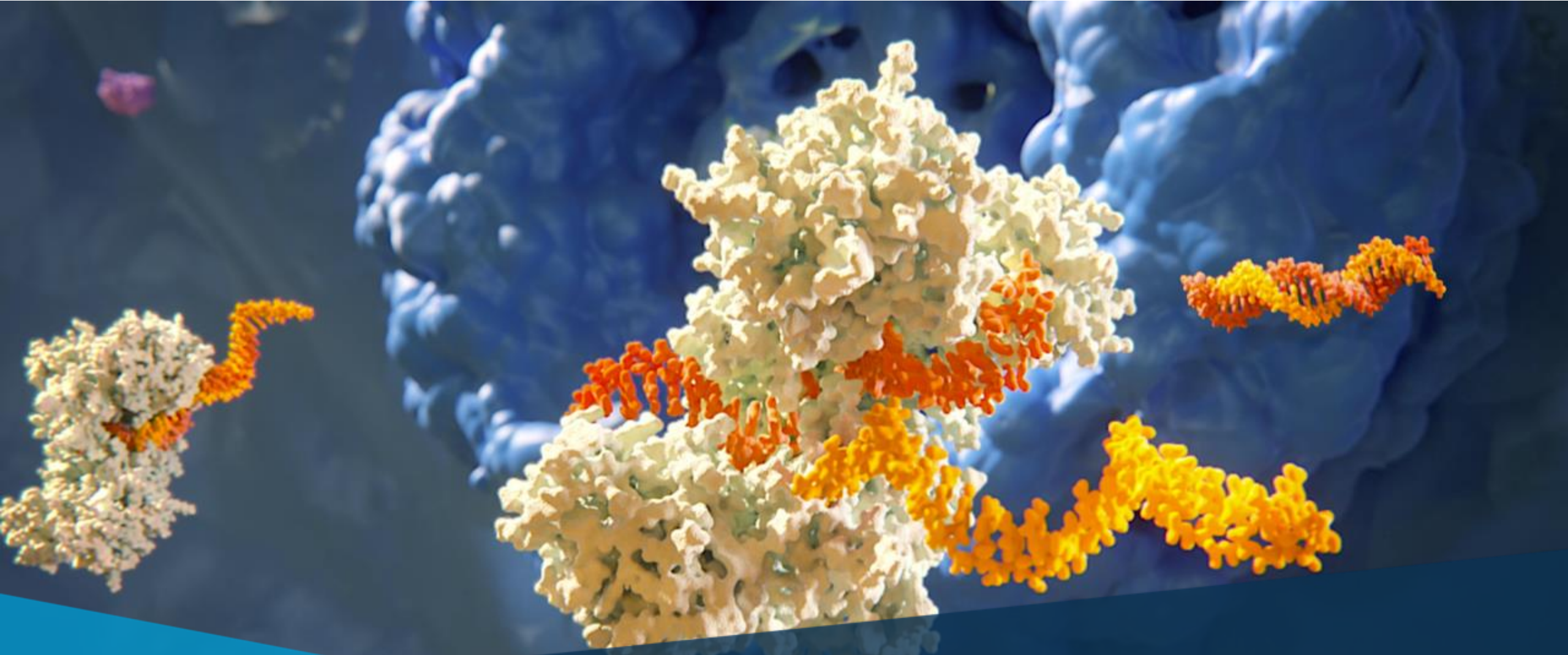


Toxicology and DMPK Assessment of siRNA Therapeutics

Joe Dybowski

October 2, 2022

Alnylam@20

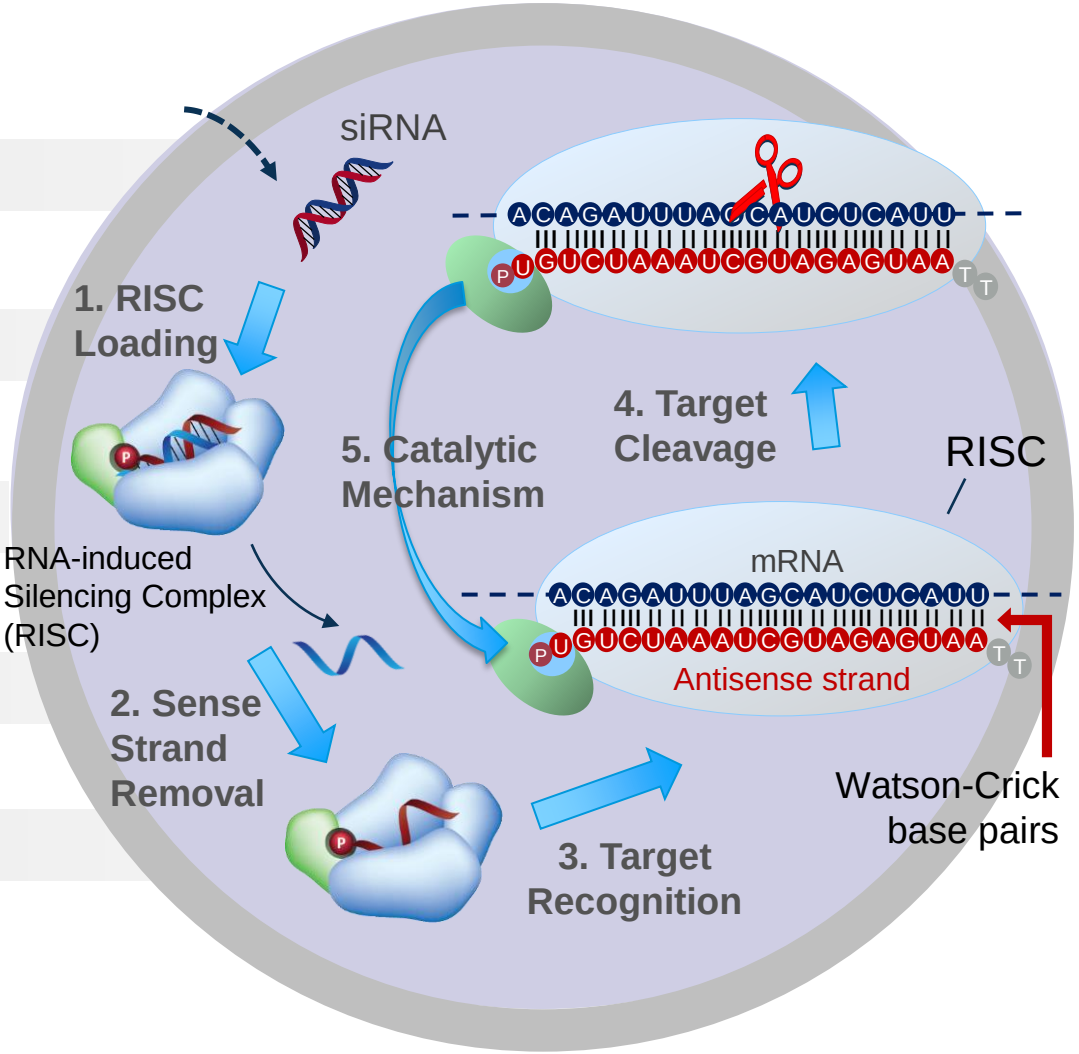
Conflict of Interest

- I am an employee of Alnylam Pharmaceuticals, Inc.

