

OCT
20-23
2024

aaps
PharmSci 360

Integrated Bioanalysis and Biotransformation Strategies for Antibody-Drug Conjugates

October 21st, 2024

Li Qu, Ph.D., Associate Director, Non-GLP Bioanalysis

Peng Li, Ph.D., Senior Scientist, MetID



Session Description and Objectives

- Understand the role of integrated bioanalysis in nonclinical pharmacokinetic evaluation of ADCs.
- Learn advanced methodologies for ADC biotransformation, including metabolite profiling and DAR analysis.
- Explore strategies to overcome challenges in ADC bioanalysis using comprehensive analytical approaches.

Biography and Contact Information



Li Qu, Ph.D.

- ◆ Ph.D. in Occupational and Environmental Health
- ◆ Expertise in high resolution mass spectrometry
- ◆ Extensive experience in large molecular bioanalysis
- ◆ Extensive experience in the preclinical development of ADC drugs
- ◆ Supported over 100 projects (ADC, Fusion Protein, Covalent Inhibitor, Chiral SM, etc.)

Business Contact

Email: qu_li0101@wuxiapptec.com

DMPK Service: <https://labtesting.wuxiapptec.com/dmpk-services/>

OCT
20-23
2024

aaps
PharmSci 360[®]

Nonclinical Pharmacokinetic Evaluation of Antibody-Drug Conjugate (ADC) Drugs with Integrated Bioanalysis Platform

October 21st, 2024

Li Qu, Ph.D., Associate Director, Non-GLP Bioanalysis



Outline

01

Introduction of ADCs

02

ADC Integrated Bioanalysis Platform and DMPK Strategies

03

Case Studies

Outline

01

Introduction of ADCs

02

ADC Integrated Bioanalysis Platform and DMPK Strategies

03

Case Studies

15 ADC drugs approved in the past decade

15 Marketed ADC drugs

Kadcyla®

Toxin: DM1
Target: HER2



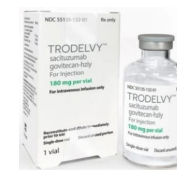
Lumoxiti®

Toxin: PE38
Target: CD22



Trodelvy®

Toxin : SN-38
Target : TROP2



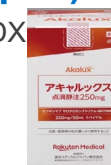
Blenrep™

Toxin : MMAF
Target: BCMA



Akalux® (Japan)

Toxin : IRDye700DX
Target: EGFR



Elahere™

Toxin: DM4
Target: FRα



2013

2018

2020

2022

2011

2017

2019

2021

Adcetris®

Toxin: MMAE
Target: CD30



Besponsa®

Toxin: Calicheamicin
Target: CD22



Mylotarg®

Toxin: Calicheamicin
Target: CD33



Polivy®

Toxin: MMAE
Target: CD79b



Padcev®

Toxin: MMAE
Target: Nectin-4



Enhertu®

Toxin: Dxd
Target: HER2



Zynlonta®

Toxin: PBD
Target: CD19



Aidixi® (China)

Toxin: MMAE
Target: HER2



Tivadak®

Toxin: MMAE
Target: TF



OCT
20-23
2024

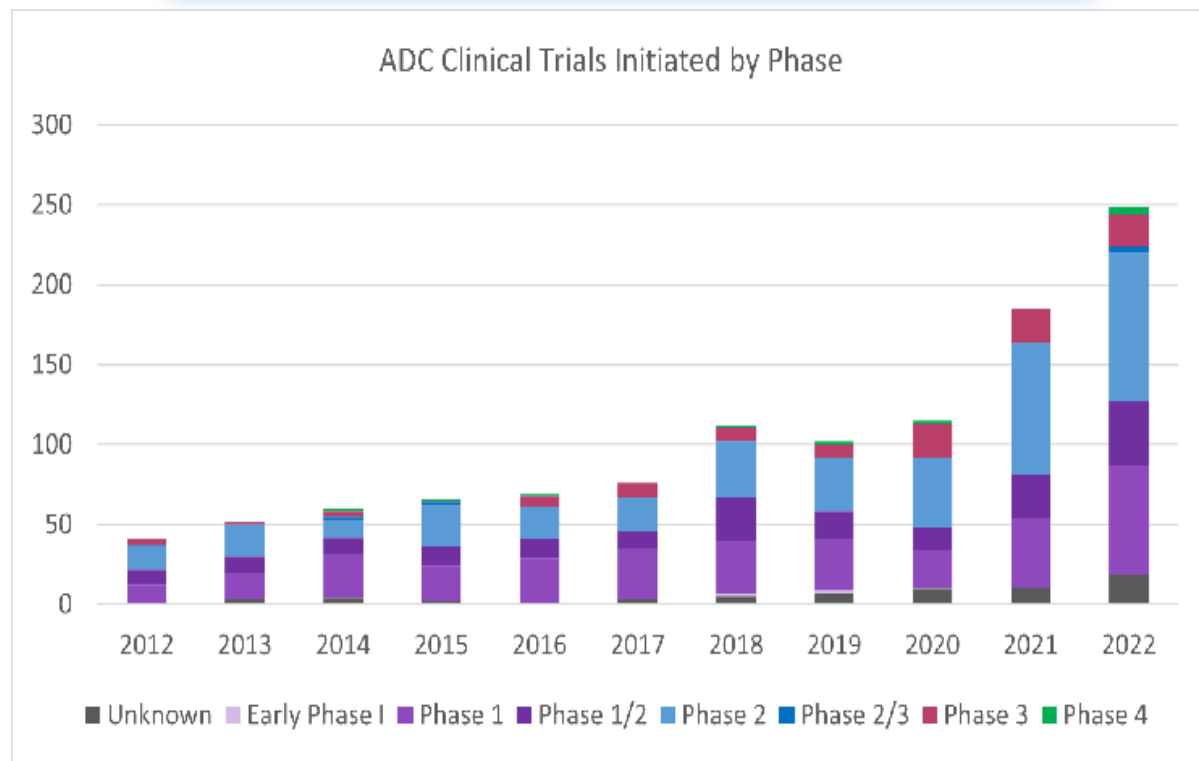
aaps
PharmSci 360

#PharmSci360

Slide 7

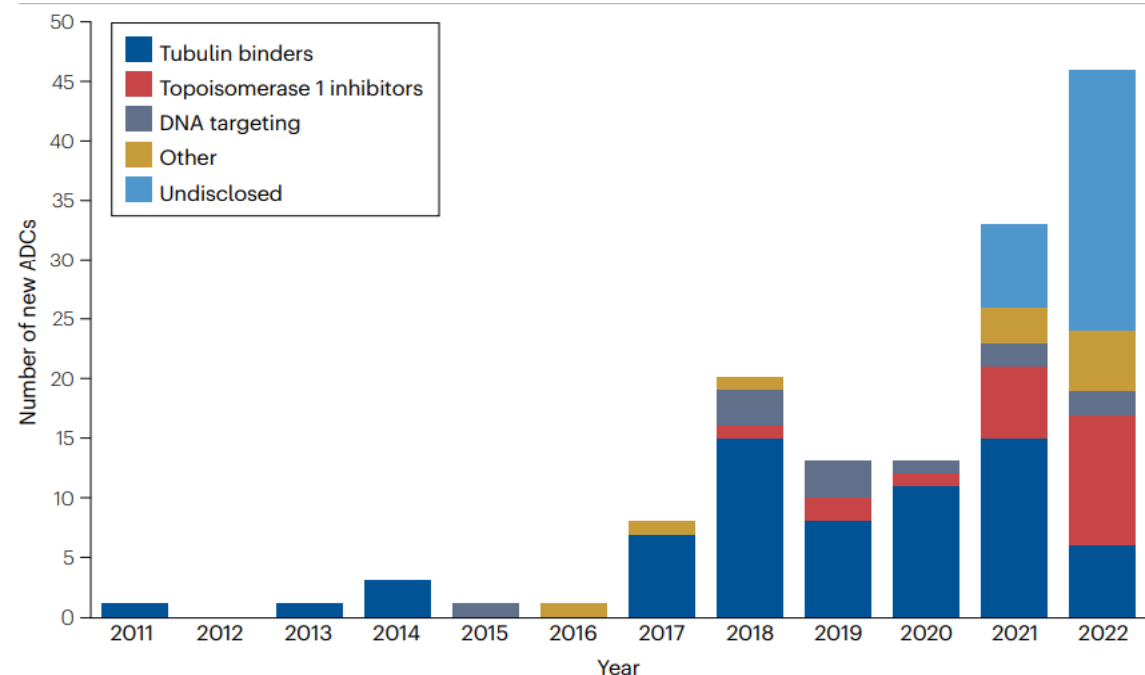
ADC Clinical Trials

Clinical trials initiated by phase per year from 2012 to 2022



Ref: The Clinical Landscape Of ADCs In 2023: Diverse Technologies, Narrow Target. *Bioprocess Online*, March 16, 2023.

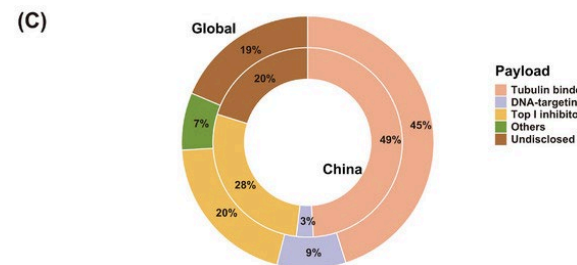
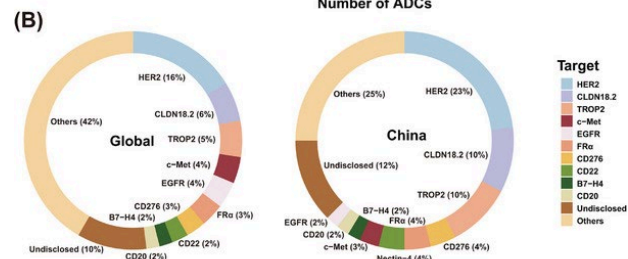
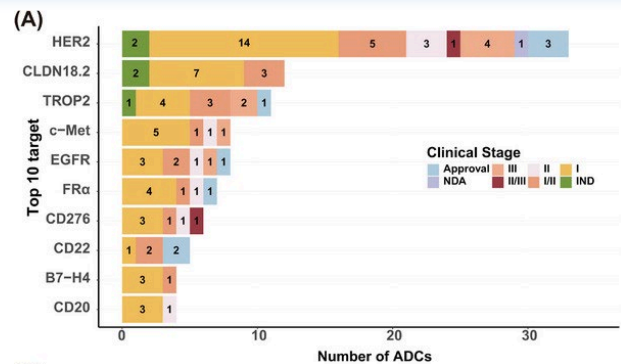
Clinical trials initiated by payload per year from 2012 to 2022



Ref: *Nat Rev Drug Discov* 22, 641–661 (2023).

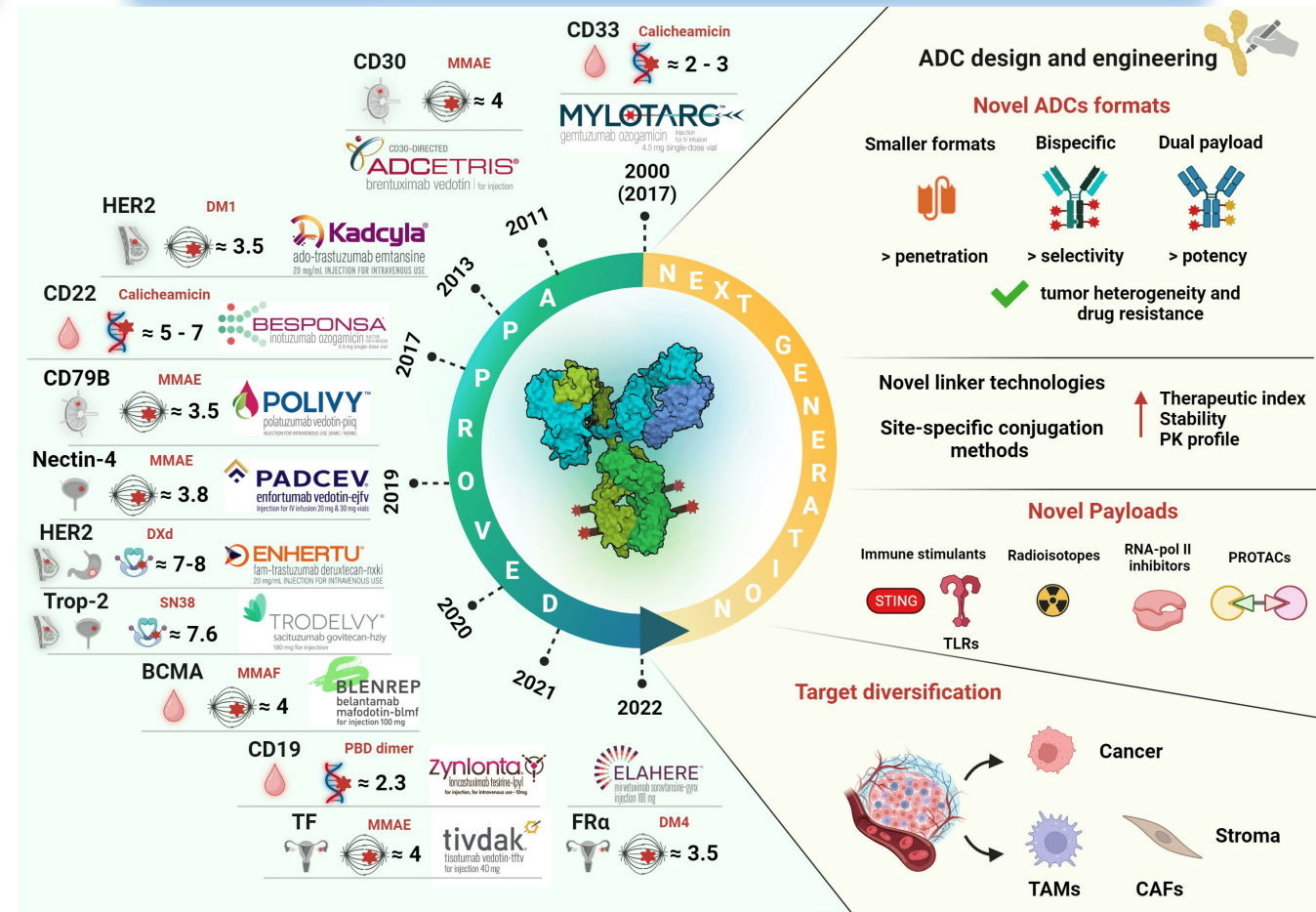
Next Generation ADCs

Continued explosive growth of the ADC pipeline



Ref: Cancer Commun (Lond). 2024 Jan; 44(1): 3–22.

