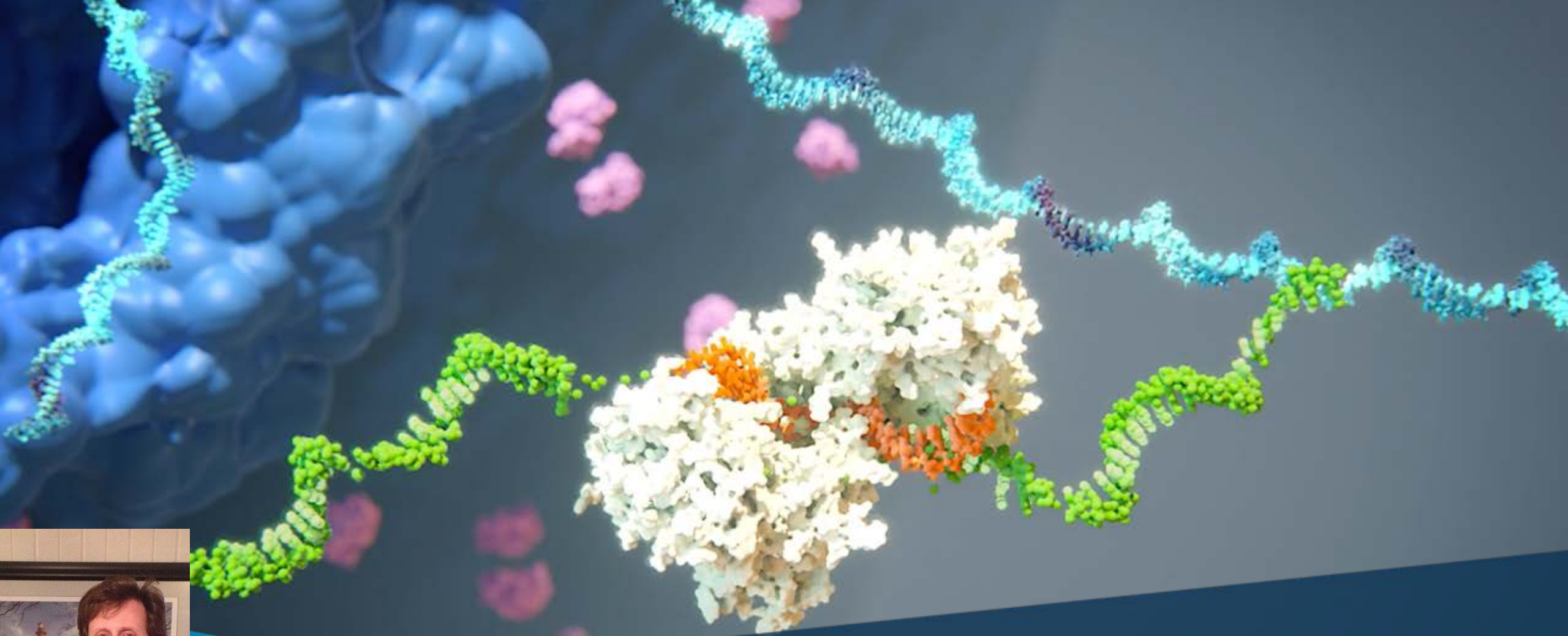


The ADME of siRNA GalNAc Conjugates

Chris MacLauchlin, PhD

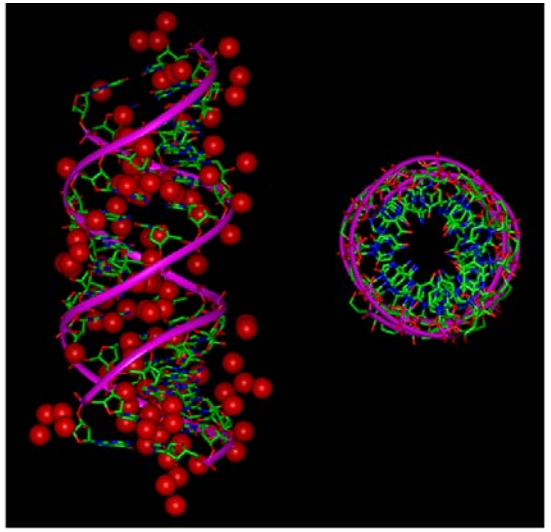
September 27, 2020

Alnylam PHARMACEUTICALS

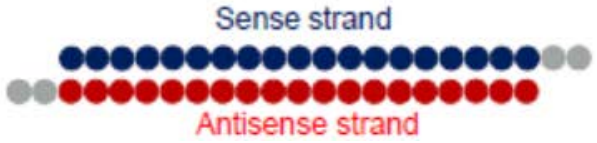
Presentation Outline

- GalNAc Absorption/PK
- GalNAc Distribution
- GalNAc Metabolism
- GalNAc Excretion
- GalNAc DDI profiles
- GalNAc PK/PD insights

Innovation in siRNA to Target Tissues



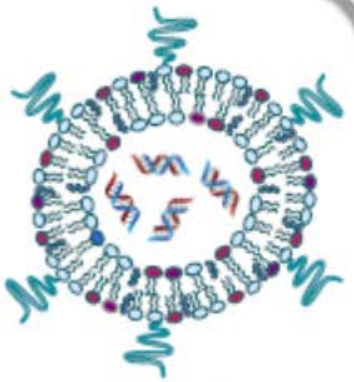
Structure adapted from Klosterman,P. S.; Shah, S. A.; Steitz, T. A. Biochemistry (1999), 38, 14784-14792.



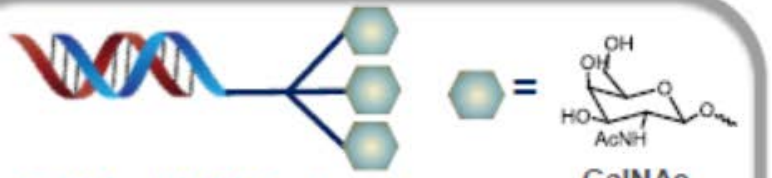
Large MW ~16K
Negatively charged (Na^+ salt)
Highly polar
Aqueous sol >200mg/ml

Lipid Nanoparticles (LNPs)

- Multi-component lipid formulation (~100 nm in size)
- Encapsulated siRNA
- Highly efficient for targeted delivery to liver
- Administered intravenously (IV)
- Clinically validated



Patisiran



GalNAc-siRNA Conjugates

- Single chemical entity
- GalNAc ligand conjugated to extensively modified siRNA
- Targeted delivery to liver
- Administered subcutaneously (SC)
- Clinically validated

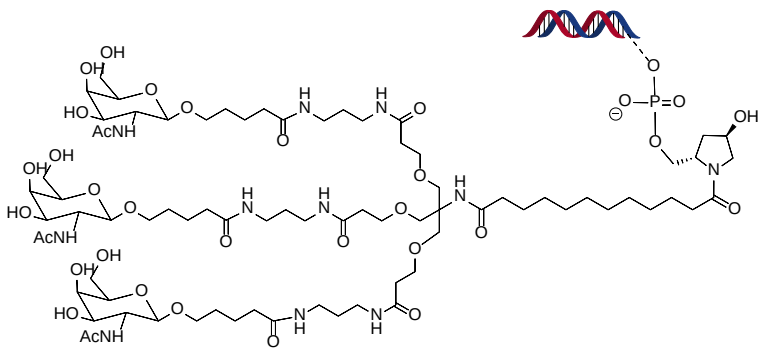
GalNAc-siRNA for Targeted Delivery to Liver