RNAi Pathway

Naturally Occurring Mechanism to Regulate Gene Expression

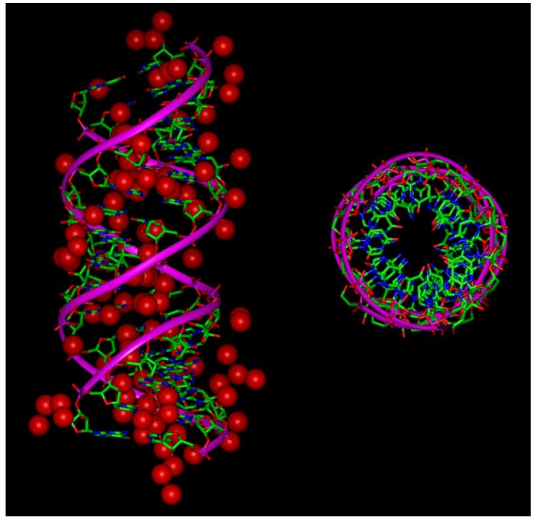
- Harnessing a natural pathway
- Catalytic mechanism
- Silence any gene in genome
- Upstream of today's medicines
- Clinically proven approach



Small Interfering RNAs as Therapeutics

Characteristics

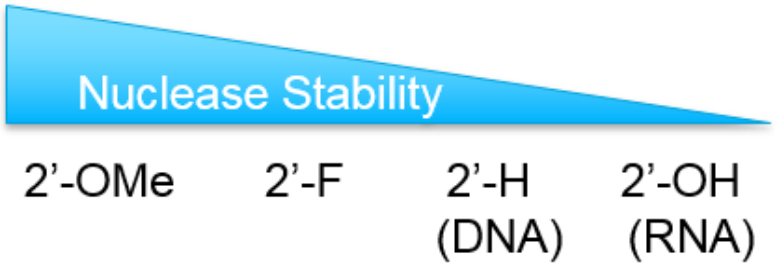
- siRNAs are chemically synthesized, double stranded oligonucleotides
- 19-25 base pairs in length
- Large molecules (~15,000 Da)
- Highly negatively charged
- Hydrophilic



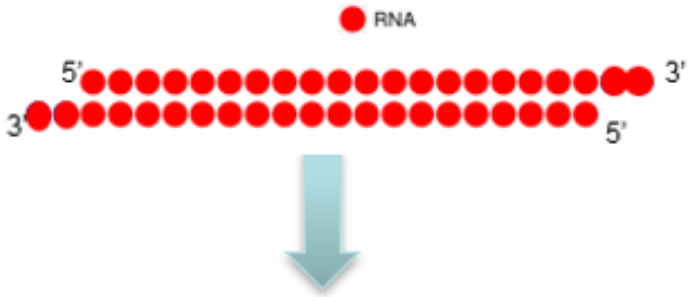
Structure adapted from Klosterman,P. S.; Shah, S. A.; Steitz, T. A. Biochemistry (1999), 38, 14784-14792.

Challenges with natural, unmodified siRNAs

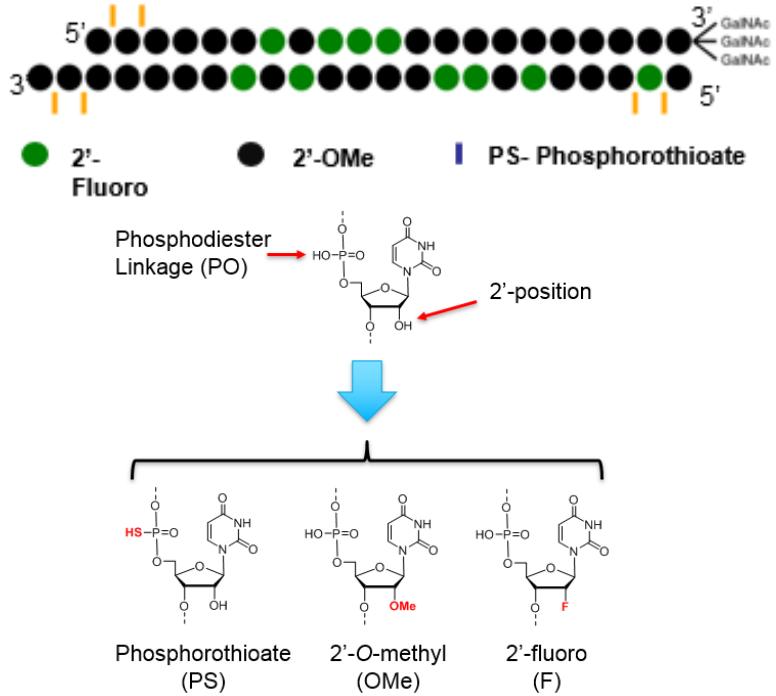
- Unstable in biological matrices
- Tissue delivery challenging due to nuclease susceptibility and lack of passive uptake across cell membranes
- Immunostimulatory



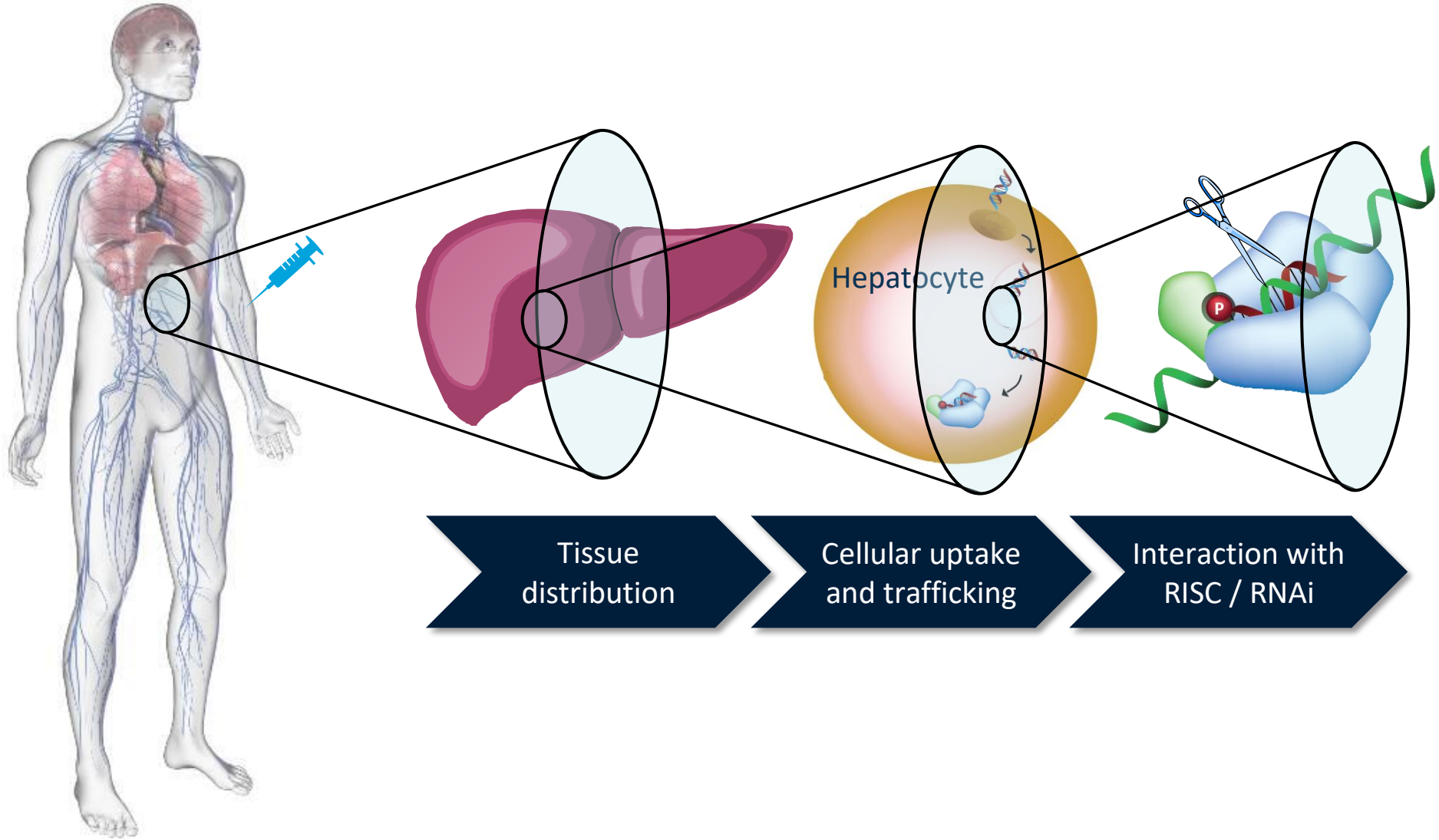
Natural, Unmodified siRNAs Lack Drug-like Properties



