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Are *in vitro* DDI Studies Needed for Oligonucleotide Therapeutics?

Mengyi Gu, Nicholas White, Xiaokun Cai, Ryan Olsen, Devin Hudson, Jovita Saquing, Lata Venkatarangan, Wenbin Liu, Zamas Lam*

DMPK, QPS LLC, Newark, DE 19711

info@qps.com | www.qps.com

Small Molecules IND-enabling Tox and ADME Studies

STRAIGHT FORWARD AND NOT CONTROVERSIAL

- Generally, 2 test species
  - 1 Rodent (mice, rat)
  - 1 Non-rodent (dog, non-human primate, mini-pig)
  - 1 Developmental & Reproductive (rat, rabbit)
- ADME
  - Absorption
    - *Rat Single or Multiple Dose*
    - *NHP Single or Multiple Dose*
  - Distribution
    - *Protein Binding (in vitro and ex vivo)*
    - **Tissue Distribution (non + radioactive ^{3}H, ^{14}C)*
  - Metabolism
    - *Metabolic Stability*
    - *Metabolite Profiling / Identification (in vitro)*
    - *Reaction Phenotyping*
    - *Drug-Drug Interactions*
    - *Drug Transporters*
  - Elimination
    - *Mass Balance / Excretion*
    - *Metabolite Profiling / Identification (in vivo)*
- Genetic Toxicology
  - *In vitro bacterial mutation test (Ames)*
  - *In vitro mammalian chromosome aberration test*
  - *In vivo rat micronucleus & TK*
- General Toxicity and TK Studies
  - *Rodent 14-day DRF*
  - **Rodent 28-day repeat dose with 28-day recovery*
  - *Non rodent 14-day DRF*
  - **Non rodent 28-day repeat dose with 28-day recovery*
- Safety Pharmacology Studies
  - *In vitro hERG*
  - *Rat Neuropharmacological Profile*
  - *Rat Respiratory Safety Pharmacology*
  - *NHP Cardiovascular & TK Study*

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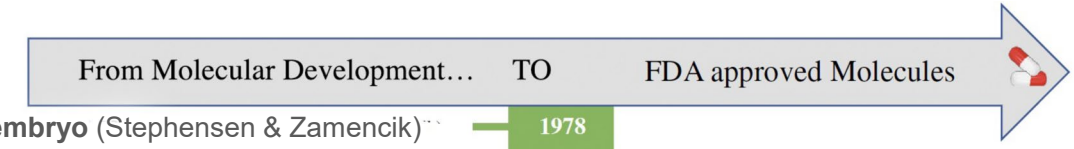
Timeline of ASO development



First ASO in Chick embryo (Stephensen & Zamencik) — 1978

First clinical trial: gene transfer into human – injection of modified tumor infiltrating lymphocytes (TILs) in melanoma (Rosenberg *et al*) — 1990

Proof of concept – Restoration of accurate slicing in thalassemia (Dominski *et al*) — 1993

RNAi discovery:
-In petunia (Napoli *et al*, 1990
-In *C. elegans* (Fire *et al*, 1998) — 1998

1999 — **Formvirsen** - first FDA and EMA approved ASO

2002 — **Formvirsen** – withdrawn from the market in USA and Europe

Prevention of protein sequestration in myotonic dystrophy
Decreased Huntingtin production in mice and monkeys (Korsasiewicz *et al*) — 2012

2013 — **Mipomersen** approved for hypercholesterolemy (SC)

2016 — **Eteplirsen** approved for DMD (SC)
Nusinersen approved for SMA (ITh)

miRNA response element (MRE) masking in mice (Messina)
Gapmer-*SAMMSON* in mice – melanoma (Leucci)
miRNA Sponge TSB-*TYRP1* in mice – melanoma (Gilot, Migault *et al*) — 2017

Inhibition of NMD in haemophilia mice (Lian et al)
Increase protein expression (Liang *et al*) — 2018