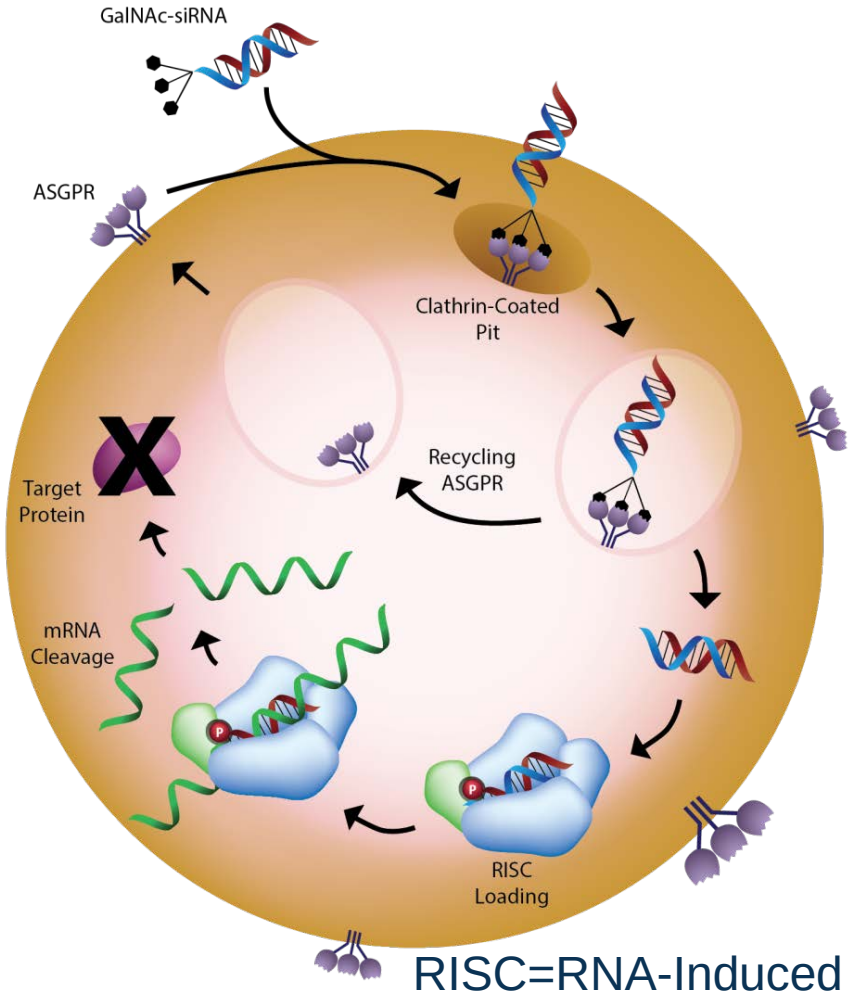
Asialoglycoprotein Receptor (ASGPR)

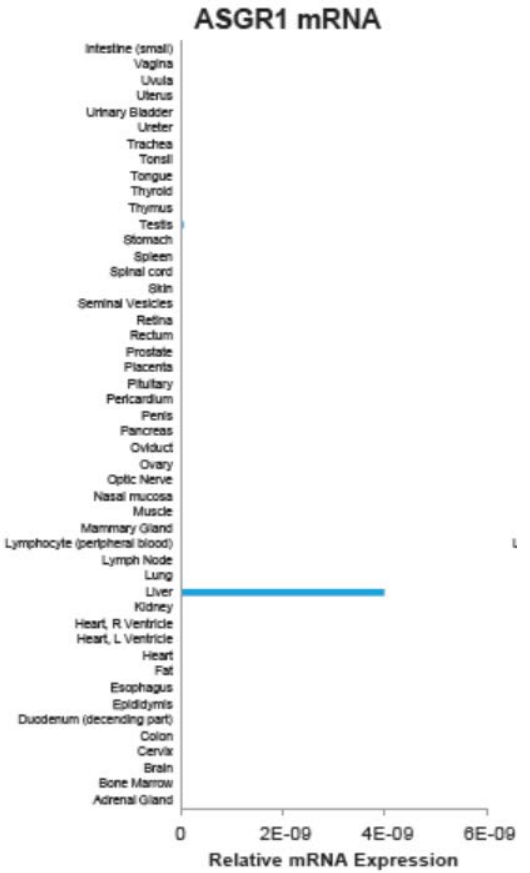
- Highly expressed on hepatocytes
- High capacity receptor
- Conserved across species

Hepatocyte specific ligand

- Trivalent GalNAc conjugated to sense strand
- High affinity and specificity to ASGPR



