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# PBPK-PD for siRNAs

An OSP implementation for Drug Disposition and Efficacy analyses

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# Acknowledgements


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- Emilie Langeskov Salim

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**Whole-Body Physiologically Based Pharmacokinetic Modeling of GalNAc-Conjugated siRNAs**


Emilie Langeskov Salim [1,2], Kim Kristensen [2] and Erik Sjögren [1,*]



https://doi.org/10.3390/pharmaceutics17010069


**Whole-Body Physiologically Based Pharmacokinetic–Pharmacodynamic Modeling for Interspecies Translation and Mechanistic Characterization of Plasma and Tissue Disposition of GalNAc-siRNAs**


Emilie Langeskov Salim [1,2], Kim Kristensen [2], Girish Chopda [3] and Erik Sjögren [1,*]



https://doi.org/10.3390/pharmaceutics17091154


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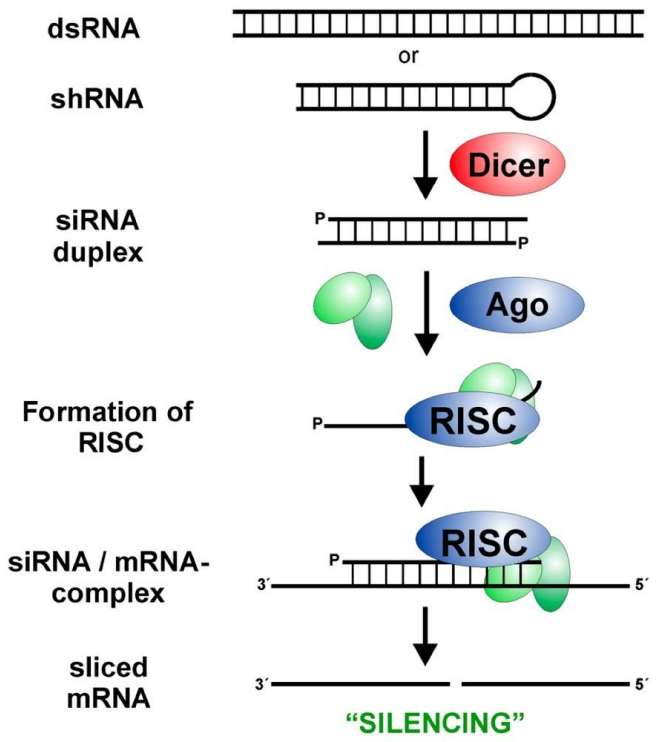
# Introduction to RNA Interference

- RNA interference (RNAi) is a natural defense mechanism
- Small interfering RNA (siRNA) is a new drug modality consisting of sense and antisense strands
- The antisense strand loads into Argonaute 2 (Ago2) proteins, forming the RNA-Induced Silencing Complex (RISC)
- RISC use the antisense strand as guide to its complementary mRNA, leading to mRNA cleavage and reduced protein translation
- The target specificity of the siRNAs make them attractive as drugs

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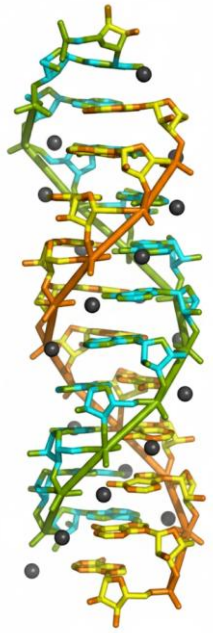
# Challenging properties limits potential for siRNAs to reach target

- Low potential for passive cell membrane translocation
  - Semi-large molecules (13-22 kDa)
  - Negatively charged
- Eliminated via endogenous nucleases
  - Systemic instability
  - Intracellular instability
  - First pass
- Renally cleared



