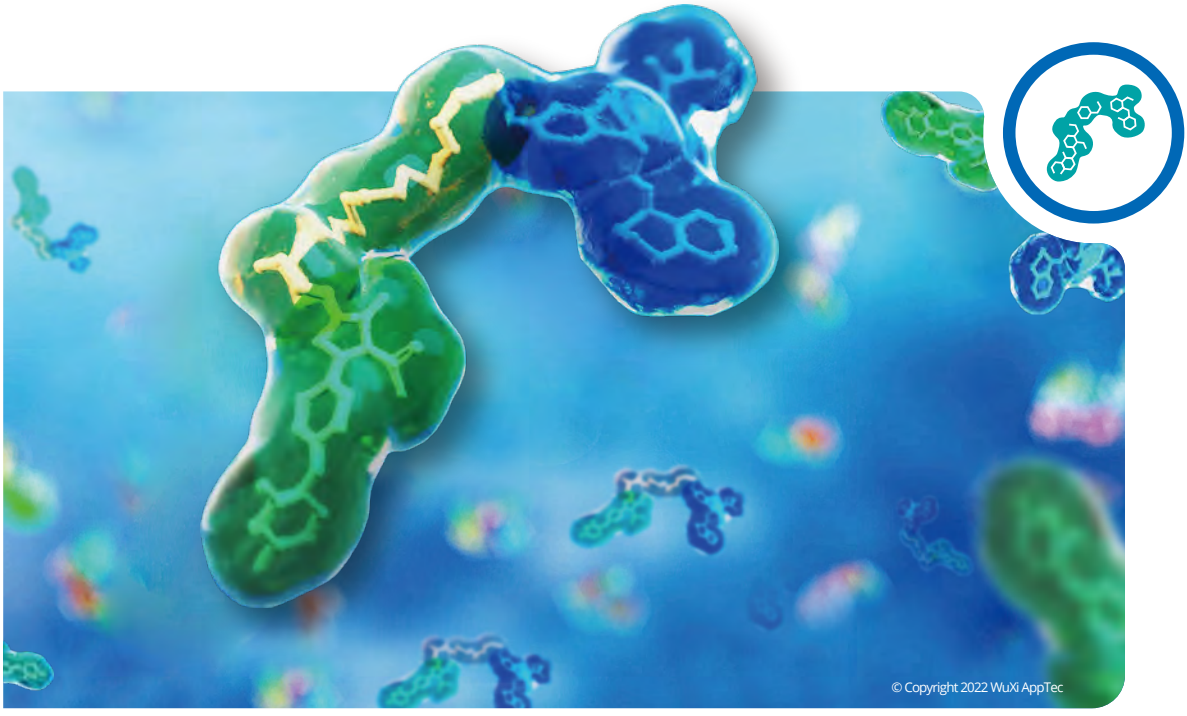
药明康德
WuXi AppTec

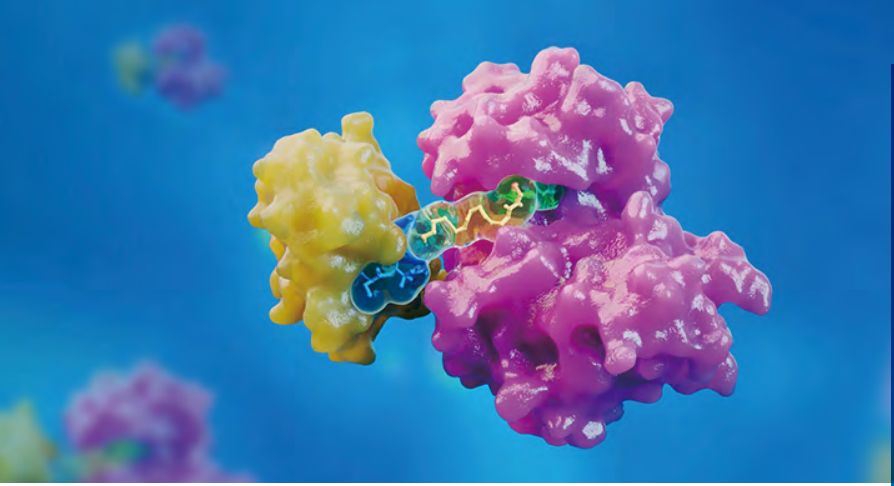
PRECLINICAL DRUG DEVELOPMENT TESTING FOR

PROTEOLYSIS TARGETING CHIMERA

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



New Modalities Series | Proteolysis Targeting Chimera



Unique Pharmacokinetics Evaluation System for **PROTAC** Drugs

PROTACs* are emerged as a novel therapeutic option for treating previously untreatable diseases. However, traditional testing methods cannot accurately evaluate the pharmacokinetics properties of **PROTAC**s. Drug developers seeking to push this exciting and important area of research forward urgently need unique pharmacokinetics evaluation systems for **PROTAC** molecules.

WuXi AppTec's Drug Metabolism and Pharmacokinetics Service Department has established a pharmacokinetics evaluation system for **PROTAC** drugs based on **PROTAC** drug study experience. This unique evaluation system relies on our complete *in vitro* and *in vivo* pharmacokinetic studies platform combined with the technical principles and characteristics of **PROTAC**.

Shortening the PROTAC Development Cycle with:

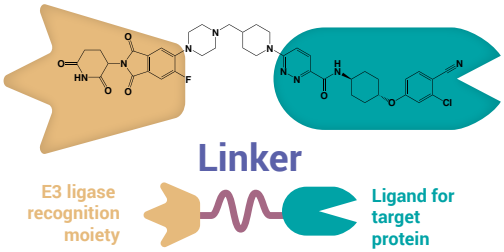
01 Pre-clinical formulation screening

02 *In vivo* pharmacokinetic study design

03 Selection of *in vitro* study methods

04 Speed of biological samples analysis

05 Ability to identify metabolites *in vitro* and *in vivo*



We have supported the screening of tens of thousands of **PROTAC** molecules and the IND applications of dozens of **PROTAC** molecules. In this process, we have accumulated extensive experience and formed a unique methodology for **PROTAC** pharmacokinetic studies – with the goal of helping drug developers quickly advance their **PROTAC** drug development projects.

