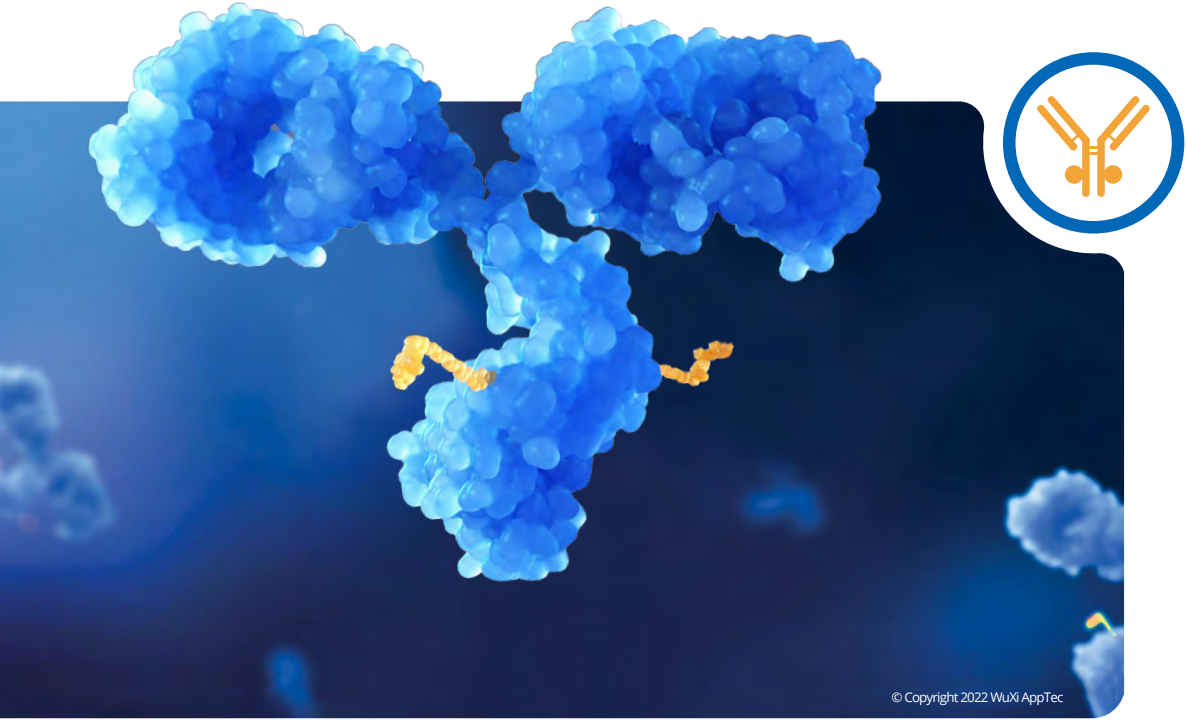
药明康德
WuXi AppTec

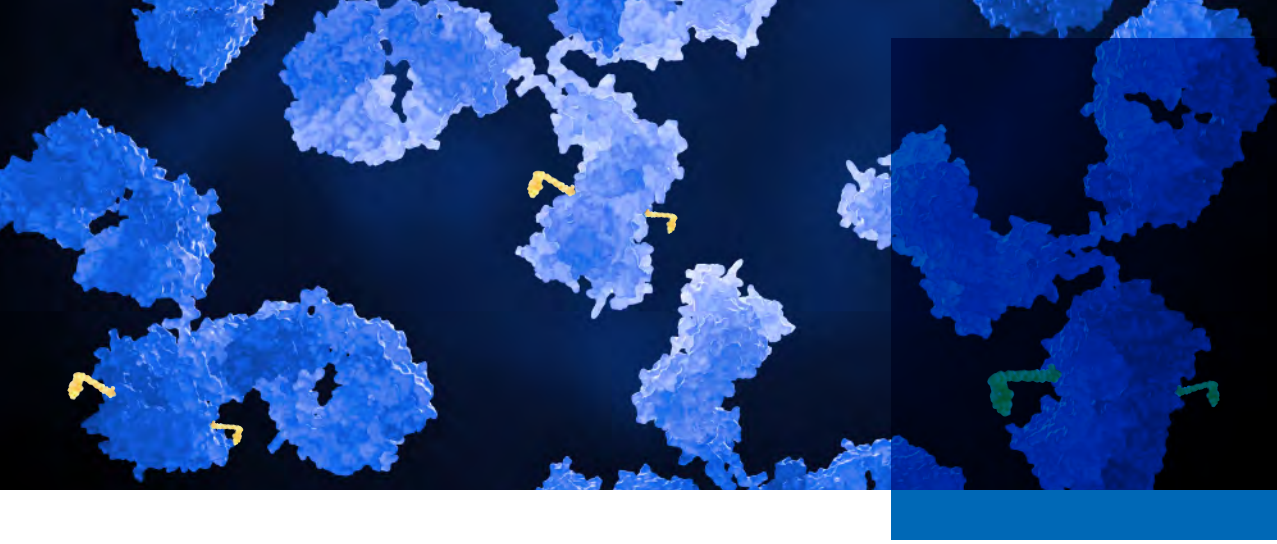
PRECLINICAL DRUG DEVELOPMENT TESTING FOR

ANTIBODY DRUG CONJUGATE

Shorten the ADC Development Cycle
with WuXi AppTec **DMPK** Services

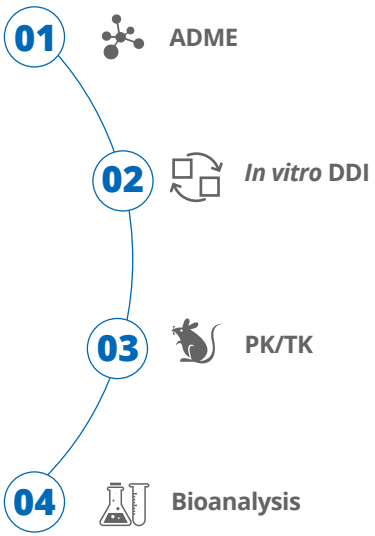


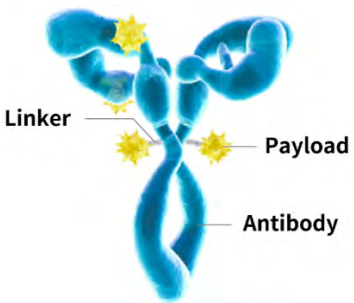
New Modalities Series | Antibody-Drug Conjugate



Unique DMPK Study Methodology to Accelerate the Development & Application of ADCs

Antibody-drug conjugates (ADCs) represent emerging biotherapeutics that are composed of cytotoxic payloads conjugated to a monoclonal antibody (mAb) via a chemical linker. Characterization of the **ADME** properties of ADCs is much more challenging. Based on our experience in **ADC** project research, we divided the **ADC** DMPK study into four main aspects: **ADME, *in vitro* DDI, PK/TK and bioanalysis**. These four aspects have their own emphasis in the discovery, preclinical and clinical stages. The WuXi AppTec **DMPK** team will propose a pharmacokinetic study strategy and study method for each **ADC** project based on their structures to accelerate the development and application of **ADCs.**





The team had successfully supported 21 **ADC** projects, including 13 **ADC** projects for IND. The team has gained extensive experience and have developed a unique methodology for **ADC** pharmacokinetic studies, which can help customers quickly advance their **ADC** drug development projects.

ADC Pharmacokinetic Study Services

ADC

- Plasma or serum stability
- *In vitro* payload release study
- *In vivo* PK study in pharmacodynamic and toxicological species
- **ADME** study of radiolabeled **ADC**s in animals
- *In vivo* identification of payload-related metabolites released by **ADC**s

Payload

- Plasma protein binding
- *In vitro* metabolite identification in liver microsomes and hepatocytes
- CYP450 enzyme inhibition and induction
- CYP450 enzyme phenotyping
- Transporter substrate and inhibition assessment
- *In vivo* PK study in pharmacodynamic and toxicological species
