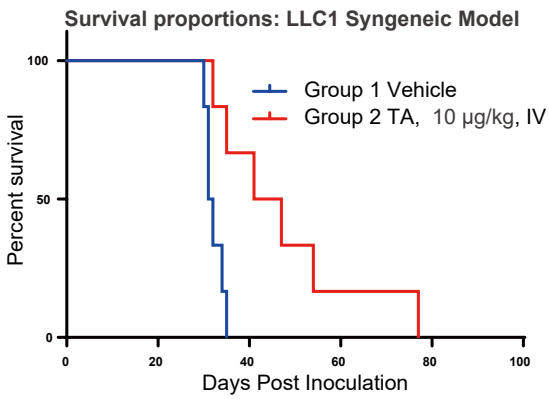




- **Animals:**
  Female C57BL/6 mice
- **Tumor Cells:**
  LLC1, 1x10^5/mouse
- **Model Establishment:**
  IV injection
- **Treatment:**
  IV injection 10 days before tumor inoculation

Medicilon Case: Cancer vaccines





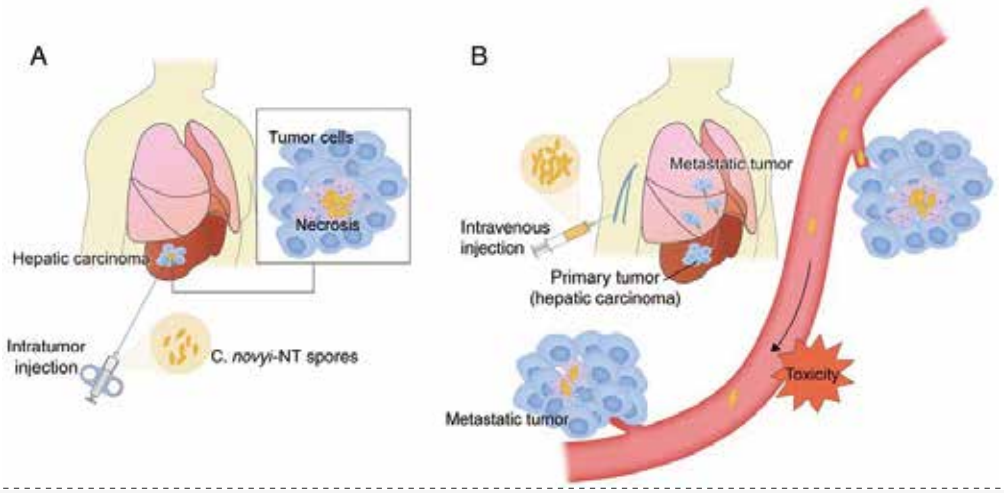
- **Animals:**
  Female C57BL/6 mice
- **Tumor Cells:**
  LLC1, 1x10^5/mouse
- **Model Establishment:**
  IV injection
- **Treatment:**
  IV injection

Intratumoral Injection (i.t.)

Intratumoral immunotherapy is a strategy that offers a unique therapeutic and exploratory setting to better understand the immune contexture across tumor lesions of patients with metastatic cancer. Intratumoral immunotherapy turns cold tumors into hot and boosts the response rates to cancer immunotherapies while decreasing their systemic exposure and toxicities. Intratumoral immunotherapy improves the immunity against tumors and changes the combination therapy currently pursued for metastatic and local cancers to extend their survival.

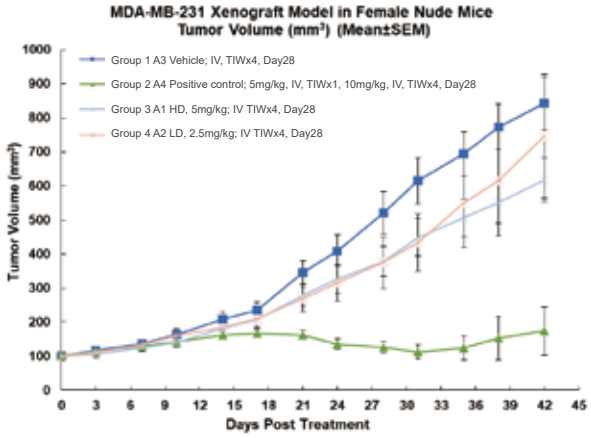
Intratumoral Injection VS Intravenous Management

Nowadays, the primary methods of administration are systemic intravenous injection and direct intratumoral injection. Direct intratumoral injection could allow relatively small doses of spores to be used, ensuring that a larger effective dose reaches the target tumor and is distributed around the tip of the needle. Systemic injection requires large doses of injected spores; however, the proportion of spores delivered to the tumor is small with lower effective dose.