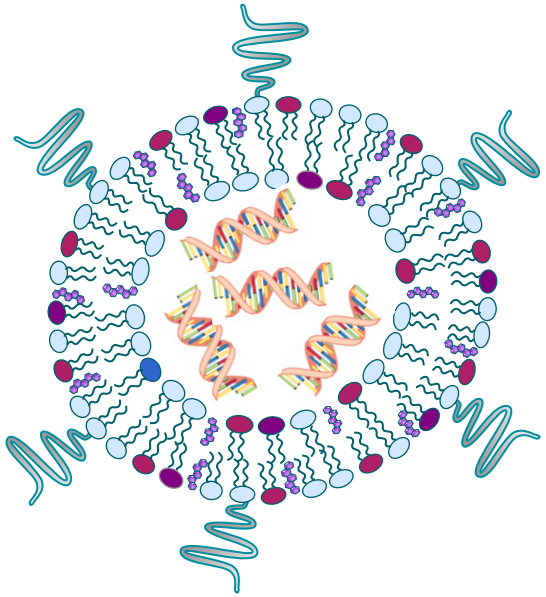
Chemical Modifications Provide Metabolic Stability and Abrogate Immune Recognition

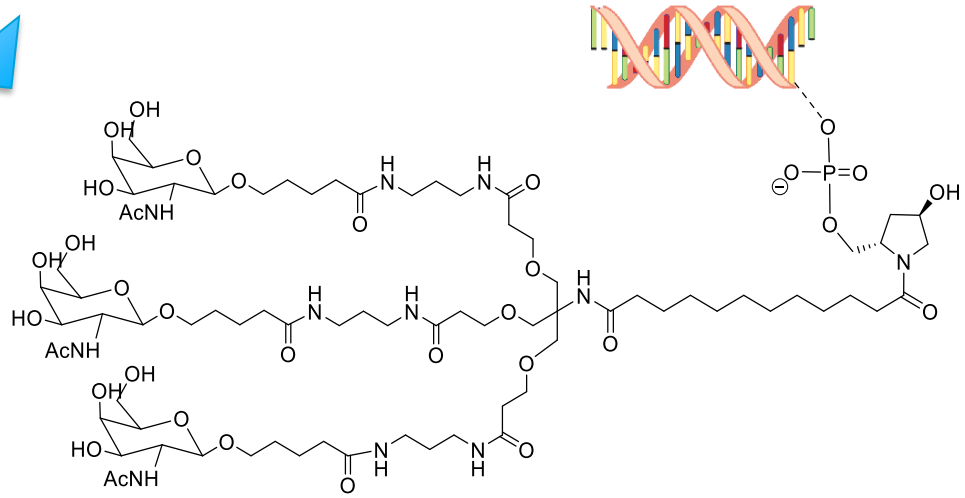


The Challenge: Functional Delivery of RNAi Therapeutics



Platforms for Functional siRNA Delivery to Target Tissue (Liver)





Lipid Nanoparticles (LNPs)

- Multi-component lipid formulation with encapsulated siRNA
- Targeted delivery to liver mediated by endogenous ApoE
- Administered intravenously

N-acetylgalactosamine (GalNAc) Conjugates

- Trivalent GalNAc ligand covalently conjugated to siRNA
- Targeted delivery to liver via cell surface receptor (ASGPR)
- Administered subcutaneously

Regulatory Challenges For Nonclinical Development of RNAi Therapeutics

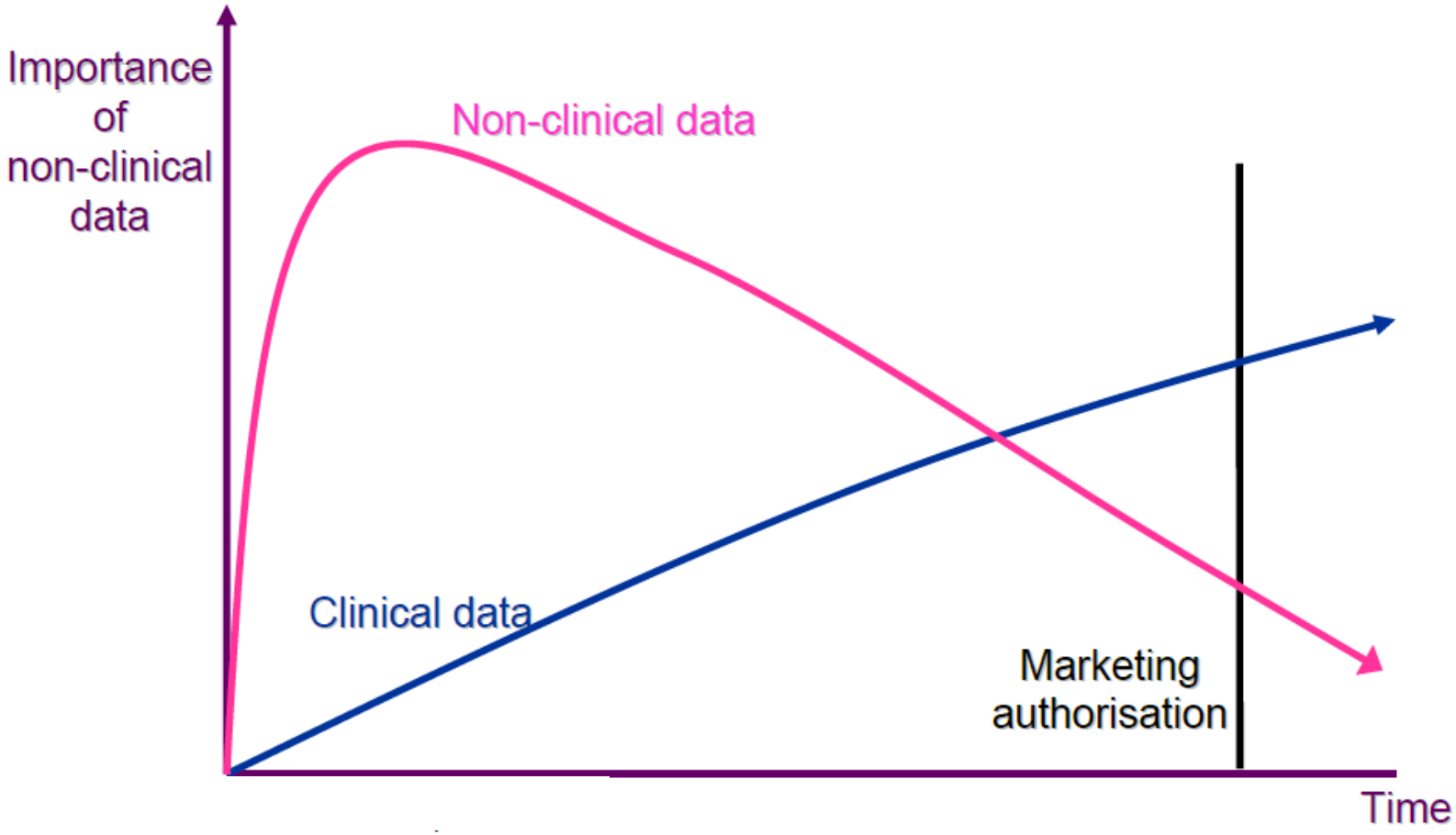
- Not a biologic….but not a traditional small molecule!
- Chemically synthesized so defined as a small molecule from a regulatory perspective
- Separate regulatory guidance not in place for RNAi therapeutic
- Approach consistent with ICH M3(R2) -Guideline on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals
- ICH S guidelines provide specific guidance on study design