- **ADME** study of radiolabeled payloads in animals

Pharmacokinetic Study Contents

	DISCOVERY	PRECLINICAL	CLINICAL
	Lead Optimization	**Support Clinical Candidate Characterization and IND Filing**	**Support Development and NDA Filing**
ADME	▪ Identify payload-related metabolites released from ADCs in S9 and/or lysosomes ▪ Identify payload-related metabolites released from ADCs in target-expressed cells ▪ Stability of ADCs in plasma or serum	▪ Metabolite identification of payload in liver microsomes and hepatocytes ▪ Plasma protein binding of payload ▪ ADME study of radiolabeled ADCs in animals ▪ ADME study of radiolabeled payloads in animals	▪ Identify payload-related metabolites in human plasma, serum, urine and/or feces
In Vitro DDI		▪ *In vitro* CYP450 inhibition and induction of payload ▪ CYP450 enzyme phenotyping of payload ▪ *In vitro* transporter inhibition of payload ▪ *In vitro* efflux transporters substrate analysis of payload	▪ Other transporter substrate analysis of payload ▪ Monitoring of CYP450 and transporter inhibition using biomarkers ▪ DDI Prediction of ADCs in human using PBPK ▪ Clinical DDI study of ADCs
PK	▪ PK study of ADCs in pharmacological model ▪ PK screening of ADCs and payload in rodents	▪ Full PK study of ADCs and payload in rodents ▪ Full PK study of ADCs and payload in non-rodents	▪ Full PK study of ADCs and payload in human ▪ Population PK study of ADCs ▪ Hepatic and renal impairment study of ADCs
Bioanalysis	▪ Evaluation of DAR of ADCs in animal plasma or serum ▪ Quantitative analysis of ADC compositions, including total antibody, ADC, unconjugated payload and active metabolites in animal plasma and/or serum	▪ Quantitative analysis of ADC compositions, including total antibody, ADC, unconjugated payload and active metabolites in animal plasma serum ▪ Evaluation of ADAs in animal plasma or serum	▪ Quantitative analysis of ADC compositions, including total antibody, ADC, unconjugated payload and active metabolites in human plasma serum ▪ Evaluation of ADAs in human plasma or serum