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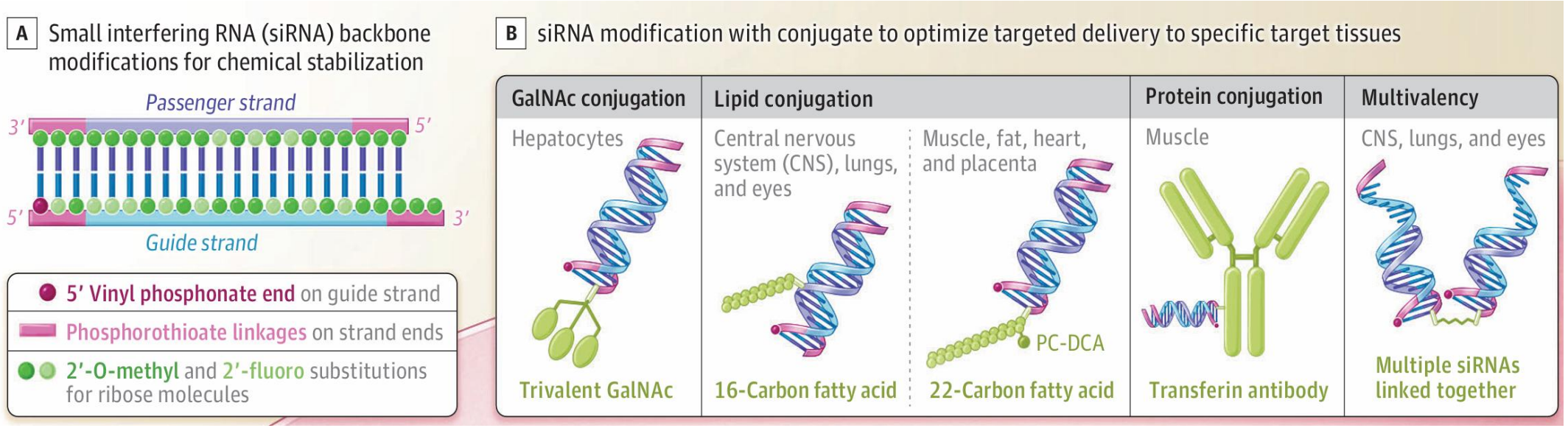
# Strategies to enhance siRNA delivery

- Increase stability [A]
- Increase cell translocation and tissue targeting [B]