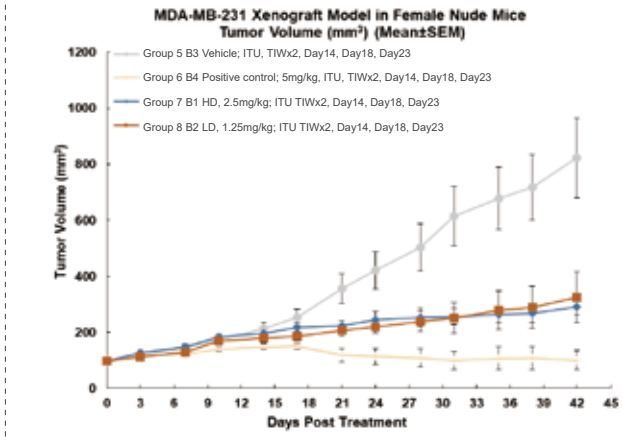


Comparison of intratumoral injection and intravenous management[1]

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.

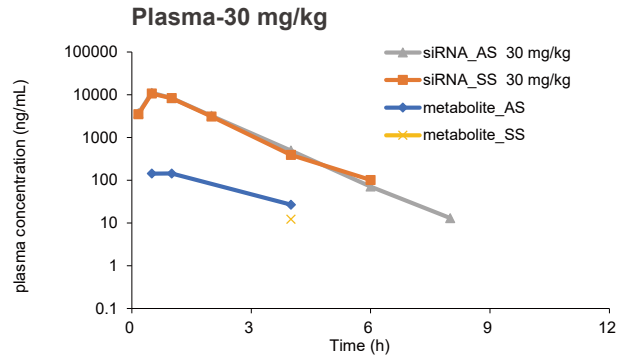


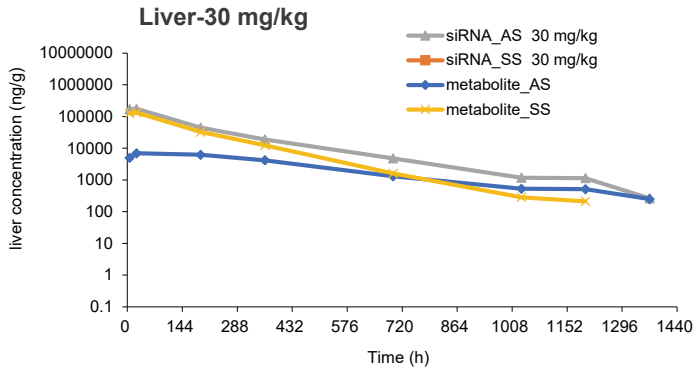
Animals: Female BALB/c Nude mice
Cells: MDA-MB-231, 5x10^6/mouse
Model Establishment: Right flank SC injection
Treatment: **Intratumor injection**; TIW (three times a week); Group 7, 8: mRNA (LNP) group.

Subcutaneous Injection (SC)

Subcutaneous injection route is used when the drug's molecular size is too large to be effectively absorbed in the intestinal tract or when better bioavailability or a faster absorption rate is needed than the oral route. It is easy to administer and requires minimal skills, so patients can often self-administer the medication. Subcutaneous administration route is widely used to administer different types of drugs given its high bioavailability and rapid onset of action. There are remarkable advantages of subcutaneous injection over the other injection types, in contrast to IV and IM administrations, SC is less painful, the risk of infection is lower in SC than in IV injection. If this occurs, the infection is generally limited to a local infection rather than a systemic infection. Furthermore, subcutaneous injection offer a broader range of alternative sites than IM injection for those patients requiring multiple doses.

Medicilon Case: siRNA and metabolite in rodent plasma and liver

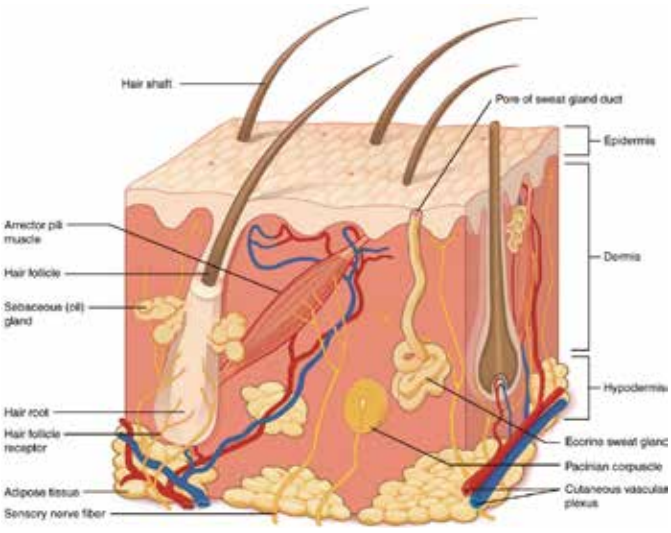




Administration Route	Dose Level mg/kg	Analyte	AUC_{last_liver} hr*ng/g	AUC_{last_Plasma} hr*ng/g	$AUC_{last\_liver}/AUC_{last\_Plasma}$
SC	30	siRNA_AS	32976645	17893	1843
SC	300	siRNA_AS	94450628	219970	429