New targets and new generation ADC technology



Ref: Cancers 2024, 16(2), 447

Suggested DMPK studies for ADCs

Recommended DMPK Studies	Object	Guidance/white page discription
<i>In vitro</i> stability	ADC	Before the clinical study, need to evaluate the ADC plasma/whole blood stability in human and relevant animal species.
<i>In vivo</i> PK	Total antibody/conjugated antibody/payload/major metabolites	<i>In vivo</i> PK: generally monitor total antibody, conjugated antibody and free payload or major metabolites.
Met ID and biotransformation	Linker-payload	For new linker-payload, necessary to understand the species of major metabolites.
Plasma protein binding	Payload/major metabolites	If the payload or major metabolite is new.
Drug metabolism phenotyping	Payload/major metabolites	
Transporters	Payload/major metabolites	
Enzyme induction/inhibition	Payload/major metabolites	
<i>In vivo</i> ADME	Payload/major metabolites	

Reference:

1. Kraynov, Eugenia, et al. "Current approaches for absorption, distribution, metabolism, and excretion characterization of antibody-drug conjugates: an industry white paper." Drug Metabolism and Disposition 44.5 (2016): 617-623.
2. Beaumont, Kevin, et al. "ADME and DMPK considerations for the Discovery and Development of Antibody Drug Conjugates (ADCs)." Xenobiotica just-accepted (2022): 1-44.
3. US Food and Drug Administration. "Clinical pharmacology considerations for antibody-drug conjugates guidance for industry: guidance for industry." (2023).
4. 《抗体偶联药物非临床研究技术指导原则》 NMPA, 2023.09

Outline

01

Introduction of ADCs

02

ADC Integrated Bioanalysis Platform and DMPK Strategies

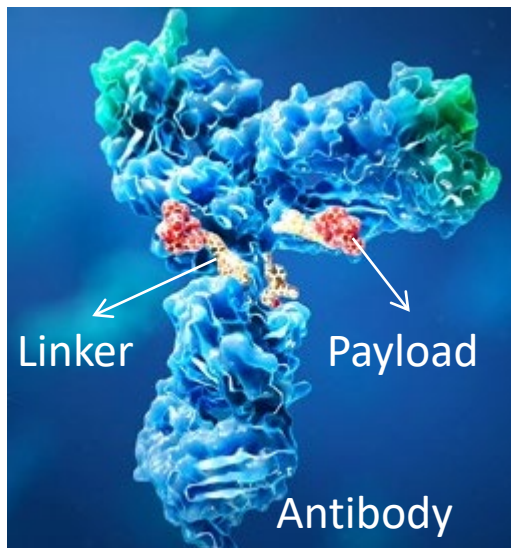
03

Case Studies

Bioanalytical Challenges for ADCs

ADC Complexity

- ❖ Antibody
- ❖ Small cytotoxic payloads



The ADCs species changed *in vivo*

- ❖ DAR (Drug-to-antibody ratio): the number of payloads conjugated to antibody
- ❖ DAR changed *in vivo* dynamically

Multiple bioanalysis platforms

- ❖ Pharmacokinetics
- ❖ Exposure-response relationships

Monitor multiple analytes

- ❖ Total antibody
- ❖ Conjugated antibody
- ❖ Free payload
- ❖ Conjugated payload
- ❖ ADA

Integrated Bioanalysis Platform for ADC Drugs

ADC bioanalysis

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

Free payload analysis

1. LC-MS/MS
2. Competitive ELISA

Payload-related metabolites

1. LC-HRMS (Payload related species identification)
2. LC-MS/MS (conjugated payload PK)

Immunogenicity detection

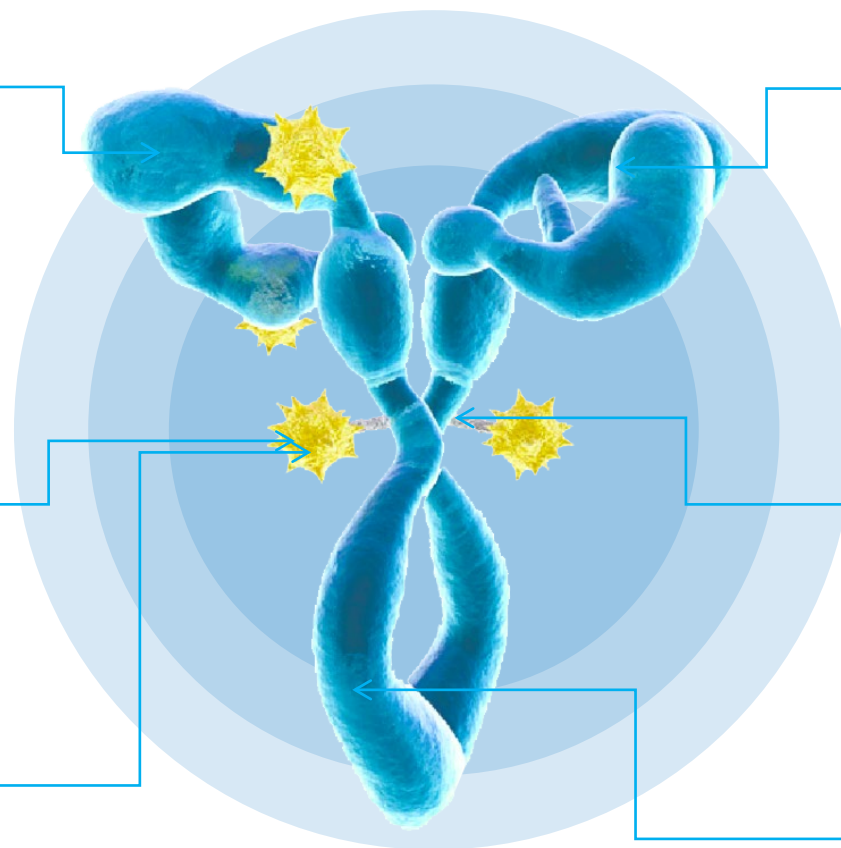
1. Total ADA
(Bridging/direct ADA assay)
2. NAb ADA
(Cell based/non-cell based)

DAR detection

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

Total antibody bioanalysis

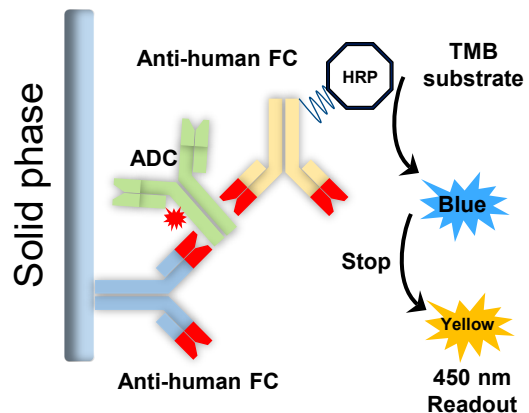
1. ELISA/ MSD (Generic/specific assay)
2. LC-MS/MS (Surrogate peptide)



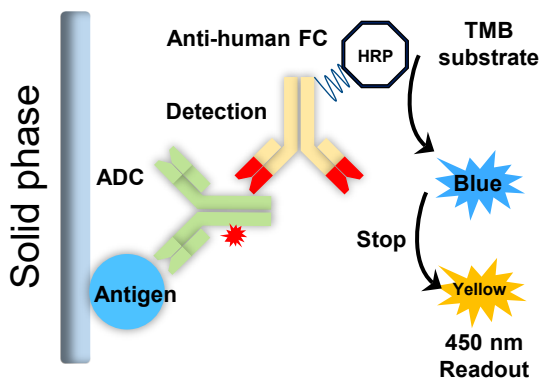
Total Antibody Bioanalysis

Ligand Binding Assay (Elisa/MSD)

Generic method

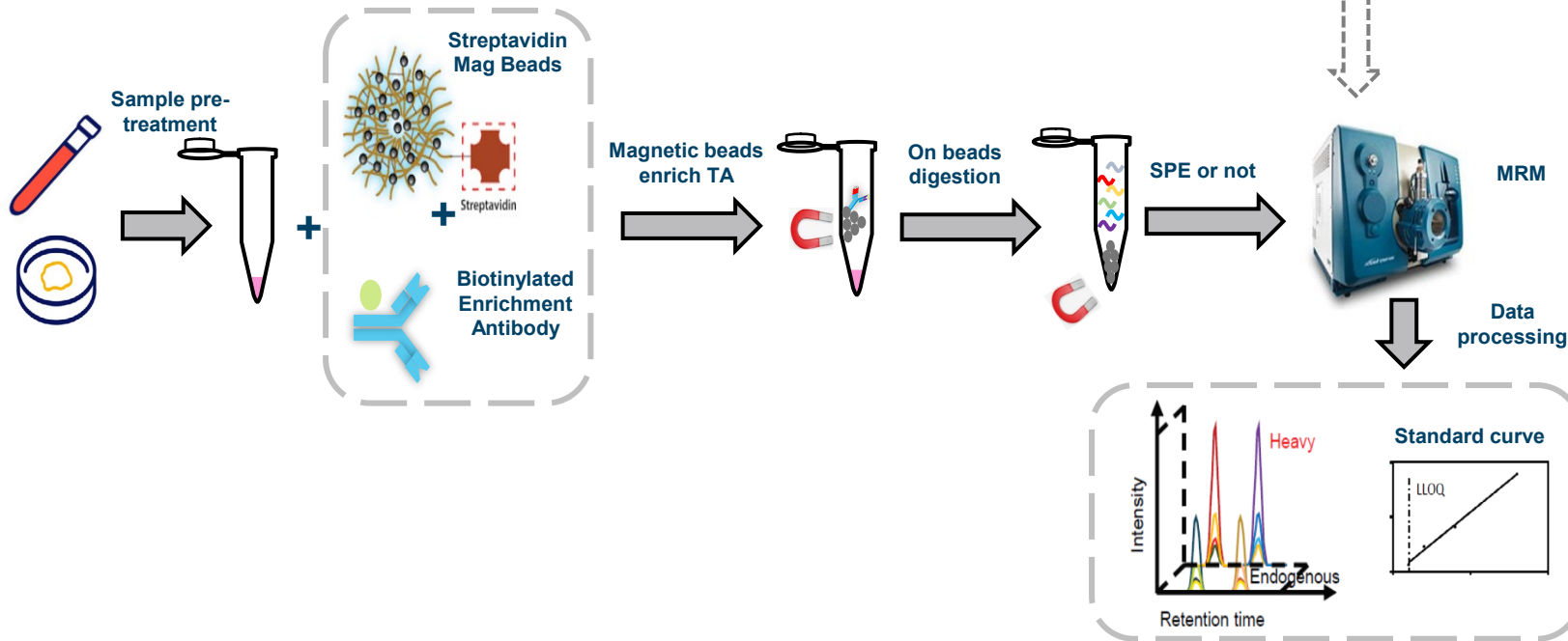
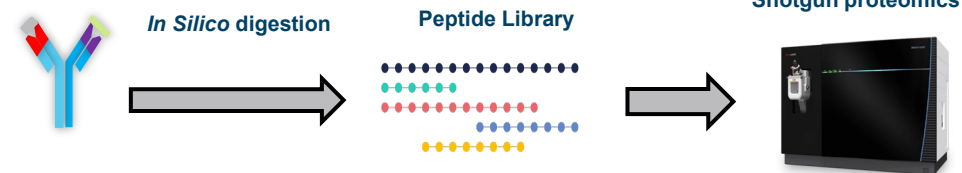


Specific method



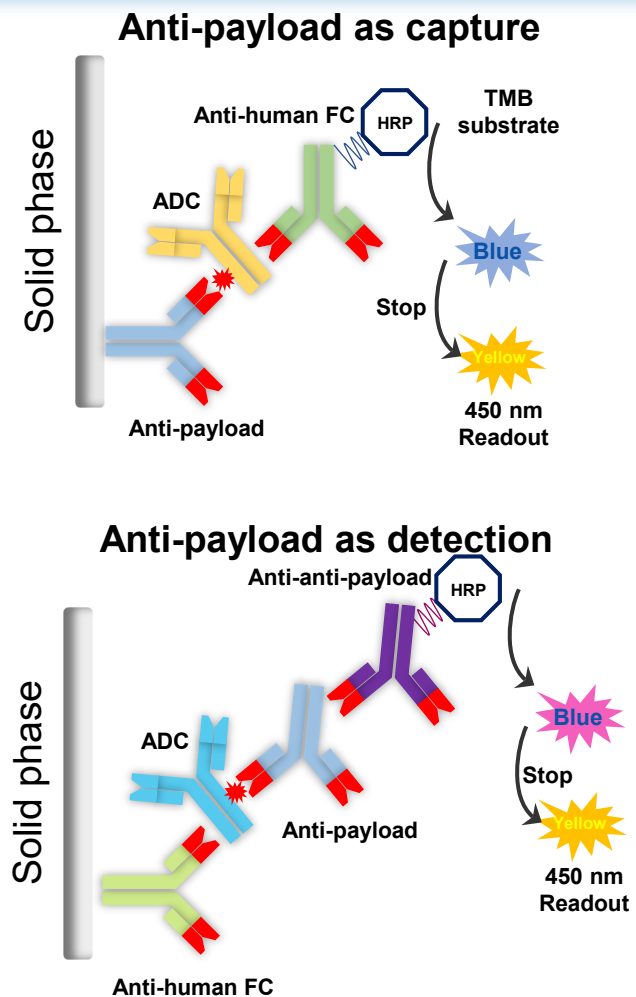
Surrogate Peptide Approach (LC-MS/MS)

Optimization for best surrogate peptides

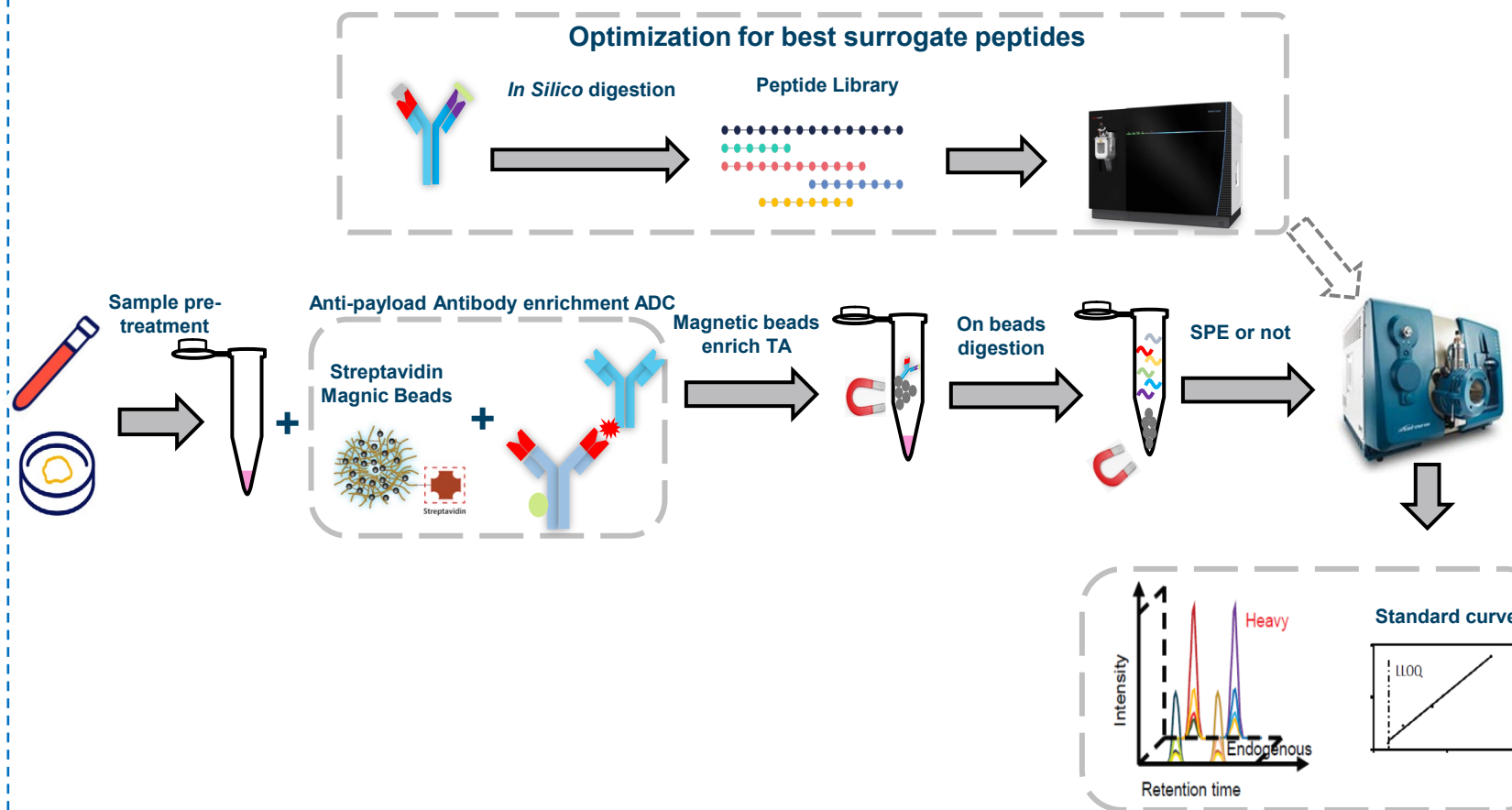


Conjugated Antibody Bioanalysis

Ligand Binding Assay (Elisa/MSD)



Surrogate Peptide Approach (LC-MS/MS)



Payload and Major Metabolite Bioanalysis

LC-MS/MS (Tri-Quad)



- ✓ Protein Precipitation
- ✓ Higher sensitivity (lower LLOQ)
- ✓ Good specificity, precision and accuracy
- ✓ Payload stability in matrix
- ✓ PK, biodistribution and excretion study

Analytes	Species	LLOQ
MMAE	Mouse/Rat/Dog/Monkey/Human	~20 pg/mL
DM1	Mouse/Rat/Dog/Monkey/Human	~50 pg/mL
SN-38	Mouse/Rat/Dog/Monkey/Human	~50 pg/mL
DXd	Mouse/Rat/Dog/Monkey/Human	~20 pg/mL
MMAF	Mouse/Rat/Dog/Monkey/Human	~20 pg/mL
DM4	Mouse/Rat/Dog/Monkey/Human	~50 pg/mL
Calicheamicin	Mouse/Rat/Dog/Monkey/Human	~50 pg/mL
Lys-MCC-DM1	Mouse/Rat/Dog/Monkey/Human	~50 pg/mL
.....		