Purpose of Nonclinical Safety Program

Ensuring patient safety is the bottom line

- **Every Safety Assessment Program has a Human Strategy. The Clinical Development Plan Essential to Design of Non-Clinical Testing Program**
  - Key considerations
    - Pattern of Use
      - Dose and exposures achieved
      - Chronic, intermittent, once, co-administration?
    - Characteristics of Target Population
      - Elderly, pediatric, gender?
    - Prognosis & Alternatives
      - Seriousness, progressivity, medical need?

Relevance of Nonclinical Studies in Drug Development



- Exceptions - embryo fetal development, peri-, post-natal development and rodent carcinogenicity studies

Figure from Nieto-Gutierrez, M, European Medicines Agency (EMA)

Target Identification for RNAi Therapeutics

- **Genetically Validated Targets**
  - **Improved likelihood of success and confidence in rationale**
    - 2-fold higher probability of success for Phase 1 to Approval vs target with no genetic correlation
  - **Rare genetic indications where silencing a mutated protein or pathway will provide benefit**
    - Broader therapeutic indications considered as well (ex: Hepatitis B)
  - **Loss of function mutations provide insight into safety of target**
    - Extensive knowledge of pharmacology of target (both canonical and non-canonical) essential
- **Target Sequence Selection**
  - **Combination of bioinformatic and in vitro screening to select both specific and potent siRNA**
    - Sequence optimized for humans but also screened for test species homology
    - Specificity is optimized for low off-target potential with both in silico and in vitro screening

Nonclinical Safety Elements of Pharmacology

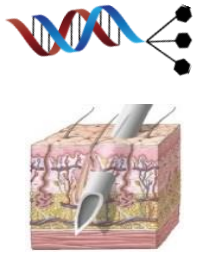
- Secondary Pharmacodynamics
  - Target Safety Assessment should include consideration of non-canonical role of target
  - Off-Target Assessment
    - In silico
      - Search against human genome
      - Predictive modeling based on sequence
    - In vitro
      - Follow-up of potential off-targets identified in silico
      - RNAseq

- Safety Pharmacology
  - Cardiovascular
    - In vitro hERG assay not typically conducted for RNAi therapeutics
    - In vivo assessment can be addressed in stand-alone study in telemeterized monkeys or as an addition to repeat-dose toxicology studies (both include a pulmonary assessment)
  - Central Nervous System
    - Stand-alone study not typically conducted. Neurobehavioral assessments incorporated in repeat-dose toxicology studies

Overview of Pharmacokinetics of GalNAc-siRNAs

Abosortion, Distribution, Metabolism, Excretion (ADME)

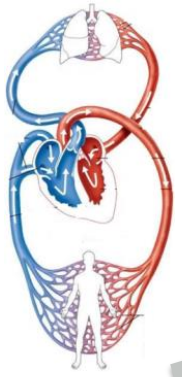
SC Injection



injection site

Near complete absorption

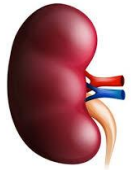
Circulation



Circulation

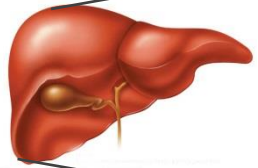
Predominantly delivered to liver

Enterohepatic Circulation



Renal Excretion

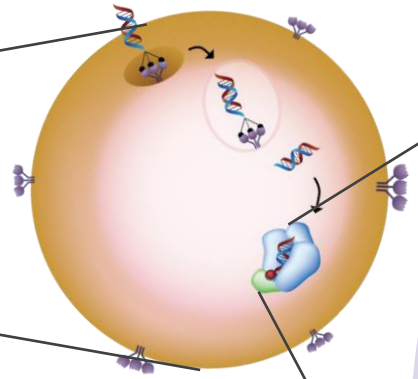
Hepatocyte Uptake



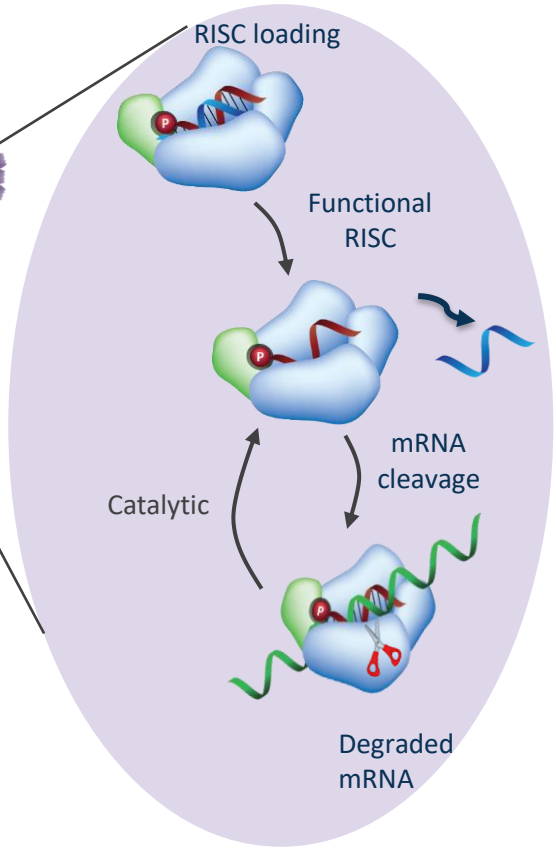
Biliary Excretion

Fecal Excretion

Intracellular: Trafficking and RISC Loading



- ASGPR-mediated hepatocyte uptake
- Ligand deglycosylation
- Intracellular trafficking and release
- Metabolism

