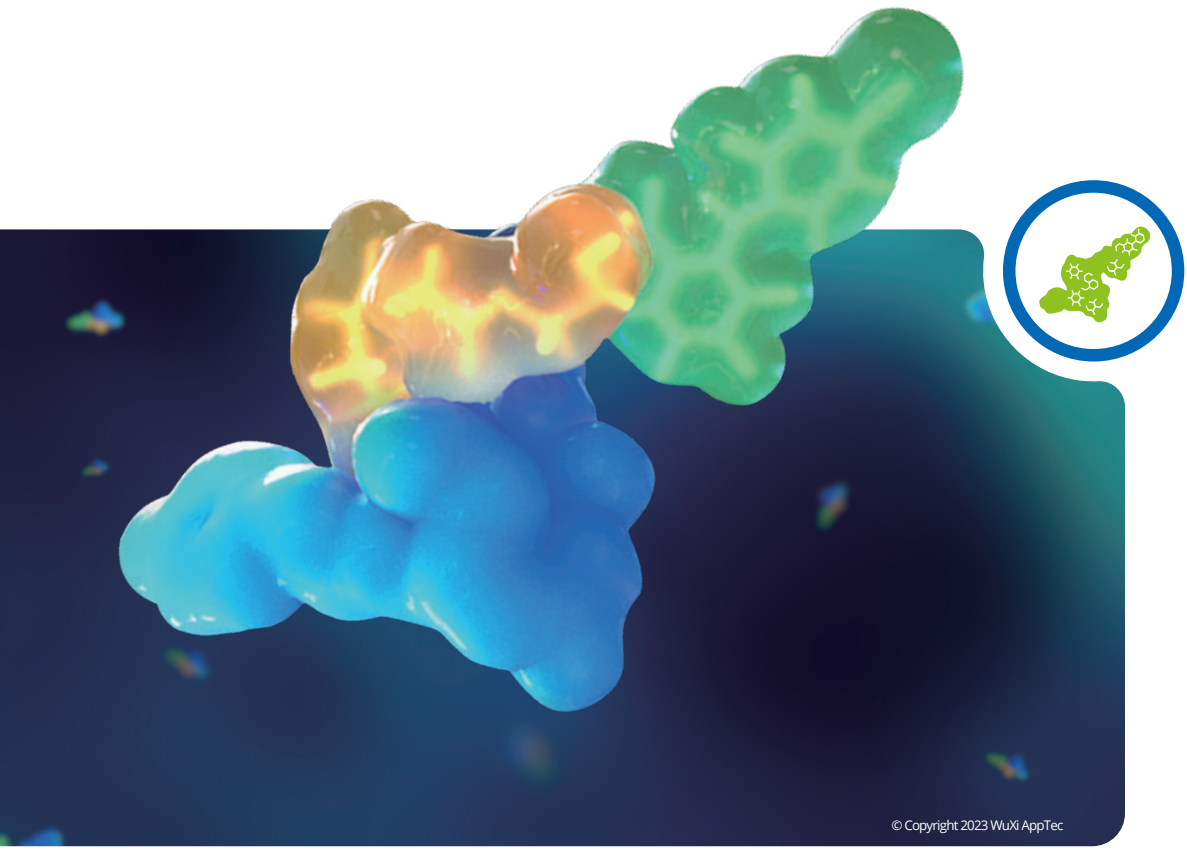
WuXi AppTec

PRECLINICAL DRUG DEVELOPMENT TESTING FOR

PEPTIDE–DRUG CONJUGATE (PDC)

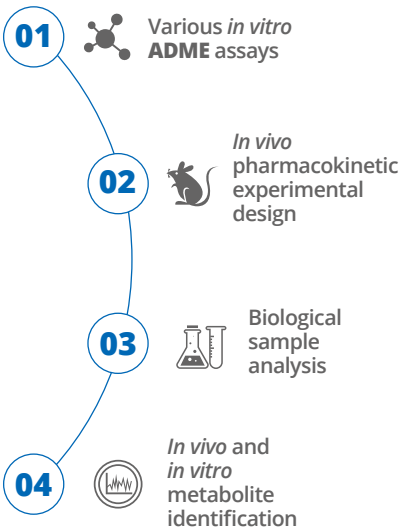
Shorten the PDC Development Cycle
with WuXi AppTec **DMPK** Services