**PROTAC® is a registered trademark of Arvinas. In this brochure, PROTAC specifically refers to the abbreviation of PROteolysis TArgeting Chimera as therapeutic modalities.*

PROTAC DMPK Study Services

Absorption

- Solubility in different medium
- Optimized *in vitro* permeability evaluation model
- *In vivo* studies to evaluate the absorption

Distribution

- Customized method to investigate plasma protein binding
- *In vivo* tissue distribution or QWBA

Metabolism

- Multiple *in vitro* metabolic models
- Explore metabolic transformation with metabolite identification

Excretion

- Investigate the excretion pathway with *in vivo* excretion experiments

Drug-drug Interaction (DDI)

- Complete DDI assessment based on drug-metabolizing enzymes
- Complete DDI assessment based on drug transporters

DMPK Study Contents

	EXPLORATORY	PRE-CLINICAL	CLINICAL
	Lead Molecule Optimization	**IND Enabling and IND Filing**	**NDA Filing**
ADME	▪ LogD ▪ Solubility (FeSSIF, FaSSIF, SGF) ▪ Caco2/MDR1-MDCK Permeability ▪ Plasma protein binding ▪ Hepatocyte stability ▪ Liver S9, microsome, AO metabolic stability ▪ Blood/plasma stability	▪ Caco2/MDR1-MDCK Permeability ▪ Plasma protein binding ▪ Liver microsome/hepatocyte stability ▪ Metabolite identification across species ▪ ADME study of radiolabeled compounds ▪ Metabolite identification in plasma, urine, feces, bile from toxicological species	▪ Metabolite identification in plasma, urine, feces from human
DDI	▪ Enzyme inhibition	▪ Enzyme inhibition and induction ▪ Enzyme Phenotyping ▪ Transporter inhibition ▪ Transporter substrate	▪ Other transporter substrates ▪ PBPK method predicts DDI risk in human ▪ Clinical DDI Research
PK/TK	▪ Testing of biomarkers in PD or PK studies in toxicological species ▪ Excretion study	▪ PK study in toxicological species ▪ Tissue distribution studies ▪ Urine, fecal and bile excretion studies ▪ PK study ▪ TK research	▪ Human PK studies ▪ Population PK study in human