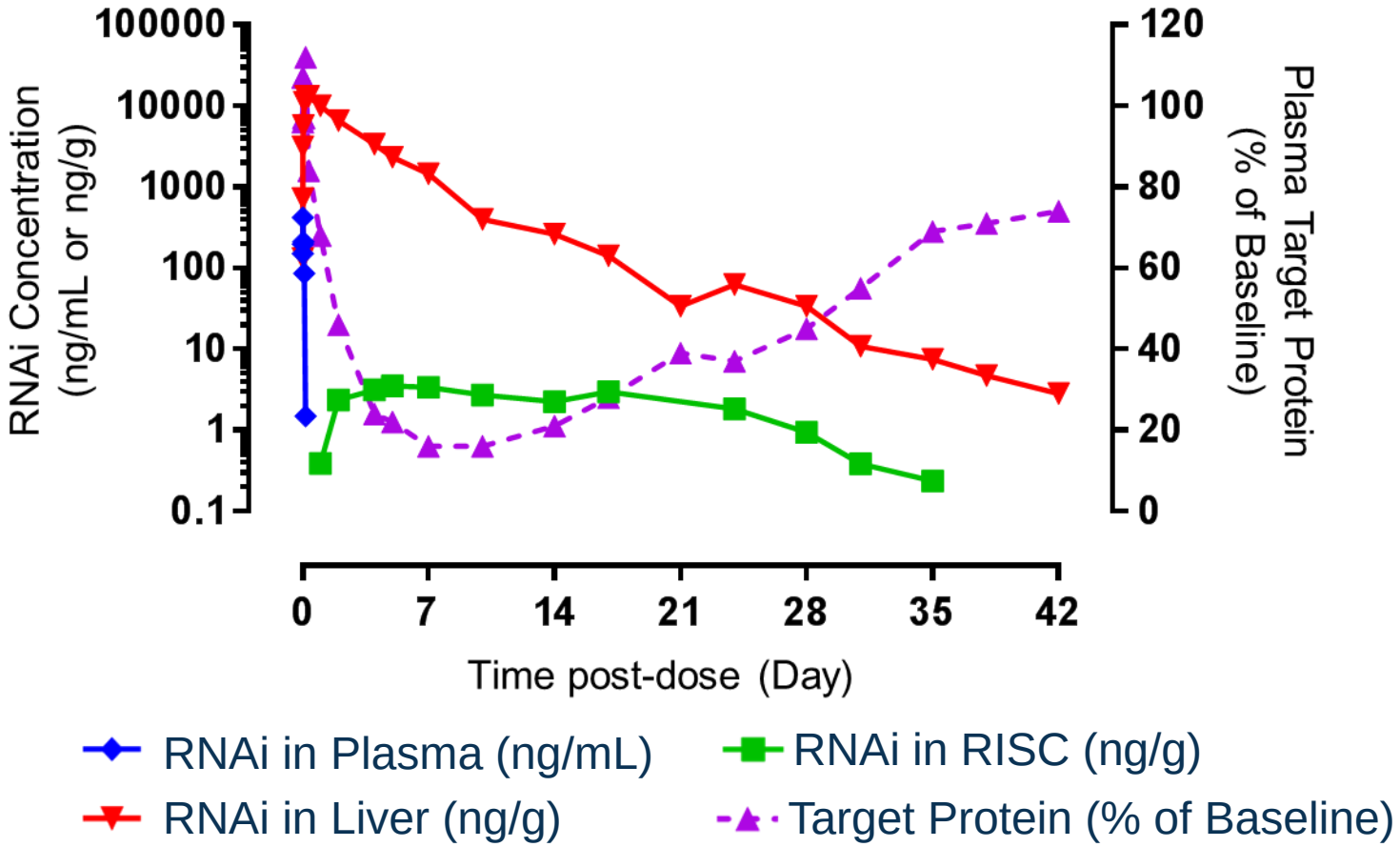


Pharmacokinetics

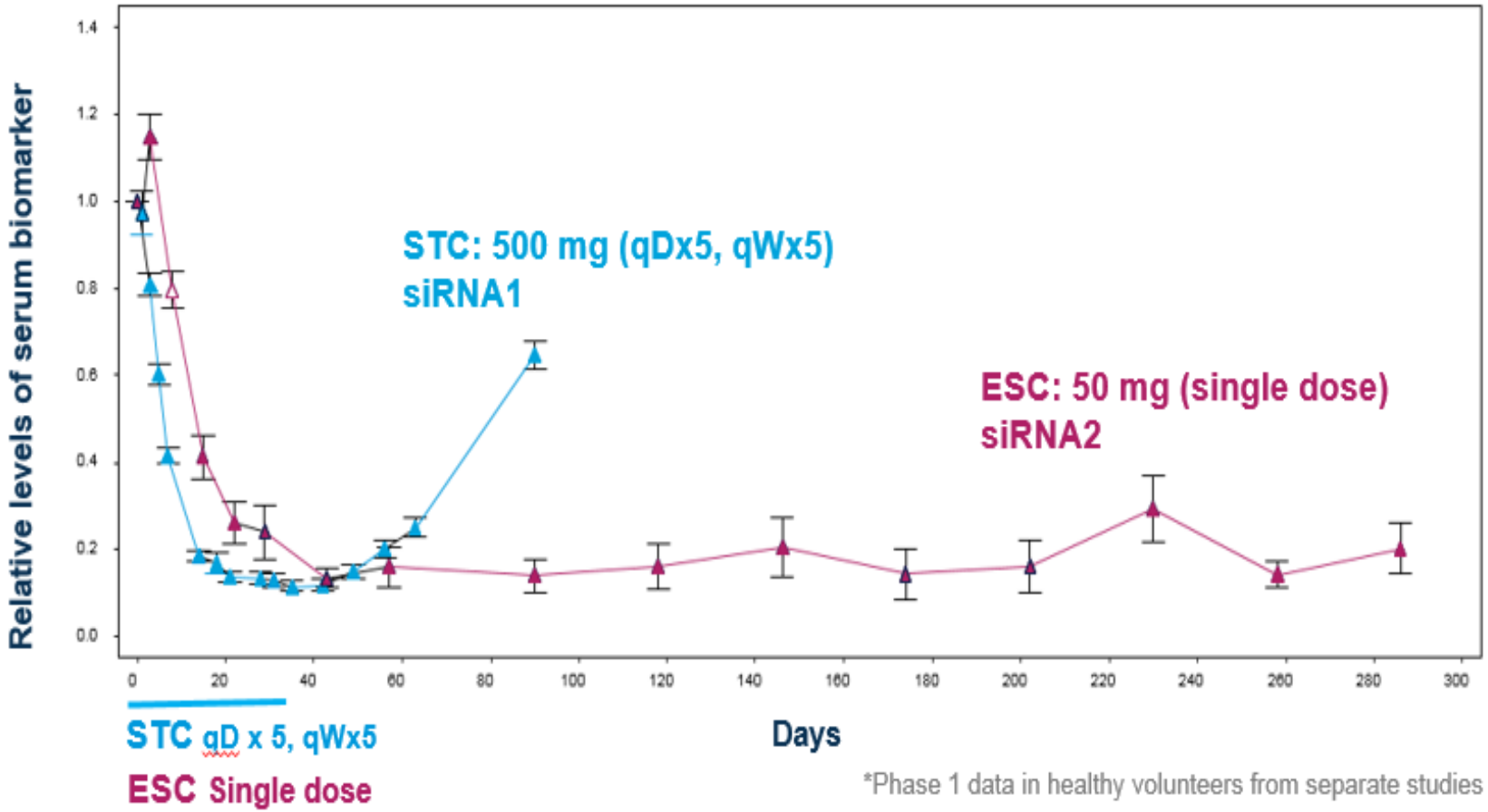
Short Plasma Half-Life Due to Rapid Liver Uptake

- Short plasma half-life due to rapid liver uptake
- Predominantly distributed to liver with long liver half-life
- Maximum PD correlates to maximum RISC loading
- Liver PK and RISC PK are the drivers for PD and PD duration, not plasma PK

