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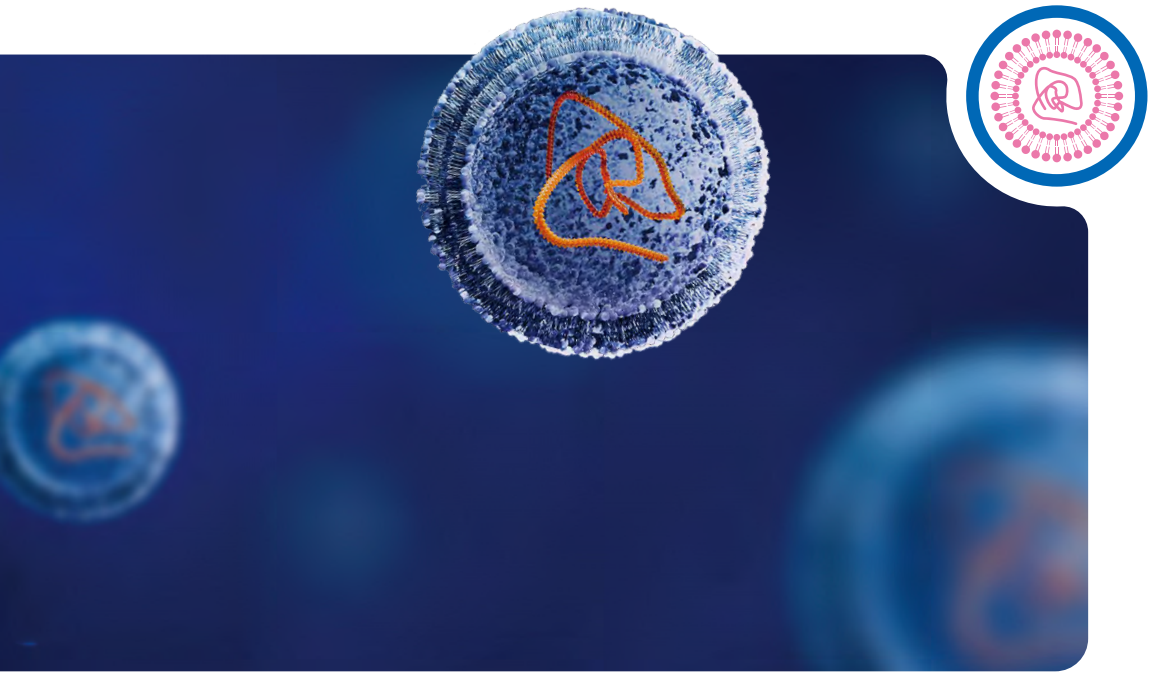
WuXi AppTec