2018 — **Inotersen** approved for hATTR (SC)

Genetic testing – ASO design – in vitro testing in cultured patient derived cells (Kim *et al*) — 2019

2019 — **Milasen** - first patient customized ASO for CLN7 (ITh)

Quemener AM, Bachelot L, Forestier A, Donnou-Fournet E, Gilot D, Galibert MD. The powerful world of antisense oligonucleotides: From bench to bedside. *Wiley Interdiscip Rev RNA*. 2020;11:e1594.
Slide courtesy of Professor Ian Blair

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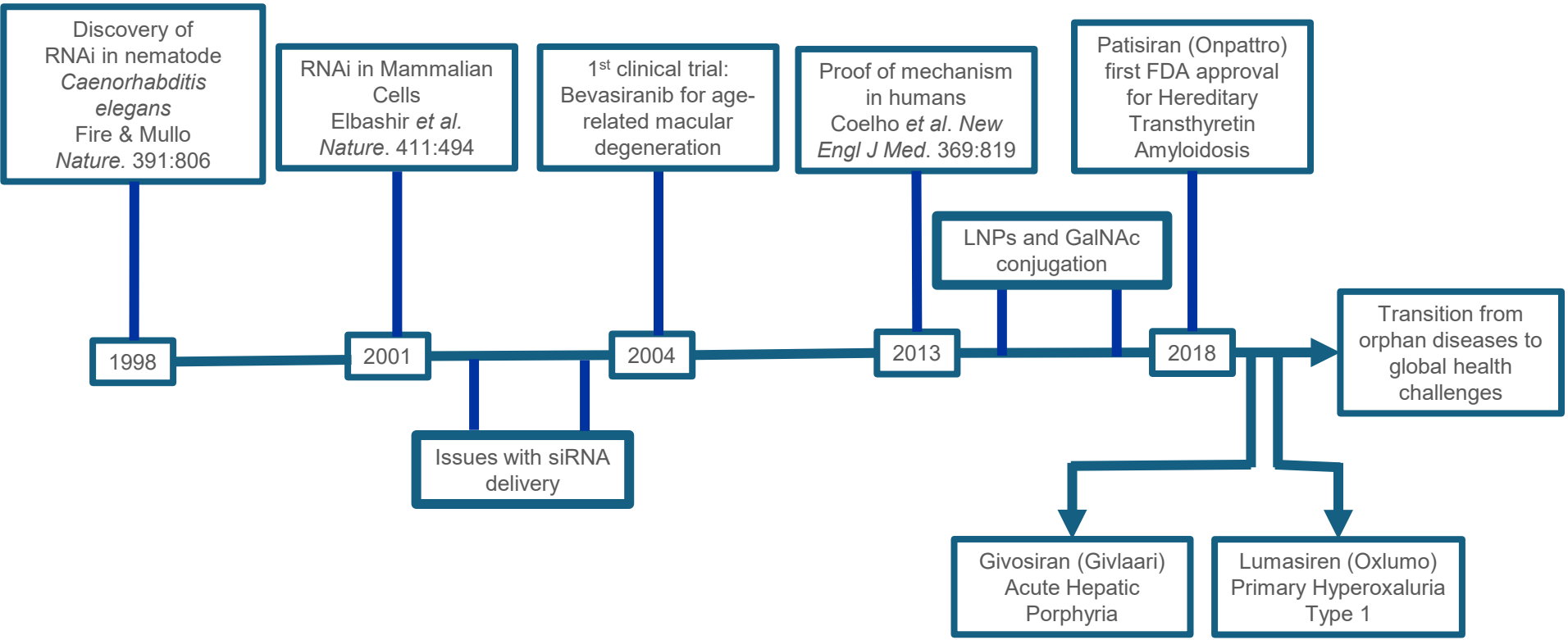
Timeline for the evolution of siRNA drugs



Slide courtesy of Professor Ian Blair

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DDI and Oligonucleotides Therapeutics