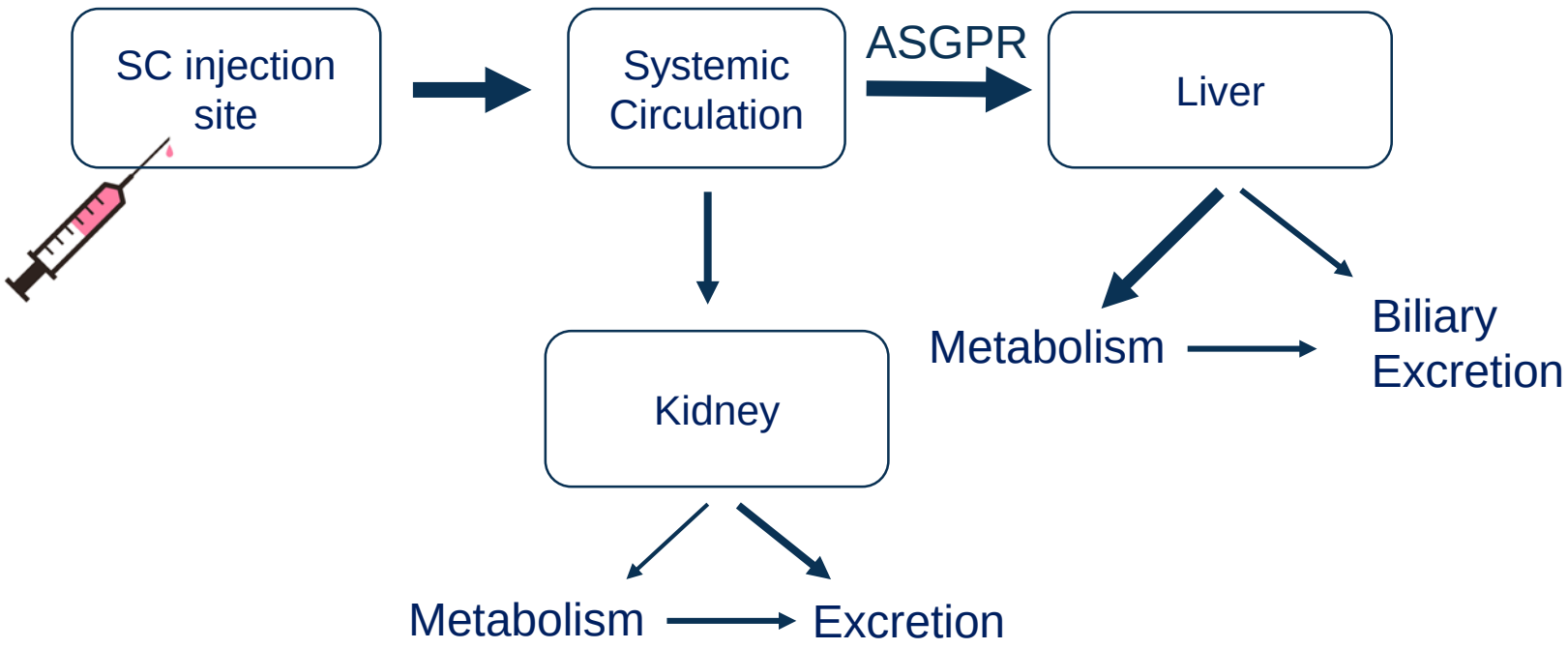




Carito et al
ACVP Meeting 2016

RISC=RNA-Induced Silencing Complex

In Vivo Fate of GalNAc-siRNA after SC Administration



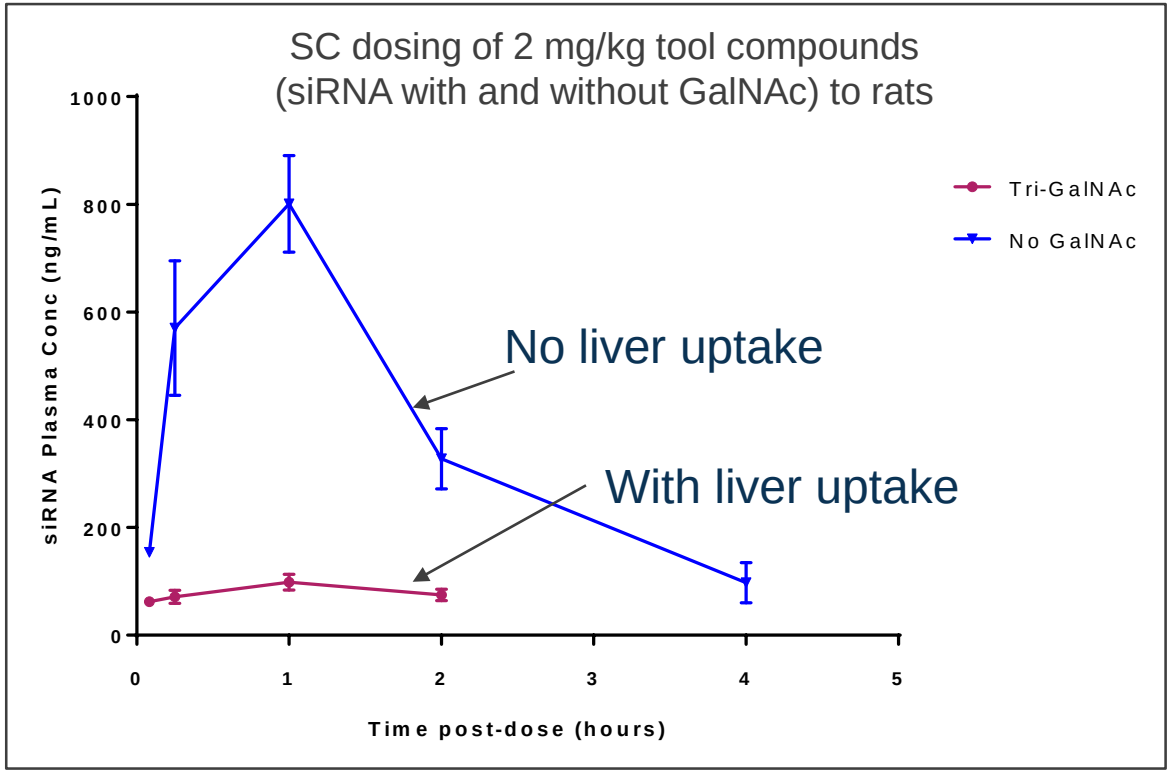
Pharmacokinetics: Transient Plasma Exposure Due to Rapid Liver Uptake

Plasma PK Parameters Across Species

Plasma PK parameter	Rat	Monkey	Human
C_{max}[1][(µg/mL)/(mg/kg)])	0.130	0.286	0.284
AUC_{last}[1] [(hr*µg/mL)/(mg/kg)])	0.373	1.31	2.95
T_{max} (hr)	1.0	1.8	4.1
$T_{1/2}$ (hr)	0.9	1.9	4.8

[1] Dose Normalized. Data from vutrisiran

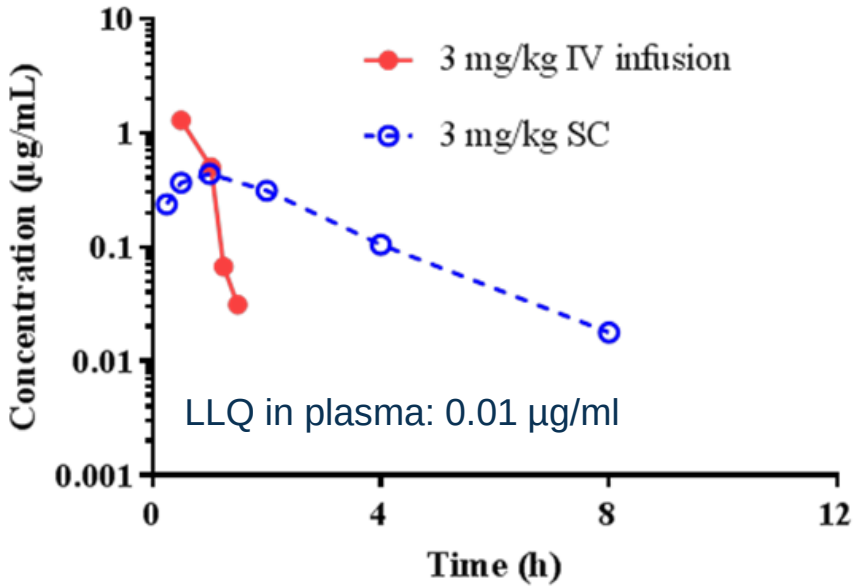
Plasma Exposure Considerably Greater in the Absence of Liver-Targeted Delivery



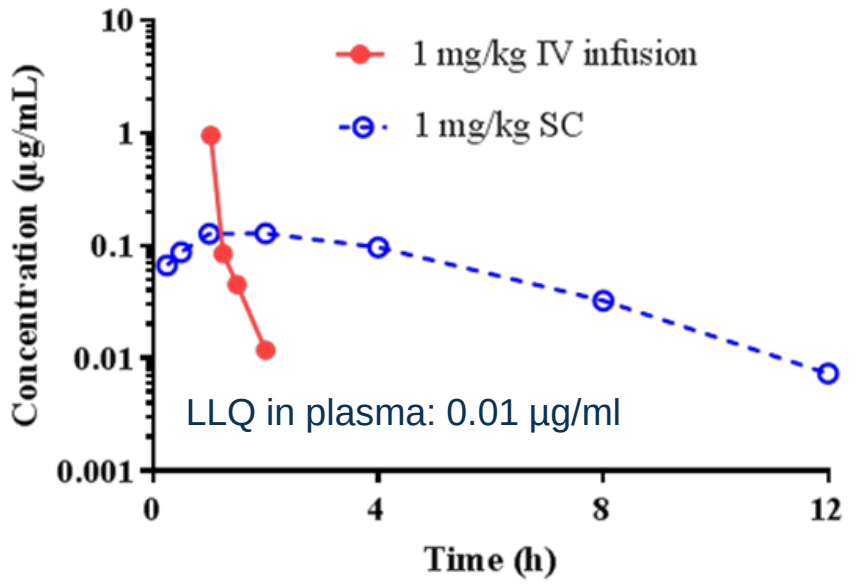
- Low plasma exposure with short half-life due to targeted rapid liver uptake
- Similar plasma PK conserved across species, scaled by allometry

Rat and Monkey PK after IV infusion and SC

Rat

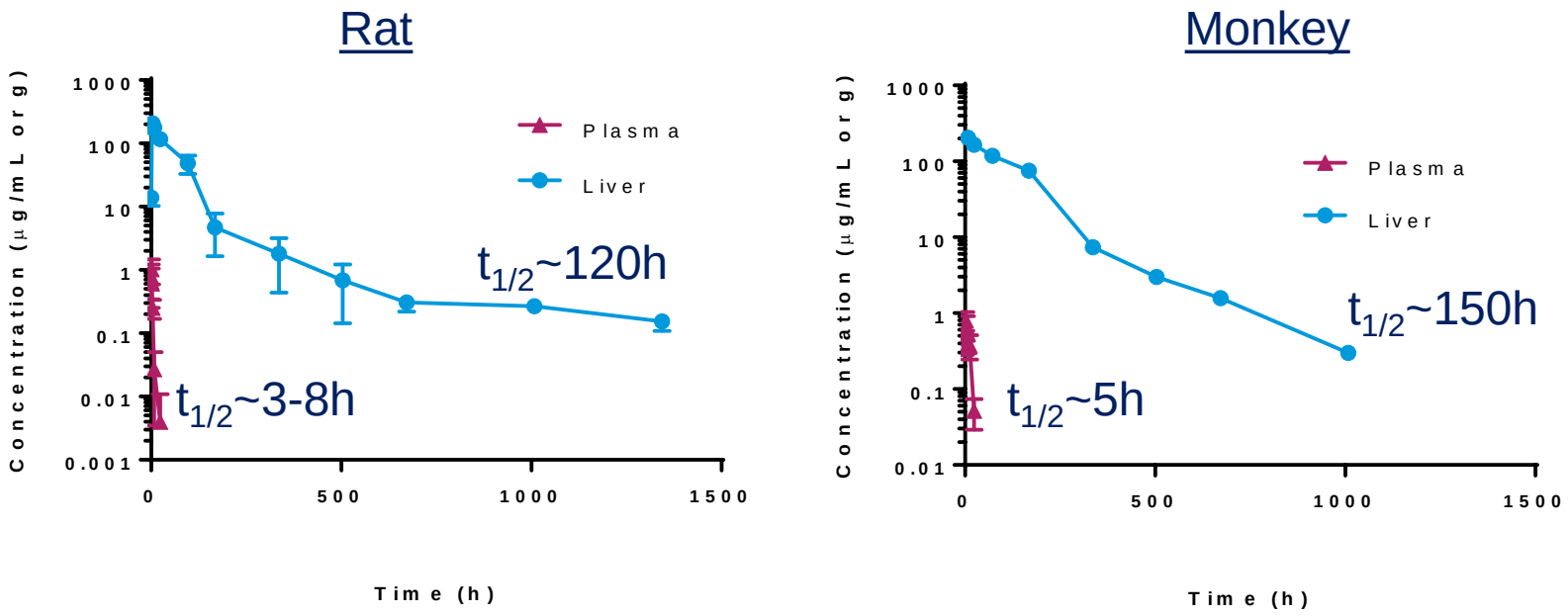


Monkey



SC administration results in a slow absorption from the SC injection site and facilitates efficient hepatic drug uptake without saturation of the ASGPR

