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WuXi AppTec

Smart Strategies of ADME-PK for Large Molecules

——Insights and solutions for ADCs and Oligos

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Outlines

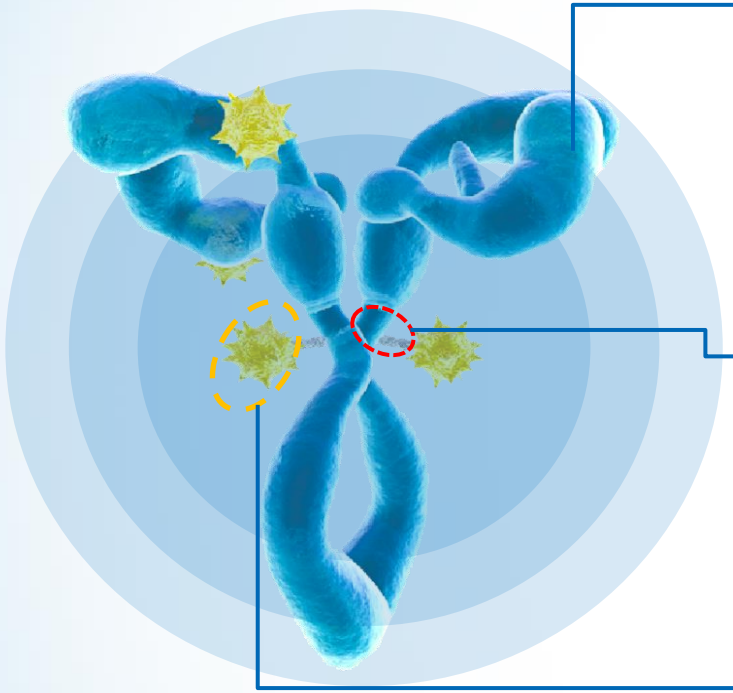
01 Biotransformation and Bioanalysis Strategies for ADCs

- Brief Introduction of ADCs
- Biotransformation of ADCs
- Bioanalysis of ADCs

02 Delivery Challenges and Strategies for CNS Oligos

- Brief Introduction of CNS Oligos
- Delivery Challenges and Strategies

Brief Introduction of ADCs



Antibody (Antigen Binding)

- High binding affinity
- Long half-life
- Rapid internalization

Linker

- Cleavable or non-cleavable
- Stable in circulatory system
- Rapid release of payload inside tumor cells
- Homogeneity

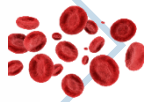
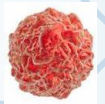
Payload

- High potency
- Various mechanisms to avoid drug resistance
- Optimal drug-antibody ratio (DAR)

ADC drugs are promising cancer treatments that enable the selective delivery of highly cytotoxic payloads to tumor cells.

Nat Rev Clin Oncol. 2024 Mar;21(3):203-223.

Biotransformation of ADCs

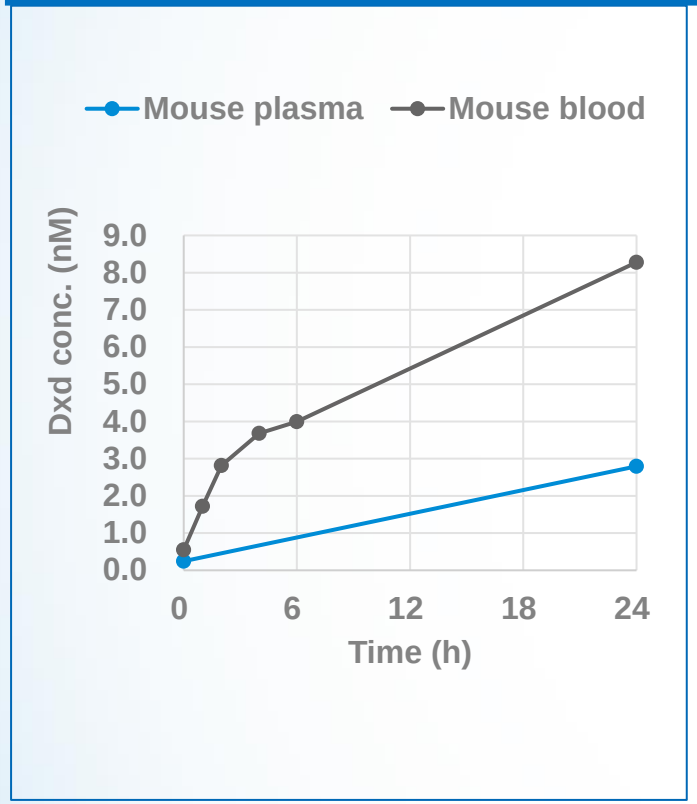
Simulate the metabolism of ADC in circulatory system	Simulate the cleavage of ADC in lysosome	Substitute for lysosome	Simulate the cleavage of ADC in tumor cells
1 Plasma/serum /blood 	2 Lysosome 	3 Liver S9 /liver homogenate 	4 Tumor cells 