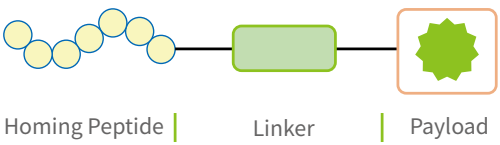


New Modalities Series | Peptide–Drug Conjugates

Well-Established WuXi AppTec **DMPK** Platform for **PDC** Drugs

Peptide-Drug Conjugates (PDCs) are composed of a cytotoxic payload, a homing **peptide**, and a linker between the **peptide** and the cytotoxic payload. After antibody-drug conjugates, they are the next generation of targeted therapeutic drugs with enhanced cellular permeability and improved drug selectivity. They pose a great opportunity to target cancer, metabolic diseases, and more. While the potential is significant, development challenges – like poor stability, inferior targeting and potential payload toxicity – can slow the preclinical and clinical development process. WuXi AppTec Drug Metabolism and Pharmacokinetics (**DMPK**) Service Department has established a robust **DMPK** research program to develop **PDC**s. This program has been built with our collective expertise of *in vitro* **ADME**, *in vivo* pharmacokinetics, bioanalysis and metabolite identification coupled with extensive research strategy, data interpretation and cross-department cooperation to significantly reduce time and costs.





We have successfully completed **PDC** projects for dozens of clients, including multiple IND filing and technical support. Through our extensive experience, we have developed a well-established **DMPK** research strategy for **PDC**, which can accelerate the progress of **PDC** projects.

DMPK Research Services for PDC

Absorption

- Solubility test in different excipients
- *In vivo* absorption study through PK

Distribution

- Protein binding in plasma and tissues
- *In vitro* partition between whole blood and plasma
- Tissue distribution with cold/hot compounds or through QWBA approach

Metabolism

- *In vitro* metabolic stability
- Bio-transformation through *in vitro* and *in vivo* metabolite identification
- Payload release study with *in vitro* models

Excretion

- *In vivo* excretion study to explore excretion pathway

Drug-drug Interactions (DDI)

- *In vitro* CYP450 DDI evaluation
- *In vitro* transporter DDI evaluation

DMPK Assay List for PDC*

	DISCOVERY	PCC STAGE	IND FILING
***In Vitro* ADME**	▪ *In vitro* metabolic stability of homing peptide and **PDC** in whole blood (or plasma) and in kidney homogenate ▪ *In vitro* metabolite identification of homing **peptide** and **PDC** in whole blood (or plasma) and in kidney homogenate ▪ *In vitro* partition of payload between whole blood and plasma ▪ Protein binding of **PDC** and payload in plasma and tissue	▪ Stability test of **PDC** in cathepsin B, or in buffers with different pH values, or in DDT (Both **PDC** and payload are monitored; specific condition is set based on the means by which the payload is cleaved) ▪ Stability of **PDC** in lysosomes (Both **PDC** and payload are monitored, suitable to non-cleavable linker) ▪ Metabolite identification of **PDC** in tumor cells	▪ Plasma protein binding of **PDC** and payload ▪ Metabolic stability and metabolite identification of payload in liver microsome and hepatocytes ▪ *In vitro* stability and metabolite identification of **PDC** in whole blood (or plasma) and kidney homogenate ▪ *In vitro* transporter and CYP450 DDI evaluation of payload and **PDC**
***In vivo* PK/other *in vivo* assays**	▪ *In vivo* metabolite identification of homing **peptide** and **PDC** in plasma and urine ▪ PK study of homing **peptide** in rodent species ▪ PK and tissue distribution study of **PDC** in rodent species (Both **PDC** and payload are bioanalyzed.)	▪ PK study of **PDC** in non-rodent species ▪ Tissue distribution of **PDC** in PD species ▪ Preliminary toxicity study of **PDC** in rodent and non-rodent species (Both **PDC** and payload are bioanalyzed in all *in vivo* assays)	▪ Bioanayltical method validation of **PDC** and payload in different biological matrices. ▪ Method validation of anti-drug antibodies (ADAs) (optional) ▪ Rodent and non-rodent PK studies of **PDC** (Both **PDC** and payload are bioanalyzed. Repeated PK study is optional with ADA detection.) ▪ PK study of payload in rodent or non-rodent species ▪ *In vivo* metabolite identification of **PDC** in plasma and excretions ▪ Tissue distribution and mass balance in rodent species

*Payload refers to cytotoxic drug.