RNAi Plasma PK, Liver PK, RISC PK and PD in Mice



Metabolic Stability Is the Key to Potency and Durability



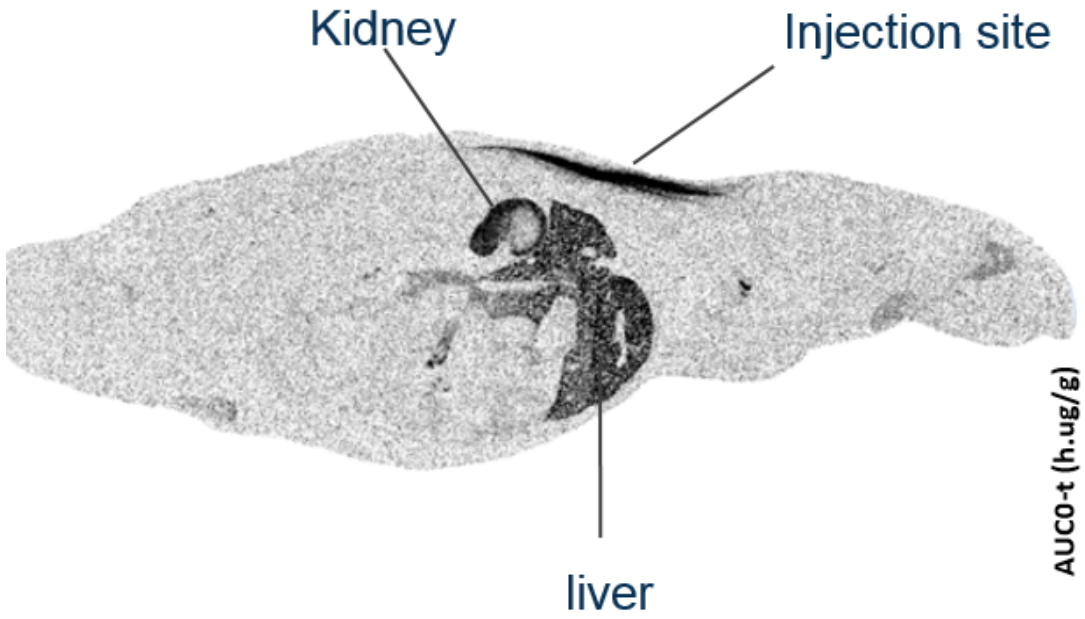
Human pharmacodynamic response with two siRNAs with <u>Same Sequence</u>

- **Different Chemical modifications**
- **100-fold lower dose of siRNA2 same level of knockdown vs cumulative dose of siRNA1**
  - **Single dose of siRNA2 vs Multiple (5) doses of siRNA1**
  - **Large effect on duration of effect observed with siRNA2 much more durable**

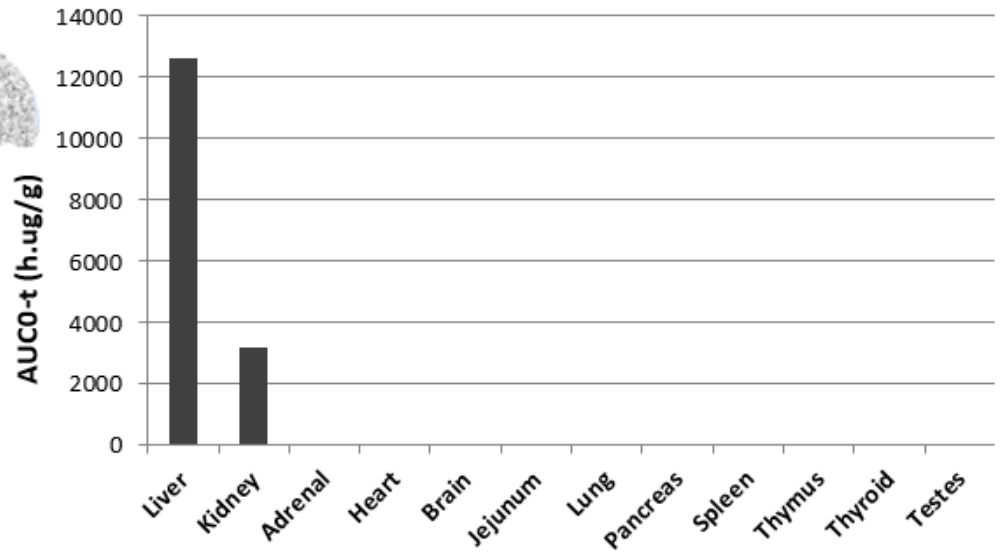
Distribution

Predominantly to Liver

Quantitative Whole-Body Autoradiography of ^{14}C labeled GalNAc-siRNA



Tissue distribution of unlabeled GalNAc-siRNA



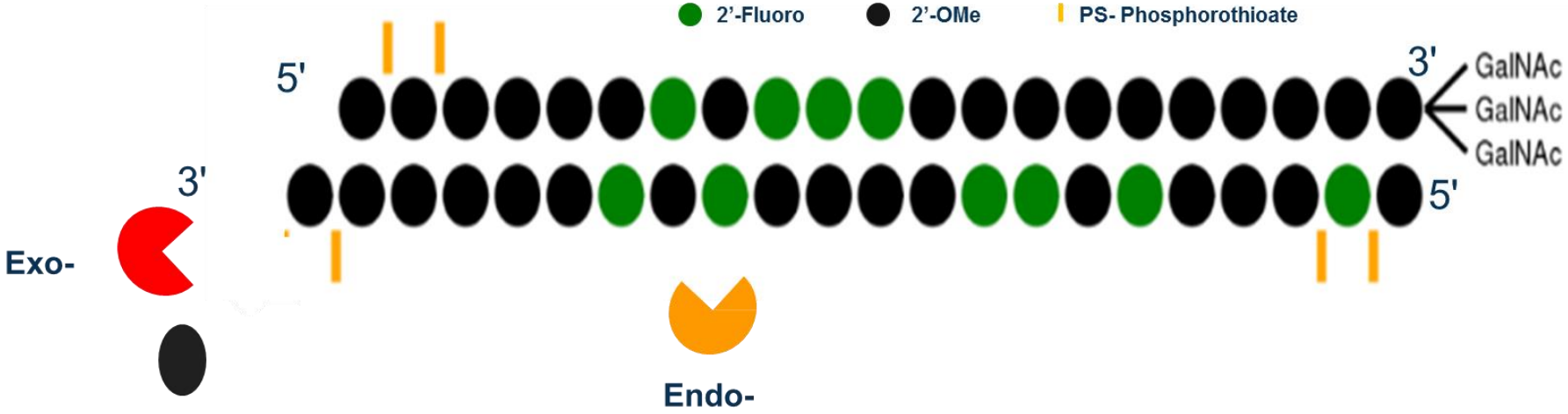
Tissue AUC_{0-t}
(h*µg/mL or h*µg/g)

Plasma	2.79
Liver	12592 (4x)
Kidney	3188
Adrenal	4.35
Heart	7.96
Brain	Not detected
Jejunum	7.90
Lung	9.19
Pancreas	25.2
Spleen	9.60
Thymus	1.72
Thyroid	9.45
Testes	6.00

- GalNAc-siRNAs predominantly distributed to the target organ - liver
- Allometrically scaled liver PK across species

Elimination

Metabolism is the primary route of elimination



3' & 5'-Exonuclease

- End products are mononucleotides

Endonuclease

- Cleaves internally

Localization

- Exo- and endonucleases are ubiquitously distributed in both plasma and tissues
- Most metabolism occurs in liver due to high metabolic stability

Species difference

- Much higher cross species similarity compared to conventional drug metabolizing enzymes