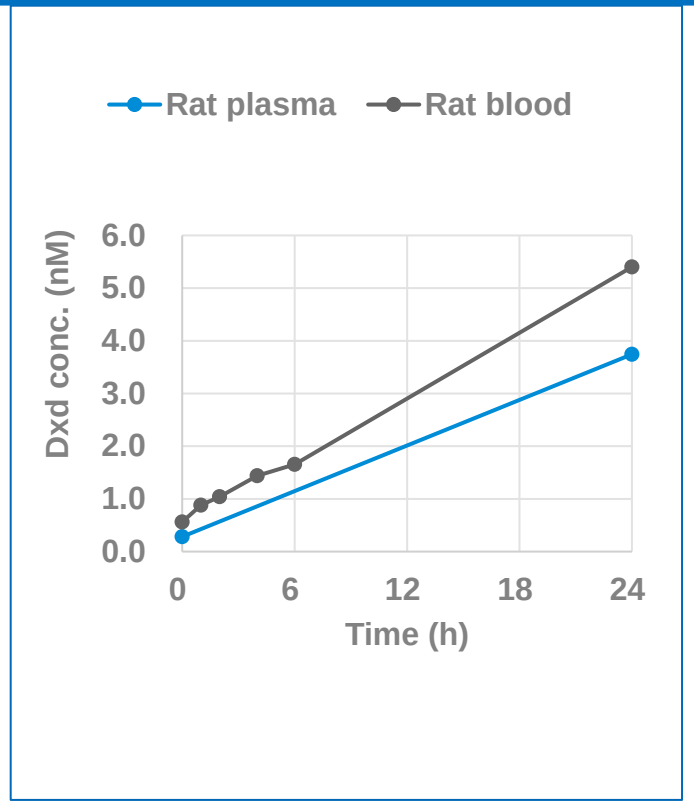
Multiple analytes

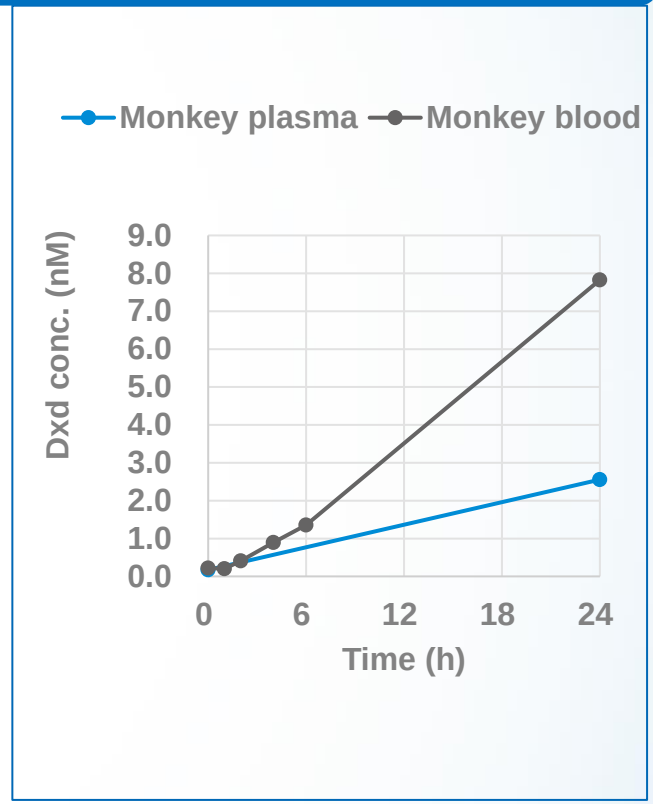
- ✓ Free payload or other metabolites
- ✓ DAR distribution
- ✓ Identification of payload-related metabolites
- ✓ ADC or total antibody
- ✓ Conjugated payload

Plasma vs Blood Stability of ADC

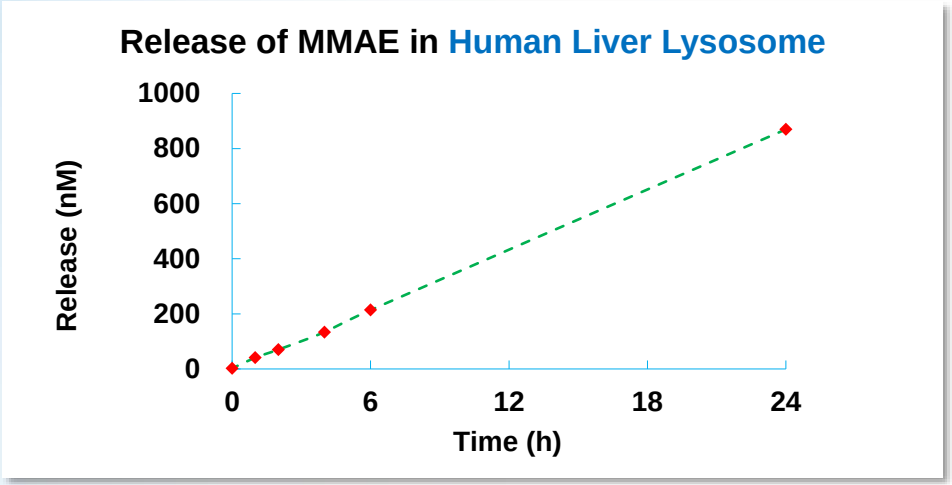
Plasma vs blood (DS-8201, 0.1 mg/mL)