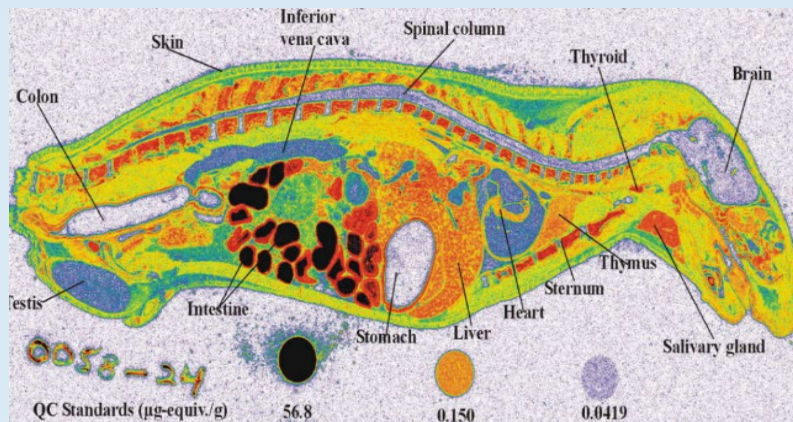
Radiolabeled ADME study for payloads of ADCs

Service

^{14}C -labeled compound synthesis
Purification
Certificate of Analysis
Support IND/NDA filings to FDA/NMPA

Experience

10+ years of synthesis experience
500+ compounds
ADC, PDC, PROTAC, PEPTIDE, etc.



- ❑ Common approach is to trace the ADME of **radiolabeled payload** *in vivo*.
- ❑ Tumor-bearing animals are often used to prove the targeted delivery of **radiolabeled ADC**.

Pharmacokinetic Evaluation of ADCs from Discovery to Development

DMPK studies		Pre-clinical Discovery	Pre-clinical Development
		Lead optimization	ADME Characterization and IND application
ADME	- Plasma/serum/blood stability of ADC - Payload release from ADC <i>in vitro</i> - Payload release from LP <i>in vitro</i> - Metabolic stability of payload		- Microsomal/hepatocyte metabolite identification of payload - Plasma protein binding of payload - ADME study of radiolabeled payload in animals - <i>In vivo</i> metabolite identification of ADC
DDI	- Study on the permeability and efflux transport 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 - PKPD study of ADC

DMPK Integrated Bioanalytical Strategy

Platform	Analyte	Notes
LBA	- Total Antibody - Conjugated Antibody (ADC) - Anti-Drug Antibody (ADA) - Neutralizing Antibody (nAB)	Conjugated Antibody(ADC) needs anti-payload antibody
Triple Quadrupole	- Total antibody(Surrogate Peptide Approach) - Conjugated Antibody (Surrogate Peptide Approach) - Free Payload - Conjugated Payload - Payload Related Metabolite	Conjugated Antibody(ADC) needs anti-payload antibody
LC-HRMS	- Monitor DAR value <i>in vitro</i> / <i>in vivo</i> - ADC Biotransformation - ADC structural characterization(Intact/Subunit MD)	- According to the ADC structure, fit for purpose design is required - Identify metabolites in plasma/tumor cells/hepatocytes as needed
QWBA	ADC payload Distribution Research	The payload needs to be pre-labeled with isotopes

Outline

01

Introduction of ADCs

02

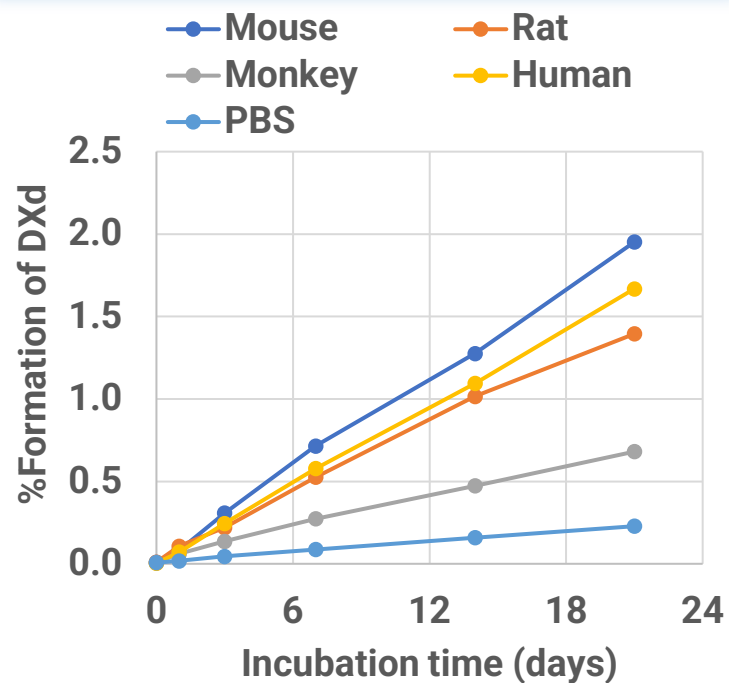
ADC Integrated Bioanalysis Platform and DMPK Strategies

03

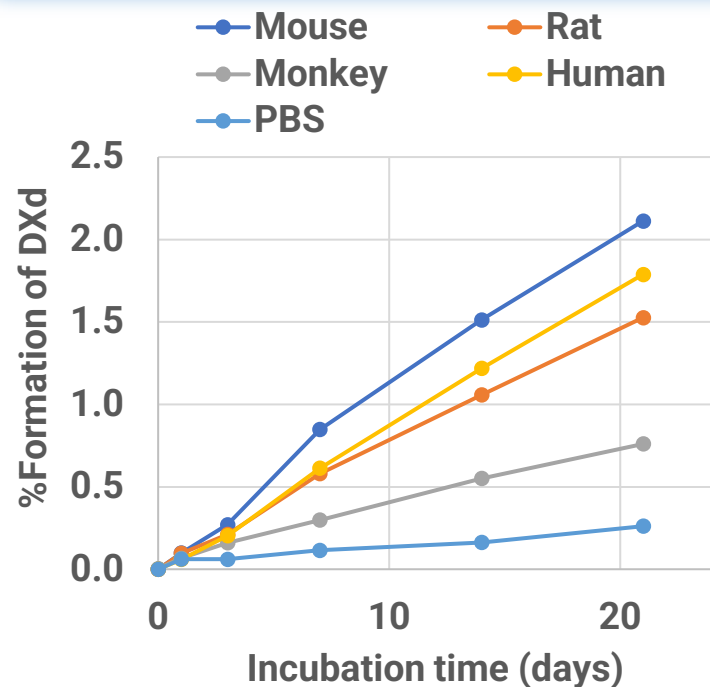
Case Studies

Case 1 *In vitro* Plasma vs Blood Stability of ADC

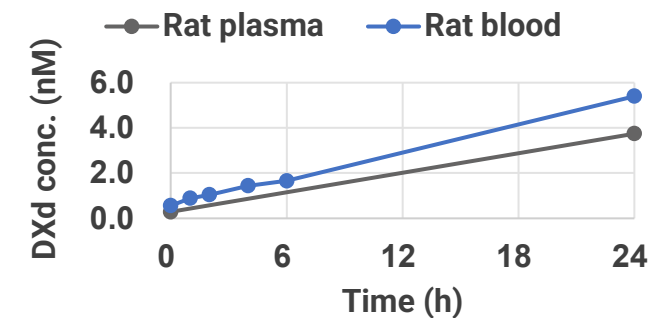
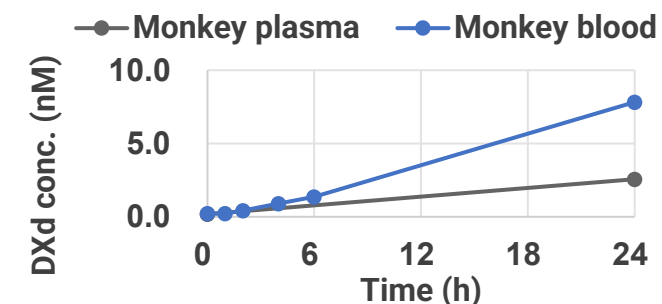
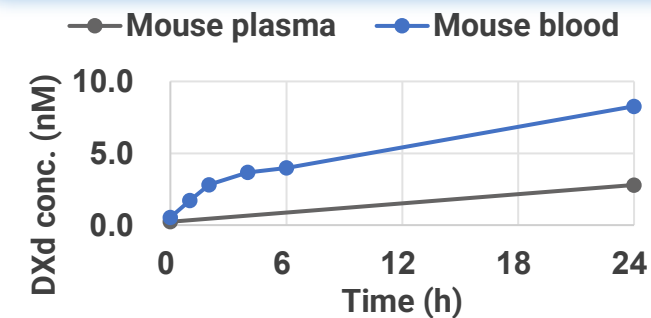
0.1 mg/mL of ADC in plasma



0.01 mg/mL of ADC in plasma

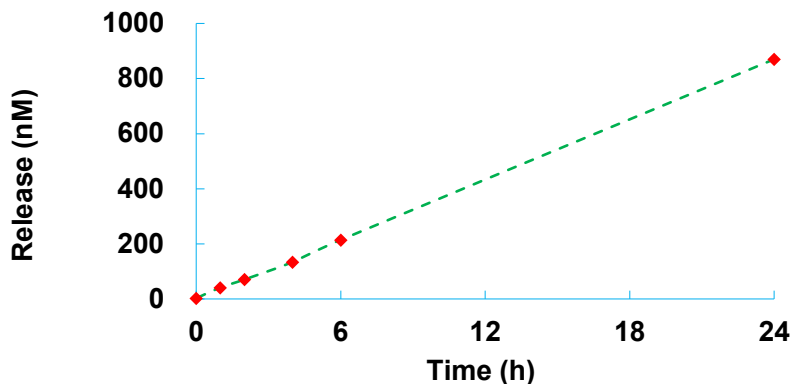


Plasma vs Blood

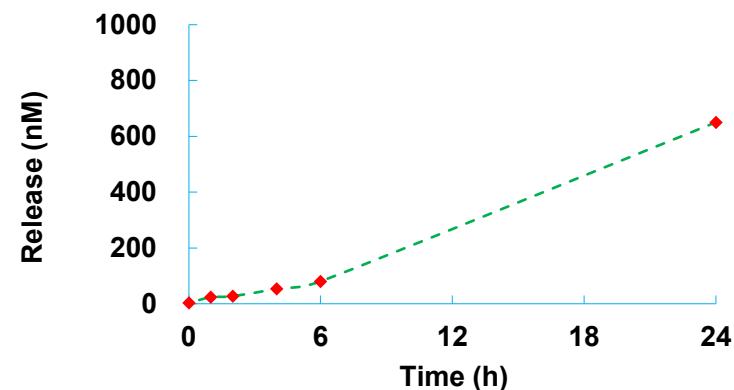


Case 2 *In vitro* Liver Lysosome, S9 and Homogenate Stability of ADC

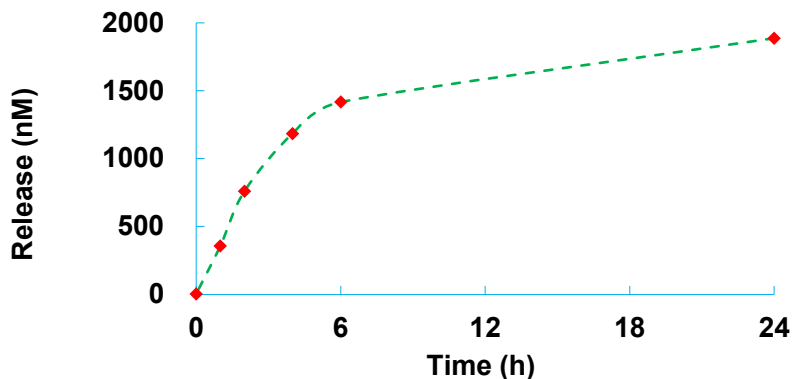
Release of MMAE in Human Liver Lysosome



Release of MMAE in Human Liver S9



Release of MMAE in Human Liver Homogenate



Protein concentration	1 mg / mL
ADC	0.1 mg/ mL
Incubation buffer	2 mM TCEP in 50 mM sodium acetate, pH=5.0, fresh prepared
Incubation temperature	37 °C
Incubation time (h)	0, 1, 2, 4, 6, 24

Case 3 ADCETRIS *In vitro* Plasma Stability

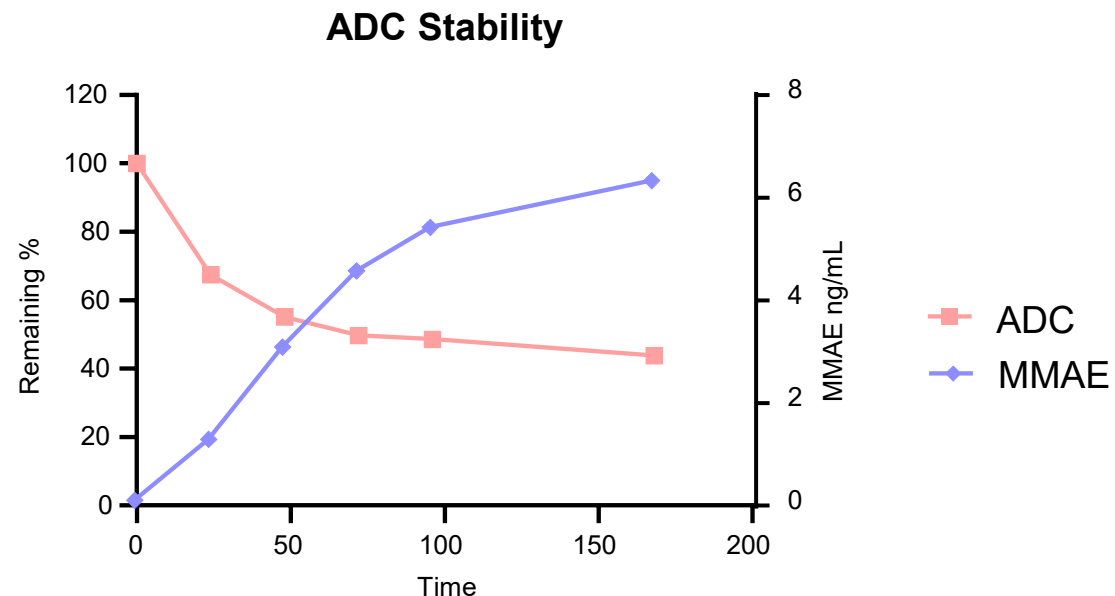
TA: ADCETRIS (Brentuximab vedotin)

Species: Human plasma (Heparin)