Liver PK in Rat and Monkey Shows Preferential Distribution



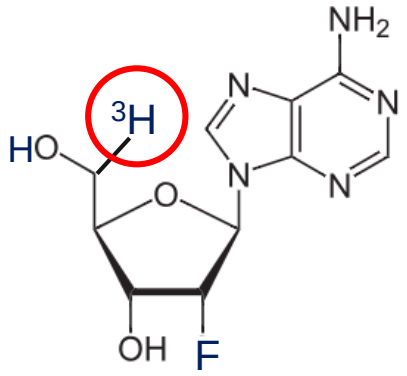
Dramatic difference in plasma PK vs. liver PK

Rat Radiolabeled ADME Study Conducted with Three GalNAc-siRNA Conjugates

Conjugate-A

Conjugate-B

Conjugate-C



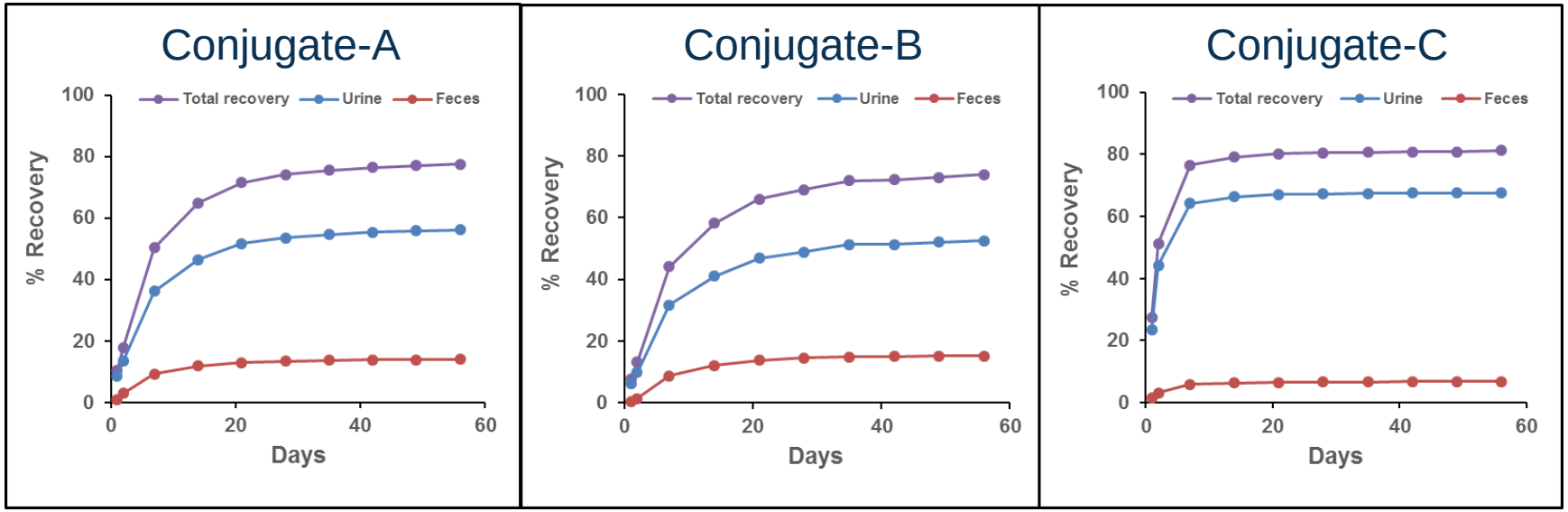
2’F-^{3}H-adenosine

Rat Radiolabeled ADME Study Design

	N (sex)	Sample Collection	Purpose
Intact Rat	4 (M)	Blood, Urine, Feces, Carcasses	Urinary/Fecal Excretion, Mass-Balance, Met ID
Intact Rat	12 (M)	Blood, Carcasses for QWBA	Tissue-Distribution, PK, Met ID
BDC Rat	6 (M)	Blood, Urine, Bile, Feces, Carcasses	Biliary Excretion, Met ID

QWBA=Quantitative whole-body autoradiography
BDC=Bile-duct cannulated (7 days only)

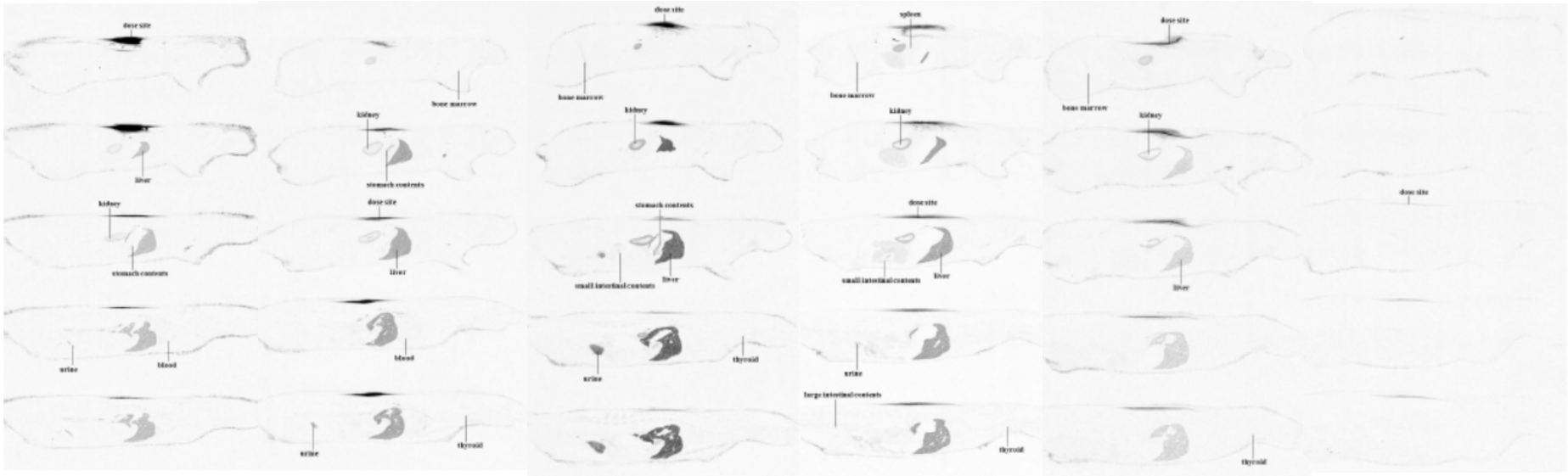
Radiolabeled ADME Study Conducted with Three Conjugates in Intact Rats



	Conjugate-A	Conjugate-B	Conjugate-C
% of Dose Excreted	81	76	82
% of Dose in Carcass	3	3	1
Total % of dose Recovered	84	79	83

Biodistribution Studies Confirm On-Target Delivery

Conjugate-A (40µm thick)



0.5hr 2hr 4hr 96hr 336hr 1344hr

- Rapid distribution to the liver with t_{max} 4-6hr
- Few other organs show significant levels of radioactivity.

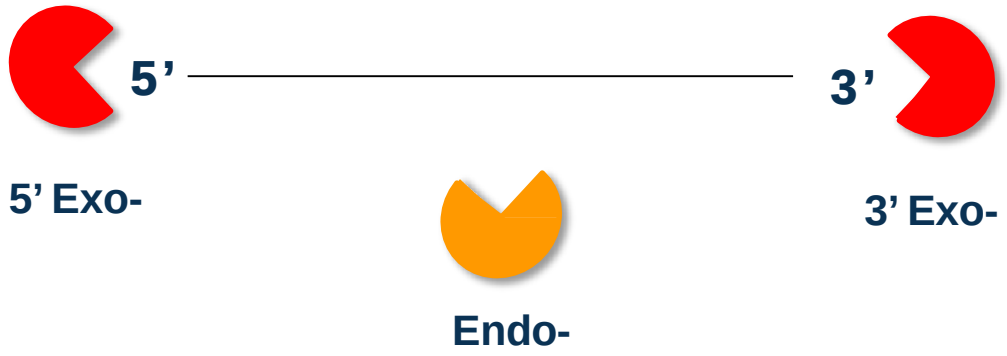
How Do GalNAc-siRNAs Get Metabolized?