Khvorova A JAMA. 2023 doi: 10.1001/jama.2023.4570

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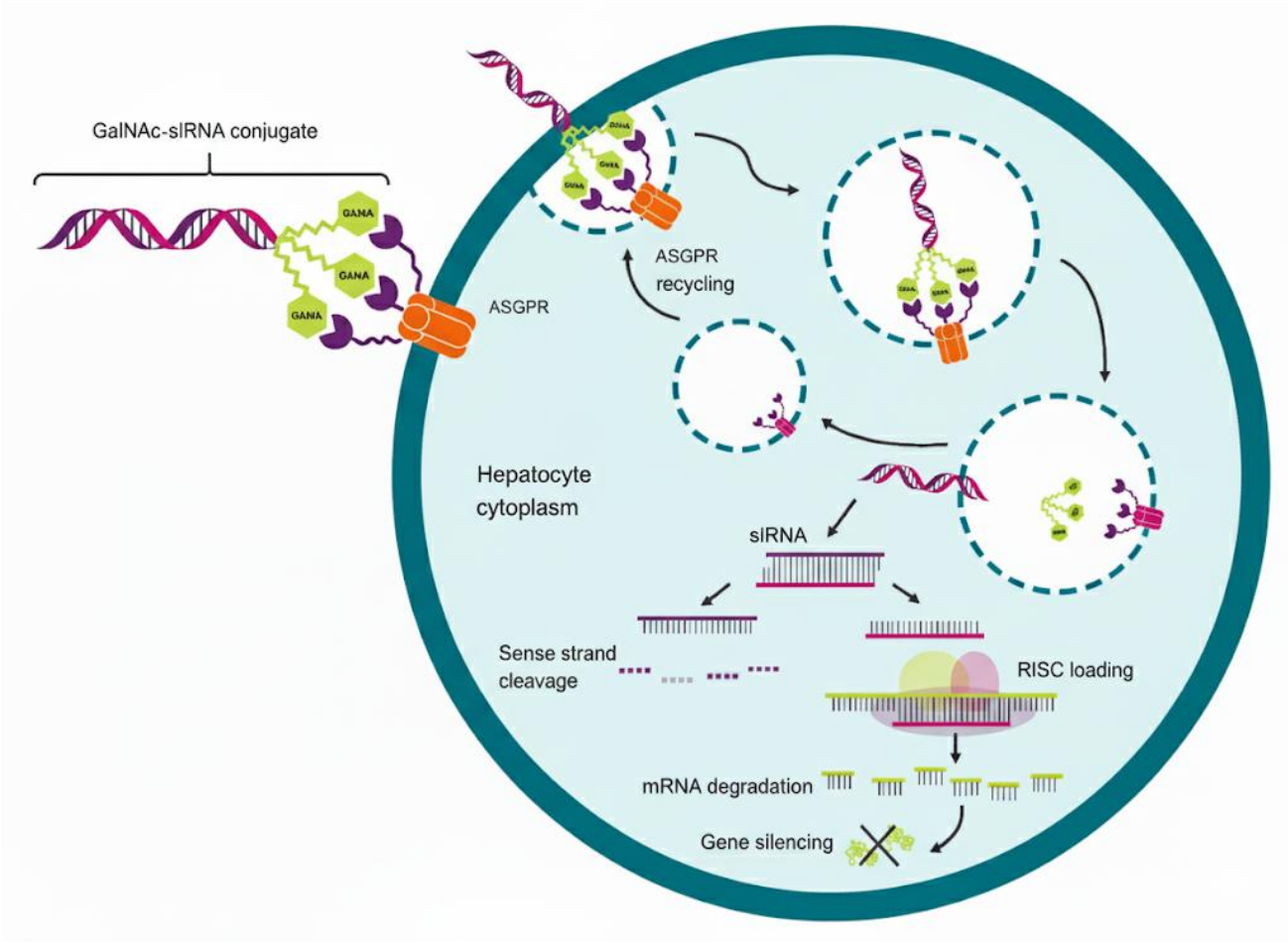
# GalNAc-siRNA for Liver Targeting via ASGPR




GalNAc-siRNA: siRNA conjugated with multivalent tris N-acetylgalactosamine

ASGPR: Asialoglycoprotein Receptor abundantly expressed by hepatocytes

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# Why do PBPK-PD modeling for GalNAc-siRNAs?

- siRNAs show transient plasma exposure and long half-life in target tissue
  - Short plasma circulation is a poor surrogate for concentrations at the target biophase
  - Traditional PK-PD and dose-response relationships are not readily applicable
- PBPK well-suited for mechanism-based translations and extrapolations
- Investigations of causality and dependency
- Enables continuous integration of knowledge supporting increased general understanding of this drug class
- Standardized structure and generic parameterization can enable supplementary model applications

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# WB-PBPK-PD Modeling: General Approach

- Models developed using the Open Systems Pharmacology Suite leveraging standard implementation for large molecules
- GalNAc-siRNA – ASGPR liver dynamics inspired by the minimal-PBPK-PD model presented by Ayyar et al. 2021
- Iterative "middle-out approach“ vs reference data
  - Commercial drugs with different stability design
  - Internal Novo Nordisk drugs
  - Data from Mouse, Monkey and Human
    - Plasma, Tissues, RISC, mRNA and Target protein