Toxicology Testing Strategy

- Follow ICH M3(R2) guidance → two species in general tox, DART, and CARC
  - Standard genotoxicity battery
  - Some aspects of ICH S6 incorporated
    - Anti-drug antibody
    - Cytokine release assay
  - All definitive toxicology studies conduct under Good Laboratory Practices (GLP) guidelines
  - Repeat-dose toxicity studies through chronic (rodent – 6 months; NHP – 9 months)

- Requires consideration of:
  - Pharmacological-relevance
  - DMPK properties
  - Off-target profile
  - Patient population and clinical dosing regimen

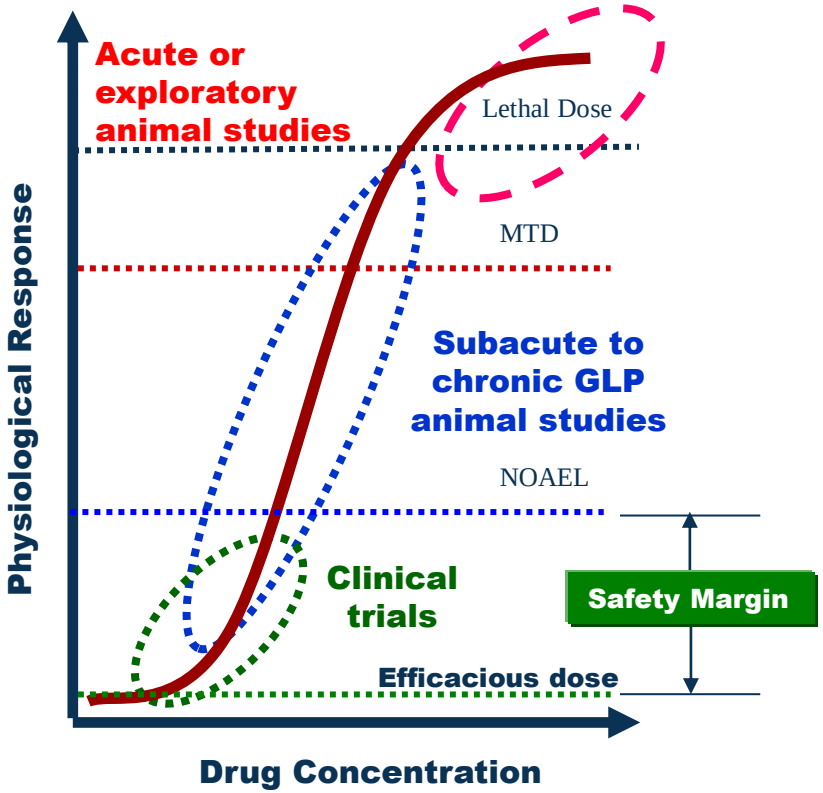
- Design and selection of human RNAi therapeutics optimized for selectivity and potency in humans
  - Typically high homology with monkey
  - Cross-reactivity with rodent as well → not always
  - Target biology could be different across species
  - Off-target profile could be very different across species

- Use of a rodent surrogate molecule not always warranted
  - Implications for DART/CARC
  - Differences in off-target profile, target biology, requires additional resources (CMC, animals, etc)

- Without rodent cross-reactivity, rat can still serve as a model for chemical toxicity while monkey can be used to assess exaggerated pharmacology and chemical toxicity.

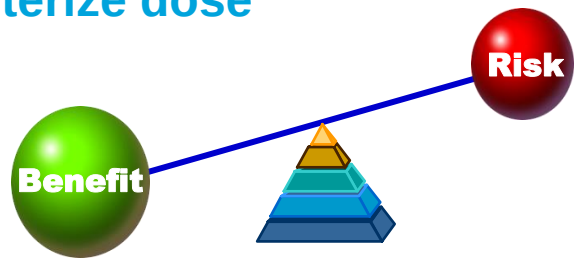
Dose Selection

All substances are poisons … The right dose differentiates a poison..." (Paracelsus,1493-1541)



Toxicity studies designed to characterize dose response curve

- Hazard Identification
  - Toleration and major toxic attributes
  - Relevant target organ toxicity



Risk = Hazard/Exposure

- Lowest dose should identify **N**o Observable **A**dverse **E**ffect **L**evel (NOAEL)

M3(R2) - animal testing should be conducted at doses that supply useful data for human risk

- Exposure margin limit accepted as 50x the anticipated maximum human exposure
- US exception—at least one study ≥1 mo at MTD for Phase III

Dosing Regimen for Toxicology Studies

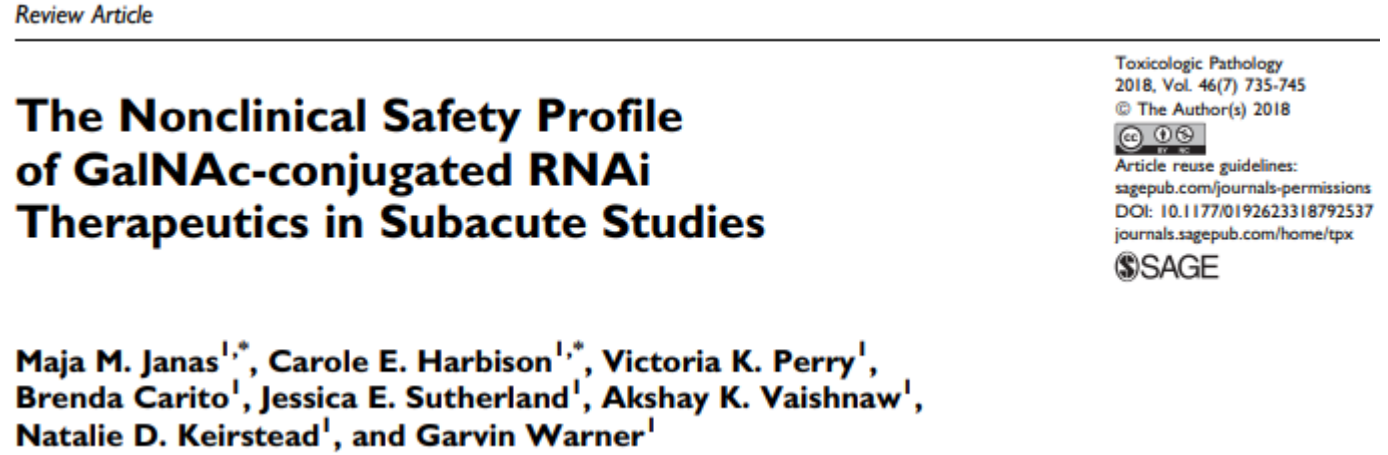
Unique PK/PD relationship impacts study design

- Extended pharmacodynamic activity results in extended dosing intervals
  - For some combination of target and modification chemistry, therapeutic efficacy can be achieved with once every 6-month dosing
  - RNAi plasma concentrations typically below the level of detection at 24 hours post dose

- Nonclinical studies design to support the clinical development strategy
  - Repeat-dose toxicity studies typically employ clinical dosing regimen or exaggerated exposure interval (ex: monthly dosing for quarterly clinical regimen)
    - Careful consideration of the DMPK properties of the compound - avoid tissue accumulation
  - Studies that require daily exposures (ex: embryo-fetal development studies), dose is fractionated to a daily dose
    - Dose-ranging studies critical for pivotal design

RNAi "Class Effects" Observed in Nonclinical Studies

- Published review of RNAi class findings in subacute studies
- Rat typically the more sensitive species (regardless of pharmacological activity)
- Findings generally not considered adverse, partially-to-fully reversible, and dose-dependent
- Findings limited to tissues of pharmacodynamic effect (liver), elimination (kidney) and the reticuloendothelial system (lymph nodes)