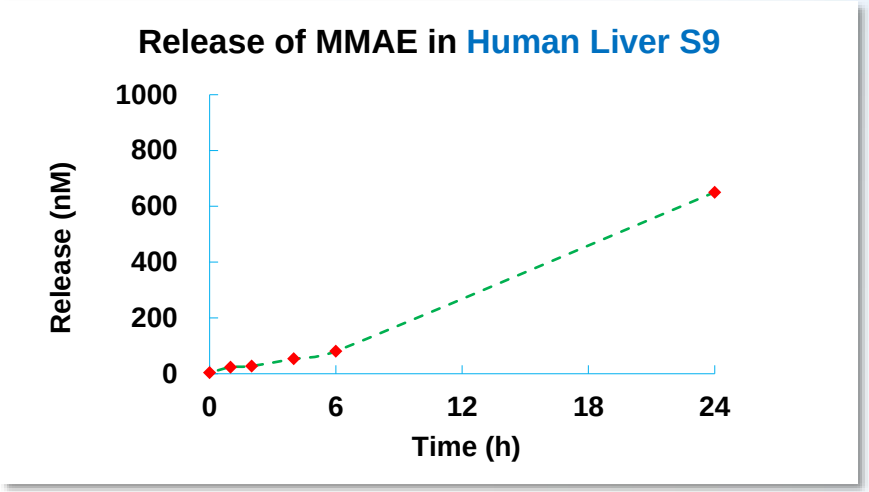


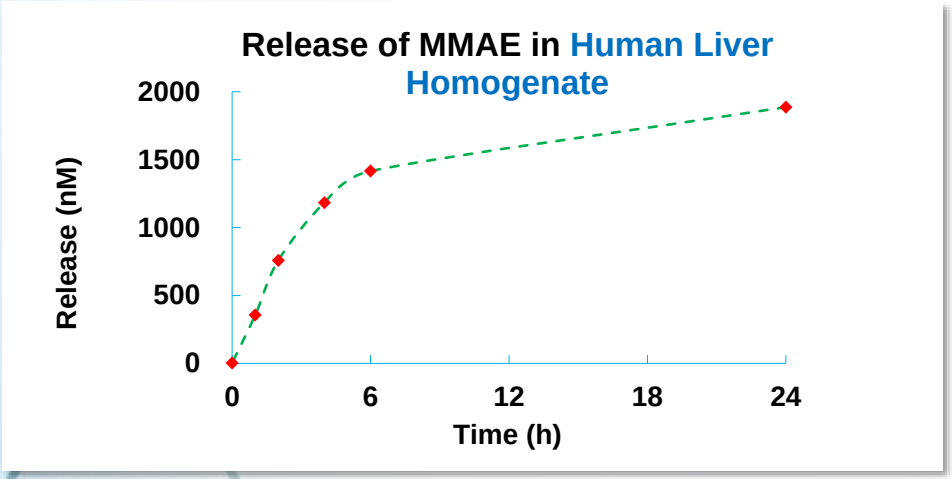




Liver Lysosome, S9 and Homogenate Stability of ADC



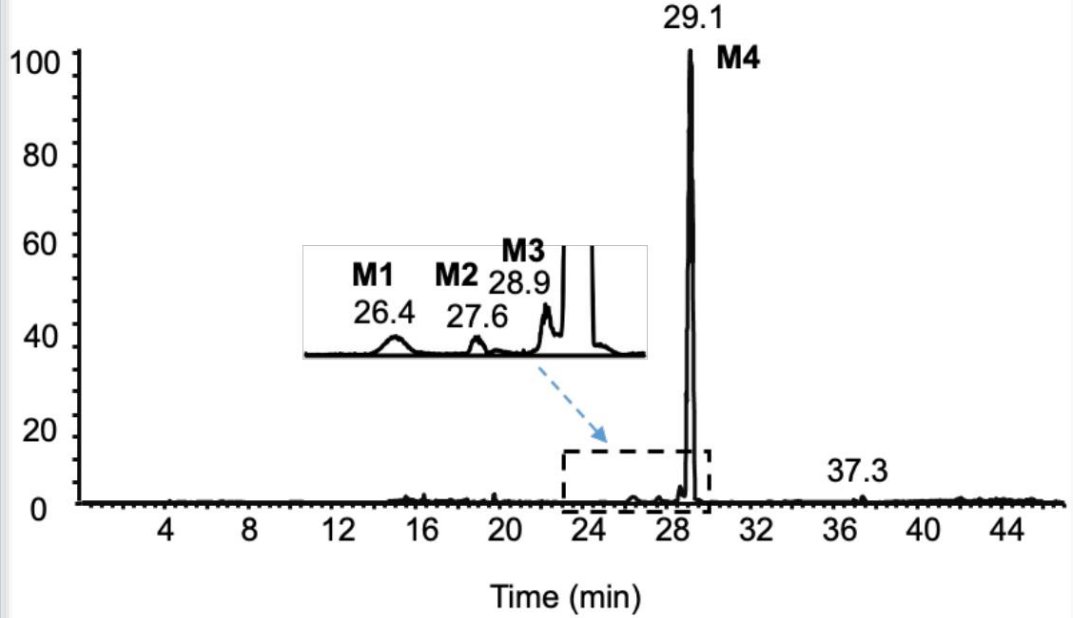




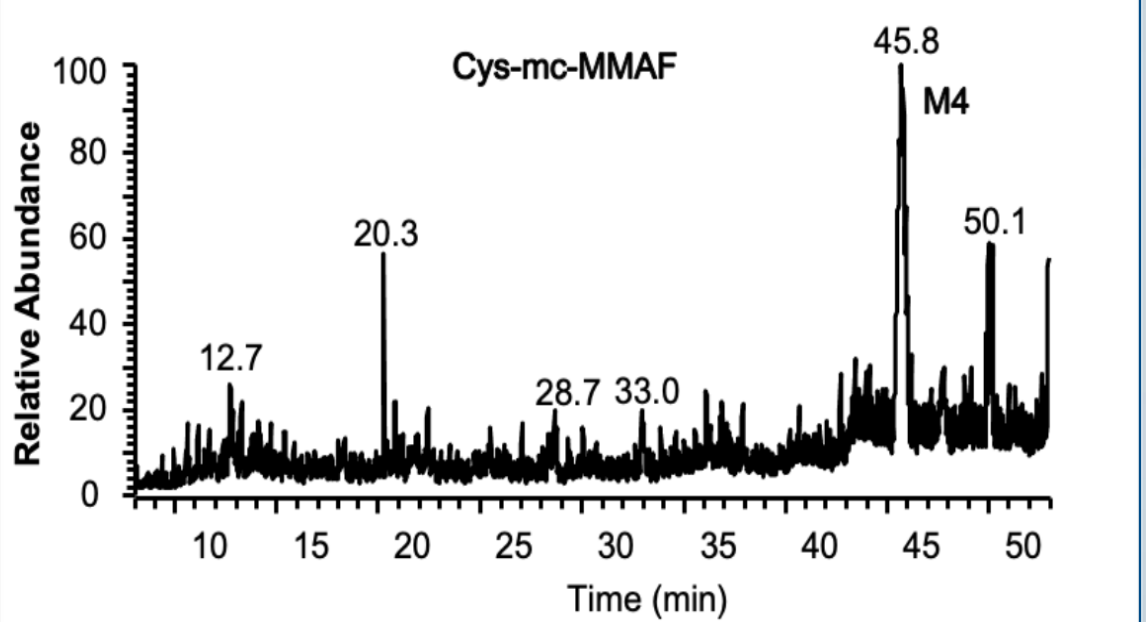
Protein Concentration	1 mg / mL
Brentuximab	0.1 mg/ mL
Incubation Buffer	2 mM TCEP in 50 mM sodium acetate, pH=5.0, fresh prepared
Incubation Temperature	37 ℃
Incubation Time (h)	0, 1, 2, 4, 6, 24

Identification of Payload-Related Metabolites

ADC (1 mg/mL) was incubated with human liver S9 in 50 mM sodium acetate (pH5.0) for 48 hours.