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# Reference Data overview

| Compound | Design | Species | Administration/Dose | Measurement |
|---|---|---|---|---|
| ALN-AT3 | ESC | Mouse | 1–5 mg/kg | Plasma, liver, liver mRNA,Target proteint |
| siAT-2 | Assumed ESC | Mouse | 2.5–25 mg/kg | Plasma, liver, liver mRNA, RISC |
| siF7-1 | ESC | Mouse | 2.5 mg/kg | Liver, liver mRNA, RISC |
| siF7-2/siF7-3 | Advanced ESC | Mouse | 0.75, 1 mg/kg | Liver, liver mRNA, RISC |
| siF9-1 | ESC | Mouse | 2.5 mg/kg | Liver, liver mRNA, RISC |
| siF9-2 | Advanced ESC | Mouse | 0.75 mg/kg | Liver, liver mRNA, RISC |
| siTTR-1/siTTR-2 | ESC | Mouse | 0.5, 1.5, 10 mg/kg* | Plasma, liver, liver mRNA**,Target protein |
| siRNA-1 | Hairpin Loop | Mouse | 3, 10, 100 mg/kg | Plasma/Liver/Kidney/Gonads/Lung/Spleen/mRNA |
| | | Monkey | 3 mg/kg | Plasma/Liver/mRNA |
| | | Human | 1, 3.5, 6.5, 13 mg/kg | Plasma |
| siRNA-2 | Hairpin Loop | Mouse | 3, 100, 300 mg/kg | Plasma/Liver/Kidney/Gonads/Lung/Spleen |
| | | Monkey | 1 mg/kg | Plasma/Liver/mRNA |
| | | Human | 0.1, 1, 3, 6, 12 mg/kg | Plasma/Target protein |
| siRNA-3 | Hairpin Loop | Mouse | 3, 100 mg/kg | Plasma/Liver/Kidney |
| | | Monkey | 3 mg/kg | Plasma/Liver/mRNA |
| | | Human | 1.5, 3, 6 mg/kg | Plasma/Target protein |
| Olpasiran© | ECS | Monkey | 10 mg/kg | Plasma/Target protein |
| | | Human | 3, 9, 30, 75, 225 mg | Plasma/Target protein |

ECS: Enhanced stabilization chemistry, * SC and IV, ** for SC dose

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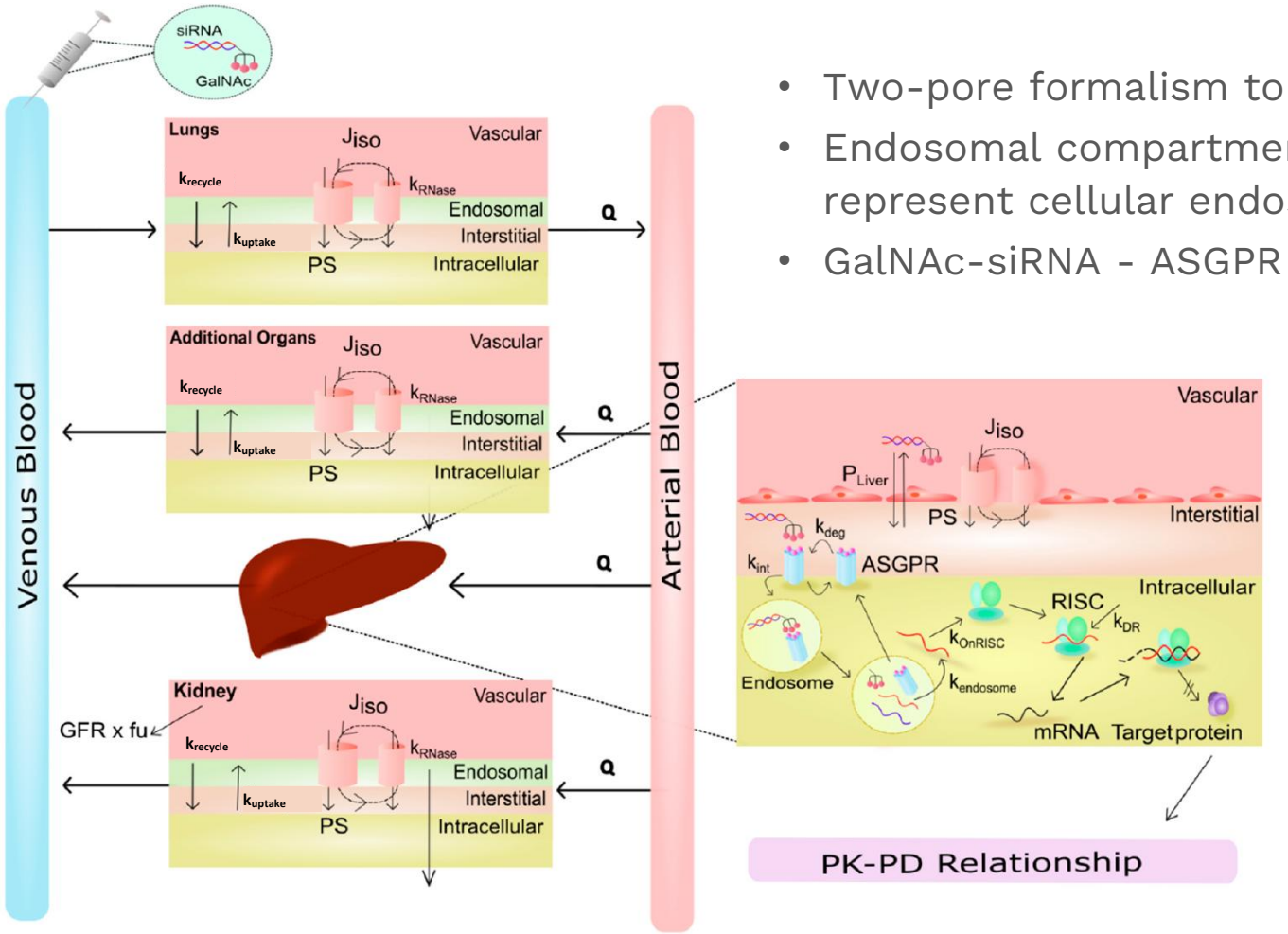
# GalNac-siRNA WB-PBPK model structure