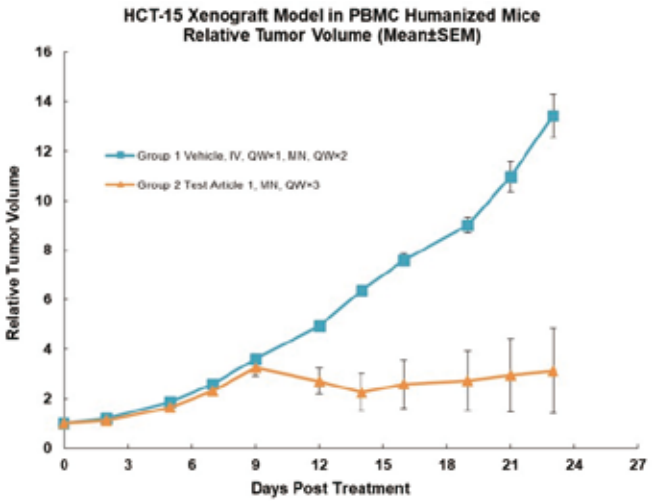
Intradermal (Intracutaneous) Injection

Intradermal injection is administered into the dermis just below the epidermis. Intradermal injection has the longest absorption time of all parenteral routes because there are fewer blood vessels and no muscle tissue. The most common anatomical sites used for intradermal injection are the inner surface of the forearm and the upper back below the scapula. Intradermal injection provides a local and very little systemic effect. Intradermal injection is commonly used for tuberculin skin testing but can also be used for allergy testing and local anesthetics because the reaction is easy to visualize, and the degree of reaction can be assessed.



Layers of Skin

Medicilon Case: mRNA in PBMC humanized mice

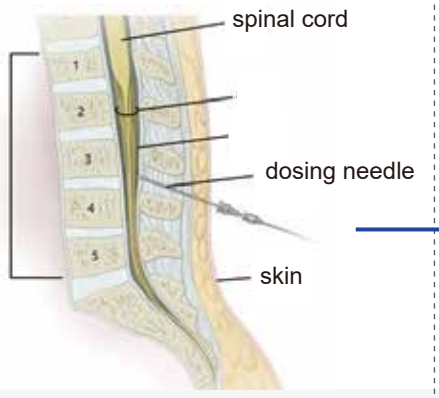


- **Animals:**
  Female NOG mice
- **Tumor Cells:**
  HCT15, $2x10^6$/mouse
- **PBMC:**
  $5x10^6$/mouse
- **Treatment:**
  Intracutaneous injection

Intrathecal Injection (IT)

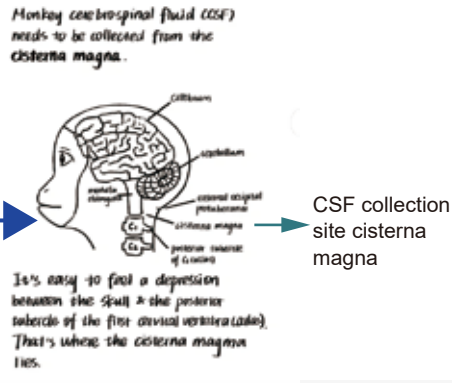
Intrathecal injection is a potential drug delivery approach with directly into cerebrospinal fluid (CSF) that fills the thecal sac. This route of administration can achieve a high concentration of therapeutic agent within the central nervous system (CNS) while minimizing off-target exposure and associated toxicity. However, the distribution of molecules following IT administration when they are provided in free form remains to be optimized. Many hydrophilic agents clear rapidly as CSF turns over, many hydrophobic agents experience delivery that is restricted near to the injection site, and many macromolecules can experience relatively limited parenchymal penetration. The potential significance of IT drug delivery for the treatment of CNS disease is amplified by deepened understanding of dynamic exchange between CSF, interstitial fluid (ISF), and peripheral tissue sites.