Challenges in ADC Pharmacokinetic Studies

High Difficulty

- **ADC** is a combination of large and small molecules and requires pharmacokinetic studies of both molecules.
- **ADC** is a novel drug with very limited understanding of its **ADME** properties.
- The payload is highly toxic, so human radiolabeled **ADME** studies cannot be performed for **ADC**s

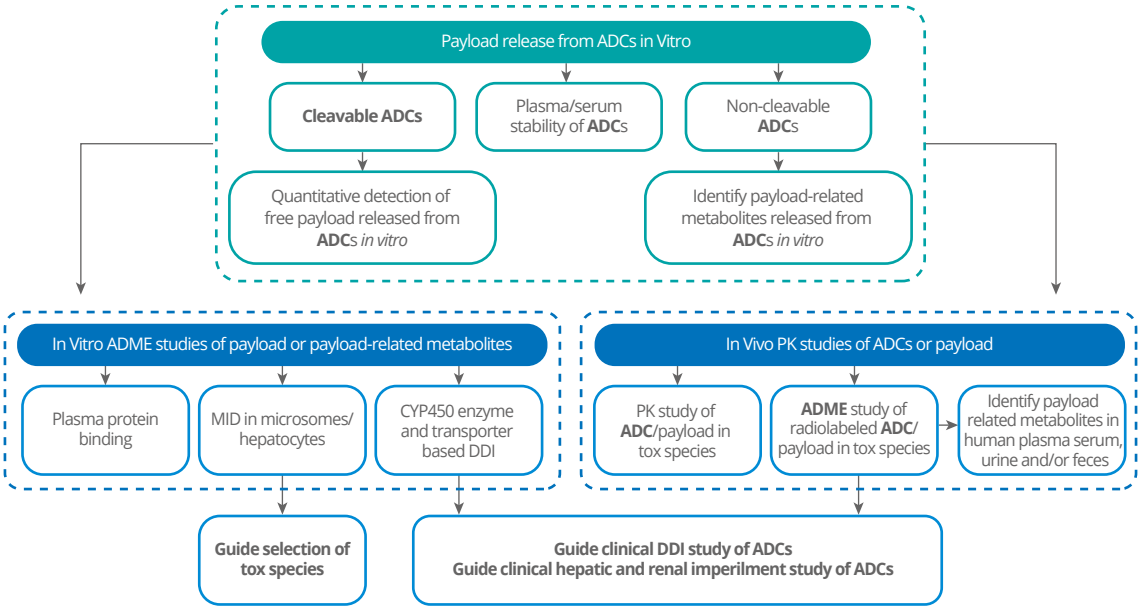
High Significance

- The efficacy of **ADC**s is directly related to the release and concentration of the payload in the target tissue.
- The toxicity of **ADC**s is directly related to the release and concentration of the payload in the non-target tissue.
- The selection of toxicological species of **ADC**s is related to the similarity of the payload in metabolism in both humans and animals.
- The DDI of **ADC**s is directly related to the exposure as well as metabolism and elimination of the payload.

Key Study Capabilities

- Investigation of the payload release from **ADC**s using different *in vitro* models.
- Detection of the payload and related metabolites released from **ADC**s using non-targeted LC/HRMS.
- Determination of total antibody and **ADC** drug concentration by LBA technique to support the **ADC** PK study *in vivo*.
- DAR analysis using HRMS.
- Study on tissue distribution of **ADC**s using QWBA technique.

ADC Pharmacokinetic Study Strategies



Our Strengths

Customer First and Customer Centric

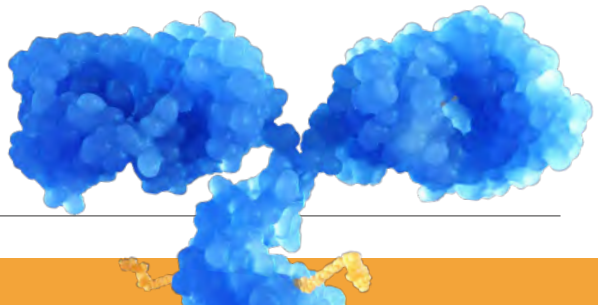
We have a specialized and dedicated service model. Each client will be connected to a dedicated study director who will provide comprehensive management services for the pharmacokinetic project from drug discovery to the clinical phase.