- Two-pore formalism to describe general extravasation
- Endosomal compartment implementation re-purposed to represent cellular endosomes
- GalNAc-siRNA - ASGPR shuttling according to Ayyar et al. 2021

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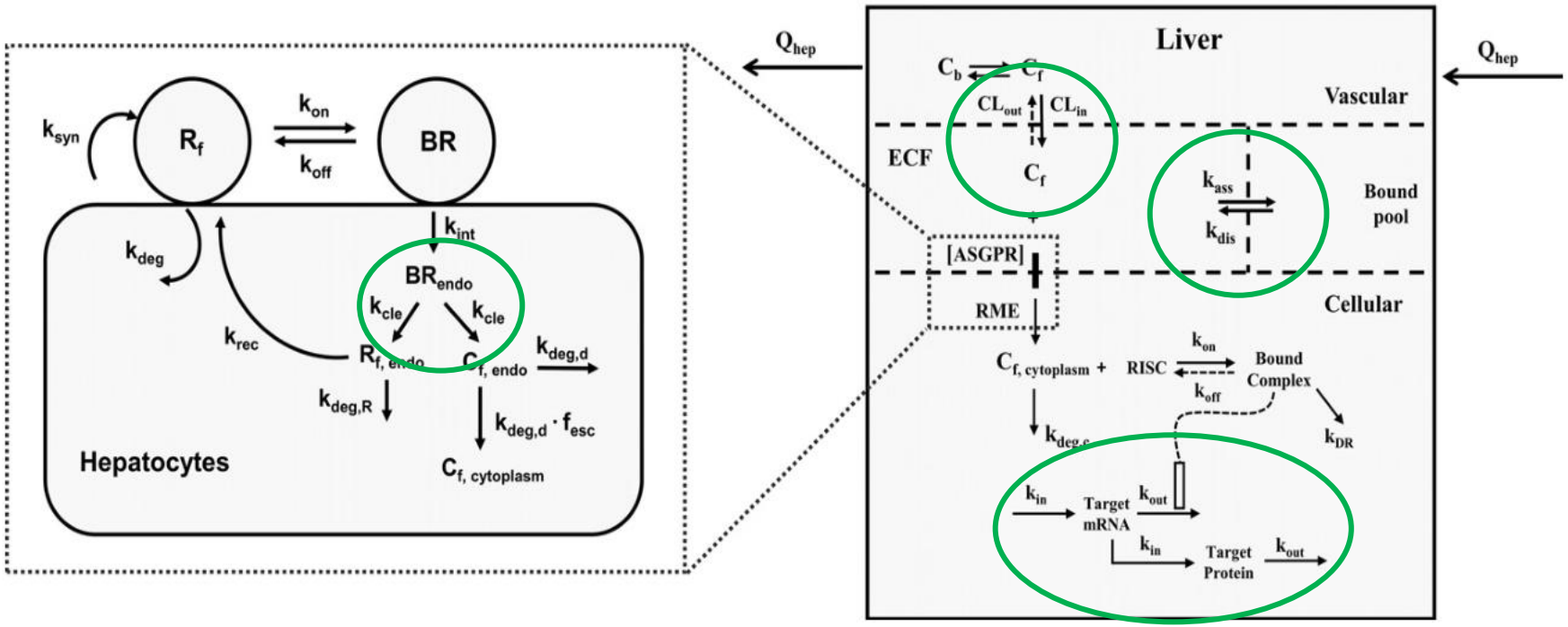