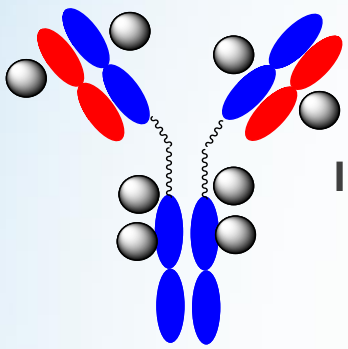
ADC (20 μg/mL) was incubated with tumor cells for 48 hours.



- Payload-related metabolites found in acidified liver S9 were consistent with those reported in tumor cells.

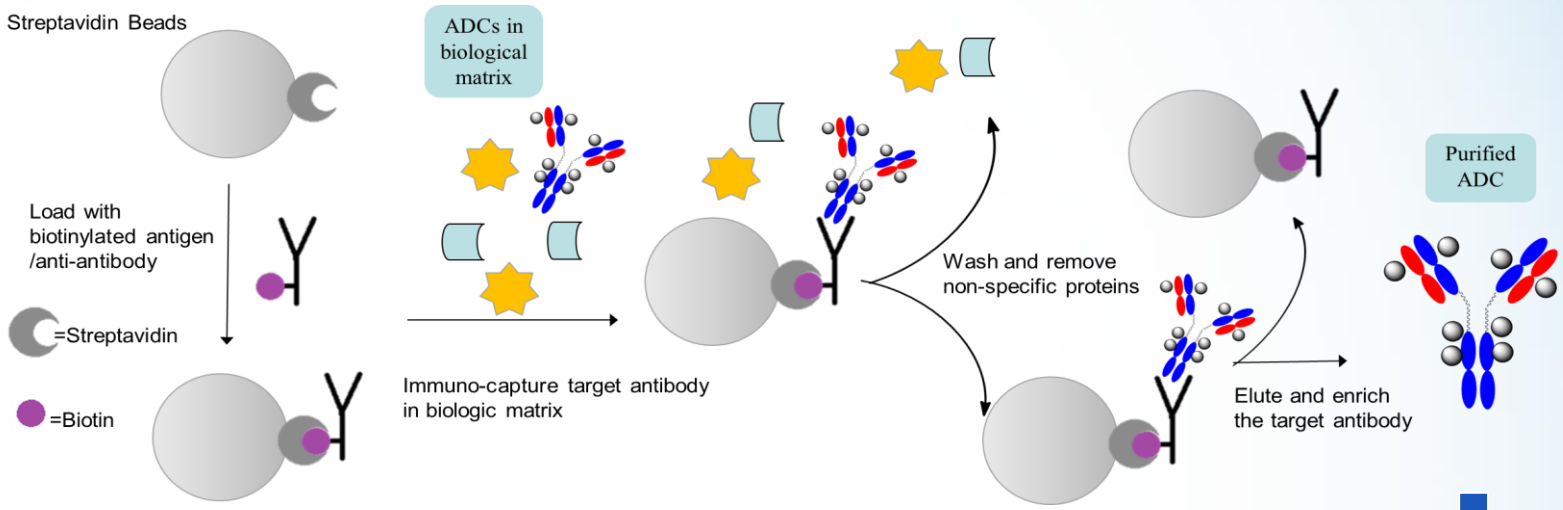
Drug Metab Dispos. 2023 May;51(5):591-598.

