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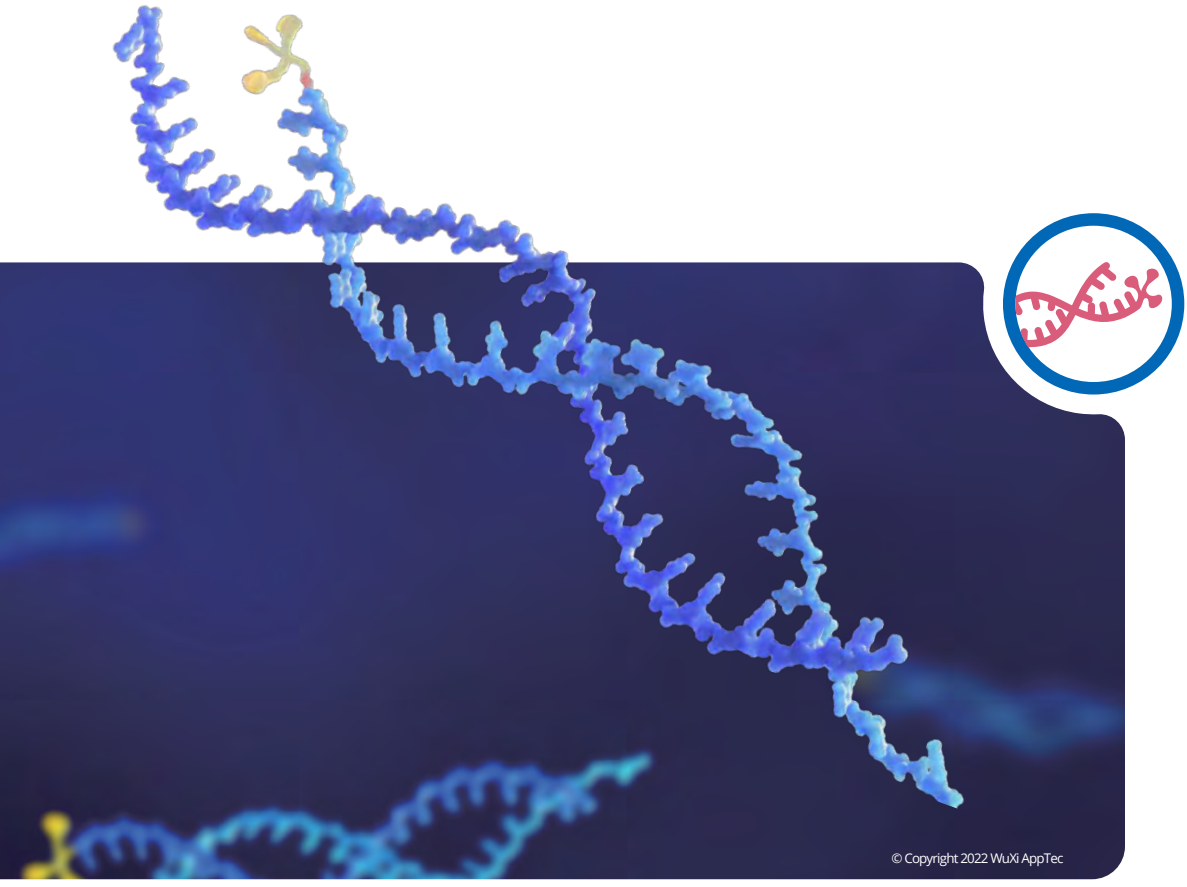
WuXi AppTec