Concentration: 100µg/mL

Duration: 7 days

Temperature: 37°C



ADC bioanalysis

Capture: Anti-MMAE Mab. clone 3F3
Detection: Goat Anti-Human IgG HRP

MMAE bioanalysis

LC: Waters Aquity UPLC
MS: SCIEX 6500+
Column: ACQUITY UPLC Protein BEH C4 300A 1.7 µm
2.1 * 50 mm Column

Case 4 Enhertu (DS-8201) *In vivo* PKPD



CDX (Cell Line-Derived Xenograft):

JIMT-1 & HCC1954 Balb/c nude

Dose: DS8201 (Enhertu), 3/10 mpk for JIMT-1; 5/10 mpk for HCC1954

Evaluate Total antibody, conjugated antibody, and free payload concentration in plasma, tumor

❖ Total antibody

Capture: Goat Anti-human IgG, ads-UNLB

Detection: Mouse Anti Human IgG Fc-HRP

❖ Conjugated antibody

Capture: Anti-DXd Mouse monoclonal antibody

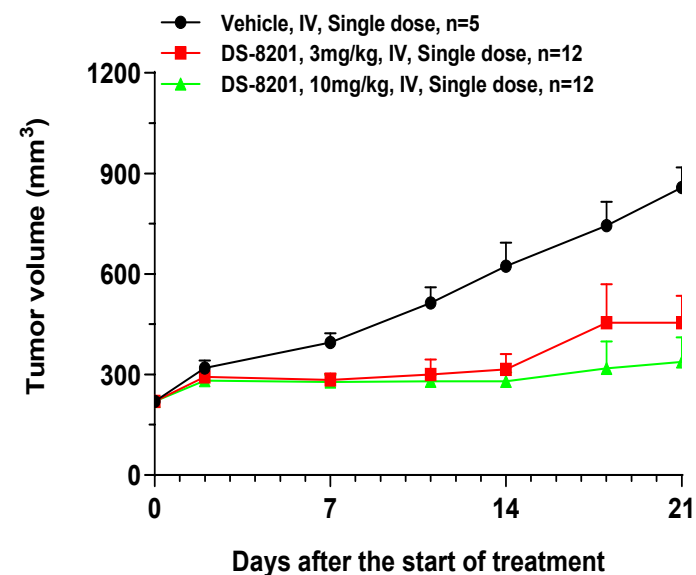
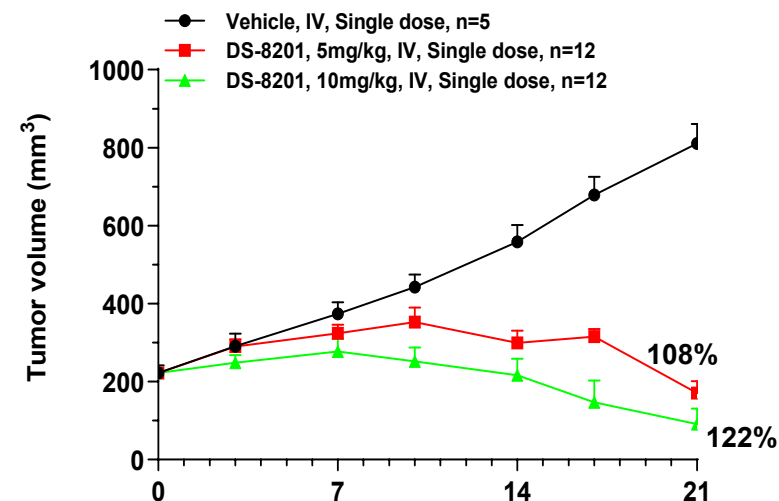
Detection: Mouse Anti Human IgG Fc-HRP

❖ Free payload

LC: Waters Acquity UPLC

MS: SCIEX 6500+

SRM Q1/Q3: DXd: m/z: 494.30/419.20



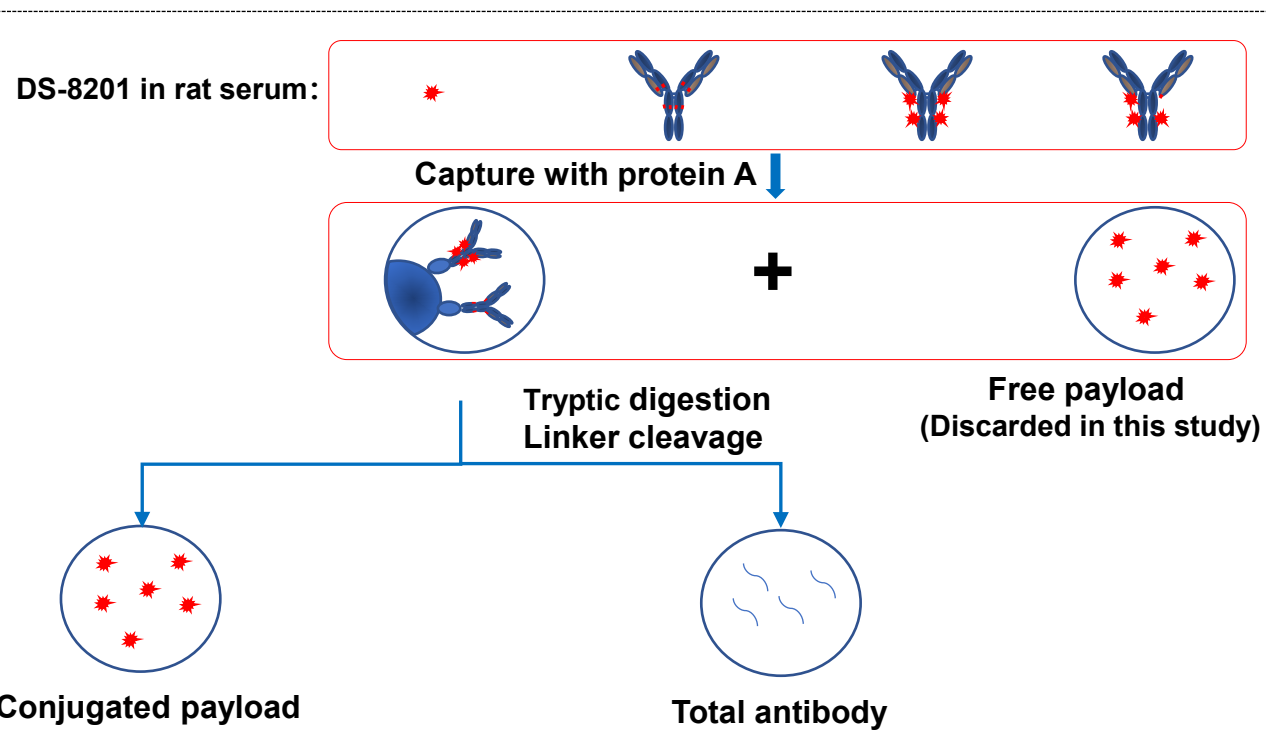
Case 5 Total Antibody & Conjugated Payload

A Novel Validated LC-MS/MS Approach for Simultaneous Determination of Total Antibody and Conjugated Payload for DS-8201 in SD Rat Serum

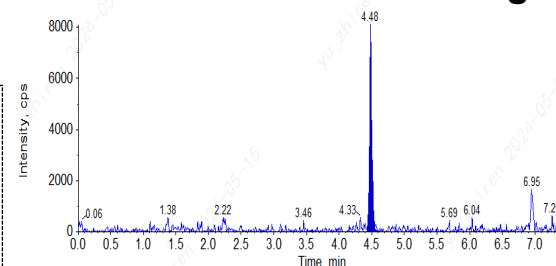
Zhiren Yu; Hefeng Zhang; Wenhan Zhang; Zhiyu Li; Hongmei Wang; Lili Xing; Yi Tao*; Liang Shen
DMPK Service Department, WuXi AppTec, 31 Yiwei Road, Waigaoqiao Free Trade Zone, Shanghai, P.R. China, 200131



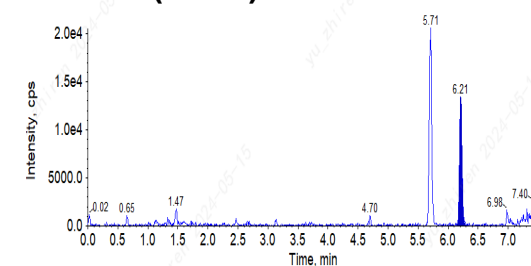
Poster#: WP023



Chromatograms at LLOQ (2 nM) level



Total antibody
("TPPVLDSDGSFFLYSK" peptide)



Conjugated payload (Calculated with
DS-8201, or 15.8 nM for DXd in theory)

Tryptic digestion efficiency (DS-8201 v.s. Trastuzumab)

Sample Name	Peak Area (of peptide)	Mean Peak Area	Efficiency (%)
Trastuzumab LQC 1,2,3	6.09E+04; 5.35E+04; 6.25E+04	5.90E+04	106.0%
DS-8201 LQC 1,2,3	6.49E+04; 5.93E+04; 6.21E+04	6.21E+04	
Trastuzumab HQC 1,2,3	4.95E+06; 5.69E+06; 5.76E+06	5.46E+06	93.6%
DS-8201 HQC 1,2,3	5.15E+06; 5.09E+06; 5.10E+06	5.11E+06	

Conjugated payload release efficiency (DS-8201 v.s. spiked-in DXd)

Sample Name	Peak Area (of Dxd)	Mean Peak Area	Efficiency (%)
DS-8201 LQC 1,2,3	8.32E+04; 7.73E+04; 7.54E+04	7.86E+04	82.0%
DXd LQC 1,2,3	8.93E+04; 9.17E+04; 1.07E+05	9.60E+04	
DS-8201 HQC 1,2,3	6.02E+06; 6.48E+06; 6.48E+06	6.33E+06	98.0%
DXd HQC 1,2,3	7.16E+06; 6.35E+06; 5.93E+06	6.48E+06	

Case 6 Conjugated Payload-MC-GGFG & MC-VC-PABC linker

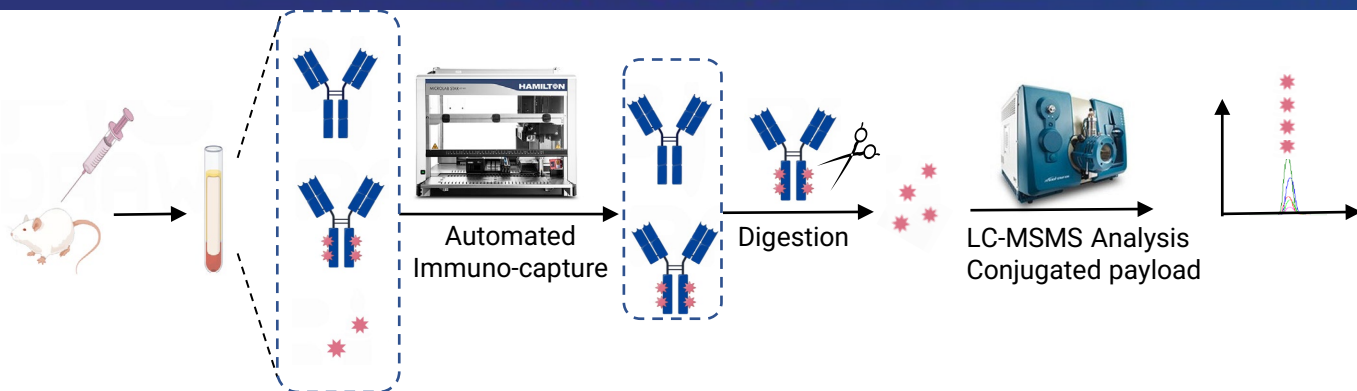
W1030-06-36

Development of a Rapid, High Throughput Quantitative Bioanalysis Method for Conjugated Payloads of Protease-Cleavable Antibody-Drug Conjugate (ADC) in Rat Serum Using Automated Immuno-Capture Based UPLC-MS/MS

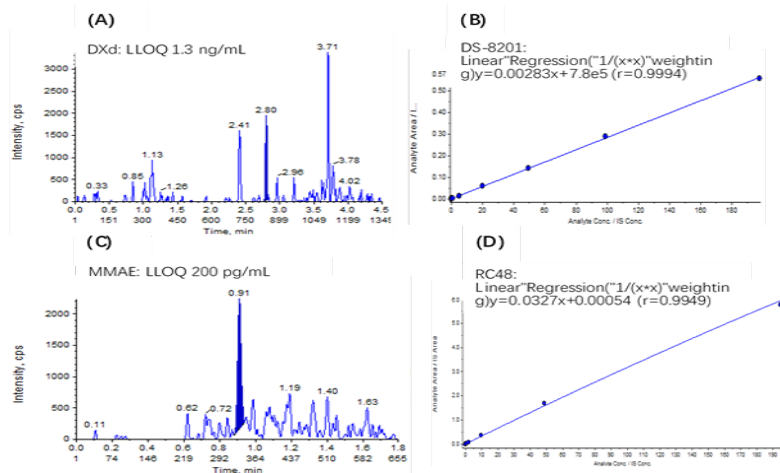
Li Qu, Rui Wang, Hongmei Wang, Zhiyu Li, Lili Xing, Yi Tao, Liang Shen

DMPK Service Department, WuXi AppTec, 31 Yiwei Rd., Waigaoqiao Free Trade Zone, Shanghai, P. R. China, 200131

aaps
PharmSci 360



Items	Method	
ADC	Trastuzumab deruxtecan (DS-8201)	Disitamab vedotin (RC48)
Linker	MC-GGFG	MC-VC-PABC
DAR	About 8	About 4
Payload	DXd	MMAE



Name	Analyte	ADC Concentration (µg/mL)	Intensity	Average Intensity	Digestion Efficiency (%)
Sample-(DS-8201) (without immunocapture, with digestion)	DXd	2.00	1.22E+05	1.17E+05	92.1%
			1.10E+05		
			1.20E+05		
Control sample (Add theoretical concentration of DXd in blank)			1.31E+05	1.27E+05	
			1.29E+05		
	1.22E+05				
Sample-(RC48) (without immunocapture, with digestion)	MMAE	0.10	1.98E+04	2.20E+04	78.0%
			2.20E+04		
			2.41E+04		
Control sample (Add theoretical concentration of MMAE in blank)			2.63E+04	2.83E+04	
			2.95E+04		
	2.92E+04				

OCT
20-23
2024

aaps
PharmSci 360

#PharmSci360

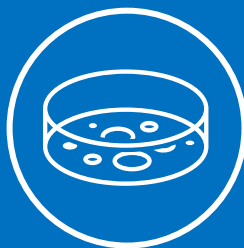
Slide 26

WuXi AppTec DMPK Figures in ADC drugs



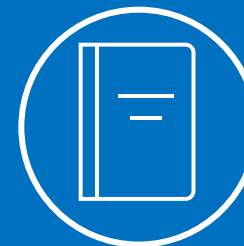
8+

Years bioanalysis experience



300+

Studies per year



90+

MID studies for ADCs



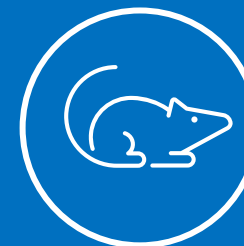
100+

IND filing projects



60+

Global clients with ADC pipelines



4+

Bioanalysis platforms:
ELISA/MSD, LC-MS, LC-
HRMS, Radioactivity, etc.

Biography and Contact Information



Peng Li, Ph.D.

- ◆ Ph.D. in Pharmaceutical (Bio-)Analysis
- ◆ Expertise in high resolution mass spectrometry
- ◆ Extensive experience in metabolomics and lipidomics
- ◆ Hands-on experience in “cold” and “hot” MetID
- ◆ Supported over 100 projects (SM, peptides, PDC, ADC, etc.)