Challenges in **PROTAC** DMPK Studies

High Difficulty

PROTACs have a large molecular weight and poor solubility, making it difficult to meet the Classical Lipinski's Rule of Five.

PROTAC drugs have poor permeability, which results in poor druggability for oral administration; the correlation between *in vitro* and *in vivo* permeability is poor, too.

Overexposure to **PROTAC** causes a 'hook effect', which renders the effective dose range difficult to control.

The metabolism of **PROTAC** involves a variety of metabolic mechanisms; thus, multiple *in vitro* metabolic models can be selected, and the metabolites are relatively complex.

Neither the **USFDA** or **ICH** has formulated specific guidelines for **PROTAC** pharmacokinetic studies.

High Significance

The physicochemical properties (solubility, lipophilicity) are closely related to its absorption properties.

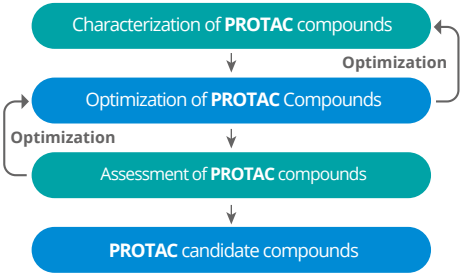
The therapeutic effect is related to its concentration in plasma and target tissues.

The selection of toxicological species is related to its similarity in metabolism, with extra attention to the linker cleavage metabolites.

It is necessary to select a suitable *in vitro* metabolic model and strategy to screen **PROTAC** molecules according to the metabolic characteristics.

PROTAC DMPK Study Strategies

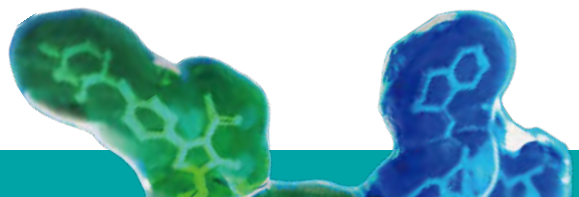
The preclinical optimization of PROTAC drugs is mainly conducted via the cascade optimization of physicochemical and pharmacokinetic properties.



Early Screening Stage: to characterize **PROTAC** molecules *in vitro* and *in vivo*. This includes physicochemical properties, permeability, protein binding, and drug interactions.

Optimization Stage: to improve the metabolic clearance and solubility of **PROTAC**. This stage involves screening **PROTAC** molecules with good oral absorption and relatively stable metabolism combined with the PK properties of oral administration.

PCC Stage: to gain a deeper exposure-response relationship. This stage uses **PROTAC** molecules with strong efficacy and sufficient oral bioavailability for further PK/PD studies.



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 and Short Turn-Around Time

We have more than 8 years experience on **PROTAC** research, with the annual study of 1,000+ **PROTAC** molecules, a variety of mature solutions, and short experiment cycle.

Comprehensive Capabilities and High-Quality Delivery

With a professional **PROTAC** study team and a complete range of instruments and equipment, WuXi AppTec **DMPK** is equipped with comprehensive **PROTAC** study and analysis capabilities to ensure the delivery of high-quality *in vivo* and *in vitro* data.

Customized Study Design

Based on flexible study concepts, we provide customized designs for pharmacokinetic study strategies for our customers' new molecules with rapid optimization and adjustment.