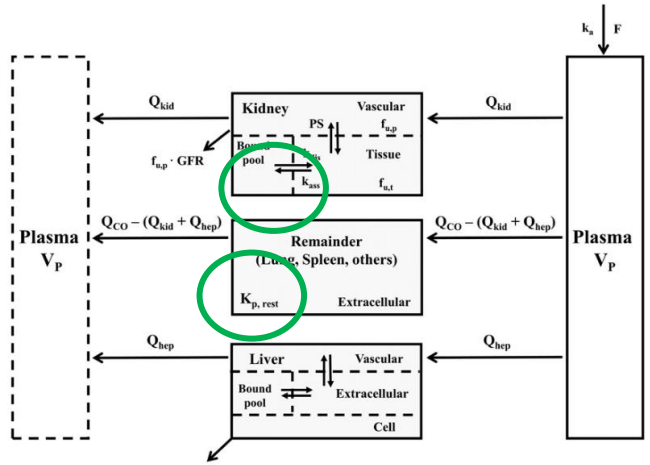
# Briefly on the Ayyar model




Ayyar et al. 2021 doi: 10.1124/jpet.121.000805

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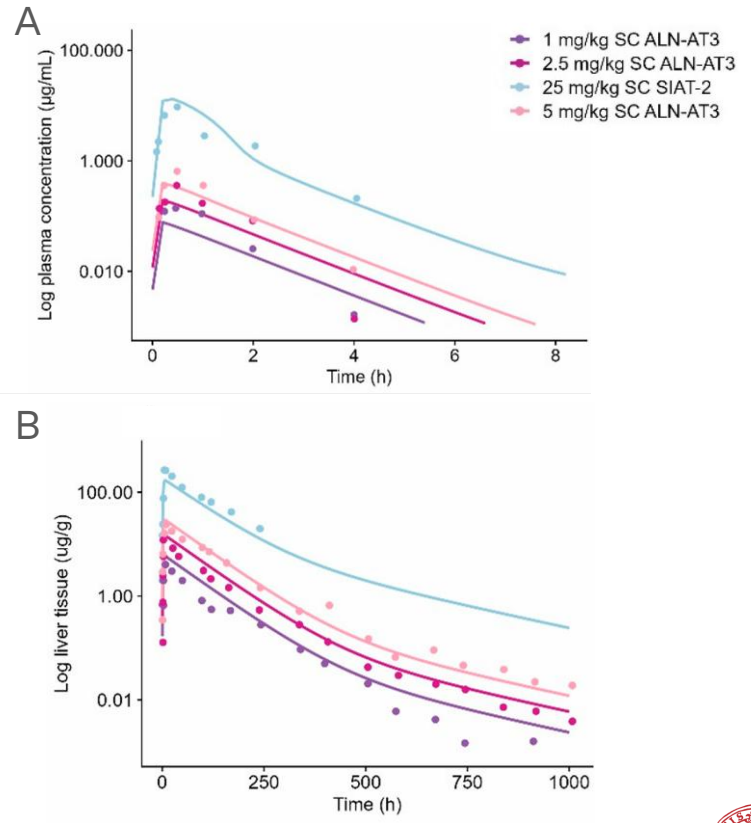
# Modeling Extravasation: A Key Challenge