Business Contact

Email: li_peng2101@wuxiapptec.com

DMPK Service: <https://labtesting.wuxiapptec.com/dmpk-services/>

OCT
20-23
2024

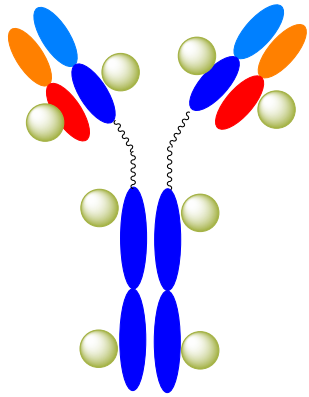
aaps
PharmSci 360

Methodologies and strategies for biotransformation study of antibody-drug conjugates

October 21st, 2024

Peng Li, Ph.D., Senior Scientist, MetID





01

Metabolic fate of ADC

02

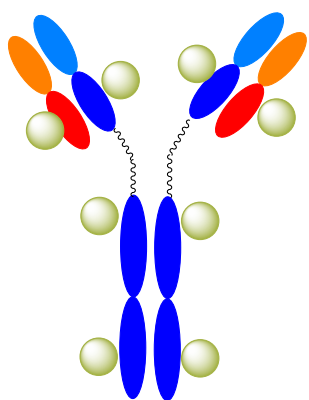
Payload-related MetID

03

DAR distribution determination

04

Conclusions



01

Metabolic fate of ADC

02

Payload-related MetID

03

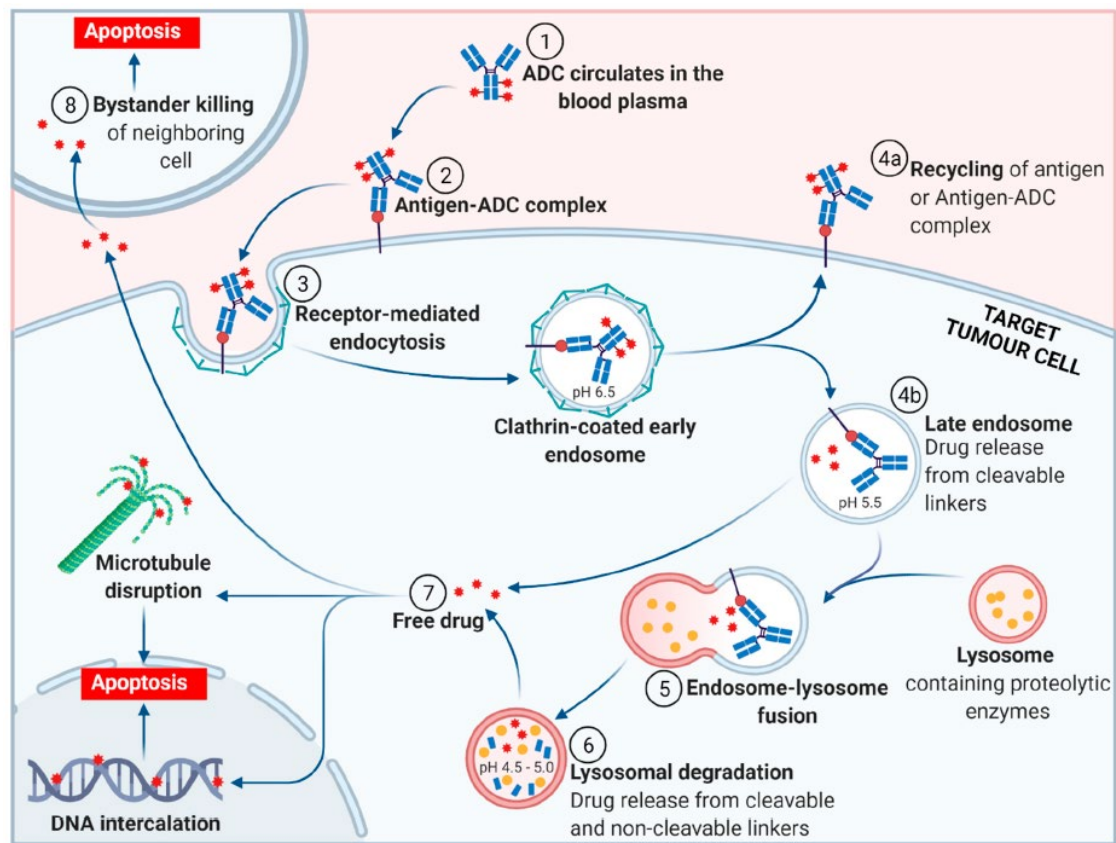
DAR distribution determination

04

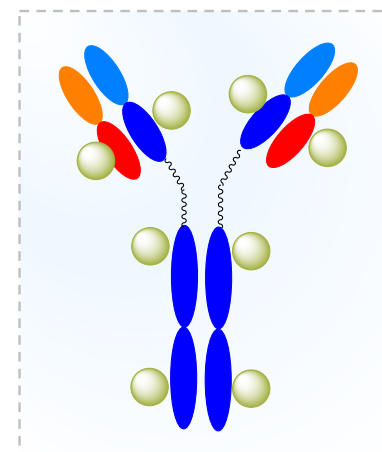
Conclusions

Mechanism of Action (MOA) and metabolic fate

MOA of ADC drugs



Biotransformation of small and large molecule



Payload release



DAR loss



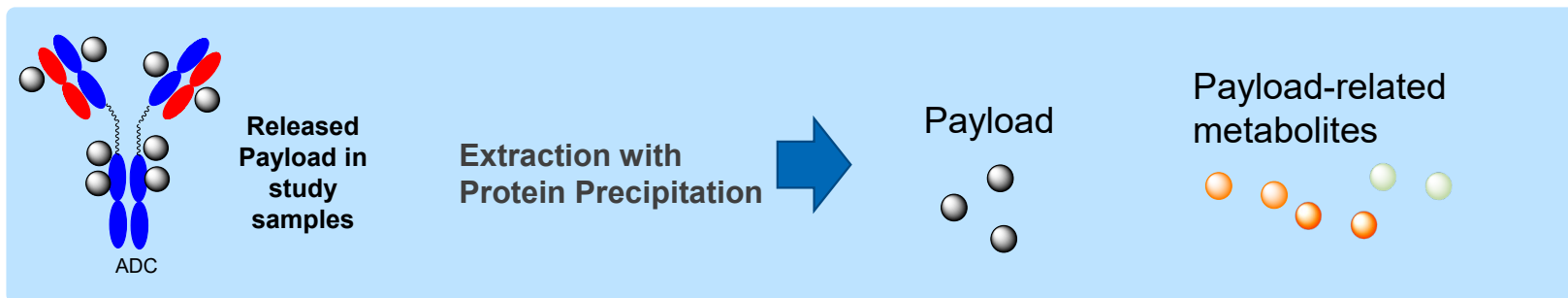
Modifications



Biotransformation characterization workflows for ADC

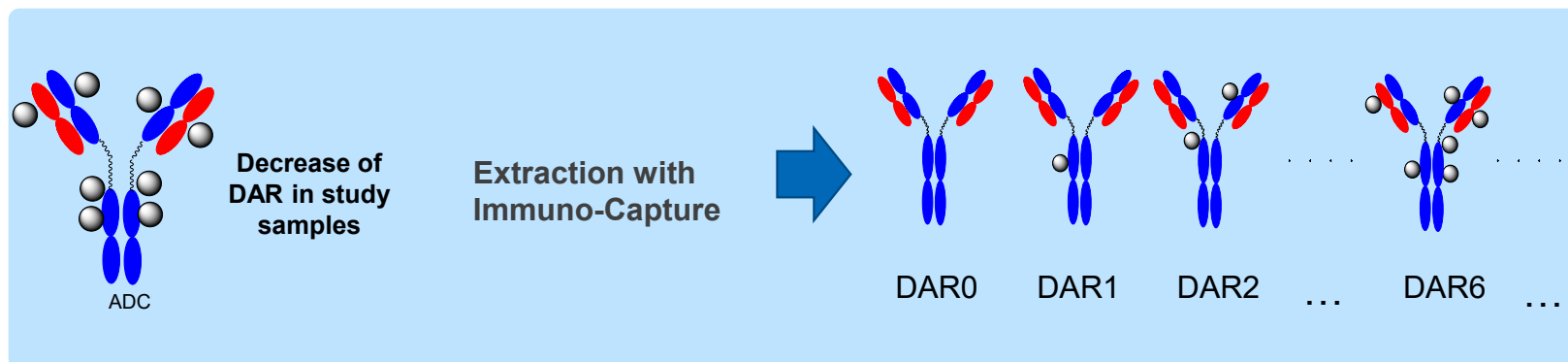
➤ Payload-related metabolite identification (MetID)

- ✓ *In vitro* plasma, S9 (Lysosome) and tumor cell
- ✓ *In vivo* plasma



➤ DAR distribution determination (various conjugation types)

- ✓ *In vitro* plasma with time course incubation
- ✓ *In vivo* plasma from PK study



LC-HRMS Platform and Software



Thermo Orbitrap
Eclipse™ Tribrid™



Thermo Orbitrap
Exploris™ 480



Thermo
Q Exactive™



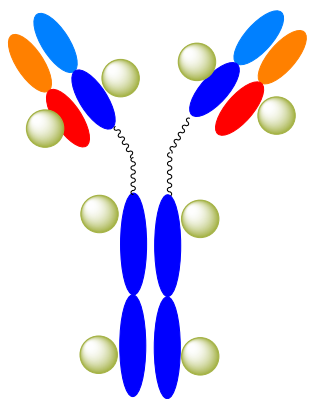
Waters VION™
IMS QTof



BioPharma
Finder



UNIFI



01

Metabolic fate of ADC

02

Payload-related MetID

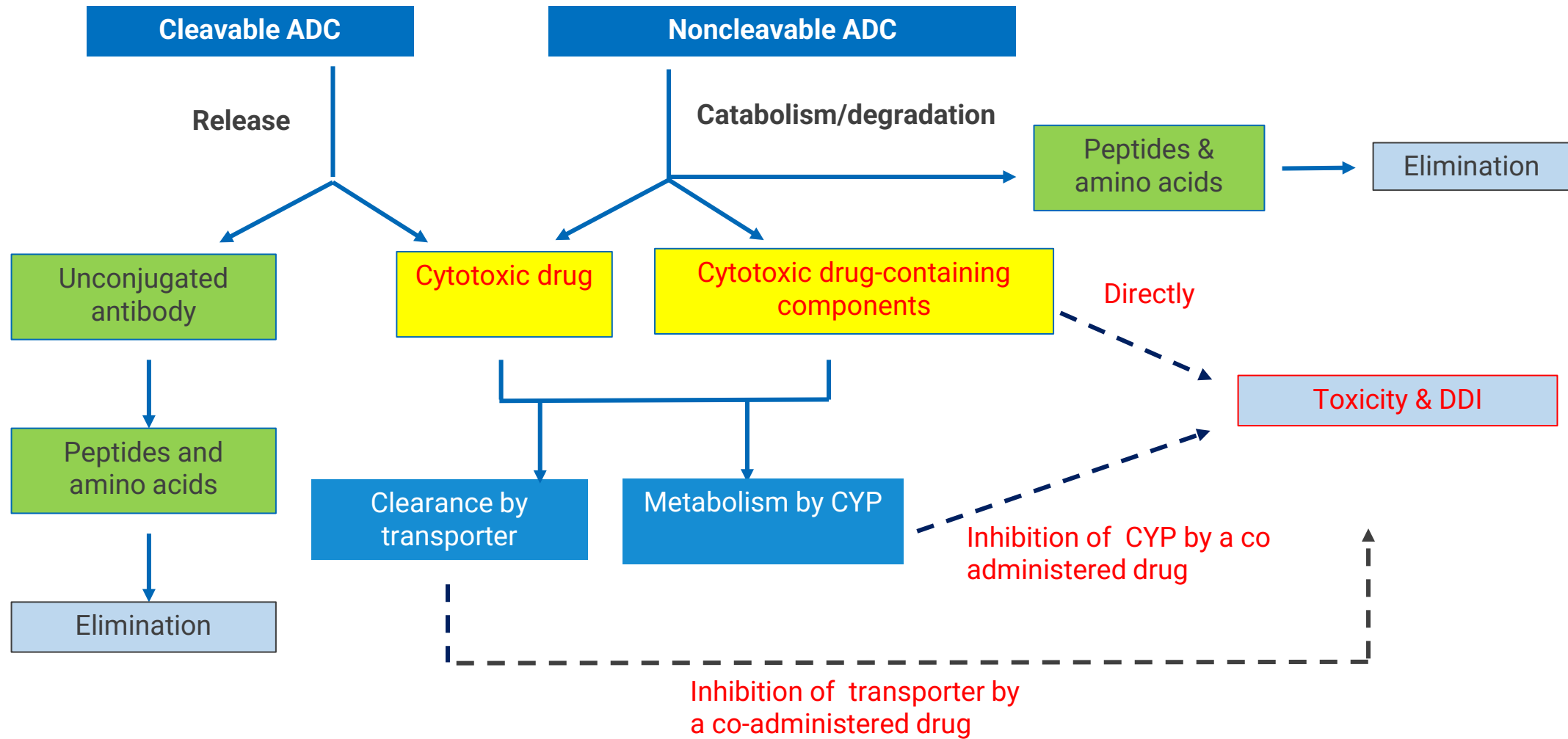
03

DAR distribution determination

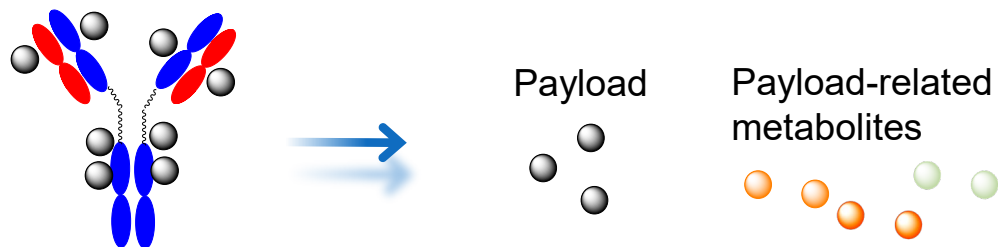
04

Conclusions

Payload-related biotransformations



In vitro systems for payload-related MetID



Cleavable: antibody-linker-**payload**

Non-cleavable: antibody**dy**-linker-**payload**

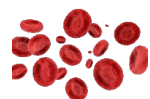
Simulate the metabolism
of ADC in circulatory
system