Medicilon Case: Monkey IT validation work flow by concentration



monkey IT dosing schematic lumbar

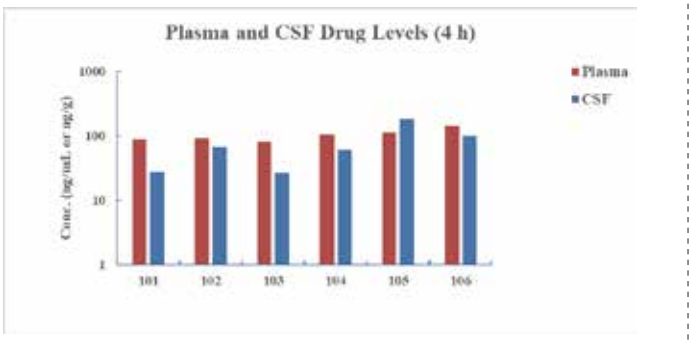
monkey dose location: lumbar
X-ray imaging monitoring

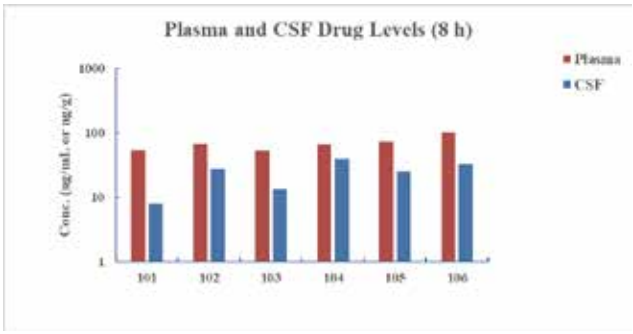


monkey CSF collection schematic

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





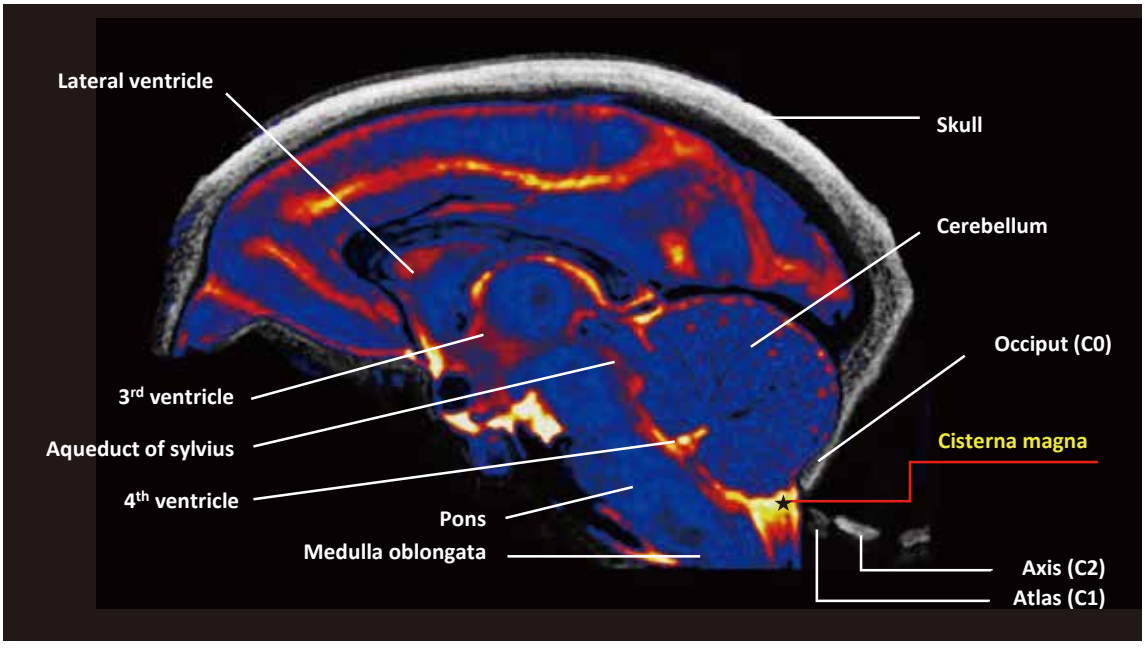
The coefficient of variation (CV%)

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

Intra-cisterna Magna Injection (ICM)

Intracisterna magna injection has been developed for enhanced CNS drug delivery. Intracisterna magna injection is widely used to bypass the blood-brain barrier and has distinct advantages for direct delivery into the CNS. An alternative to CSF-mediated delivery routes are lumbar IT injection and ICV infusion. Lumbar puncture used to obtain CSF or for chemotherapy is a highly skilled procedure that requires practical experience and specific knowledge of the relevant anatomy. In addition, cranial puncture site infections and intracerebral hemorrhage after ICV infusion are inherent surgical risks. Therefore, ICM administration has a clear advantage, given that it is widely used in animal models. In addition, the ICM route has been used extensively, particularly in NHPs, because it offers the easiest entry into the ventricles of the brain and subarachnoid space around the brain and spinal cord, except for craniotomy.