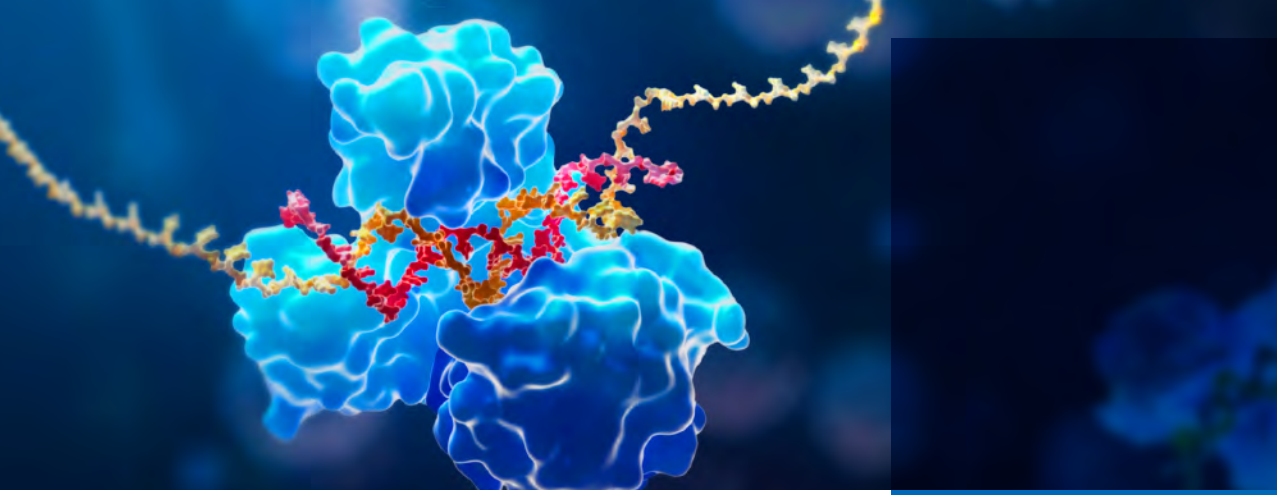
PRECLINICAL DRUG DEVELOPMENT TESTING FOR

OLIGONUCLEOTIDE DRUGS

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



New Modalities Series | Oligonucleotide



Unique PK Study Methodology to Accelerate the Development & Application of **Oligonucleotides**

Since the first siRNA drug was approved in 2018, **oligonucleotide** therapeutics have increased as an emerging drug modality. **Oligonucleotides** are short single or double-stranded fragments of DNA or RNA molecules that have a wide range of potential applications. However, due to its novelty and complexity, **oligonucleotides** have more technical challenges and unknown consequences for drug developers compared to many more conventional drugs. As such, **oligonucleotides** require pharmacokinetic evaluation systems that differ from traditional testing methods. WuXi AppTec's Drug Metabolism and Pharmacokinetics (**DMPK**) Service Department's extensive experience with **oligonucleotide** studies – combined with guidelines issued by various drug regulatory authorities on **oligonucleotide** development and studies in frontier literatures[1][2] – has enabled us to build a set of **ADME** evaluation systems for **oligonucleotides** that significantly shorten development cycles.

Shortening the Oligonucleotide Development Cycle with:

01 Preclinical *in vitro* study methods

02 *In vivo* pharmacokinetic experimental design

03 Quantitative Non-GLP analysis methods

04 Metabolite identification

05 Radiolabeled tissue distribution and mass balance studies

We have helped dozens of clients successfully screen and evaluate **oligonucleotide** pipelines. And have enabled dozens of **oligonucleotide** molecules' IND applications, including ASO, siRNA, miRNA, and more. We not only have extensive experiences to study **oligonucleotides** but also have developed a specific methodology for pharmacokinetic study on **oligonucleotide** drugs. Our program enables faster and more efficient development for new **oligonucleotides** for our customers.

Oligonucleotide Pharmacokinetic Study Services

Absorption

- Investigate multiple special routes of administration in pharmacokinetic studies.

Distribution

- Determine plasma protein binding rates through customized experimental procedures.
- Conduct studies on tissue distribution through non-radiolabeled or radiolabeled **oligonucleotides**.
- Investigate drug distribution with a quantitative whole-body autoradiography (QWBA) study.

Metabolism

- Evaluate drug metabolism through various *in vitro* metabolic models.
- Evaluate metabolic transformation through *in vivo* and *in vitro* metabolite identification.

Excretion

- Investigate excretion pathways through *in vivo* excretion experiments.

Drug-drug Interactions (DDI)

- Well-established DDI evaluation based on drug metabolic enzymes and transporters.