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

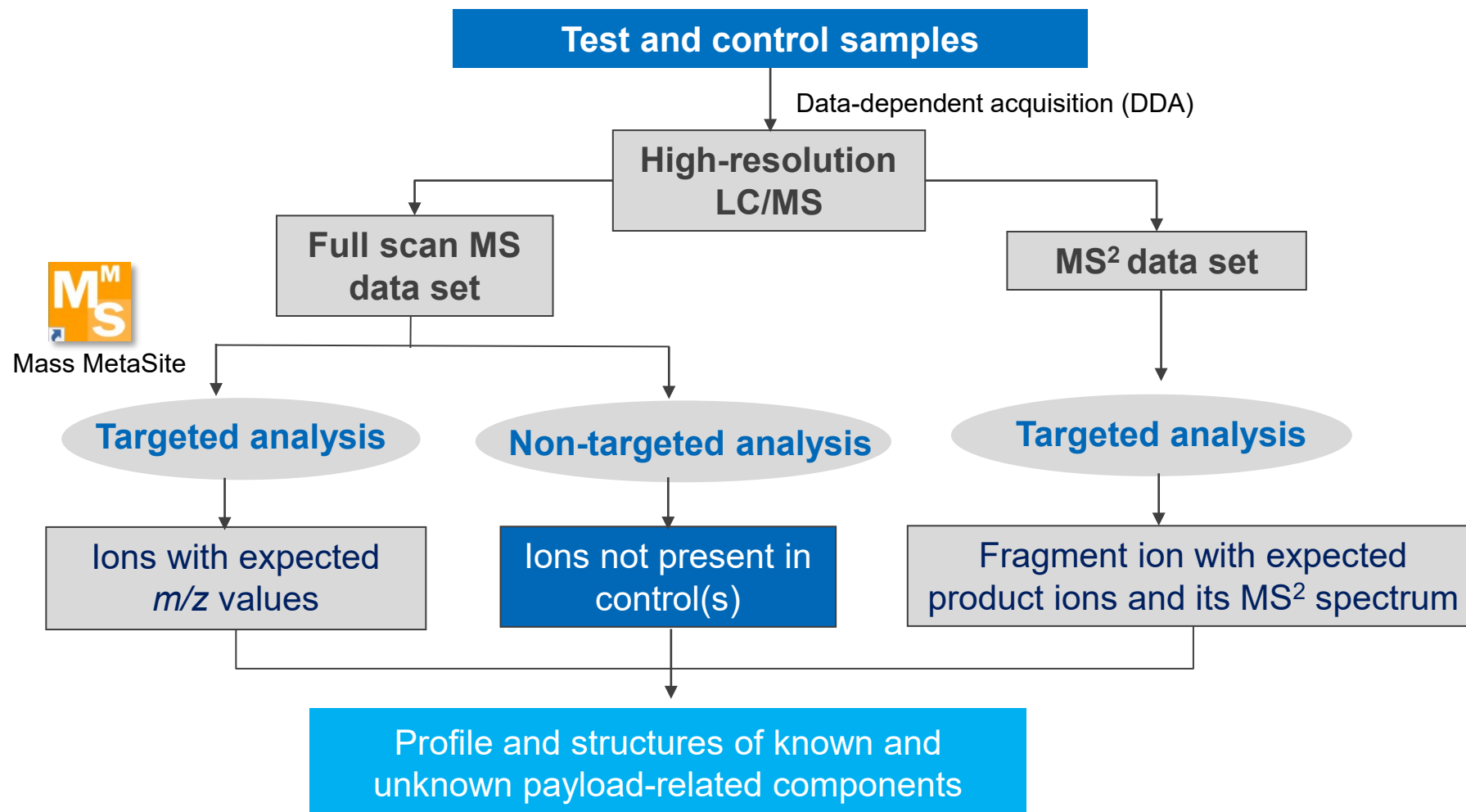


Established workflow for payload-related MetID

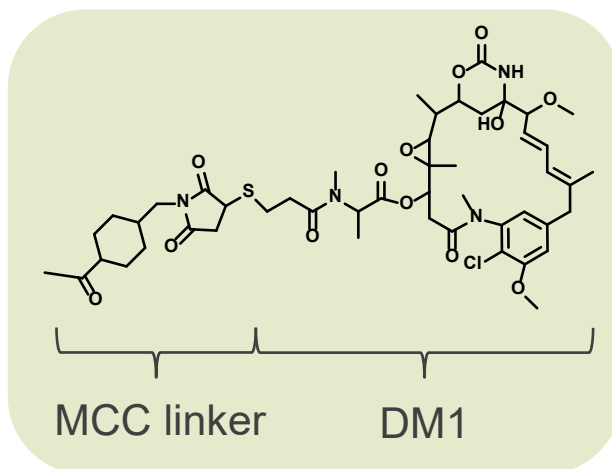
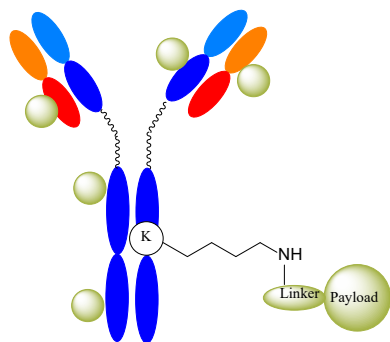
1. Data Acquisition

2. Data Mining

3. Data Interpretation



Payload-related MetID of Kadcylya (TDM1)



Liver lysosome incubation

TDM1 (0.5 mg/mL), human liver lysosomes (0.2 mg/mL) in 50 mM sodium acetate (pH 5.0),

Time points: 0, 4, 24 and 48 hours.

Naked antibody was incubated as a negative control.

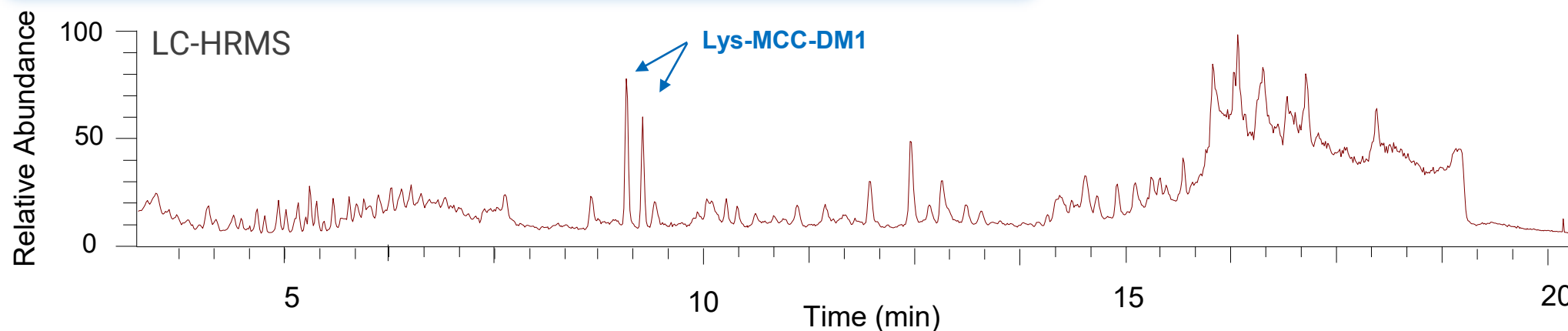
Liver S9 incubation

TDM1 (0.5 mg/mL), human liver S9 (1 mg/mL) in 50 mM sodium acetate (pH 5.0),

Time points: 0, 4, 24 and 48 hours.

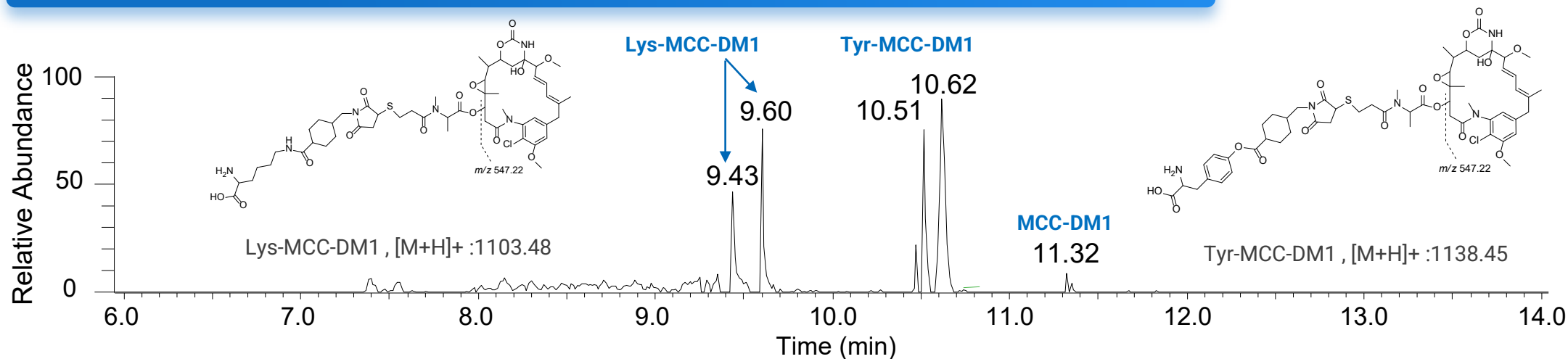
Naked antibody was incubated as a negative control.

A. Targeted analysis – expected m/z in full mass scan data

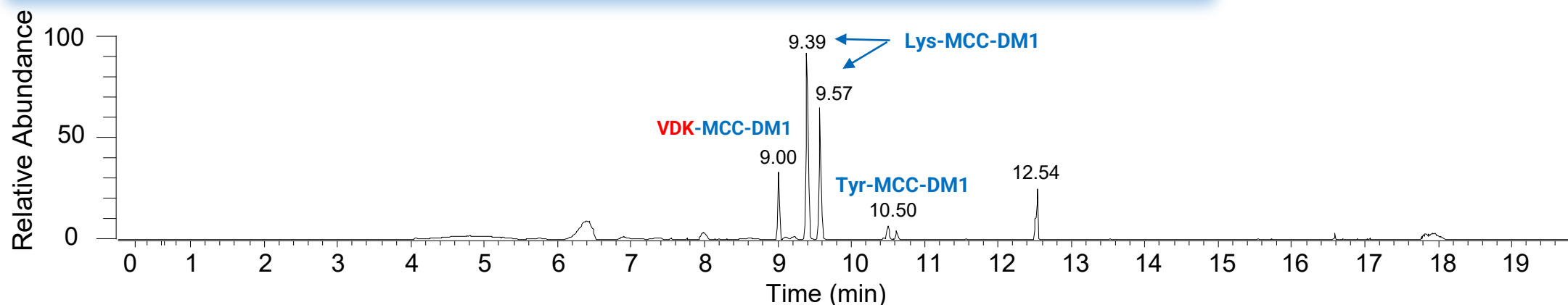


Payload-related MetID of Kadcylya (TDM1)

B. Targeted analysis – expected fragments in MS² data



C. Untargeted analysis – background subtraction with naked antibody



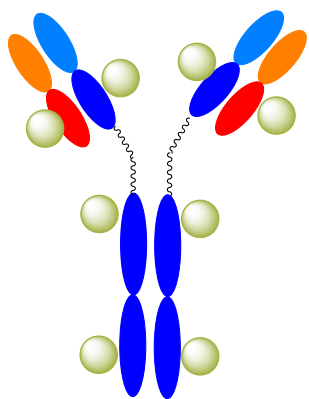
Identified metabolites of Kadcylla (TDM1)

Metabolites in liver lysosome

RT(min)	[M+H] ⁺	Payload related species
6.65, 6.78	1368.63	(KHK)-MCC-DM1
7.14, 7.28	1346.61	(KSR)-MCC-DM1
7.27, 7.40	1341.59	(KTH)-MCC-DM1
7.67, 7.78	1532.69	(KSRW)-MCC-DM1
7.83, 7.91	1482.63	(YTRS)-MCC-DM1
7.89, 7.97	1395.59	(YTR)-MCC-DM1
8.16, 8.32	1404.64	(KTIS)-MCC-DM1
9.26, 9.39	1317.57	(VDK)-MCC-DM1
9.43, 9.61	1103.48	Lys-MCC-DM1
10.55, 10.56	1138.45	Tyr-MCC-DM1
11.68, 11.80	975.38	MCC-DM1

Metabolites in liver S9

RT(min)	[M+H] ⁺	Payload related species
7.92, 8.06	1190.51	Ser-Lys-MCC-DM1
7.96, 8.10	1204.53	Thr-Lys-MCC-DM1
8.00, 8.13	1232.52	Glu-Lys-MCC-DM1
8.09, 8.21	1160.50	Gly-Lys-MCC-DM1
8.11, 8.24	1174.51	Ala-Lys-MCC-DM1
8.20, 8.33	1206.49	Cys-Lys-MCC-DM1
8.2, 8.31	1200.53	Pro-Lys-MCC-DM1
8.31, 8.46	1103.47	Lys-MCC-DM1
8.32, 8.44	1266.54	Tyr-Lys-MCC-DM1
10.31, 10.41	975.38	MCC-DM1



01

Metabolic fate of ADC

02

Payload-related MetID

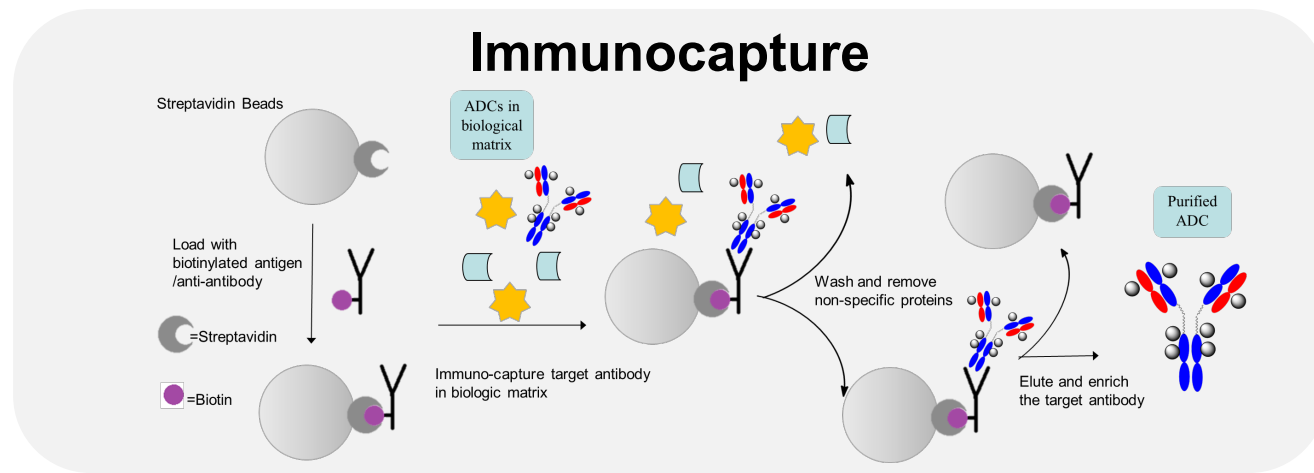
03

DAR distribution determination

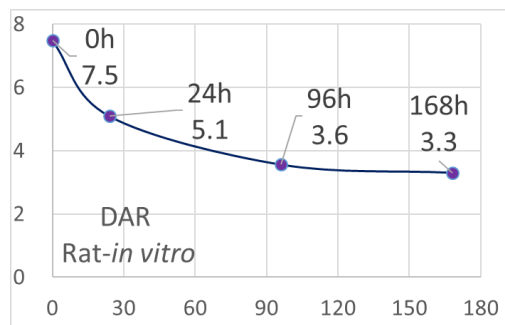
04

Conclusions

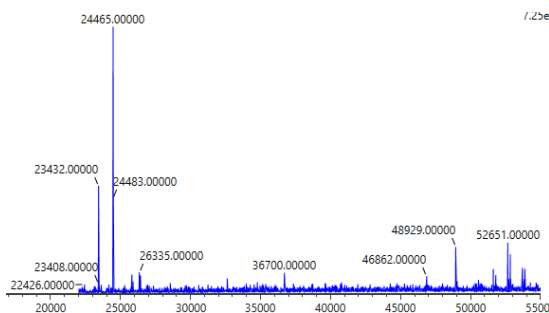
Established workflow for DAR determination



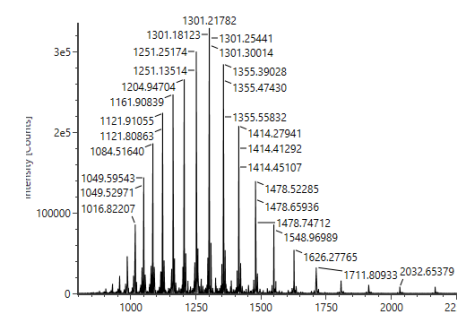
DAR distribution



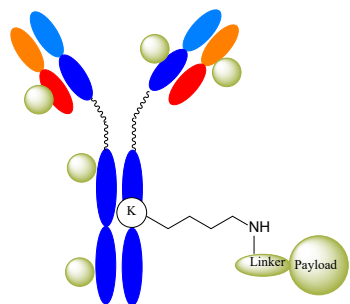
Deconvoluted MS data



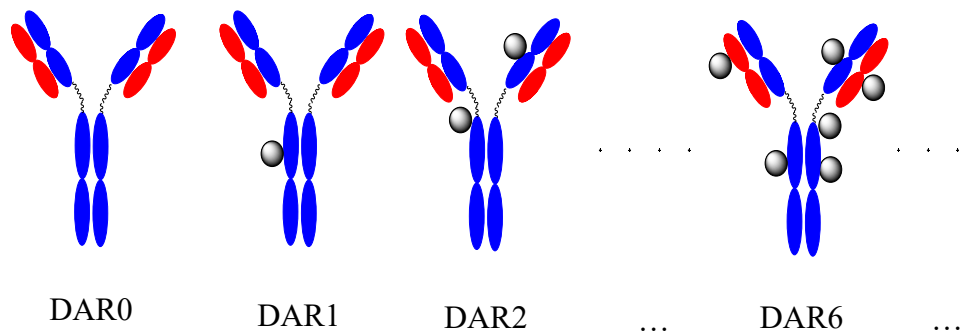
Isotopic Envelopes



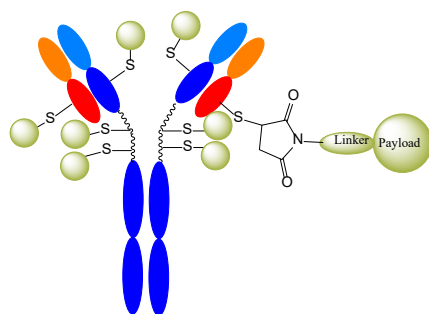
DAR determination for ADC of different conjugation types