Review Article

The Nonclinical Safety Profile of GalNAc-conjugated RNAi Therapeutics in Subacute Studies

Maja M. Janas[1,*], Carole E. Harbison[1,*], Victoria K. Perry[1], Brenda Carito[1], Jessica E. Sutherland[1], Akshay K. Vaishnaw[1], Natalie D. Keirstead[1], and Garvin Warner[1]

Toxicologic Pathology
2018, Vol. 46(7) 735-745

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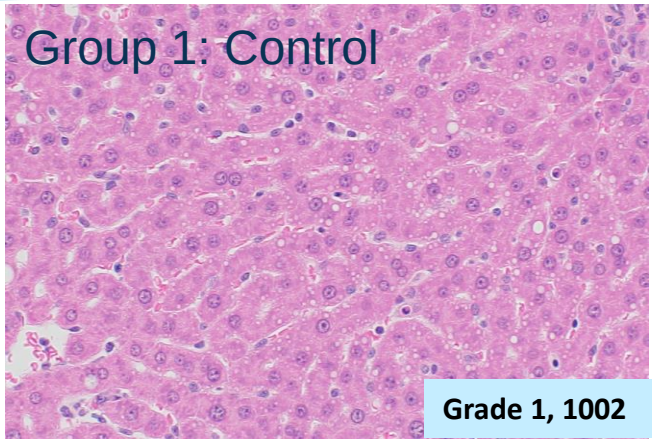
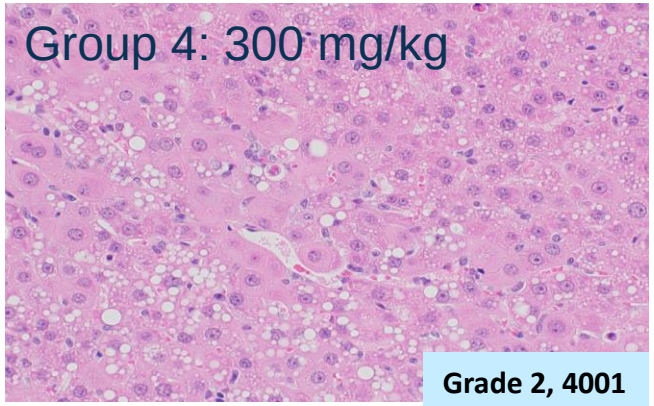
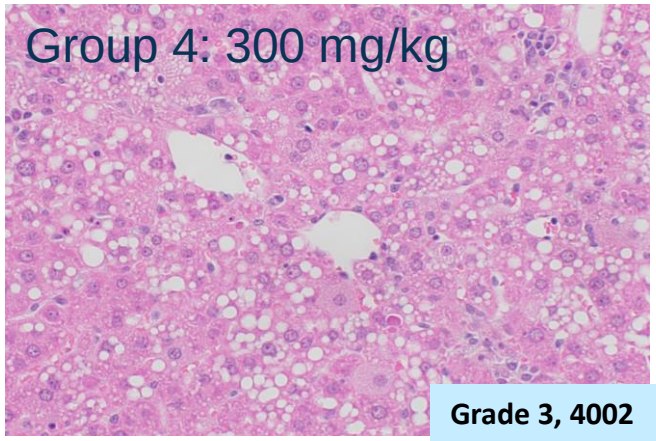






RNAi Class effect - Hepatocellular Findings in Rats

Hepatocellular vacuolation is the most consistent finding in rats

- Not always observed in other species
- Content confirmed as lipid droplets
- Dose dependent incidence and severity

Other hepatocellular findings commonly observed in the rat

- Minimal to mild single cell necrosis
  - +/- Increased mitoses & regeneration

Hepatocellular findings are typically not considered dose limiting and show evidence of recovery

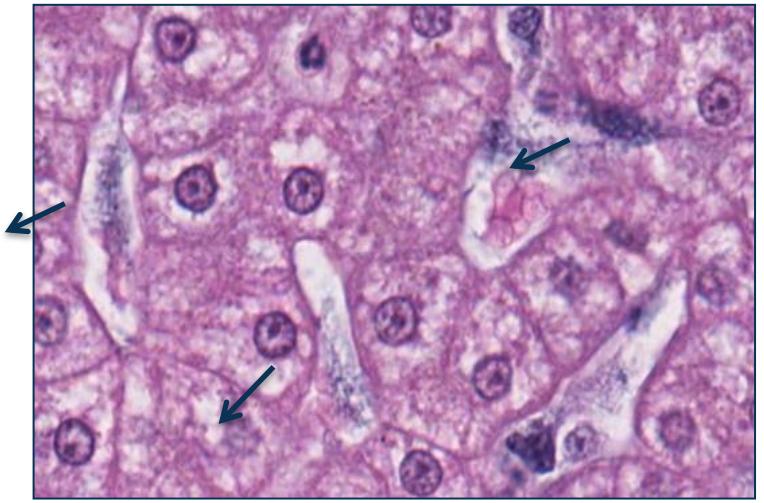
RNAi Class-Wide Findings:

Test Article Accumulation, NHPs

Basophilic granules in macrophages +/- vacuolation

- Kupffer cells, lymph nodes
- Severity: Minimal to moderate, dose dependent
  - Partially reversible in recovery

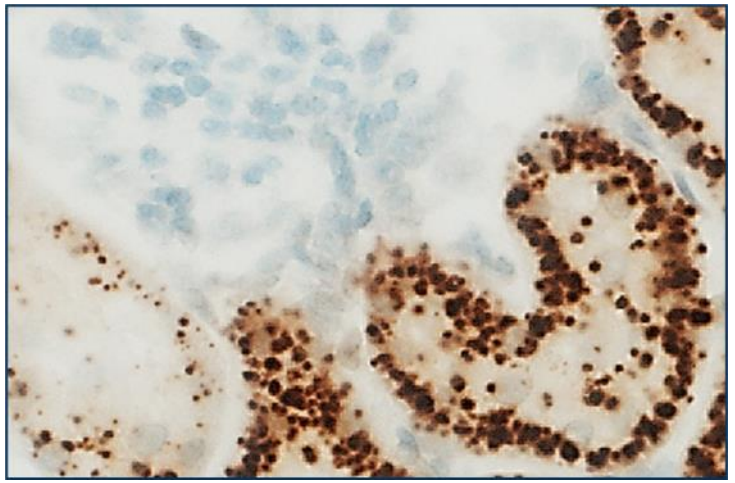
- Not related to setting NOAEL
  - No degenerative or necrotic changes
  - Cytokine panels negative



Liver NHP (RNAi)

Basophilic granules in renal tubule epithelium

- Rat proximal renal tubules
  - Rare in other species, only at extremely high doses
    - not observed in NHP studies
- Not associated with inflammation, degeneration or renal dysfunction
- No evidence of pharmacological activity
- Not related to setting NOAEL



Rat Kidney - Test-article ISH

Summary

RNAi therapeutics are a novel class of medicines with applicability for varied diseases

- Various genetically validated liver targets in active development

RNAi chemistry advances lead to enhanced potency, stability, reduced immune stimulation, and reduced off-target activity

Not a typical small molecule

- RNAi nonclinical safety strategies designed to support clinical development plan and therapeutic use

Despite the highly regulated nature of non-clinical study design, each development strategy is unique to the program and intended indication

RNAi-related nonclinical “class effect” findings generally non-adverse, partially-to-fully reversible, and dose-dependent