Localization of the cisterna magna (CM) in monkey brain[2]

Medicilon Case: Preliminary Test of a Single Dose of MK-8931 in Cynomolgus Monkeys

Group	Number of Animals	Test Substance	Conc. (ng/mL)	Dose Volume mL/animal	Route/Frequency
1	1	MK-8931	240	1	sub-occipital puncture into the cisterna magna (1 min±10 s), once

Cerebrospinal Fluid (CSF) Analysis

MK-8931 Pharmacokinetics Analysis

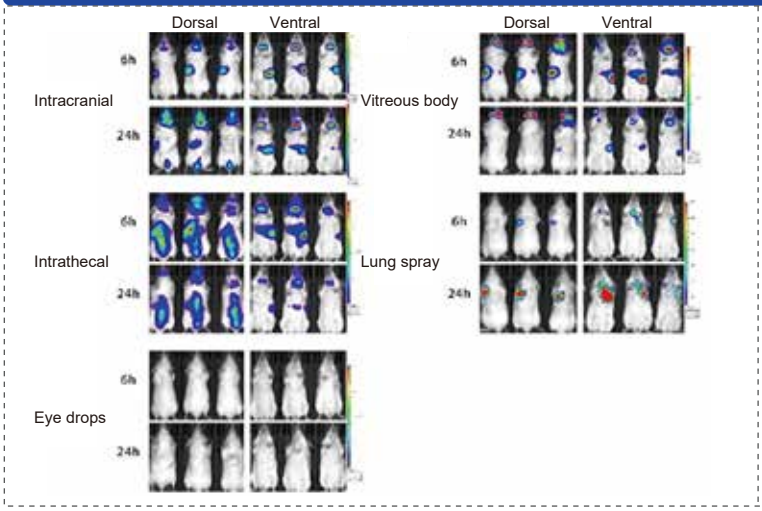
The LC-MS/MS analytical method for analyzing MK-8931 concentrations in CSF will be developed by ligand binding analysis laboratory in testing facility. The analytical results will be confirmed using quality control samples for intra-assay variation. The accuracy of at least 66.7% samples and 50% quality control samples at each concentration level should be within 80%-120%.

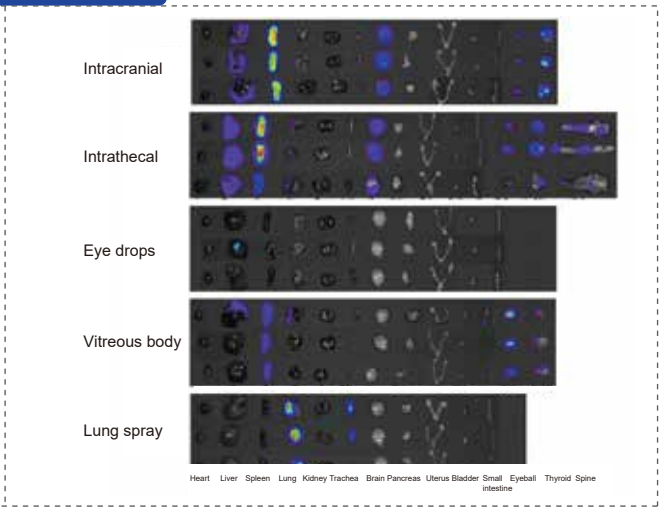
Aβ40/ Aβ42 Analysis

The Aβ40/Aβ42 level in CSF will be tested by bioanalysis laboratory in testing facility by using an ELISA kit.

Multiple Administration Routes

Medicilon Case: Nucleic acid drug delivery efficiency: siRNA





References:

[1] Xu Feng, et al. Novel insights into the role of Clostridium novyi-NT related combination bacteriolytic therapy in solid tumors. Oncol Lett. 2021 Feb;21(2):110. doi: 10.3892/ol.2020.12371.

[2] Junghyung Park, et al. XperCT-guided Intra-cisterna Magna Injection of Streptozotocin for Establishing an Alzheimer's Disease Model Using the Cynomolgus Monkey (Macaca fascicularis). Exp Neurobiol. 2022 Dec 31;31(6):409-418. doi: 10.5607/en22027.

MEDICILON

Email: marketing@medicilon.com

Address: 50 Soldiers Field Place, Boston, MA 02135

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