Workflow for the DAR (Drug-Antibody Ratio) Detection

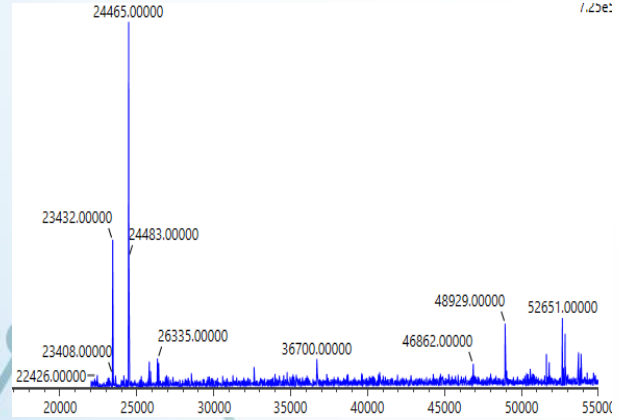


ADC

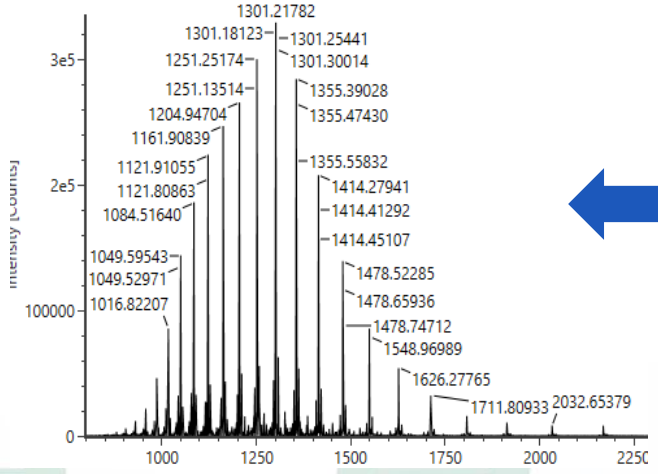
Immunocapture



Deconvoluted MS



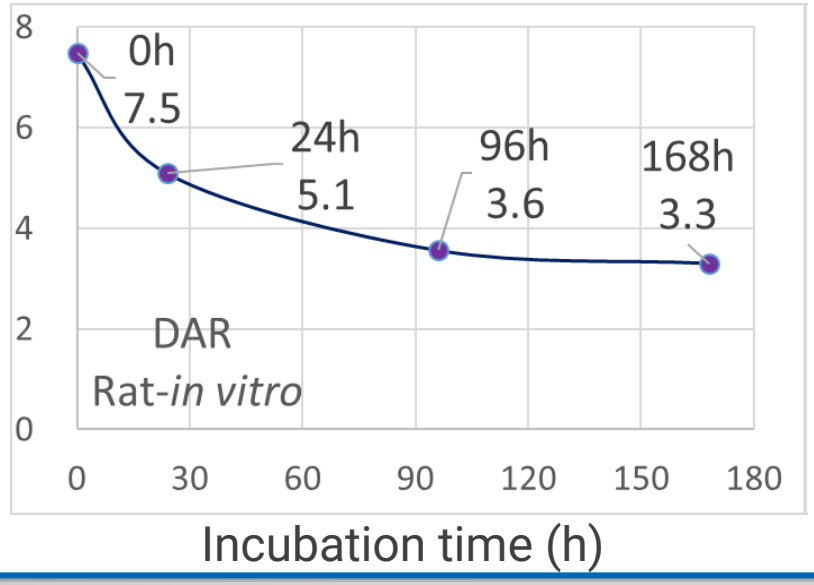
Multiple Charged MS



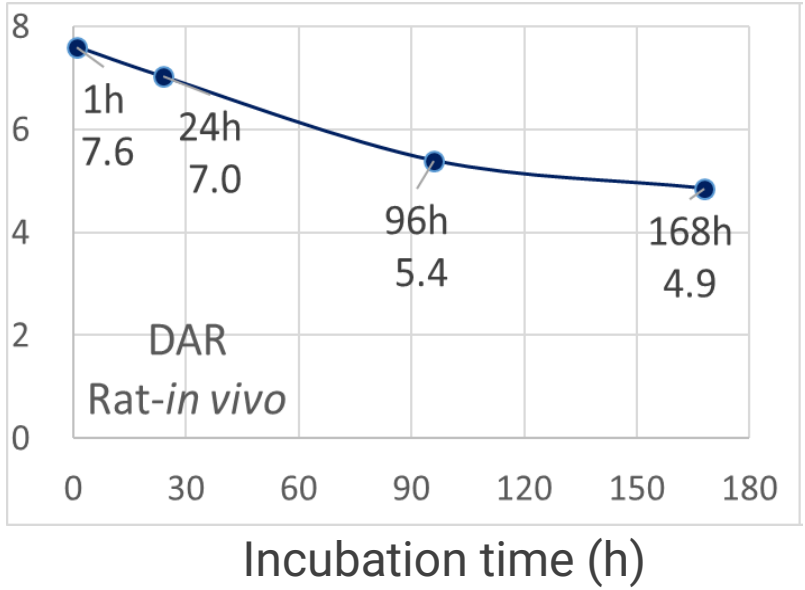
LC-HRMS

DAR Detection for ADC

DAR of DS-8201 in *in vitro* plasma



DAR of DS-8201 in *in vivo* plasma



Integrated Bioanalysis Platform for ADC Drugs

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ADC

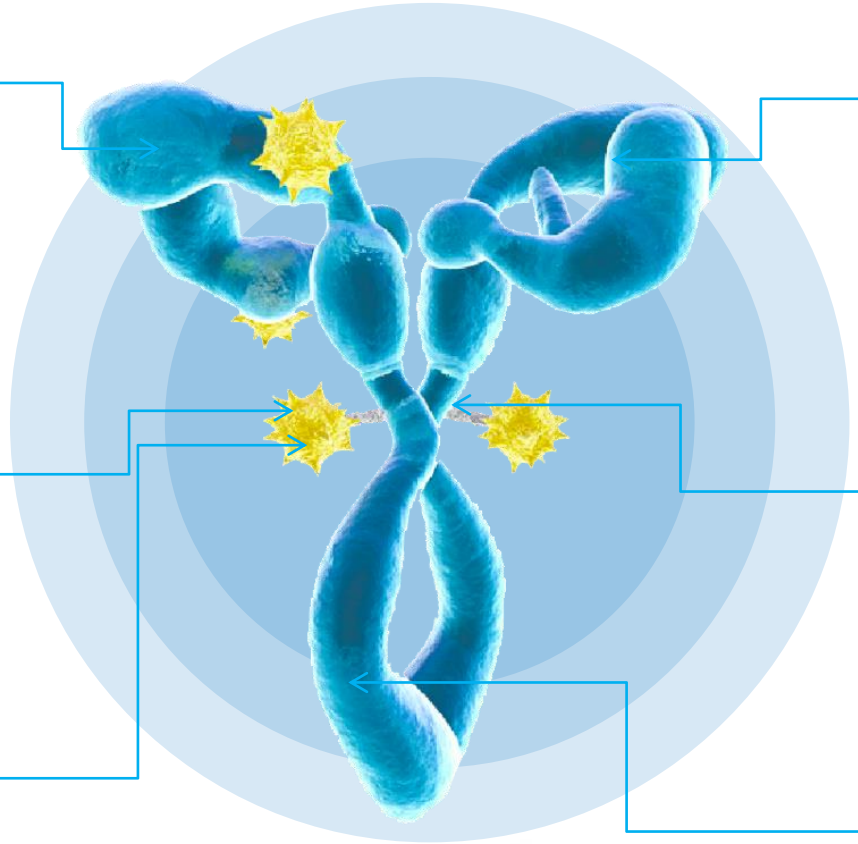
1. ELISA/MSD
   (Antibody specific to payload)
2. Immunocapture+LC-MS
   (Intact/surrogate)