FDA GUIDANCE FOR INDUSTRY JUNE 2024 CLINICAL PHARMACOLOGY

- Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics
- Section II D Considerations for Assessing Drug-Drug Interactions
  - (1) Pharmacokinetic Interactions with Cytochrome P450 Enzymes and Transporters
    - *(a) Oligonucleotide therapeutics as substrates for cytochrome P450 enzymes and transporters*
      - Oligonucleotide therapeutics are not typically metabolized by cytochrome P450 (CYP) enzymes. These drugs are primarily metabolized by endonucleases and exonucleases or are chemically modified to resist degradation. Therefore, the disposition of oligonucleotide therapeutics is not anticipated to be affected by inhibitors or inducers of CYP enzymes. Additionally, modulation of efflux transporters such as P-gp and BCRP, hepatic uptake transporters such as OATP1B1 and OATP1B3, or renal uptake or efflux transporters such as OAT1, OAT3, OCT2, MATE1, and MATE2/K are generally not anticipated to have a significant impact on the pharmacokinetics of oligonucleotide therapeutics.

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DDI and Oligonucleotides Therapeutics

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- Section II D Considerations for Assessing Drug-Drug Interactions
  - (1) Pharmacokinetic Interactions with Cytochrome P450 Enzymes and Transporters
  - (b) Oligonucleotide therapeutics as modulators of CYP enzymes and transporter
    - *Evaluating the drug-drug interaction liability of oligonucleotide therapeutics as inhibitors and inducers of CYP enzymes or drug transporters usually begins with in vitro assessments. Refer to the FDA guidance entitled In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions (January 2020) for general considerations when conducting in vitro experiments and interpreting data. Because differences among the various in vitro systems have been reported, sponsors should carefully select the appropriate in vitro systems to evaluate drug-drug interactions. Based on current experience, oligonucleotide therapeutics either do not modulate or minimally modulate the major CYP enzymes and drug transporters. However, an overall recommendation for specific types of oligonucleotide therapeutics (e.g., based on chemistry or delivery strategies) cannot be provided at this time. The sponsor should provide justification if in vitro assessments of the potential of oligonucleotide therapeutics to affect CYP enzymes or transporters are **not** conducted.*
    - *Other possible mechanisms for interactions between oligonucleotide therapeutics and CYP enzymes or transporters or their modulators (e.g., by interfering with the synthesis or degradation of heme or cytokines) should be considered based on the pharmacology of the oligonucleotide therapeutic.*
    - *If studies indicate that the oligonucleotide therapeutic could modulate CYP enzymes or transporters, the sponsor should consider clinical studies to evaluate in vivo drug interactions. For general considerations on study design and conduct, refer to the FDA guidance entitled Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry (January 2020).*

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DDI and Oligonucleotides Therapeutics

FDA GUIDANCE FOR INDUSTRY JUNE 2024 CLINICAL PHARMACOLOGY

- Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics
- Section II D Considerations for Assessing Drug-Drug Interactions
  - (2) Pharmacodynamic Interactions
    - *Oligonucleotide therapeutics can exhibit pharmacodynamic interactions with a concomitant drug when the pharmacological effect of one drug is altered by that of another drug (e.g., drugs with shared mechanism of action pathways). Because such interactions may be unique to individual therapeutics, the sponsor is encouraged to consult with the relevant review Division regarding assessment of pharmacodynamic drug interactions.*

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ONT IND-enabling Tox and ADME Studies

WHAT ADME STUDIES ARE NEEDED?