3' & 5'-Exonuclease

- End products are mononucleotides

Endonuclease

- Cleaves internally

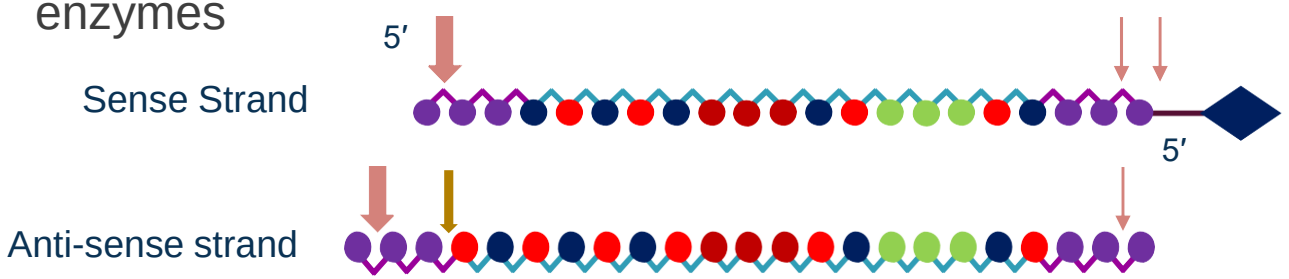


Localization

- Exo- and endonucleases are ubiquitously distributed in both plasma and tissues

Species difference

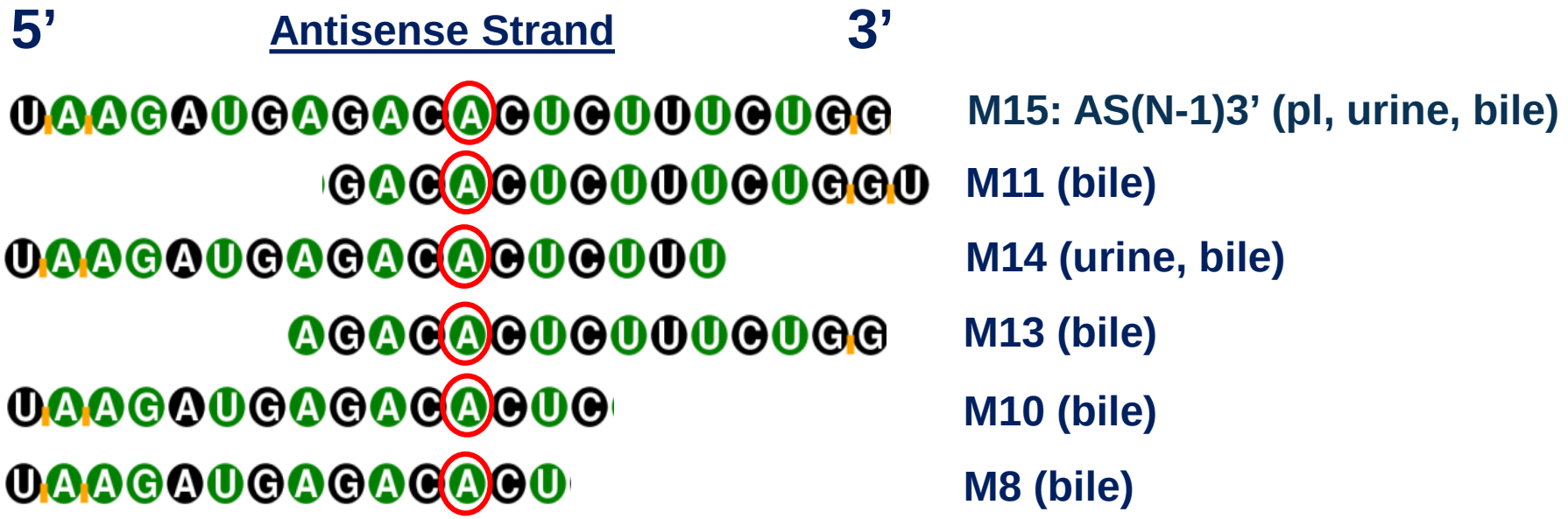
- Much higher cross species similarity compared to conventional drug metabolizing enzymes



Metabolites from 3' -end antisense strand are typically active with comparable potency

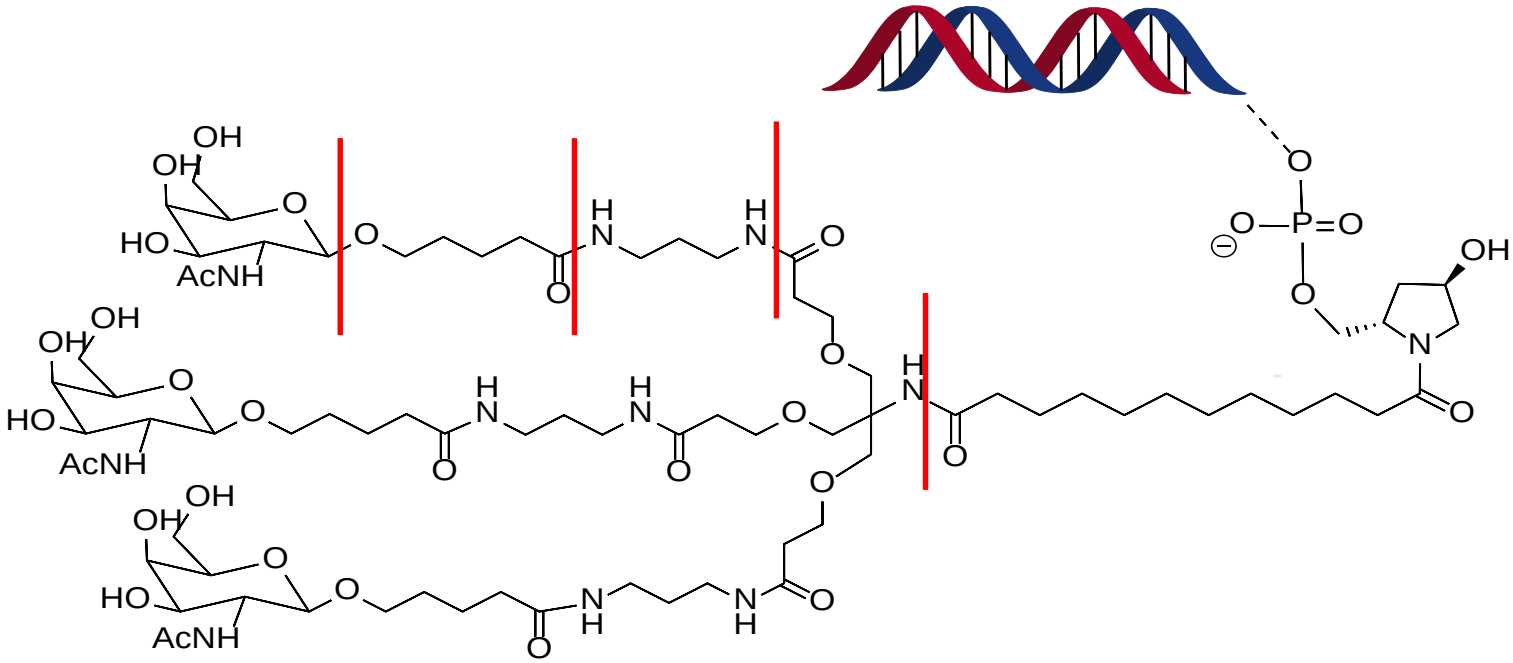
Metabolite Profiles

Metabolism of ^{3}H-labeled (O) siRNA in rats



- M15: AS(N-1)3’ metabolite is the major metabolite in all matrices

Sense Strand Metabolism



- GalNAc moieties are quickly cleaved after uptake into hepatocytes
- The linkers are metabolized by hydrolysis of the amide bonds

Excretion: Minimal Renal Elimination

Species	Conjugate-A	Conjugate-B	Conjugate-A
Rat	15-22% (58% total radioactivity)	11% (53% total radioactivity)	9% (68% total radioactivity)
Monkey	1%	19%	11-24%
Human	6 – 32%	3 – 17%	10 - 25%