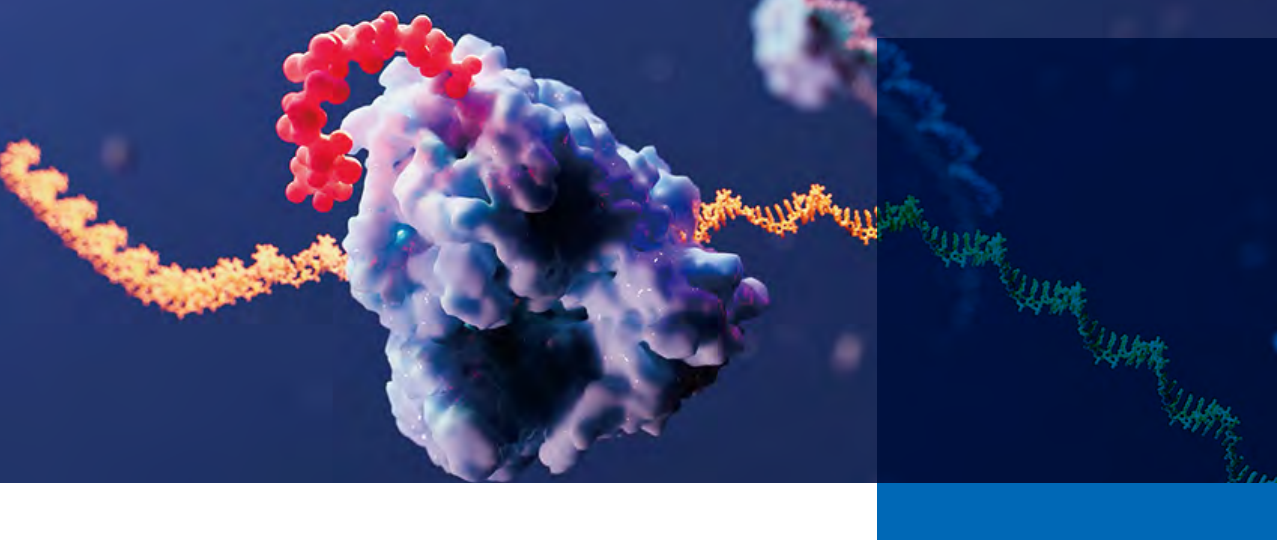
PRECLINICAL DRUG DEVELOPMENT TESTING FOR

mRNA-BASED VACCINES AND THERAPEUTICS

Shorten the mRNA Development Cycle
with WuXi AppTec **DMPK**



New Modalities Series | mRNA



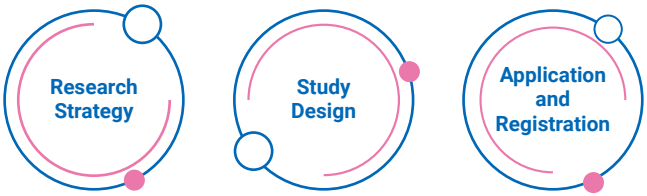
Unique Pharmacokinetics Evaluation System for mRNA-Based Vaccines and Therapeutics

Adhering to the stringent guidelines set by the FDA, ICH, and CDE for vaccines, gene therapies, pharmaceutical excipients, and adjuvants, the DMPK Service Department of WuXi AppTec has meticulously compiled pharmacokinetic study designs and research methodologies for mRNA-based vaccines and therapeutics in both clinical stages and the market. Our comprehensive review provides a detailed interpretation of preclinical pharmacokinetic data from FDA-approved mRNA vaccines. Given the substantial differences in mechanisms of action and molecular types, distinct evaluation objectives and requirements emerge in preclinical pharmacokinetic studies. Consequently, tailored pharmacokinetic research strategies are crucial to address these specific needs effectively.

01 Research strategies and study designs for IND applications

02 Early-stage *in vitro* and *in vivo* assessments and screenings for delivery systems

03 Integrated bioanalytical platform



To facilitate the research and development of mRNA-based vaccines and therapeutics, WuXi AppTec DMPK has established an integrated bioanalytical platform. This platform includes qPCR, branched DNA (bDNA), ligand binding assays (LBA), liquid chromatography-mass spectrometry (LC-MS), and flow cytometry, enabling quantitative and semi-quantitative analysis of mRNA. We determine the pharmacokinetic study strategy based on the unique characteristics of each client's products and provide an appropriate study design, accelerating the research and IND application of mRNA-based vaccines, therapeutics, and delivery systems.

Pharmacokinetic Research Services for mRNA-Based Vaccines and Therapeutics

mRNA Vaccines

Biodistribution study of mRNA

mRNA Therapeutics

Biodistribution study of mRNA/translated proteins

PK study of mRNA/translated proteins in relevant species

PK/Pharmacodynamics (PD) study of mRNA/translated proteins in model animals

Immunogenicity of translated proteins in relevant species

Delivery Systems

PK, distribution, metabolism, and excretion of novel lipids in relevant species

Drug interactions (DDIs) of novel lipids

Immunogenicity of PEG lipids

mRNA Pharmacokinetic Research Contents

mRNA Vaccines

Biodistribution

- Tissue distribution study of mRNA

mRNA Therapeutics

In Vivo PK

- Pharmacokinetic study of mRNA/translated proteins
- PK/ PD study of mRNA/translated proteins
- Immunogenicity study of translated proteins

Biodistribution

- Tissue distribution study of mRNA/translated proteins

Lipid Nanoparticle (LNP) Delivery Systems

In Vivo PK

- Plasma pharmacokinetic study of LNPs
- Immunogenicity of PEG lipids

Biodistribution

- Tissue distribution study of LNPs

Excretion

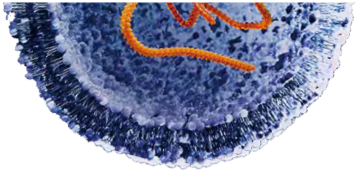
- Excretion study of LNPs

In Vitro and *In Vivo* Metabolism

- Stability of novel lipids in plasma, liver microsomes, and hepatocytes
- Metabolite identification of novel lipids in liver microsomes and hepatocytes
- *In vivo* metabolite identification of novel lipids

Drug Interactions

- Inhibition and induction effect on metabolic enzymes by novel lipids
- Inhibition effect on transporters by novel lipids



Challenges in Pharmacokinetic Studies for mRNA-Based Vaccines and Therapeutics

High Difficulty

- The diverse mechanisms of action of mRNA therapeutics hinder the establishment of a unified research strategy. Therefore, a tailored study design is necessary.
- Pharmacokinetic study of mRNA therapeutics involves analysis of various molecular modalities, necessitating support from a comprehensive and professional bioanalysis platform.
- Quantitative analysis of mRNA is challenging. Currently, there are no specific guidelines on qPCR analysis methods for mRNA.
- Lipid molecules are prone to issues such as non-specific binding, making sample handling and analysis challenging.