Payload

1. LC-MS/MS (free payload)
2. Immunocapture+cleavage+
   LC-MS/MS (conjugated payload)

Metabolite identification

1. LC-HRMS (Payload related metabolite identification)



Immunogenicity

1. Total ADA
   (Bridging/direct ADA assay)
2. NAb ADA
   (Cell based/non-cell based)

DAR evaluation

1. LC-MS/MS
   (Cleaved payload analysis)
2. LC-HRMS (Intact ADC analysis)

Total antibody

1. ELISA/MSD (Generic/specific assay)
2. LC-MS (Surrogate peptide)

Summary

- WuXi AppTec supports the pharmacokinetic evaluation of ADC from discovery to development

DMPK studies	Preclinical Discovery	Preclinical Development
	Lead optimization	**ADME Characterization and IND application**
ADME	- *In vitro* stability of ADC - Payload release from ADC *in vitro* - Payload release from LP *in vitro* - Metabolic stability of payload - *In vitro* permeability of payload	- *In vitro* stability of ADC and DAR evaluation - Microsomal/hepatocyte metabolite identification of payload - Plasma protein binding of payload - ADME study of radiolabeled payload in animals - *In vivo* metabolite identification/DAR evaluation of ADC
DDI	- Efflux transport substrate evaluation of payload	- Study on inhibition and induction of P450 enzyme by payload - Metabolic enzyme phenotyping of payload - Study on the inhibition of drug transporters by payload
PK	- PK study of ADC or payload in rodents - Screening PKPD study of ADC	- PK studies of ADC or payload in non-rodents (monkey) - PKPD study of ADC

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- Delivery Challenges and Strategies

Brief Introduction of CNS Oligos

CNS-Targeted Oligonucleotide Drugs Launched and Under Clinical Development

Phase 1	Phase 1/2	Phase 2	Phase 3
ION541/BIIB105	GTX-102	IONIS-MAPTRx/BIIB080	ION363
ION260/BIIB132	ION582	ION859/BIIB094	Zilganersen
ION464/BIIB101	WVE-003	STK-001	Tominersen
ALN-APP	WVE-004		

Launched

SPINRAZA®
(Nusinersen)
Spinal muscular atrophy

QALSODY®
(Tofersen)
Amyotrophic Lateral Sclerosis

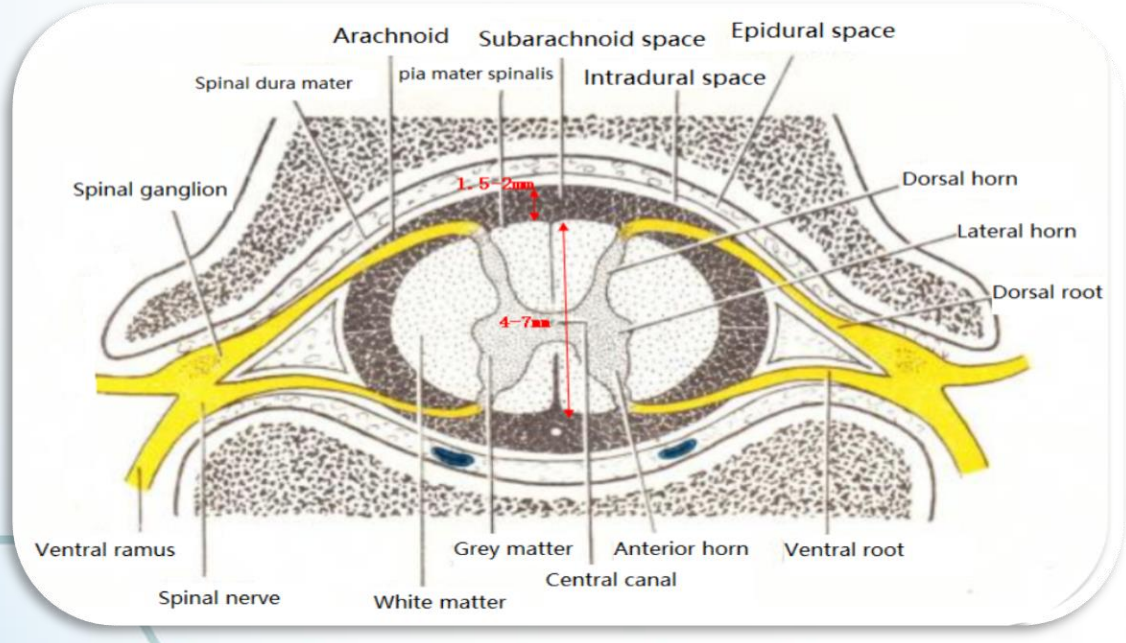
- **Delivery method to CNS:**
  Intrathecal injection (IT)
- **Preclinical animal species:**
  Rodent and Monkey

Biopharm Drug Dispos. 2023;44(1):26-47

Challenges for Intrathecal Injection (IT)

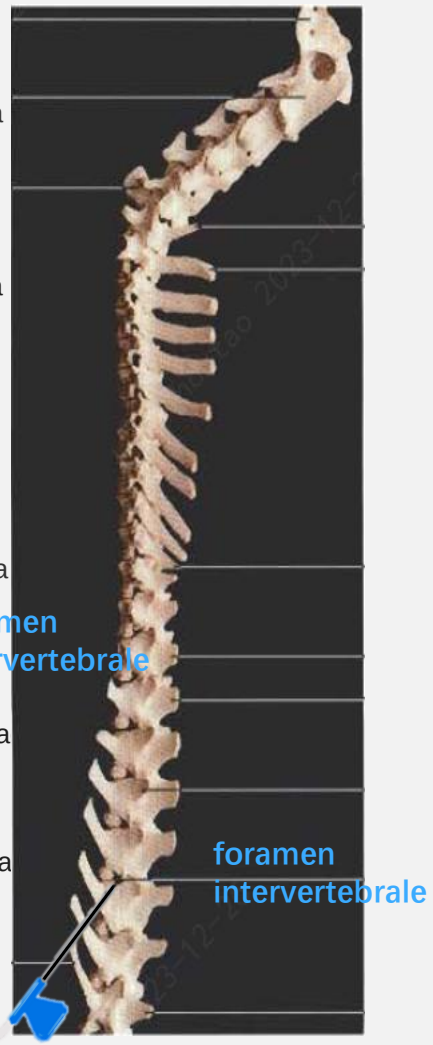
Challenges

- Ensure right position for IT
- Ensure serial CSF collection
- Evaluate success of IT