Challenges in **PDC** Pharmacokinetic Studies

High Difficulty

- A **PDC** is a conjugate of a **peptide** and payload. It is necessary to conduct **DMPK** research for both the **PDC** and payload.
- Neither the FDA nor the ICH has specific guidelines for **DMPK** research on **PDC**s.
- If the payload is highly toxic, it is impossible for the **PDC** to perform absorption, metabolism and excretion (AME) studies with a radiolabeled **PDC** in a human.

High Significance

- The efficacy of a **PDC** is closely related to the release and concentration of the payload in the target tissue.
- The toxicity of a **PDC** is directly related to the release and concentration of the payload in the non-target tissues.
- Toxicological species of a **PDC** is determined by the metabolism similarity of a **PDC** in humans and other species.
- Drug-drug interactions of a **PDC** are closely associated with the exposure, metabolism, and elimination of a **PDC** and payload in the tissues.

Key Study Capabilities

- Various *in vitro* payload release models.
- Metabolite identification of a **PDC** and payload in different matrices using high-resolution mass spectrometry.
- *In vivo* PK study on a **PDC** utilizing LC/MS and MSD platforms.
- Tissue distribution and excretion studies with a radiolabeled **PDC**.
- Tissue distribution of a **PDC** in healthy or tumor-bearing rodent species using QWBA method.

PDC Pharmacokinetic Study Strategies

01. Early Screening Stage:

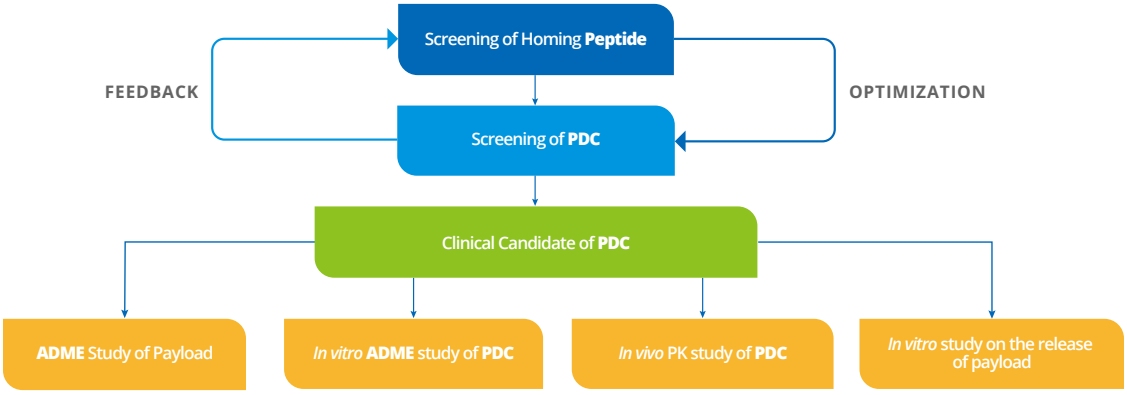
The screening stage mainly focuses on the *in vitro* and *in vivo* stability optimization of homing **peptides** and **PDC**, including the stability in whole blood (or plasma) and kidney homogenate. In addition, *in vitro* and *in vivo* metabolite identification is often conducted to further optimize **PDC** structures, thus improving the stability and prolonging the *in vivo* half-life. Tissue distribution studies in rodent species are often recommended in early stages to assess the potential toxicity of payloads.

02. Preclinical candidate (PCC) stage:

Depending on whether and how the linker is cleaved, payload release is suggested to perform *in vitro* in different matrices to mimic *in vivo* release. When conducting *in vivo* experiments, the concentrations of both **PDC** and payload in the samples will be detected. Detecting ADA levels in the repeated-dose experiments is recommended as well as tissue distribution in the PD species to evaluate the targeting capabilities of the **PDC**.