- The generic two-pore formalism too restrictive for liver extravasation, limiting ASGPR-mediated liver uptake, and failing to capture the fast onset of liver concentrations
- Passive permeability for the liver was introduced to characterize liver distribution
- Better mechanistic understanding of GalNAc-siRNA extravasation warranted




Model-simulated vs. observed **A)** plasma and **B)** liver concentration data in mouse for ALN-AT3/SIAT-2

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# Modeling ASGPR & Endosomal Dynamics

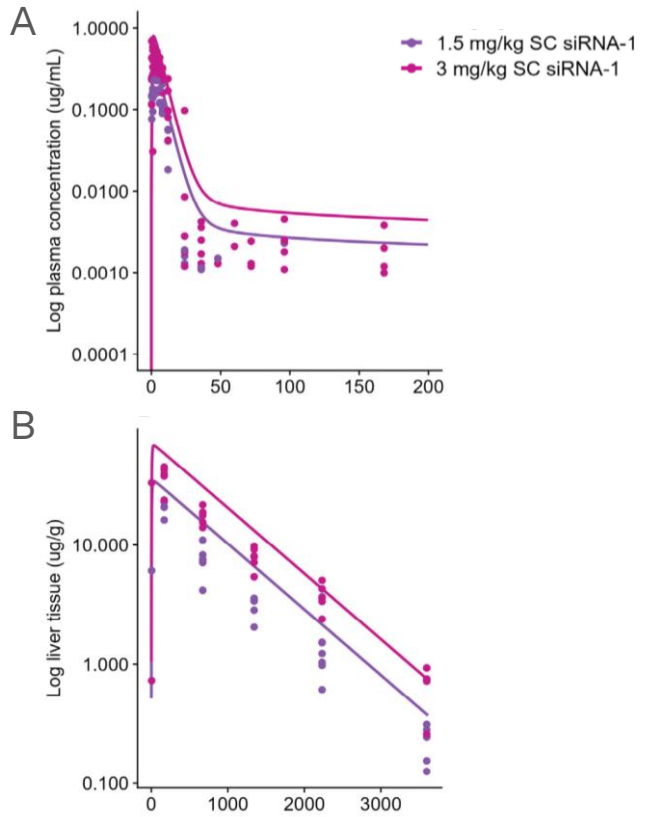
## Liver:

- ASGPR dynamics and GalNAc-siRNA shuttling
  - Structure from Ayyar applied
  - GalNAc-siRNA liver disposition
  - Additional high dose reference data
- Non-specific endosomal uptake and recycling

## Other tissues:

- Endosomal uptake and recycling applied to describe tissue distribution/retention.
  - Tissue specific parameters when data available

Data driven process including iterative parameters optimization to accurately describe ASGPR-mediated non-linear uptake and tissue exposures




Model-simulated vs. observed **A)** plasma and **B)** liver concentration data in monkey for siRNA-1

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# Interspecies Translation: PK and PK-PD

## PK

- The WB-PBPK-PD model was first established in mouse and then scaled to monkey and human
- Some species-specific optimization beyond physiologically based scaling was applied