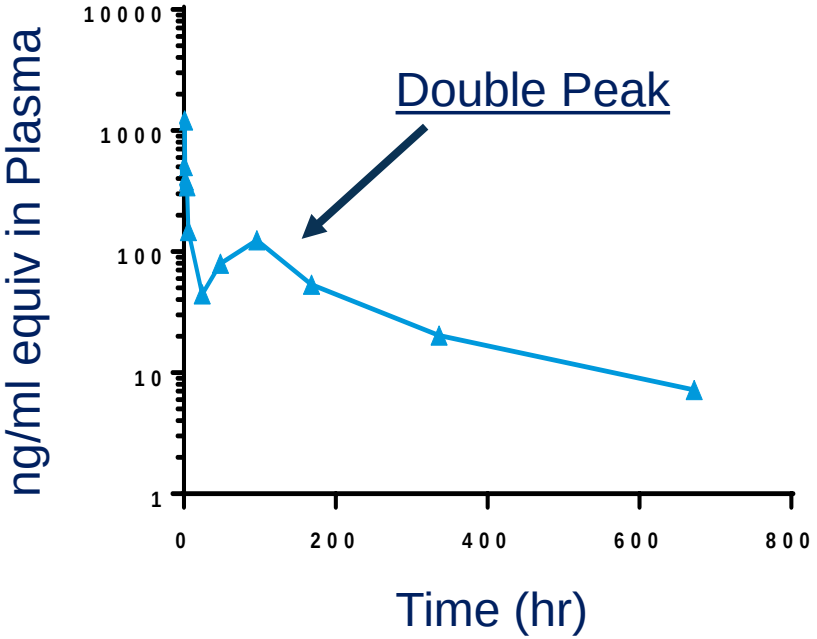
- Renal elimination across compounds is low (<25%)
- Similar data across species

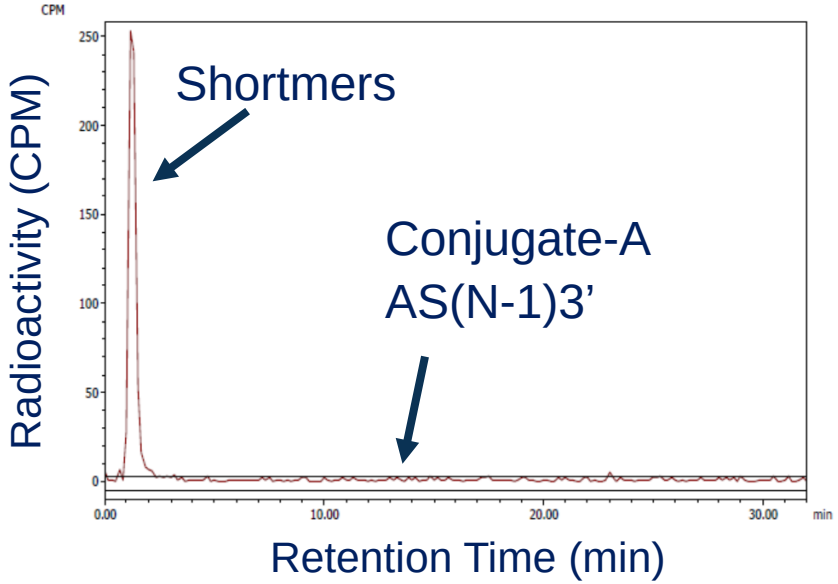
Biliary Excretion

	Conjugate-A		Conjugate-B		Conjugate-C	
	% of Radioactivity Dose Recovered					
	Bile	Feces	Bile	Feces	Bile	Feces
Intact Rat		14		15		7
BDC Rat	27		24		17	

- Total radioactivity recovered in feces (intact rat) was lower than the total radioactivity excreted in bile (BDC rat), suggesting reabsorption of radioactivity via enterohepatic recycling
- Mostly full length and AS(N-1)3’ metabolite found in the bile

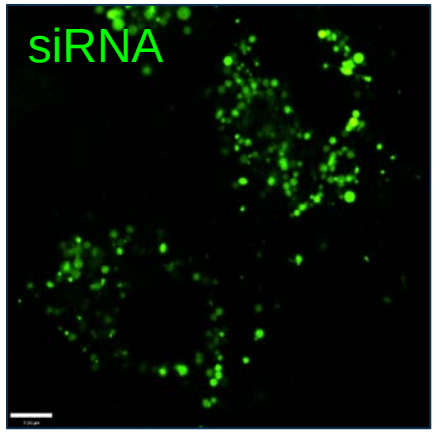
Biliary Excretion (Conjugate-A)

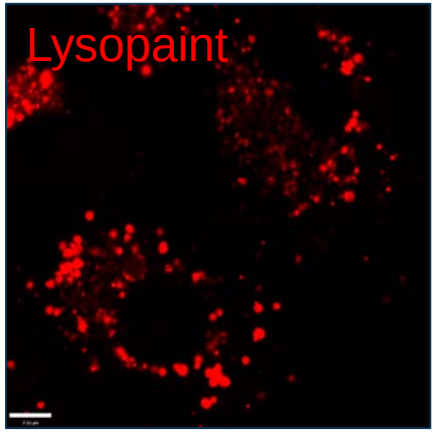


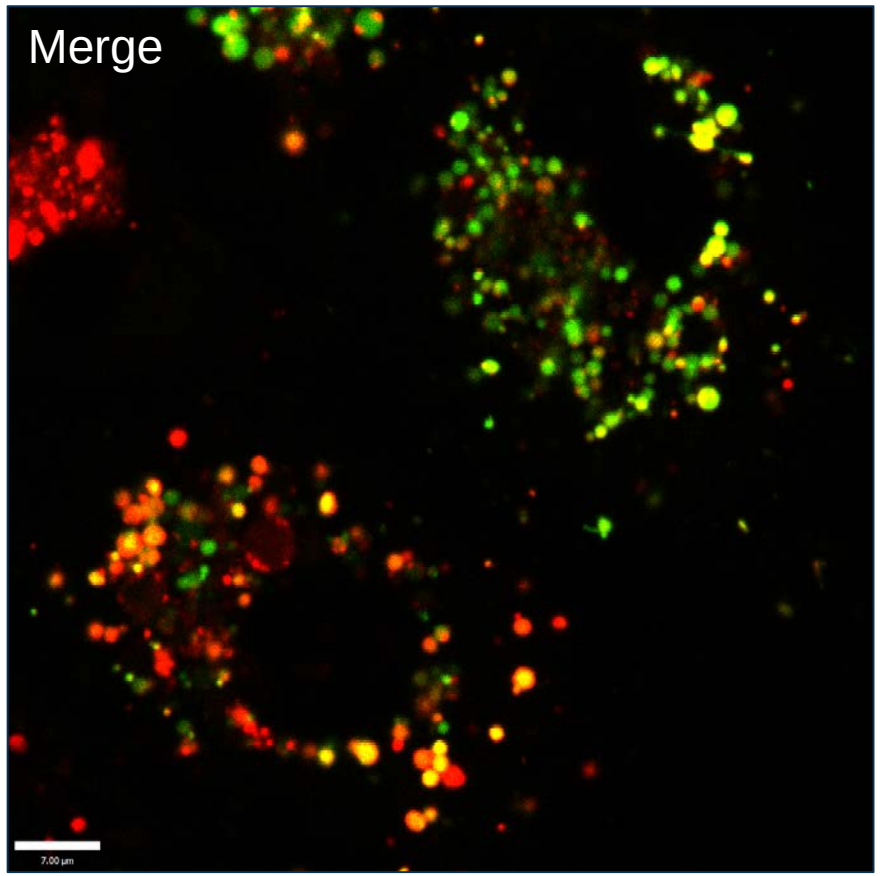


- Classical double peak observed (enterohepatic recycling)
- No Conjugate-A or AS(N-1)3' metabolite detected in plasma
- Full length and AS(N-1)3' metabolite are further cleaved in the GI tract to shortmers, and get reabsorbed