- Generally, 2 test species
  - 1 Rodent (mice, rat)
  - 1 Non-rodent (non-human primate, mini-pig)
  - 1 Developmental & Reproductive (rat, rabbit)
- ADME
  - Absorption
    - *Rat Single or Multiple Dose*
    - *NHP Single or Multiple Dose*
  - Distribution
    - ~~*Protein Binding (in vitro and ex vivo)*~~
    - **Tissue Distribution (non + radioactive ^{3}H, ^{14}C, ^{35}S)*
  - ~~Metabolism~~
    - ~~*Metabolic Stability*~~
    - ~~*Metabolite Profiling / Identification (in vitro)*~~
    - ~~*Reaction Phenotyping*~~
    - ~~*Drug-Drug Interactions*~~
    - ~~*Drug Transporters*~~
  - Elimination
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DDI and Oligonucleotides Therapeutics

FDA GUIDANCE FOR INDUSTRY JUNE 2024 CLINICAL PHARMACOLOGY

- Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics
- Section I Introduction
  - …
  - Oligonucleotide therapeutics include a wide variety of synthetically modified RNA or RNA/DNA hybrids that are specifically designed to bind to a target RNA sequence to alter RNA expression and/or downstream protein expression. Even within the therapeutic modality, oligonucleotide therapeutics can differ in several ways, including but not limited to:
    - *Mechanism of action (e.g., splice modulating, RNA interference, RNase H-mediated cleavage)*
    - *Structure (e.g., single-stranded RNAs, double-stranded RNAs, RNA/DNA hybrids) [DNA enzymes]*
    - *Chemical modifications to the base and/or backbone*
    - *Size*
    - *Sequence*
    - *Delivery strategy (e.g., lipid nanoparticles, liposomes, other polymeric nanoparticles, polyethylene glycol (PEG), N-acetylgalactosamine (GalNAc) conjugation) [C16 conjugation, C22 conjugation, linker, …]*
    - *Conjugation with other moieties (e.g., small molecules, proteins, antibodies) [peptides, aptamer]*

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DDI and Oligonucleotides Therapeutics

WHAT ADME STUDIES ARE REALLY NEEDED?

- Absorption
  - Rodent Single or Multiple Dose
  - Non rodent Single or Multiple Dose
- Distribution
  - Protein Binding (*in vitro* and *ex vivo*)
  - *Tissue Distribution (non and/or radioactive)
- Metabolism
  - Metabolic Stability
  - Metabolite Profiling / Identification (A to I conversion by ADARs)
  - Reaction Phenotyping
  - Drug-Drug Interactions
  - Update and efflux transporters

  } Linkers (most common – L96), conjugates, LNP components …
- Elimination
  - Mass Balance / Excretion
  - Metabolite Profiling / Identification

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Twenty-three US FDA / EMA approvals • 9/23 approvals • 9/17 approvals in the past 8 years

OLIGONUCLEOTIDE THERAPEUTICS APPROVALS & QPS MILESTONES

10/30/2025

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QPS

2003
Oligonucleotides PK *via* LC-MS/MS

2006
CLIA Assay - Inclusion/ Exclusion Criteria
Biomarkers - Primary End-point

2007
Oligonucleotides PK *via* Hybridization-ELISA

2009
Oligonucleotides PK *via* Hybridization-LC-FLD
Oligonucleotides PK *via* LC-UV

2010
LNP *in vitro* Protein Binding
LNP *in vitro* Metabolic Stability
LNP Reaction Phenotyping
LNP Metabolite Identification
LNP *in vitro* CYP Inhibition

2010
LNP *in vitro* CYP Induction
Oligonucleotides Biodistribution *via* Autoradiography
Oligonucleotides *in vitro* Metabolic Stability
Oligonucleotides Reaction Phenotyping
Oligonucleotides Metabolite Identification
Oligonucleotides *in vitro* CYP Inhibition