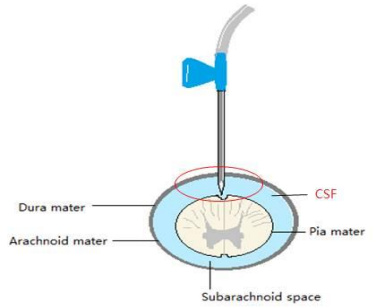
Cross-sectional image of lumbar spine

Anatomic diagram of monkey dorsal spine



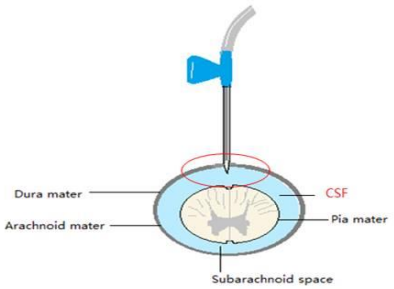
Anatomic diagram of dog dorsal spine

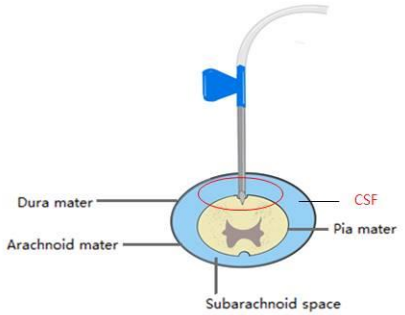
Ensure Right Position for IT and Inject Accurately



Right Position

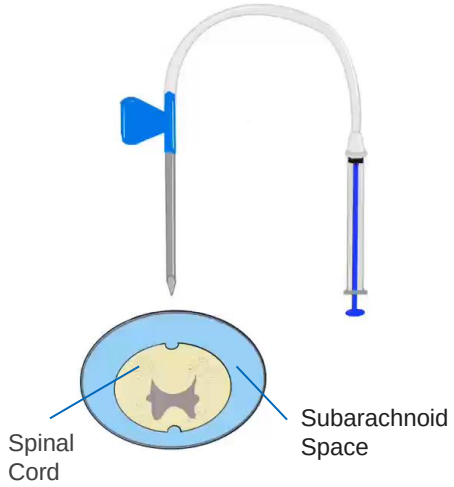
Vertical injection with slope of needle tip immersed in subarachnoid space



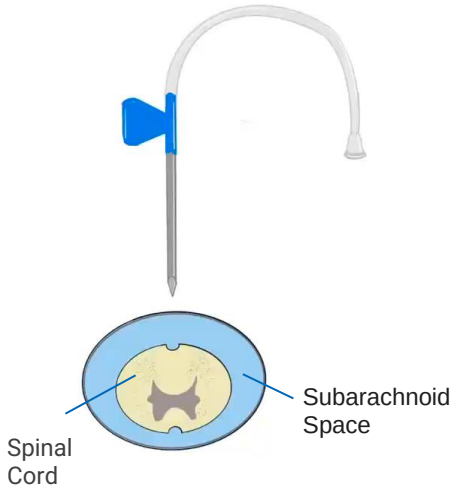


Wrong Position

Vertical injection with slope of needle tip partially immersed in Arachnoid mater or Pia mater



Pre-Optimized Method



Optimized Method

How to ensure success rate to find the right position

- Choose L4-L5 or L5-L6
- Injection tip with proper height of slope and ensure the tip totally immersed in subarachnoid space
- Observe CSF flow out instead of CSF drawn out
- Observe the CSF could flow out post injection