GalNAc-siRNAs Co-localize With Lysosomes





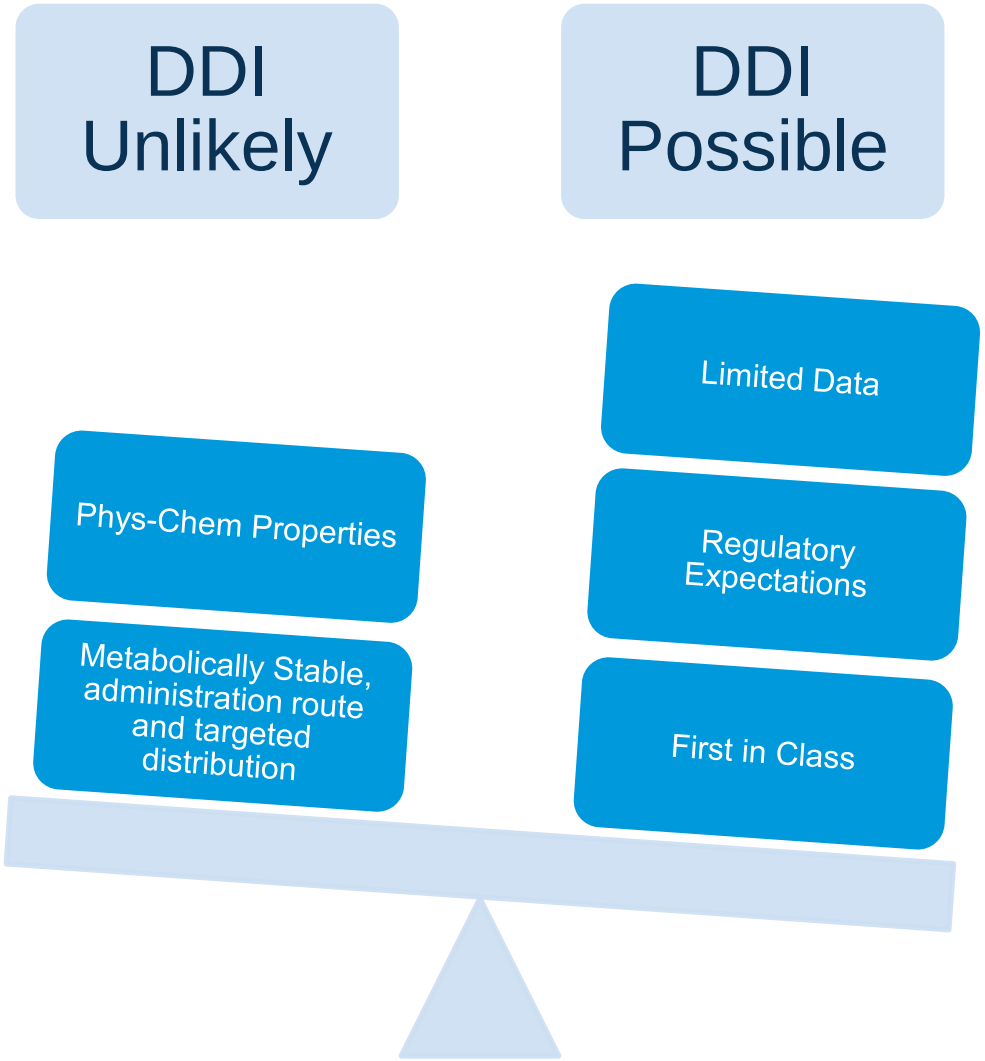


Mouse Hepatocytes (Live cells)

- Free uptake (image taken 4 hours post-dose)
- Lysosomes (Lysopaint)
- siRNA (AD-326763, 10 nM)
- Knockdown not seen until 6 hours

Brown, Nucleic Acids Research, 2020: 1-18.

DDI Strategy at Alnylam



- Mechanistically siRNA DDIs seem unlikely
- Given the limited amount of data and unknown regulatory expectations a traditional approach to DDI evaluation was taken
- Retrospective analysis of the data has allowed a shift in amount of DDI data needed (data has been published: Ramsden D et al., DMD 2019)

Drug-Drug Interaction Potential: Summary

GalNAc-siRNA	CYP substrate	CYP inhibitor		CYP inducer	Transporter substrate	Transporter inhibitor
		Direct	Time dependent			
cemdisiran	X	X (CYP2B6 = 583 µM, CYP2C8 = 224 µM) [clinical] = 0.048 µM	X	X	Not evaluated	Not evaluated
HBV01	X	X	X	X	Not evaluated	Not evaluated
vutrisiran	X	X	X	Not evaluated		
AAT01	X	X	X	Not evaluated		
fitusiran	X	X (CYP2C8 = 56 µM) [clinical] = 0.0092 µM	Not evaluated	X	X	X
givosiran	Not evaluated	X	X	X	X	X (P-gp = 4.2 µM) [clinical] = 0.02 µM
revusiran	X	X	Not evaluated		X	X
lumasiran	X	X (CYP2C8 = 416 µM) [clinical] = 0.061 µM	X	Not evaluated		
inclisiran	Not evaluated	X	X	X	X	X
AGT01	Not evaluated	X	X	Not evaluated		
AAT02	Not evaluated	X	X	Not evaluated		
HBV02	Not evaluated	X	X	Not evaluated		

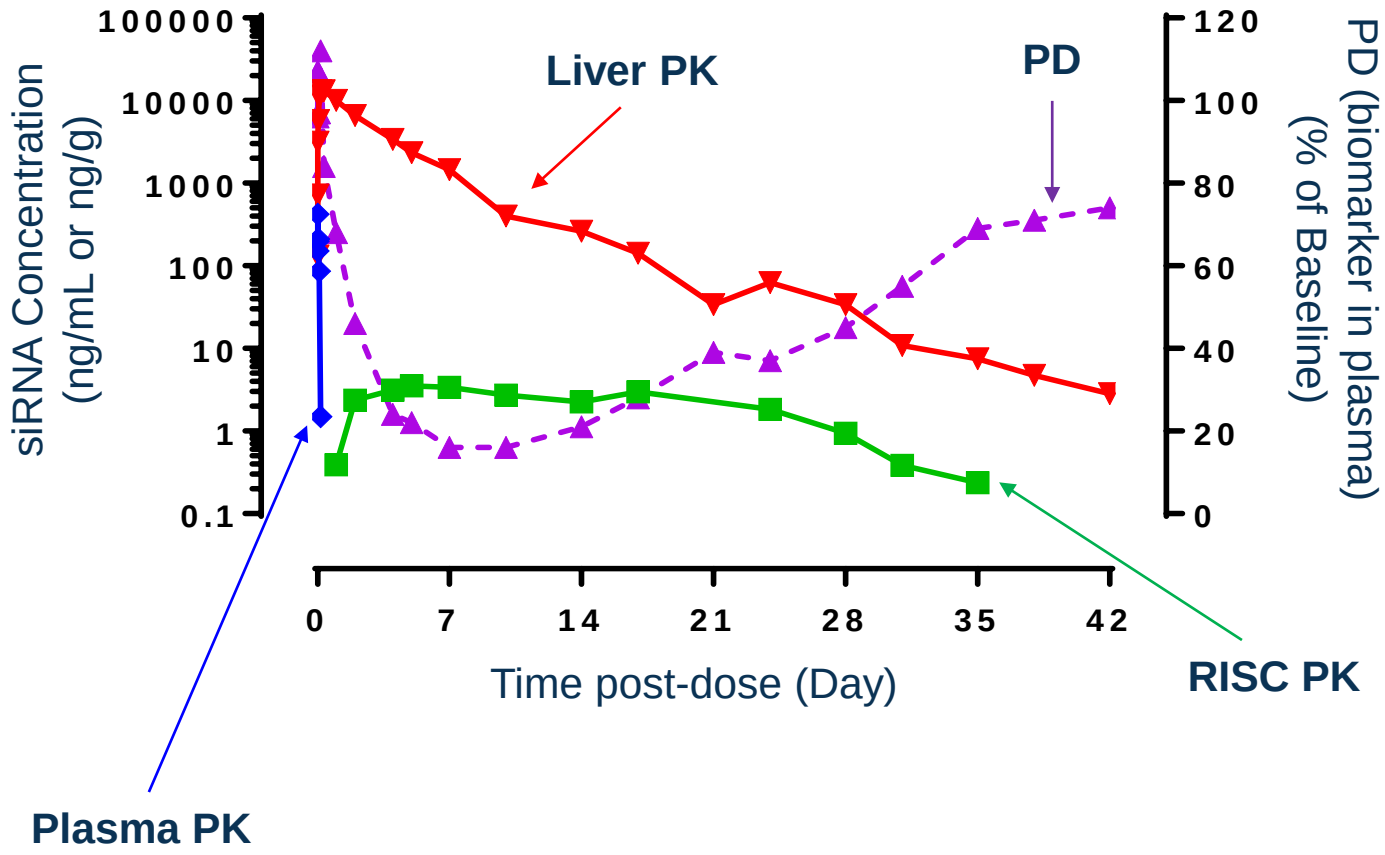
GalNAc-siRNAs are not:

- Substrates of major CYPs or transporters
- Inducers of drug metabolizing enzymes
- Likely to cause clinically meaningful inhibition of CYPs or transporters

Major CYP isoforms (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4/5) Major transporters (P-gp, BCRP, BSEP, MATE1, MATE2-K, OATP1B1, OATP1B3, OAT1, OAT3, OCT1 and OCT2)