2011
Oligonucleotides Biodistribution *via* QWBA
Oligonucleotides Mass Balance/Excretion

2012
Oligonucleotides Biodistribution *via* Microautoradiography

2013
Oligonucleotides (ASO, siRNA, miRNA) ADA
Oligonucleotides *in vitro* CYP Induction

2014
Oligonucleotides PK *via* LC-HRMS
Oligonucleotides *in vitro* Protein Binding

2015
Oligonucleotides *in vitro* Reaction Phenotyping

2016
Oligonucleotides *in vitro* Drug Transport

2021
AOC/POC PK *via* Immunocapture LC-MS/MS
AOC/POC PK *via* LBA
AOC/POC ADA *via* LBA

2025
Oligonucleotides PK *via* Hybridization-LC-MS/MS
Downstream Protein Quantitation *via* Immunocapture LC-MS/MS
Immunophenotyping *via* Flow Cytometry
Cellular Immunogenicity *via* Flow Cytometry
Cellular Immunogenicity *via* ELISpot

1998
ASO
Vitravene™
(fomivirsen)

2004
Aptamer
Macugen®
(pegaptanib)

2013
ASO
Kynamro®
(mipomersen)

2016
Polydisperse Natural Oligonucleotides
Defitelio®
(defibrotide)

ASO
Spinraza®
(nusinersen)

ASO
Exondys 51®
(eteplirsen)

2018
siRNA
Onpattro®
(patisiran)

ASO
Tegsedi®
(inotersen)

2019
siRNA
Givlaari®
(givosiran)

ASO
Waylivra®
(volanesorsen)

ASO
Vyondys 53™
(golodirsen)

2020
ASO
Viltepso®
(viltolarsen)

siRNA
Oxlumo®
(lumasiran)

2021
ASO
Amondys 45™
(casimersen)

siRNA
Leqvio®
(inclisiran)

2022
siRNA
Amvuttra®
(vutrisiran)

2023
ASO
Qalsody™
(tofersen)

Aptamer
Izervay™
(avacincaptad pegol)

siRNA
Rivfloza™
(nedosiran)

ASO
Wainua™
(eplontersen)

2024
ASO
Tryngolza™
(olezarsen)

2025
siRNA
Qfitlia™
(fitusiran)

ASO
Dawnzera™
(donidalorsen)

KEY

BIOANALYSIS ●
ADME ●
OLIGONUCLEOTIDE THERAPEUTICS APPROVALS ●

DDI and Oligonucleotides Therapeutics

WHAT ADME STUDIES ARE REALLY NEEDED?

- QPS conducted an internal ADME characterization of 50+ oligonucleotide therapeutics with functionalities covering different:
  - **Mechanisms**: splice modulating, RNA interference, RNase H-mediated cleavage
  - **Structures**: aptamers, single stranded RNAs, double strands RNAs, but no DNA
  - **Modifications**: Gen 1 (phosphorothioate), Gen 2 (2'-O-alkyl), and Gen 3 (LNA, PNA, PMO, …)
  - **Sizes**: 15-mer to 50-mer
  - **Delivery systems**: LNP, PEG, conjugation
  - **Conjugations**: galNAc, lipids, peptides, proteins, mAb
- Methodologies followed the FDA Guidance documents
  - 2012: Draft guidance on *in vitro* and clinical studies, covering both Cytochrome P450 (CYPs) and transporters
  - 2017: Final guidance published regarding clinical pharmacology labeling for DDI
  - 2020 Finalized:
    - *In Vitro Metabolism- and Transporter-Mediated Drug-Drug Interaction Studies*
    - *Clinical Drug Interaction Studies—Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions*
  - 2024: Adopted the ICH M12 Guidance on Drug Interaction Studies

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DDI and Oligonucleotides Therapeutics

HISTORICAL DATA

- Applied Assay types
  - Plasma, serum, liver S9 fractions
  - Liver microsomes, Tritosomes, cryopreserved human hepatocytes
  - Suspension, plated, long-term co-culture
  - cDNA-expressed enzymes
  - Polarized cell monolayers (ABC efflux transporters), and HEK-transfected cells (SLC transporters)
- Assay types “pros and cons” needs to be considered at assay or method selection

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DDI and Oligonucleotides Therapeutics