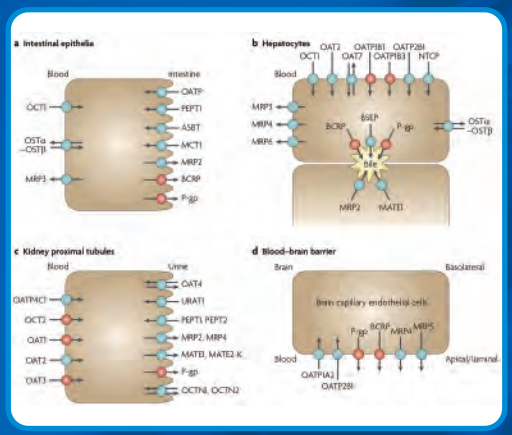
Cross-Department Cooperation and High Efficiency

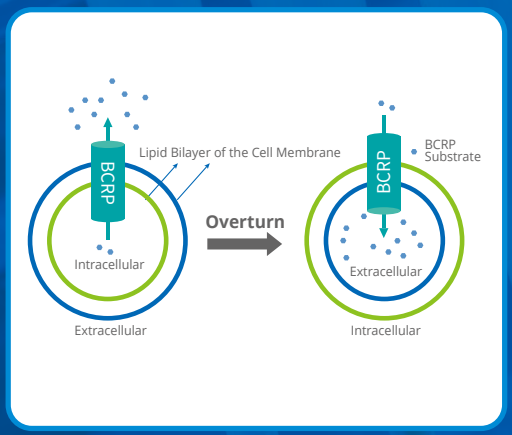
Cross-department organization and coordination to promote the smooth operation of the project, which shortens the research period.

Case Study PROTAC Transporter Inhibition Evaluation

Background: **BCRP** (Breast Cancer Resistance protein) is an important ATP-binding cassette (ABC) transporter. **BCRP** is mainly expressed in the apical membrane of the intestinal epithelia, in the bile duct side of hepatocytes, and in the blood-brain barrier near the blood, which can have a significant impact on restricting the entry of **BCRP** substrates into intestinal epithelia, mediating drug biliary excretion and the blood-brain barrier. A client needed to evaluate the inhibition of **BCRP** by **PROTAC** molecules. Preliminary results showed that the molecule had no significant inhibition of **BCRP** (IC 50 > 100 μM). However, the **PROTAC** molecule was reported in the literature to inhibit **BCRP**, so the client sought assistance and explanation.

Customized Design: After receiving questions from the client, we evaluated the study methods and data. Based on what we know about **PROTAC** molecules, **PROTAC** generally has poor permeability. With the routine cell model the extracellular **PROTAC** concentration is high, while the concentration of drugs entering the cell may be very low. Therefore, we recommended the BCRP-expressing inside-out membrane vesicles model for the evaluation of **BCRP** inhibition. The transport direction of the BCRP-mediated substrates is from the outer membrane to the inner membrane, and thus **PROTAC** could directly bind to the **BCRP** transporter outside the membrane.





Results: The vesicle method showed that the **PROTAC** molecule had a strong inhibitory effect on **BCRP**, which was consistent with the previous literature reports. Compared with conventional methods, which cannot obtain an inhibitory window, the vesicle method is more sensitive in the evaluation of efflux transporter inhibition. In addition to the **BCRP** transporter, the vesicle method was also recommended for the evaluation of the inhibition of other efflux transporters by **PROTAC** molecules.

References

[1] Cantrill C, Chaturvedi P, Rynn C, Petrig Schaffland J, Walter I, Wittwer MB. Fundamental aspects of DMPK optimization of targeted protein degraders. Drug Discov Today. 2020 Jun;25(6):969-982. doi: 10.1016/j.drudis.2020.03.012. Epub 2020 Apr 13. PMID: 32298797.

[2] Giacomini, Kathleen M.; Huang, Shiew-Mei; Tweedie, Donald J.; Benet, Leslie Z.; Brouwer, Kim L.R.; Chu, Xiaoyan; Dahlin, Amber; Evers, Raymond; Fischer, Volker; Hillgren, Kathleen M. (2010). Membrane transporters in drug development. , 9(3), 215–236. doi:10.1038/nrd3028