High Significance

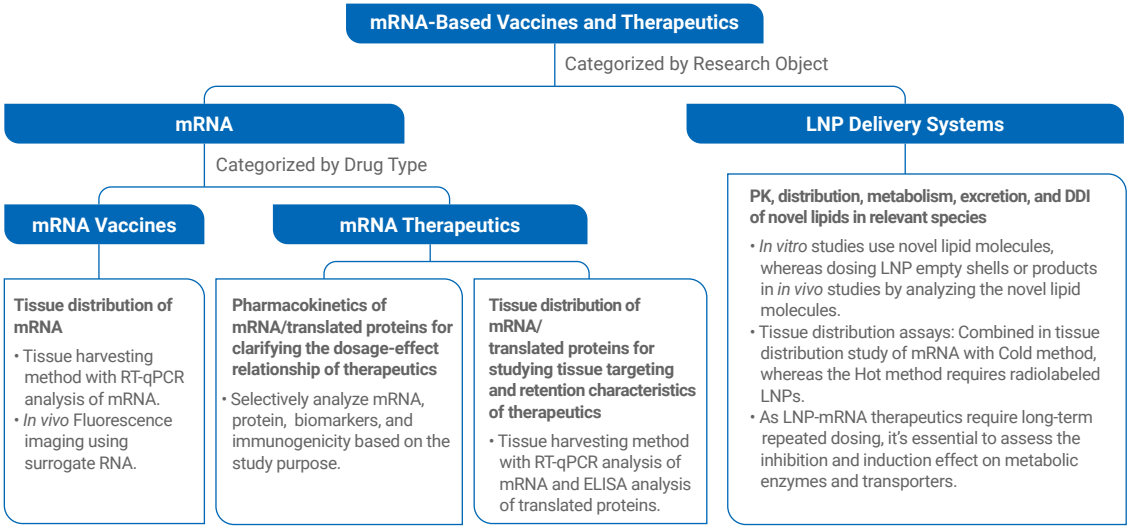
- The efficacy and safety of mRNA therapeutics are directly related to the biodistribution and pharmacokinetics of mRNA and translated proteins.
- Owing to the cytotoxicity of cationic lipids in LNPs, screening of metabolizable cationic lipids is crucial.
- The immunogenicity of PEG lipids in LNPs could lead to the Accelerated Blood Clearance (ABC) effect, which may impact the pharmacokinetic profile of mRNA after multiple administrations.

Key Study Capabilities

- Extensive DMPK research experience in nucleic acid drugs and lipid-based delivery systems.
- An integrated bioanalytical platform could enable quantitative and semi-quantitative analysis of lipids, mRNA, proteins, PD biomarkers, and immunogenicity.
- Radioisotope and QWBA platform could support the study of biodistribution in rodent animals and cynomolgus monkeys.
- Liver biopsy techniques could support the study of target-tissue distribution of liver-targeted mRNA products in large animals.

Pharmacokinetic Research Strategies

mRNA products comprise two major categories: mRNA vaccines and therapeutics. mRNA vaccines activate the immune system by expressing antigens to achieve preventive or therapeutic purposes, with low dosage and relatively low administration frequency. mRNA therapeutics exert therapeutic effects by expressing functional proteins, while drug efficacy is related to protein expression levels and duration directly. Additionally, the delivery system is an integral component of mRNA products. In preclinical studies, it is necessary to combine the mechanism of action of products and develop appropriate study strategies for different components of the product to characterize the pharmacokinetic profile comprehensively.



Our Strengths

Committed to Your Program

We offer 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.

Cross-Department Cooperation and High Efficiency

We work closely and share resources with the chemistry and biology departments internally to promote a project's smooth operation.

Extensive Experience and Customized Research Strategies

We have years of experience screening and supporting IND applications. This allows us to offer customized pharmacokinetic study strategies for our clients' mRNA products. We optimize study designs based on the properties of the products.