Pharmacokinetic Study Contents

	EXPLORATORY FINDINGS	PRECLINICAL	CLINICAL
	Leading Molecule Optimization	**Support Clinical Candidate Molecule Characterization and IND Applications**	**Support Clinical Development and NDA Applications**
ADME	▪ Plasma protein binding ▪ S9 metabolic stability ▪ Plasma/serum stability ▪ Tissue homogenate metabolic stability ▪ Cytosol metabolic stability	▪ Plasma protein binding ▪ Tissue homogenate metabolic stability ▪ Plasma/serum stability ▪ S9 metabolic stability and metabolite identification ▪ **ADME** study with radiolabeled compounds in toxicological species ▪ Identification of metabolites from *in vivo* samples (tissue, plasma, urine, feces, bile, cerebrospinal fluid, etc.)	▪ Identification of metabolites in human plasma, urine, and feces, etc. ▪ Identification of metabolites from *in vivo* samples such as tissue, plasma, urine, feces, and bile in toxicological species
DDI		▪ Substrate and inhibition of drug transporters ▪ CYP enzyme inhibition (reversible and time-dependent inhibition) ▪ CYP450 enzyme induction	▪ Prediction of DDI risks in human through PBPK modelling ▪ Clinical DDI study
PK	▪ Tissue distribution in rodents ▪ Biomarker detection in PK or PD species	▪ PK study and tissue distribution in rodents ▪ Study on urine, feces, and bile excretion in rodents ▪ PK study and tissue distribution in non-rodents	▪ *In vivo* PK study ▪ Immunogenicity evaluation ▪ *In vivo* population PK study

Challenges in **Oligonucleotide** Pharmacokinetic Studies

High Difficulty

- **Diverse types:** The different chemical modifications, sequences, or delivery systems of **oligonucleotides** may have their respective specificities, thus requiring different pharmacokinetic evaluation methods.
- **Complex sample pretreatment:** It is difficult to establish a proper mass spectrometry analysis method due to its poor stability, matrix effect, ion inhibition, and metabolite interference.
- **Multivalent in mass spectrometry:** Both **oligonucleotides** and their metabolites tend to be multivalent in mass spectrometry, and there are various metabolites in the tissue matrix, which makes it difficult to identify metabolites.
- **Non-final regulatory guidelines:** Neither FDA nor ICH has formulated final specific guidelines for the preclinical pharmacokinetics study of **oligonucleotides**.

High Significance

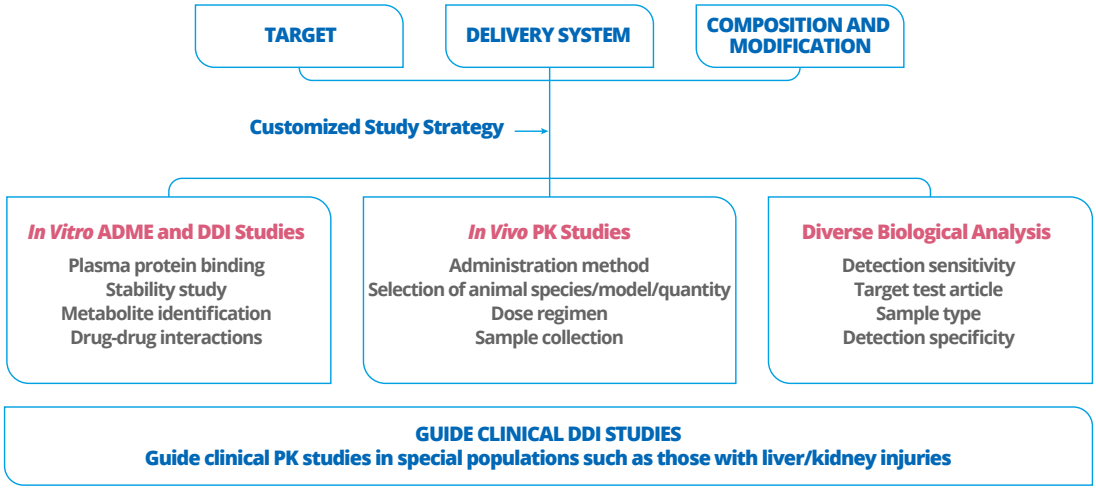
- An appropriate *in vitro* metabolic system needs to be selected in the screening stage.
- Appropriate bioanalytical methods are critical for PK studies of **oligonucleotides**.
- Metabolite identification facilitates early detection of active metabolites.
- Exposure to the drug in target tissues rather than plasma exposure is more relevant to drug efficacy.