Extensive Experiences and Custom-Designed ADC Study Strategies

With years of accumulated experience, we provide customized designs for pharmacokinetic study strategies for our customers' new molecules based on flexible study concepts with rapid optimization and adjustment.

Comprehensive Capacities and the Executive Capability to Successfully Respond to the Challenges in ADC Studies

We are capable of carrying out both small and large molecule **DMPK** studies, comprehensive **ADC** bioanalysis, metabolite identification of payloads *in vivo* and *in vitro*, and radiolabeled **ADME** studies.



Case Study

Background: Take preclinical PK/TK study of TIVDAK (Tisotumab vedotin-tftv) as an example. TIVDAK, the latest **ADC** approved by the FDA (marketed on September 20, 2021), targets Tissue Factor (TF), is used for the treatment of adult patients with recurrent or metastatic cervical cancer that progresses during or after chemotherapy. The antibody to TIVDAK is Tisotumab, an unmarketed antibody drug. The payload is MMAE, which has been used in many **ADC**s (ADCETRIS, POLIVY and PADCEV). The Linker is a commonly used cleavable valine-citrulline structure.

Study Design: The preclinical pharmacokinetic study of TIVDAK was summarized according to the published literature.

(1) Payload release study: Plasma stability was mainly investigated.

(2) *In vivo* PK/TK studies of ADC and payload: According to the homology of amino acid sequence and tissue cross reaction results of monoclonal antibodies, the relevant animal species selected was cynomolgus monkey. So, PK/TK studies on **ADC** and monoclonal antibodies were carried out in cynomolgus monkeys. Tissue distribution of radiolabeled monoclonal antibodies were carried out in cynomolgus monkeys and tumor-bearing models. Tissue distribution of radiolabeled **ADC** was carried out in tumor-bearing models. The PK/TK studies of MMAE were carried out in rats and cynomolgus monkeys. The mass balance study of MMAE was carried out in rats.

(3) *In vitro* ADME studies of payload: The plasma protein binding, metabolite identification and CYP450 reaction phenotyping, transporter substrate and inhibition studies were mainly investigated.

(2) *In Vivo* PK/TK Studies of ADC and Payload

Administration	Trial Deisgn	Animal Species	Testing
Tisotumab Vedotin	Repeated IV dosing for 13 weeks Dose: 1,3,5 mg/kg	Cynomolgus monkey	Total antibody, Tisotumab vedotin, MMAE
Tisotumab	Repeated IV dosing for 13 weeks Dose: 25 mg/kg	Cynomolgus monkey	Total antibody
^{89}Zr-Labeled Tisotumab	Single IV dosing Dose: 0.4,1, and 3 mg/kg	Cynomolgus monkey	Positron emission tomography (PET)
^{89}Zr-Labeled Tisotumab	Single IV dosing Dose: 1 mg/kg	Pancreatic cancer bearing mice with high or low/no TF expression	Positron emission tomography (PET)
^{89}Zr-Labeled Tisotumab Vedotin	Single IV dosing Dose: 1 mg/kg	Pancreatic cancer bearing mice with high or low/no TF expression	Positron emission tomography (PET)
cAC10-vc-[3H]-MMAE or [3H]-MMAE	Single IV dosing	Rat	Radioactivity in feces or urine
MMAE	Repeated IV dosing for 4 weeks Dose: 0.0097, 0.097 and 0.194 mg/kg	Rat	MMAE
MMAE	Repeated IV dosing for 11 weeks Dose: 0.058 mg/kg	Cynomolgus monkey	MMAE

(1) Payload Release from ADC

Assay Type	Assay Condition
Plasma Stability	Matrix: mouse, cynomolgus monkey, human plasma; 1% BSA in PBS Concentration: 50μg/mL Time: Incubate at 37°C for 14 days

(3) *In Vitro* ADME Studies of Payload

Assay Type	Assay Condition
Plasma Protein Binding	Matrix: mouse, cynomolgus monkey, human plasma Concentration: 1, 10, 100 nM Method: Ultracentifugation
Metabolite Identification	Matrix: Human liver microsomes; rat, cynomolgus monkey, and human hepatpcytes
Enzyme Phenotyping	CYP450 enzyme phenotyping
Drug-Drug Interaction	Substrate study: P-gp, BCRP, MRP2, OCT2, OAT1, OAT3, OATP1B1, OATP1B3 Inhibition study: P-gp, BCRP, BSEP, MRP2, OCT1, PCT2, OAT1, OAT3, OATP1B1 , OATP1B3

References

[1] Drug Metab Dispos 44: 617-623, May 2016;

[2] https://www.accessdata.fda.gov/drugsatfda_docs/nda/2021/761208Orig1s000MultidisciplineR.pdf