Ensure Serial CSF Collection

CMC, Cisterna Magna Cannulation

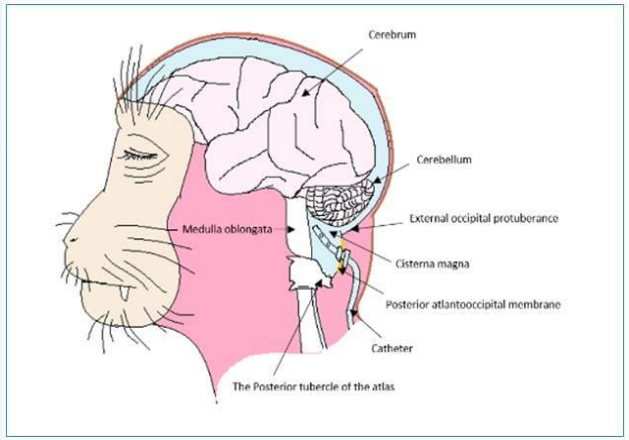


Fig 1. Anatomical Structure-Monkey Head

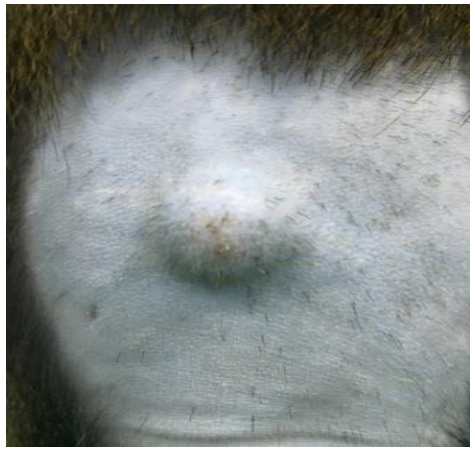


Fig. 2 Cisterna Magna Cannulation in a Monkey

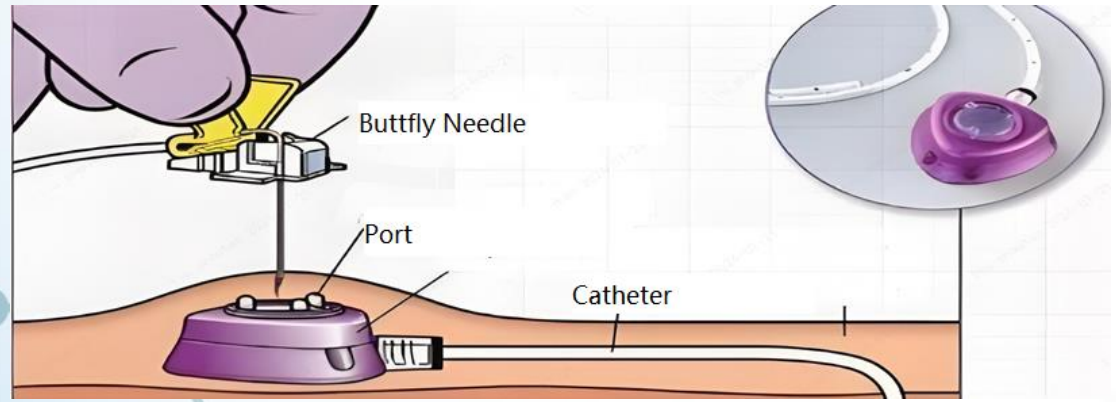


Fig 3. Subcutaneous Port

Correct CSF for CNS Evaluation

- CSF from CM is close to brain and could reflect free concentration in brain
- Support long term and serial sampling of CSF
- No anesthesia before CSF collection
- No contamination by blood

How to Evaluate Success of IT

Industrial Criteria （A patent from Alnylam）

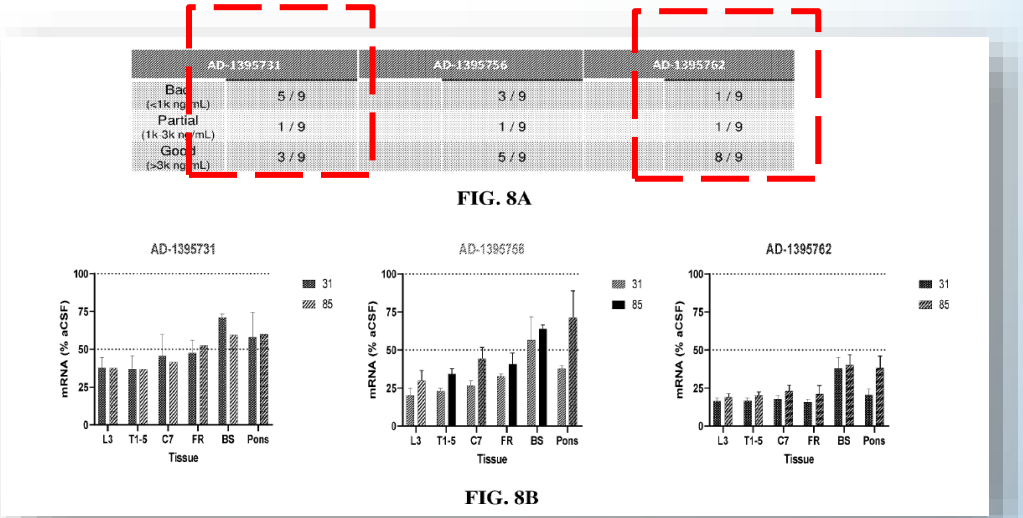
Definition: 70 mg/animal, siRNA, duplex levels in CSF at 24 hours post dosing

- **Bad exposure** < 1000 ng/mL
- **Good exposure** > 3000 ng/mL
- **Partial exposure:** 1000-3000 ng/mL

Success Rate: The patent mentioned that IT success rate ranged from 30 to 90% based on CSF exposure

Figure 8A is a Table depicting the numbers of non-human primates considered to have received "bad exposure" (duplex levels in a CSF sample <1,000 ng/mL at 24 hours), "good exposure" (duplex levels in a CSF sample >3,000 ng/mL at 24 hours), or "partial exposure" (duplex levels in a CSF sample 1,000-3,000 ng/mL at 24 hours) to a single 70 mg intrathecally administered dose of AD-1395762, AD-1395756, or AD-1395731.

Figure 8B are graphs depicting the level of SOD1 mRNA in lumbar spinal cord (L3), thoracic spinal cord (T1-T5), cervical spinal cord (C7), frontal cortex (FC), brainstem (BS), or pons samples in non-human primates following intrathecal administration of a single 70 mg dose of the indicated duplexes at Days 31 and 85 post-dose.



International Publication Number QO 2022/174000 A2, Fig 8A and Fig 8B

How to Evaluate Success of IT

Criteria at DMPK, Laboratory Testing Division:

- Dose Level: 60 mg/animal, Oligos
- Criteria of Success of IT: Initial CSF concentration of test article post IT dosing ≥ 1mg/mL at 1 hour post dosing

  (The CSF concentration at 1 hour of injection is considered as the initial concentration)

Success Rate: ~90%

An Example: Oligo concentration in CSF (ng/mL)	
Time (h)	C1001
1	3564212
6	460053
24	23869
336	64.5
672	48.8
1008	40.3
1344	30.2

Large Animal PKs to Support ADC and Oligos

Abundant Animal Resources

Sufficient large animals
(naïve or non-naïve monkey, dog and others) in stock, no lead time

Extensive Surgery Experience

Equipped with state-of-the-art animal surgery rooms
15+ years of surgery experience

Animal Welfare

Well trained and executed
Accredited by AAALAC and OLAW
European standard caging system

Comprehensive Capabilities

Various dose administration routes, extensive sample collections, and readily available large animal surgical models for cistern magna, bile duct, and more

Intelligent Facilities

Intelligent cage cards are utilized to manage animals

Intelligent Scheduling System

Full-process experiment nodes are made transparent and real-time, each step verification record is traceable

Questions

Scan to visit our website or contact an expert

Email: DMPK_Service@wuxiapptec.com