Key Study Capabilities

- **DMPK** study capabilities for multiple types of **oligonucleotides** such as ASO, siRNA, microRNA, and aptamer.
- Comprehensive bioanalysis (mass spectrometry, qPCR, fluorescent probes, etc.), biomarker analysis (target mRNA, target protein, or downstream biomarkers), *in vitro* and *in vivo* metabolite identification, and radiolabeled non-clinical **ADME** and clinical AME study capabilities.
- Diverse *in vitro* metabolic study models.
- Special administration routes, such as intrathecal injection and intravitreal injection.
- Liver biopsy for large animals to support PK study of **oligonucleotides**.
- Study on tissue distribution of **oligonucleotides** using QWBA technology.

Oligonucleotide Pharmacokinetic Study Strategies

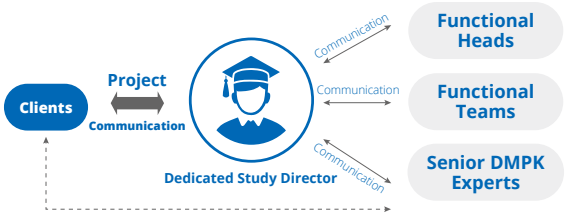
There are various types of **oligonucleotides**, and their pharmacokinetic properties vary depending on mechanism, delivery system, and chemical modification. In the preclinical development stage, study strategies should be customized according to the specific characteristics of the **oligonucleotide** to accelerate its development. For *in vitro* **ADME** study, it is necessary to select an appropriate *in vitro* metabolic model based on the structural characteristics of the drug; for *in vivo* PK study, different administration methods can be used to investigate the PK properties. An appropriate detection method should be developed in accordance with the properties of the **oligonucleotide**.



Our Advantages

Committed to Your Program

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 Custom-Designed

We have more than four years of **oligonucleotide** study experience. We provide customized designs of pharmacokinetic study strategies for our customers' new molecules, together with rapid optimization and timely adjustment based on flexible study concepts.

Cross-Department Cooperation and High Efficiency

Our team works closely with the chemistry and biology departments internally to promote the smooth operation of the project, reduce the wait time, and ensure data security.

Comprehensive Capabilities and Diverse Bioanalytical Methods

With a professional **oligonucleotide** study team and comprehensive instruments and equipment, WuXi AppTec's **DMPK** team can conduct extensive **oligonucleotide** studies and analysis, capable of delivering high-quality *in vitro* and *in vivo* data – all to accelerate the drug development process.

Triple Quad 6500 System

Q-Exactive™ Plus

Fluorescence Detectors

Molecular Devices SpectraMax M5e

QuantStudio™ 7 Flex

Integrity Bioanalytical Platforms for Oligonucleotides

LC-MS/MS	LC-HRMS	LC-FL	LBA	qPCR
▪ Pharmacokinetic study ▪ Tissue distribution ▪ *In vitro* **ADME** study	▪ PCC Screening ▪ Sequence confirmation ▪ Impurity measurment ▪ Metabolite profiling ▪ Pharmacokinetic study ▪ Tissue distribution	▪ Pharmacokinetic study ▪ Tissue distribution	▪ Pharmacokinetic study ▪ Tissue distribution ▪ Immunogenicity	▪ Pharmacokinetic study ▪ Tissue distribution ▪ siRNA-RISC loading ▪ Exon skipping ▪ Knockdown target mRNA

Case Analysis: Inclisiran DMPK study

Background: Take the preclinical PK study of Inclisiran (Novartis Leqvio®) as an example. In December 2020, the European Union granted market approval for Inclisiran. It is an innovative gene-based therapy, the world's first small interfering nucleic acid (siRNA) in cholesterol-lowering field, targeting the liver proprotein convertase subtilisin/kexin type 9 (PCSK9) protein for the treatment of hypercholesterolemia or mixed dyslipidemia.

Inclisiran has undergone multiple chemical modifications, such as replacement of the phosphodiester bond with thiophosphate ester bond, and ribose modification by 2'-OMe (2'-O-methyl) or 2'-F(fluorine), which covalently links to three GalNAc molecules at the 3'-end of sense strand.[3]

Experimental design: Based on relevant published literatures, the contents of the preclinical pharmacokinetic study of Inclisiran are summarized as follows:

(1) *In vitro* ADME studies: Electrophoretic mobility shift assay (EMSA) on plasma protein binding rate, serum and liver S9 stability, CYP enzyme inhibition and induction, and drug transporter substrate and inhibition, etc.