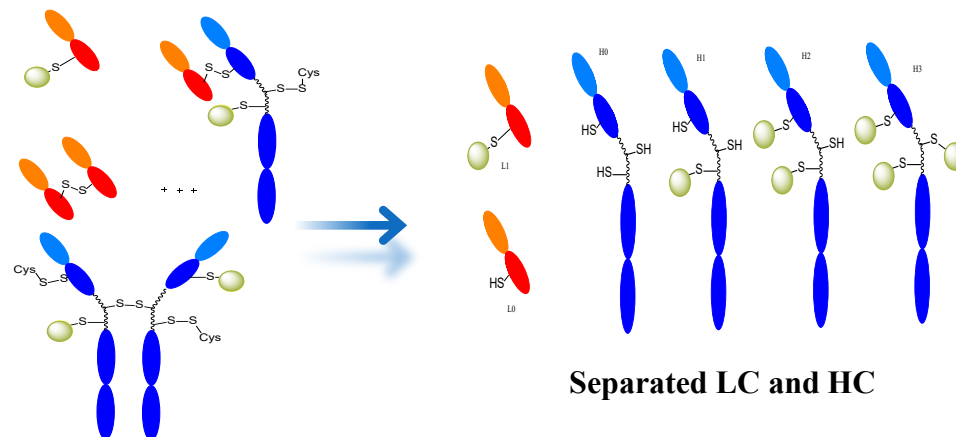
Lysine conjugation



$$DAR = \frac{\sum n \times DAR_n}{\sum DAR_n}$$



Cysteine conjugation



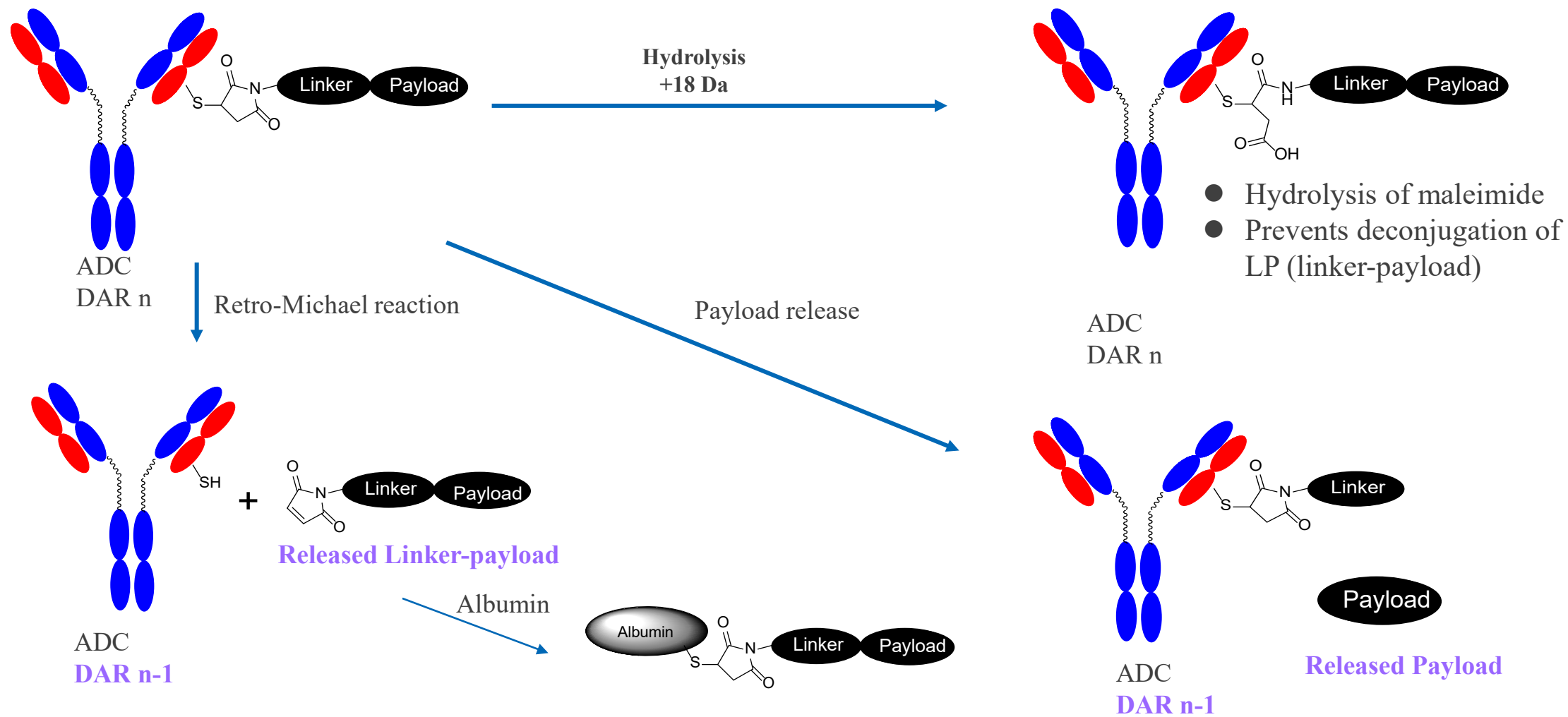
Separated LC and HC

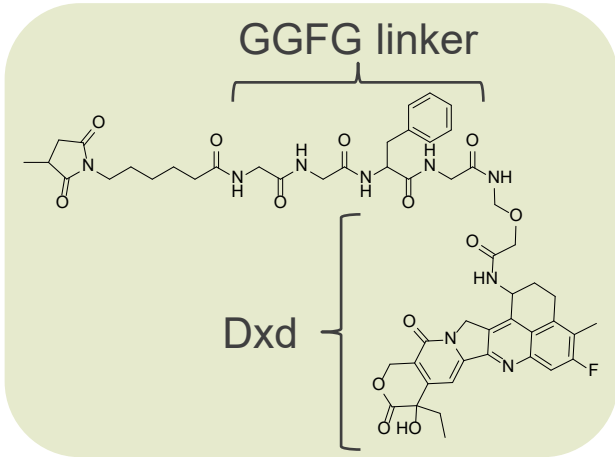
$$DAR (LC) = \frac{L1}{L1+L0}$$

$$DAR (HC) = \frac{3 \times H3 + 2 \times H2 + 1 \times H1}{H3 + H2 + H1 + H0}$$

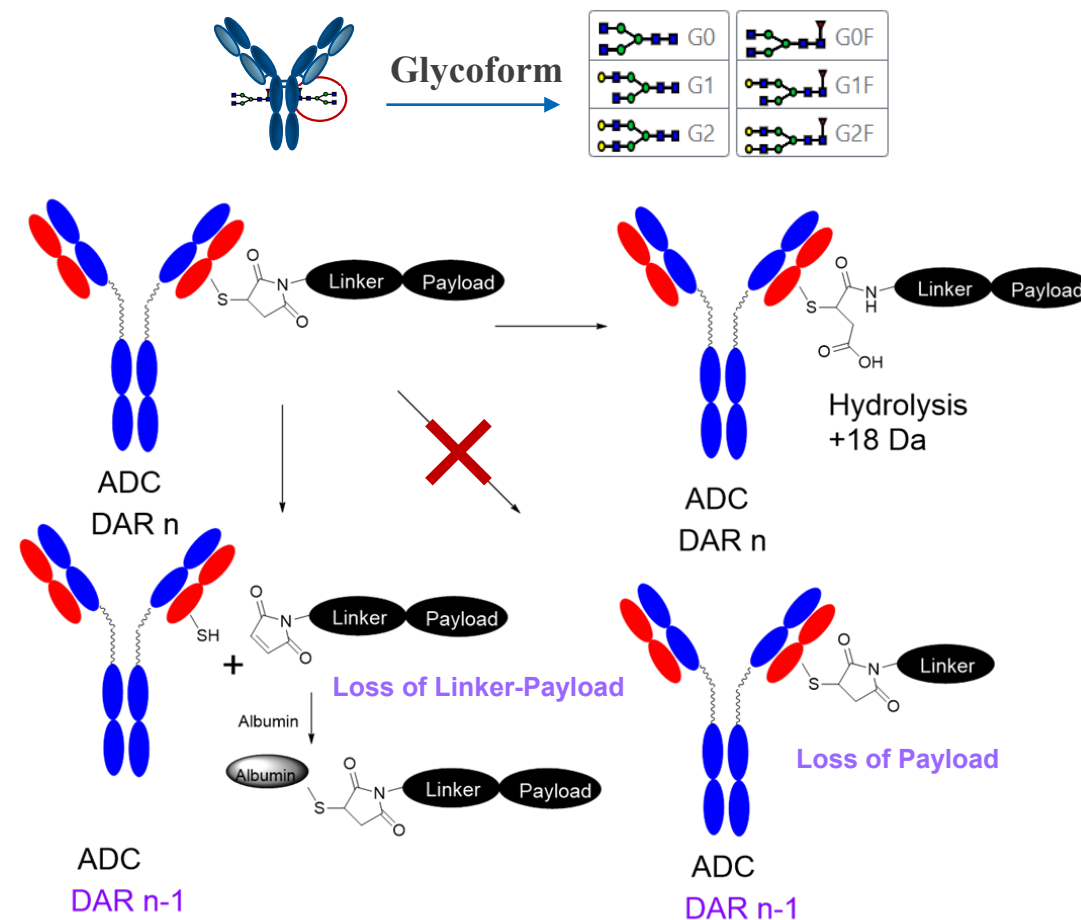
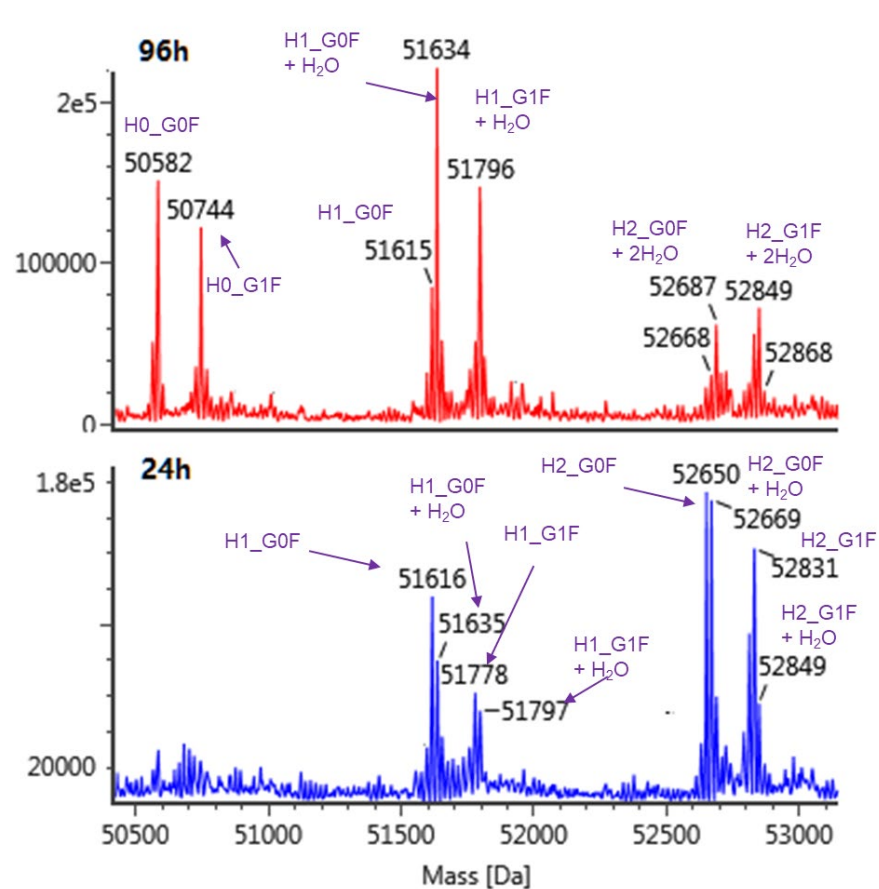
$$DAR = 2 \times (DAR (LC) + DAR (HC))$$

Proposed biotransformation of cysteine conjugated ADC





Biotransformation of TDxd observed



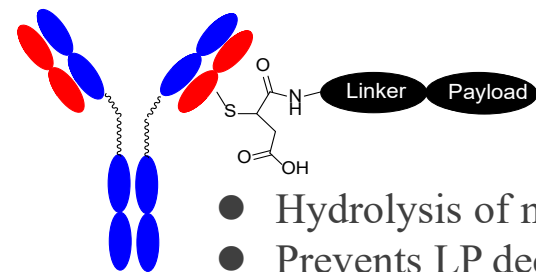
Deconvoluted mass

Structure

Biotransformation

Calculation of DAR value

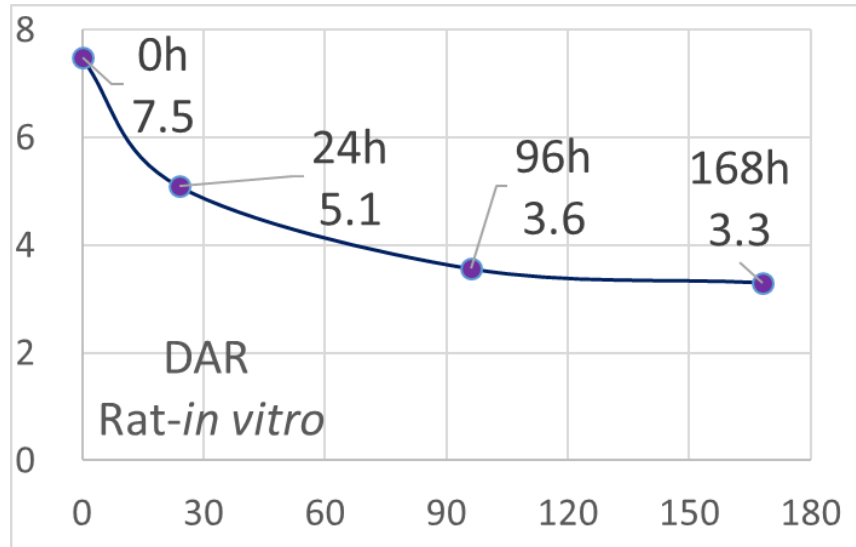
DAR Distribution	Identification	Mass	MS Intensity			
			1h	24h	96h	168h
L0	LC	23432	2.41E+05	6.93E+05	1.83E+06	1.40E+06
L1	LC + 1*LP	24466	2.66E+07	1.13E+07	4.62E+06	1.57E+06
	LC + 1*LP+H2O	24484	3.56E+06	2.10E+06	2.13E+06	1.52E+06
H0	HC	50582	1.31E+05	0.00E+00	2.21E+05	1.99E+05
	HC+Gal	50744	6.48E+04	0.00E+00	5.92E+04	1.93E+05
H1	HC + 1*LP	51616	5.27E+04	1.23E+05	5.68E+04	4.01E+05
	HC + 1*LP + H2O	51634	0.00E+00	1.07E+05	3.06E+05	3.71E+05
	HC+Gal + 1*LP	51778	1.21E+05	2.14E+05	3.87E+05	3.08E+05
	HC+Gal + 1*LP+H2O	51796	1.39E+05	2.07E+05	3.13E+05	3.28E+05
H2	HC + 2*LP	52650	5.31E+05	1.11E+06	1.38E+05	3.11E+05
	HC + 2*LP+H2O	52668	3.51E+05	8.07E+05	9.18E+04	4.03E+05
	HC + 2*LP+2H2O	52686	0.00E+00	0.00E+00	4.34E+05	2.47E+05
	HC+Gal + 2*LP	52812	4.80E+05	8.75E+05	1.14E+05	3.41E+05
	HC+Gal + 2*LP+H2O	52830	3.42E+05	5.38E+05	9.77E+04	4.35E+05
	HC+Gal + 2*LP+2H2O	52848	2.85E+05	2.59E+05	2.94E+05	2.43E+05
H3	HC + 3*LP	53684	4.24E+06	1.71E+06	2.88E+05	1.47E+05
	HC + 3*LP+H2O	53702	2.53E+06	1.42E+06	5.12E+04	1.73E+05
	HC + 3*LP+2H2O	53720	7.41E+05	5.30E+05	2.02E+05	1.72E+05
	HC + 3*LP+3H2O	53738	3.25E+05	2.02E+05	0.00E+00	0.00E+00
	HC+Gal + 3*LP	53846	3.26E+06	1.28E+06	2.67E+05	1.16E+05
	HC+Gal + 3*LP+H2O	53864	2.04E+06	1.11E+06	6.32E+04	1.78E+05
	HC+Gal + 3*LP+2H2O	53882	5.94E+05	3.76E+05	2.38E+05	1.62E+05
	HC+Gal + 3*LP+3H2O	53900	2.77E+05	2.02E+05	1.62E+05	0.00E+00