WHAT WE OBSERVED

- CYP450 Reaction Phenotyping
  - No ONT tested shown as substrates
- CYP450 inhibition
  - ~16% of ONT tested showed inhibitory effects on at least one CYP450 isoform
  - No ONT tested showed time dependent inhibition
- CYP450 induction
  - ~35% of ONT tested induce at least one CYP450 isoforms
- Drug Transport
  - No ONT tested shown as efflux or SLC substrates
  - No ONT tested shown inhibitory effect on efflux transporters
  - ~33% of ONT tested showed inhibitory effects on uptake on at least one SLC transporters
- Plasma Protein Binding
  - *~0.2 % to ~20% unbound in human plasma*
- *In vitro* Metabolic Stability
  - Driven by ss or ds, combination of Gen 1 and 2 chemistry, conjugation
- Metabolite Profiling and Identification
  - AS and SS have different stability
  - A to I conversion if located at the end
  - PS stable, non-PS and non-2'-O-alkyl not stable
  - GalNAc conjugates – progressive/sequential loss of triantennary GalNAc
  - Lipid conjugates …

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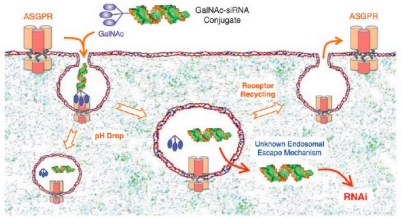
DDI and Oligonucleotides Therapeutics

OTHER CONSIDERATION?

- PPB is not consistent across the board
  - Concentration dependency?
- [Liver] >> [Plasma]
- Metabolic Stability
  - Plasma $T_{½}$ is >4h, effective *in vitro* systems?
    - *Microsomes, S9 fractions, serum enzymes not 'fully active' after 1.5, 4, & 48 hours, respectively*
    - *Only rat tritosomes are available*
    - *Limited 'co-culture' models*
- Metabolite Profiling and Identification
  - Phosphorothioate creates chiral center
    - *e.g. AS of inclisiran has*
      - 4 chiral centers and A to I conversion
      - Potential 16 (2^4) chiral structures each with 0 to 3 Da difference from 'parent' AS

- CYP450 inhibition
  - Small molecules T/DI protocol well established
  - ONT are not small molecules
    - *via endocytosis and 'the endosomal trap'*



Springer AD, Dowdy SF. GalNAc-siRNA Conjugates: Leading the Way for Delivery of RNAi Therapeutics. *Nucleic Acid Ther.* 2018;28:109.

    - *Hepatocytes vs. Microsomes*
      - Enzyme kinetic needed for every ONT drug candidates or just per 'platform'?
        - *Specific conjugates to specific liver receptor?*
      - Metabolism is slow and low
        - *Pre-incubation for <4 hours may not be relevant*
    - *Relevant test concentration*
      - Clinical relevant concentration or >>
      - Well stirred model may not be appropriate

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    - *Reaction Phenotyping*
    - *Drug-Drug Interactions*
    - *Drug Transporters*
  - Elimination
    - *Mass Balance / Excretion*
    - *Metabolite Profiling / Identification*
- Toxicology and Safety Studies
  - Genetic Toxicology
    - ~~*In vitro bacterial mutation test (Ames)*~~
    - ~~*In vitro mammalian chromosome aberration test*~~
    - *In vivo rat micronucleus and TK*
  - General Toxicity and TK Studies
    - *Rat 14-day DRF*
    - *Rat 28-day repeat dose with 28-day recovery (GLP)*
    - *NHP 14-day DRF*
    - *NHP 28-day repeat dose with 28-day recovery (GLP)*
  - Safety Pharmacology Studies
    - ~~*In vitro hERG*~~
    - ~~*Rat Neuropharmacological Profile*~~
    - ~~*Rat Respiratory Safety Pharmacology*~~
    - *NHP Cardiovascular & Toxicokinetic Study*
- Bioanalytics
  - Exposure: Plasma, Excreta (urine, feces), target organs (liver, kidney, …)
  - Immunogenicity and neutralizing antibodies

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