03. IND filing stage:

It is necessary to conduct comprehensive *in vitro* **ADME** assays and a PK study in at least one species for payload if the **DMPK** properties are unknown. In addition, sponsors often find it helpful to evaluate the tissue distribution and mass balance in rodents with a radiolabeled **PDC** or using a QWBA approach. We also recommend drug-drug interaction and metabolism studies in other matrices of **PDC**.



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 Experience in Customizing Study Designs

We have extensive experience in completing many successful **PDC** projects – allowing us to offer customized study design for clients.

Cross-Department Cooperation and High Efficiency

Our **DMPK** team works closely with chemistry and biology departments internally to facilitate smooth execution of the project and shorten the turnaround time.

Comprehensive Capabilities to Deal with PDC Challenges

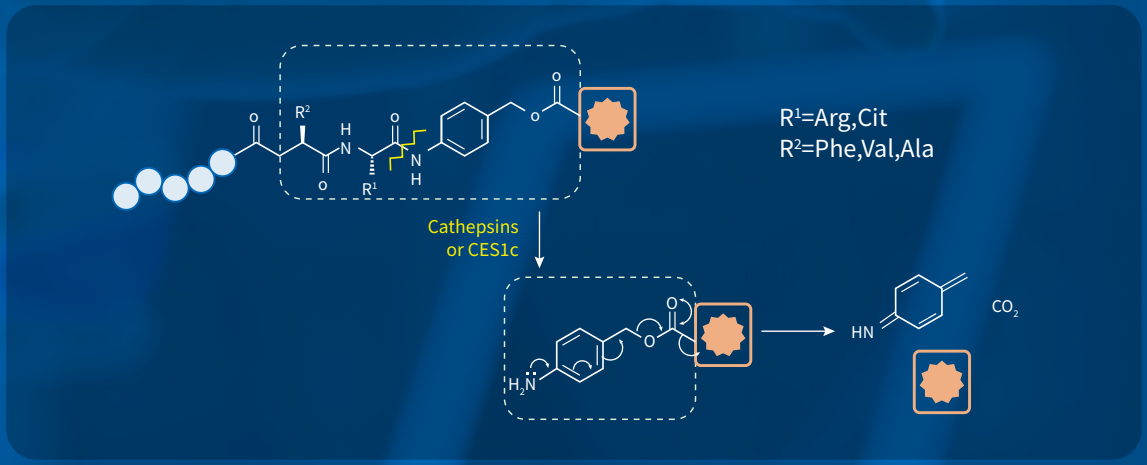
We are able to conduct **DMPK** studies for the payload and **PDC**. In addition, we have the comprehensive capacity to carry out bioanalysis, *in vitro* and *in vivo* metabolite identification, and radiolabeled **ADME** experiments of **PDC**.

Case Study

Case: PK/PD bioanalysis of **PDC** in mouse plasma

Customized design: After we received the request from the client, we took the initiative to ask the client about the linker type of the **PDC**. According to our experience, special attention needs to be paid to linker type. For example, attention should be given to the pH value of the mobile phase on the detection instrument in the case of pH-sensitive linker. For this **PDC**, the homing **peptide** was linked to p-aminobenzyl carbamate (PABC) and MMAE via Val-Cit dipeptides. To our knowledge, the CES1c enzyme from mouse plasma probably hydrolyzes this linker to release MMAE. If the mouse plasma is directly collected following routine procedure, freeze-thaw stability issue probably arises during bioanalysis, resulting in unreliable concentration of MMAE and the **PDC**. Due to the release of MMAE hydrolyzed by CES1c enzyme from mouse plasma, the concentration of MMAE will increase while the concentration of **PDC** will decrease during the time of plasma sample processing. Therefore, we suggested that our client began with method development. Based on method development, direct protein precipitation in the plasma afforded the supernatant immediately after collection, and specific absorption inhibitor Triton X-100 was added to remove non-specific binding.

Test results: Reliable analysis results were attained after following our suggestion. During the bioanalysis of the **PDC** samples, special attention should be given to the issues such as stability and non-specific binding.



References

[1] Bethany M. Cooper et al. Peptides as a platform for targeted therapeutics for cancer: peptide–drug conjugates (PDCs). *Chemical Society Reviews.* 2021, 50, 1480. f