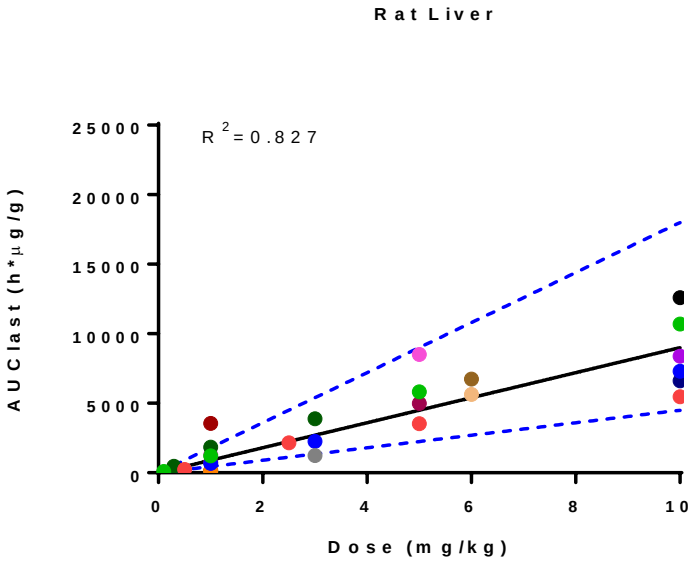
Ramsden D et al., DMD 2019

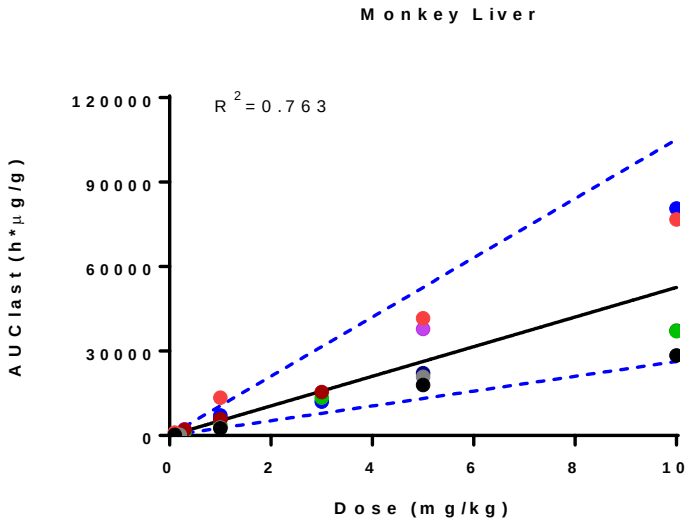
Plasma PK, Liver PK, RISC PK and PD in Mice



Cross Platform PK/PD Insights

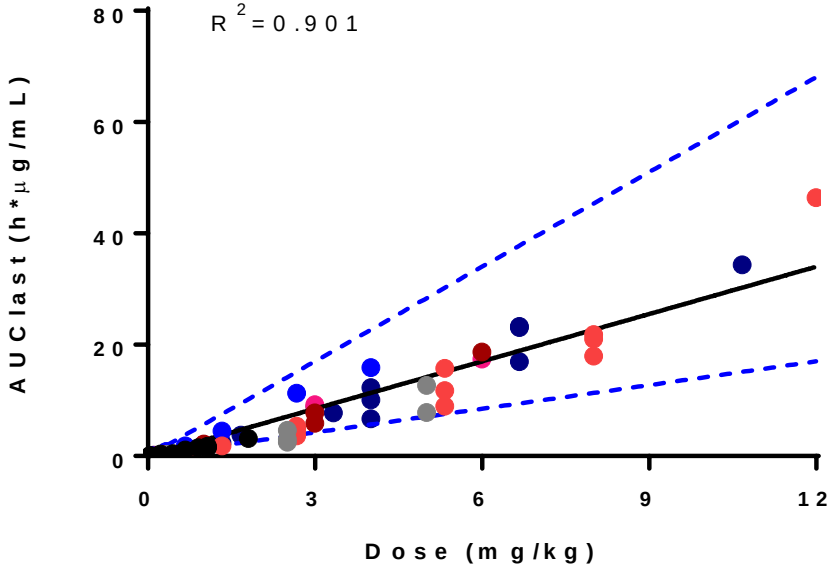
(8 Conjugates)





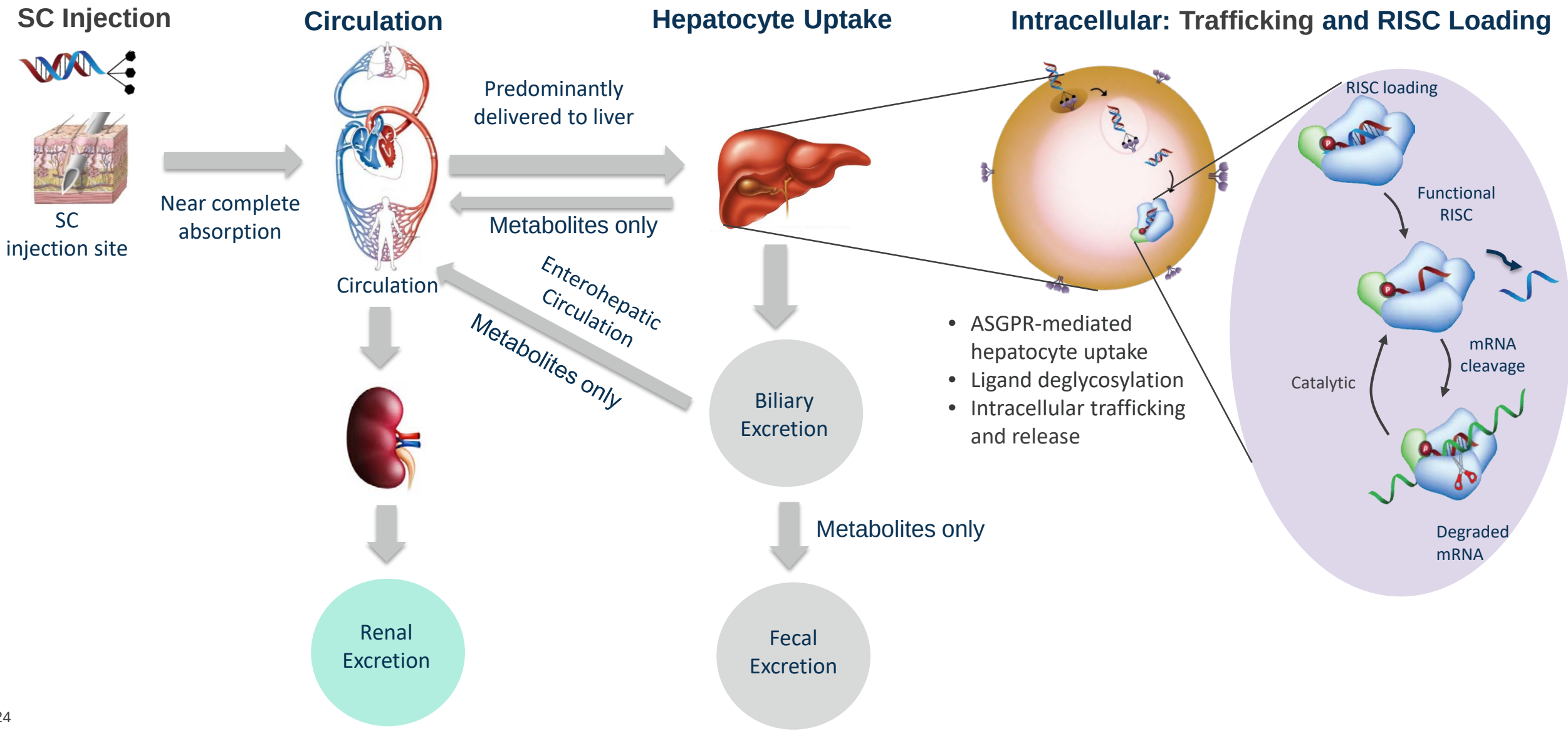
Monkey and rat liver exposure is similar across platform

Human Plasma Exposure



Human plasma exposure is similar across platform

ADME Summary of GalNAc-siRNAs



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Kirk Brown

Chris Brown
Martin Maier
Ivan Zlatev
Varun Goel
Pete Smith

Alnylam Early Development Group

Alnylam RNAi Platform Team

To those who say “impossible, impractical, unrealistic,” we say:

CHALLENGE ACCEPTED

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Alnylam PHARMACEUTICALS