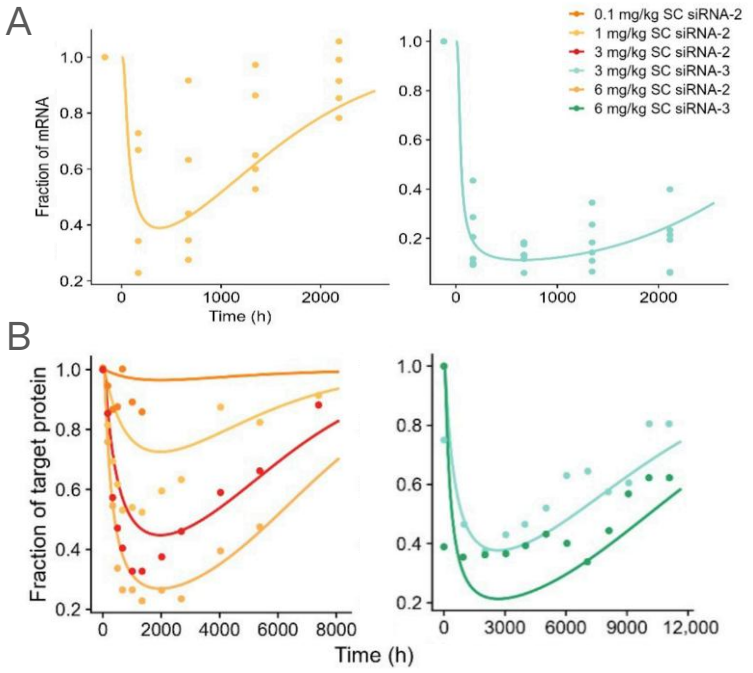
  A general tendency was observed for slower processes in humans compared to mice and monkey

## PK-PD

- Conserving PD effect parameters across compounds and species
- Optimizing RISC parameters across compounds and species

  Significant species-specific differences in RISC dynamics

  Clinical data gaps limits full characterization and comparison




Model-simulated vs. observed data,
**A)** Knockdown of target mRNA in monkey and **B)** downstream effect on target protein in human for siRNA-2, and siRNA-3

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# Conclusions: WB-PBPK-PD Model

- A generic WB-PBPK-PD model for GalNAc-siRNAs established
  - Implemented on basis of the default large molecule model in OSP
  - Adequate description of GalNAc-siRNA PK-PD relationships across diverse compounds and species
  - Distinguished between compound- and species-specific parameters
- A tool for characterization of novel GalNAc-siRNAs in drug development
  - Supporting drug safety and dose assessments
  - Investigations of disposition mechanisms
- Learnings and structure can be leveraged in model activities of similar drug classes

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# Conclusions: Identified knowledge gaps

- Increase mechanistic understanding of extravasation

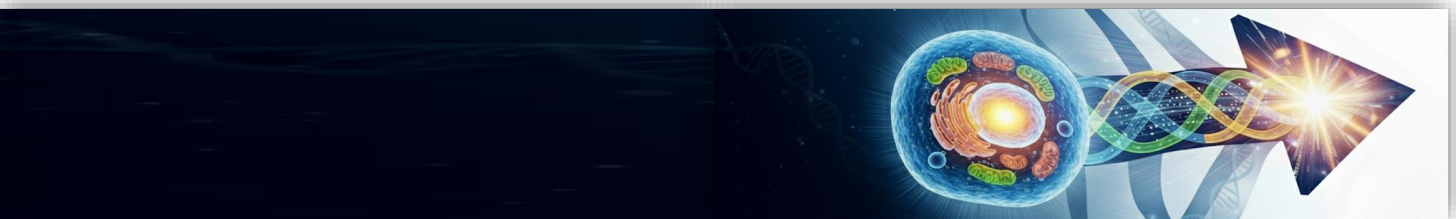
  More detailed insights into the processes for GalNAc-siRNAs vascular-extra cellular space distribution is needed

- Deeper understanding of RISC dynamics

  Significant variations in RISC association and degradation across species and compounds highlight a critical knowledge gap concerning the siRNA-Ago2 interaction

- Information on species-specific ASGPR variations

  Species differences in ASGPR subunits may influence liver distribution predictions, leading to miss-informed translations based on expression level



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# Thank you for your attention!

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