(2) *In vivo* PK studies: Pharmacokinetic characteristics in rats and monkeys under subcutaneous or intravenous administration conditions. In some studies, multiple samples were taken from the same animal through liver biopsy to analyze the drug concentrations in the liver. ^{14}C labeled Inclisiran was used to investigate the tissue distribution and excretion pathway in rats and monkeys; the sampling periods lasted up to 98 days (rats) and 42 days (cynomolgus monkeys) by utilizing QWBA technology.

(3) Metabolite identification studies: Thorough identification of metabolites was conducted. *In vitro* evaluation mainly included the metabolite identification of Inclisiran in human, mouse, rat and monkey serum and liver S9. *In vivo* evaluation included the metabolite identification of ^{14}C labeled Inclisiran in the plasma, urine, bile (rat only), feces, livers, kidneys and injection sites (rat only) of rats and cynomolgus monkeys after administration; the metabolites in the plasma and liver tissues of rats and cynomolgus monkeys after the administration of Inclisiran were also identified.

In Vivo Studies

Administration	Trial Deisgn	Animal Species	Testing
Inclisiran	Single IV administration: Dosage: 6 mg/kg	Cynomolgus Monkey	Drug concentrations in liver, kidney, and plasma
Inclisiran	Single SC administration: Dosage: 1,3, and 6 mg/kg	Cynomolgus Monkey	Drug concentrations in liver, kidney, and plasma
Inclisiran	Single SC administration: (Intravenous injection or infusion) Dosage: 5 mg/kg	Rat	Drug concentrations in plasma
Inclisiran	Single SC administration: Dosage: 1,5, and 25 mg/kg	Rat	Drug concentrations in plasma and tissue
^{14}C Labeled Inclisiran	Single SC administration: Dosage: 65 mg/kg	Rat	QWBA
^{14}C Labeled Inclisiran	Single SC administration: Dosage: 20 mg/kg	Cynomolgus Monkey	QWBA
Inclisiran	Repeat SC administration: Dose: A loading dose of 6 mg/kg is administard firstly, a dose of 3 mg/kg is then administered once a month for 3 consecutive months. Dose: A loading dose of 6 mg/kg is administered firstly, a dose of 3 mg/kg is then administered every 2 weeks for 6 consecutive months.	Cynomolgus Monkey	Drug concentrations in liver, kidney, and plasma

Metabolite Identification Studies

Study Type	Analysis Matrix
In vitro metabolite identification study	Serum and liver S9 of human, mouse, rat, cynomolgus monkey
In vivo metabolite identification study	Plasma, urine, bile (rat only), feces, liver, kidney, and injection site (rat only) of rats and cynomolgus monkey after the administration of ^{14}C labeled Inclisiran Plasma and liver of rats, and cynomolgus monkeys after administration of Inclisiran

In Vitro Studies

Test Type	Test Condition
Plasma Protein Binding	Matrix: Mouse, rat, cynomolgus monkey, and human plasma Method: Electrophoretic mobility shift assay (EMSA)
Stability in Liver S9	Matrix: Human, mouse, rat, cynomolgus monkey liver S9
Stability in Serum	Matrix: Human, mouse, rat, cynomolgus monkey serum
Drug-Drug Interactions	CYP Inhibition study (reversible and time-dependent inhibition): CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 CYP Induction study: CYP1A2, CYP2B6, and CYP3A4 Substrates and Inhibition study of Drug Transporters: P-gp, BCRP, OAT1, OAT3, OCT1, OCT2, OCT3, OATP1B1, OATP1B3, MATE1, MATE2-K and BSEP.

References

[1] Yu, S. S., Hu, X. M., Wang, H. X., Wang, Y. & Wang, Q. L. An overview of nonclinical study and evaluation of therapeutic single-stranded oligonucleotides drugs. Chinese J. New Drugs 27, 1122–1129 (2018).

[2] Guidance, D. Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics Guidance for Industry. (2022)

[3] https://www.ema.europa.eu/en/documents/assessment-report/leqvio-epar-public-assessment-report_en.pdf. (2020)