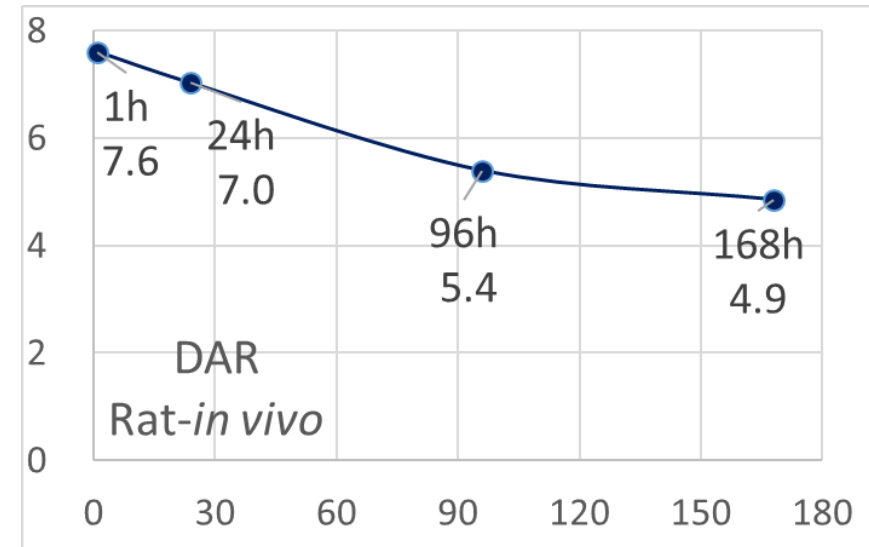
DAR	1h	24h	96h	168h
DAR LC	0.99	0.95	0.79	0.69
DAR HC	2.81	2.56	1.91	1.74
DAR	7.60	7.02	5.39	4.85

DAR distribution of TDxd in plasma over time

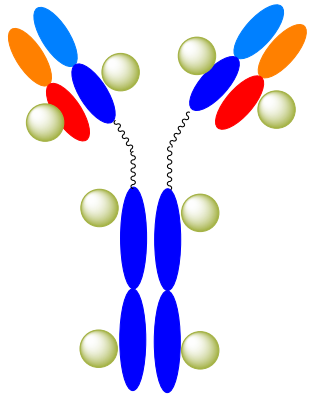
TDxd in rat plasma (in vitro)



TDxd in rat plasma (in vivo)



- ❑ The DAR values calculated at each in vitro incubation time point and in vivo dosing sampling time point indicate the changes in ADC DAR values in vitro and in vivo.
- ❑ The DAR value of TDxd decreased to 3.3 after 168 hours of in vitro plasma incubation in rats, while the DAR value decreased to 4.9 after 168 hours of in vivo dosing in rats.



01

Metabolic fate of ADC

02

Payload-related MetID

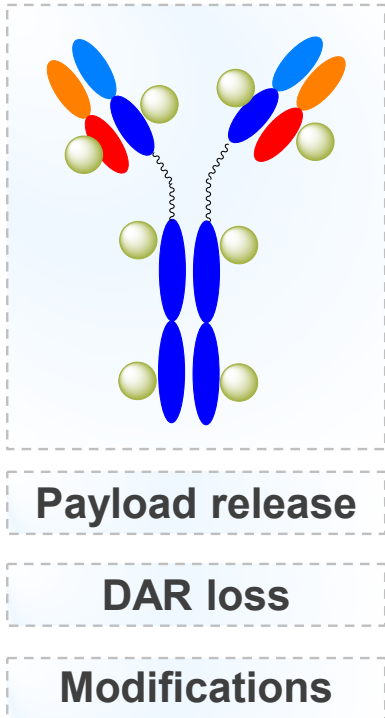
03

DAR distribution determination

04

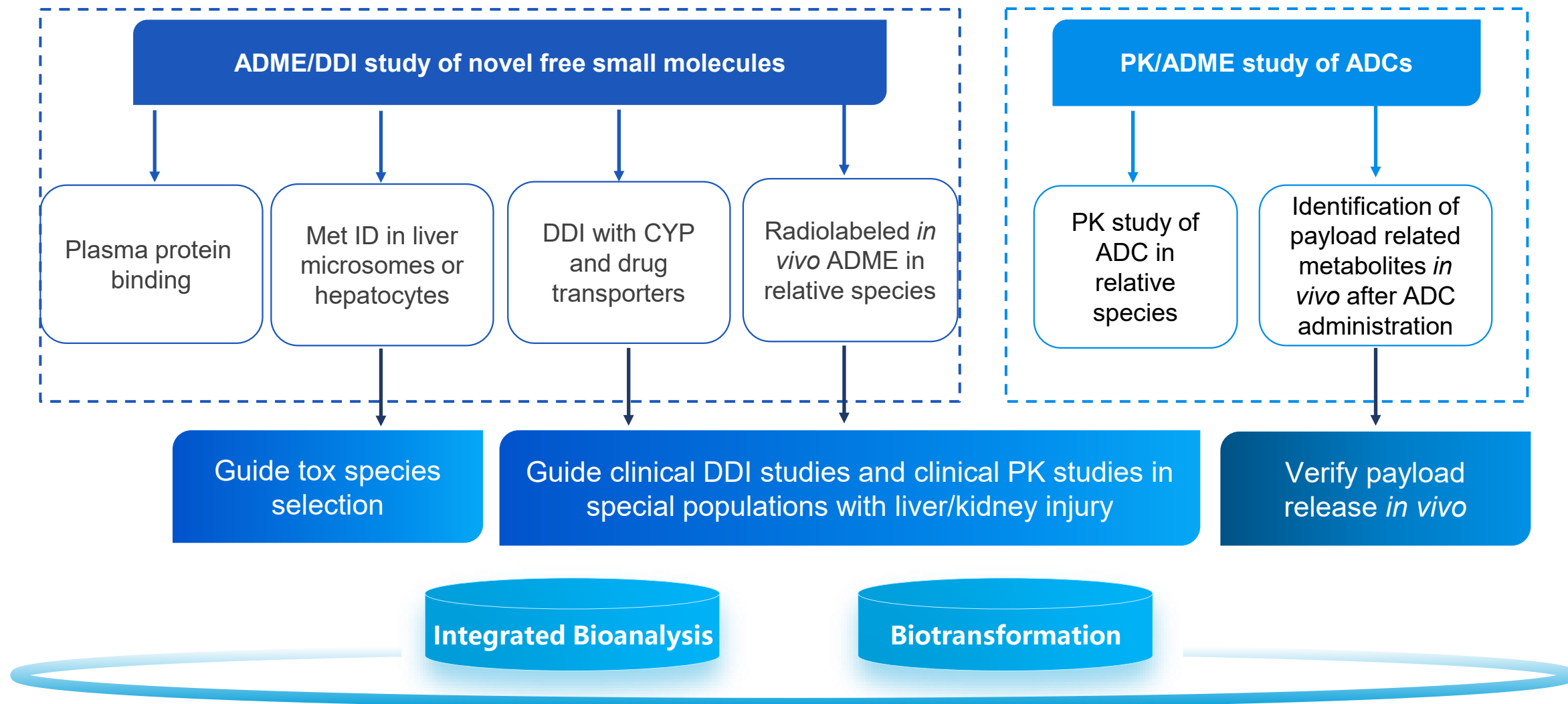
Conclusions

Established workflow for DAR determination



- ❑ ADC is an emerging therapeutical modality
- ❑ **Small molecule metabolism** and **intact protein modifications**
- ❑ Biotransformation study is critical for release mechanism:
 - Payload-related MetID** verifies from the small molecule side
 - DAR determination** verifies from antibody side
- ❑ Combination of workflows gives a full picture of biotransformation

Proposed preclinical DMPK strategies for ADC drugs



Drug Metabolism and Pharmacokinetic (DMPK) Service

In Vitro ADME Study

In Vivo PK Study

Metabolite Identification

Radiolabeled Compound Synthesis
& ADME Study

Non-GLP Bioanalysis

New Modality & Therapeutic
Platforms

Compound Management &
Import and Export Support



High-Standard Instruments and Facilities

Animal facilities are AAALAC accredited
High-precision bioanalytical instruments
High-throughput automation platforms



Reliable and Fast Data Delivery

Meet IND and NDA requirements of the FDA, NMPA, and EMA
No lead time
Short turnaround time for screening and IND assays

18

Years of experience

1,000+

Employees

1,600+

Global clients

1,500+

Successful IND filings
supported

OCT
20-23
2024

aaps
PharmSci 360

#PharmSci360

Slide 52

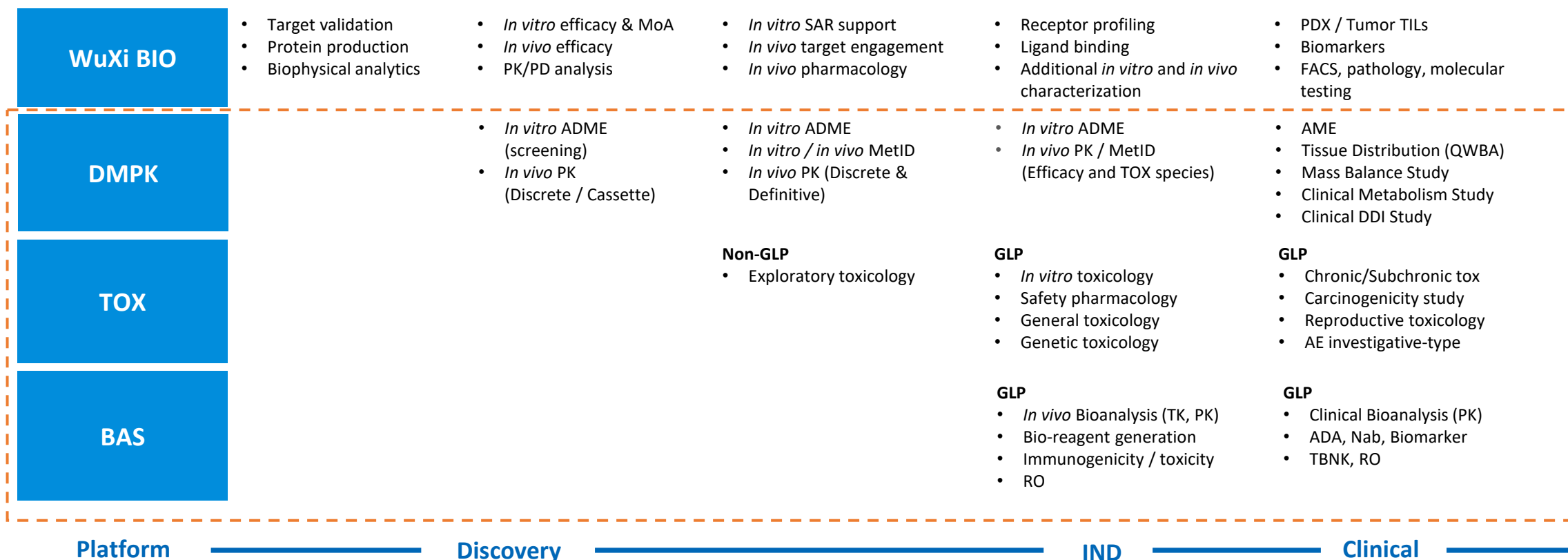
Drug R&D Testing Platform

CUSTOMIZED SERVICES

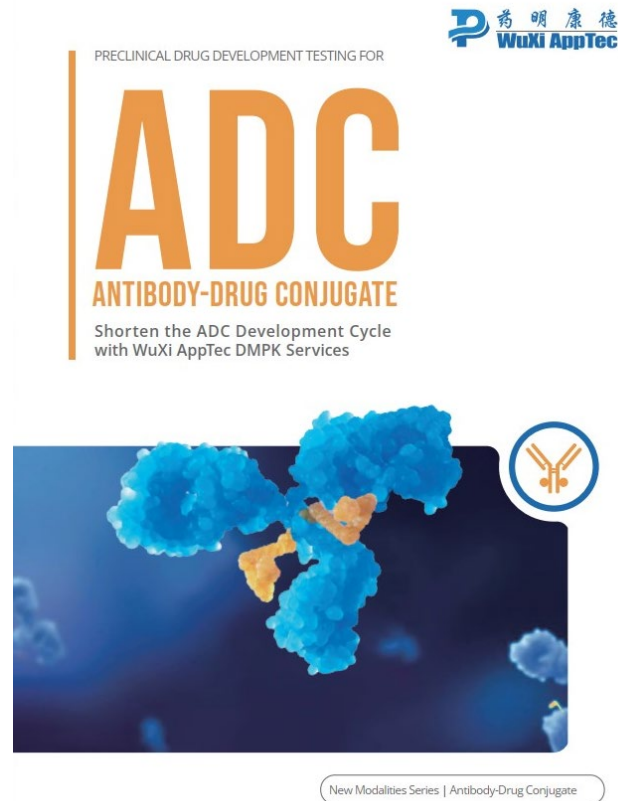
From Discovery to Candidate Selection and Beyond

Drug R&D Roadmap

Lab Testing Division



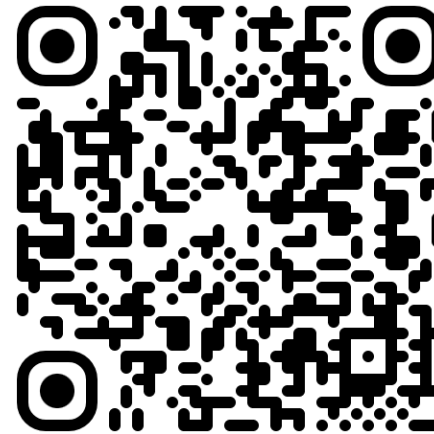
Questions



Scan to Visit WuXi AppTec DMPK Service Website

<https://dmpkservice.wuxiapptec.com/>

<https://labtesting.wuxiapptec.com/>



Email: DMPK_Service@wuxiapptec.com