Comprehensive Capabilities with an Integrated Bioanalytical Platform

Bioanalytical Platform for mRNA-Based Vaccines and Therapeutics

mRNA Quantification	Delivery System Quantification	Protein Quantification	Biomarker Analysis	Immunogenicity Analysis	Radioactive Assay
qPCR, bDNA	LC-MS/MS	LBA, LC-MS/MS	LC-MS/MS, LBA, Flow Cytometry	LBA, Flow Cytometry	QWBA or Liquid Scintillation Counting

Triple Quad 7500 System

Orbitrap Eclipse™ Tribrid™

QuantStudio™ 7 Pro

Molecular Device M5e

MESO QuickPlex SQ 120

BD Fortessa X-20

Leica CM3600 XP

Amersham Typhoon RGB

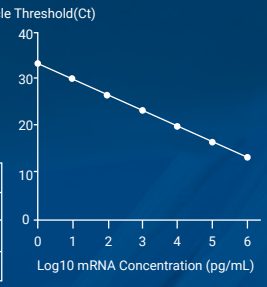
Case Study

Background : For IND applications of mRNA-based vaccines and therapeutics, a robust and validated bioanalysis method for mRNA absolute quantification is required. Currently, there is no official guidance for mRNA bioanalysis validations. Here, we reference the consensus qPCR validation guidelines on the whitepaper published by the Global CRO Council in Bioanalysis (GCC) and generate our internal mRNA bioanalysis validation guidance.
We developed and validated the mRNA bioanalysis method in rat whole blood and solid tissue (such as liver) by using a mRNA mimic (EGFP mRNA). The validated qPCR method is suitable for FDA/NMPG/TGA IND application. The standard curve and inter/intra accuracy and precision evaluation from the validation results are shown below.

RT-qPCR Absolute Quantification Method for EGFP mRNA in Whole Blood

Test mRNA: EGFP mRNA
Matrix: SD Rat Whole Blood
Lower Limit of Quantitation: 1 pg/mL
Linear Range: 1 pg/mL–1,000 ng/mL

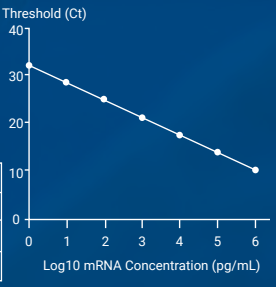
Parameters	Value
Slope	-3.364
R^2	0.9995
Efficiency (%)	98.3



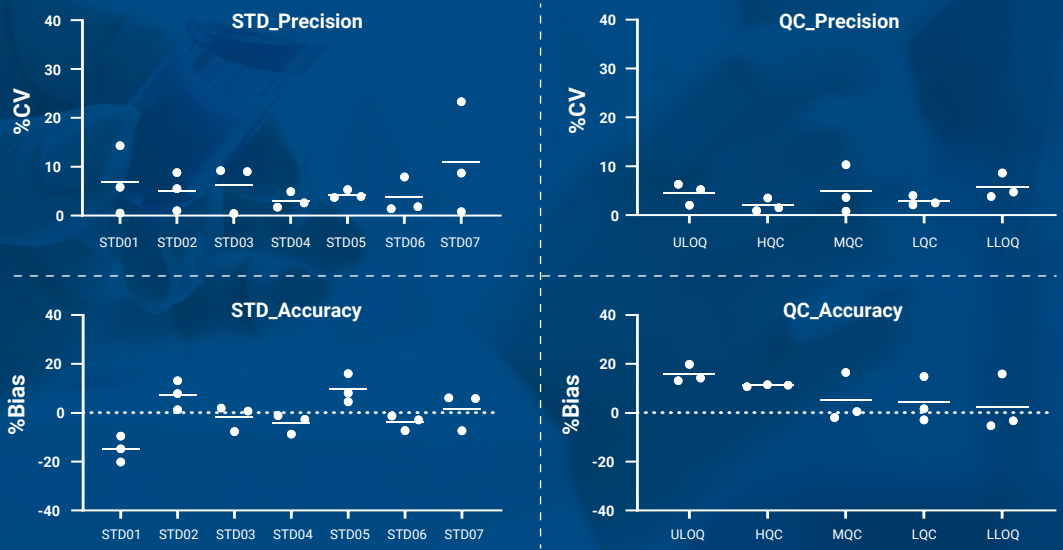
RT-qPCR Absolute Quantification Method for EGFP mRNA in Liver

Test mRNA: EGFP mRNA
Matrix: SD Rat Liver
Lower Limit of Quantitation: 1 pg/mL
Linear Range: 1 pg/mL–1,000 ng/mL

Parameters	Value
Slope	-3.369
R^2	0.9998
Efficiency (%)	98.1



RT-qPCR Inter/Intra A&P Evaluation for Whole Blood



Improving Health.
Making a Difference.

Talk to our experts today about a drug development program tailored specifically to your needs.

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